Université de Liège

GIGA

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Wallonie

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The GIGA: an innovative concept!

«The researchers have long ago understood the virtues of cooperation, synergies, exchanges and networking. But there's sometimes many a slip between the cup and the lid, so that between the recognition of the benefits of this mode of organization and work and its implementation, strong bridges are still to build.

So, there are some who speak and some who do!

The initiators, researchers and heads of the GIGA have truly sublimated this process of working together, which catalyses success and fertilizes the emergence of knowledge.

It's spectacular! Even the structure of the premises is the result of a thinking marked by this willingness to collaborate. The researchers are mixing together, the experimenters are meeting one another, the test pieces are colliding and the scientific results are abounding.

The landscape of the research and innovation in Belgium is characterized by two elements: first, the high quality of the researchers who are part of it, secondly, the atomization and fragmentation of the means agencies and the sources of funding. The availability of credits suffers from it. The GIGA model shows, not only in this context, but generally, that we can do much better with as much, if the resources are mobilized intelligently.

The GIGA is a concept and a method. A source of inspiration too: the researchers are constantly subject to international peer-review which leads to a salutary questioning that is sometimes lacking elsewhere. And the results are spectacular. The number of publications and their quality are only increasing.

As a provider of funds for research in all the countries, I can only welcome this approach as innovative as the result of your work.»

Philippe Mettens
Chairman of the Executive Committee
Federal Public Service – Science Policy

Board of Governance
Chairman: J. Martial

Coordination - Administration

GIGA-Research
Chairman: J. Boniver
Director: J. Piette

- GIGA-Cancer
- GIGA-Development, Stem cells & Regenerative medicine
- GIGA-Genetics
- GIGA-Infection, Immunity & Inflammation
- GIGA-Neurosciences
- GIGA-Signal Transduction
- GIGA-Systems biology and Chemical biology

GIGA-Platforms
Coordinator: C. Sadzot
Business developer: M. Maurin

- GIGA-GenoTranscriptomics
- GIGA-Proteomics
- GIGA-Imaging & Flow Cytometry
- GIGA-Bioinformatics
- GIGA-Mice facility & Transgenesis
- GIGA-Zebrafish facility & Transgenesis
- GIGA-ImmunoHistology (new - 2011)

GIGA-Tech transfer

GIGA-Business

GIGA-Training Center

- Highlight : Dentin matrix protein 1 induces membrane expression of VE-cadherin on endothelial cells and inhibits VEGF-induced angiogenesis by blocking VEGFR-2 phosphorylation
- Roles of the siblings proteins during tumor development: regulation of the cancer stem cell phenotype by osteopontin
- Roles of the siblings proteins during angiogenesis: mechanism (s) of action and characterization of dmp1 as an anti-angiogenic factor
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- Role of HDAC5 in DNA replication process
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- Therapeutic evaluation of the use of peptides derived from the prolactin/growth hormone family
- Understanding the mechanism of action of 16K fragments of the prolactin/growth hormone
- Identification of new modulators of angiogenesis
- VEGF111, alternative splicing and cancer
- Functions of aminoprocollagen peptidases (ADAMTS2, 3 and 14)
- Intracellular signaling by the RhoGTPase network
- Mechanisms of thoracic cancers: epigenetic therapy and identification of biomarkers
- Epigenetic regulation of angiogenesis
- Mechanisms and novel therapies of deltaretrovirus induced diseases
- Personalized medicine for gliomal tumors
- HPV and the sequence "metaplasia-dysplasia-cancer" sequence
- Genetic alterations and YY1 activity in colon adenocarcinoma
- Biomarker discovery based on DNA methylation and validation for endometrial and cervical cancer diagnosis
- Neuropilin-1, a newly identified coreceptor for leptin: molecular Characterization and pathophysiological applications

- Highlight : Neuregulin-1 modulates the differentiation of neural stem cells in vitro through an interaction with the Swi/Snf complex
- Role of transcription factor Pax6b in the differentiation of pancreatic endocrine cells
- Nkx6.1 and nkx6.2 regulate α and β cell formation in zebrafish by acting on pancreatic endocrine progenitor cells
- Cartilage and bone development and homeostasis
- The anterior pituitary in zebrafish
- Zebrafish as model system for toxicology and pharmacology
- Contribution to deciphering Oct4 regulation in Human embryonic stem and carcinoma cells in response to hypoxic culture conditions

- Highlight : CCDC39 is required for assembly of inner dynein arms and the dynein regulatory complex as well as normal ciliary motility in humans and dogs
- Searching for rare susceptibility variants for Crohn's disease by massive parallel resequencing.
- Towards multi-tissue genetic, epigenomic, transcriptomic and microbiomic annotation of loci associated with inflammatory bowel disease (IBD).
- Towards a meta-GWAS for IBD
- Positional identification of genes predisposing to familial forms of complex diseases
- Deciphering the molecular origins of brain damage in phenylketonuria (PKU)
- Forward genetic dissection of monogenic disorders in the dog
- Positional identification of genetic determinants of variation in blood pressure, glucose and lipid metabolism in healthy dogs
- Genomic surveillance and management of inherited defects in livestock
- Positional identification of QTL and QTN in livestock
- Development and application of statistical tools for genomic selection in livestock
- Genetic analysis of male recombination and segregation distortion in cattle
- Analysis of Copy Number Variation (CNV) in livestock
- Towards molecular understanding of polar overdominance at the ovine callipyge locus
- Patrocles: polymorphic miRNA-mediated gene regulation
- Engineering male-specific double-muscling
- Towards semen sexing using a transgenic approach

- Highlight : Discovery and biochemical characterization of four novel biomarkers for osteoarthritis
- Viral induction of Zac1b through TLR3- and IRF3-dependent pathways
- Generation of high and long term efficient hematopoietic stem cell engraftment and mounting of HIV specific cellular and humoral immune response in humanized NOD/LtsZ-scidIL-2Rγnull mice
- Insights into the role of the dual-specificity phosphatase VHR (Vaccinia Virus H1-related) in inflam-

GIGA-Research

«The extraordinary acceleration in knowledge brought by whole genomes sequencing, the global and dynamic apprehension of the protein interactome and the fantastic progress in cellular imaging methods have already and will continue to open novel diagnostic and therapeutic options in treating the major pathologies afflicting the human population. The design and development of appropriate cellular and animal models are also more then ever required to apprehend and investigate the integrated nature of biological processes and molecular pathways, both in normal development, tissue homeostasis and in pathologies that emerge when these processes go awry.

The GIGA concept, that associates multi-disciplinary research of high quality in the context of permanently ongoing scientific exchanges, top-level teaching and education, close association and collaborations with the hospital, fostering of young biotech companies and contacts with established industry, already allowed to accompany or even anticipate these major mutations in biological sciences.

Continue to build this concept by finding novel ways to even more facilitate the interactions between seemingly distant research fields, adapt it to emerging technologies by insuring high-standart platforms and devoted personnel, welcome talented researchers must remain major objectives of this endeavour. The enthousisasm, dynamism and willingness to go forward that can easily be perceived from the GIGA personnel are fertile grounds to the determined pursuit of these objectives. They are the guarantee to see GIGA pursue its well-engaged way to become a research pole of reference and excellence at the international level.

This challenge is no small one, but the potential to succeed is clearly there. I wish you the best possible journey to meet this challenge.»

Jacques Ghysdael
Institut Curie, France

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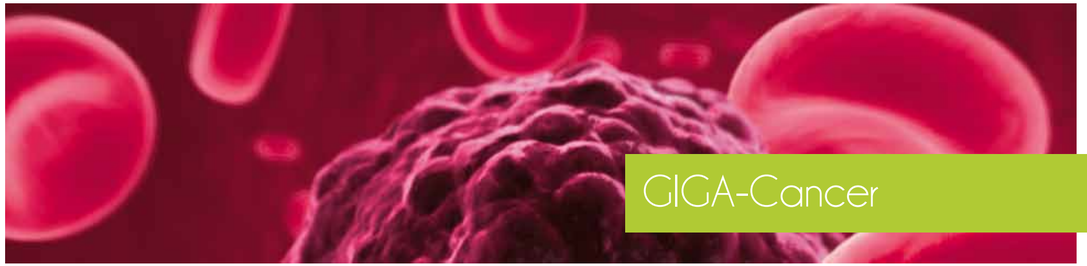

GIGA-Cancer

GIGA-Cancer is a thematic research unit resulting from the association of 8 laboratories aiming at studying several aspects of cancer. No less than 130 seniors scientists, postdoctoral researchers and graduate students collaborate to investigate the molecular mechanisms that intervene at different steps of cancer development and progression. In general, synergies are developed among the 8 laboratories with the ambition to share materials, models, equipment and ideas.

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COMPOSITION OF THE THEMATIC RESEARCH UNIT

8 laboratories
134 scientists
- 22 Principal Investigators
- 31 PhD (postdoc)
- 56 PhD students
- 25 Technicians

Highlight

DENTIN MATRIX PROTEIN 1 INDUCES MEMBRANE EXPRESSION OF VE-CADHERIN ON ENDOTHELIAL CELLS AND INHIBITS VEGF-INDUCED ANGIOGENESIS BY BLOCKING VEGFR-2 PHOSPHORYLATION.

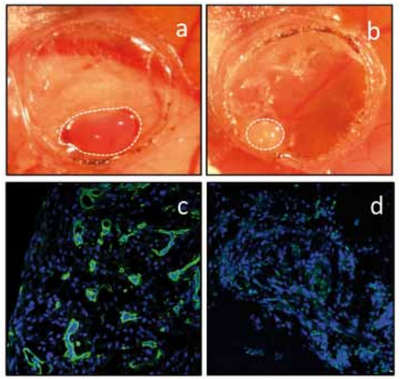
Blood. 2011;117:2515-2526

Dentin matrix protein 1 (DMP1) is a member of the Small Integrin-Binding Ligand N-linked Glycoproteins (SIBLINGs) family, a group of proteins initially described as mineralized extracellular matrices components. More recently, SIBLINGs have been implicated in several key steps of cancer progression, including angiogenesis. We demonstrated that this DMP1 induced the expression of VE-cadherin, a key regulator of intercellular junctions and contact inhibition of growth of endothelial cells that is also known to modulate VEGFR-2 activity, the major high affinity receptor for VEGF. DMP1 induced VE-cadherin and p27 expression followed by cell cycle arrest in human umbilical vein endothelial cells (HUVEC), in a CD44-dependent manner. VEGF-induced proliferation, migration and tubulogenesis responses were specifically blocked upon DMP1 pre-treatment of HUVEC. Indeed, subsequently to VE-cadherin induction, DMP1 inhibited VEGFR-2 phosphorylation and Src-mediated signaling. However, DMP1 did not interfere with bFGF-induced angiogenesis. *In vivo*, DMP1 significantly reduced laser-induced choroidal neovascularization lesions and tumor-associated angiogenesis. These data enable us to identify this protein as a new specific inhibitor of VEGF-induced angiogenesis.

[1]Sophie Pirotte, [1]Virginie Lamour, [2,3]Vincent Lambert, [3]Maria-Luz Alvarez Gonzalez, [4]Sandra Ormenese, [2]Agnès Noël, [1]Denis Mottet, [1]Vincent Castronovo, [1]Akeila Bellahcène.

[1]Metastasis Research Laboratory, GIGA-Cancer, [2]Laboratory of Biology of Tumor and Development, GIGA-Cancer, [3]Department of Ophthalmology, CHU, [4]GIGA-Imaging and Flow Cytometry, University of Liège, Belgium

In this model, an experimental tumor develops (panel a, dashed line) from tumor cells implanted on the chick embryo chorioallantoïc membrane (CAM). The volume of the tumor is significantly reduced when cancer cells overexpress DMP1 (panel b). The control tumor presents with numerous blood vessels stained in green (panel c) while the vascularization is barely visible in the tumor formed by tumor cells overexpressing DMP1 (panel d).



LABORATORY OF METASTASIS RESEARCH (MRL)

The Metastasis Research Laboratory (MRL) is involved in three main areas of research that are interconnected and aims not only to unveil the molecular mechanisms involved in the development and progression of malignant cells but is also eager to develop new tools for the early detection, prognostic evaluation and innovative therapies that could benefit to patients suffering from cancer. Noteworthy in 2010 is the creation of a spin-off company Targetome SA which will in practice commence with the development of the discovered biomarkers into real therapeutic targets.

Roles of the siblings proteins during tumor development: regulation of the cancer stem cell phenotype by osteopontin

Small Integrin-Binding Ligand N-linked Glycoproteins (SIBLINGs), a family of five soluble integrin-binding glycophosphoproteins comprising osteopontin (OPN), bone sialoprotein (BSP), dentin matrix protein 1 (DMP1), dentin sialophosphoprotein (DSPP) and matrix extracellular phosphoglycoprotein (MEPE), represent an emerging group of molecular tools that cancer cells use to facilitate their expansion. SIBLINGs are soluble secreted proteins that can act as modulators of cell adhesion as well as autocrine and paracrine factors by their interaction with specific cell surface receptors such as integrins. In 2010, the team headed by A. Bellahcène and V. Castronovo examined the role of the SIBLINGs family in glioblastoma (GBM). Both DSPP and OPN are expressed in GBM. They studied further the link between OPN expression and the acquisition of a cancer stem cell phenotype. They observed that OPN plays a key role in this process. Indeed, OPN knock-down in these cells induced the inhibition of essential stem-cell associated transcription factors. These data bring the first demonstration of OPN implication in glioma pathogenesis to a greater degree than anticipated. Indeed, OPN new effects on cancer stem cells let us foresee anti-OPN based therapy as a potential way for impairing tumor progression from initiation to probable recurrence in glioma patients.

Akeila Bellahcène, Virginie Lamour, Aurélie Ory, Manuela Dewald, Mathieu Fontaine, Olivier Peulen, Bernard Rogister, Jérôme Kroonen, Alain Chariot, Tieu-Lan Chau and Vincent Castronovo.
Collaborators: Manuel Deprez (Belgium), Larry W. Fisher (USA) and Zofia von Marschall (USA).

Roles of the siblings proteins during angiogenesis: mechanism (s) of action and characterization of dmp1 as an anti-angiogenic factor

We have recently demonstrated that DMP1 plays a key negative regulatory role during angiogenesis (see GIGA-Cancer «highlight article»). In the continuity of this observation, we plan to identify the endothelial cell receptor(s) mediating DMP1 anti-angiogenic effect and to characterize the bioactive region/peptide of DMP1 that is responsible for this activity. Most solid tumors are known to exhibit highly enhanced vascular permeability, similar to the one observed in the inflammatory tissues. Therefore, the potential use of DMP1 as an anti-vascular permeability agent will be evaluated *in vitro* and *in vivo*.

Akeila Bellahcène, Aurélie Ory, Mathieu Fontaine, Manuela Dewald, Virginie Lamour, Vincent Castronovo
Collaborator: Julie Gavard (France)

Role of HDAC7 in tumoral angiogenesis

Angiogenesis is the proliferation of a network of blood vessels that supplies nutrients and oxygen and removes waste products. This complex biological process requires the activation of endothelial cells, their proliferation, their migration and their ability to form new blood vessels. In a recent work, using small interfering RNAs strategy, we demonstrated that HDAC7 is a key regulator of angiogenesis. The specific silencing of HDAC7 altered endothelial cell morphology, their motility and prevented their assembly in tube-like structures *in vitro*. However, the mechanism by which HDAC7 silencing inhibits endothelial cell migration and disturbs cell morphology was poorly known.
The current study aims at elucidating the key pathways involved in the anti-angiogenic effect following the knockdown of HDAC7. Experimentally, this MRL team has knocked down HDAC7 in HUVEC using siRNA, extracted the soluble proteins, identified and quantified them employing nano-UPLC-MS technique. The data indicated modulation of several biological process related to cell adhesion, cell migration and cell-cell communication. In particular, HDAC7 appears to control the transcription of AKAP12, a anchor protein which interacts with PKA/PKC and target these kinases phosphorylates. The role of AKAP12 protein in angiogenesis as well as the identification of its different substrates in endothelial cells are currently addressed.

Denis Mottet, Nicolas Matheus, Vincent Hennequière, Andrei Turtoi and Vincent Castronovo
Collaborators: Eric Verdin (USA) and Franck Dequiedt (GIGA)

Role of HDAC5 in DNA replication process

In order to achieve the high level of control required to coordinate nuclear processes such as DNA replication, repair and transcription, cells have developed a variety of means to locally and specifically modulate chromatin structure and function. Among different changes in chromatin, post-translational modifications of histone proteins such as acetylation and deacetylation represent one of the major molecular mechanisms to alter DNA packing. HDACs are enzymes which regulate the balance between acetylated /deacetylated states of histones. Several broad spectrum HDAC inhibitors have been shown to alter DNA replication process but the role of individual HDAC is still largely ignored.
In this project, we aimed to identify which member(s) of the HDAC family can control this biological process. We found that subnuclear localization of HDAC5 dynamically changed during S phase progression, with HDAC5 co-localizing with heterochromatic regions during late S phase and we demonstrated that its specific depletion by siRNA slowed down ongoing replication fork and induced DNA damage checkpoint pathways which delays S phase progression, decreases DNA synthesis and consequently, decreased cell

proliferation. The precise mechanism of action of HDAC5 during DNA replication process is currently investigated.

Denis Mottet, Paul Peixoto and Vincent Castronovo
Collaborators: Franck Dequiedt (GIGA), Marc Thiry (GIGA), Christian Herens (Belgium) and Arturo Londono-Vallejo (France)

Antibody derivatives as molecular agents for neoplastic targeting

In the frame of FP7 EU project «ADAMANT», we have been implicated in the effort to characterize several human carcinoma with respect to their accessible, tumor associated antigens. These specific tumor markers have to be abundant in the tumor, not expressed in the normal tissues and accessible by bloodstream. This latter property is essential for their application in antibody-based therapy which can be more specific and versatile compared with classical therapies. Accordingly, we have completed an extensive study focused on pancreas cancer. Within the project, they have identified several novel proteins never reported in cancer studies so far. Their differential expression was validated in a collection of normal and tumor pancreas samples using IHC. Current efforts are focused on establishing their function in cancer. Recently, they have dedicated a significant part of resources in studying CRC (colorectal cancer) and have concluded an extensive study of accessible proteins in a broad collection of human CRC liver metastases. Finally, we have also begun with establishing the mouse CRC liver metastases model and collected preliminary data on LS174T and SW1222 cell lines which are able to initiate metastasis *in vivo*.

Andrei Turtoi, Bruno Dumont, Arnaud Blomme, Edwin De Pauw, Philippe Delvenne and Vincent Castronovo
Collaborators: Raffaella Giavazzi (Italy), Dario Neri (Switzerland), Alex Berndt (Germany), Matteo Zanda (Italy), Eveline Trachsel (Switzerland), Silvia Marsoni (Italy), Barbara Pedley (United Kingdom) and Guus Van Dongen (Netherlands)

Identification of accessible proteins suitable for development of tumor-targeted therapies

In the context of ARC-ULG project «IDEA», we further devised and refined adequate methods for accessible proteome analysis. These concern the combination of glycoproteomics and biotinylation to scan for accessible tumor proteins and the reduction of the required sample amount for respective proteomics analysis in order to exploit scarce and valuable clinical material (e.g. biopsies) or stem cells. Along these lines, they have developed a novel, comprehensive and efficient method allowing the identification of accessible proteins in precious pathological samples. The demonstration of the value of the method was performed on human breast cancer tissue samples. The approach consisted of three main successive steps during which they firstly isolated the potentially accessible proteins via linkage of biotin molecules to free lysine and N-terminal amino groups followed by streptavidin purification. The remaining proteins were digested and subjected to glycopeptide isolation. All samples were analyzed using mass spectrometry. In comparison to the biotinylation alone, the sequential approach significantly increased the number (+45%) of identified proteins of potential therapeutic and/or diagnostic value. The approach highlighted to light several novel biomarkers which were successfully validated using IHC. Currently, this MRL team is evaluating the possibility to use the same approach on cells, especially on cancer stem cells.

Andrei Turtoi, Bruno Dumont, Arnaud Blomme, Philippe Delvenne, Edwin De Pauw, Agnès Noel and Vincent Castronovo

Collaborators: Eugène Nzaramba Mutijima (Belgium), Eric Lifrange (Belgium) and Gabriel Mazzucchelli (GIGA)

LABORATORY OF BIOLOGY OF TUMOR AND DEVELOPMENT (LBTD)

The Laboratory of Biology of Tumor and Development is co-headed by Jean Michel Foidart, Agnes Noel and Didier Cataldo. We have longstanding expertise in proteases (MMPs and serine proteases), cell matrix biology, angiogenesis, lymphangiogenesis, tumor-fibroblast interactions and metastatic dissemination. We study the impact of steroids and the tumor microenvironment (angiogenesis, inflammation and fibrosis) on the progression of breast, lung and cervical carcinomas. We have developed tools and models to investigate the important vascular remodelings associated not only with cancer, but also with several pathologies such as endometriosis, ocular diseases and abnormal embryonic implantation.

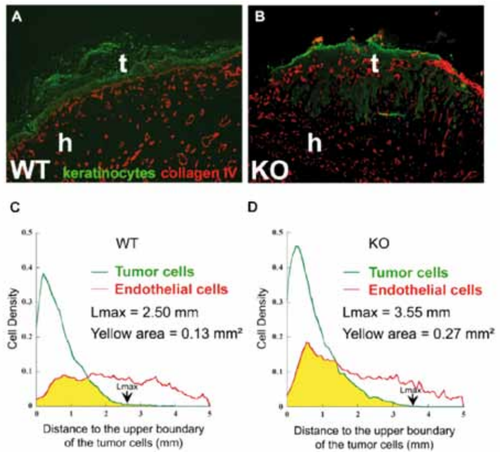
Angiogenesis and lymphangiogenesis in pathological processes

Lymphangiogenesis or *de novo* lymphatic formation, is primarily an embryonic event and in adults, it is closely associated with several pathologies such as cancer, endometriosis, psoriasis and graft rejection. One of the lymphatic vessel features is the close association of lymphatic endothelial cells (LEC) with the underlying interstitial matrix. During lymphangiogenesis, LEC has to deal with interstitial collagen known to be degraded by interstitial collagenases belonging to the matrix metalloprotease family (MMP). Recently, we provided evidences that MMP-2 is involved in the development of lymphatic vessels as a collagenase by using several *in vivo* and *in vitro* models (in mice and zebrafish).
Endometriosis, defined as the development of endometrial tissue outside the uterus, is a benign disease responsible for infertility and pelvic pain. The role of lymphangiogenesis has not been fully clarified. We demonstrated that endometriotic cells, like cancer cells, could hijack angiogenesis and lymphangiogenesis to disseminate in distant organs. We have also developed experimental animal models that are useful for testing different therapeutical strategies to overcome the problem of endometriosis and also endometrial bleeding.
We are also exploring the importance of lymphangiogenesis in cervical cancers.

Benoit Detry, Jenny Paupert, Charlotte Erpicum, Catherine Maillard, Sandra Ormenese, Hélène Pendeville, Ingrid Struman, Agnes Noel, Cedric Detry, Frederic Coffin, Frederic Kridelka, Michèle Nisolle, Aude Béliard, Maria Luz Alvarez, Céline Gérard, Géraldine Brichant, Carine Munaut, Mélanie Mestdagt, Jean-Michel Foidart
Collaborators: Peter Carmeliet (Belgium), Etienne Marbaix (Belgium), Jacques Donnez (Belgium)

Contribution of soluble Metalloproteinases (MMP) in cancer progression

Serine proteases (uPA, tPA), metalloproteinases (MMP, ADAM, ADAM-TS) are major effectors in tissue remodeling and in the elaboration of a permissive tumor environment for cancer dissemination. To elucidate the unknown *in vivo* functions of ADAMTS-12, we generated a knockout mouse strain (Adamts12-/-) in which Adamts12 gene was deleted in the mice transgenic platform of the GIGA. By applying three different *in vivo* models of angiogenesis to Adamts12-/- mice, we provided evidence for a protective effect of this host enzyme towards angiogenesis and cancer progression. In the absence of Adamts-12, both the angiogenic response and tumor invasion into the host tissue were increased. This angioinhibitory effect of ADAMTS-12 was independent of its enzymatic activity. The implication of this ADAMTS in inflammatory processes is currently under investigation.



Angio-inhibitory effect of Adamts-12 on tumor. Malignant keratinocytes were and transplanted on the back of Adamts12+/+ (WT) (A,C) or Adamts12-/- (KO) (B,D) mice). A, B: Double immunostaining identify tumor cells (green) and vessels (red guinea); t = tumor, h= host tissue. C,D: Tumor and endothelial cell density distributions determined by computerized image analysis. Intermingling of tumor cells and vessels is shown in yellow.

By using the transgenic mouse model of multistage epithelial carcinogenesis (K14-HPV16 mice), we also showed that PAI-1 deficiency did not impair tumor development, inflammation and the (lymph)angiogenic switch suggesting the presence of functional redundancy masking the effect of PAI-1 deficiency in this long-term model of carcinogenesis.
We are currently studying the impact of Mesenchymal stem cell-derived MMP on metastatic dissemination.

Julie Lecomte, Anne Masset, Mehdi El Hour, Geneviève Paulissen, Carine Munaut, Didier Cataldo, Agnès Noel, Fabian Ectors (GIGA Mouse Transgenics platform)
Collaborators: Gunilla Hoyer Hansen (Danemark), Carlos Lopez-Otin (Spain), Lisa Coussens (USA), Thomas Rheneckel (Germany)

Analysis of the contributions of MT1-MMP during tumour progression

As invading breast carcinoma cells breach their underlying basement membrane, they become confronted with a dense three-dimensional reactive stroma dominated by type I collagen. To develop metastatic capabilities, invading tumour cells must acquire the capacity to negotiate this novel microenvironment. Collagen influences the fate of epithelial cells by inducing apoptosis. However, the mechanisms used by invading tumour cells to evade collagen-induced apoptosis are yet be defined. We demonstrate that membrane type-1 matrix metalloproteinase (MT1-MMP/MMP-14) confers breast cancer cells with the ability to escape apoptosis when embedded in a collagen gel and after orthotopic implantation *in vivo*. In the absence of MMP-14-dependent proteolysis, type I collagen triggers apoptosis by inducing the expression of the pro-apoptotic Bcl-2-interacting killer (BIK). MMP-14 has been implicated in four out of the six hallmarks of cancer, including self-sufficiency in growth signals, insensitivity to antigrowth signals, tissue invasion/metastasis, and angiogenesis. Our observations shed light on a new mechanism whereby MMP-14 activity promotes tumour progression by circumventing apoptosis.

Erik Maquoi, Delphine Assent, Julien Detilleux, Nor Eddine Sounni
Collaborators: Luuk Hawinkels (Netherlands), Alex Strongin (USA)

Deciphering the molecular pathways regulated by the (GIP)-anchored MMP (MT4-MMP) in metastatic breast cancer cells

We previously demonstrated that MT4-MMP (MMP17), a membrane-anchored MMP primarily expressed by breast tumor cells, promotes primary breast cancer growth and metastases. We also provided evidence that MT4-MMP expression by cancer cells induce (i) an enlargement of blood vessels, (ii) a detachment of mural cells from the vascular tree and (iii) increased vessel permeability. Through site directed mutagenesis, the inactivation of MT4-MMP catalytic functions inhibited the pro-angiogenic and pro-metastatic effect of MT4-MMP suggesting that MT4-MMP activity is required for metastatic dissemination of breast carcinoma cells to the lung. By using a global proteomics phosphoantibody array approach we determined the signaling pathways activated by MT4-MMP in promoting proliferation of breast carcinoma cells *in vivo* and in 3D Matrigel matrix *in vitro*. Among the

targets found, Rb pathway has been identified as a major one. Ongoing investigations aim at deciphering the unknown molecular and cellular mechanisms of this enzyme in promoting cancer metastasis through a genomic profiling and the establishment of functional assays.

Nor Eddine Sounni, Alexandra Paye, Host Lorin, Carine Munaut, Christine Gilles, and Agnès Noël

Implication of Epithelial-to-mesenchymal transtions in the metastatic progression

Epithelial-to-mesenchymal (EMT) processes endow epithelial cells with enhanced migratory/invasive properties and are therefore likely to contribute to the metastatic progression. *In vitro*, we have shown that the reorganisation of cell-cell junctions associated with EMTs can directly modulate the expression of mesenchymal genes through the relocalisation of specific molecules such as **β**-catenin or ZO-1 and enhance migration/invasion.

Because of the difficulty in following dynamic EMT processes in human tumors, we have developed mice models. In a first axis, the expression of our genes of interest (ZOs, SIP-1) was modulated in luciferase-expressing cells by shRNA or cDNA transfection and the modification of the *in vivo* metastatic behaviour was analyzed. Preliminary results showed that SIP-1 cDNA transfectants display an enhanced ability to metastasize in the lung, whereas ZO-1 shRNA transfectants are less metastatic. In a second axis, we developed and characterized an animal model with transplantable human breast tumor cells showing spontaneous EMT events to occur and reverse *in vivo*. Our data uniquely demonstrates that EMT occurs in the primary tumors, providing the cells with an enhanced ability to intravasate and generate circulating tumor cells (CTCs). They further suggest that mesenchymal-to-epithelial (MET) phenomena occur in secondary organs, facilitating the metastatic growth.

Anne Brysse, Laidya Syne, Meggy Suarez-Carmona, Christine Gilles
Collaborators: Myriam Polette (France), Philippe Birembaut (France), Arnaud Bonnomet (Belgium/France), Rik Thompson (Australia), Geert Berx (Belgium), Walter Hunziker (Singapore)

New insights into abnormal angiogenesis associated with Age-related Macular Degeneration (AMD)

Age-related macular degeneration (AMD) is the leading cause of vision loss in the western world among people aged 50 or older. Ninety percent of all vision loss due to AMD results from the exsudative form, which is characterized by choroidal neovascularization (CNV). However, vascular endothelial growth factor (VEGF)-A has emerged as a key regulator of CNV formation. The successful application of anti-VEGF approach in the clinic has obviously changed the face of ophthalmology. Recently, placental like growth factors (PlGF) emerged as well as a key regulator to be targeted. Inflammation is also an important factor in AMD development and macrophage depletion in mice inhibits CNV formation. At present, the laser-induced Bruch's membrane photo-coagulation model is the most widely accepted and most frequently utilized experimental murine CNV model. By applying this model to different transgenic mice, we contributed to demonstrating the key contribution of matrix metalloproteinases (MMPs), serine proteases and PlGF in CNV. Despite important advances in our understanding of the molecular mechanisms underlying AMD, and in the development of new therapies currently used in clinics, age-related changes that induce abnormal neovascularization are not completely understood. Since nothing is known about the metabolic changes occurring in patients with AMD, we applied a metabolomic approach to AMD patients and the experimental CNV laser-induced mice model.

Vincent Lambert, Julie Lecomte, Sandrine Bekaert, Agnes Noël
Collaborators: Jean-Marie RAKIC (Belgium), Pascal de Tullio (Belgium), Peter Carmeliet (Belgium)

Implication of soluble VEGFR1 and soluble VEGFR2 in physio-pathological processes

Two soluble forms of vascular endothelial growth factor (VEGF) receptors, sVEGFR-1 and sVEGFR-2, are physiologically released and overproduced in several pathologies such as cancer and pre-eclampsia. They are known to act as anti-VEGF agents. We have reported that these soluble receptors contribute to vessel maturation by mediating a dialogue between endothelial cells and mural cells that leads to blood vessel stabilization. In addition, little is known about the mechanisms modulating the expression and the release of these soluble receptor forms from endothelial cells. We evaluated the impact of PKC activation on the release of sVEGFR1 and sVEGFR2 from EC. While PKC activation increased sVEGFR1 secretion through an alternative splicing of its mRNA, it increased full-length VEGFR2 expression without modulating sVEGFR2 release. In VEGFR overexpressing cells, PKC activation increases both sVEGFR1 and sVEGFR2 release through metalloproteinase-dependent ectodomain shedding. Considering the crucial impact of sVEGFR1 on hemangiogenesis and of sVEGFR2 on lymphangiogenesis regulation, the modulation of PKC activity may contribute to regulating these fundamental biological processes in pathological contexts.

Carine Munaut, Christel Péqueux, Sophie Lorquet, Emily Gengoux, Agnès Noël, Jean-Michel Foidart
Collaborators: Kypros Nikolaides (UK), Johannes Waltenberger (The Nedderlands)

Fertility/Infertility

The endometrium is remodelled throughout the menstrual cycle and exhibits only a short period of receptivity, known as the "implantation window", which is crucial both for implantation and gestation and still remains poorly explored in routine reproductive medicine. Our project aims to a better understanding of the implication of several cytokines, cytokine receptors in normal and pathological implantation such as preeclampsia. We are also examining whether IVF/ICSI repeated implantation failures (IF) or recurrent miscarriages (RM) could be related to pre-conceptional endometrial deregulations.

In the context of cancer, a correlation between the number of young cancer survivors and patients confronting infertility is well established. The gonadotoxic effect of chemo and radiotherapy on premature ovarian failure (POF) is well documented. As a consequence, there has been a surge in the number of patients seeking fertility preservation. Ovarian tissue freezing and transplantation represent the only feasible option for children. However, ischemia of the grafted ovarian fragment affects follicular pool, graft survival and fertility restoration and thus represents one of the main limitations. We are currently setting an original xenotransplantation model of cryopreserved ovarian tissue and evaluating the local effect of various factors on angiogenesis and follicle preservation by using this model.

Soraya Labied, Laurie Hardy, Maïté Fransolet, Alain Colige, Carine Munaut, Michèle Nisolle, Jean-Michel Foidart
Collaborators: Sophie Perrier d'Hauterive (Belgium), Nathalie Kirschvink (Belgium), Nathalie Lédée (France)

Improvement of image analysis for experimenal and clinical applications

Advances in imaging and in the computerized quantification of images are transforming our exploration of complex biological processes. To improve the objectivity and reproducibility in image quantifications, we have set up different computerized methods applicable to complex biological systems including 2D or 3D cultures, tissue slides and mammograms.

We first focused on blood and lymphatic vessels that form two interconnected vascular systems involved in various pathological conditions. Unfortunately, their quantification is a complex matter because images of vascularization usually do not have well defined morphology. We are proposing new tools to characterize these complex structures in addition to typical global parameters such as area, length, end-point and branching densities. These methods have been validated on complex 3D cultures of lymphatic or blood endothelial cells, on tissue sections of breast and cervical tumors issued from human patients or xenografted mice. In addition, in order to develop a clinical tool to detect breast pathologies, the Hessian-based method was used to describe changes in digital mammograms. It is of great interest that these methods are available to investigate various structures in complex biological systems or tissue sections.

Silvia Blacher, Nicolla Signol, Cédric Balsat, Vincent Bataille, Agnès Noël, Jean-Michel Foidart
Collaborators: Eric Lifrange (Belgium), Joëlle Desreux (Belgium)

A new formulation of solubilized curcumin affect cell proliferation and apoptosis *in vitro* and is active against lung cancer in an orthotopic mouse model

Treatments available for advanced lung cancer still remain very disappointing. Curcumin is the main curcuminoid derivative and has been reported as potentially active against colon and pancreatic cancer. As curcumin solubility is very poor, new formulations have been used in this study to obtain aqueous solutions therefore dramatically increasing bioavailability. Effects of curcumin have been evaluated *in vitro* as well as *in vivo* in an orthotopic lung tumor mouse model.

Cell proliferation was reduced while apoptosis was enhanced if lung epithelial tumor cells (Lewis Lung carcinoma or BZR cells) were cultured in presence of our formulation as compared to treatment of cells with vehicle only.

In vivo, C57Bl/6 mice were xenografted with Lewis Lung carcinoma cells into lungs and treated daily by an oral administration of the newly designed formulation.. We found that solubilized curcumin reduces the incidence and size of orthotopically-implanted lung tumors as compared to vehicle only or to a non soluble form of curcumin. In conclusion, our data demonstrate that a soluble form of curcumin is active against cell proliferation and increases tumor cells apoptosis *in vitro* while decreasing tumor size *in vivo*.

Natacha Rocks, Sandrine Bekaert, Gilles Sarlet, Geneviève Paulissen, Maud Guéders, Jean-Michel Foidart, Didier Cataldo.
Collaborator : Brigitte Evrard (Belgium)

Cigarette smoke-induced inflammation promotes melanoma cell metastasis in lung parenchyma

It is only during the last decade that clear evidence has been obtained that inflammation plays a critical role in different stages of tumor development, including initiation, promotion, malignant conversion, invasion and metastasis. There is increasing evidence that an inflammatory microenvironment is an essential component of all tumors.

We assessed *in vivo* the impact of cigarette smoke (CS) on tumor cell extravasation in lungs after tail vein injection of B16F10 melanoma cells. We first characterized airway inflammation obtained after smoke exposure (reference cigarettes 3R4F) for 1, 2, 4, 8 and 12 weeks. Neutrophils, alveolar macrophages, interstitial macrophages, dendritic cells, T cells and natural killer T (NKT) cells, were characterized in lung tissues of mice exposed to CS and AIR using flow cytometry. *In vitro*, the direct effect of cigarette smoke extract (CSE) on proliferation of B16F10 melanoma cells was determined for 1 to 5 days. *In vivo*, mice exposed for 2 weeks to cigarette smoke or air were injected with B16F10 melanoma cells in the tail vein. After 3 weeks, hematoxylin-eosin staining allowed quantification of lung metastasis (tumor area/total lung area). An increase of metastasis and implantation site in lungs was observed in CS exposed group. Conceivably, CS constituents significantly promote extravasation of melanoma cells in lung tissues. The mechanism or signaling pathway responsible for this dissemination needs to be further investigated.

Sandrine Bekaert, Natacha Rocks, Geneviève Paulissen, Maud Guéders, Agnès Noel, Didier Cataldo
Collaborators : Fabrice Bureau et Christophe Desmet (CIGA), Muriel Pichavant (France)

LABORATORY OF MOLECULAR BIOLOGY AND GENETIC ENGENEERING: ANGIO TEAM

Blood vessels are formed by vasculogenesis (differentiation of endothelial cells from progenitors) and angiogenesis (formation of blood vessels from pre-existing ones). The process of blood vessel formation is critical for embryonic development, growth and reproduction. Undesired or excessive vasculogenesis/angiogenesis contributes to numerous disorders including cancer, retinopathy... The general goal of the research of the laboratory is to understand the mechanism of angiogenesis in order to develop new antiangiogenic inhibitors for therapeutic purposes. Research is conducted to pursue the evaluation inhibitors of angiogenesis previously identified by us and to discover new ones.

Therapeutic evaluation of the use of peptides derived from the prolactin/growth hormone family

We previously showed that recombinant 16-kDa N-terminal fragment of human prolactin (16K prolactin) and its derived peptides have angiostatic properties both *in vitro* and *in vivo*. Recently we showed that 16K prolactin affects the morphology of tumor blood vessels; ongoing research is being conducted in order to determine whether 16K prolactin affects vessel maturation and to identify the mechanism involved. Our work also attempts to answer the following questions: Does 16K prolactin prevent lymphangiogenesis, the process of lymphatic vessel development shown to be involved in some tumorigenic processes and more importantly in metastasis? Does 16K prolactin affect initial steps in tumor progression like vasculogenesis and recruitment of endothelial precursors?

Ngoc-Quynh-Nhu Nguyen, Karollen Castermans, Virginie Kinet, Michelle Lion, Ingrid Struman and Joseph Martial
Collaborators: Vincent Goffin (France), Birthe B. Kragelund (Danmark), Denise Hilfiker-Kleiner (Germany)

Understanding the mechanism of action of 16K fragments of the prolactin/growth hormone

The functional pleiotropy of 16K prolactin makes it important to identify one or more target molecule(s) mediating these activities. Although a high-affinity specific binding site is present in endothelial cells, so far, and despite much research performed by several laboratories, this «receptor» has remained undetermined. Using the yeast two hybrid approach, we recently identified a binding partner for 16 prolactin. Research has been performed in order to validate the requirement of this binding partner for antiangiogenic activity of 16K prolactin using, among others, si-RNA interference, knock-out mice.

Khalid Bajou, Salvino D'Amico, Mohammed Srahna, Stéphanie Herkenne, Jean -Yves Carabin, Ingrid Struman and Joseph Martial
Collaborators: Arjan W. Griffioen (The Nederlands), Paul Declerck (Belgium)

Identification of new modulators of angiogenesis

Following on from our previous research performed on inhibitors of angiogenesis, we have taken advantage of our expertise to identify new modulators of angiogenesis. On the one hand, new candidates are targets of angiostatic agents. Some new candidates were selected from data obtained during our previous transcriptomic and/or proteomic analysis performed on endothelial cells or tumor samples. We recently identified sprouty-1 as an important regulator of angiogenesis. Among others, we also address a special interest in microRNAs. We have taken advantage of the bioinformatic and zebrafish tools available in our lab and in the GIGA-Research center to identify new microRNAs involved in angiogenesis. We performed functional studies after having modulated their expression *in vitro* in endothelial cells and *in vivo* in zebrafish and mice. We have already identified 3 microRNAs that potentially regulate angiogenesis using this approach.

Hélène Pendeville, Sébastien Tabruyn, Céline Sabatel, Ludovic Malvaux, Julie Halkein, Nicolas Bovy, Olivier Nivelles, Ingrid Struman and Joseph Martial

LABORATORY OF CONNECTIVE TISSUES BIOLOGY (LCTB)

The LCTB has a longstanding interest in the field of connective tissues biology and pathology. This includes (i) biochemical and molecular characterizations of the main extracellular matrix (ECM) components and of the enzymes regulating their deposition and remodeling and (ii) studies of cell-ECM interactions and determination of the reciprocal regulations operated by mechanical forces developed by cells and issued from the ECM. Built on promising data obtained in these research areas, more recent projects aim at determining the therapeutical significance of a new variant of VEGF (VEGF111) and characterizing the regulation affecting the alternative splicing machinery during chemotherapy

VEGF111, alternative splicing and cancer

Studies investigating the regulation of cell phenotypes by their physical and chemical environment lead to the original discovery of VEGF111, a new splice variant of the VEGF-A transcript. Its synthesis results from modifications of the alternative splicing machinery induced in many different cell types by genotoxic agents, including chemotherapeutic drugs and ionizing radiations. VEGF111 possesses specific proangiogenic properties that might be adverse during chemotherapy by promoting tumoral angiogenesis. On the contrary, its use would be beneficial for the treatment of ischemic diseases or conditions. Based on preliminary promising data, this hypothesis is currently being investigated in a number of models, such as cutaneous wound healing, tissue grafting, tendon repair and coronary infarction.
Besides VEGF, genotoxic agents induce modifications of the splicing of several other primary transcripts coding for proteins crucial for the regulation of cell proliferation and survival. A new project in this field has recently been initiated. It aims at evaluating whether modifications of the alternative splicing repertoire can influence cell sensitivity to chemotherapy or can be correlated to the resistance appearing during the treatment.

Romain Delcombel, Laurianne Janssen, Charles Lambert, Maude Gabriel, Yves Delforge, Johanne Dubail
Collaborators: Yvette Habraken, Jacques Piette, Philippe Delvenne, Frédéric Baron, Yves Beguin (CIGA) Luc Bertrand and Jean-Louis Vanoverschelde (Belgium), Chantal Dessy and Jean-Luc Balligand (Belgium), David Bates (UK)

Functions of aminoprocollagen peptidases (ADAMTS2, 3 and 14)

In the ADAMTS family, ADAMTS2, 3 and 14 form the aminoprocollagen peptidase subfamily. Besides cleaving the aminopropeptide of fibrillar collagens, we have shown that ADAMTS2 is also able to cleave in amino acid sequences different from those involved for procollagen processing, clearly suggesting the existence of a substrate repertoire much larger than initially thought. Absence of ADAMTS2 activity *in vivo* has consequences related to the incomplete processing of procollagen molecules, such as extreme skin fragility and relative protection against fibrosis. It also induces unexpected features such as male sterility, by interfering with post-natal development of testis, and is also a potent antiangiogenic factor. Regarding ADAMTS3 and ADAMTS14, most of the *in vivo* characterizations remain to be done, largely because of the absence of relevant KO animals.
Extensive determination of the role of these 3 enzymes is currently being performed by complementary approaches. ADAMTS3 and ADAMTS14 KO mice have been generated and their phenotype is being characterized. Their functions during embryogenesis are more specifically determined in the zebra fish model. Finally alternative substrates for these enzymes will be identified by wide screening (N-terminal labeling of cleavage sites and mass spectrometry) and by studying specifically non-fibrillar collagens, proteins containing triple helical domains (such as those involved in innate immunity) and proteins containing TSP1 repeats.

Johanne Dubail, Antoine Heyeres, Audrey Hoffmann
Collaborators: Marianne Voz & Bernard Peers (CIGA) Suneel Apte (USA), Linda Sandel (USA)

Intracellular signaling by the RhoGTPase network

The small GTPases of the Rho family form a major branch of the Ras superfamily of small GTPases. These enzymes and their regulatory partners are key integrators of various extracellular signals, such as those mediated by growth factor receptors and integrins. Two fields of research are more specifically developed.
The first one addresses the specific role of individual RhoGTPases, especially RhoA, Rac1 and Cdc42, in the cytoskeletal mechano-sensing machinery allowing different types of connective tissue cells to sense and react to the mechanical forces (gravity, stretching, ...). The second project aims at investigating the respective contribution of specific members of the RhoGTPases network of regulation and their cross-talk in the acquisition of an invasive phenotype. The silencing of RhoC, but not of RhoA, increased the expression of gene encoding tumor suppressors, such as NAG-1, and decreased the migration and the anchorage-independent growth of PC-3 cells *in vitro*. *In vivo*, siRhoC impaired tumor growth. In addition of being induced by RhoC silencing, NAG-1 was also largely up-regulated in cells overexpressing RhoA. These data and others demonstrate that the levels and activity of RhoA, RhoC and closely related enzymes are mutually dependent and highly sensitive to modifications of their relative abundance. Their cross-talk involves RhoGDI and is established in a hierarchical order between closely related homologues.

Christophe Deroanne, Audrey Stultiens, Thibaut Neutelings, Nancy Garbacki, Audrey Courtois
Collaborators: Nathalie Jacobs, Virginie Renoux, Ingrid Struman, Eric Maquoi, Alain Chariot (GIGA), Alain Guignandon & Laurence Vico (France), Yves Poumay (Belgium)

LABORATORY OF MOLECULAR AND CELLULAR EPIGENETICS

We aim at understanding the basic mechanisms of cancer and at investigating novel therapeutic approaches. The current research topics include leukemia (HTLV associated adult T-cell leukemia ATLL, chronic lymphocytic leukemia and bovine leukemia) and thoracic cancers (non-small cell lung cancer NSCLC, small cell lung cancer SCLC and mesothelioma MPM). The following projects are currently ongoing: 1) DNA replication and mitotic catastrophe, 2) gene activation therapy of cancer, 3) design of novel epigenetic inhibitors for cancer therapy, 4) epigenetic regulation of angiogenesis, 5) viral glycomics, 6) genomic markers for NSCLC response to therapy.

Mechanisms of thoracic cancers: epigenetic therapy and identification of biomarkers

Lung cancer is the number one cause of cancer-related death worldwide, with more than 1 million deaths per year. Malignant pleuralmesothelioma (MPM) is a neoplastic disease of the pleura strongly associated with exposure to asbestos fibers. Present chemotherapeutic regimens are marginally efficient, tumor cells being particularly resistant to radiotherapy and/or chemotherapy. We hypothesized that unresponsiveness of tumors to conventional therapeutic agents is due to inappropriate gene expression resulting from epigenetic modifications and leading to transcriptional silencing. We have demonstrated that valproate, an HDAC (histone deacetylase) inhibitor, improves the evectiveness of standard treatments of different thoracic cancers: MPM, non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). Ongoing projects include (1) development of targeted therapies of circulating tumor cells in NSCLC patients to reduce occurrence of metastases, (2) design of novel epigenetic inhibitors interfering concomitantly with HDAC and DNMT (DNA methyltransferase) activities, (3) identification of biomarkers for response to chemotherapy of non-small cell lung cancer and (4) study of the role of retrotransposons in NSCLC.

Chrisostome Costa, Roland Hubaux, Sathyanarayana N.S. Reddy, Fabian Vandermeers, Nicolas Gillet, Jean-Philippe Cosse, Stéphane Georges and Luc Willems
Collaborators: Pascale Hubert, Philippe Delvenne, Renaud Louis, Lionel Bosquée, Bernard Duysinx (GIGA), Didier Allaer (Belgium), Thierry Berghmans (Belgium), Jean-Paul Scutier (Belgium)

Epigenetic regulation of angiogenesis

Several studies have shown that histone deacetylase (HDAC) and DNA methyltransferase (DNMT) inhibitors are potent antiangiogenic compounds. Though combination of HDAC and DNMT inhibitors are now being examined in clinical trials of hematological malignancies, very little work has been done to understand the effect of this combination on normal and tumoral angiogenesis. We have designed and tested a family of twin drugs with intrinsic HDAC and DNMT inhibitory activities in relevant models of angiogenesis *in vitro* (cultures of endothelial cells and pericytes, 3D aortic ring assay) and *in vivo* (chick chorioallantoic membrane assay). We have identified a lead compound that has a quantifiable antiangiogenic effect without the cytotoxicity associated with increased global acetylation and decreased DNA methylation levels. This compound is presently used to develop effective approaches to treat cancer by modulating the process of angiogenesis.

T.V. Shiva Shankar, Béatrice Sulka and Luc Willems
Collaborators: Catherine Maillard, Silvia Blacher, Agnès Noël (GIGA), Didier Lambert (Belgium), Johan Wouters (Belgium)

Mechanisms and novel therapies of deltaretrovirus induced diseases

It is estimated that 20 million are infected with human T-lymphotropic virus type 1 (HTLV-1) worldwide. Among them, 2-5% will develop one of the major HTLV-1 associated diseases (ATLL Adult T cell leukaemia/lymphoma or HAM/TSP HTLV-associated myelopathy/Tropical Spastic Paraparesis). Bovine leukemia virus (BLV) is a closely related δ-retrovirus infecting B lymphocytes and inducing leukemia/lymphoma in cattle. A major feature of the infection induced by both viruses is transcriptional silencing which allows proviruses to escape recognition and destruction by the host immune response. We have evaluated the effect of a novel therapeutic strategy based on transient activation of viral expression using epigenetic modulators, thereby exposing infected cells to the immune response and resulting in significant reductions in proviral loads. We have also studied another therapeutic approach that includes the ability of checkpoint inhibitors in controlling HTLV-1 infection and preventing malignant disease. We have characterized the fundamental mechanisms pertaining to these therapies, which offer an option for lymphoma and relapsing acute ATLL patients. In the BLV model, we study the kinetics of viral replication and the role of the host immune response.

Mathieu Boxus, Alix de Brogniez, Arnaud Florins, Fanny Boulanger, Sabrina Rodriguez, Nicolas Gillet, Alexandre Carpentier, Pierre-Yves Barez, Thibaut Masy and Luc Willems

LABORATORY OF HUMAN GENETICS

Our laboratory performs translational cancer research aiming at the exploration of cellular signaling pathways that could be targeted by specific agents and the definition of resistance mechanisms.

Personalized medicine for gliomal tumors

We perform genomic and functional studies on gliomal tumors in order to:
- sub-classify the tumors
- identify the tumor heterogeinity through the presence of different genetic populations
- study activated signaling pathways responsible for the response or the resistance to targeted treatments

Pierre Robe, Vincent Bours, Valérie Caprara, Olivier Piérard, Maria Artesi, Christophe Poulet
Collaborators: Emmanuel Deprez (Belgium), Markus Bredel (USA), Arnab Chakravarti (USA)

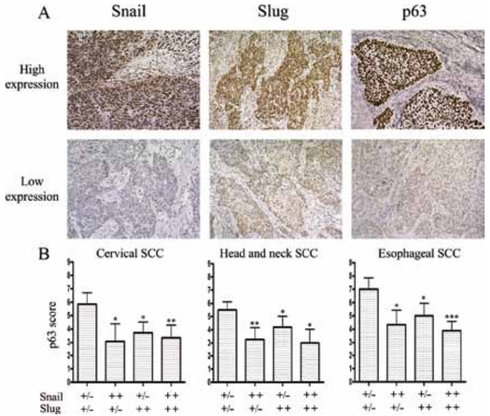
LABORATORY OF EXPERIMENTAL PATHOLOGY (LEP)

Our research team has accumulated a large expertise in the diagnosis/prognosis of cancers and in the fundamental knowledge of tumor development, with a special interest for early steps of carcinogenesis. The LEP has an expertise to reproduce and manipulate (pre)neoplastic lesions *in vitro* (organotypic cultures), to generate antigen presenting cells, to produce virus-like particles (VLP) of Human papillomavirus (HPV) and to isolate lymphoid and other immune cells for functional studies. We also collaborate with the Human Tissue Bank of the University of Liège which includes several thousands of normal and cancer samples.

HPV and the sequence "metaplasia-dysplasia-cancer" sequence

HPV infection, particularly type 16, is causally associated with cancer of the uterine cervix. The progression of cervical lesions suggests that viral antigens are not adequately presented to the immune system. This hypothesis is reinforced by the observation that most preneoplastic lesions (SILs) show quantitative and functional alterations of dendritic/Langerhans cells (DC/LC). One of the main goals of the LEP is to determine the effects of HPV proteins on DC/LC recruitment and functions. We demonstrated that HPV16 VLP are able to enhance DC motility towards lymph nodes whereas HPV16 VLP-treated keratinocytes induce a lower LC migration via the secretion of PGE2, which may be responsible *in vivo* for the reduced LC recruitment into the SILs.
Another factor potentially important for the density and function of LC is the process of epithelial metaplasia (EpM) which is observed in the uterine cervix. EpM is an adaptative process resulting from the transformation of one epithelium into another. This process is associated with an increased frequency of (pre)cancerous lesions. We demonstrated that Snail and Slug transcription factors reduce the expression of ΔNp63 which is important for the establishment of metaplastic epithelia and their malignant transformation. Another objective for LEP is to integrate the respective roles of inflammatory and immunosuppressive factors in the epithelial metaplasia-dysplasia-cancer sequence with a particular attention to DC and pDC.

Pascale Hubert, Anca Reschner, Michaël Herfs, Ludivine Herman, Stéphanie Demoulin, Joan Somja
Collaborators: Francis Frankenne (Belgium), Sven Saussez (Belgium), Véronique Fontaine (Belgium), Anton Hopman (The Netherlands), Mohammad Arafa (Egypt), Jean-Michel Foidart (GIGA)



Loss of p63 immunoreactivity is observed in human SCC overexpressing Snail and/or Slug. A: Snail, Slug, and p63 expressions in paraffin-embedded sections of human SCC surgical specimens were assessed by immunohistochemistry. Variable degrees of Snail, Slug, and p63 expression were detected. B: Semiquantitative evaluation of p63 expression in 38 cervical, 32 head and neck, and 35 esophageal paraffin-embedded SCC specimens. The tissue samples were classified into four groups according to Snail and Slug immunoreactivity.

Genetic alterations and YY1 activity in colon adenocarcinoma

During colorectal tumorigenesis, several successive events, including genetic and epigenetic alterations, are involved in the transformation of the normal epithelium to a cancerous tissue with invasive and metastatic potential. The detection of these alterations is important for the diagnosis and prognosis of colorectal cancer (CRC), as well as for the administration of targeted therapies.
We showed, on samples from around 200 patients, that KRAS mutations (G->A) are strongly correlated to MGMT promoter methylation. Statistical analysis showed that these alterations occur after the methylation of H-cadherin (early marker) and before the methylation of E-cadherin (late marker). We used a new technique called MLPA to analyze amplification and deletion of different genes in the same samples. We observed, in 197 cases of colorectal tumors, EGFR gene amplification (32,5%), amplification of ERBB2 (7.6%), deletion of TP53 (32,5%), PTEN (12%) and CDKN2A (10%). Results also showed that EGFR gene amplification is correlated to deletion of TP53 and PTEN. We also analyzed the role of YY1 transcription factor in colorectal tumorigenesis. IHC results showed a link between YY1 expression and tumor differentiation status. Using shRNA transfection, we showed that YY1 inhibition leads to a decreased proliferation *in vitro* and in tumor development in mice.

Dominique Begon, Julie Mardaga
Collaborators: Thomas Wenner (Luxemburg), Marc Pauly (Luxemburg), Mario Dicato (Luxemburg), Denis Mottet (GIGA), Vincent Castronovo (GIGA), Cécile Oury (GIGA), Vincent Bours (GIGA)

Biomarker discovery based on DNA methylation and validation for endometrial and cervical cancer diagnosis

Cancer is one of the most common causes of death worldwide. Therefore the development of reliable and cost effective early detection methods is a priority in translational cancer research. Aberrant DNA methylation pattern may constitute valuable diagnostic tools. A platform for DNA methylation biomarker discovery was set up to differentiate cancer tissue from normal counterpart. A protocol of affinity on the MBD2 protein which binds specifically the hypermethylated DNA was employed. After hybridization on microarray slides and bioinformatic analysis, specific biomarkers were selected and a validation step using Methylation Specific PCR and bisulfite sequencing was undertaken.
By performing high-throughput DNA methylation screening, we have identified 30 genes with a strongly increased DNA methylation in cervical cancer compared to normal exocervix. We have also identified miRNA which could play a role in tumor development. Due to intellectual property issues, lists of methylated sequences, miRNA or genes which have been identified and selected as potential biomarkers have to be kept confidential. In collaboration with F. Fucks (ULB), we are currently triyng to confirm the identified methylation patterns by bisulfite pyrosequencing. In parallel, we are currently studying the function of some of these genes during the carcinogenesis or metastasis steps. The relationship with tumor proliferation, invasion, angiogenesis induction or control of immune system will be unraveled.

Pierre Dehan, Gaëlle Kustermans, Julie Horion, Samuel Guénin, Christine Canon, Moustapha Mouallif
Collaborators: François Fuks and Xavier Lampe (Belgium), Sven Saussez (Belgium), Agnès Noel (GIGA), Lucas Willems (CIGA), Jean-Michel Foidart (CIGA), Michaël Rehli (Germany), Dorian Pamart (Belgium), Wim van Criekinge (Belgium), Michèle Nisolle (Belgium).

LABORATORY OF HISTOLOGY-CYTOLOGY (LHC)

Our research topic is related to the analysis of the cross-talk between hematopoietic- and stromal- cells in the bone marrow in normal and pathological conditions, with a special interest in bone marrow adipocytes.

Neuropilin-1, a newly identified coreceptor for leptin: molecular Characterization and pathophysiological applications

We have shown that adipocytes negatively regulate granulopoiesis by inhibiting the production of G-CSF by macrophages. This inhibition required Neuropilin-1 (NP-1) expression and occurred independently of the two known NP-1 ligands, VEGF and Sema3A. We also demonstrated that leptin secreted by adipocytes was involved in this inhibition. These findings prompted us to make the hypothesis that NP-1, which is deficient in intrinsic signaling properties, forms a stable complex with OB-R and can contribute to the regulation of OB-R functions. Preliminary results showed that indeed NP-1 forms a complex with OB-R. Therefore, NP-1 could participate in the regulation of OB-R/leptin functions in the same way that NP-1 regulates plexin/SEMA and VEGF-R/VEGF functions by interacting with both the receptor and the ligand. We plan to study the new interaction that we have identified between NP-1 and OB-R as a new receptor complex for leptin and the consequences in OB-R biological functions. We intend to: 1. Characterize the molecular properties of the NP-1/OB-R/leptin complex (in collaboration with the team of Ralf Jockers) 2. Extend our study of the role of OB-R/NP-1/leptin in granulopoiesis 3. Study the importance of OB-R/NP-1/leptin complex chronic myeloid leukemia (CML).

Marie-Paule Defresne, Chantal Humblet, Géraldine Poncin, Aurore Beaulieu, Delphine Delneuville
Collaborators: Olivier Hermine (France), Ralf Jockers (France), Geoges Lognay (Belgique)

GIGA-Development, Stem Cells and Regenerative Medicine

Development is the study of various cell types and organs that are generated from the zygote. A better understanding of the molecular and cellular processes involved in generating an animal or a human being from a single cell is very important not only for basic science, but this understanding can also be fruitful to imagine and/or to design new therapeutic approaches to cure and stimulate regeneration in adulthood after a disease.

In a few words, a better understanding of how we are built could help us to rebuild after a lesion, whatever the aetiology is. For this reason, this unit is called «Development, Stem Cells and Regenerative Medicine». It encompasses about 30 scientists, students and technicians working on several model systems including human and animal embryonic stem cell (ES cell) cultures, zebrafish, chick and mouse.

Contact

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→ www.giga.ulg.ac.be/development

COMPOSITION OF THE THEMATIC RESEARCH UNIT

4 laboratories
34 scientists
- 5 Principal Investigators
- 5 PhD (postdoc)
- 18 PhD Students
- 6 Technicians

Highlight

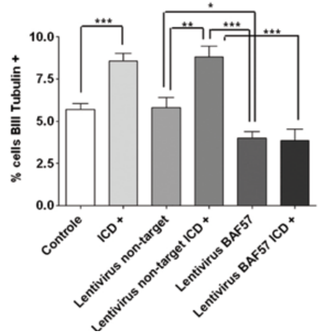
Neuregulin-1 modulates the differentiation of neural stem cells *in vitro* through an interaction with the Swi/Snf complex

Molecular and Cellular Neuroscience. 2010;43:72-80

During development of the central nervous system, neural stem cells proliferate and differentiate into neurons, then into precursors of oligodendcytes (OPC), and finally into astrocytes. OPC become mature oligodendrocytes around and after birth. Both the proliferation and the differentiation of neural stem cells are highly regulated processes that are dependent of intrinsic influences (i.e. transcription factors) but also extrinsic stimulation (i.e. growth factors). A family of growth factors already described as playing a role in these processes is neuregulin encoded by four genes. The neuregulin-1 (Nrg-1) gene is translated into several protein isoforms, which are either secreted or membrane-anchored. *In vitro*, neural stem cells express mainly the cystein-rich-domain NRG (CRD- NRG) isoform, a membrane-anchored type III form. This isoform exhibits a cystein-rich-domain, which constitutes a second transmembrane domain and can be cleaved to release both a signaling EGF-containing domain (ECD) at the cell surface and an intracellular domain (ICD). The main goal of this paper was to determine the exact role of ECD and ICD in neural stem cell survival, proliferation and differentiation. Using a siRNA approach, we demonstrated that CRD-NRG inhibition was followed by a decrease in neural stem cells proliferation and of neuronal or oligodendroglial differentiation. Overexpression of ICD but not ECD was followed by a decrease in neural stem cell proliferation and an increase in neuronal and oligodendroglial differentiation. Moreover, using several approaches (double hybrid, TAP-Tag and co-immunoprecipitation), we showed that ICD physically interacted in cultured neural stem cells with BRM and BAF57, two members of the Swi/Snf remodeling complex, and that ICD stimulation of neuronal cell differentiation is dependent on the presence of BAF57.

Dorothée Pirotte[1], Sabine Wislet-Gendebien[1], Jean-Michel Cloes[2,3] and Bernard Rogister[1, 4, 5]

[1]Laboratory of Developmental Neurobiology, GIGA-Neurosciences Research Center, University of Liège, Belgium [2]Department of Biotechnology, Haute Ecole de la Province de Liège, Belgium; [3]Laboratory of Virology and Immunology, GIGA-Infection, Immunity and Inflammation, University of Liège, Belgium; [4]GIGA_Development, Stem Cells and Regenerative Medicine, University of Liège, Belgium ; [5]Department of Neurology, University of Liège, C.H.U. (B35) Sart Tilman, B-4000 Liège, Belgium



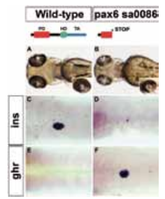
*ICD induced neuronal differentiation of neural stem cells in a BAF57-dependent manner. Dissociated neural stem cells were transduced by lentiviral particles for 1 h. The transfection reagent with ICD-expression plasmid was then added and cells were transferred onto coverslips after 1 h of culture in suspension. Five days after transduction/transfection, cells were paraformaldehyde-fixed and processed for immunolabeling using anti-βIII-tubulin antibodies allowing us to recognize and count newly differentiated neurons. Inhibition of BAF57 expression by shRNA induced a decrease in the number of neurons and this decrease was not compensated by the expression of ICD. Twenty fields per coverslip were randomly counted, two coverslips per transfection and a total of eight transfections (N=8). Statistical analysis were performed using Student's t-test method; *, ** and *** respectively represent p<0.05, p<0.01 and p<0.0005.*

LABORATORY OF MOLECULAR BIOLOGY AND GENETIC ENGINEERING (LMBGE-A)

We use the zebrafish as an animal model to study the molecular mechanisms controlling pancreas development. Our objective is to elucidate the regulatory molecules and processes involved in pancreas morphogenesis and controlling pancreatic cell differentiation. During 2010, we could decipher the role of pax6b and nkx6 in pancreatic cell fate determination. Such studies could have an important impact on the design of novel therapies for human pancreatic diseases such as diabetes or pancreas tumorigenesis.

Role of transcription factor Pax6b in the differentiation of pancreatic endocrine cells

Pax6 is a well conserved transcription factor found in all vertebrates which plays crucial functions in the development of eyes, brain and pancreas. This transcription factor has the peculiarity of possessing two distinct DNA binding domains, a paired domain and a homeodomain. Zebrafish has two pax6 genes, named pax6a and pax6b paralogs, and previous studies from LBMGE-a have demonstrated that only pax6b is expressed during pancreatic development. This year, LBMGE-a has shown that pax6b plays a crucial function in the cell fate choice of pancreatic endocrine progenitors. Indeed, in homozygous zebrafish mutants harboring a null mutation in the pax6b gene (the sa0086 mutant line), the pancreatic progenitor cells cannot differentiate into insulin-expressing β cells and become ghrelin-expressing ε cells.



Surprisingly, LBMGE-a discovered that the homeodomain of Pax6b was dispensable for its function in the pancreas. Indeed, a targeted deletion of pax6b homeodomain or a mutation in this homeodomain (present in the zebrafish sunrise mutant line) had no obvious effect on pancreatic cell differentiation, while it caused severe anomalies in the formation of the eyes. These results suggest that the Pax6b protein is binding to DNA through different mechanisms and domains in the eye and in the pancreas.

Vincianne Verbruggen, Olivier Ek, François Delporte, Virginie Von Berg, Nathalie Detry, Joseph Martial, Marianne Voz, Isabelle Manfroid and Bernard Peers
Collaborators: Daphné Georlette (USA), Frédéric Biemar (USA), Pedro Coutinho (UK)

Nkx6.1 and nkx6.2 regulate α and β cell formation in zebrafish by acting on pancreatic endocrine progenitor cells

In mice, the Nkx6 genes are crucial to α and β cell differentiation, but the molecular mechanisms by which they regulate pancreatic subtype specification remain elusive. LBMGE-a has shown that in zebrafish, nkx6.1 and nkx6.2 are co-expressed at early stages in the first pancreatic endocrine progenitors, but that their expression domains gradually segregate into different layers, nkx6.1 being expressed ventrally with respect to the forming islet, while nkx6.2 is expressed mainly in β cells. Knockdown of nkx6.2 or nkx6.1 expression leads to nearly complete loss of α cells but has no effect on the other pancreatic cell type. In contrast, nkx6.1/nkx6.2 double knockdown leads additionally to a drastic reduction of β cells. Synergy between the effects of nkx6.1 and nkx6.2 knockdown on both α and β cell differentiation suggests that nkx6.1 and nkx6.2 have the same biological activity, the required total nkx6 threshold being higher for α cell than for β-cell differentiation. Finally, we demonstrate that the nkx6 act on the establishment of the pancreatic endocrine progenitor pool whose size is correlated with the total nkx6 expression level. On the basis of our data, we propose a model in which nkx6.1 and nkx6.2, by allowing the establishment of the endocrine progenitor pool, control α- and β-cell differentiation

Anne-Catherine Binot, Lydie Flasse, Aurélie Ghaye, Isabelle Manfroid, Marianne Voz, Bernard Peers, Joseph Martial
Collaborator: Patrick Motte (Belgium)

LABORATORY OF MOLECULAR BIOLOGY AND GENETIC ENGINEERING (LBMGE-B)

The research team LMBGE-b uses the zebrafish model to study cartilage and bone development and homeostasis. The function of several transcription factors as well as specific signal transduction pathways are investigated. In addition, several factors involved in the development of the anterior pituitary are characterized and medium scale screening methods are developed to investigate various biological properties of chemical compounds on zebrafish.

Cartilage and bone development and homeostasis

Our approach to studying bone metabolism in zebrafish concentrates on the earliest stages (up to 10 days post-fertilization) and mainly on the head skeleton. We studied the function of the egr1 gene, coding for a Zn-finger transcription factor, in cartilage formation. We showed that egr1 is expressed in the pharyngeal endoderm, adjacent to the developing chondrocytes, and that it is part of a regulatory cascade in this tissue. Activation of this cascade modulates extracellular signalling through the BMP pathway precisely, which is required for differentiation and morphogenesis of the head cartilage. We also investigate the precise roles of the Fgf pathway in these processes, by studying the Fgf receptors, their expression profile and their signalling in the endoderm and developing chondrocytes.

A different approach to identifying genes that are involved in bone metabolism is to apply treatments that perturb or enforce bone formation and to identify genes that are affected by these treatments. As a proof of principle, we used the drugs «parathyroid hormone» and «vitamine D3» to study affected genes by whole genome expression microarrays. Other drugs will be tested. We also identified several specific factor inhibitors that perturb cartilage formation, and we use experiments in decreased or increased gravity to manipulate bone formation.
Our final aim is to obtain clues about the causes for bone pathologies such as osteoporosis or osteopetrosis

Marc Muller, Julia Dalca, Jessica Aceto, Arnaud Larbuisson, Benoist Pruvot, Joseph Martial
Olivier Stern, Raphael Marée, Louis Wehenkel
Collaborators: Patrick Motte (Belgium), Peter Alestrom (Norway), Silvia Bradamante, Jeannette Maier (Italy), Roland Goerlich (Germany), Manfred Schartl (Germany), Stefan Schulte-Merker (Netherlands), Christoph Winkler (Singapore), Klaus Slenzka (Germany), Jack van Loon (Netherlands), Till Eisenberg (Germany), Richard Hill (UK)

The anterior pituitary in zebrafish

The pituitary gland is composed of several cell types which produce each a specific hormone that, taken together, control many aspects of development and growth in vertebrate organisms. We previously characterized an additional cell type specific to fish that produces somatolactin. We then investigated the function of the transcription factor Sox4b in pituitary development and we showed that this factor is required for the expression of thyroid-stimulating-hormone and gonadotropins in the developing pituitary. Additional factors, identified by the genetic analysis of mice and cattle, are also presently being investigated.

Marc Muller, Yobhana Quiroz , Joseph Martial
Collaborators: Patrick Motte (Belgium), Matthias Hammerschmidt (Germany), Zoltan Varga (USA)

Zebrafish as model system for toxicology and pharmacology

The zebrafish is increasingly recognized as an interesting model system for vertebrate embryology and physiology and is thus increasingly used to study the biological (toxic and pharmacologic) effects on an entire organism. In this project, we set up standard toxicological tests for lethality, developmental defects, cardiac activity, cell death, oxidative stress and behavior. We extended the test panel by developing new specific tests, also using transgenic zebrafish lines and by studying gene expression by RT-PCR, in situ hybridization and expression microarrays.

Marc Muller, Benoist Pruvot, Yobhana Quiroz, Nathalie Jeanray, Audrey Voncken, Amandine Piot, Joseph Martial, Raphael Maree, Pierre Geurts, Louis Wehenkel
Collaborators: Yves-Jacques Schneider (Belgium), Dominique Lison (Belgium), Jean-Paul Noben (Belgium), Bert Smeets (Netherlands), Kurt Hofmann (Germany)

EMBRYOLOGY

The laboratory of Embryology has now two major research interests: (i) development of novel transgenesis tools in the chicken by establishing, characterizing and engineering primordial germ cell lines and (ii) contributions to the understanding of human embryonic stem cell pluripotency and the effort to optimize their cryopreservation in a bio-safe and high throughput format. Work on human embryonic stem cells is carried on at the GIGA-R, the research on chicken primordial germ cells being performed at the Faculty of Veterinary Medicine.

Contribution to deciphering Oct4 regulation in Human embryonic stem and carcinoma cells in response to hypoxic culture conditions

The Oct4 transcription factor is known to play a central role in the maintenance of the pluripotent state of embryonic stem-cells (ESCs), and is a key component of the «cocktails» allowing successful reprogramming of differentiated cells towards an embryonic-like pluripotent state. Yet its own regulation is still poorly understood. Culture under low oxygen tension, mimicking the prevailing conditions during early embryonic development, has been shown to favour pluripotency and self-renewal of murine ESCs by sustaining Oct4 expression via Hypoxia-Inducible Factors (HIFs). Our aim is to contribute to the understanding of the cis-transcriptional regulation of Oct4, especially in response to hypoxia. We have confirmed the growth-promoting effect of hypoxic conditions on human embryonic carcinoma cells (hECCs, good models for human ESCs, but easier to work with for preliminary work). Yet, we did not observe any change in Oct4 nor Hif2a levels, which is still to be confirmed. In that perspective, we have generated hECC lines carrying a promoter-reporter transgene that will allow us to study various regions of the Oct4 promoter in response to hypoxia or to overexpression of HIFs. ChIP-Seq targeting Hif2a will also be performed. The same approach is translated on one hESC line.

Delphine Connan
Collaborators: Paul De Sousa (UK), Steve Pells (UK)

GIGA-Genetics

The Genetics thematic unit (GTU) regroups three ULg laboratories: the laboratory of Human Genetics (Vincent Bours), the laboratory of Hepatology and Gastroenterology (Edouard Louis), and the laboratory of Animal Genomics (Michel Georges). The GTU focuses on the forward genetic dissection of Mendelian and complex traits in human and domestic animals. It has a strong epigenetics program, rooted in unique animal models, with special emphasis on microRNA biology. It develops approaches for the utilization of molecular information in livestock breeding, including genomic selection and transgenics.

The GTU totals about 50 members, of which more than halve are post-doctoral fellows or senior scientists. In addition to wet-lab genomicists, the unit has a strong bioinformatics/statistical genetics component. Women make up more than 50% of the unit, and nine nationalities are represented. Members of the GTU cluster in seven teams: genetics of inflammatory bowel disease, human genetics, dog genetics, livestock genetics, genomic selection, epigenetics and transgenics.

Contact

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michel.georges@ulg.ac.be
→ www.giga.ulg.ac.be/genetics

COMPOSITION OF THE THEMATIC RESEARCH UNIT

3 laboratories
42 scientists
- 6 Principal Investigators
- 17 PhD (postdoc)
- 12 PhD Students
- 7 Technicians

Highlight

CCDC39 is required for assembly of inner dynein arms and the dynein regulatory complex as well as normal ciliary motility in humans and dogs

Nature Genetics. 2011;43:72-78

Primary Ciliary Dyskinesia (PCD) is an inherited disorder characterized by recurrent infections of the upper and lower respiratory tract, reduced fertility in males, and *situs inversus* in ~50% of patients (Kartagener syndrome). It is caused by motility defects of respiratory cilia responsible for airway clearance, of flagella propelling sperm cells, and of nodal monocilia determining left-right asymmetry. Recessive mutations in genes encoding components of the outer dynein arms, radial spokes and cytoplasmic pre-assembly factors of axonemal dyneins have been identified but only account for ~50% of cases. We exploit the unique properties of dog populations to positionally clone a novel PCD gene: CCDC39. We demonstrate that loss-of-function mutations in the human ortholog underlie a significant fraction of PCD cases with axonemal disorganization and abnormal ciliary beating. Functional analysis indicate that CCDC39 localizes to ciliary axonemes, and is essential for assembly of inner dynein arms and the dynein regulatory complex.

Anne-Christine Merveille[1,*], Erica E. Davis[2,*], Anita Becker-Heck[3,4,5,*], Marie Legendre[6,*], Israel Amirav[7], Géraldine Bataille[1], John Belmont[8], Nicole Beydon[9], Frédéric Billen[10], Annick Clement[11], Cécile Clercx[10], André Coste[12], Rachelle Crosbie[13], Jacques de Blic[14], Stephane Deleuze[10], Philippe Duquesnoy[6], Denise Escalier[6], Estelle Escudier[6], Manfred Fliegauf[3], Judith Horvath[3], Kent Hill[13], Mark Jorissen[15], Jocelyne Just [16], Andreas Kispert[17] , Mark Lathrop[18], Niki Tomas Loges[3,5], June K. Marthin[19], Yukihide Momozawa[1], Guy Montantin[6], Kim G. Nielsen[20], Heike Olbrich[3,6], Jean-François Papon[6,12], Isabelle Rayet[19], Gilles Roger[21], Miriam Schmidts[3], Henrique Tenreiro[6], Jeffrey A.Towbin[8], Diana Zelenika[18], Hanswalter Zentgraf[22], Michel Georges[1], Anne-Sophie Lequarré[1,#], Nicholas Katsanis[2,#], Heymut Omran[3,5,#], Serge Amselem[6,#].

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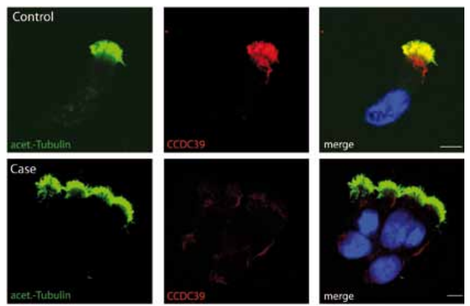
[14]AP-HP, Groupe Hospitalier Necker-Enfants Malades, Service de pneumologie et d'allergologie pédiatriques, France. [15]Department of Otorhinolaryngology, Head and Neck Surgery, University Hospitals Leuven, Leuven, Belgium. [16]AP-HP, Hôpital Armand-Trousseau, Centre d'investigation de l'asthme et des allergies, France. [17]Institut für Molekularbiologie, Medizinische Hochschule Hannover. [18]Centre National de Génotypage, France. [19] Hôpital Nord, Service de réanimation pédiatrique, Centre Hospitalier Universitaire de Saint-Etienne, France. [20] Copenhagen University Hospital, Rigshospitalet, Danish PCD Center, Pediatric Pulmonary Service, Denmark. [21]AP-HP, Hôpital Armand-Trousseau, Service d'ORL et de chirurgie cervico-faciale pédiatrique, France. [22]Department of Tumor Virology, German Cancer Research Center, Germany. * Contributed equally to this work. # These authors jointly supervised this work.



Sub-cellular localization of CCDC39 in respiratory epithelial cells from PCD patients carrying CCDC39 mutations. As control, axoneme-specific antibodies against acetylated α-tubulin (green) were used. Nuclei were stained with Hoechst 33342 (blue). In respiratory epithelial cells from healthy probands (a), CCDC39 (red) localizes predominantly along the entire length of the axonemes, as well as to the apical cytoplasm. In respiratory epithelial cells from patients (b), CCDC39 is absent from the axoneme and markedly reduced in the apical cytoplasm. White scale bars (a-e) are 5µm. (Image courtesy of Heymut Omran).

GENETICS OF CROHN'S DISEASE

Searching for rare susceptibility variants for Crohn's disease by massive parallel resequencing.

Genome-wide association studies (GWAS) have identified tens of risk loci for many complex disorders including Crohn's disease (CD). However, common disease-associated SNPs explain at most 20% of the genetic variance. Several factors may account for the missing heritability, including rare risk variants not adequately tagged in GWAS. The fact that rare susceptibility variants contribute to the variation of multifactorial phenotypes has been demonstrated for colorectal cancer, plasma levels of HDL cholesterol, blood pressure, type I diabetes, hypertriglyceridemia and - in the case of CD – for the NOD2 gene. We have used high-throughput resequencing of DNA pools to search for rare coding variants influencing susceptibility to CD in 63 GWAS-identified positional candidate genes. We have identified low frequency coding variants conferring protection against inflammatory bowel disease (IBD) in the IL23R gene, yet we conclude that rare coding variants in positional candidates do not make a large contribution to inherited predisposition to CD.
Funding: Walloon Region, FNRS, Belgian Science Policy.

Yukihide Momozawa, Myriam Mni, Kayo Nakamura, Wouter Coppieters, Cécile Libioulle, Catherine Reenaers, Edouard Louis, Michel Georges.
Collaborators: Leila Amininejad, Isabelle Cleynen, Peter de Rijk, Olivier Dewit, Dirk Goossens, Debby Laukens, Paul Rutgeerts, Jurgen Del-Favero, Martine de Vos, Denis Franchimont, Severine Vermeire (Belgium), Diana Zelenika, Mark Lathrop, Jean-Pierre Hugot (France).

Towards multi-tissue genetic, epigenomic, transcriptomic and microbiomic annotation of loci associated with inflammatory bowel disease (IBD).

GWAS have identified more than 100 loci harboring common variants conferring inherited predisposition to IBD. However, for the majority of risk loci, causative gene(s) and variant(s) remain unknown. To help identify the underlying molecular defects, we are collecting samples of nine cell types/tissues and corresponding "adherent" fecal samples (ileal, colonic, rectal) from ≥400 healthy Caucasians. We genotype each individual for ≥700K SNPs, analyze the transcriptome of each tissue sample using expression arrays, analyze the methylome of a subset of the tissue samples using a genome-wide methylation assay, and study the microbiome composition of the fecal samples using high throughput sequencing (Roche FLX) of 16S rRNA amplicons. Using the resulting data, we are searching for (i) associations between the gene expression level and SNP alleles/haplotypes, particularly those associated with IBD risk loci, (ii) associations between the methylation status of specific CpG and SNP alleles/haplotypes, (iii) correlations between the methylation status and expression levels, (iv) associations between the microbiome composition and SNP alleles/haplotypes, and (v) associations between microbiome composition, gene expression and methylation. This project will identify genes and pathways underlying inherited predisposition to IBD, and will examine the heritability of intestinal microbiome composition.
Funding: Walloon Region, Belgian Science Policy.

Valérie Deffontaine, Yukihide Momozawa, Emilie Théâtre, Benoît Charloteaux, Myriam Mni, François Crins, Ann-Stephan Gori, Alex Kvasz, Catherine Reenaers, Edouard Louis, Michel Georges

Towards a meta-GWAS for IBD

It is increasingly apparent that inherited predisposition to common complex diseases, including IBD, involves a vary large number of «polygenes» with individually small effects. Detecting such minor gene effects is possible but requires very large case-control cohorts, which can only be assembled by means of collaborative efforts involving many laboratories. With that goal in mind, the International IBD Consortium was formed, bringing together samples of more than 10,000 IBD patients and an equivalent number of controls. During 2010, all these have been genotyped with the «Immunochip», an infinium array with close to 200,000 SNPs focusing on genes that are related to the immune response and its disorders. More than 2,000 samples have been genotyped at GIGA-R.
Funding: Walloon Region, Belgian Science Policy.

Emilie Théâtre, Benoît Charloteaux, Myriam Mni, Naima Ahariz, Nadine Cambisano, Alex Kvasz, Wouter Coppieters, Michel Georges, Edouard Louis.
Collaborators: International IBD Consortium

HUMAN GENETICS

Positional identification of genes predisposing to familial forms of complex diseases

We have conducted linkage analysis in two large multiplex pedigrees segregating for (i) an autosomal dominant form of strabismus, and (ii) familial isolated pituitary adenoma without mutations in the AIP gene. Regions of genome-wide significant linkage have been found in both cases. Resequencing of positional candidates is being conducted to identify the causative genes. In addition, we have studied the relevance of mutations in the IAP gene in sporadic cases of AIP.
Funding: French-speaking community and ULg.

Kayo Nakamura, Anouk Georges, Naima Ahariz, Nadine Cambisano, Carole Charlier, Michel Georges, Vincent Bours
Collaborators: Adrian Daly, Emilie Castermans, Albert Beckers, Vincent Paris (Belgium)

Deciphering the molecular origins of brain damage in phenylketonuria (PKU)

We are studying the process of myelin formation in the central nervous system in a mouse model of PKU as well as in *in vitro* primary co-cultures.

Claire Josse, Renaud Schoemans, Vincent Bours
Collaborator : Catherine Lubetzki (France)

CANINE GENETICS

Forward genetic dissection of monogenic disorders in the dog

We are studying two monogenenic defects in the dog. The first is primary ciliary dyskinesia (PCD) for which we have identified the causative gene (CCDC39), which we have shown to cause PCD in a significant proportion of human patients with axonemal disorganisation. CCDC39 was shown to be essential for assembly of the inner dynein arms and dynein regultory complex. The second is progressive juvenile glomerulonephropathy. A candidate locus has been identified and is presently being scrutinized.
Funding: European Union (LUPA project).

Anne-Christine Merveille, Géraldine Bataille, Tom Druet, Carole Charlier, Marilou Ramos Pamplona, Anne-Sophie Lequarre, Michel Georges.
Collaborators: Cécile Clercx, Dominique Peeters, Frédéric Farnir, France: Serge Amselem, Catherine Andre (Belgium), Erica Davis, Nico Katsanis (USA), Heymut Omran (Germany).

Positional identification of genetic determinants of variation in blood pressure, glucose and lipid metabolism in healthy dogs

We have collected a battery of phenotypes pertaining to cardiovascular physiology, glucose and lipid metabolism, in a cohort of more than 500 healthy dogs representing 10 breeds. The cohort has been genotyped with the 180K SNP panel. QTL influencing any of the measured traits will be mapped using an approach that exploits within breed segregation yet combines the signal across breeds (accounting for between-breed heterogeneity). Participating groups have agreed on a common clinical protocol and have initiated the actual data collection.
Funding: European Union (LUPA project).

Anne-Christine Merveille, Géraldine Battaille, Marilou Ramos Pamplona, Anne-Sophie Lequarre, Michel Georges.
Collaborators: Kathleen McEntee (Belgium), LUPA consortium

LIVESTOCK GENETICS

Genomic surveillance and management of inherited defects in livestock

Intense selection for improved performances in livestock reduces the effective population size, causing regular outburst of recessive defects that are a major cost to the industry. We have established a heredosurveillance platform that (i) collects samples from emerging defects, (ii) genetically maps the causative genes using genome-wide high density SNP arrays, (iii) identifies the causative mutations by resequencing positional candidates, (iv) develops and offers diagnostic tests for the benefit of the breeders. Our laboratory developed the first high density SNP array for livestock and demonstrated its effectiveness for positional cloning. We have pursued these efforts and have now mapped 13 inherited defects in cattle and identified the causative mutation for eight of these including by means of whole genome resequencing. Diagnostic tests have been developed for the 13 defects and are being offered to the breeders. Large-scale utilization of the tests has allowed the eradication of the defects with major benefits to breeders.
Funding: Walloon Ministry of Agriculture.

Arnaud Sartelet, Corinne Fasquelle, Sarah Geron, Nico Tamma, Latifa Karim, Tom Druet, Michel Georges, Wouter Coppieters, Carole Charlier.
Collaborators: Daniel Desmecht, Frédéric Rollin, Marc Dive (Belgium)

Positional identification of QTL and QTN in livestock

The UAG is involved in several projects aimed at identifying QTL influencing economically important traits in livestock. In one of these, we mapped a Quantitative Trait Locus (QTL) with major effect on bovine stature in a ~780kb chromosome interval using a Hidden Markov Model-based approach that simultaneously exploits linkage and linkage disequilibrium. We resequenced the ~780 kb interval in six sires with known QTL genotype, and identified 13 clustered candidate Quantitative Trait Nucleotides (QTN) out of > 9,572 discovered variants. We eliminated five candidate QTN by studying the phenotypic effect of a recombinant haplotype identified in a breed diversity panel. We demonstrated that QTN genotype influences the expression level of seven of the nine genes mapping to the ~780Kb in multiple fetal tissues. We demonstrated that two of the eight candidate QTN, mapping to the PLAG1-CHCHD7 intergenic region, influence bidirectional promoter strength and affect the formation of complexes with nuclear factors. By performing expression QTL (eQTL) analysis, we identified a splice site variant and exploited this naturally occurring null-allele to exclude CHCHD7 as single causative gene.
Funding: Unit of Animal Genomics and Boviquest (New Zealand).

Latifa Karim, Haruko Takeda, Li Lin, Tom Druet, Denis Baurain, Nadine Cambisano, Naima Ahariz, Michel Georges, Wouter Coppieters
Collaborators: Frédéric Farnir, Bernard Grisart (Belgium), Juan Arias, Stephen Davis, Bevin Harris, Mike Keehan, Mathew Littlejohn, Richard Spelman (New Zealand).

Development and application of statistical tools for genomic selection in livestock

We have developed a novel method based on hidden markov models for the phasing of high density SNP data that allows imputation of missing genotypes, as well as haplotype-based mapping and genomic selection. The performances of the method has been evaluated for imputation and for genomic selection. It has been used within the Eurogenomics project to allow integration of SNP genotypes across European partners. The method has been extended to allow for the analysis of binary traits while correcting for stratification. We are applying genomic selection methodology for the estimation of genome-wide relative risk to develop common complex diseases such as Crohn's disease.
Funding: Walloon Ministry of Agriculture, CRV (The Netherlands), Belgian Science Policy.

Zhiyan Zhang, Birgit Debrabant, Michel Georges, Tom Druet
Collaborators: Frédéric Farnir (Belgium), Sande de Roos, Chris Schrooten (Netherlands).

Genetic analysis of male recombination and segregation distortion in cattle

We exploited available genome-wide SNP data for more than 15,000 bulls to perform a detailed analysis of male recombination in cattle. We studied genome-wide recombination rate, chromosome-specific recombination rates, locus-specific recombination rate, recombination hotspot usage, and chromosome interference. For each trait we evaluated the degree of interindividual variation, as well as the repeatability and heritability of the trait. We performed genome-wide QTL scans for each trait and identified a number of genome-wide significant and suggestive QTL encompassing strong candidate genes, which are being pursued. The same data set is being used to study the phenomenon of segregation distortion.

Cynthia Sandor, Wanbo Li, Amabelle Tenge, Nadine Cambisano, Naima Ahariz, Sarah Geron, Wouter Coppieters, Tom Druet, Carole Charlier, Michel Georges

Analysis of Copy Number Variation (CNV) in livestock

We have used a variety of complementary approaches (SNP genotypes, array CGH, while genome sequencing and FISH) to identify and characterize CNV in livestock. Several hundreds of CNV have been identified. One of these was shown to be associated with the color sided phenotype. A detailed analysis of this CNV revealed an entirely novel mechanism of duplicative transposition involving circular shuttling intermediates. We are searching for similar events in other organisms including human. We postulate that this mechanism may contribute to exon shuffling.

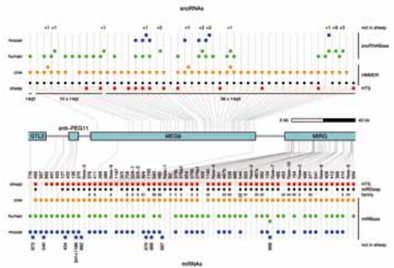
Keith Durkin, Denis Baurain, Wouter Coppieters, Michel Georges, Carole Charlier
Collaborator : Tosso Leeb (Switzerland)

EPIGENETICS

Towards molecular understanding of polar overdominance at the ovine callipyge locus

The callipyge muscular hypertrophy is characterized by a unique mode of inheritance (polar overdominance) in which only heterozygous animals receiving the CLPG mutation from their father express the phenotype. We have shown that the CLPG mutation is a gain-of-function mutation that causes ectopic expression of the paternally expressed imprinted DLK1 gene in skeletal muscle of $+^{Mar}/CLPG^{Pat}$ animals, hence causing the phenotype. We hypothesized that the absence of phenotypic expression in CLPG/CLPG animals, is due to post-transcriptional silencing of DLK1 due to ectopic expression of miRNAs processed from the maternally expressed non-coding RNA genes GTL2/antiPEG11/RIAN/MIRG. To identify the responsible miRNA we generated exhaustive miRNA catalogues of ovine skeletal muscle by high throughput sequencing, including 111 miRNAs processed from 66 precursors in the DLK1/GTL2 domain. We demonstrated that these miRNAs are imprinted, subject to the cis-effect of the CLPG mutation, jointly account for 20% of the miRNA population in CLPG/CLPG animals, and that some of them undergo A to I editing. We are testing whether these miRNA target DLK1 use both bioinformatic and wet-lab approaches.
Funding: European Union, Belgian Science Policy, FNRS, French-speaking Community and ULg.

Florian Caiment, Huijun Chen, Xuewen Xu, Carole Charlier, Michel Georges, Denis Baurain, Haruko Taked
Collaborator : Noelle Cockett (USA)



Comparative map of the small RNA genes in the DLK1-GTL2 domain: snoRNAs (upper panel) and miRNA (lower panel)

Patrocles: polymorphic miRNA-mediated gene regulation

In 2006, our laboratory identified the first polymorphism that caused a mutant phenotype by perturbing miRNA-mediated gene regulation in any vertebrate: a mutation creating a potential illegitimate miRNA target site in the myostatin gene was shown to affect musculority in sheep. We subsequently established a database (www.patrocles.org) that compiles polymorphisms predicted to perturb miRNA mediated gene regulation in vertebrates. Patrocles lists tens of thousands of polymorphisms that are predicted to affect miRNA targets, miRNA precursors or components of the silencing machinery. Contextual information is provided to assess the relevance of the predictions. To allow confirmation of the Patrocles predictions we have developed a biochemical procedure to identify polymorphic miRNA-target interactions *in vivo*. The method is based on the coimmunoprecipitation of miRNA targets using anti-argonaut antibodies from tissues of individuals that are heterozygous for the SNPs of interest. The effect of the SNP on miRNA mediated gene regulation is then assessed from the degree of allelic imbalance in the immunoprecipitated material.
Funding: European Union, Belgian Science Policy, FNRS, French-speaking Community and ULg.

Haruko Takeda, Denis Baurain, Samuel Hiard, Carole Charlier, Michel Georges
Collaborator: Tomi Pastinen (Canada)

TRANSGENIC ENGINEERING OF LIVESTOCK

Engineering male-specific double-muscling

We have previously shown that "double-muscling" in cattle is caused by loss-of-function mutations in the myostatin gene. We have subsequently shown that it was possible to integrate dominant negative myostatin alleles onto the Y chromosome by homologous recombination, thereby generating male-specific double-muscling in a mouse model. We are in the process of extending the same approach to livestock. To that end we have extensively characterized the bovine Y chromosome and pseudoautosomal region and have developed the necessary vectors to target the bovine Y chromosome by homologous recombination followed by integration of dominant negative myostatin alleles by recombinase mediated cassette exchange. Experiments in fetal fibroblasts are underway as are the optimizations for cloning by nuclear transfer.

Funding: Walloon Ministry of Agriculture

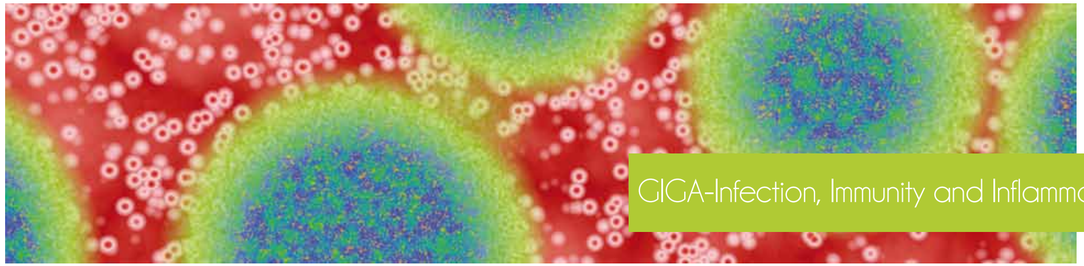
Mallory Draye, Anne-Sophie Van Laere, Fabien Ectors, Michel Georges

Towards semen sexing using a transgenic approach

The ability to predetermine the sex of offspring remains a very important objective in animal production. We are attempting to develop transgenic approaches towards semen sexing using the mouse as model. Our aim is to induce gene expression specifically in Y-bearing sperm. To achieve this goal, two major problems are to be solved: (i) we need to overcome meiotic sex chromosome inactivation (MSCI), and (ii) we need to block diffusion of gene products between syncitial spermatids.

Funding: Walloon Ministry of Agriculture.

Muralidhar Metta, Fabien Ectors, Michel Georges



GIGA-Infection, Immunity and Inflammation

In order to cure or improve the treatment of many human diseases, it is essential to understand the mechanisms by which the immune system maintains the organism integrity against internal or external aggressions.

The Infection, Immunity and Inflammation (I^3) thematic unit of the GIGA-Research Institute aims at stimulating synergies between research groups studying various but complementary aspects of immunity. These aspects include immune cell development, hematology, inflammation, allergy, cancer immunology and viral infections. Created in 2008, the I^3 Unit is composed of 10 laboratories and 93 researchers. Research themes span the spectrum from bench-to-bedside. Indeed, studies range from cell culture systems and animal models of disease to clinical studies, all using state-of-the-art laboratory techniques.

During the year 2010, members of the Unit produced or contributed to an appreciable number of significant scientific publications.

Contact

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COMPOSITION OF THE THEMATIC RESEARCH UNIT

10 laboratories
93 scientists
- 17 Principal Investigators
- 21 PhD (postdoc)
- 42 PhD Students
- 13 Technicians

Highlight

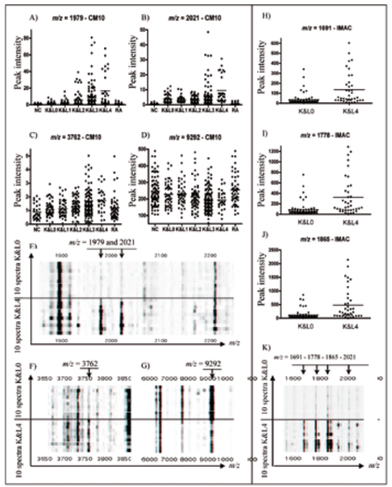
Discovery and biochemical characterization of four novel biomarkers for osteoarthritis

Annals of the Rheumatic Diseases. 2011

Knee osteoarthritis (OA) is a heterogeneous, complex joint pathology of unknown etiology. Biomarkers have been widely used to investigate OA but currently available biomarkers lack specificity and sensitivity. Therefore, novel biomarkers are needed to better understand the pathophysiological processes of OA initiation and progression. We have used the SELDI-TOF-MS proteomic technique to analyze protein expression levels in 284 serum samples from patients with knee OA classified according to Kellgren & Lawrence (K&L) score of severity (0 to 4). OA serum samples were also compared to serum samples provided by healthy individuals (normal control; NC; n=36) and rheumatoid arthritis (RA) patients (n=25). Proteins that gave similar signal in all K&L groups of OA patients were ignored, while proteins with increased or decreased levels of expression were selected for further studies. Protein profiles were analyzed by two statistical approaches: a univariate analysis (Mann-Whitney test) and a decision-tree multivariate analysis (a machine-learning algorithm called Extra-Trees). Consequently, two proteins were found to be expressed at higher levels in sera of OA patients at all 4 K&L scores compared to NC and RA, and were identified as V65 vitronectin fragment and complement C3f peptide. Of the two remaining proteins one showed increased expression (unknown protein at m/z of 3762) and the other (identified as CTAPIII protein) was decreased in K&L scores >2 subsets compared to NC, RA and K&L scores 0 or 1 subsets. These novel biomarkers showed correlations with a number of OA biomarkers (aggrecan, keratan sulfate, YKL-40, CTX-II, pyridinoline and deoxypyridinoline, TNF-α and MMPs) associated with cartilage loss, bone remodelling and local inflammation. These biomarkers, except the CTAPIII protein, were also detected in synovial fluids when present in serum samples from OA patients. The proteomics study reported here is based on samples obtained from a cross-sectional prospective study. There is a need for further studies using serum samples from new cohorts, a wider range of disease control subjects, as well as longitudinal studies to establish the usefulness of the four novel biomarkers identified.

Dominique de Seny[1], Mohammed Sharif[2], Marianne Fillet[3], Gaël Cobraiville[1], Marie-Alice Meuwis[4], Raphaël Marée[4], Jean-Philippe Hauzeur[1], Louis Wehenkel[5], Edouard Louis[6], Marie-Paule Merville[3], John Kirwan[7], Clio Ribbens[1] and Michel Malaise[1].

[1] Laboratory of Rheumatology, GIGA Research, University of Liège, CHU Liège, Belgium [2] Department of Anatomy, University of Bristol, United-Kingdom [3] Laboratory of Clinical Chemistry, GIGA Research, University of Liège, CHU Liège Belgium [4] GIGA Proteomics and Bioinformatics platform, University of Liège, Belgium [5] Bioinformatics and Modeling Unit, Department of Electrical Engineering Computer Science, GIGA Research, University of Liège, Belgium [6] Laboratory of Hepato-Gastroenterology, University of Liège, CHU Liège, Belgium [7] Rheumatology Unit, University of Bristol, United Kingdom



A),B),C),D) - Peak intensity distribution of serum biomarkers detected by the SELDI-TOF approach: V65 vitronectin fragment (m/z = 1979 , C3f peptide (m/z = 2021) , the unknown protein (m/z = 3762) and CTAPIII protein (m/z = 9292) through the 7 groups of patients (NC, K&L0 1, 2, 3, 4 and RA). E), F) and G) - Gel view spectra provided by 20 patients with OA (10 with K&L0 and 10 with K&L4) representing V65 vitronectin fragment (m/z = 1979) and C3f peptide (m/z = 2021), the unknown protein (m/z = 3762) and CTAPIII protein (m/z = 9292), respectively. H),I) and J) - truncated variants (m/z = 1691, 1778 and 1865) from m/z 2021 (C3f) through K&L0 and K&L4 groups . K) - Gel view spectra provided by 20 patients with OA (10 with K&L0 and 10 with K&L4) representing these variants: 1691, 1778, 1865 and 2021 m/z markers .

LABORATORY OF CELLULAR AND MOLECULAR PHYSIOLOGY

Our research laboratory has vast experience in the study of the cellular and molecular mechanisms involved in immune responses. Our laboratory is working in tight cooperation with several laboratories in Belgium and abroad. We also collaborate with the ULg University Hospital (CHU) to translate our experimental results in mice to humans.

Viral induction of Zac1b through TLR3- and IRF3-dependent pathways

Zinc finger protein regulator of apoptosis and cell cycle arrest (Zac1) is a transcription factor able to induce apoptosis or cell cycle arrest through independent pathways. In spite of the important potential functions attributed to Zac1, little is known of its physiological regulation and biological function. We discovered that variant Zac1b was expressed in murine embryonic fibroblasts (MEFs) treated with polyriboinosinic polyribocytidylic acid [poly(I:C)], a synthetic double-stranded RNA. This regulation occurred mainly through Toll-Like Receptor 3 (TLR3)- and Interferon Regulatory Factor 3 (IRF3)- dependent pathways. As TLR3 and IRF3 are central activators of antiviral immunity, we hypothesized that Zac1 may be implicated in antiviral responses. In line with this notion, we observed that Zac1b was expressed in MEFs infected with the Encephalomyocarditis virus (EMCV). We also observed that Zac1-deficient MEFs were less sensitive to EMCV-induced cell death than wild-type MEFs. However, Zac1 gene inactivation had no effect on the survival of mice infected with EMCV. In conclusion, this study describes for the first time a transcriptional regulation of Zac1b, induced by synthetic dsRNA and RNA viruses. Although the exact physiological function of Zac1 remains to be further investigated, Zac1b could thus play a role in antiviral responses.

Barbara Warzée, Claire Mesnil, Dimitri Pirottin, Pierre-Vincent Drion, Pierre Lekeux, Fabrice Bureau, Christophe Desmet

Collaborators: Didier Hober (France), Didier Caloone (France), Michel Garigliany (France), Daniel Desmecht (Belgium), Laurent Journot (France)

LABORATORY OF IMMUNOLOGY AND INFECTIOUS DISEASES

We have good expertise in the investigation of the cellular and molecular pathways leading to immunosuppression, in *in vitro, ex vivo* and *in vivo* animal models. We are mainly interested in the HIV infection and immunopathogenesis. We are also focused on understanding the role of several protein phosphatases in the negative regulation of T cell activation and cancer. We are collaborating with different laboratories (in house and international) and with the ULg University Hospital (CHU).

Generation of high and long term efficient hematopoietic stem cell engraftment and mounting of HIV specific cellular and humoral immune response in humanized NOD/LtsZ-scidIL-2Rγnull mice

Long and efficient HIV-1 replication studies in cord blood hematopoietic stem cells (CBHSCs) transplanted new generation immunodeficient NOD/LtsZ-scidIL-2Rγnull (NSG) and NOD/SCID/IL2Rγnull (NOG) mice have been hindered by preconditioning strategy constraints. Total body irradiation regime (TBI) limits the duration of HIV-1 infection study by limiting the survivability and life span of mice, amputation lowers engraftment levels of human CD45+ cells in a particular number of human T cells which are essential for replication of HIV-1. IMI new protocol of busulfan pre-treatment and fresh CBCD34+ cell transplantation in 4 week old mice resulted in efficient engraftment of approximately 92% of human CD45+ cells in peripheral blood, which was not reported with CBHSCs transplanted adult or new born NSG mice. Augmented engraftment also promoted early and elevated CD3+ levels with better lymphoid structure genesis and prolonged human cell reconstitution for 300 days with 100% survivability of mice. These Humanized NSG mice showed marked long-lasting viremia after infection with HIV-1 JRCSF and HIV-1Bal isolates for around 100 days, a gradual decline in CD4+ T cell count and wide spread HIV-1 induced immune activation.

Maneesh Singh, Morgane Bourcy

Collaborators: Guido Vanham (Belgium), Kjetil Tasken (Norway)

Insights into the role of the dual-specificity phosphatase VHR (Vaccinia Virus H1-related) in inflammation and neo-angiogenesis

VHR is a small dual-specificity phosphatase (21 kDa) belonging to the cysteine-based-motif phosphatase family. VHR has been reported to dephosphorylate Erk and Jnk, but not p38 on pTyr and pThr residues. VHR is considered as an atypical MAPK phosphatase, however, unlike many MKPs (MAPK phosphatases), VHR expression is not induced in response to activation of MAP kinases, but is instead regulated during cell cycle progression. IMI has recently reported that this phosphatase regulates cell cycle and is upregulated in several human cancers. IMI has recently generated VHR knockout mice in collaboration with T. Mustelin. These mice are healthy and have no apparent pathologies. However, IMI's preliminary data show that VHR deficiency in mice prevented neo-vascularization of transplanted malignant cells in matrigel. On the other hand, these mice are more resistant to sceptic shock induced by injection of lethal doses of LPS. IMI is currently investigating the role of VHR phosphatase in endothelial tumour angiogenesis and in innate immune response.

Mathieu Amand, Pratibha Singh, Lisbeth Maurissen

Collaborator: Lutz Tautz (USA)

TCR modulation and negative regulation of mature T cell responses. Involvement of the protein tyrosine phosphatase LYP/PEP in the development of type 1 diabetes (T1D)

A nonsynonymous single nucleotide polymorphism (nsSNP) in the human PTPN22 gene is associated with an increased risk of type 1 diabetes (T1D) and with other systemic and organ-specific autoimmune diseases. The gene encodes for the lymphoid-specific-phosphatase, LYP, which is mainly expressed in lymphoid cells. The less common allelic variant LYPW620 presents a tryptophan (W) in position 620 of the LYP protein instead of an arginine (R). There are many biochemical and epidemiological arguments in favour of the involvement of LYP in autoimmune diseases, therefore the PTPN22 locus is now well recognized as the «newest confirmed autoimmunity susceptibility locus». Biochemical studies of the LYPW620 variant have revealed that the LYPW620 versus LYPR620 protein *in vitro* is a more potent negative regulator of T lymphocyte activation, because it is a more potent phosphatase (gain-of-function variant). Moreover it was shown that LYPW620 fails to bind Csk tyrosine kinase (change-of-function variant). Our research project focused on the understanding of the molecular mechanisms leading to autoimmunity when the genetic variant LYPW620 is present, even in one copy, in people with T1D. We proposed two approaches, one is based on the hypothesis that LYPW620 gain-of-function phenotype causes autoimmunity. The second one is based on the hypothesis that LYPW620 change-of-function is important in the development of autoimmunity.

Lucia Musumeci, Fairouz Mehennaoui
Collaborator: Lutz Tautz (USA)

LABORATORY OF VIROLOGY AND IMMUNOLOGY

We study Varicella Zoster Virus (VZV) which is a human herpesvirus responsible for chickenpox and zoster. Our research is focused on the molecular level mechanisms that regulate the infectious cycle and on the mechanisms developed by VZV to interfere with the cellular machinery in order to increase its replication efficacy. We also collaborate with other groups on a mouse model of asthma with the aim to better understand gene expression in this disease.

Characterization of the functions of VZV proteins by engineering.

Identification of cellular partners VZV encodes for about 70 proteins among which only the regulatory proteins as well as some structural proteins have been intensively studied. Some proteins remain thus poorly characterized despite their high level of expression. Moreover their posttranslational modifications as well as their interaction with cellular partners are generally poorly documented although they could deeply impact on the biology of the virus. Our work is currently focused on some structural proteins or glycoproteins that appear to be highly posttranslationally modified and to be essential for the viral replication in some tissue. Their functions as well as the nature of their posttranslational modifications are under investigation. In parallel, interactomic experiments have been performed and have allowed us to identify new cellular partners that could give new clues concerning their functions.
It is crucial to validate the data obtained in transient expression by analysis in a viral context. Such studies require viruses expressing WT or mutated tagged proteins. Therefore, using the BAC technology that was optimized for our purpose, we have generated simple- and double-mutants in which the proteins of interest have been mutated and/or tagged. Thanks to these mutants that constitute an essential tool, we have analyzed more precisely the subcellular localisation of some of the structural proteins. Time lapse microscopy has allowed to decipher the process of virion assembly.

Marielle Lebrun, Emmanuel Di Valentin, Laura Riva, Isabelle Gluckmann, Xavier Rambout, Frank Dequiedt, Jean-Claude Twizere, Jacques Piette and Catherine Sadzot-Delvaux
Collaborators: Marc Vidal and David hill (USA)

Identification of new asthma biomarkers: mRNA and miRNA profiling in murine models of acute and chronic asthma

miRNAs are now recognized as key regulator elements in gene expression. Although they have been associated with a number of human diseases, their implication in acute and chronic asthma and their association with lung remodelling have never been thoroughly investigated. In order to establish miRNA expression profile in lung tissue, mice were sensitized and challenged with ovalbumin mimicking acute, intermediate and chronic human asthma. Levels of lung miRNAs were profiled by microarray and in silico analysis were performed to identify potential mRNA targets and to point out signalling pathways and biological processes regulated by miRNA-dependent mechanisms. 58, 66 and 75 miRNAs were found to be significantly modulated at short-, intermediate- and long-term challenge, respectively. These data allow us to use the transcriptomic data from our previous study to relate mRNA targets with the miRNAs identified. Inverse correlation with the expression of mRNA targets identified mmu-miR-146b, -223, -29b, -29c, -483, -574-5p, -672 and -690 as the best candidates for an active implication in asthma pathogenesis. The bioinformatics analysis identified miRNA-linked regulation of several signalling pathways, as matrix metalloproteinases, inflammatory response and TGF-β signalling, and biological processes, including apoptosis and inflammation. This study highlights that specific miRNAs are likely to be involved in asthma disease.

Emmanuel Di Valentin, Jacques Piette, Nancy Garbacki, Vân Anh Huynh-Thu, Pierre Geurts, Alexandre Irthum, Céline Crahay, Didier Cataldo, Alain Coligе.
Collaborators: Thierry Arnould and Johan Grooten (Belgium)

LABORATORY OF BIOLOGY OF TUMOR AND DEVELOPMENT

We are mainly focused on the study of the roles played by proteases and extracellular matrix components (including mediators) on cancer and inflammatory diseases. We participate in the GIGA-cancer and GIGA-I³.
Many different models are currently used to induce inflammatory conditions mimicking human diseases. Asthma models have been developed by using different allergens (OVA and house dust mite extracts); COPD models are induced by exposure of animals to cigarette smoke; bronchial hyperresponsiveness is also studied by the mean of an ozone-exposure model. Various specific techniques have been developed: inExpose®, Flexivent®, Penh measurements, powder inhalators, CTscan.

Characterization of roles played by MMP-12 in asthma and development of new therapeutic strategies based on its inhibition

Based on the results of a microarray study pointing MMP-12 as a key mediator in asthma and taking into account the various properties of this enzyme including elastolytic properties and pro-TNF-α converting capacities, the role played by MMP-12 in the processes leading to airway inflammation has been studied in MMP-12-/- mice. These animals displayed less airway inflammation, hyperresponsiveness and significantly less remodeling after long term allergen exposures. MMP-12 silencing by intratracheal instillation of anti-MMP-12 siRNA in wild type mice led to a significant decrease of asthmatic features showing that MMP-12 should be considered as a potential therapeutic target.

Céline Crahay, Maud Guéders, Jonathan Hacha, Natacha Rocks, Geneviève Paulissen, Nancy Garbacki, Alain Coligе, Emmanuel Di Valentin, Didier Cataldo.

Characterization of roles played by MMP-19 in asthma

LBTD reported that MMP-19 expression is increased in the lungs of animals during the course of asthma. LBTD therefore applied a mouse model of allergic asthma to MMP-19 deficient mice (MMP-19-/-) and corresponding wild-type mice. LBTD has shown that upon allergen exposure, genetic deletion of MMP-19 in a mouse model of asthma led to the development of extensive eosinophilic inflammation. LBTD was able to demonstrate that MMP-19 plays a role in lung homeostasis by modulating the Th2 cytokine levels and deposition of extracellular matrix components, including tenascin-C. Tenascin-C is thought to be the main actor responsible for eosinophil accumulation in LBTD experiments and could accumulate in MMP-19-/- animals following a lack of cleavage by MMP-19 that occurs in physiological conditions to restore the homeostasis of extracellular matrix and prevent inflammation.

Maud Guéders, Geneviève Paulissen, Jonathan Hacha, Didier Cataldo
Collaborator: Carlos Lopez Otin (Spain)

Characterization of roles played by ADAM-8 in asthma and development of new therapeutic strategies based on their inhibition

As ADAM-8 was overexpressed in lungs of human asthmatics, LBTD has specifically investigated ADAM-8 expression and production in a mouse model of allergen-induced airway inflammation. In allergen-exposed animals, increased expression of ADAM-8 was found in lung parenchyma and in purified dendritic cells from the lungs. The role of ADAM-8 in the development of allergen-induced airway inflammation was further investigated by using an anti-ADAM-8 antibody and ADAM-8 knock-out mice. A decrease in allergen-induced acute inflammation was observed both in BALF and peribronchial tissues in anti-ADAM-8 antibody-treated mice and in ADAM-8 deficient mice after allergen exposure. ADAM-8 depletion led to a significant decrease of lung dendritic cell populations. Moreover, CCL11 and CCL22 production was lower in anti-ADAM-8 antibody-treated mice and in ADAM-8 deficient mice, which might be explained by a decreased eosinophilic inflammation and lower numbers of dendritic cells, respectively. Taken together, these results suggest that ADAM-8 is not only a marker of allergen-induced acute inflammation, but probably also an effector, since its depletion impairs the development of eosinophilic inflammation through decreasing lung dendritic cells and alveolar macrophage recruitment and modulating CCL11 and CCL22 production.

Geneviève Paulissen, Maud Guéders, Natacha Rocks, Florence Quesada, Denis Bedoret, Fabrice Bureau, Christophe Desmet, Didier Cataldo.
Collaborator: Andrew Docherty (UK)

LABORATORY OF HUMAN GENETICS - CARDIOVASCULAR RESEARCH GROUP

We are interested in the identification of new molecular mechanisms regulating the inflammatory response and associated thrombotic events. Special focus is made on extracellular ATP and its receptors. ATP, released from cells as a result of platelet or leukocyte activation or upon multiple stress stimuli or tissue damage, interacts with cell surface P2 receptors belonging to two subclasses, the G protein-coupled P2Y receptors (P2Y1,2,4,6,11-14) and the P2X ion channels (P2X1-7). Identifying the receptors mediating the ATP effects constitutes a real challenge that could lead to the discovery of novel therapeutic targets.

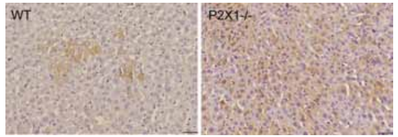
Lack of P2X1 ion channels increases endotoxemia-associated coagulation and organ damage through neutrophil hyperresponsiveness

ATP-gated P2X1 ion channels contribute to arterial thrombosis by amplifying platelet activation. In the search for novel anti-platelet strategies, targeting P2X1 ion channels is appealing. However, in our study we found that lack or inhibition of P2X1 channels enhanced neutrophil respiratory burst activity *ex vivo*. To study the consequence of P2X1 deficiency on neutrophil function *in vivo*, P2X1-/- mice were used in a model of endotoxin-

induced sepsis. Upon injection of lipopolysaccharides (LPS), plasma myeloperoxidase (MPO) concentrations reached higher levels in the P2X1-/- mice, and circulating neutrophils expressed higher levels of surface CD11b compared to wild-type mice. Neutrophil relocalization into the lungs of LPS-treated P2X1-/- mice was also significantly augmented, reflecting a higher activation state of P2X1-/- neutrophils under conditions of sepsis. Accordingly, more extensive lipid peroxidation was observed in the liver of LPS-treated P2X1-/- mice, indicative of exaggerated oxidative damage (Figure). Concomitantly, the levels of thrombin-antithrombin complexes were higher in the plasma of LPS-treated P2X1-/- mice and thrombocytopenia was worsened as compared to wild type mice. Our results strongly suggest that P2X1 ion channels play a protective role in sepsis by negatively regulating systemic neutrophil activation, thereby limiting oxidative damage, activation of coagulation and platelet accumulation in the lungs.

Christelle Lecut, Céline Faccinetto, Vincent Bours, Cécile Oury

Collaborators: Richard Evans (UK), Chantal Dessy (Belgium), Jean-Luc Balligand (Belgium)



Increased lipopolysaccharide-induced lipid peroxidation in the liver of P2X1-/- mice. Wild-type and P2X1-/- knock-out mice were injected with lipopolysaccharide (E.coli, O111:B4, 20 mg/kg, 15 hours). Liver sections were stained with 4-hydroxy-2-nonenal. Magnification 40X.

Key contribution of P2 receptors to immune cell recruitment into the lungs: insights from mouse models

The ecto-nucleoside triphosphate diphosphohydrolase (E-NTPDase) CD39 has been shown to play a central role in the regulation of mucosal nucleotide concentrations, and chronic lung diseases are characterized by higher rates of nucleotide elimination, E-NTPDase expression and activity. In order to investigate, *in vivo*, the role of CD39, mucosal ATP and P2 receptors in airway inflammation, we have generated transgenic mice over-expressing CD39 in the airway epithelia. The phenotype of these mice has been analyzed in mouse models of lung diseases such as endotoxin-induced acute lung injury, Pseudomonas aeruginosa infection and cystic fibrosis. It appears that, in these models, overexpression of CD39 increases inflammatory cell recruitment in the lungs by enhancing P2 receptor activity, which demonstrates an important role for this E-NTPDase in the control of inflammation in diseased states.

Emilie Théâtre, Kim Frederix, Lucien Bettendorf, Vincent Bours, Cécile Oury

Collaborators: Rudi Beyaert (Belgium), Johan Grooten (Belgium)

LABORATORY OF RHEUMATOLOGY

PPAR-γ-regulated genes promote storage of fatty acids and repress lypolysis, but are also involved in the proliferation, differentiation, and apoptosis of different cell types. These last few years, the rheumatology department showed the prominent role of PPAR-γ in chondrocyte and synovial fibroblast. Our current studies are concentrated on mechanisms of leptin expression considered as a pro-inflammatory adipokine in rheumatic diseases. Our laboratory is also focusing its research on biomarker identification by the SELDI-TOF and 2D DIGE proteomic approach that differentially investigates levels of proteins present in the serum of patients with rheumatic diseases (cfr highlight project).

Mechanism of leptin expression in mesenchymal stem cells

Leptin plays a central role in maintaining energy balance with multiple systemic effects. Regarding the importance of this hormone, there is a need to understand its expression regulation. Here, we investigated leptin production in other mesenchymal stem cells (MSC) from bone marrow (BM-MSC), cord matrix (UMSC), and primary and dedifferentiated chondrocytes (DCH). Results showed that BM-MSC produced leptin that was dramatically enhanced by glucocorticoids. Interestingly, UMSC did not produce leptin, in the absence or presence of glucocorticoids, whereas chondrocytes acquired the ability to produce leptin progressively with dedifferentiation. This dedifferentiation process was correlated with down-regulated Smad2 phosphorylation (p-Smad2) and the gain of β-catenin expression. Furthermore, UMSC presented an enhanced level of pSmad2. TGF-β1 induced an increased p-Smad2 expression and markedly inhibited endogenous and glucocorticoids induced leptin production in BM-MSC, while SB 431542 (TGF-β receptor I inhibitor) restored this expression. Glucocorticoids down-regulated p-Smad2 in BM-MSC but not in UMSC. β-catenin was expressed in both BM-MSC and UMSC, however, glucocorticoids induced an increased nuclear p-β-catenin accumulation in BM-MSC but not in UMSC. These results showed that down-regulated pSmad2 and increased β-catenin phosphorylation are required for leptin production in MSC.

Mustapha Zeddou, Biserka Relic, Dominique de Seny, Michel Malaise

Osteogenic mesenchymal stem cells differentiation

The field of cell therapy is experiencing plenty of cell types for different protocols. Bone marrow mesenchymal stem cells (BM-MSC) are widely used for osteogenic differentiation studies. Here, we investigated and compared the osteogenic differentiation capacity between umbilical cord matrix derived mesenchymal stem cells (UMSC), considered as an emerging source of MSC, synovial fibroblast (SVF) and BM-MSC. Results showed that SVF have an important potential for osteogenic differentiation, whereas UMSC do not express phosphatase alcalin (PAL) activity in the absence or presence of osteogenic differentiation mix. Interestingly, SVF culture supernatant was able to induce PAL activity in UMSC, and increased this activity in BM-MSC. SVF culture supernatant analysis showed the presence of an important amount of leptin. We previously demonstrated the implication of TGF-β pathway in leptin expression in MSC. Therefore, leptin expression inhibition with TGF-β1 induced a decreased PAL activity, whereas TGF-β RI inhibition drastically increased PAL activity in BM-MSC. Further analysis on SVF culture supernatant should be performed to identify additional compounds that could be implicated in osteogenic differentiation.

Mustapha Zeddou, Biserka Relic and Michel Malaise

Synovial fibroblast chondrogenesis *in vitro*

The main objective of this research is to improve an *in vitro* mesenchymal stem cell model of chondrogenesis, the process by which joint cartilage is formed. During this period we have studied *in vitro* effects of two basic active substances present in the chondrogenenic medium: dexamethasone and TGF-β1 on synovial fibroblast chondrogenesis and leptin production. We have recently shown that synovial fibroblasts produced leptin when stimulated with dexamethasone. Leptin is considered as a pro-inflammatory adipokine in rheumatic diseases whereas it is not expressed in healthy cartilage. Therefore, we have tested leptin production of *in vitro* cartilages obtained in micromass cultures from synovial fibroblasts. Results showed that *in vitro* cartilage produces leptin. Moreover, leptin was detected, but at a markedly less amount, in cultures that were grown in chondrogenic medium without dexamethasone. TGF-β1 markedly inhibited glucocorticoid-induced leptin production in synovial fibroblast monolayer cultures, and partially during *in vitro* chondrogenesis. In our future work, we would like to improve this model in order to obtain for the first time *in vitro* cartilage that is devoid of leptin production.

Biserka Relic, Mustapha Zeddou, Michel Malaise

LABORATORY OF HEMATOLOGY

The hematology laboratory is involved in research related to biology and the graft of hematopoietic and mesenchymal stem cells. We also study the reconstitution of the immune system after a graft and its impact on the elimination of the patient's cancerous cells.

Evaluation of the supportive activity of mesenchymal stem cells toward hematopoietic cells

Bone marrow (BM) mesenchymal stem cells (MSC) support proliferation and differentiation of hematopoietic progenitor cells (HPC) *in vitro*. Since they represent a rare subset of BM cells, MSC preparations for clinical purposes involve a preparative step of *ex vivo* multiplication. The aim of our study was to analyze the influence of culture duration on MSC supportive activity. MSC were expanded for up to 10 passages. MSC and CD34+ cells were seeded in cytokine-free co-cultures after which the phenotype, clonogenic capacity and *in vivo* repopulating activity of harvested hematopoietic cells were assessed. Early passage MSC supported HPC expansion and differentiation towards both B lymphoid and myeloid lineages. Late passage MSC did not support HPC and myeloid cell outgrowth but maintained B cell supportive ability. *In vitro* maintenance of NOD/SCID mouse repopulating cells cultured in contact with MSC was effective until the fourth MSC passage and declined afterwards. CD34+ cells achieved higher levels of engraftment in NOD/SCID mice when co-injected with early passage MSC; however MSC expanded beyond 9 passages were ineffective in promoting CD34+ cell engraftment. Non-contact cultures indicated that MSC supportive activity involved diffusible factors. Among these, interleukin (IL)-6 and IL-8 contributed to the supportive activity of early passage MSC but not of late passage MSC.

Alexandra Briquet, Sophie Dubois, Yves Beguin, André Gothot

Optimization of hematopoietic stem cell transplantation following non-myeloablative conditioning regimen

Allogeneic hematopoietic cell transplantation (HCT) following myeloablative conditioning is associated with transplant-related morbidity and mortality, limiting its use to younger patients without co-morbidities. However, median age at the time of diagnosis of leukemia or lymphoma ranges from 60 to 70 years. Alloreactivity of donor immunocompetent cells in the graft against the host tumor plays a major role in eradicating malignancies after allogeneic HCT (graft-versus-tumor effects). Several groups of investigators have thus developed nonmyeloablative conditioning regimens allowing the use of allogeneic HCT in older patients, and those with co-morbidities. These approaches rely nearly exclusively on graft-versus-tumor effects for tumor eradication. The aims of the current project are to improve our understanding it and to learn how to better manipulate alloreactivity following nonmyeloablative HCT. The project includes a randomized study comparing 2 non-myeloablative conditioning regimens; a prospective randomized study analyzing the co-infusion of mesenchymal stem cells (MSC) and hematopoietic stem cells to prevent graft rejection and acute GVHD after nonmyeloablative HCT with partially HLA-mismatched donors; and a prospective study assessing MSC infusion as treatment of GVHD or poor graft function after HCT. Special emphasis is put on immune reconstitution after transplantation, using a variety of sophisticated techniques.

Muriel Hannon, Ludovic Belle, Catherine Menten, Coline Daulne, Sophie Dubois, Olivier Dengis, Sylviane Simar, Sophie Servais, France Bruck, Stéphanie Humblet-Baron, Chantal Lechanteur, André Gothot, Yves Beguin, Frédéric Baron

Collaborators: Pierre Zachée (Belgium), Dominik Selleslag (Belgium), Philippe Lewalle (Belgium), Tessa Kerre (Belgium), Carlos Graux (Belgium), Xavier Poiré (Belgium), Johan Maertens (Belgium), Koen Theunissen (Belgium), Rik Schots (Belgium), Michel Van Gelder (The Netherlandts), Rémi Cheynier (France)

Progenitor cell mobilization by G-CSF is associated with improved heart perfusion and function after myocardial infarction in mice

We demonstrated that G-CSF administration in healthy mice induced the mobilization in the peripheral blood of hematopoietic (HSC) and mesenchymal (MSC) stem cells, as well as endothelial progenitors (EP), with specific kinetics for each cell type. Using an array of delicate techniques evaluating cardiac function (echocardiography, conductance sensors) and vascularisation (immunohistology, microSPECT), we showed that G-CSF impaired cardiac relaxation, as well as compliance and deformability of the heart. However, cardiac perfusion was significantly improved. After myocardial infarction (MI), HSC numbers decreased in the bone marrow and blood, through inflammatory inhibition of hematopoiesis. CSM and CPE numbers remained stable in bone marrow but increased considerably in blood after MI, but not in sham-operated animals. MSC and EP in MI mice were further mobilized in the blood by G-CSF. G-CSF improved left ventricular remodelling and mouse survival after MI. Perfusion defects and infarct size were significantly improved. Cardiac output increased, while left ventricular ejection fraction remained unchanged. Our data point to altered cardiac muscle relaxation and reduced ventricular compliance. These results confirmed the potential of G-CSF to mobilize progenitor cells into the circulation and their possible contribution to cardiac repair after MI.

Marie Delgaudine, Sophie Dubois, Olivier Dengis, André Gothot and Yves Beguin
Collaborators: Véronique Roelandts (Belgium), Pierre Gianello (Belgium)

LABORATORY OF MEDICAL CHEMISTRY

We are involved in biomarker discovery for the diagnosis, prognosis, and response to pharmacological treatments of chronic inflammatory diseases using proteomic strategies. We also aim at understanding the biological role of new biomarkers in inflammatory processes.

New methods for low molecular weight proteins SELDI-TOF-MS differential analysis

In most diseases, the clinical need for serum/plasma markers has never been so crucial, not only for diagnosis, but also for the selection of the most efficient therapies as well as exclusion of ineffective or toxic treatment. Due to the high sample complexity, prefractionation is essential to explore the deep proteome and find specific markers. Three different sample preparation methods (i.e. highly abundant protein precipitation, restricted access materials (RAM) combined with IMAC chromatography and peptide ligand affinity beads) were investigated to select the best starting point for further differential proteomic experiments focusing on the LMW proteome (MW inferior to 40000 Da). Indeed, the aim was not to cover the entire plasma/serum proteome, but to enrich for potentially interesting tissue leakage proteins. These three methods were evaluated on their reproducibility, on the SELDI-TOF-MS peptide/protein peaks generated after fractionation and on the information supplied. Peptide ligand affinity beads were found to provide efficient depletion of HMW proteins and peak enrichment in protein/peptide profiles.

Muriel De Bock, Dominique de Seny, Marie-Alice Meuwis, Anne-Catherine Servais, Jean-Paul Chapelle, Edouard Louis, Michel Malaise, Marie-Paule Merville, Marianne Fillet
Collaborators: Tran Quang Minh, Jean Closset

Biomarker discovery in biological fluids

Protein profiling using SELDI-TOF-MS has gained an increasing interest in the field of biomarker discovery over the past few years. The technology presents great potential if some parameters, such as sample handling, SELDI settings and data analysis, are strictly controlled. Practical considerations to set up a robust and sensitive strategy for biomarker discovery were discussed. Biological fluids generally available, including their peculiar properties and the pre-analytical challenges inherent to sample collection and storage were described. Finally, some new insights for biomarker identification and validation challenges were provided.

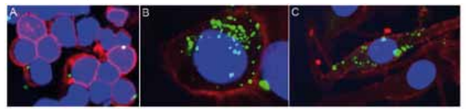
Muriel De Bock, Dominique de Seny, Marie-Alice Meuwis, Anne-Catherine Servais, Jean-Paul Chapelle, Edouard Louis, Michel Malaise, Marie-Paule Merville, Marianne Fillet

LABORATORY OF EXPERIMENTAL PATHOLOGY - INNATE LYMPHOCYTE GROUP

We study the immune response induced by innate lymphocytes such as Natural Killer (NK) and γδ T cells. These lymphocytes are in the intersection between innate and adaptive immunity. Despite a recent new interest, due to a better characterization of innate lymphocyte mechanism of action, knowledge about these cells is still limited.
Our research model is cervical cancer associated with human papillomavirus (HPV) infection. This cancer is an important public health problem since it is the third most common cancer in women. Moreover, this model allows us to study both anti-tumoral and anti-viral immune response. Our group has several years of expertise in this model.

Role of NK cells in HPV-associated lesions

The importance of the immune system in the control of uterine cervical cancers was highlighted by the fact that the incidence of this cancer is significantly increased in women with an immunodefiency.



Confocal microscopy of fluorescent HPV-VLP internalization in natural killer cells (A), epithelial cells (B) and dendritic cells (C).

Additionally, more than 80% of HPV infections are cleared within 2 years post-infection, but the cellular effectors of the immune response against HPV are not clearly identified. In this context, we studied if NK cells are able to induce an immune response against HPV infection. Since HPV could not be produced *in vitro*, we used virus-like particles (VLP) to study the interaction between this virus and NK cells. HPV-VLP and the native virus share the same morphology and the same immunological epitopes. HPV-VLP have recently been licenced as a vaccine against cervical cancer. Our results showed that HPV-VLP were internalized in NK cells by macropinocytosis. The entry mechanism is different from the one described for dendritic or epithelial cells (see figure). We also identified CD16 as the co-receptor responsible for the HPV-VLP uptake. Interestingly, in response to HPV-VLP internalisation, NK cells secreted cytokines and increased their cytotoxic activity against HPV+ cell line. These results suggest that NK cells can participate to virus elimination.
Our future plans are to assess the molecular mechanisms of this response and to study the activation of NK cells by VLP-HPV can help dendritic cells induce an adaptive immune response against HPV.

Nathalie Jacobs, Virginie Renoux, Inge Langers, Estelle Dortu
Collaborators: Marc Thiry (GIGA), Christophe Deroanne (GIGA), Béatrice Clémenceau (France), Pierre Coursaget (France)

LABORATORY OF PNEUMOLOGY

The research activities of our laboratory have been dedicated to three diseases including asthma, chronic obstructive lung disease and malignant pleural diseases.

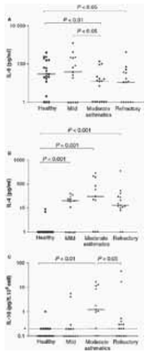
Exhaled nitric oxide thresholds associated with a sputum eosinophil count >3% in a cohort of unselected asthmatics

It has been claimed that exhaled nitric oxide (FeNO) could be regarded as a surrogate marker for sputum eosinophil count in patients with asthma. However, the FeNO threshold value that identifies a sputum eosinophil count ≥3% in an unselected population of patients with asthma has been poorly studied. We conducted a retrospective study in 295 patients with asthma aged 15-84 years recruited from the asthma clinic of the University Hospital of Liège. Receiver-operating characteristic (ROC) curve and logistic regression analysis were used to assess the relationship between sputum eosinophil count and FeNO, taking into account covariates such as inhaled corticosteroids (ICS), smoking, atopy, age and sex. Based on ROC curve, FeNO ≥41 ppb gave 65% sensitivity and 79% specificity (AUC=0.777) for identifying a sputum eosinophil count ≥3%. Using logistic regression analysis, a threshold of 42 ppb was found to discriminate between eosinophilic and non-eosinophilic asthma. Patients receiving high doses of ICS (≥1000 µg beclometasone) had a significantly lower FeNO threshold (27 ppb) than the rest of the group (48 ppb). Atopy also significantly altered the threshold (49 ppb for atopic vs 30 ppb for non-atopic patients) and there was a trend for a lower threshold in smokers (27 ppb) compared with non-smokers (46 ppb). Age and sex did not affect the relationship between FeNO and sputum eosinophilia. When combining all variables into the logistic model, FeNO, high-dose ICS and smoking were independent predictors of sputum eosinophilia, while there was a trend for atopy. FeNO is able to identify a sputum eosinophil count ≥3% with reasonable accuracy and thresholds which vary according to the dose of ICS, smoking and atopy.

Florence Schleich, Maïté Manise, Renaud Louis
Collaborators: Laurence Seidel, Jocelyne Sele, Valérie Quaedvlieg, Alain Michils (Belgium)

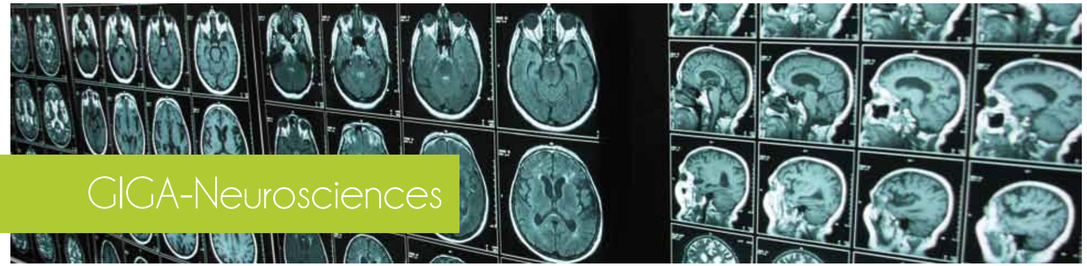
Cytokine production from sputum cells and blood leukocytes in asthmatics according to disease severity

Although mild to moderate asthma is known to be Th2 driven, cytokines produced in refractory asthma might not fit the classical Th2 pattern. The aim of our study was to assess the cytokine production by sputum and blood cells from 15 refractory asthmatics (American Thoracic Society Criteria) compared to 15 mild untreated and 17 moderate treated asthmatics and 22 healthy subjects. Spontaneous production of interleukin (IL)-4, IL-6, IL-10, interferon-γ, and tumor necrosis factor α was measured by immunotrapping after 24 h sputum or blood cell culture. Moderate and refractory asthmatics were both characterized by a lower production of IL-6 from their airway cells compared to healthy subjects. However, the difference was no longer significant when expressing the results per gram of sputum. No significant difference between the three groups was found regarding other cytokines. As for cytokine production from blood, the three groups of asthmatics exhibited raised production of IL-4 when compared to healthy subjects, and this was true when results were expressed per blood volume or after normalization for total leukocyte cell count. Moderate asthmatics exhibited a greater production of IL-10 when compared to refractory asthmatics and healthy subjects when results were normalized for total leukocyte cell count.Sputum cells from moderate and refractory asthmatics release less IL-6. While the systemic overproduction of IL-4 was observed through the all spectrum of asthma severity, moderate asthmatics exhibited greater systemic IL-10 production compared to refractory asthmatics.



Spontaneous production of IL-6 by sputum cells and of IL-4 and IL-10 by blood leukocytes in asthma according to disease severity. Bars represent median.

Maïté Manise, Florence Schleich, Monique Henket, Jean-Louis Corhay, Renaud Louis
Collaborators: Natacha Gusbin, Laurent Godinas, Nicolas Antoine (Belgium)



GIGA-Neurosciences

The GIGA-Neurosciences is composed of 7 Units that are affiliated to the Faculty of Medicine and the Faculty of Sciences. This thematic research unit is dedicated to study a broad range of topics in Neurosciences.

More than 80 senior scientists, post-doctoral researchers and graduate students collaborate to investigate the cellular and molecular aspects of the development and activity of the central and peripheral nervous systems in health and disease.

The interdisciplinary structure of the GIGA-Neurosciences provides a framework for students to tackle a wide range of topics in Neurosciences including neuronal development and plasticity, neuronal degeneration and repair, neurophysiology and neuropharmacology.

The major techniques used in our laboratories include molecular and cellular biology, neurophysiology, neuroanatomy, and animal behavior

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COMPOSITION OF THE THEMATIC RESEARCH UNIT

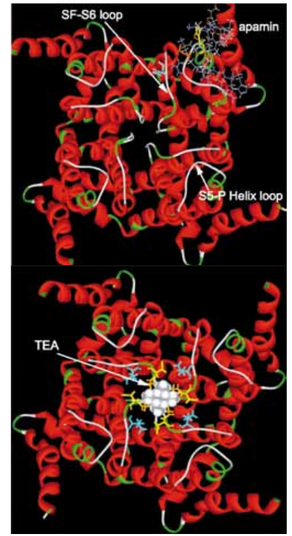
7 laboratories
83 scientists
- 19 Principal Investigators
- 20 PhD (postdoc)
- 30 PhD Students
- 14 Technicians

Allosteric block of KCa2 channels by apamin

The Journal of Biological Chemistry. 2010;285:27067-27077

Small conductance calcium-activated potassium (SK or KCa2) channels underlie the afterhyperpolarization which follows action potentials in many types of neurons and thereby controls their excitability. Modulation of these channels may be useful in various types of disorders such as cognitive impairment, depression and dopamine-related disorders. In order to make progress in the design of blockers of these channels, a thorough understanding of their mechanism of action is needed. We have used a multidisciplinary approach (patch clamp, binding and mutagenesis on transfected cell lines, as well as molecular modeling) to study the action of the protypical peptidic SK blocker apamin and of non-peptidic blockers. We found that these agents are not pore blockers, contrary to the classical blocker tetraethylammonium (TEA), but allosterically modulate their opening. In order to demonstrate this, we used a fast-application system and showed that TEA did not affect the onset of channel block by apamin, whereas another blocker synthesized by our group, N-methyl-laudanosine, did. An outer pore histidine residue was shown to be critical both for binding and block by apamin, but not TEA. Interestingly, block of the channels by apamin was observed at concentrations higher than those needed for the toxin to bind, suggesting that additional steps are needed after binding for the block to occur, another argument for an allosteric modulation. Molecular modelling confirmed that apamin does not physically obstruct potassium ion flow through the selectivity filter, but binds more laterally and interacts with amino-acid residues in the turret region of the channel. Taken together, these experiments increase our understanding of how SK channel blockers work, which may help to design more efficient and/or selective agents in the future.

Cédric Lamy[1*], Samuel Goodchild[2*], Kate Weatherall[2], David Jane[2], Jean-François Liégeois[3], Vincent Seutin[1•] and Neil Marrion[2•]
[1]GIGA Neurosciences and Laboratory of Pharmacology, University of Liège, Belgium. [2] Department of Physiology and Pharmacology, School of Medical Sciences, University of Bristol, Bristol BS8 1TD, United Kingdom, [3]Laboratory of Medicinal Chemistry and Centre Interfacultaire de Recherhce du Médicament, University of Liège Belgium
*equal contribution of the two first authors; •equal contribution of the two last authors



Top down view of the pore region of four SK channel subunits (modelled by homology starting from the structure of the voltage-dependent Kv1.2 channel) showing that apamin binds to the turret region of the channel, whereas TEA is a pore blocker.

LABORATORY OF BEHAVIORAL NEUROENDOCRINOLOGY

Our research aims at identifying the neuroendocrine and neurochemical mechanisms that mediate the activation and sexual differentiation of reproductive behavior in higher vertebrates using a variety of mammalian and avian species. We are also interested in the metabolism of sex steroids in the brain, in particular the aromatization of testosterone into estradiol and the interaction of steroids with neurotransmitters. Neurochemical and neuroanatomical sex differences are analyzed to determine to what extent they explain sex differences in behavior and physiology. We also investigate the control of neural plasticity by steroids observed during ontogeny, as well as in adulthood.

Rapid changes of brain aromatase activity as a function of stress or social interactions

Testosterone (T) activates male sexual behavior largely through its aromatization into estrogens. The enzyme aromatase that catalyzes this transformation is expressed in several hypothalamic nuclei. Its activity is regulated in a sex-specific anatomically discrete manner in two different time domains: (1) in the long term (hours to days) via changes in enzyme concentration (controlled by the genomic action of steroids) and (2) in the short term (within minutes) through post-translational modifications (phosphorylations) of the enzymatic protein. We demonstrated this year that sexual interactions with a female restraint stress affect aromatase activity within 5-15 min. The changes observed were different in males and females and varied from one hypothalamic nucleus to another, but in general, the effects of stress were diametrically opposite to those observed after exposure to a female. Rapid controls of aromatase activity by phosphorylations are not limited to the quail brain enzyme but are also observed in the ovary and in human aromatase expressed in a variety of mammalian cell lines. Site-directed mutagenesis of six serine or threonine residues to alanine affected the basal enzymatic activity but did not interfere with the rapid changes in activity observed in phosphorylating conditions. Changes in activity must therefore result from phosphorylations at other sites, from simultaneous phosphorylations of multiple residues or from phosphorylations of another regulatory protein.

Jacques Balthazart, Charlotte A. Cornil, Thierry D. Charlier, Molly F. Dickens, Aurore Seredynski, Catherine de Bournonville
Collaborators: Gregory F. Ball (USA), Nobuhiro Harada (Japan)

Steroid-dependent seasonal brain plasticity and singing behavior in songbirds

Steroid-dependent seasonal variation in the brain of songbirds, such as canaries, has emerged as one of the best model systems for the study of naturally occurring brain plasticity. We investigate the cellular mechanisms underlying this plasticity focusing on the role of doublecortin (DCX), a microtubule-associated protein that plays a key role in the migration of newborn neurons. Seasonal changes in the volume of the vocal control nucleus HVC are regulated by testosterone (T) and its androgenic (5α-dihydrotestosterone; DHT) or estrogenic metabolites (17β-estradiol; E2). We examined the effect of T and its two metabolites alone or in combination on DCX expression in adult female canaries. T or DHT+E2 increased HVC volume and neuron numbers as well as the total numbers of fusiform (migrating) and round (differentiating) DCX neurons in the nucleus, but generally not in adjacent areas. DHT or E2 alone did not increase these measures, but increased the density of fusiform DCX cells per section. No effect of any treatment on DCX cell densities was detected in other parts of the nidopallium. DHT and E2 by themselves, thus increase the density of DCX cells migrating through HVC but are not sufficient in isolation to induce the recruitment of these newborn neurons in the nucleus. These effects are not observed in the rest of the nidopallium, implying that steroids act on the attraction and recruitment of new HVC neurons without having any effects on their production at the ventricle wall.

Jacques Balthazart, Thierry D. Charlier, Jennifer M. Barker
Collaborators: Gregory F. Ball (USA), Takashi Yamamura (Japan)

Hormonal and genetic mechanisms underlying the sexual differentiation of the brain and behavior using transgenic mouse models

A central tenet of contemporary theories of mammalian brain and behavioral sexual differentiation is that an organizational action of testosterone, secreted perinatally by the male's testis, controls male-typical facets of brain and behavioral development, whereas no active perinatal sex hormone signaling is required for female-typical differentiation. However, evidence of a possible contribution of estradiol to female-typical behavioral development involved our observation that aromatase knockout female mice showed significantly lower levels of female sexual behavior than wild-type controls following adult treatment with ovarian hormones. We have now confirmed an active role of prepubertal estradiol in female-typical differentiation of brain mechanisms controlling both appetitive and consummatory aspects of female sexual behavior, since female sexual behavior was rescued in aromatase knockout female mice when treated with estradiol over a specific prepubertal period. These results indicate that the classic view of a default organization of female typical neural and behavioral characteristics must be revised.

Julie Bakker, Olivier Brock, Laura Szymanski, Christelle De Mees
Collaborators: Michael Baum (USA), Roberto Melcangi (Italy), Ulrich Boehm (Germany)

LABORATORY OF DEVELOPMENTAL NEUROENDOCRINOLOGY

The laboratory of Developmental Neuroendocrinology aims at uncovering the fetal origin of some adult diseases. Our team focuses on interactions between mechanisms regulating energy balance, reproduction and cerebral cortex development as well as the early effects of endocrine disrupters on those aspects.

Early effects of endocrine disrupters on cererbral cortex development

Endocrine disrupting chemicals (EDCs) are exogenous substances able to alter endocrine functions. In utero exposure to Polychloro biphenyls (PCBs) has been shown to alter memory, learning and audition in rodents and humans possibly through thyroid function alterations. PCBs were widely used in lubricating oil and flame retardant. Even though they are forbidden in Europe and USA, they are still present in our environment because of their long half-life. We have shown that prenatal exposure to PCBs causes a decrease of total thyroxin serum level in pregnant rats. It does not affect progenitor proliferation in the periventricular zone of the fetal cortex but appears to affect progenitor cell cycle exit and radial migration.We are now focusing on the effects of prenatal exposure to PCBs on neuronal proliferation and dendritogenesis in the hippocampus because of its implication in memory and learning.

Elise naveau, Arlette Gérard, Anne-Simone Parent, Jean-Pierre Bourguignon
Collaborators: Gary Westbrook (USA), Thomas Zoeller (USA)

Early effects of endocrine disrupters on sexual development and energy balance

The hypothalamus is a single place for regulation of homeostasis including reproduction and energy balance. Both prenatal underfeeding and early exposure to EDCs cause alteration of reproduction and energy balance later in life. Several peptides regulating appetite and energy balance play a role in the control of puberty and reproduction. Thus it appears important to study the effects of EDCs on the interactions between both systems. Leptin is not only an anorexigenic hormone produced by adipocytes, but also a structural organizer of the hypothalamic circuitry controlling energy balance during a critical period perinatally. We have shown that early exposure to an estrogenic endocrine disrupter such as diethyl stilbestrol (DES), alters the hypothalamic sensitivity to leptin. While most studies focused on the effects of endocrine disrupters on peripheral organs such as the testis or ovaries, these data suggest a neuroendocrine disruption by DES. We are now focusing on the combined effect of prenatal malnutrition and early exposure to EDCs such as DES or bisphenol A on the hypothalamic control of energy balance and reproduction. We hypothesize that the observed alterations could be explained by early re-programming of homeostatic processes at the hypothalamic level.

Elise Naveau, Arlette Gérard, Anne-Simone Parent, Jean-Pierre Bourguignon
Collaborators: Sébastien Bouret (France), Vincent Prévot (France), Sabine Heger (Allemagne)

LABORATORY OF BIOENERGETICS, EXCITABILITY AND CEREBRAL PLASTICITY

The work of our unit is aimed at understanding the interplay between neuronal bioenergetics, excitability and cell survival. We have three main research projects with multiple international collaborations.
We are interested in new phosphorylated thiamine (vitamin B1) derivatives and the understanding of their implications in neurodegenerative diseases.
The second project aims at discovering and characterizing the genes involved in various forms of epilepsies.
The last project of our unit is dedicated to a peptide called melanin-concentrating hormone. Our aim is to understand how it controls the behavior and how it affects brain excitability.

The physiopathological basis of epilepsy: is juvenile myoclonic epilepsy a developmental disease ?

Juvenile myoclonic epilepsy (JME) is the most common form of genetic generalized epilepsy. EFHC1 (EF-hand containing one) is a promising causative gene for JME as different missense mutations have been found in several unrelated patients suffering from the disease. We previously showed that EFHC1 is a Microtubule-Associated Protein involved in the regulation of cell division. On a model of brain development, EFHC1 loss of function dramatically impairs neuronal migration. Therefore, we proposed that abnormal neuronal migration during brain development could lead to abnormal brain circuitry which, in turn, will produce JME.
By using a specific antibody, we observed a stronger expression of EFHC1 at the embryonic stages as compared to postnatal stages. At E16 a clear immunostaining is observed at the borders of ventricles and corresponds to the radial glia cells, which confirms that EFHC1 plays a role during neuronal migration.
Ongoing experiments 1) investigate the effect of pathological and non-pathological mutations of EFHC1 on cell division and neocortical development 2) aim at determining the cellular role of EFHC1 by the identification of its partners.

Thierry Grisar, Bernard Lakaye, Laurence de Nijs, Nathalie Wolkoff
Collaborators : Antonio Delgado-Escueta (USA), Andreas Daga (Italy)

Role of melanin-concentrating hormone in goal-oriented behaviors

The hypothalamus is a complex brain structure, important for body homeostasis as it controls goal-oriented behavior such as eating, drinking, arousal, reproduction or stress. Melanin-concentrating hormone (MCH), a 19 amino-acid cyclic peptide produced in that structure, is a promising new target to treat obesity and depression.
Previously, we demonstrated some cognitive defects in mice lacking MCH receptor (MCHR1-KO). We have now extended these data and we have confirmed MCH plays

a role in aversive motivated learning. In accordance with these observations, we have detected major impairments of glutamatergic transmission and long term synaptic plasticity in the hippocampus of MCHR1-KO animals.
Based on the above observations and because astrocytes are critical for the control of glutamate homeostasis, we hypothesized MCH could modulate their properties. We have demonstrated for the first time that *in vitro*, astrocytes do express functional MCHR1 receptors and that their activation by MCH impairs Glt-1 mediated glutamate uptake. These data are interesting as there is now increasing evidence for an implication of astrocyte in the control of several physiological and pathological processes such as obesity, sleep, depression and learning/memory, all of which are also modulated by MCH.

Thierry Crisar, Bernard Lakaye, Sophie Harray
Collaborators: Emmanuel Hermans (Belgium), Helmutt Kettenmann (Germany)

Thiamine and cellular bioenergetics

Thiamine (vitamin B1) is an essential molecule for all life forms. The main thiamine compound is thiamine diphosphate, an indispensible cofactor in cell energy metabolism. Other thiamine compounds are thiamine triphosphate (ThTP) and adenosine thiamine triphosphate, the latter only recently discovered in our laboratory. We have shown that ThTP is synthesized in brain mitochondria by a chemiosmotic mechanism. It may then be transported into the cytoplasm. Cytosolic ThTP concentrations are controlled by a 25-kDa thiamine triphosphatase. A much smaller activity in human tissues compared to rodent tissues explains the relatively high ThTP concentrations observed in human tissues. ThTP is involved in the regulation of cellular energy metabolism. The brain is particularly sensitive to energy failure and this research may help us understand how neurons adapt to conditions of energy stress, a situation occurring in neurodegenerative diseases. Recent work has shown the beneficial effects of certain thiamine precursors in animal models of Alzheimer's disease. Understanding the way how ThTP regulates cellular processes will be important for the design of new drugs reducing the progression of the disease in Alzheimer's patients.

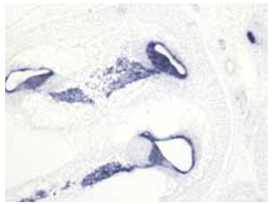
Lucien Bettendorff, Bernard Lakaye, Marjorie Gangolf, Tiziana Gigliobianco
Collaborators: Victoria Bunik (Russia), John Walker (Great-Britain), C.L. Keen (USA), Pascale Frey-Klett (France)

LABORATORY OF DEVELOPMENTAL NEUROBIOLOGY

Our overall goals are to uncover new cellular and molecular mechanisms involved in key developmental processes such as the production of neurons and glial cells, their differentiation and their migration in the central and peripheral nervous system in health and disease.

Differentiation, protection and regeneration of the auditory portion of the inner ear

Deafness commonly results from a lesion of the sensory cells and/or of the neurons of the auditory part of the inner ear and there is currently no treatment designed to interrupt or reverse the progression of hearing loss. Our work is focusing on the molecular mechanisms that regulate cell proliferation, differentiation and apoptosis during the development of the inner ear. We report that Sox10, a high-mobility group DNA-binding domain transcription factor is specifically expressed in postmigratory neural crest cells in the cochleo-vestibular ganglion. Using Sox10-deficient mice, we demonstrate that this transcription factor is essential for the survival, but not the generation, of glial cells within the inner ear. Surprisingly, auditory neuron differentiation, sensory target innervation and survival are conserved despite the absence of glial cells. Indeed, an increase trophic support from hair cells promotes the survival of spiral ganglion neurons in Sox10 mutant mice. In parallel, prevention of auditory sensory loss was also investigated. The maintenance of normal hearing involves the protection of auditory neurons but also their synaptic connections with hair cells. To identify new otoprotective molecules we decided to develop an *in vivo* model of deafness using mice and specific ototoxic molecules. However, mice were resistant to ototoxic treatment, contrary to guinea pigs.



E17.5 sox10LacZ/+ cochlea stained with X-gal showing that sox10 is specifically expressed in the spiral ganglion and the cochlear duct

Morgan Bodson, Ingrid Breuskin, Nicolas Thelen, Marc Thiry, Laurence Borgs, Laurent Nguyen, Brigitte Malgrange, Anne-lise Poirier, Priscilla Van den Ackerveken
Collaborato : Michael Wegner (Germany)

Usefulness of bone marrow mesenchymal stem cells as a source of new neurons for cell therapy protocols of neurological diseases

First clinical trials of cell therapy in Parkinson's disease stressed out the problem of the cellular source used in grafting protocols. In these trials, human neuroblasts isolated from aborted fetuses were grafted in adult patients, but this approach raised ethical, immunological and technical questions. A few years ago, our laboratory demonstrated that some adult bone marrow Mesenchymal Stem Cells (MSC) could express nestin, an intermediate filament protein initially characterized in neural stem cells, and that those nestin-expressing MSC can differentiate *in vitro* into fully functionnal neurons, able to trigger action potentials. We recently described and characterized the MSC sub-population able to express nestin and then to adopt a neuronal fate. Using a double transgenic approach (cell fate-mapping), we were able to demonstrate that this MSC population derives embryonically from neural crest stem cells. Moreover, we also reported that these neural crest-derived MSC could be propagated *in vitro* upon stimulation with Wnt1 and BMP2. We are now trying to set up an isolation protocol of these neural crest-derived MSC which are able to express nestin and to differentiate into functional neurons. We think that this cell population could be an interesting alternative cell source considering cell therapy protocols in neurological diseases, because they avoid ethical and also immunological problems as they could be used in autologous graft.

Aneta Gleizer, Emerence Laudet, Pierre Leprince, Sabine Wislet-Gendebien , Bernard Rogister
Collaborators: Lukas Sommer (Switzerland), Serge Schiffmann (Belgium), Emmanuel Hermans (Belgium).

Relationships between glioblastoma-initiating cells and adult neurogenic zones

Glioblastoma Multiformans is the most frequent primary tumor in the central nervous system. The pronostic of this tumor is very bad as the median survival curve after treatment is 16 months. This situation is a direct consequence of a relapse or tumor recurrence even after surgical ressection combined with radiotherapy and chemotherapy. This tumor recurrence is a consequence of the glioblastoma-initiating cells (GIC), a subpopulation in the tumor. Indeed GIC are the only cells able to start a new tumor when grafted in a host animal. As these GIC are thus regarded as «stem cells», we addressed the question of a possible relation of these GIC with the adult neurogenic zones which house the adult neural stem cells in the suventricular zone (SVZ) in the dentatus gyrus. Therefore, we grafted 50 000 human glioblastoma cells into the right striatum of adult athymic mice and we observed a preferential migration of human cells to the homo- and heterolateral SVZ of the host but not into the dentatus gyrus. In the animal SVZ, human cells start to express immature cells markers (nestin and sox2) and migrate rostrally with normal newly-formed host neuroblasts to the olfactory bulbs. With several experimental approaches, we were able to demonstrate that the human cells present in the SVZ are GIC. We are now trying to unravel the molecular regulation of this specific migration of GIC to the SVZ and to understand the benefits of such a relocalisation.

Jérôme Kroonen, Jessica Nassen, Pierre-Yves Boulanger, Bernard Rogister, Vincent Castronovo, Akeila Bellahcene, Vincent Bours, Pierre Robe, Manuel Deprez

Cellular and molecular regulation of cerebral cortical development

The cerebral cortex contains neurons that are distributed within layers and that are regionally organized into specialized areas that underlie sophisticated motor, cognitive and perceptual abilities. Cortical lamination follows an « inside-out » sequence of neuronal placement and maturation that arises from the sequential birth and orderly migration of pyramidal projection neurons born in the dorsal telencephalon and, GABAergic interneurons generated in the ganglionic eminences. Both populations of neurons actively migrate into the developing cortex, with projection neurons engaging primarily in radial migration, while interneurons undergo tangential migration, travelling along oblique paths via specified routes to reach the cortical plate. The development of the cortex progresses through several stages including, neural proliferation, neuroblast migration and neuronal differentiation. Disrupting the completion of one or several of these steps often cause cortical malformations that lead to severe learning disabilities, mental retardation and epilepsy. The objective of our project is to elucidate new molecular pathways that regulate cerebral cortical neurogenesis with an emphasis on the contribution of: 1/ cell-cycle independent properties of Cip/Kip proteins; 2/ specific postranscriptional/postranslational modifications of cytosolic substrates.

Juliette Godin, Sophie Laguesse, Ariel Avila-Macaya, Noémie Thomas, Marie-Laure Volvert, Laurent Nguyen
Collaborators: Arnaud Besson (France), Eleanor Coffey (Finland), Julian Heng (Australia), Sandrine Humbert (France), Christine Métin (France)

LABORATORY OF AXONAL REGENERATION AND CEPHALIC PAIN

Our unit includes two groups: firstly, the «axonal regeneration» group steered by Rachelle Franzen and Jean Schoenen studies cellular and molecular facets of post-lesional neuroplasticity using spinal cord and peripheral nerve injuries as models. Therapeutic strategies to improve axonal regrowth and functional recovery are explored including stem cell therapy. Secondly, the «cephalic pain» group led by Sylvie Multon and Jean Schoenen focuses on the neurobiological aspects of trigeminal nociception exploring the effects of sex hormones, tryptophan metabolism and various therapeutic strategies. The objective is to better understand the pathophysiology of headaches and migraine, and to pave the way for more effective therapies.

The Placental Growth Factor (PlGF): A novel actor in the Wallerian degeneration inflammatory process

Wallerian degeneration (WD) is an inflammatory process of axonal degeneration. It includes axonal fragmentation, myelin breakdown and debris clearance by Schwann cells and macrophages, providing a favourable environment to successful axonal regeneration after nerve injury. Placental growth factor (PlGF) is a glycoprotein of the VEGF (Vascular Endothelial Growth Factor) family. Originally identified in the placenta, it has also been detected in the brain, especially when cells are activated or stressed. In addition to its pro-angiogenic action, PlGF has various properties like monocyte activation and attraction, ability to increase expression of pro-inflammatory cytokines and chemokines, as well as axonal guidance that could make it a candidate involved in the WD process, and in the success of axonal regeneration. To test this hypothesis, we have compared WD after sciatic nerve complete section in Pgf knock-out and wild-type mice. We showed that in absence of PlGF, WD events, like macrophage invasion, Schwann cell dedifferentiation, axonal regeneration are delayed compared to wild-type mice. As a consequence, motor recovery is impaired. We also showed that PlGF transcription is regulated by NF-κB, and MCP-1, a NF-κB-dependent gene, is not properly expressed in Pgf null mice.

Toluidine blue staining on semi-thin cross-section of uninjured sciatic nerve

Linda Chaballe, Rachelle Franzen, Jean Schoenen, Alain Chariot, Pierre Close
Collaborator: Peter Carmeliet (Belgium)

Migraine preventive drugs differentially affect cortical spreading depression (CSD) in rats

CSD is most likely the culprit for the neurological aura of migraine. It is suggested that all preventive anti-migraine drugs may act by suppressing CSD. We therefore explored the effect of chronic administration of lamotrigine, valproate or riboflavin in the rat model of KCl-induced CSD. Rats received daily ip injections of one of the 3 drugs for 4 weeks. After treatment, CSDs were elicited for 2h by occipital KCl application. We measured CSD frequency, its propagation between a posterior and an anterior electrode, and the number of Fos-IR nuclei in the frontal cortex. Our study shows that preventive anti-migraine drugs have differential effects on CSD. Lamotrigine suppressed CSD, while valproate had no effect on CSD induction, but reduced CSD propagation along the cortex. Riboflavin had no significant effect. In line with the electrophysiological results, frontal CSD-induced Fos expression was decreased after lamotrigine and valproate, but not after riboflavin. In addition, we quantified Fos-IR nuclei in the periaqueductal grey matter (PAG), known to be involved in pain and possibly in migraine. We discovered for the first time that CSD significantly reduced Fos expression in PAG and this reduction was further enhanced by lamotrigine treatment. In a translational perspective this may explain how CSD can promote central sensitization and headaches and why lamotrigine is effective on the migraine aura but not on the migraine headache.

Vladimir Bogdanov, Sylvie Multon, Virginie Chauvel, Jean Schoenen
Collaborator: Arpad Pardutz (Hongria)

LABORATORY OF ELECTROPHYSIOLOGY

Our laboratory is dedicated to cellular and molecular electrophysiology. We work on transfected cell lines, rodent brain slices, anaesthetized rats and awake rats using various recording techniques (patch clamp, intracellular, extracellular single-cell and multicellular recordings). In collaboration with J.-F. Liégeois, we have also developed binding assays on membranes from transfected HEK293 cells. Using these techniques and preparations, we study various aspects of ion channel physiology and pharmacology in CNS neurons. Broadly speaking, we ask whether it is possible to obtain therapeutic effects in CNS diseases by modulating various types of ion channels, besides the rare ones that have already been targeted.

Physiology and pharmacology of K+ channels

SK (or KCa2) channels are K+ channels that are sensitive only to the intracellular concentration of Ca2+ (EC50 ~ 300 nM) and have a small unitary conductance (10-15 pS). In neurons, they contribute to the afterhyperpolarization (AHP) that follows action potentials and underlies the "refractory period". Thereby, they play a major role in the control of neuronal excitability. We used transfected cell lines to study the physiology and the pharmacology of these channels. Besides the experiments on the prototypical blocker apamin, which are described in the «highlight» section, we also showed that the sigma receptor agonist 1,3-di-o-tolyl-guanidine is able to block SK currents, an action that was previously unknown.

Serotonergic and dopaminergic neurons are endowed with an AHP which is mediated by SK channels. Using patch clamp recordings in slices, we demonstrated using a pharmacological analysis that activation of these channels is mostly mediated by Ca2+ entering through N- and T-type channels in serotonergic neurons.

In collaboration with the group of Rodolphe Sepulchre (Systems and Modeling), we developed and validated a simplified mathematical model of a prototypical dopaminergic neuron. This model allowed us to make predictions on the effect of SK channels, as well as other channels, on its excitability. One of these predictions was verified experimentally.

Cédric Lamy, Philippe Alix, Jacqueline Scuvée-Moreau, Guillaume Drion, Stanislav Koulchitsky, Vincent Seutin, Rodolphe Sepulchre
Collaborators: Jean-François Liégeois (Belgium), Sébastien Dilly (Belgium), Neil Marrion (UK), David Jane (UK), Kate Weatherall (UK)

Excitability of midbrain dopaminergic and GABAergic neurons

In 2010 we completed the description of the functional properties of voltage-gated Na+ channels in Dopamine (DA) neurons and GABA neurons of the rat substantia nigra (SN). DA neurons are implicated in the control of movement and in reward and their dysfunction has been related to Parkinson's disease. Action potentials of SN neurons differ in their rise time and amplitude, two parameters largely dependent on Na+ channel activation. In somatic patches, peak amplitudes of macroscopic Na+ currents were smaller in DA neurons than in GABA neurons. The mean peak Na+ conductance density was twice as large in GABA neurons in comparison to DA neurons. The voltage dependence of Na+ channel activation was not different between the two types of neurons. Na+ channels in DA and GABA neurons differed, however, in the voltage dependence of inactivation, the mean mid-point potential of steady-state inactivation curve being more positive in DA neurons than in GABA neurons. The results suggest that specific Na+ channel functional properties may have consequences on somatic synaptic signal integration in both types of neurons and on somatodendritic release of dopamine from DA neurons.

In collaboration with Arnaud Ruiz (UCL, London), we started to describe the effects of zinc on passive and active properties of rat hippocampal mossy fiber buttons using direct patch-clamp recordings.

Dominique Engel, Vincent Seutin
Collaborator: Arnaud Ruiz (United Kingdom)

GIGA-Signal Transduction

The unit of Signal Transduction is interested in deciphering the mechanisms underlying NF-κB activation through the classical or the alternative pathway in order to better understand how this transcription factor is deregulated in chronic inflammatory diseases as well as in solid and haematological cancers. We also investigate the roles of phosphatases in cell apoptosis. The molecular mechanisms allowing cells to sense and to react to DNA damage or to pathogens are investigated. Protein acetylation in health and diseases is also investigated in order to better understand the physiological significance of this post-translational modification.

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COMPOSITION OF THE THEMATIC RESEARCH UNIT

3 laboratories
43 scientists
- 9 Principal Investigators
- 7 PhD (postdoc)
- 20 PhD Students
- 7 Technicians

The repressing function of the oncoprotein BCL-3 requires CtBP, while its polyubiquitination and degradation involve the E3 Ligase TBLR1

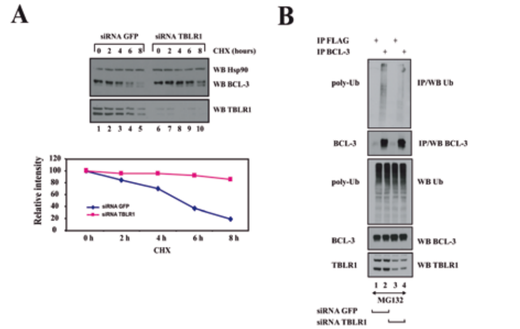
Molecular and Cellular Biology. 2010;30:4006-4021

The nuclear and oncogenic BCL-3 protein activates or represses gene transcription when bound to NF-κB proteins p50 and p52, yet molecules that specifically interact and drive BCL-3-mediated effects on gene expression remain largely uncharacterized. Moreover, GSK3-mediated phosphorylation of BCL-3 triggers its degradation through the proteasome but proteins involved in this degradative pathway are poorly characterized. A biochemical purification of interacting partners of BCL-3 led to the identification of the E3 ligase TBLR1 as a protein involved in the degradation of this oncogenic molecule through a GSK3-independent pathway. TBLR1-depleted cells were defective in BCL-3 polyubiquitination and subsequent degradation. As a result, BCL-3 half-life is extended in these cells. Surprisingly, TBLR1 is also required for the repressing abilities of BCL-3, which suggests that polyubiquitination is not exclusively required for BCL-3 degradation. Thus, our data establish a functional link between the E3 ligase TBLR1 and NF-κB.

Aurore Keutgens[1,2,3], Kateryna Shostak[1,2,3,6], Pierre Close[1,2,3,6], Xin Zhang[1,2,3], Benoît Hennuy[1,4], Marie Aussems[1,2,3], Jean-Paul Chapelle[1,2,3], Patrick Viatour[1,2,3], André Gothot[1,5], Marianne Fillet[1,2,3] and Alain Chariot[1,2,3]

[1]Interdisciplinary Cluster for Applied Genoproteomics (GIGA-Research), [2]Unit of Medical Chemistry and [3]GIGA-Signal Transduction, [4]GIGA Transcriptomics Facility, [5]Department of Medicine /Hematology, University of Liège, CHU, Sart-Tilman, Liège, Belgium.

[6]These authors equally contributed to this work



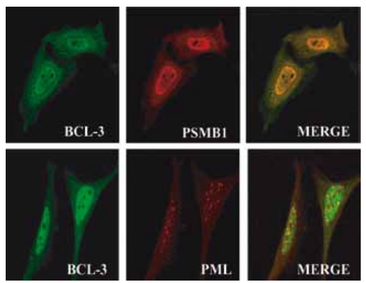
TBLR1 is required for the polyubiquitination of BCL-3. A. Extended BCL-3 half-life in TBLR1-depleted cells. TBLR1-depleted Karpas cells were left untreated (lanes 1 and 6) or stimulated with CHX (50 microg/ml) (lanes 2 to 5 and 7 to 10) and cell extracts were subjected to anti-Hsp90, -BCL-3 and -TBLR1 western blots, as indicated. At the bottom, a quantification of BCL-3 levels in control conditions or upon TBLR1 depletion in Karpas cells is illustrated. The signal intensity in siRNA GFP Karpas cells is set to 100%. B. Impaired polyubiquitination of BCL-3 upon TBLR1 depletion. Karpas cells transfected with siRNA GFP or TBLR1 were stimulated with MG132 and cell extracts were subjected to anti-FLAG (negative control) or -BCL-3 immunoprecipitations followed by anti-Ub or -BCL-3 western blots (top and second panel from the top, respectively). Cell extracts were subjected to anti-BCL-3, -Ub and -TBLR1 western blots as well (bottom panels).

LABORATORY OF MEDICAL CHEMISTRY

Our laboratory is interested in deciphering the molecular mechanisms underlying NF-κB activation in health and diseases, with a special focus on solid and haematological disorders. We actually investigate why the transcription factor NF-κB is constitutively activated in most human tumors. We focus our studies on the BCL-3 protein which is known to transform cells once overexpressed. The elucidation of the BCL-3-dependent pathways includes the identification of new interacting partners as well as the characterization of kinases or E3 ligases that modulate its function. Another project is dedicated to the better understanding of the physiological relevance of protein acetylation in cancer development and progression.

Insight into the mechanisms underlying BCL-3 degradation through interactomic studies

BCL-3 is an oncogenic protein that regulates gene transcription as a nuclear molecule constitutively bound to the NF-κB proteins p50 and p52. BCL-3 is degraded via a phospho- and GSK3-dependent pathway. However, the mechanisms underlying its degradation remain poorly understood. Yeast-two-hybrid analysis led to the identification of the proteasome subunit PSMB1 as a BCL-3-associated protein.



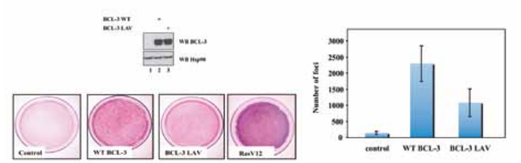
PMSB1 and BCL-3 mainly colocalize in the nucleus but not in the PML bodies. HeLa cells were transfected with FLAG-BCL-3 and with Myc-PMSB1 and their localization was revealed through anti-FLAG and -Myc immunofluorescences, respectively. PML bodies were also visualized using the corresponding anti-PML antibody.

The binding of BCL-3 to PSMB1 is required for its degradation through the proteasome. Indeed, PSMB1-depleted cells are defective in degrading polyubiquitinated BCL-3. The N-terminal part of BCL-3 includes lysines 13 and 26 required for the Lys48-linked polyubiquitination of BCL-3. Moreover, the E3 ligase FBW7, known to polyubiquitinate a variety of substrates phosphorylated by GSK3, is dispensable for BCL-3 degradation. Thus, our data defined a unique motif of BCL-3 that is needed for its recruitment to the proteasome and defined PSMB1 as a new protein required for the degradation of a nuclear and oncogenic IκB protein.

Aurore Keutgens, Xin Zhang, Jean-Paul Chapelle and Alain Chariot
Collaborators: André Gothot (GIGA), Alain Vanderplasschen (Belgium), Françoise Bex (Belgium)

Insight into the mechanisms underlying BCL-3 oncogenic potential

The molecules that specifically interact and drive BCL-3-mediated effects on gene expression remain largely uncharacterized. Although the NF-κB proteins p50 and p52 appear to be required for BCL-3 oncogenic potential, it is currently unclear to which extent other interacting proteins are also involved. Biochemical purification of interacting partners of BCL-3 led to the identification of the co-repressor CtBP as an essential molecule for the ability of BCL-3 to repress gene transcription. BCL-3 actually harbors a motif within its N-terminal domain that is critical for binding to CtBP. Point mutations within this motif disrupts the ability of BCL-3 to transform cells and to inhibit UV-mediated apoptosis in keratinocytes. Taken together, our data defined CtBP as a new signaling molecule required for the oncogenic potential of BCL-3 and for its capacity to promote cell survival in keratinocytes.



CtBP is required for the transforming potential of BCL-3. On the left, cell extracts from NIH3T3 cells infected with a control pBabe retrovirus or a WT or BCL-3 LAV-expressing pBabe construct were subjected to anti-BCL-3 and -Hsp90 western blots, as indicated. The BCL-3 LAV mutant harbors point mutations within the motif required for binding to CtBP. On the right, foci formation assays with NIH3T3 cells infected with the control vector, the BCL-3 vector, the LAV mutant or RasV12 (positive control). Foci were visualized after Giemsa coloration.

Aurore Keutgens, Kateryna Shostak, Xin Zhang, Pierre Close, Marianne Fillet and Alain Chariot, André Gothot

LABORATORY OF VIROLOGY AND IMMUNOLOGY

We aim at a better understanding of the relationship between the Varicella Zoster Virus (VZV) and the host, with a focus on the mechanisms developed by the virus to interfere with cellular response. In addition, the LVI is involved in several research projects dealing with the molecular characterization of signal transduction pathways leading to NF-κB activation.

The Varicella-Zoster Virus ORF47 kinase interferes with the host innate immune response by inhibiting the activation of IRF3

The innate immune response constitutes the first line of host defence that limits viral spread and plays an important role in the activation of adaptive immune response. Viral components are recognized by specific host pathogen recognition receptors triggering the activation of IRF3. IRF3, along with NF-κB, is a key regulator of IFN-β expression. Until now, the role of IRF3 in the activation of the innate immune response during Varicella-Zoster Virus (VZV) infection has been poorly studied. In this work, we demonstrated for the first time that VZV rapidly induces an atypical phosphorylation of IRF3 that is inhibitory since it prevents subsequent IRF3 homodimerization and the induction of target genes. Using a mutant virus unable to express the viral kinase ORF47p, we demonstrated that (i) IRF3 slower-migrating form disappears; (ii) IRF3 is phosphorylated on serine 396 again and recovers the ability to form homodimers; (iii) amounts of IRF3 target genes such as IFN-β and ISG15 mRNA are greater than in cells infected with the wild-type virus and (iv) IRF3 physically interacts with ORF47p. These data led us to hypothesize that the viral kinase ORF47p is involved in the atypical phosphorylation of IRF3 during VZV infection, which prevents its homodimerization and subsequent induction of target genes such as IFN-β and ISG15.

Patricia Vandevenne, Marielle Lebrun, Nadia El Mjiyad, Isabelle Ote, Emmanuel Di Valentin, Yvette Habraken, Estelle Dortu, Jacques Piette, Catherine Sadzot-Delvaux

DNA damage signaling cascade

Genome integrity is continuously challenged by exogenous as well as endogenous agents that react with DNA. Cellular responses elicited by DNA double-strand breaks (DSB), the most dangerous of all DNA lesions, are controlled by the ATM kinase. DSB induce: repair, cell cycle arrest, alternate splicing, activation of transcription factors such as p53 and NF-κB. Together these responses determine the fate of the damaged cells (survival or death). We are studying the cellular responses to DSB induced by antitumor radio- or chemotherapies (Cs137, DNA topoisomerase I and II inhibitors). Genotoxic treatments are frequently used to eradicate cancerous cells that are proliferating rapidly and often present altered cellular response to DSB; i.e. loss of function of p53 and constitutive or exacerbated activation of NF-κB. These two transcription factors favor apoptosis and survival respectively. A few years ago, we identified, in a yeast two-hybrid experiment, sixteen unknown ATM substrates that could be used as therapeutic targets. The ATM targeted serine were identified in two of them and mutated to prevent their phosphorylation. The physiological functions of these mutant proteins were assessed. We focused especially on their affect on p53 and NF-κB and on the viability. A transcriptomic analyze was conducted. In parallel, this year, we started a study of the molecular mechanism underlying the alternative splicing of mRNAs induced by genotoxic stress.

Hélène Sabatel, Céline Pirlot, Kamila Rzewucka, Emmanuel Di Valentin, Alain Colige, Charles Lambert, Luc Willems, Jacques Piette, Yvette Habraken
Collaborators : Olivier De Backer (Belgium), Wim Vanden Berghe (Belgium)

LABORATORY OF VIROLOGY AND IMMUNOLOGY - MOLECULAR IMMUNOLOGY AND SIGNAL TRANSDUCTION GROUP

We are interested in understanding signalling pathways emerging downstream of TNFR (Tumor Necrosis Factor Receptor) family members. TNFR are expressed as membrane anchored or decoy soluble proteins and control a plethora of biological functions ranging from immune system development and homeostasis, osteoclastogenesis, inflammation or cell fate decisions through survival or cell death. These functions are controlled by two main pathways called the classical and the alternative NF-κB pathways. We are currently undertaking different biochemical and genetic approaches to decipher in detail how NF-κB fulfills its multiple tasks and how its subversion can lead to inflammatory disorders and cancer.

The ubiquitin system and NF-κB

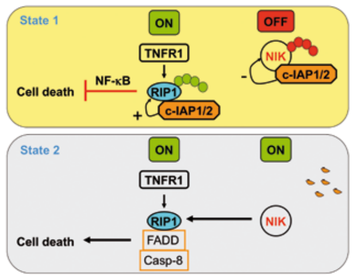
The ubiquitin system plays important roles in the control of cell cycle, signal transduction as well as transcriptional regulation. This system relies on the enzymatic activities of a triad of enzymes called E1, E2 and E3 whose function is to transfer ubiquitin moieties to a specific substrate. E3 ligases are classified into two main classes on the basis of structural similarities. So far, the main E3 ligase that is linked to the degradation of IκB family members is β-TrCP1/2. We are currently focusing on two types of polyubiquitination, which are K63- and K48-linked, and the role of these modifications at the level of mediators that control either the classical or the alternative NF-κB pathway. Abnormalities in ubiquitin-mediated processes have been shown to cause pathological conditions, including malignant transformation or inflammatory disorders. Thus, the identification of new E3 ligases may help to better understand the link between NF-κB and these pathologies.

Caroline Remouchamps, Barbara Maldonado, Corinne Ganeff, Jacques Piette, Emmanuel Dejardin

The hidden function of NIK/MAP3K14 in cell death

The NF-κB-Inducing Kinase (NIK) has been known to be involved in the alternative NF-κB pathway, inducing phosphorylation of IKKα and NF-κB2/p100 processing. We demonstrated that NIK acts as a pro-death kinase through RIP1 in a TNF-dependent manner. We showed that the c-terminal region of NIK mediates RIP1 recruitment through its kinase and intermediate domains. Depletion of NIK neither prevented TNFR1-dependent RIP1 recruitment nor c-IAP1/2 degradation. However, RIP1/Caspase-8 complex formation relied on NIK's kinase activity. Interestingly, induction of this NIK-dependent pro-death pathway did not involve downstream mediators of the alternative NF-κB pathway. In line, NIK deficiency prevented cell death in a mouse model of constitutively activated TNFR1/LTβR, while nfkb2 deficiency did not. In summary, we identified a novel function and pathway for NIK in cell death downstream of non-death receptors in conjunction with RIP1. Therefore, NIK not only contributes to inflammation and cancer, but also to cell death.

Layla Boutaffala, Caroline Remouchamps, Jacques Piette, Emmanuel Dejardin
Collaborators : Mathias Heikenwalder (Germany), Jorge Caamano (United Kingdom), Jean-Ehrland Ricci (France), Peter Vandenabeele (Belgium), Mathieu Bertrand (Belgium)



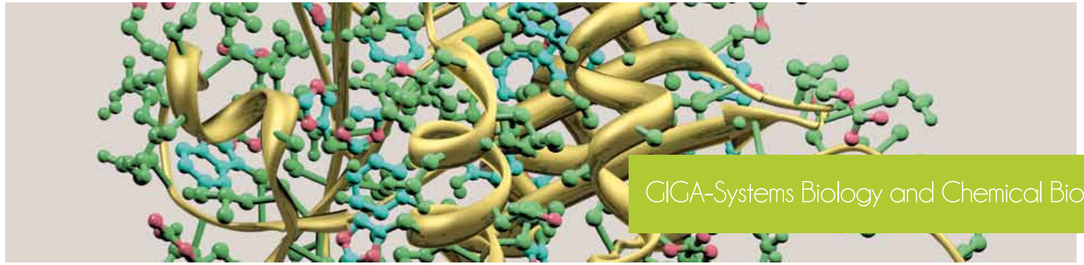
LABORATORY OF PROTEIN SIGNALING AND INTERACTIONS

The laboratory of Protein Signaling and Interactions focuses its research efforts on the study of protein post-translational modifications and protein-protein interaction networks in various physiological and pathological processes.

Identification of a key developmental function for PP2A Bα regulatory subunit: Bα regulates adhesion-related angiogenesis through the control of class IIa HDAC7 activity

Class IIa histone deacetylases (HDACs) are key transcriptional repressors involved in several major developmental programs. In response to specific signals, their repressive activity is neutralized through phosphorylation and nuclear exclusion. Recently, we have identified PP2A, a trimeric enzyme as the phosphatase responsible for dephosphorylating class IIa HDACs and promoting their repressive activity. Functional specificity of PP2A is achieved through incorporation of specific B subunits into the holoenzyme complex. This project aimed at identifying the B subunit specifically involved in the regulation of HDAC7 during angiogenesis. Using an unbiased siRNA approach, we observed that silencing Bα but not other PP2A regulatory subunits specifically impairs endothelial cell (EC) angiogenic capacities. These angiogenic defects are due to alteration in the dynamics of the adhesion process, which prevents PP2A-Bα silenced ECs from migrating properly, a central event in the process of angiogenesis. PP2A-Bα suppression in Zebrafish impaired proper vessel formation, which supports a key role for PP2A-Bα in vascular development. Microarray analysis reveals that PP2A-Bα controls the expression of several key genes involved in EC adhesion and unravels a strong functional link between Bα- and the class IIa HDAC member HDAC7-regulated vascular signaling pathway. For the first time, these results associate a specific PP2A regulatory subunit with a major developmental program.

Maud Martin, Anouk Bleuart, Jonathan Bruyr, Denis Mottet, Marielle Lebrun, Jean-Claude Twizere, Franck Dequiedt
Collaborators: Michale Potente (Germany), Marina Mione (Italy), Nicolas Simonis (Belgium), Veerle Janssens (Belgium), Wolf-Karsten Hofmann (Germany)



GIGA-Systems Biology and Chemical Biology

The overall goal of the Thematic Research Unit of Systems Biology and Chemical Biology is to develop and apply novel quantitative methods in order to contribute to ongoing and future research in biology, medicine and biotechnology. The research carried out in the unit is based on the blending of principles from computer science, applied mathematics, physics and chemistry, and the unit gathers a highly interdisciplinary panel of about 65 researchers, with expertise in molecular biology, proteomics, image analysis, machine learning, statistics, control theory, and optimization.

The research questions addressed by the four laboratories participating in the unit include the development of high resolution molecular imaging techniques, the design of macromolecules targeting specific structural or functional properties, the study of collective behaviors of ensembles of genes/proteins/cells, the quantification of cytologic/histologic/physiomic alterations, and the modeling and analysis of the behavior of higher organisms in their environment. The very transversal nature of the research unit is highlighted by its many collaborations with all other thematic research units of the GIGA-R.

Contact

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COMPOSITION OF THE THEMATIC RESEARCH UNIT

4 laboratories
65 scientists
- 10 Principal Investigators
- 31 PhD (postdoc) or senior scientits
- 19 PhD Students
- 5 Technicians

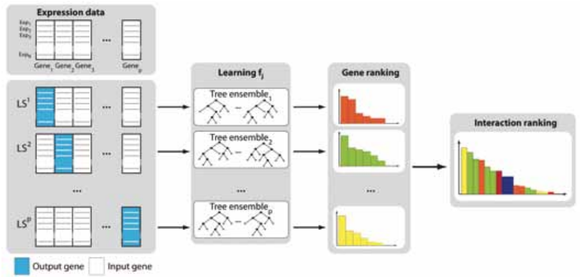
Highlight

Inferring gene regulatory networks from expression data using tree-based methods

PLoS One. 2010;5:e12776

One of the pressing open problems of computational systems biology is the elucidation of the topology of genetic regulatory networks (GRNs) using high throughput genomic data, in particular microarray gene expression data. This paper introduces GENIE3 an original algorithm for the inference of GRNs. GENIE3 decomposes the prediction of a regulatory network between p genes into p different regression problems. In each of the regression problems, the expression pattern of one of the genes (target gene) is predicted from the expression patterns of all the other genes (input genes), using tree-based ensemble methods, Random Forests or Extremely Randomized Trees. The importance of an input gene in the prediction of the target gene expression pattern is taken as an indication of a putative regulatory link. Putative regulatory links are then aggregated over all genes to provide a ranking of interactions from which the whole network is reconstructed. The resulting method does not make any specific prior assumption about the nature of gene regulation mechanisms, it can deal with combinatorial and non-linear interactions, it produces directed GRNs, and is it fast and computationally scalable. Our method was evaluated twice in the context of the Dialogue for Reverse Engineering Assessments and Methods (DREAM) challenge, which is the annual international competition aiming at the evaluation of GRN inference algorithms on benchmarks of simulated and real data. The GENIE3 method was ranked as best performer both on the DREAM4 In Silico Multifactorial challenge in 2009 and on the DREAM5 Network Inference challenge in 2010. Two benchmarks of this latter challenge corresponded to real datasets related to two microorganisms, e coli and s aurus. As partner of the DREAM Consortium, our predictions, as well as those of the best performing teams of the other subchallenges, are currently being experimentally validated. Together with researchers from INRA Toulouse (Jimmy Vandel) and from the VIB at University of Ghent (Dr. Yvan Saeys), we are now working on several further improvements of our approach, as well as on its extensions to handle genotype datasets and other kinds of molecular interactions.

Vân Anh Huynh-Thu [1,2], Alexandre Irrthum [1,2], Louis Wehenkel [1,2], Pierre Geurts [1,2]
[1] GIGA-Research, Systems Biology and Chemical Biology, University of Liège.
[2] Department of Electrical Engineering and Computer Science, Systems and Modeling, University of Liège.



Schematic representation of the GENIE3 procedure: For each gene j = 1, . . . , p, a learning sample LSj is generated with expression levels fj of gene j as output values and expression levels of all other genes as input values. A function fj is learned from LSj and a local ranking of all genes except j is computed. The p local rankings are then aggregated to get a global ranking of all regulatory links.

LABORATORY OF SYSTEMS AND MODELING

Our laboratory belongs to the Department of Electrical Engineering and Computer Science. Our members (about 60) work on mathematical modeling, simulation, optimization, statistics, control theory, machine learning and image analyses, and their applications. About a third of them are involved in the research at GIGA-R. We work on the design of methods for analysis, modeling, and optimization targeted towards problems in biology, such as the elucidation of molecular networks from large-scale genomics annotations and experimental datasets, the modeling of dynamics of biological systems at various scales, and the design of algorithmic and statistical approaches to exploit heterogeneous datasets composed of images, genoproteomics measurements.

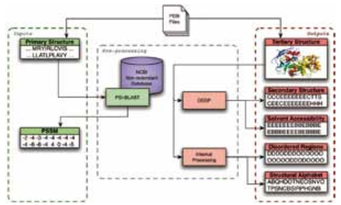
Biological network inference using statistical and machine learning techniques

In biology, relationships between biological entities such as genes, proteins, transcription factors, micro-RNAs, tissues or diseases are often represented by graphs or networks. These networks may represent physical interactions (e.g., protein-protein or miRNA/mRNA interactions) or functional interactions (e.g., gene co-expression or cooperation of proteins inmetabolic pathways). Building on our expertise in machine learning, our objective is to develop original statistical techniques to infer or complete these networks by integrating various kinds of experimental data and then to collaborate with biologists to indentify novel interactions for wet lab validation. In 2010, our team has won the DREAM5 contest, whose aim is to challenge techniques to infer regulatory networks from expression data. We have also pursued our collaboration with the laboratory of Molecular Biology, MRC Cambridge, UK (Dr. M. Mohan Babu) for the application of our techniques to epigenetic interactions in the Yeast S.cerevisiae. Several collaborations with teams from GIGA-R concern the inference of microRNA-mRNA interactions at work in some biological condition by the joint analyses of miRNA and mRNA expression data. This research is supported by the BioMAGNet project funded by the Interuniversity Attraction Poles Programme, initiated by the Belgian State, by the EU FP7 PASCAL2 network of excellence, and by FRIA and FNRS.

Pierre Geurts, Van Anh Huynh-Thu, Alexandre Irrthum, Marie Schrynemackers, Louis Wehenkel
Collaborators: Florence d'Alché-Buc (France), Madan Mohan Babu (UK), Yvan Saeys (Belgium), Jimmy Vandel (France)

In-Silico prediction of functional and structural properties of proteins

This project started end of 2009 and aims at developing novel techniques to infer structural and functional properties of macromolecules such as large proteins, by combining the evidence gathered in experimental databases with physicochemical ground knowledge and ad hoc optimization and machine learning methods. Our research has two main threads, namely on the one hand, the simultaneous prediction of structural properties of proteins at growing levels of detail (from secondary to tertiary structure), and on the other hand, the detailed study of partially unstructured proteins. During the last year, we have developed a 'multitask iterative supervised learning approach' that is able to jointly solve several related structure prediction problems; in particular we have shown that this approach leads to better predictive accuracy when simultaneously trying to predict secondary structure, solvent accessibility, disordered regions, and structural alphabets of proteins, than the methods addressing these problems separately.



Database collection and pre-processing for the prediction by machine learning of structural properties of proteins

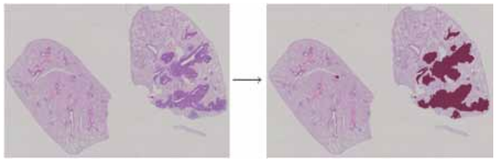
Our long-term goal aims at combining this approach with direct optimization methods for structural energy minimization in order to develop a state-of-the-art pipeline for in-silico protein structure prediction. This research is supported by the BioMAGNet project funded by the Interuniversity Attraction Poles Programme, initiated by the Belgian State, by the EU FP7 PASCAL2 network of excellence, and by FRIA and FNRS.

Francis Maes, Julien Becker, Fabien Heuze, Alexandre Irrthum, Pierre Geurts, Louis Wehenkel
Collaborators: Madan Mohan Babu (UK), Dominique Dehareng (Belgium)

Computational methods to explore and quantify high-throughput imaging data

With the recent advent of digital (2D, 3D, 3D+Time) image acquisition technologies (such as high-content screening, whole-slide virtual microscopy, etc.), scientists routinely generate larger and larger amounts of imaging data. Human interpretation of images is time-consuming and often biased. This stresses the need for reproducible and efficient computational methods to extract useful and quantitative information from these datasets. In this project, we design machine learning algorithms for automated image analysis, including methods to index and search similar images in large distributed databases, classify them into known phenotypes, and segment them into meaningful regions. With the recent financial support of the Walloon Region (CYTOMINE WIST3 project), we now develop a web-based software platform composed of easy-to-use services for domain experts to visualize, annotate and analyze high-resolution tissue and cell images with a specific focus on lung-cancer. Other ongoing collaborations with local and foreign industries and academic research

laboratories concern cell sorting for screening and diagnosis in anatomopathological cytology, tissue quantification of mice lungs in the context of cancer and asthma treatment evaluation, zebrafish phenotype classification for toxicology studies, detection of protein crystals in droplets for structure-based drug discovery, and automatic cell counting in Boyden chemotaxis assays.



Automatic recognition and quantification of tumor patterns in hematoxylin and eosin stained images of mice lungs

Raphaël Marée, Benjamin Stévens, Loïc Rollus, Olivier Stern, Nathalie Jeanray, Didier Cataldo, Marc Muller, Pierre Geurts, Louis Wehenkel
Collaborators: Isabelle Salmon (Belgium), Kurt Hoffmann (Germany)

Dynamic modeling of learning in the Morris water maze

The Morris water maze is an experimental procedure in which animals learn to escape swimming in a pool using environmental cues. Despite its success in neuroscience and psychology for studying spatial learning and memory, the exact mnemonic and navigational demands of the task are not well understood. Here, we provide a mathematical model of rat swimming dynamics on a behavioural level. The model consists of a random walk, a heading change and a feedback control component in which learning is reflected in parameter changes of the feedback mechanism. The simplicity of the model renders it accessible and useful for analysis of experiments in which swimming paths are recorded. Here, we used the model to analyze an experiment in which rats were trained to find the platform with either three or one extramaze cue. Results indicate that the 3-cues group employs stronger feedback relying only on the actual visual input, whereas the 1-cue group employs weaker feedback relying to some extent on memory. Because the model parameters are linked to neurological processes, identifying different parameter values suggests the activation of different neuronal pathways.

Eric Bullinger, Dirk Fey
Collaborator: Sean Commins (Ireland)

Dynamic modeling of pro- and antiapoptosis signalling

Pro- and anti-apoptotic signalling is inherently dynamical and dosis dependent. In this project, we develop and analyze a mathematical model of a cell population stimulated by TNF-α. Based on experimental data by our partners, we collaborate on the model development and on the analysis of the mathematical model.

Eric Bullinger
Collaborators: Monica Schliemann (Germany), Rolf Findeisen (Germany)

Statistical approaches for the study of gene-gene and gene-environment interactions

The semi-parametric gene-gene and gene-environment interaction detection method Model-Based Multifactor Dimensionality Reduction (MB-MDR) harbors many possibilities for future enhancements to deal with several (statistical) hurdles in the field of genetic epidemiology. In this project these possibilities are explored in more detail, leading to different studies investigating the performance of MB-MDR in a variety of settings, including the presence of multivariate and categorical outcomes, (unmeasured) confounding factors, influential noise factors, distortions leading to skewed and non-symmetrical trait distributions.

Tom Cattaert, Elena Gusareva, Jestinah M Mahachie John, François Van Lishout, Kristel Van Steen
Collaborators: Malu Calle (Spain), Nuria Malats (Spain), Marylyn Ritchie (USA), Benjamin Raby (USA)

Reclassification of Inflammatory Bowel Disease based on genetic marker data

Crohn's Disease (CD) has a heterogeneous presentation, and is typically being classified according to extent and location of disease. The genetic susceptibility to CD is well known and genome-wide association scans (GWAS) and meta-analyses thereof both have identified over 100 susceptibility loci. Except for the association between ileal CD and NOD2 mutations, efforts in trying to link CD genetics to clinical sub-phenotypes have not been very successful. In this project we investigate the utility of latent variable models to identify patient sub-groups using genetic marker data. New patient classes are then further described using additional clinical and non-clinical measurements via machine learning and data-mining techniques.

Jestinah M Mahachie John, Kristel Van Steen, Michel Georges
Collaborator: Ben Spycher (Switzerland)

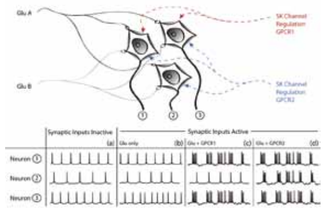
Comparison of genetic association strategies in the presence of rare alleles

In the quest for the missing heritability of most complex diseases, rare variants have received increased attention. The advances in large-scale sequencing have led to a shift from the Common Disease Common Variant to the Common Disease Rare Variant Hypothesis, or at least have re-opened the debate about the relevance and importance of rare variants for gene discoveries. The investigation of modeling and testing approaches to identify significant disease - rare variant associations is in full motion. New methods to better deal with parameter estimation instabilities, convergence problems or multiple testing corrections in the presence of rare variants or effect modifiers of rare variants are only at their infancy. In this project we compare several genetic association strategies to detect main effects caused by rare alleles. Since the rarity of certain marker alleles hampers the validity of model assumptions, the distributional properties of test statistics, as well as assumptions underlying some commonly used measures to correct for multiple testing or to control false positive rates, this project also envisages the development of novel methodologies.

Tom Cattaert, Elena Gusareva, Jestinah M Mahachie John, François Van Lishout, Alain Empain, Kristel Van Steen
Collaborator: Marylyn Ritchie (USA)

Mathematical and Experimental Analysis of the Mechanisms Underlying the Electrical Activity of Pacemaker Neurons of the Central Nervous System

A common feature of many pacemaker (i.e. spontaneously active) cells of the central nervous system (CNS), such as dopaminergic (DA) and seretoninergic (5-HT) neurons, is that they are able to exhibit two different electrical modes (or firing patterns) in physiological configuration (*in vivo*): irregular and burst firing. The signaling of these neurons strongly relies on these firing patterns as well as on the degree of synchronization between neurons of subpopulations. Therefore, a mathematical analysis of the mechanisms underlying these phenomena would help to understand the behavior of these cells in physiological and pathological conditions, as well as to isolate targets for the treatment of diseases affecting these neurons, such as Parkinson's disease. For this purpose, and in collaboration with the group of V. Seutin (Electrophysiology), we developed a minimal mathematical model exhibiting the firing patterns observed in these pacemaker cells in similar conditions, initially focusing on the DA neuron. Inter alia, the analysis of this model and experimental validations of key features allowed us to reconcile 17 years of discrepant experimental results concerning the transmembrane proteins engaged in the spontaneous electrical activity of DA neurons. We also made novel predictions on the role of particular transmembrane proteins, called small conductance calcium-activated (SK) potassium channels, in the regulation of the firing pattern and synchrony of many pacemaker cells. These predictions are on their way to be verified experimentally using the patch clamp technique.



Potential mechanism underlying the concomitant switch of firing pattern and synchrony in pacemaker cells.

Rodolphe Sepulchre, Vincent Seutin, Guillaume Drion
Collaborators: Peter Wellstead (Ireland), Terrence J. Sejnowski (USA)

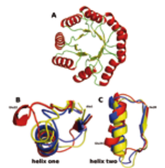
LABORATORY OF MOLECULAR BIOLOGY AND GENETIC ENGINEERING (LBMGE-PROTEIN ENGINEERING GROUP)

We study the sequence-structure-function relationships in proteins and peptides. For this, we *de novo* design, produce, purify and analyze artificial proteins (1). We also focus on the relationships between the protein surface structure and its ability to yield high quality crystals in different conditions (2). In projects relating to biomimetics, we study the structural determinants involved in the highly specific binding properties of natural (3) or genetically engineered (4) peptides to inorganic surfaces; we also characterize the relationship between the conformation of peptides immobilized onto inorganic surfaces and their activity (5).

De novo design of artificial α/β barrel proteins: computational design and directed evolution.

One way to gain insight into the sequence-structure-function relationship in proteins is to *de novo* design artificial proteins. Six generations of Octarellins, *de novo* polypeptides of more than 200 amino acids modelled on the TIM α-barrel fold, have been built and structurally characterized using biophysical and spectroscopical methods. The fifth generation of Octarellins was designed following a hierarchical method combining the specificity of rational design with the power of computational design. The resulting protein was expressed in fusion to GFP, used as reporter of solubility, and the protein was improved by directed evolution. Bioinformatic analyses on the evolutionary optimization process was performed. Statistical analysis was used to characterize the relevance of the different mutations from the parental Octarellin until the last variant obtained from the directed evolution process. Molecular dynamic studies were performed to evaluate the influence of the different mutations in the protein structures as well as to predict regions in the parental Octarellin with particular errors in the design. Co-crystallographic tests with camel antibodies and HEAT like proteins are in progress to verify the structure of our designed proteins.

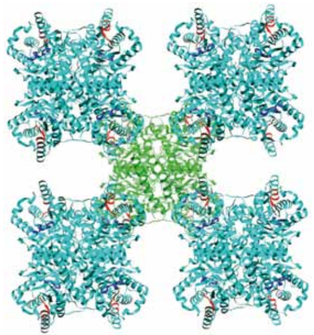
Maximiliano Figueroa, Alexandre Irthum, Christine Evrard, Anabelle Lejeune, Cécile Van de Weerdt and Joseph Martial
Collaborators : Mireille Dumoulin (Belgium), André Matagne (Belgium), Christian Damblon (Belgium), Philippe Minard (France), Else Pardon (Belgium)



A) Overview of the Octarellin V designed in an (alfa/β)8 fold.
B) and C) Effect of the optimization by Error Prone PCR in the helix one and two of Octarellin V analyzed by Molecular Dynamic simulations. Original design (yellow) has a discrepancy in the relationship between amino acid sequence and tertiary structure, as is showed after Molecular Dynamic simulation (red), however the introduction of mutations by EPP could revert this problem (blue), andthis final model has more similarity with the original 3D design.

Influence of mass transport and surface growth processes on protein crystal perfection

The protein crystallization process is still the major bottleneck for the technique allowing the determination of protein structure by crystallography. Indeed, the structural resolution critically depends on the quality of the crystalline lattice; in addition, crystal size is also an important factor. Both lattice quality and crystal size are believed to be related to gravity dependent processes. In a work supported by the European Space Agency (ESA) and in collaboration with Belgian and international groups, our laboratory studies the relationships between protein crystal growth conditions and the crystal quality, as well as the effect of microgravity on the latter. For this purpose, we used the Protein Crystallisation Diagnostics Facility (PCDF), an instrument developed by the European Space Agency (ESA) for the investigation of protein crystallization in microgravity. The PCDF instrument allows *in-situ* observation of nucleation and growth behavior of the crystallizing proteins. A previous study showed that the structuring of the water molecules around the protein molecules in solution is the main component of the thermodynamics of crystallization and largely determines the kinetics of crystal growth. In another part of the project, we started a mutational surface engineering study on glucose isomerase in order to get more insight on how the composition of the protein surface and the interactions between water and amino acid residues determine the assembly.



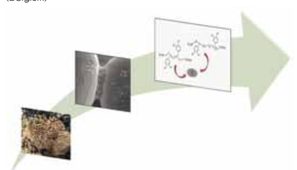
The glucose isomerase homotetramer (4x388aa). View of the central tetramer (green) with the 4 tetramers involved in crystal packing contacts.

Christine Evrard, Cécile Van de Weerdt and Joseph Martial
Collaborators: Dominique Maes (Belgium), Mike Sleutel (Belgium), Klass Decanniere (Belgium), Gregoire Nicolis (Belgium), Fermin Otorolla (Spain)

From marine adhesive to new bioglue: cloning, production and characterization of unusual proteins (Biomad)

Naturally produced adhesives are common in many biological systems and are known for their capability to strongly bind many surfaces at ambient temperature, even in a humid and salted environment. The goal of the project is to isolate, produce and characterize the features of several marine adhesives in order to develop biomimetic adhesives for industrial applications. Three animal models were selected, representing three different types of adhesion: the sea star, Asteria rubens, for temporary adhesion, the honeycomb worm, Sabellaria alveolata, for permanent adhesion and the sea cucumber, Holothuria forskali, for instantaneous adhesion. In these three systems, the adhesive secretion is mainly composed of proteins. The characterization of the proteins reveals special features like repeated sequences with bias in amino acid composition and post-traductionnal modification specific of their adhesive properties like the hydroxylation of tyrosine in 3,4-dihydroxyphenylalanine (DOPA). We cloned and expressed the cDNA of these proteins in E. coli, under different forms: full length, with their original sequence, or in a multimeric form composed of several sets of the repetitive peptides. Following purification, the proteins are modified to mimic the natural adhesives. To transform the tyrosine residues in DOPA, two approaches are under investigation: a chemical track and an enzymatic track. For the enzymatic modifications, we are working on the isolation of mussel phenoloxydases.

Anabelle Lejeune, Aline Joris, Aurélie Radoux, Cécile Van de Weerdt and Joseph Martial
Collaborators: Patrick Flammang (Belgium), Pierre Becker (Belgium), Elise Hennebert (Belgium), Pascal Damman (Belgium)



From marine adhesive to new bioglue : Cloning, production and characterization of unusual proteins of Sabellaria alveolata glue system.

Study of the determinants involved in the highly specific binding properties of inorganic binding peptides

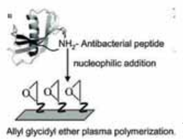
Genetically Engineered Peptides for Inorganic (GEPI) are peptides - selected by a genetic engineering method such as phage display-displaying a specific and optimal affinity for an inorganic material. Hundreds of GEPI specific for different kinds of surfaces have been identified. We know that this specificity depends on (i) the chemical composition, (ii) the texture (powder or flat surface), and (iii) the crystallographic form of the inorganic substrate, but we do not understand the recognition mechanisms at the molecular level.This specificity gives them interesting properties and several applications, using GEPI as linkers between organic or inorganic compounds and inorganic surfaces, have been highlighted. We are focusing on understanding the fundamental aspects of the molecular interactions between peptides and inorganic materials. The carbon nanotubes (CNT) have been chosen as inorganic model because of their physical properties which make them potentially useful in many applications in various fields of biological or material science. We have used different random peptide libraries displayed on the phage M13 surface for the selection of GEPI on CNT. After several rounds of panning, we have identified the best GEPI-candidates and synthesised them for further binding, absorption and conformational studies. We will use AFM, RMN, HPLC and spectroscopy methods combined with bioinformatics and alanine scanning to better understand the peptides recognition mechanisms.

Alexis Genin, Christelles Vreuls, Germaine Zocchi, Cécile Van de Weerdt and Joseph Martial
Collaborator: Julien Amadou (Belgium)

Development of new bio-inspired functional coatings for inorganic surfaces (Biocoat)

For years, functional biological systems have inspired man-made advanced material design and engineering. The unique molecular/nano-architectural features underlying the biological material properties is accomplished in Nature via specific interactions between biomolecules and inorganic materials. Following that same idea, we have designed bifunctional peptides displaying both an inorganic material binding site and a desired biological activity in order to create bio-inspired functional coatings for inorganic surfaces. We have isolated metallic surface-binding peptides by phage display technology and used these GEPI (Genetically Engineered Peptide for Inorganic) as linkers for anchoring antimicrobial peptides onto the inorganic substrate. The antibacterial effect of the functionalized surfaces was demonstrated against Gram+ and/or Gram- bacteria. It is well known that immobilisation of bio-active proteins/peptides often leads to partial or total loss of biological activity due to a random orientation of the immobilized biomolecule, a modification of its conformation or a poor accessibility of the protein for its target. In order to optimize the biological activity of the anchored antibacterial peptides or proteins, we are initiating a fundamental study on the interactions at the molecular scale, of the GEPI alone or coupled to an antibacterial peptide in interaction with the inorganic surface.

Christelle Vreuls, Germaine Zocchi, Hélène Vandegaart, Fabrice Farina, Geoffrey Garitte, Alessandro Gallo, Cécile Van de Weerdt and Joseph Martial
Collaborators: Catherine Archambeau, Michel Beguin, Jacques Pélerin, Christophe Detrembleur, Christine Jérôme, Anne-Sophie Duwez (Belgium)



Stainless steel bio-functionalization via antimicrobial peptide grafting

LABORATORY OF MASS SPECTROMETRY

The Mass Spectrometry Laboratory is a multidisciplinary laboratory, given the versatility of mass spectrometric techniques. Our objective is to contribute to the development of new fundamental concepts and to enlighten their applications in the fields of biomolecular sciences. Basic research deals with the intrinsic properties of complex biomolecules and supramolecular systems. It leads to a better understanding of their reactivity and the role of their environment. The Characterization of molecular systems includes their mass, their structure, their shape and since the introduction of mass spectrometry imaging, their spatial localization especially in biological samples.

Probing DNA and RNA supramolecular assemblies using novel gas phase methods

The advantages of using mass spectrometry-based techniques to study supramolecular assemblies are the sensitivity of the method and its ability to detect and separate minor species present in solution. Mass spectrometry is a unique structural investigation technique for biomolecules because, once extracted from the solution, each complex is isolated from its environment. A mass spectrum is therefore like a snapshot view of the composition of the solution: the peak positions provide information on the stoichiometry of the complexes formed in solution, while the peak intensitites provide information on their relative abundance. Moreover, once the complexes are isolated (i.e. in the gas phase), a variety of methods can be used to get more insight into the structure of each species. In addition to using the collision-induced dissociation and ion mobility spectrometry to study interaction between subunits and ion shape, we also develop novel spectroscopy-based approaches to probe the structure of the complexes in the gas phase. This requires to modify commercial instruments and couple them with powerful wavelength-tunable lasers, and to study the effects of laser irradiation on the fragmentation of the complexes. We use these mass-spectrometry based techniques for example to investigate G-quadruplex formation and assembly, and their interaction with small molecule ligands that inhibit telomerase activity through binding to telomeric G-quadruplexes.

Frédéric Rosu, Valérie Gabelica, Nicolas Smargiasso, Gabriel Mazzucchelli, Denis Morsa, Anastasia Burmistova, Edwin De Pauw
Collaborators: Philippe Dugourd (France), Jean-Louis Mergny (France), Stephen Neidle (UK), Giorgia Oliviero (Italy), Marie-Paule Teulade-Fichou (France), Jean-François Riou (France).

Nanovenomics

Many naturally peptides such as animal toxins are structured by disulfide bridges. These bridges are essential since they allow the decreasing of the toxin immunogenicity's (increasing their efficiency), but they also provide the adequate conformation to bind to their receptor with high affinity and selectivity. Moreover, the number of cysteins and the arrangement of disulfides can give access to the family and to the potential biological target of the studied toxin, as in the case of conotoxins. The study of disulfide arrangements

as well as tridimensional structures of toxins appears then crucial to understand the toxin-receptor interactions at the molecular level. Ion mobility mass spectrometry (IMMS) is an emergent field of mass spectrometry. The addition of a mobility cell in a classical Q-TOF spectrometer allows separating molecules according their gas-phase conformations, like a real "gas-phase electrophoresis". In this project, we investigate the possibility of determining cysteine pairing with a combination of partial disulfide reduction, ion mobility separation and fragmentation. This leads to a rapid full characterization of toxins in a single animal venom and could lead to a high troughput search for new compounds with high potential as lead drugs.

Loic Quinton, Julien Echterbille, Edwin De Pauw
Collaborators: Pierre Escoubas (France), Nicolas Gilles (France)

Glyco

Initiated in 2008, the project GLYCO is focused on the develoment of new tools for rapid characterization of glycans and glycoproteins and especially antibodies for pharma applications. The Laboratory is associated with the CER Marloie for producing the antibodies under various cell culture conditions. Purification methods based on affinity and sequencing methods using mass spectrometry allowed to identify a specific petide sequence bearing eight different glycan moieties that can be quickly characterized using electron transfer dissociation (ETD). In source decay, a very fast top down sequencing method usually used for proteins has been extended to glycans. Finally, ion mobility measurements are now performed to characterize the shape of the biomolecules and refine their characterization by folding data.

Nicolas Smargiasso, Pierre Jonlet, Edwin De Pauw
Collaborators: Alfred Collard, Sophie Waxweiler (Belgium)

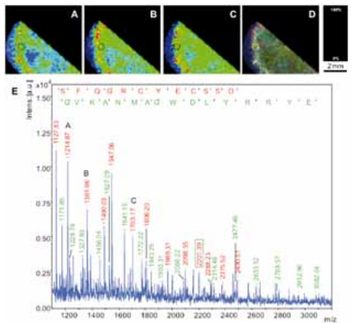
LABORATORY OF HISTOLOGY - CYTOLOGY

The laboratory of Histology-Cytology, within the CART and GIGA-R, develops tools for the cellular and tissue analysis, in the fields of the biomaterials and in experimental cancerology. The two axis are:
1- Cellular or tissular interactions with various biomaterials (artificial implants, polymers, metal, textiles or nanoparticules) are analyzed in relation to substrate surface properties and specific biocompatibility tests *in vitro* and *in vivo* are developped.
2- Techniques of proteomics, in particular differential profile of finite or continuous lines, exposed to xenobiotics or drugs and the molecular imagery on tissues are realized in collaboration with the laboratory of mass spectrometry.

Innovative technique of implementation of intraoculaires flexible lenses from a material of nanocomposite type (LIONEL)

The most common complication after implantation of intraocular lenses (IOLs) is the posterior capsular opacification (PCO) or secondary cataract. This is the result of lens epithelial cells proliferation and their transition to mesenchymal cells. IOLs are elaborated from polymers of various hydrophobic to hydrophilic character. Their degree of biocompatibility must be very high while maintaining optimal optical properties. The project aims to compare hydrophobic and hydrophilic IOLs in order to evaluate the resulting interactions with lens epithelial cells. Complementary investigation techniques and methods are used to study the surface properties and the biocompatibility of IOLs, in order to define performance signs related to cell adhesion and PCO. Search of biomarkers on lenses is performed by immunohistochemistry and by mass spectrometry imagery.

Virginie Bertrand, Delphine Debois, Edwin De Pauw, Marie-Claire De Pauw-Gillet
Collaborators: Christophe Pagnoulle, Dimitriya Bozukova (PhysIOL, Belgium), C. Jerôme (CERM, Belgium)



MALDI-in source decay applied to mass spectrometry imaging: a new tool for protein identification. Spatial distribution of lens soluble crystallins on porcine lens slice. D Debois et al., Anal Chem. 2010 May 15;82(10):4036-45

In vitro bioassay using MCF-7/BOS line and 17β-estradiol to quantify by 2D DIGE and identify by mass spectrometry concentration dependent biomarkers of environmental estrogen exposure

The aim of this work is the development of a screening test allowing the detection of estrogens and estrogen like compounds by monitoring protein biomarkers of estrogenic activity. The use of differential proteomic techniques such as 2D-DIGE (Two Dimension Difference Gel Electrophoresis) conjugated with principal component analysis and mass spectrometry constitutes a powerful tool for the study of the protein abundance changes. MCF-7/BOS cell line, a hormonal dependent cell line, and 17β estradiol are used as reference for the discovery and the characterization of biomarkers. MCF-7/BOS cells were treated for 24hrs with concentrations from 1pM to 500pM of 17β-estradiol, the most active estrogenic compound, in order to identify potential estrogen biomarkers for further cell based screening. The cytosolic fraction of the cells was analyzed by 2D-DIGE (Two Dimension Difference Gel Electrophoresis), a differential proteomic technique using 2D PAGE (PolyAcrylamide Gel Electrophoresis). This strategy enabled us to identify several proteins showing variations in their expression depending on the concentration of estradiol. 17 significantly regulated proteins were identified and 10 were selected as potential biomarkers. A first set of potential biomarkers induced by estradiol was confirmed by MRM (multiple reaction monitoring) analyses.

Mike Collodoro, Pascale Lemaire, Virginie Bertrand, Rowan Dobson, Gabriel Mazzucchelli, Joëlle Widart, Edwin De Pauw, Marie-Claire De Pauw-Gillet.
Collaborator: Gauthier Eppe (Belgium)

Functionalized gold nanoparticles for optoacoustic cancer detection: identification and targeting of specific prostatic cancer cells surface proteins

A major challenge in oncology is to develop more accurate imaging assessments. The ADONIS Project intended to prove the concept of using optoacoustic imaging with biologically functionalized gold nanoparticles as an integrated biosensor based imaging system for the production of specific and sensitive data for accurate diagnosis of prostate cancer. One of the main objectives of this project is to achieve and validate a versatile lab system composed of functionalized nanoparticles for diagnosis of different superficial and accessible cancers, e.g. prostate cancer. Gold nanorods with specific spectral properties have been synthesized and functionalized with antibodies targeting specific antigens of cancerous cell-lines. Initially, the biosensor system has been developed targeting the Prostate Specific Membrane Antigen (PSMA), a suitable biomarker of the prostate cancer already described in the literature. In order to identify new potential cancer targets for the biosensor, novel quantitative proteomic techniques have been developed focusing on the purification of phosphorylated and glycosylated proteins.

Maximilien Fleron, Daureen Schol, David Waltregny, Yohan Greffe, Anne-Cécile Massart, Vincent Hennequière, Davide Musmeci, Gabriel Mazzucchelli, Marie-Claire De Pauw-Gillet, Vincent Castronovo, Edwin De Pauw, Andrei Turtoi
Collaborator: Christine Jérôme (Belgium)

Translational Research : GIGA/CHU

Translational research is based on a permanent interaction between basic and clinical research and aims at a rapid transfer of prognostics, diagnostics and therapeutics beneficial for the patient.

Thanks to the proximity of GIGA-R and the University Hospital of Liège (CHU), many translational research programs have been initiated. More than 200 research projects focused on a better understanding of the functioning of human beings, in particular in case of dysfunctions, are currently performed at the GIGA-R and CHU.

The cellular and gene therapy illustrates the efficacy of the strong interactions between researchers, clinicians and companies.

DEVELOPING NEW TECHNIQUES APPLIED TO CELLULAR AND GENE THERAPY IN THE FIELD OF HEMATOLOGY AND ONCOLOGY, AND IN REGENERATIVE MEDICINE AND IMMUNOLOGY

The Laboratory of Cell and Gene Therapy (LTCG), integrated both in the University Hospital of Liège and in GIGA-R is operational since 2002 and constitutes the most active cellular therapy unit in the Walloon region and in French-speaking Belgium.

LTCG activities are focused on:
- Processing of cellular products,
- Clinical studies
- Research and Development on new techniques applied to cellular and gene therapy

Four issues are addressed:
- Hematopoietic stem cells (HSC)
- Umbilical Cord Blood Bank (CBB)
- Mesenchymal stem cells (MSCs)
- Cellular immunotherapy

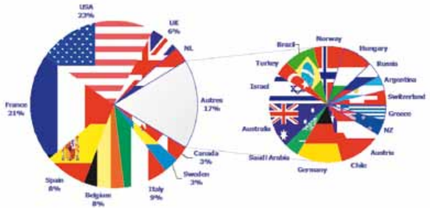
The LTCG facilitates partnerships with the pharmaceutical industry within clinical research protocols and has strong collaborations with companies such as Cardio3BS or Bone Therapeutics.

Hematopoietic stem cells (HSC)

The LTCG ensures the collection, processing, cryopreservation, and storage of autologous or allogeneic HSCs derived from the bone marrow, peripheral blood or cord blood. These cells are used for HSC transplantation («bone marrow transplantations») for the treatment of patients suffering from leukaemia or other severe blood disorders in Belgium or abroad. It also performs positive cell selection (i.e. selection of cells bearing the CD34 or AC133 antigens) or selective depletion of some cell populations with monoclonal antibodies and immunomagnetic methods (i.e. depletion of CD8+ lymphocytes).

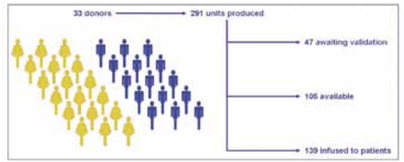
Umbilical Cord Blood Bank (CBB)

Constituted mainly with the support of the Televie, the National Fund for Scientific research (FNRS), and the Belgian Foundation against Cancer (FBC), the bank operates the recruitment, collection, processing, and storage of allogeneic cord blood units collected by a 5-maternity accredited network within the Liège Province. It has built up an inventory of more than 2500 validated units, allowing selection and distribution of CB HSC units to transplant centers in Belgium but mainly abroad (more than 140 transplants distributed to more than 20 countries).



Mesenchymal stem cells (MSCs)

MSCs are mostly present in the bone marrow where they differentiate into bone, cartilage, and fat. One MSC subset is said to have the ability to differentiate into other cells, such as nerve, muscle, or liver cells. MSCs also have impressing immunosuppressive properties. They have low immunogenic potential, which allows considering transplantation without strict HLA match. The LTCG is building an allogeneic MSC bank from healthy volunteer bone marrow donors, putting cells into culture for 4 weeks in the laboratory, and finally cryopreserving them. Over 290 MSC products from 35 healthy volunteer donors have been prepared, and 139 infused to patients.
These cells are used within clinical research protocols for the prevention of graft rejection or of Graft-Versus-Host disease (GVHD) in the HSC transplantation program, and for the treatment of GVHD and poor HSC graft function. Immune suppression protocols with MSC are in preparation for the treatment of autoimmune diseases and organ transplantation.



The LTCG also prepares autologous pre-osteoblasts from cultures of bone marrow-derived MSCs, according to a process licensed by the Bone Therapeutics Company. These cells are used in Rheumatology and orthopaedic surgery for the regenerative therapy of bone disorders. 50 patients have been treated with this method between 2006 and 2010.

Cellular immunotherapy

Cellular immunotherapy consists in using cells from the immune system (lymphocytes and dendritic cells) as therapeutic weapons, notably against cancer or infectious diseases. Such cells can be autologous or allogeneic, from related or unrelated donors.
The LTCG is involved in several clinical research protocols using immunotherapy with lymphocytes in the allogeneic HSC transplantation setting. Moreover, activity in the field of processing dendritic cells from culture of blood-derived monocytes allows the development of anti-cancer or anti-viral vaccination protocols.

All production activities performed in the LTCG are controlled by a performing quality management system. (GMP and GTP). Clinical research protocols are performed according to GCP (Good Clinical Practice) and cellular products in use in those protocols are approved by the Federal Agency for Drugs and Health Products (AFMPS). The LTCG regularly does internal and external audits and has received certification from the Belgian Ministry of Health as a tissue bank for its activities regarding HSCs, Cord Blood, and other cells (MSCs, Dendritic cells).
It has also obtained a license from the FDA to export cellular products to the USA. In 2005, it has been the 8th bank in the world to obtain international accreditation for the Cord Blood Bank from the American institution FACT (Foundation for the Accreditation of Cellular Therapy) and from the Netcord international network of cord Blood Banks. So far it has been the only bank in Belgium to have been granted that accreditation.

SUCH CLINICAL ACTIVITIES RESULT FROM INTENSIVE RESEARCH PROGRAMS PERFORMED AROUND THE WORLD INCLUDING IN LIÈGE AT GIGA-R.

The Laboratory of Hematology (GIGA-R) is dedicated to fundamental and applied research in hematology and cellular therapy. The laboratory currently includes 3 physicians with Professorship (Yves Beguin, André Gothot and Frédéric Baron), 3 post-doc researchers, 8 PhD students and 3 lab technicians. The pursued themes of research are as follows:
- Marrow homing of HSCs produced from *ex-vivo* expansion
- Characterization of hemato-supportive properties of MSCs
- Roles of Erythropoietin and of Iron in Erythropoiesis in Cancer patients
- Preparation of cellular vectors with anti-angiogenic effects
- Optimisation of HSC grafts after non-myeloablative conditioning
- Anti-tumor and anti-viral vaccination with dendritic cells
- Role of Treg cells in experimental transplantation
- Study of the immunomodulatory properties of MSCs: application to prevention and treatment of GVHD
- Study of immune reconstitution after HSC transplantation
- Cellular therapy of myocardial ischemia with bone marrow stem cells mobilised from blood
- Cellular therapy of liver metabolic diseases with cord blood stem cells

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CIM: A NEW CENTER OF MEDICAL INNOVATIONS

The University Hospital of Liège, Meusinvest and the Walloon Region have created a start-up named CIM (Center of Medical Innovations) to speed-up and secure new activities in the new fields of medicine such as translational research and cellular therapy.

CIM will propose its services to pharmaceutical or biotech companies, to research groups and to clinicians. It will participate to the detection of the biomedical projects with high industrial potentials. It will help coordinate scientific research programs, ensuring a scientific expertise and biological resources management. Thematic such as Neurology, inflammatory diseases, oncology that are very active in Liège through research or clinical activities will be targeted by CIM.

This start-up that will begin its activity in 2011, will be located within the GIGA (B34, 5th floor) to ensure efficient interactions between all the partners involved. A new facility will be built to offer offices and clean rooms to the CIM as well as to private companies which need to start phase 1-2 trials in patients with innovative cellular products.

GIGA Key numbers 2010

Members	**546**
PhD	216
PhD Students	193
Technicians	98
Administration	27
Logisticians	12
Foreign Researchers	95
Nationalities	35
FNRS positions	**90**
(Belgian Fund for Scientific Research)	
Research Fellows	22
Postdoctoral Researchers	19
Research Associates	27
Senior Research Associates	9
Research Directors	6
Scientific Research Workers	4
Logisticians	3
FRIA Fellowships	**37**
Televie Fellowships	**38**

Publications	**181**
IF < 3	61
IF between 3 and 5	68
IF between 5 and 10	41
IF > 10	11
PhD Thesis	**27**
Seminars	**37**
ULg speakers	7
Belgian speakers (non-ULg)	8
International speakers (Europe)	15
International speakers (outside UE)	7
Patents	
Patent applications filed	16
Patent applications published	17

Technology Platforms

GIGA-Platforms

More than ever, to stay competitive, the scientists need to have access to up-to-date technologies that evolve rapidly and require specific equipments and expertise.

Resulting from the pooling of technological and human resources, GIGA-Technology platforms meet this need and contribute to speeding up the biomedical research. Each technology platform is managed by experts who are fully dedicated to the platform and can give advice and/or perform the experiments in close collaboration with the scientist.

All platforms are open to academic researchers as well as to the private sector. Since their creation, the technology platforms have regularly diversified their range of services to answer the researchers needs.

A Business Developer and a Quality Manager have been hired to facilitate the relationship with the researchers and to set up a Quality Policy which is required, particularly by the private sector. Traceability, documentation, procedures, equipment monitoring are progressively implemented to assure the ISO 90001 or GLP-like compliance in a near future, depending on the customers request. In the meantime, some platforms have already been positively evaluated by external customers.

Technology platforms at the researchers service...that's not only our goal but also our reality!

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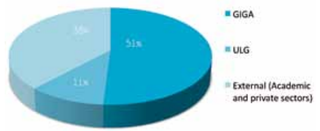


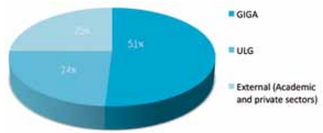
GIGA-Genotranscriptomics

The diversity of the services offered by the GenoTranscripomics platform and the new developments offered by the high throughput sequencing technologies require a solid expertise. Five persons with an important background of Genomics and Transcripomics make this platform a very efficient tool, not only for basic experiments such as 3730 sequencing or genotyping but also for the development of new applications supported by the ILLUMINA iScan, the ROCHE GS FLX 454 and the ILLUMINA Genome Analyzer IIx.

Facts

23 501 Sanger sequencing reactions and runs
42 High Throughput Sequencing runs (454 GS FLX or Illumina GAIIx)
1182 Expression profilings (Affymetrix or Illumina iScan)
3278 Human genotyping microarrays (Affymetrix or Illumina)
6831 Non-human genotyping microarrays (Affymetrix or Illumina iScan)
2078 DNA or RNA Quality controls

This Platform is extensively used by both academic researchers and private companies. In 2010, its sequencing activities have been positively evaluated twice by external QM services.



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USERS FEEDBACK

Quality, time-lines and flexibility…

«Eppendorf Array Technologies (EAT) uses the sequencing service offered by the GIGA since May 2010. From this time, more than 600 mini-preps and sequencing reactions were performed by the GIGA for us with orders of more than 100 sequencing reactions per run. The delivery of the results was always respected. The use of sequencing process controls for each run and the delivery of the results of these controls in the final report is one point which was taken into high consideration and that gives an added value to the service offered by the GIGA. Less than 1 % of the sequences could not be analyzed and this probably due to the sequence.
As ISO 9001 and ISO 13485 certified company, EAT is obliged to strictly follow all its suppliers and subcontractors. We had the opportunity to visit the sequencing service twice since May 2010. During those audits, we noted the real goodwill of all team members to implement quality principles.
Within 6 months, traceability, documentation and equipment monitoring have been clearly improved. All those elements lead us to conclude that the service offered by the GIGA meets our requirements in terms of quality, time-line.»
Sandrine Hamels, EAT, R&D Project Manager,
Muriel Art, EAT, Quality Manager

High throughput sequencing: a tool for the identification of single nucleotide polymorphisms

«The genotranscriptomic platform sequenced the complete genomes of several strains of Brucella abortus, our favorite bacterium, using 454 technology (Roche). Thanks to these data, and particularly their help in providing the softwares necessary to analyze the genomic sequences, we have been able to quickly identify the suppressive mutations allowing a thermosensitive strain to grow at non-permissive temperature. The whole sequence analysis was performed in less than 3 hours, and allowed identification of 2 single nucleotide polymorphisms.»
Xavier Debolle, FUNDP, Namur

High throughput sequencing as an efficient approach to identify mutations in the zebrafish genome: Proof of principle

«Zebrafish provide a valuable resource to study vertebrate gene function notably through the screening of mutants obtained after chemical mutagenesis. However, the traditional positional cloning techniques (SSLP mapping) for identifying the mutated genes are arduous, time consuming and expensive. Our group, in collaboration with the Animal Genomics laboratory and the Genotranscriptomics platform, developed a new strategy to bypass the laborious classical SSLP mapping and they succeeded to identify the causal mutation responsible for the loss of pancreatic exocrine tissue observed in a zebrafish mutant line. High-throughput sequencing of the whole genome of a pool of mutated zebrafish embryos was performed and allowed to define the region containing the mutation to an interval of about 9 Mb on the zebrafish chromosome 5 through the loss of polymorphisms found in this region. Furthermore, careful examination of the coding sequences of all genes in this interval highlighted a non-sense mutation in the SNAPC4 coding sequence. SNAPC4 knock-down experiments further confirmed that the SNAPC4 mutation is the causal event. This result constitutes a proof-of-principle for using whole-genome sequencing as efficient approach to identify mutations in the zebrafish genome.»
Marianne VOZ, LBMCG, GIGA-R

EXAMPLES OF PUBLICATIONS INCLUDING RESULTS OBTAINED PARTLY THANKS TO THE GIGA-GENOTRANSCRIPTOMICS PLATFORM

Takeda H, Charlier C, Farnir F, Georges M.
Demonstrating polymorphic miRNA-mediated gene regulation in vivo: application to the g+6223G->A mutation of Texel sheep.
RNA. 2010 16(9):1854-63.

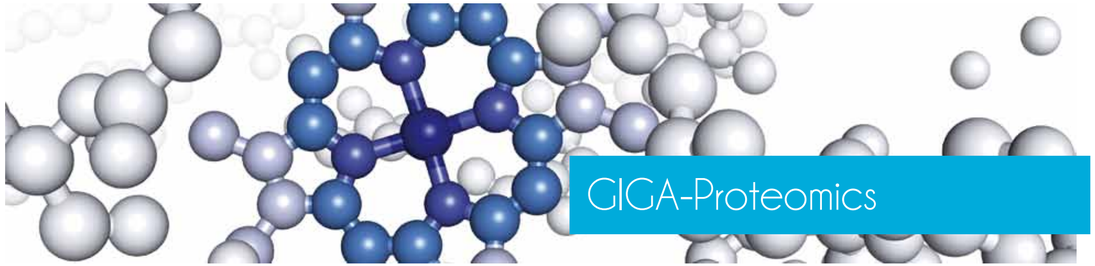
Keutgens A, Zhang X, Close P, Robert I, Hennuy B, Aussems M, Vanderplasschen A, Chapelle JP, Viatour P, Merville MP, Bex F, Gothot A, Fillet M, Chariot A.
The repressing function of the oncoprotein BCL-3 requires CtBP while its polyubiquitination and degradation involve the E3 ligase TBLR1.
Mol Cell Biol. 2010 ;30(16):4006-21.

Caiment F, Charlier C, Hadfield T, Cockett N, Georges M, Baurain D.
Assessing the effect of the clpg mutation on the microrna catalog of skeletal muscle using high-throughput sequencing.
Genome Res. 2010;20:1651-1662

NEWSLETTERS 2010

Focus on the GenoTranscriptomics services

- *De Novo* sequencing and resequencing via GS FLX Titanium kit, on 454-Roche (January 2010)
- Human methylation profiling with Illumina BeadArray technology (April 2010)
- For which projects use Roche 454 ? (July 2010)
- Evolution of the GenoTranscriptomics platform at GIGA (October 2010)

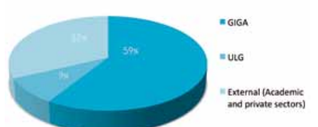


GIGA-Proteomics

The Proteomics platform is highly used by external users, reflecting the excellent visibility of the team. While a high number of routine analyses were performed both for academic researchers and for the private sector, new techniques requiring a high expertise such as posttranslational modification characterization and molecular imaging which have been developed and are currently being optimized.

Facts

8589	MALDI-TOF protein identification
1672	LC-MSMS protein digest identification
269	Mass determinations by FT-MS or ESI-Q-TOF-MS
149	Quantifications of proteins in complex mix
63	MALDI Mass spectrometry pr manual sequencing
30	Multidigestion for maximum recovery of pure protein
50	SELDI profiling
17	Label free quantitative differential proteomics (nanoUPLC-Synapt HDMS)



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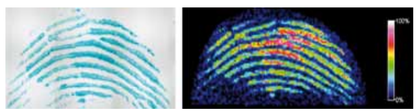
The new «SolariX FTMS Based MALDI System»

Developers: Delphine Debois & Tyler Zymmerman

In 2010, the platform acquired the new SolariX FTMS Based MALDI System financed by the EU funds and the Walloon Region. This unique powerful platform addresses Molecular Imaging (spatial in situ distribution) of small molecules, like drugs, metabolites, small peptides or lipids, in slices of tissue samples.
This system and the laboratory provide:
- complete workflow know-how on sample slice preparation (with frozen specimen) using the ImagePrep(TM) station
- full automation and fast data acquisition at up to 1 pixel/sec with 1 kHz laser speed
- spatial resolution of 20 µm.
- ultra-high mass resolution, sensitivity small molecule MALDI-MS imaging, without a loss in sensitivity due to MS/MS for selectivity
- high mass accuracy (<1ppm) for assigning unambiguous elemental formula on detected peaks for their identification
- sensitivity necessary to detect drugs and metabolites at therapeutic dosages
- highly effective treatment workflow including data acquisition, image processing with FlexImaging(TM) software and small molecules statistical class imaging utilizing ClinProTools(TM).

This system shows high opportunity for clinicians and researchers interested in *in situ* localization of their specific targeted small molecules in tissue samples.



Fingerprint picture (A) and Molecular Imaging (solariX FTMS Based MALDI System) of a specific ion (m/z 666.4774) on this fingerprint (B). The color represents the relative abundance of that ion.

USERS FEEDBACK

Analysis of a Radioactive labeled biomolecule

«In the framework of a research project held at the Nuclear Energy Study Center and aiming to create a new treatment for cancers, our team contacted the GIGA Proteomics platform. With their help and their expertise, our project found rapid and spectacular progress. Indeed, the project aims to fix a radioactive element to a specific biomolecule of a type of cancerous tumor. The contribution of the team, under the scientific direction of E.De Pauw,proved vital both for protein analysis and their purification and for the scientific research.»
Dr. Nathalie Impens, Project Leader, Radiobiology Unit, SCK•CEN

«I am a senior scientist working for a young start-up company called NOKAD. We are located on the Genopole's incubator established in Evry, in the south of Paris. NOKAD has developped innovative technology to generate new in vivo target validation tools for pharmaceutical industry and research. In parallel to this activity, we conduct our own research programs in the haematology field to identify new biomarkers capable of easing diagnostic and to adapt customised treatment to patients suffering of haematological disorders. We have used the proteomic plateform of the Giga for two of our research programs, one to characterize by mass spectrometry a purified compound used for our immunisation technology, and a second time to try to identify a protein band extracted from a SDS page gel. In both cases, we have been highly satisfied by this collaboration with the proteomic team. We appreciated the technical skills and professionalism of the scientist we interacted with. For both projects we elaborated technical solutions with the team of proteomic to define the best way to analyze our samples. I would recommend this platform and NOKAD will continue to collaborate with it for our future projects.»
Olivier Vidalin, NOKAD-Pharma, Evry, France

EXAMPLES OF PUBLICATIONS INCLUDING RESULTS OBTAINED PARTLY THANKS TO THE GIGA-PROTEOMICS PLATFORM

Debois D, Bertrand V, Quinton L, De Pauw-Gillet MC and De Pauw E.
MALDI-In Source Decay applied to Mass spectrometry imaging: a new tool for protein identification.
Anal. Chem., 2010, 82: 3969-4304

Smargiasso N, De Pauw E.
Optimization of matrix conditions for the control of MALDI in-source decay of permethylated glycans.
Anal Chem. 2010, 82(22):9248-53.

Turtoi A, Mazzucchelli G and De Pauw E.
Isotope coded protein label quantification of serum proteins--comparison with the label-free LC-MS and validation using the MRM approach.
Talanta (2010), 80(4), 1487-95

Kischel P, Waltregny D, Dumont B, Turtoi A, Greffe Y, Kirsch S, De Pauw E and Castronovo V.
Versican overexpression in human breast cancer lesions: Known and new isoforms for stromal tumor targeting.
International Journal of Cancer, 2010, 126(3), 640-50

NEWSLETTERS 2010

Focus on the Proteomics services

- Label free quantitative differential proteomics with nano Acquity UPLC, on line with Synapt HDMS G1 (January 2010)
- Automated high throughput pure protein identification by MALDI-TOF-MS and MS/MS (April 2010)
- 2D-DIGE strategy for quantitative differential proteomics (July 2010)
- Clinical differential proteomics by Surface Enhanced Laser Desorption Ionisation-Time of Flight-Mass Spectrometry (SELDI-TOF-MS) (October 2010)

GIGA-Imaging and Flow Cytometry

The GIGA-Imaging and flow cytometry platform includes 2 confocal microscopes both equipped with time-lapse devices, an all-in-one epifluorescence microscope, 4 flow cytometers and a laser microdissector. The platform has also acquired the licence of Imaris, for 3D and 4D real-time data visualization, analysis, segmentation and interpretation of microscopy datasets.

After a specific training performed by the logistician, the researchers can use most of these equipments on their own. Thanks to the high availability of both the logistician and the technician, this platform has regularly increased its activity since its creation in 2008.

Facts

1100 Confocal analyses (on Leica SP2 and/or Olympus FV1000) including time lapse
150 More than 150 users
1600 Flow cytometry analyses (CBA, cell viability, calcic flow, phenotyping,...)
145 Cell sortings (Facs Vantage or FacsAriaII)
26 Laser microdissections

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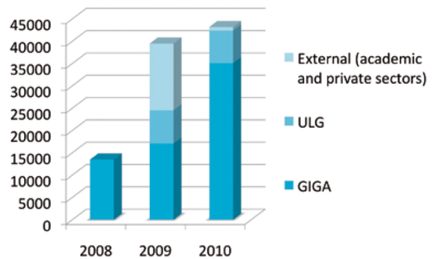

To meet the needs of the scientists and to increase the accessibility to imaging tools and services, 2 confocal microscopes will be purchased in 2011.
The platform has also acquired a BD Pathway 855 system that offers a high flexibility for high-content imaging of live and fixed cells, with the possibility to select among 16 different excitation filters suitable for a broad range of applications. Thanks to an environmental control and a liquid handling, the BD pathway will allow a wide range of fluorescence-based kinetic and endpoint biological assays.
In june 2011, all these equipments will move to a new infrastructure (GIGA B34, 4th floor) that will facilitate the access to the imaging and cytometry technologies as well as to image analysis.

USERS FEEDBACK

Stimulating discussions lead to new ideas…

«One of the researches of the laboratory of Bioenergetics (F. Franck, Ulg) is focusing on the production of the valuable antioxidant astaxanthin by the green micro-algae Haematococcus pluvialis. By optimising a growing medium for this organism, we found that an unusually high phosphate supply was beneficial to cell productivity and vegetative stage persistence. The running hypothesis to explain this response to phosphate is related to the capacity of H. pluvialis to accumulate phosphate as intracellular polyphosphate granules. Since these granules can be observed by fluorescence microscopy after staining with DAPI, we first intended to study the relationship between phosphate supply and polyphosphate occurrence by confocal microscopy. The GIGA Imaging platform was definitely the good place to start this research: as newbies in imaging technologies, we received there all the technical assistance needed to address our specific question. Moreover, from stimulating discussions with the platform team, new ideas emerged and we now plan to study polyphosphate metabolism in H. pluvialis by a mutagenesis approach and a screening by flow cytometry.»
Pierre Tocquin, PhD, Laboratory of Bioenergetics, ULg

The high skill of the team has been very helpful

«The aim of our project is to study the immune reconstitution after allogeneic hematopoietic stem cell transplantation (HSCT). In that context, we need to detect and quantify the CD8+ T cell population specific for various pathogen such as CMV, EBV, FLU, … and to evaluate if they come from the donor or from the recipient. Using the BDBioSciences FACSARIA II cytometer from the GIGA Imaging and flow cytometry platform, we were able to sort 4 distinct cell populations.
However, the high skills of the logisticians, who know their equipment perfectly, have been very usefull to sort with a high purity cells whose frequency was sometimes very low.».
Catherine Menten, PhD, Hematology unit, GIGA-R

EXAMPLES OF PUBLICATIONS INCLUDING RESULTS OBTAINED PARTLY THANKS TO THE GIGA IMAGING AND FLOW CYTOMETRY PLATFORM

Dubail J, Kesteloot F, Deroanne C, Motte P, Lambert V, Rakic JM, Lapière C, Nusgens B, Colige A.
ADAMTS-2 functions as anti-angiogenic and anti-tumoral molecule independently of its catalytic activity.
Cell Mol Life Sci 2010, 4213-4232

Guelluy PH, Fontaine-Aupart MP, Grammenos A, L'ecart S, Piette J and Maryse Hoebeke.
Optimizing photodynamic therapy by liposomal formulation of the photosensitizer pyropheophorbide-a methyl ester: In vitro and ex vivo comparative biophysical investigations in a colon carcinoma cell line.
Photochem & Photobiol Sci 2010, 1252-1260

Soyer J, Flasse L, Raffelsberger W, Beucher A, Orvain C, Peers B, Ravassard P, Vermot J, Voz ML, Mellitzer G and Gradwohl G.
Rax6 is an Ngn3-dependent winged helix transcription factor required for pancreatic islet cell development.
Development 2010, 137, 203-212.

NEWSLETTERS 2010

Focus on the Imaging and Flow Cytometry services

- Presentation of the new FACS Aria II cell Sorter (January 2010)
- Bringing phase contrast, bright field and fluorescence imaging to everyone with world's first all-in-one epifluorescence microscope (April 2010)
- How to manage and analyze your data in the GIGA Cell imaging and flow cytometry ? (July 2010)
- IMARIS: explore the third dimension (October 2010)

GIGA-Bioinformatics

The GIGA-Bioinformatics platform works in close collaboration with other platforms and with researchers of the Bioinformatics and Modeling research unit to offer data analysis and software development services. It has a broad range of activities to help life science researchers explore and analyze various types of data including transcriptomics data, microRNAs, DNA methylation, protein and genomic sequences, and microscopy images, related to different pathologies or biological processes.

In 2010, the platform significantly increased the computing capacity of its hardware infrastructure which is the foundation of provided services. Its computing capacity reached a total of 400 computing cores and a total of 2Tb of fast memory. Its storage capacity reached 40Tb of disk storage and it will be further expanded in 2011 and 2012 to the Petabyte scale. Thanks to the Alma In Silico (InterReg) project and Cytomine project (DGO6), new softwares are being developed and installed on this infrastructure, and the platform is benefiting from these developments to increase and improve its services.

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More precisely, during 2010, the platform performed various tasks in collaboration with the Bioinformatics and Modeling research unit, including, among others:

- Joint analysis of miRNA and mRNA expression data, ie. identification of differentially expressed mRNAs and miRNAs, prioritization of miRNA-mRNA interactions, visualization of the interaction networks around mRNAs and miRNAs, ... for cancer and asthma studies,

- SELDI-TOF mass spectra data analysis ie. identification of potential biomarkers for various diseases and processes including fibromyalgia, pseudopolyarthritis, colon cancer,..

- Microscopy image analysis ie. automatic cell counting in Boyden chemotaxis assays and in soft agar assays for studies on angiogenesis, cell sorting in digitized cytology slides for anatomopathological screening, tumor quantification in digitized histology slides for drug discovery, high-throughput detection of protein crystals in crystallization droplets for structural genomics, zebrafish measurements and phenotype recognition for toxicological studies,...

USERS FEEDBACK

«Since 2007, the GIGA Bioinformatics team carried out several biostatistical studies for our SELDI-TOF mass spectrometry data related to the high-density lipoprotein (HDL) proteome. Differents sets of patients challenged by toxins (e.g. Lipopolysaccharides) or with a specific disease (e.g. familial hyper cholesterolemia) were analyzed using statistical methods. The bioinformatic analyses allowed us to confirm our hypothesis, identify novel markers, and better understand the dynamics of proteins associated with HDL.»
PhD J.H.M. Levels, AMC (Academic Medical Center, Department of Experimental Vascular Medicine, Amsterdam).

EXAMPLES OF PUBLICATIONS INCLUDING RESULTS OBTAINED PARTLY THANKS TO THE GIGA BIOINFORMATICS PLATFORM

Schoemans R, Aigrot MS, Wu C, Marée R, Hong P, Belachew S, Josse C, Lubetzki C and Bours V.
Oligodendrocyte development and myelinogenesis are not impaired by high concentrations of phenylalanine or its metabolites,
J Inherit Metab Dis. 2010, 33(2):113-20

Sabatel C., Malvaux L, Bovy N, Deroanne C, Lambert V, Alvarez Gonzalez ML, Colige A, Rakic JM, Noël A, Martial JA and Struman I.
MicroRNA-21 Exhibits Antiangiogenic Function by Targeting RhoB Expression in Endothelial Cells
PLoS One. 2010, 10, 2011.

Sabatel C., Cornet AM, Tabruyn SP, Malvaux L, Castermans K, Martial JA and Struman I.
Sprouty1, a new target of the angiostatic agent 16K prolactin, negatively regulates angiogenesis.
Molecular Cancer 2010, 9:231.

NEWSLETTERS 2010

Focus on the Bioinformatics services

- Bioconductor for gene expression profiling analysis (January 2010)
- Grid computing made easy (April 2010)
- Automated large-scale bioimage analysis (July 2010)
- Joint analysis of miRNA and mRNA expression data (October 2010)

GIGA-Mice Facility and Transgenesis

More and more, the results obtained *in vitro* have to be validated *in vivo*. Access to a high quality animal facility is thus critical for the biomedical researchers. The GIGA mice facility can house mice in a conventional zone including biosafety level 2 and 3 labs as well as a zone dedicated to experimental neurosciences, large surgical rooms. This facility is equipped with a Xenogen capable of imaging bioluminescense and fluorescence in living animals. A Specific-Pathogen-Free (SPF) zone can house mice whose health is monitored every three months according to the Federation of Laboratory Animal Science Associations (FELASA) recommendations.

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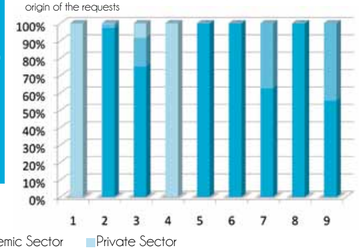
FACTS AND FIGURES

977	Mouse Embryo Assays (1)
178	Colonies housed (2)
12	Translational research programs (3)
1	Bottle-feeding program (4)
1	Pronucleus injection (5)
2	mES cells injections (6)
16	Lines Cryopreservation (7)
5	Lines Revitalisation (8)
9	Lines Sanitisation (9)

Repartition of the services proposed by the platform according to the origin of the requests



Various pronucleus or mES cells injections have been performed and related transgenesis programs are ongoing. Resulting from a collaborative research program (FP7), the first transgenic mice, deficient for the ADAMTS-12 protease have been produced in 2010.

In order to limit the housing of the numerous transgenic mice that have been generated for a particular purpose, the platform has optimized the embryo cryopreservation. This service will assure that the strains that are no more necessary due to research evolution would remain available for further use.

Sixteen lines have been cryopreserved in 2010, making these embryos available for future research programs or future collaborations. The facility has also performed 980 MEA (Mouse Embryo Assays) for quality control of culture media used for human *in vitro* fertilization. Customers for this specific application have audited the platform and the GIGA Quality Manager is currently implementing the procedures to assure the GLP-like compliance.

USERS FEEDBACK

An international collaboration for generating a Adamts-12 deficient mouse

«In the context of the FP7 MicroEnviMet program, a european consortium coordinated by A. Noel (GIGA-Cancer) aims at interfering with the protease network that controls the properties of cancer cells, cancer stem cells and/or host cells. These extracellular or membrane-associated proteases emerged as key regulators of the elaboration of a specific microenvironment in primary or secondary tumors that are permissive or not for cancer growth and dissemination. Pr Lopez-Otin (University of Oviedo, Spain), a partner of the FP7 MicroEnviMet program, has initiated a study on ADAMTS-12, a soluble metalloprotease with enigmatic functions. In collaboration with this team, Adamts-12-deficient mice have been generated with the help of the GIGA-Transgenic platform. Mutant embryonic stem cell (ES) clones in which exons 6 and 7 of Adamts-12 gene were deleted have been generated in Spain through homologuous recombination and used in the GIGA platform for mutant mice generation. Homozygote mutant mice obtained in pure C57/Bl6 background led to a mice colony displaying an apparent normal phenotype. The different models of angiogenesis and cancer that have been applied to the mutant mice revealed an acceleration of tumoral angiogenesis in the absence of Adamts-12 gene expression, suggesting an in vivo protective effect of ADAMTS-12 towards cancer progression and emphasizing the complex functions of proteases in cancer.»
A. Noel, MicroEnviMet Coordinator

EXAMPLES OF PUBLICATIONS INCLUDING RESULTS OBTAINED PARTLY THANKS TO THE GIGA MICE FACILITY AND TRANSGENESIS PLATFORM

El Hour M, Moncada-Pazos A, Blacher S, Masset A, Cal S, Berndt S, Detilleux J, Host L, Jobaya A, Maillard C, Foidart JM, Ectors F, Noël A and Lopez-Otin C.
Higher sensitivity of Adamts12-deficient mice to tumor growth and angiogenesis.
Oncogene. 2010, 20;29(20):3025-32

Lambert F, Léonard A, Drion P, Sourice S, Layrolle P, and Rompen E.
Influence of space-filling materials in subantral bone augmentation: blood clot vs. Autogenous bone chips vs. bovine hydroxyapatite.
Clinical Oral Implants Research. 2010 [Epub ahead of print].

Warzée B, Mesnil C, Hober D, Caloone D, Pirottin D, Garigliany M-M, Desmecht D, Drion P, Journot L, Lekeux P, Desmet C, and Bureau F.
Viral induction of Zac1b through TLR3- and IRF3-dependent pathways.
Mol. Immunol., 2010, 48(1-3):119-27.

NEWSLETTERS 2010

Focus on the Mice facility and transgenesis services

- Cryopreservation of embryos : a way to reduce the number of housed animals and the genetic drift (January 2010)
- KI/KO mice : the gene you want to target is probably already trapped ! (July 2010)
- A new approach for the production of KO/KI mice (October 2010)

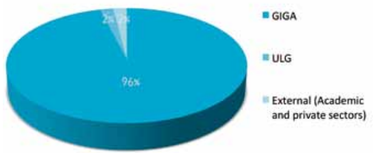
GIGA-Zebrafish Facility and Transgenesis

Zebrafish (*Danio rerio*) are small freshwater tropical fish whose biological characteristics make them an appropriate model for various uses in biology.

Their genome has been almost completely sequenced revealing that zebrafish contain homologues for more than 80% of human genes. In addition, most cell signalling pathways are identical in both species.

Facts

630 Strains housed
2700 fixed embryos provided
365 000 live embryos provided
220 adult zebrafish provided
8 new transgenic or mutant strains/ morpholino injection



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USERS FEEDBACK

Zebrafish as a good tool for ecophysiology lab classes

«Two years ago, I came to the Giga platform and its scientific team as an assistant in Biological Sciences, to organize laboratory sessions dedicated to Master I students in Biological Sciences to illustrate the 'Ecophysiology, ethology' course. The zebrafish biological model was chosen as particularly appropriate to illustrate the morphological and physiological changes in response to acute exposure to various chemicals. In addition the effect of an external stressor – in the case the temperature – on the early development of a genetically modified Zebrafish line was tested. From feed-back session organized right after the laboratory sessions, it appeared that the students particularly appreciated having access to this novel biological material and the surrounding facilities developed at the platform as well as exchanging knowledge with experienced researchers.»
Virginie Debacker, Assistant in Biology, ULg

Zebrafish as a model for angiogenesis

«We have been working with the Giga zebrafish platform for several years. Under the supervision of Marie Winandy we have been able to learn basic zebrafish handling techniques and microinjections of different morpholinos into one-cell stage embryos of various transgenic strains (kdr:EGFP and fli:EGFP). The goal of our project was to identify novel regulators of physiological angiogenesis. During several visits which have been organized in a very professional manner, we have been able to show that one of our candidate genes is required for branching of the intersomitic vessels. In situ hybridization expression studies have been performed in collaboration with the confocal microscopy facility.»
Martin Hagedorn, U920, INSERM, Bordeaux, France

Quality of the services and availabe expertise

«In 2010 the unit of Clinical Genomics at Maastricht UMC+ has worked extensively with the scientists and service providers of GIGA within the context of the Euregional Alma In Silico project. Together with the Zebrafish facility a model for genetic mitochondrial disease is being constructed. The Illumina Genome Analyzer of the Genotranscriptomics platform is used for exome sequencing of patients from autosomal recessive families and, finally, collaborations in various aspects of Bioinformatics were established. We were highly impressed by the quality of the services, the expertise available at GIGA and the friendly and positive attitude of the people working there. It is a pleasure to work together in such fruitful and productive collaborations. It is the aim of the Alma In Silico project to establish partnerships within systems biology and bioinformatics across the borders in the Euregio and we have clearly shown to skeptical Dutchmen that Liège is closer to Maastricht than to the Mediterranean and that there is a world to gain in excellent life sciences research by working together.»
Bert Smeets, Maastricht University, The Netherlands

EXAMPLES OF PUBLICATIONS INCLUDING RESULTS OBTAINED PARTLY THANKS TO THE GIGA ZEBRAFISH AND TRANSGENESIS PLATFORM

Verbruggen V, Ek O, Georlette D, Delporte F, Von Berg V, Detry N, Biemar F, Coutinho P, Martial JA, Voz ML, Manfroid I and Peers B.
The Pax6b homeodomain is dispensable for pancreatic endocrine cell differentiation in zebrafish.
J Biol Chem. 2010, 30;285(18):13863-73.

Soyer J, Flasse L, Raffelsberger W, Beucher A, Orvain C, Peers B, Ravassard P, Vermot J, Voz ML, Mellitzer G and Gradwohl G.
Rfx6 is an Ngn3-dependent winged helix transcription factor required for pancreatic islet cell development.
Development. 2010;137(2):203-12.

NEWSLETTERS 2010

Focus on the Zebrafish facility and transgenesis services

- Zebrafish motility and activity analysis with Zebrabox© (April 2010)

The Biobank

Biomedical, medical and pharmaceutical research can't be undertaken without access to biological materials of human origin. Because of the evolution of medicine, the needs of human body material in these areas continue to grow. It is thus important to provide mechanisms to collect, store samples of human body material and make these samples available to researchers in appropriate ethical, technical and logistical conditions. The «Biothèque Universitaire de Liège» results from a partnership between the University Hospital of Liège and the University of Liège (GIGA) and makes a link between clinicians and researchers.

The Biobank is a collection of human cells and tissues. It can also collect biological liquids (sera, blood,…) or derivatives such as DNA, RNA or proteins. All these samples coming from residual material after clinical surgery can be either frozen or embedded in paraffin and are documented with clinical data in the strict respect of the legislation and the ethics committee of the University Hospital. So far, the biobank is composed of tumoral and neuronal tissues, although these thematics will evolve in the near future.

CHU de Liège — GIGA

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Communication and networking

The technology platforms are accessible to academic researchers as well as SMEs or companies. As the technology evolves rapidly, it is important to keep the researchers informed. A Newsletter sent by email to anyone interested by the technological platforms activity is published 4 times a year, with an emphasis on one service/equipment per technology platform.

GIGA technology platforms participate actively in Biowin projects and have been represented at different events:

- AWEX mission in Bilbao, Spain (March 2010)
- Biomedica, Aachen, Germany (March 2010)
- CIEL, Centre d'Innovation Entreprise de Lille, France (May 2010)
- Business meets Research, LuxInnovation (May 2010)
- WBI, Wallonie-Bruxelles International-AWEX mission in Stockholm, Uppsala, Linköping, Sweden (May 2010)
- Fish Day, Liège, Belgium (June 2010)
- Rencontres Euro-Régionales de la biotechnologie et de la Santé, Montpellier, France (July 2010)
- Luxemburg Clinical Proteomics Center, Luxemburg (September 2010)
- Meet & Match (Wallon clusters), Charleroi, Belgium (October 2010)
- Rencontres de l'Innovation, Liège, Belgium (October 2010)
- Science and Technology Parks: a driving force for the knowledge-based economy in Europe, Brussels, Belgium (November 2010)
- Master class Skills 3 (Interreg program), Liège, Belgium (November 2010)
- B4Bio Connexions: Medical Diagnostics, Montpellier, France (November 2010)
- Biowin Day, Louvain-La-Neuve, Belgium (December 2010)
- Bioproduction Symposium, Genopole, Evry, France (December 2010)
- Pharma Connexion , Gembloux, Belgium (December 2010)

They are also involved in transnational programs

FASILIS

Facility Sharing in Life Sciences (Interreg IV), a transnational pilot project, aims at creating a framework for durable cooperation between SMEs and facilities in Northwest-Europe (South East England, Øresund Region in Denmark and South Sweden, BioRegion STERN around Stuttgart, Bio-Liège in Wallonia, Health Valley and the LifetecZONe area in the Netherlands). An e-catalogue listing all the technology platforms available in the partners' regions has therefore been created. In two calls, more than 70 vouchers have been distributed in 2010 to SMEs to help them getting access to one of the partners' platform.

Among these generated collaborations, the GIGA-Genotranscriptomics platform has been solicited by a Dutch start-up in the frame of the FASILIS project. That company, active in the field of tissue engineering and cell culture needed to better characterize their cell lines. After some discussions and advice from the platform logisticians, an optimal solution was finally chosen among the panel of services the platform provides.

ABCEUROPE

ABCEurope is a European project aiming at reinforcing active and structured partnerships between EU bio clusters and their companies. This project gathers 13 partners all around Europe and tends to provide better quality and more efficient services to biotech companies. The key driver behind this program is the development and delivery of services in a fully integrated mechanism. Indeed, one of the work-package of ABCEurope is devoted to the elaboration of a portfolio (web portal) of state-of-the-art technology platforms, together with strict specification rules (standard contracts, clearly defined services and prices, quality management, …). GIGA-Technology platforms have been one of the models for setting up specifications.

ALMA IN SILICO

In 2009, the Bioinformatics unit has launched as coordinator the Alma-in-Silico Interreg IV project, in collaboration with the Universities of Aachen, Maastricht and Hasselt. This 7.5M€ project, aims at creating the platform for bioinformatics and systems biology in the Euregio Meuse-Rhine.

The project is organized around three main actions, namely (i) the establishment of a bioinformatics infrastructure based on open software using web-services, grid computing and mass data storage systems in connection with a bottom layer connected to technological platforms for sequencing, transcriptomics, imaging and proteomics, and a top layer allowing the management of workflows and services requested by end-users, (ii) the integration and expansion of the technological platforms of the four centers into a single systems biology oriented experiment center, and (iii) the establishment of scientific and technical collaborations among academy and industry in the Euregio Meuse-Rhine for running projects, specially in the fields of neurological, mitochondrial, cardiovascular and inflammatory diseases, as well as for the development of high-throughput pipelines for toxicology and pharmacology based on Zebrafish models.

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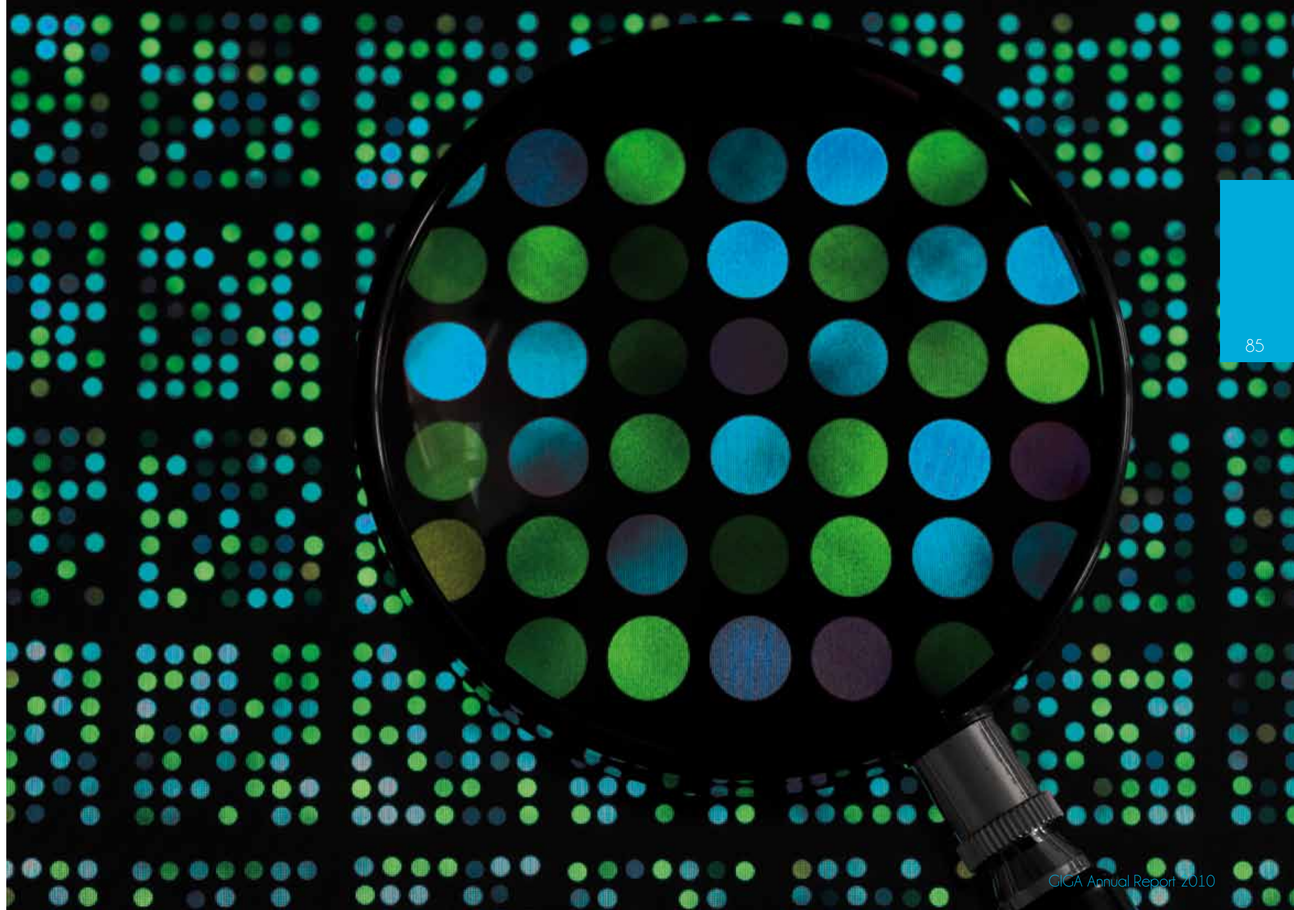
Team
Diane Zander, Alain Thiré, Alain Empain, Xavier Tordoir, Pierre-Yves Gilson, Florence Lemahieu, François Van Lishout, Yannick Schutz, Amandine Piot, Yobhana Quiroz O'Donova, Marc Muller, Louis Wehenkel
In collaboration with Jean-Paul Noben (Belgium), Bert Smeets (Netherlands), Kurt Hoffman (Germany)



Business

Business

«The CIGA-Business division represents a great growth potential and high quality location choice for both start-up businesses and more accomplished companies.

Located in the heart of the CIGA Research facilities, the CIGA-Business division provides privileged access to:
- *World-class Biotech Science and Research & Development,*
- *Top Level Equipment,*
- *and Highly Educated Workforce.*

This division also provides a perfect business environment dedicated to help companies develop and accelerate their projects.

It offers top facilities to enterprises, with equipped laboratories and offices for rent, but moreover a unique working environment, i.e.:
- *Immediate proximity with the R&D departments of the CIGA Research and with the University Hospital (CHU)*
- *Easy access to a vast array of technological platforms and outstanding equipments with the required experts to run them*
- *Stimulating interactions and exchanges – both formal and informal – with scientific and business partners who share the same location*
- *Business services provided by experienced teams, among others : virtual incubation, market studies and business plans*
- *Funding opportunities through close partnership with the MEUSINVEST Group.*

The dynamism of the companies integrated in the CIGA-Business departments together with their projects for further development, on the one hand, and the growing cooperation between academics, business and partners providing business services and financing, on the other hand, are key to success for the CIGA-Business Division.

Our business space in the CIGA Building has welcomed a new company this year and is currently full.

New premises in a brand new nearby building will be available at the end of the first semester of 2011.»

Freddy Meurs
Chief Executive Officer, Meusinvest Group

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WALLONIA BIOTECH COACHING

WBC is the biotechnology incubator of the Belgian Region of Wallonia. WBC aims to stimulate the creation and maturation of biotech spin-offs, start-ups or spin-outs based on research and development activities performed in academic institutions and industrial companies.

Its mission consists in giving a continuous support to entrepreneurs via a panel of financial, commercial and managerial services. In 2010, WBC spent 200.000€ on its incubees.

WBC has now 14 Master Partnership Agreements (MPA) with 14 projects (9 spin-offs, 3 start-ups, 1 spin-out and 1 subsidiary). Last year, we added 6 new projects; 3 incorporated companies and 3 spin-off/start-up projects. These projects develop products/services in different fields such as medical device (3), bio logistics (1), human therapy (1), diagnostics (1).

As we look at the figures for the first 6 incubees, we noticed a 28% increase of the number of employees (37 people) and a 22% increase of the turnover.

In addition to the support of its incubees, WBC is involved in many activities such as:
- Consulting mission for the BioLogEurope project;
- Development of a managerial software dedicated to entrepreneurs;
- Economic missions with Awex in the USA.

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MDx HEALTH

In 2010, MDxHealth made a number of organizational changes to implement its new strategy.
These changes involved centralizing all European lab activities in Belgium, changing the company's name from OncoMethylome Sciences to MDxHealth and renewing its management and board with a number of seasoned executives.

The company's new focus is predominantly the development of clinical diagnostic tests for prostate, lung and colon cancer. These products will be sold directly to physicians in the US using a dedicated sales force, and the tests will be performed in the company's own CLIA laboratory.

MDxHealth entered into 3 agreements with major pharmaceutical companies for the development of companion diagnostics:
- with GSK for its immunology oncology program,
- with MERCK SERONO for brain cancer,
- with PFIZER for PARP inhibition in breast and ovarian cancers

MDxHealth has continued to out-license its non-core assets, entering agreements with companies that have a dedicated focus on one cancer indication. MDxHealth out-licensed certain biomarkers to Exact Sciences for use in their test for colorectal cancer screening, and out-licensed other biomarkers to Predictive Biosciences for bladder cancer.

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DIAGENODE

Diagenode is a leading developer and marketer of innovative life-science tools and integrated systems for epigenetics, genomics and diagnostics.

Diagenode is the only company providing a complete solution for epigenetics research, including state-of-the-art products and technologies for DNA sonication, best-in-class antibodies (ChIP grade and ChIP-seq grade), and high-quality kits for chromatin immunoprecipitation (ChIP) and methylation studies, and now an automated epigenetics system, the IP-Star™.

Diagenode's customers include leading epigenetics researchers, academic institutions, high-profile genome centers and core labs, life sciences tools companies, molecular diagnostics players, and pharmaceutical and biotechnology companies.

Our products provide researchers with the best quality and performance and allow scientists to produce consistent, cost-effective and robust results in their research. We expect that our technologies will enable our customers to discover cutting-edge breakthroughs in the field of epigenetics, leading to a better understanding of health, disease and development.

In 2010 Diagenode launched several new products, including new equipment for DNA shearing optimized for next generation DNA sequencing, several new kits and antibodies for epigenetic research and new CE marked molecular diagnostic kits for gastric infections. The company signed several important marketing agreements including distribution agreement with Ion Torrent Inc Ca USA and Sonicbio Japan.

Thanks to a strong R&D effort supported by several new EU grants from the FP7 program, the company is building a pipeline of innovative products which should be able to support the growth of the following 2 to 3 years.

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ARTIALIS

Founded in 2010 by Yves Henrotin, Artialis is a spin-off company of the University of Liège specialized in the discovery and commercialization of new biochemical markers for the diagnostic, prognostic and follow-up of osteoarthritis and its related disorders.

Osteoarthritis is a progressive degenerative disease characterized by a gradual loss of cartilage, an inflammation of the synovial membrane, the deterioration of the synovial fluid lubricating joints and a knobbly bony deformity. Patients with osteoarthritis show severe mechanical pain that affects many aspects of their daily lives. Osteoarthritis affects nearly 40 million people in Europe. The diagnostic of osteoarthritis is based on a detailed patient clinical history, a complete physical examination and a standard radiography. Unfortunately, when radiographic signs are visible, the cartilage lesions are already irreversible. This is why it is necessary to diagnose the disease before the appearance of these radiological signs. The early diagnosis of osteoarthritis is the key to success for a good therapeutic treatment of the disease.

Artialis offers a range of innovative patented biochemical markers for the early detection of osteoarthritis. The most popular ones are short circulating peptides corresponding to degrading components of the cartilage generated by the destruction of the local collagen. As well as this, Artialis has also identified nitrated forms of these peptides that allow us to follow inflammatory process that occurs in diseased joints. The main advantage of these biomarkers is the fact that they can be directly and specifically measured in serum, synovial fluid and urine. These biomarkers meet the criteria of the NHI-recommended BIPED classification and are validated for the diagnosis, prognosis and evaluation of the efficacy of some drugs.

Set up in the University hospital of Liège, Artialis has its own Good Laboratory Practice certified Laboratory to offer a personalized approach to help human and veterinary pharmaceutical companies, along with the food industry, in their development of innovative drugs and food supplements. Artialis also commercializes its innovative biomarkers under the form of ELISA kits for helping researchers in their understanding of the pathology.

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ARLENDA

Arlenda is a company specialized in Applied «Modeling & Statistics» for informed and timely decision making in Life Sciences. Our consulting services cover the areas of Early Clinical Phases, Non-Clinical development and specialized software development.

Arlenda Services
In Early clinical Phases :
- Statistics for clinical studies from protocol to reporting
- Applied Model based drug development
- Effective translational sciences
- Clinical trial simulation and prediction
- Adaptive and optimal designs

In Non-Clinical Development :
- Statistics for (Bio)Analytical methods
- Quality by Design and Design Space for processes and methods
- Biomarker validation
- In silico optimization

Arlenda Software
Software as a Services (SaaS) in life-cycle of (bio)analytical methods: validation, transfer, stability (Shelf-Life), laboratory Inventory, method comparison

Arlenda Training
Introductive to advanced and Standard to dedicated training in life-cycle of (bio)analytical methods, advanced modeling, bayesian statistics, statistical programming (R, SAS, Winbugs, JMP), design of Experiments, QBD and Design Space

In 2010, Arlenda opened a new office in Louvain-la-Neuve dedicated to clinical service activities. Arlenda has also signed a commercial agreement with Pharmaceutical Development Services for representation in UK.

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PROBIOX

In 2010, PROBIOX has conducted several clinical trials. The SOCO study investigating the company's proprietary nutraceutical product ProPill® was successfully completed during this year. The results confirm the efficiency of ProPill® in reducing oxidative stress in women under oral contraception and thus preserve them from associated cardiovascular risks.

In parallel the company has pursued the development of its services activity aimed at validating the health benefits of nutraceutical products (in vitro studies and clinical studies) for the nutraceutical and cosmetic industry.

End of the year, the french holding company «Territoires et Croissance» invested in Probiox in order to participate actively in the development of the company.

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LABAGE

Labage is dedicated to the development of new software and web-based tools for the management of technical expertise and R&D data and resources, using collective intelligence technologies.

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Training
Center

Training center

The Biotechnology Training Center Forem-GIGA was created in 2005 by the Walloon Office for Jobs and Training of Liège (Le FOREM) in partnership with the Interdisciplinary Cluster in Applied Genoproteomics (GIGA) of the University of Liège, supported by the European Funds and the Walloon Region.

Our aims are to develop and organize biotechnology training for job hunters and companies staff, in response to market needs and to complement the training offered by technical colleges in terms of techniques and specific expertise. Most of the companystaff training is validated by Biowin, the Health Cluster of Wallonia. To achieve these goals, the Biotechnology Training Center works in close collaboration with both the academic and the industrial world of biotechnology.

Current topics approached are: molecular biology, molecular diagnostic, immunology, protein production and purification, gas and liquid chromatography, cell culture, quality control, quality assurance, validation, biosafety, GxP's, bioinformatics, regulatory affairs and project management. Beside these subjects, training sessions can be tailored to customer needs.

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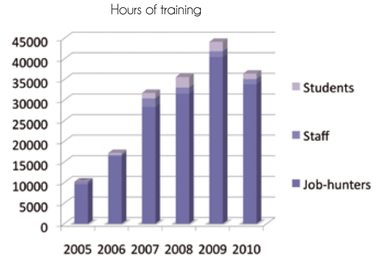
RESULTS FROM YEAR 2010



1. JOB HUNTERS

In 2010, six long-term training courses were organized for 73 people coming from the entire Walloon Region. Besides the transverse skills (biosafety, GxP's, QA, QC, validation, regulatory affairs, scientific English, good communication and team work) included in each session, the technical subjects were related to:

- Project and team management in biotechnology
- Molecular biology, in biosafety level 1 to 3 environments
- Analytical techniques: HPLC-GC-CE
- Immunology
- Protein production and characterization

Most of the trainees performed a 2-month-long immersion program within biotech companies to achieve their training. This led to a high rate of job insertion, since 88 % of them founded a job in less then 6 months after the end of their training period (final data will be available by the end of 2011).

«The GIGA-Forem training course enabled me to become more familiar with the business world and to gain enough hands-on working experience to arouse the interest of recruiters. Thanks to this training, I now work as a researcher within a rapidly growing company, as part of a young, motivated, dynamic team performing studies for large bipharma companies.»
S. Ralet, Master in Biology

We have also developed a new partnership with Culture *in vivo* ASBL which organizes two-month long biotech training session in Nivelles. We set up three 5-day-long modules focusing on:

- Western blotting
- Animal cell culture
- Real-Time-Q-PCR techniques

These modules were designed to strengthen specific technical skills of job hunters and increase their professional insertion. First results are encouraging.
Finally, two modules focused on biosafety and animal cell culture were given for 24 people following a long-term training at the Cefochim skills center.

«For several years, we have established a collaboration with Forem-Biotech based on them welcoming trainees. This collaboration appears to be very enriching and instructive. Enriching by the exchange of points of view between the Zentech team and the trainee but also instructive because it is possible to evaluate the professional quality of trainees. This collaboration is embodied by the company taking on several trainees.»
A. Bosselair, Chief Science Officer, Zentech S.A.

2. COMPANIES STAFF

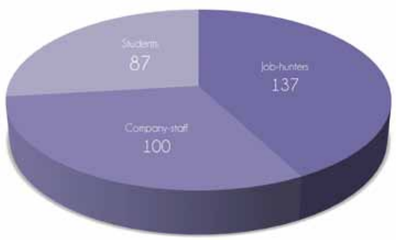
Eight training modules dedicated to specific needs of the biotech companies (biosafety, realtime-Q-PCR, microbiology, bacteriology, PCR) were followed by 106 people (including 9 job hunters, 28 students from technical colleges (Hautes Ecoles)). Most of the sessions were given through the Biowin program.

In addition, a tailormade session focused on Cell culture was again organized for 27 employees of the leading pharmaceutical company.

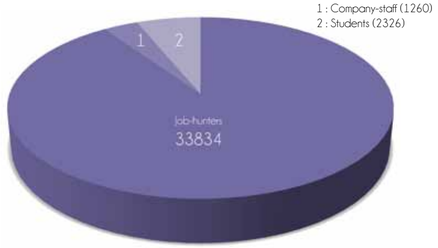
3. HIGHER EDUCATION STUDENTS - TECHNICAL COLLEGES

Five short-term modules designed for 80 students were organized in 2010, partly through Biowin, partly in collaboration with Le Forem. The aim is to complete their academic courses and give them access to the state-of-the art technology unaffordable for technical colleges, promoting interactions with experts in the field and with researchers.

Numbers of trainees (2010)



Hours of training (2010)



This year, we have asked some of our clients, 2010's trainees and biotech companies, to tell us what they think of our activities and how these can help them.

«One of the main interests of our collaboration with the Forem Formation Biotechnologie Center is the opportunity to have an eight week traineeship period during which the trainee and our company can become familiar with one another.
We've been able to select trainees in accordance with our normal recruitment procedure and take advantage of the work experience period to start their training and integration into the company. This period has also helped us back up our choice before going on to employing the trainees definitively.
The training given in the center is also advantageous for Quality Assistance in that it introduces not only several of the techniques we use, but also the regulatory aspects linked to our sector of activity.
We've been working with the center for a few years already and, during that time, potential trainees have been able to visit our premises and discover in the field some facets of the job that they might take on in the future …»
S. Rovillard - Director, Training Department, Quality Assistance S.A.

«In today's world, job-hunting is a major challenge for graduates. Although I hold a master's degree in animal-behavior-oriented biology, unfortunately I did not obtain a grant for a doctoral thesis. The only remaining possibility was to find a laboratory job. I applied to various companies without getting any job interviews, notably because I lacked experience. The GIGA-FOREM training course enabled me to become more familiar with the business world and to gain enough hands-on working experience to arouse the interest of recruiters. The training included an internship in a company. Thanks to this immersion, I was able to land my first job in biotechnology. My internship supervisor hired me on a one-year contract, renewable as a permanent one.
I now work as a researcher within a rapidly growing company, as part of a young, motivated, dynamic team performing studies for large biopharma companies.»
S.Ralet – Master in Biology

«When I started the training on protein production and purification coordinated by the Forem Formation Biotechnologie, I expected to add additional expertise to go along with my scientific background. I was still intent on continuing my job search close to my home within the Luxembourg province and within my then targeted domain: the Agro industrial/ Agro biotechnological sector.
However, I soon discovered that the program involved much more than scientific training of the highest quality. During the four months I spent travelling daily between Arlon and Liège, I also discovered a group of professionals committed to providing myself and my fellow course mates with additional tools aimed at improving our possibilities of obtaining a job.

Whether it was by delivering tips on how to better prepare a curriculum vitae or the ensuing interview, or by introducing us to previously unknown Biotech jobs, or by motivating us to expand our geographical search for a job, the training instructors opened up a whole new array of possibilities to us.

Personally, I discovered the area of Regulatory Affairs in the Pharmaceutical Industry, a field in which I completed a two-month internship in a leading Pharmaceutical company, before being hired immediately by the host company.
Thanks to the Forem Formation Biotechnologie, I now have a career in a field that I genuinely enjoy, where my strengths are maximized and learning never stops, even if it means having a long commute to work!»
B. Harrison – Master in Sciences

«I wish to thank the whole team of trainers and organisers of GIGA-FOREM's training course in immunological techniques. Thanks to them all, my life and professional experience have changed enormously.
In four months of training, I gained quality know-how and social skills in demand on the job market.
With generosity and professionalism, the GIGA-FOREM staff provided us with the necessary tools and taught us how to use them. Everything was organised to support and motivate each trainee in his or her job hunt and/or professional re-orientation. Yet without the will-power and perseverance of the trainee, the goal of this training could not readily be reached.
To crown those four months of intense training, I was offered an internship in a ULB spin-off involved in cell therapy. This led to a permanent job as supervisor of a laboratory whose implementation was in progress: the Quality Control laboratory.
Since then, although my experience in this department is limited to a few months of work, I have been promoted to the position of QC Manager to replace my departing boss. This would not have been possible without the techniques and complementary skills gained in the course of my GIGA-FOREM training.
Thank you all once again!»
I.Obreja – Master in Biochemistry

«Upon returning to Belgium after several years spent abroad, I soon received focused assistance from the Forem, which suggested a training course in biotechnology and immunological techniques organised by the Forem Formation Biotechnologie Center. After a written test and an interview, I became a member of a small group of trainees selected for the session that was about to begin.
This training, organised within the GIGA, is given by active professionals on state-of-the-art equipment. Alternating theory and practice, it lasts four months, followed by an eight-week internship in a company. As a finishing touch, the trainees receive extra support: courses in communication and writing a resume, advice from the instructors.
In my case, the internship led to a job within the company. I would still be working there, had there not arisen an opportunity to do a PhD thesis at GIGA. I chose to seize this opportunity, so I am now back in the laboratory and in the team that trained me.»
I. Gluckmann – Master in Biology

«A year ago, I took a training course in biotechnology/protein production and purification at the GIGA-Forem Training Center (University of Liège).
Thanks to this training, I was hired in March 2010 as a Quality Technician in the Quality Assurance and Regulatory Affairs Department of the company Lonza Braine SA.

My job was to write summaries of Active Pharmaceutical Ingredient (API) analytical procedures for registration files.
In June 2010, I got in charge of an outsourcing project aiming to archive CMP documentation. This involved organising the transfer and managing four operators. Another task was to review batch records and laboratory control records of critical process steps before releasing the APIs.
In January 2011, I joined the Qualification and Validation team at Lonza Braine and that is where I work today.
I wish to thank the GIGA center and all the instructors. Thank you for your energy!»
Y. Lamti - Post-master's Degree in Biotechnology

«The Biotechnology Training Center gave me the opportunity to increase my technical and transverse skills related to the laboratory world. Tips delivered to better prepare my resume and jobinterviews were very helpful for finding a job. Thanks to this training, my motivation and rigour at work has returned helping me better conquer the job market. Finally, this training allowed me to find a job as a lab technician within the GIGA and now, in my day-to-day work, I can practise newly trained laboratory techniques such as cell culture.»
M. Tshiamalenge – Bachelor in Medical Laboratory

Technology Transfer

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Spin-off : Targetome

TARGETOME, A NEW BIOTECHNOLOGY SPIN-OFF FROM GIGA

Targetome is a new biotechnology spin-off company that aims to develop, validate and bring to the clinics innovative tools for the early detection, imaging and therapeutic targeting of human liver metastases.

Liver metastases represent a major public health problem that affects tens of thousands patients in the world (650 000 patients/year worldwide). Most liver metastases originate from colon adenocarcinoma but also from breast and ovary cancers. Liver metastases is a disease with highly unmet needs since it is considered as an incurable disease with almost all patients dying within two years following diagnostic.

The core business of the company is based on the use of a patented technology that accelerates significantly and effectively the identification of protein biomarkers that have a high potential to be targetable because they are discovered based on their accessibility. One unique strength of Targetome is based on the fact that the initial discovery phase of the biomarkers starts directly from the patients. Indeed, the proteins are identified through a path that is the same that the one used by the therapeutic agent that will target them. This strategy is therefore a «from the clinic to the laboratory and back» concept.

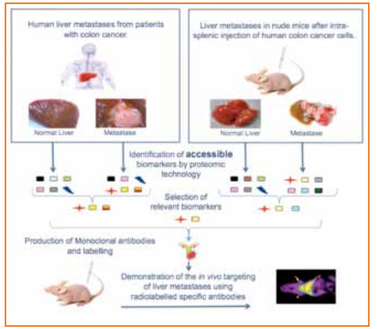
The strategy used by Targetome to achieve its objective is:
1. Identify biomarkers accessible in human liver metastases using the protected technology.
2. Selection of biomarkers of potential interest based on their high expression in liver metastases, their lack of expression or accessibility in normal tissues, their presence as accessible proteins in a mouse model of human liver metastases (discovered using the same technology in 1)
3. Validation of the selected biomarkers based on: their consistent expression in a large collection of human liver metastases (from colorectal cancer and other malignancies such as breast, ovary cancer), their lack of expression in normal human tissue
4. Production of specific epitopes corresponding to the selected biomarkers.
5. Generation and characterization of humanized monoclonal antibodies directed against the specific epitopes.
6. Preclinical validation of the monoclonal antibodies using the mouse model of human colon cancer liver metastases.

Once validated in the animal model, the antibody will be ready for human clinical trial with a high probability that the antibody will be as effective in human than in the animal model since it was first identify in the patient.

It is clear that the technology developed by Targetome regarding the liver metastases will be applicable, mutatis mutandis, to any other malignancies that suffer from non effective therapy such as bone metastases, pancreas cancer and glioblastoma.

Innovative technology for targetable biomarker discovery

Targetome owns an innovative patented technology based on the biotinylation of accessible biomarkers from tissues as described in the figure below.
The technology applied to several types of cancer has already led to a repertoire of biomarkers that have been already patented.



Schematic representation of the various step of the Targetome strategy for liver metastases biomarkers discovery and preclinical validation.

Patents

Filed and granted patents include:
«*In vitro* method for screening accessible biological markers in pathological tissues»
Priority date: January 7, 2006
International Ref: WO2007/080039A1
European Patent Ref (granted 07/07/2010) : EP1969376
US and Japan demands in process
A divisionary of this patent was filed on June 1, 2010 on 5 biomarkers with highest potential for detecting breast cancer
Three patents have been deposited on December 13, 2010 with a list of 38 biomarkers focused on breast, pancreas and liver cancers with diagnostic, therapy and imaging applications.
Title : «Biomarkers, uses of biomarkers and a method of identifying biomarker»

Patents

In 2010, GIGA-R has contributed to the filling of 16 patent applications.
In 2010, 17 patents or patent applications involving GIGA researchers have been published.

Title	Inventors		Publication Number	TRU
Pharmaceutical compositions of pyrimidine-2,4,6-triones	Pierre Bartsch Didier Cataldo* Richard Endele Brigitte Evrard Jean-Michel Foidart* Han-Willi Krell Gerd Zimmermann	US	US 2010/0261672 (A1)	GIGA-I3
An *in-vitro* for method screening accessible biological markers in pathologic tissues and biological marker for detecting breast cancer	Vincent Castronovo* David Waltregny* Philippe Kischel*	EP	EP 2226638 (A2)	GIGA-Cancer
Double-Muscling in mammals	Michel Georges* Dimitri Pirottin* Luc Grobet*	US	US 2010/0107265 (A1)	GIGA-Genomics
Method for determining the genotype at the Crohn's disease locus	Michel Georges* Edouard Louis* Cécile Libioulle* Mark Lathrop	US	US 2010/0136543 (A1)	GIGA-Genomics
Method for determining the genotype at the Crohn's disease locus	Michel Georges* Edouard Louis* Cécile Libioulle* Mark Lathrop	JP	JP2010519895 (A1)	GIGA-Genomics
Inorganic-binding peptides and quality control methods using them	Christelle Vreuls* Cécile Van de Weerdt* Catherine Archambeau André Renard	PCT	WO 2010/000493 (A2)	GIGA-Systems Biology and Chemical Biology
A genetic marker test for brachyspina and fertility in cattle	Michel Georges* Wouter Coppieters* Carole Charlier* Jorgen Steen Agerholm Merete Fredholm	PCT	WO 2010/012690 (A1)	GIGA-Genomics
Method and kit for detecting genetic predisposition for crooked tail syndrome (CTS) in bovine individuals	Michel Georges* Carole Charlier*	EP	EP 2186915 (A1)	GIGA-Genomics
Use of a trioxopyrimidine for the treatment and prevention of bronchial inflammatory diseases	Hans-Willi Krell Luc Delattre Jean-Michel Foidart* Didier Cataldo* Brigitte Evrard	AU	AU2005230379 B2	GIGA-I3
Use of a trioxopyrimidine for the treatment and prevention of bronchial inflammatory diseases	Didier Cataldo* Luc Delattre Brigitte Evrard Jean-Michel Foidart* Hans-Willi Krell	JP	JP4584981 B2	GIGA-I3
Use of a trioxopyrimidine for the treatment and prevention of bronchial inflammatory diseases	Didier Cataldo* Luc Delattre Brigitte Evrard Jean-Michel Foidart* Hans-Willi Krell	RU	RU2402329 B2	GIGA-I3
Antiangiogenic peptides	Joseph Martial* Ingrid Struman* Ngoc-Quynh-Nhu Nguyen* Robert Brasseur Laurence Lins	EP	EP 1778724 B1	GIGA-Cancer
Antiangiogenic peptides	Joseph Martial* Ingrid Struman* Ngoc-Quynh-Nhu Nguyen* Robert Brasseur Laurence Lins	US	US 7,655,626 B2	GIGA-Cancer
Use of cyclodextrin for treatment and prevention of bronchial inflammatory diseases	Didier Cataldo* Brigitte Evrard Agnès Noël* Jean-Michel Foidart*	EP	EP 1799231 B1	GIGA-I3
Use of cyclodextrin for treatment and prevention of bronchial inflammatory diseases	Didier Cataldo* Brigitte Evrard Agnès Noël* Jean-Michel Foidart*	US	US 7,829,550 B2	GIGA-I3
Bis1,2,3,4-tetrahydroisoquinoline derivatives and their uses as pharmaceuticals	Amaury Graulich Jean-François Liégeois Jacqueline Moreau* Vincent Seutin*	EP	EP 1998775 B1	GIGA-Neurosciences
An in-vitro method for screening accessible biological markers in pathologic tissues	Vincent Castronovo* David Waltregny* Philippe Kischel*	EP	EP 1969376 B1	GIGA-Cancer

* = GIGA members

PhD Thesis

Etude de l'endomètre normal et pathologique à partir d'un modèle expérimental murin
Alvarez-Gonzalez Marie-Luz, Laboratory of Biology of Tumor and Development, GIGA-Cancer

Implication de SIP1 (Smad Interacting Protein 1) dans la transition épithélio-mésenchymateuse associée à la progression métastatique.
Bindels Sandrine, Laboratory of Biology of Tumor and Development, GIGA-Cancer

Involvement of the nkx6 factors in the zebrafish pancreas development
Binot Anne-Catherine, Laboratory of Molecular Biology and Genetic Engineering, GIGA-Development, Stem cells & Regenerative medicine

Role of estrogens in brain and behavioral development
Brock Olivier, Laboratory of Behavioural Neuroendocrinology, GIGA-Neurosciences

Contribution à la compréhension du phénomène de surdominance polaire au locus callipyge du mouton
Caiment Florian, Unit of Animal Genomics, GIGA-Genetics

Role of SHIP-1 in the regulation of CD95/APO-1/Fas-induced apoptosis in T lymphocytes
Charlier Edith, Laboratory of Virology, GIGA-Signal Transduction

Etude du rôle de la métalloprotéase MMP-12 dans l'asthme.
Crahay Céline, Laboratory of Biology of Tumor and Development, GIGA-Infection, Immunity and Inflammation

Thérapie cellulaire de réparation tissulaire cardiaque par cellules souches hématopoïétiques et mésenchymateuses.
Delgaudine Marie, Laboratory of Haematology, GIGA-Infection, Immunity and Inflammation

Caractérisation d'EFHC1, une protéine mutée dans l'épilepsie myoclonique juvénile
De Nijs Laurence, Laboratory of Bioenergetics and Cerebral Excitability, GIGA-Neurosciences

Mécanismes d'implantation des cellules souches hématopoïétiques amplifiées *ex vivo*.
Foguenne Jacques, Laboratory of Hematology, GIGA- Infection, Immunity and Inflammation

Etude du mécanisme de synthèse du triphosphate de thiamine dans le cerveau de mammifères
Gangolf Marjorie, Laboratory of Bioenergetics and Cerebral Excitability, GIGA-Neurosciences

Etude des mécanismes moléculaires et cellulaires responsables de la métaplasie épidermoïde cervicale et de sa susceptibilité au développement cancéreux
Herfs Michaël, Laboratory of Experimental Pathology, GIGA-Cancer

Insight into the mechanisms underlying the oncogenic potential of BCL-3 through interactomic studies
Keutgens Aurore, Laboratory of Medical Chemistry, GIGA-Signal Transduction

Evaluation de la capacité de la prolactine 16K humaine de prévenir la croissance tumorale et métastatique par inhibition de l'angiogenèse et de la lymphangiogenèse
Kinet Virginie, Laboratory of Molecular Biology and Genetic Engineering, GIGA-Cancer

Propriétés biophysiques et pharmacologie des canaux SK
Lamy Cédric, Laboratory of Electrophysiology, GIGA-Neurosciences

Exploration des propriétés d'une protéine dont le gène est muté dans certaines familles atteintes d'épilepsie myoclonique juvénile (EMJ) : EFHC1 ou myoclonine 1
Léon Christine, Laboratory of of Bioenergetics and Cerebral Excitability, GIGA-Neurosciences

Intérêts des antagonistes de la protéine kinase A dans le traitement des syndromes immunodéficitaires acquis.
Nayjib Btissam, Laboratory of Immunology and Infectious Diseases, GIGA-Infection, Immunity and Inflammation

Characterization of the post-transcriptional regulation by the IE4 protein of the Varicella-Zoster virus (VZV)
Ote Isabelle, Laboratory of Virology, GIGA-Infection, Immunity and Inflammation

Rôle des protéases de la famille des ADAMALYSINES (ADAMs et ADAMTS) et leurs inhibiteurs dans l'asthme
Paulissen Geneviève, Laboratory of Biology of Tumor and Development, GIGA-Infection, Immunity and Inflammation

Etude de l'antagonisme MIF-glucocorticoïdes dans les glioblastomes : mécanismes pathogéniques et modulations thérapeutiques
Piette Caroline, Laboratory of Biology of Tumor and Development, GIGA-Cancer

Mécanismes d'action des androgènes sur l'expression des récepteurs de la famille du récepteur à l'EGF dans les cellules cancéreuses prostatiques: implication dans l'évolution vers des tumeurs hormono-indépendantes.
Pignon Jean-Christophe, laboratory of Molecular Oncology, GIGA-Cancer

Mécanismes et thérapies des surdités neurosensorielles
Poirrier Anne-Lise, Laboratory of Developmental Neurobiology, GIGA-Neurosciences

Identification of the gene Sprouty1 and the microRNA miR-21 as angiogenic modulators
Sabatel Céline, Laboratory of Molecular Biology and Genetic Engineering, GIGA-Cancer

Contribution au développement d'approches statistiques en vue d'identifier des gènes affectant des caractères complexes ayant un intérêt médical ou agronomique
Sandor Cynthia, Unit of Animal Genomics, GIGA-Genetics

La phénylcétonurie : étude de la myélinisation du système nerveux central et contribution à la thérapie génique
Schoemans Renaud, Laboratory of Human Genetics, GIGA-Cancer

Study of the expression and the function of zac1, a transcription factor and tumor suppressor
Warzee Barbara, Laboratory of Cellular and Molecular Physiology, GIGA-Infection, Immunity and Inflammation

Contribution à l'étude des mini-allogreffes de cellules souches hématopoïétiques.
Willems Evelyne, Laboratory of Hematology, GIGA-Infection, Immunity and Inflammation

Seminars

Francesco Argenton
University of Padova, Italy
Mother-of-snow-white (msw): a maternal effect allele affecting behavior and the formation of the left-right body axis in zebrafish

Jean-François Arnal
Université Paul Sabatier, Toulouse, France
Uncoupling the beneficial and the undesirable actions of estrogens : molecular dissection of the functions of estrogen receptor α in vivo

Christophe Bernard
Université de la Mediterranée, Marseille, France
Constructing an epileptic brain: epigenetics mechanisms

Luc Bertrand
UCL , Louvain-la-Neuve, Belgium
The AMP-activated protein kinase, a putative therapeutic target to treat myocardial pathologies like ischemia, hypertrophy and insulin-resistance

Anne-Catherine Binot
GIGA-R, ULg, Belgium
Régions non-codantes conservées et déserts génétiques

Roger Butterworth
McGill University, Montréal, Canada
Mechanisms of Selective Neuronal Cell Death in Wernicke's Encephalopathy

Patrice D. Cani
UCL , Louvain-la-Neuve, Belgium
The role of the gut microbiota in the development of inflammation associated with obesity and metabolic disorders.

Marie-Christine Chartier
Inserm, Institut Pasteur, Lille, France
New discoveries in autosomal dominant parkinsonism

Sébastien Couillard-Despres
Paracelcus Medical University Salzburg, Austria
TGF-β signaling in the neurogenic niche : implications for neurodegenerative disorders

Valérie Gailus-Durner
German Mouse Clinic, Munich, Germany
Systemic phenotyping in the German Mouse Clinic

Julien Hanson
Chimie pharmaceutique, Ulg, Belgium
Involvement of keratinocytes and COX-2-mediated prostanoid formation in the nicotinic acid-induced flushing

Bassem Hasan
KUL, Leuven, Belgium
Integration, competition and robustness in brain wiring

Damien Hermand
FUNDP, Namur, Belgium
A gene-specific pattern of RNA polymerase II CTD phosphorylation controls cell differentiation

Sandrine Horman
UCL, Louvain-la-Neuve, Belgium
Calcium-dependent functions of AMP-activated protein kinase: more than a metabolic sensor

Carsten Janke
Institut Curie, Paris, France
Regulation of microtubule functions by posttranslational modifications

Sebastian Jessberger
Institute of Cell Biology, Zurich, Switzerland
Molecular mechanisms and functional significance of adult neurogenesis

Randy Jirtle
Department of Pathology, Duke University, USA
Epigenetics, Imprinting, and the Developmental Origin of Disease Susceptibility

Virginie Kinet
GIGA-R, Ulg, Belgium
Le rôle du système lymphatique dans le cancer

Thomas A. Kufer
Institute for Medical Microbiology, Uniklinik Köln, Germany
New insights into the biology of NLR proteins

Patrick Laurent
University of Cambridge, UK
From genes to neural circuits, how C. elegans generates its behaviour

Adrian Liston
KUL, Leuven, Belgium
Assymetric immunity in response to the loss of regulatory T cell

Ludovic Malvaux
GIGA-R, Ulg, Belgium
Nouveaux concepts autour des microARN

Isabelle Ote
GIGA-R, Ulg, Belgium
Viral diversion of the post-transcriptional machinery

Tomi Markku Pastinen
McGill University, Montréal, Canada
Genome-wide allele-specific analysis and role of non-coding genetic variants in human phénotypes

Anselme Perrier
Institute for stem cell therapy, Inserm, Evry, France
Human pluripotent stem cells for Huntington's disease cell therapy

Muriel Pichavant
Institut Pasteur, Lille, France
NKT cells and lung function

James Putney
Signal Transduction, NIEHS, USA
Regulation and Function of Store-operated Calcium Channels

Margaret E Rice
Department of Neuroscience, New York University, USA
How H2O2 mediates and modulates neurotransmission in the basal ganglia

Céline Sabatel
GIGA-R, Ulg, Belgium
Mécanismes de répression post-transcriptionnelle des microARN

Ekaterina Tskitishvili
Department of Obstetrics and Gynecology, Osaka University, Japan
Tumor-associated antigen RCAS1 and its role in pregnancies complicated by preeclampsia

Ludovic Vallier
Centre of stem cell biology and régénérative médicine, University of Cambridge, UK
Similarities and divergences between human embryonic stem cells and induced pluripotent stem cells

Benoît Van den Eynde
De Duve Institut, UCL, Bruxelles, Belgium
Tumor antigens and preclinical models for cancer immunotherapy

Patricia Vandevenne
GIGA-R, ULg, Belgium
Réponse immunitaire innée et stratégies d'interférence développées par les virus

Luc Vanhamme
Service de parasitologie moléculaire, ULB, Bruxelles, Belgium
How African trypanosomes survive in their human host: an example of host-parasite arms race.

Jean-Claude Voegel
UMR 977 Strasbourg, France
Multilayer polyelectrolyte films and their «bio» functionalization

Andreas Ziegler
University of Lübeck, Germany
Analysis of global gene expression and genome-wide association data: From theory to practice

Thomas Zoeller
Biology Department, University of Massachusetts, USA
Thyroid Hormone, Brain Development and the Environment

Perspectives

In 2011...

LAB HOTEL

Equipped space available for scientists willing to collaborate with GIGA-R team's or GIGA technology platforms.

CENTER FOR MEDICINE INNOVATIONS (CIM)

A new Center for Medicine Innovations (CIM) hosted in the GIGA building to speed-up and secure new activities in new fields of medicine such as translational research and cellular therapy.

NEW TECHNOLOGY PLATFORMS

New technology platforms or new technologies to answer the need of the scientists:
- Immunohistology with fully automatized stations for treating a variety of samples from biopsies to immunostaining
- Interactomics (funded by the « Fondation contre le Cancer ») with 3 automatized stations for Yeast-2-Hybrid experiments
- High Throughput mutagenesis and protein purification (in collaboration with the Center for Protein Engineering, CIP)
- High Content Analysis

GIGA2

A new building for Biomedical or Biotechnology companies (in Partnership with SPI+, SPS)

MORE SPACE FOR THE GIGA-TRAINING CENTER

More space for the GIGA-Training center (in Partnership with FOREM) to face the increasing numbers of both training sessions and trainees:
- 2 multimedia equipped classrooms
- 1 fully equipped lab,
- 2 small rooms for pre and post-PCR experiments
- 1 meeting room

AND CERTAINLY MUCH MORE...

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