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Société Belge de Biologie Cellulaire et du Développement
Belgische Vereniging voor Cel- en Ontwikkelingsbiologie

Protein Modifications in Developmental Signalling and Disease

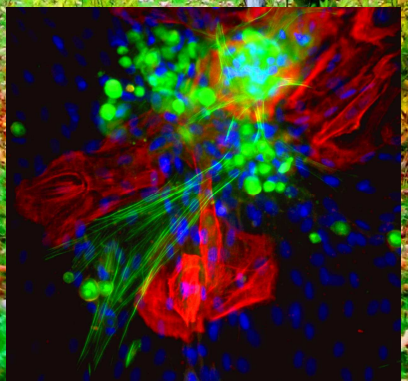
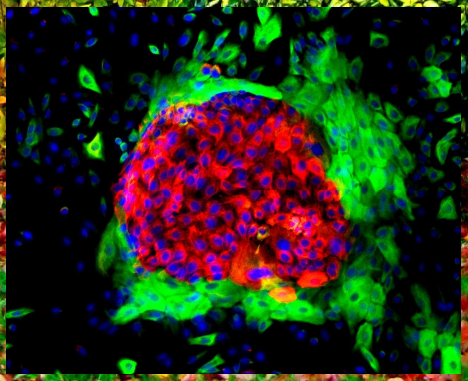
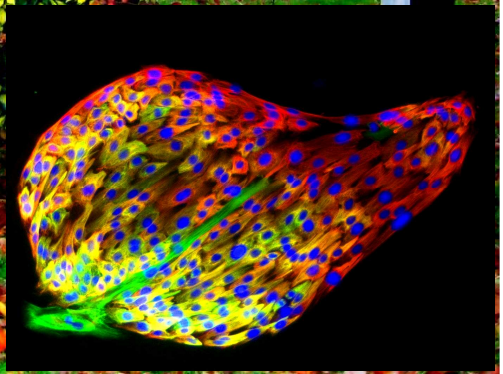
Autumn 2009
October 17th, CHU, Sart-Tilman, Liège, Belgium

Invited speakers:

LUKE O'NEILL (Trinity College, Dublin, Ireland)
MOHAMED BENTIRES-ALJ (FMI, Basel, Switzerland)
GILLES COURTOIS (Hôpital Necker, Paris, France)
JEAN-CHRISTOPHE MARINE (VIB, Ghent, Belgium)
FRANCOIS FUKS (ULB, Brussels, Belgium)

Deadline for abstract submission:
September 15th 2009
Information on meeting and award:
http://bscdb.ugent.be

Organizer: Alain Chariot,
GIGA-R, ULG, Liege
Alain.chariot@ulg.ac.be
Tel: 04/366 24 72

The BSCDB Autumn Meeting 2009

"PROTEIN MODIFICATIONS IN DEVELOPMENT AND DISEASES"

in Liège, 17th October 2009

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The Belgian Society of Cellular Biology and Development

"PROTEIN MODIFICATIONS IN DEVELOPMENT AND DISEASES"

in Liège, 17th October 2009

Program

8h30-9h15	Registration and display of posters
9h15-9h30	Welcome and Introduction (Alain Chariot)

MORNING SESSION

9h30-10h15	MOHAMED BENTIRES-ALJ (FMI, Basel, Swtizerland) "*Roles of the protein tyrosine phosphatases (PTPs) in breast cancer"*
10h15-1100	GILLES COURTOIS (Hôpital Necker, Paris, France) "*Protein ubiquitination in NF-κB activation"*
11h00-11h30	**Coffee break, visit of posters and commercial exhibition**
11h30-12h15	LUKE O'NEILL (Trinity College, Dublin, Ireland) *"Protein phosphorylation in TLR signalling"*
12h15-12h30	MATHIEU BERTRAND, VIB, Gent "*Cellular inhibitor of apoptosis cIAP1 and cIAP2 are E3 ubiquitin ligases required for innate immunity signaling by the pattern recognition receptors NOD1 and NOD2"*
12h30-12h45	ANNE BRYSSE, ULG, Liege "*Zonula occludens-1 (ZO-1) contributes to the metastatic progression of breast tumor cells"*
12h45-14h00	**Lunch, visit of posters and commercial exhibition**

AFTERNOON SESSION

14h00-14h45	JEAN-CHRISTOPHE MARINE, VIB, Gent "*E3 ubiquitin ligases and cancer development*"
14h45-15h00	SASKIA LIPPENS, VIB, UGent "*Unravelling RIP4 signalling in the skin"*
15h00-15h15	HUIYU TIAN, VIB, UGent "*Ocular anterior segment dysgenesis following deletion of p120CTN in neural-crest stem cells"*
15h15-15h30	Coffee break, visit of posters and commercial exhibition
15h30-16h15	FRANCOIS FUKS, ULB, Brussels "*Mechanisms of DNA methylation: from chromatin to cancer*"
16h15-16h45	CATHERINE CREPPE, ULG, Liege (PhD award winner)
16h45-17h00	Poster award & End of the meeting-Agnès Noël

BSCDB Autumn meeting 2009
Liège

POSTER PRIZES

At the end of the Autumn Meeting of 2009, poster prizes were awarded to

- Irene Kahr
- Fanelie Bauer

for their posters:

FUNCTIONAL ANALYSIS OF THE CANDIDATE TUMOR SUPPRESSOR PROTEIN PROTOCADHERIN-10

Irene Kahr (1,2), Uta Fuchs (1,2), Katrien Staes (1,2) and Frans van Roy (1,2)

1: Department for Molecular Biomedical Research, Molecular Cell Biology Unit, VIB, Technologiepark 927, B-9052 Ghent

2: Department of Biomedical Molecular Biology, Ghent University, Technologiepark 927, B-9052 Ghent

and

STUDY OF THE ROLE OF tRNA WOBBLE URIDINE MODIFICATIONS BY REVERSE PROTEIN ARRAYS

Fanelie Bauer (1), Akihisa Matsuyama (2), Minoru Yoshida (2) and Damien Hermand (1)

1: Academie Universitaire Louvain (FUNDP), Laboratoire de Genetique Moleculaire, Namur

2: Chemical Genetics Laboratory, RIKEN Advanced Science Institute, Wako, Saitama, Japan

The Belgian Society of Cellular Biology and Development

"PROTEIN MODIFICATIONS IN DEVELOPMENT AND DISEASES"

in Liège, 17th October 2009

Abstracts

1: O'Neill Luke
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TOLL-LIKE RECEPTOR SIGNAL TRANSDUCTION

Luke O'Neill

School of Biochemistry and Immunology, Trinity College, Dublin, Ireland

The IL-1R/TLR superfamily was first defined in detail 10 years ago. Since then, there has been remarkable progress in our understanding of both branches of the superfamily. Ligands have been described for most receptors. Within the IL-1R subfamily, notable examples include IL33 for ST2 and IL-1F6 for IL-1Rrp2. The role of TLRs in the sensing of microbial products has led to a renaissance of interest in innate immune mechanisms. For investigators interested in signal transduction, the area has proved very fruitful in terms of the discovery of new signalling pathways and processes. MyD88 is the universal adapter for the superfamily and its central role in inflammation, host defence and even in certain cancers, has been confirmed from studies in knockout mice. We now have a good understanding of the major components activated by TLRs, notably the TIR domain- containing adapters that initiate signalling following recruitment to TIR domains within the TLRs themselves, the IRAK family of protein kinases that are then recruited, and a series of ubiquitination and phosphorylation reactions that ultimately lead to the activiation of transcription factors such as NF-kappaB and IRF family members. The structural basis for signalling is still poorly understood however, and we have no appreciation of the kinetics involved in the pathways. Additional components and regulatory cross-talk from multiple signals also continue to be discovered. Genetic variation in signalling components such as in IRAK4, Mal and Unc93b however highlight the importance of these pathways in human health and disease. I will discuss our recent findings of a novel component in TLR4 signaling termed TAG, which specifically inhibits TLR4 signaling from endosomes, and the emerging role of miRNAs as key regulators of TLR signalling events.

2: Bentires-Alj Mohamed
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ROLES OF THE PROTEIN TYROSINE PHOSPHATASES (PTPs) IN BREAST CANCER

Mohamed Bentires-Alj

Friedrich Miescher Institute for Biomedical Research (FMI), Basel, Swtizerland

Each year 1.1 million new cases of breast cancer will occur among women worldwide and 400,000 women will die from this disease. Although progress has been made in understanding breast tumor biology, most of the relevant molecules and pathways remain undefined. Their delineation is critical to a rational approach to breast cancer therapy. We focus on the roles of the family of protein-tyrosine phosphatases (PTPs) in the normal and neoplastic breast. Virtually all cell signaling pathways are modulated by reversible protein tyrosine phosphorylation, which is regulated by two classes of enzymes: protein-tyrosine kinases (PTKs) and PTPs. Not surprisingly, tyrosine phosphorylation has an important role in breast development and cancer. Whereas the role of specific PTKs, like the HER2 receptor, in breast cancer is well studied, almost nothing is known about the function of specific PTPs in this disease. Our data suggest that PTP1B has an important role in breast differentiation and that both PTP1B and SHP2 play positive roles in breast cancer.

3: Courtois Gilles
presenting author ; e-mail : gilles.courtois@inserm.fr

PROTEIN UBIQUITINATION IN NF-kappaB ACTIVATION

Gilles Courtois

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NF-kappaB transcription factor plays a critical role in inflammation, immunity and cancer. In resting cells, it is maintained in the cytoplasm through association with inhibitory molecule IkappaBalpha. Upon cell stimulation, IkappaBalpha is phosphorylated by IkappaBalpha kinase (IKK) and degraded. This allows NF-kappaB to enter the nucleus and to regulate its target genes. A major feature of the NF-kappaB signaling pathway is its ability to be activated by a large collection of stimuli in many different physiological settings. Very often, ligand binding to receptors induces recruitment of adaptor molecules and activation of various kinases, eventually resulting in IKK activation. Over the last years, it has been demonstrated that these different steps recurrently require post-translational modifications involving ubiquitin. Such modications not only generate platforms for assembling active macromolecular complexes participating in NF-kappaB activation, through specific recognition of ubiquitin moities by ubiquitin-binding domains, but also provide an additional layer of regulation controled by enzymes of the deubiquitinase family. Here, I will provide a general overview of the ubiquitination process before describing the components of the NF-kappaB pathway that are ubiquitinated and the molecular machineries involved. Hypothesis about how modifications through ubiquitination may result in IKK activation will also be presented.

4: Marine Jean-Christophe
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COP1 REGULATES C-JUN PROTEIN STABILITY IN VIVO AND PROTECTS AGAINST TUMORIGENESIS

Domenico Migliorini (1), Sven Bogaerts (1), Alberto Vitari (2), Enrico Radaelli (3,4), Geertrui Denecker (1), Tino Hochepied (5) and Jean-Christophe Marine (1)

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2: Genentech, 1 DNA Way, South San Francisco, CA, USA
3: Department of Veterinary Pathology, Hygiene, and Public Health, University of Milan, Faculty of Veterinary Medicine, Via Celoria, 10 – 20133 Milano, Italy
4: Animal Model Systems, Fondazione Filarete, Viale Ortles, 22-4 – 20139 Milano, Italy
5: Transgenic Mice Core Facility, Department of Molecular Biomedical Research, VIB and Ghent University, B-9052 Gent

We have recently provided clear genetic evidence that constitutive degradation of p53 in various embryonic and adult tissues occur in a strict Mdm2-dependent manner. However, transfection studies suggest the existence of additional p53 ubiquitin ligases, including the constitutively photomorphogenic protein Cop1. These Biochemical data support the potential of Cop1 as target for cancer therapy. To explore the role of Cop1 in the etiology of cancer in vivo we generated an allelic series of Cop1 in mice using an innovative genetic approach. We show that Cop1 is required for embryonic development and, unexpectedly, that Cop1 protects against tumorigenesis. Cop1 hypomorphic mice are viable but spontaneously develop malignancy at a high frequency in their first year of life and are highly susceptible to radiation-induced lymphomagenesis. Biochemically, Cop1 regulates the stability of the C-Jun oncoprotein and thus modulates c-Jun/AP1 transcriptional activity in vivo. Importantly, Cop1-deficiency stimulates cell proliferation in a c-Jun-dependent manner. We conclude that Cop1 functions as a tumor suppressor, at least in part, by antagonizing C-Jun oncogenic activity. Moreover, our data fail to support a role of Cop1 in the regulation of the p53 pathway, thus arguing against the use of Cop1-inhibitory drugs for cancer therapy. Our observations have therefore implications for cancer etiology as well as for cancer therapy.

5: Fuks François
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MECHANISMS OF DNA METHYLATION IN MAMMALS

François Fuks

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In mammals, DNA methylation plays an important role in development and cancer and is associated with transcriptional silencing. The goal of our current work is to elucidate the mechanisms by which the DNA methylation machinery - the DNA methyltransferases (DNMTs) and the MBDs- functions. In particular, we are currently addressing the following questions:

1. How are the DNMTs targeted to specific genomic sequences?
2. How do the DNMTs lead to gene silencing?
3. How are the DNMTs, and in particular their enzymatic activity, regulated?

1. Our previous work suggests that the DNMTs can be recruited to particular loci through their association with specific transcription factors. Our studies indicate that targeting of DNA methyltransferases by transcription factors could be a general mechanism by which specific DNA methylation patterns are generated (1).

2. One mechanism by which the DNA methylation machinery brings about transcriptional repression is through recruitment of HDAC and histone H3 Lys9 methyltransferase activities (2). Our recent work also indicates that the DNA methylation machinery is mechanistically linked to the Polycomb Group (PcG) proteins. Recent genome-wide studies show that a significant number of CpG islands marked by H3K27 trimethylation undergo de novo methylation, indicating that Polycomb-directed de novo methylation might play an important part in carcinogenesis. Mechanistically, while there are not always coupled, the DNA methylation machinery can be linked to the Polycomb Group (PcG) proteins. More precisely, our results suggest that EZH2 serves as a recruitment platform, at least in some situations, for DNA methyltransferases (4) and this might impact on PML-RAR-mediated leukemia (5).

3. We considered whether the enzymatic activity of the DNMTs could be regulated by post-translational modification. We observed that DNMT3A is phosphorylated both in vitro and in vivo by the CK2 kinase. Data will be presented suggesting a new mechanism for the regulation of DNA methylation by post-translational modification.

1. Brenner et al. EMBO J. (2005) 24:336-46.
2. Brenner & Fuks. Developmental Cell (2007) 12(6):843-4.
3. Epsztejn-Litman et al. Nature Struct Mol Biol. (2008) 15(11):1176-83.
4. Vire et al. Nature (2006) 439:871-4.
5. Villa et al. Cancer Cell (2007) 12(6):843-4.

1: Bauer Fanelie
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STUDY OF THE ROLE OF tRNA WOBBLE URIDINE MODIFICATIONS BY REVERSE PROTEIN ARRAYS

Fanelie Bauer (1), Akihisa Matsuyama (2), Minoru Yoshida (2) and Damien Hermand (1)

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2: Chemical Genetics Laboratory, RIKEN Advanced Science Institute, Wako, Saitama, Japan

Modifications of transfer RNA (tRNA) are widespread and numerous but their function is not well understood. Particularly, three tRNA (lysine, glutamine and glutamate) show universal modifications of the 2nd and 5th carbon of their wobble base.

The «elongator» complex (Elp1-6) is proposed to modify carbon 5 while we have recently shown that the Ctu1-Ctu2 complex is responsible for the thiolation of carbon 2. In both cases, the modification is thought to be required to optimize the codon-anticodon interaction. The deletion of either complexes results in thermosensitivity associated with genome instability.

A key step in understanding how this phenotype results from a defect in tRNA modifications is the identification of proteins whose level is decreased in these strains.

The fission yeast integrated ORFeome, combined to reverse protein arrays, allows the determination of relative expression level proteome-wide. With this technology, we are currently comparing the proteomes of a wild type strain and strains lacking either one or the other tRNA modification. This should highlight the protein set affected in the mutants and allow us to link it to the phenotype.

The conserved Wobble uridine tRNA thiolase Ctu1-Ctu2 is required to maintain genome integrity. Dewez M, Bauer F, Dieu M, Raes M, Vandenhaute J, Hermand D. Proc Natl Acad Sci U S A. 2008,105(14):5459-64

ORFeome cloning and global analysis of protein localization in the fission yeast Schizosaccharomyces pombe. Matsuyama A, Arai R, Yashiroda Y, Shirai A, Kamata A, Sekido S, Kobayashi Y, Hashimoto A, Hamamoto M, Hiraoka Y, Horinouchi S, Yoshida M. Nat Biotechnol. 2006 Jul,24(7):841-7

2: Bergiers Isabelle
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SEARCHING FOR HOXA2 INTERACTORS

Isabelle Bergiers (1), Emmanuelle Abgueguen (1), Jean-Claude Twizere (2), Olivier De Backer (3) and René Rezsöhazy (1)

1: Unité des Sciences Vétérinaires, Institut des Sciences de la Vie, Université catholique de Louvain, Louvain-la-Neuve
2: Cellular and Molecular Biology Unit, FUSAGx, Gembloux
3: Unité de Recherche en Physiologie Moléculaire, FUNDP School of Medicine, University of Namur, Namur

Initially identified in Drosophila, Hox genes encode homeodomain-containing transcription factors essential during embryonic morphogenesis. Although the developmental roles of Hox proteins have been extensively documented, their mechanisms of action still remain to be defined. In this study, we focus on the molecular role Hoxa2 plays during hindbrain development in the mouse.

In that prospect, our goal is to identify and characterize molecular partners interacting with the Hoxa2 protein. Two complementary approaches are currently developed to find out Hoxa2 interactors.

As a first approach, we are searching for Hoxa2 protein partners by screening for interactions between Hoxa2 and the nearly complete human ORFeome by the well-known Gal4-based yeast two hybrid system (Y2H). “Prey” and “bait” yeast expression vectors for Hoxa2 have been obtained with the Gateway® technology. Three screens against the pooled AD-hORFeome library have been accomplished and one screen against the DB-hORFeome library is still ongoing. More than a hundred putative Hoxa2 interactors have been identified so far.

The second approach, the tandem affinity purification (TAP) method, consists in recovering a tagged protein, Hoxa2, along with its interactors, by sequential immunoprecipitation steps. This protein-protein interaction analysis can be performed under near-physiological conditions. It will be carried out from engineered mouse embryonic stem (ES) cells during differentiation along the neurectodermal lineage. These ES cells possess a mutant Hoxa2 knockin allele carrying a targeted EGFP gene useful to label cells expressing Hoxa2 upon differentiation. The second allele has been replaced by a TAP-tag-Hoxa2 chimeric gene we have obtained and controlled for its proper expression and for the subcellular localisation of the resulting fusion protein. Upon retinoic acid mediated differentiation we will be able to follow the expression of the tagged Hoxa2 allele and perform the tandem affinity purification. The retrieved interactors of Hoxa2 will be identified by mass spectrometry.

Then putative interactors will be confirmed by cross-validation between the Y2H and TAP methods, co-immunoprecipitation and BiFC methods.

3: Bertrand Mathieu
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CELLULAR INHIBITOR OF APOPTOSIS cIAP1 AND cIAP2 ARE E3 UBIQUITIN LIGASES REQUIRED FOR INNATE IMMUNITY SIGNALING BY THE PATTERN RECOGNITION RECEPTORS NOD1 AND NOD2

Bertrand M.J. (1,4), Doiron K. (2), Labbé K. (2), Korneluk R.G. (3), Barker P.A. (1) and Saleh M. (2)

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3: Apoptosis Research Centre, Children's Hospital of Eastern Ontario, 401 Smyth Road, Ottawa, Ontario K1H 8L1, Canada
4: Present address : Department for Molecular Biomedical Research, Molecular Signalling and Cell Death Unit, VIB and Ghent University, B-9052 Ghent

Inhibitor of apoptosis proteins (IAPs) form a family of genetically conserved proteins characterized by the presence of 1-3 baculovirus IAP repeat (BIR) motifs. Previous studies have shown that three family members (termed XIAP, cIAP1 and cIAP2) are potent suppressors of cell death. XIAP, the most studied IAP, inhibits apoptosis by binding and inhibiting caspases and it has been broadly assumed that cIAP1 and cIAP2 block apoptosis through a similar mechanism. However, recent structure-function analyses have indicated that these IAPs are not direct caspase inhibitors, suggesting that their anti-apoptotic function must involve alternative mechanisms. An interesting feature of several IAPs, including cIAP1 and cIAP2, is the presence of a C-terminal RING-finger domain with E3 ubiquitin ligase activity. We recently demonstrated an unexpected new function of cIAP1 and cIAP2 in the TNFR1 signaling pathway. These proteins act as K63 E3 ubiquitin ligases for RIP1. cIAP-mediated polyubiquination of RIP1 allows it to bind to the pro-survival kinase TAK1 and facilitates activation of NF-kappaB to promote cell survival.

Cell death and innate immunity are ancient evolutionary conserved processes that utilize a great number of related molecular effectors and parallel signal transduction mechanisms. NOD1 and NOD2, two cytosolic NOD-like receptors (NLRs), function as intracellular sentinels against bacterial infection. Here, we report that cIAP1 and cIAP2 are E3 ubiquitin ligases that are required for receptor-interacting protein 2 (RIP2) ubiquitination and for nucleotide-binding and oligomerization (NOD) signaling. Macrophages derived from Birc2(-/-) or Birc3(-/-) mice, or colonocytes depleted of cIAP1 or cIAP2 by RNAi, were defective in NOD signaling and displayed sharp attenuation of cytokine and chemokine production. This blunted response was observed in vivo when Birc2(-/-) and Birc3(-/-) mice were challenged with NOD agonists. Defects in NOD2 signaling are associated with Crohn's disease, and muramyl dipeptide (MDP) activation of NOD2 signaling protects mice from experimental colitis. Here, we show that administration of MDP protected wild-type but not Ripk2(-/-) or Birc3(-/-) mice from colitis, confirming the role of the cIAPs in NOD2 signaling in vivo. This discovery might provide therapeutic opportunities in the treatment of NOD-dependent immunologic and inflammatory diseases.

4: Bex Françoise
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POST-TRANSLATIONAL MODIFICATIONS OF THE TAX ONCOPROTEIN OF HUMAN T-CELL LEUKEMIA VIRUS CONTROL CYTOPLASMIC AND NUCLEAR STEPS IN TAX-MEDIATED ACTIVATION OF THE NF-kappaB PATHWAY

Françoise Bex, Carla Sampaio, Isabelle Lamsoul and Julie Lodewick

Université Libre de Bruxelles and Institut de Recherches Microbiologiques JM Wiame, Brussels

Background:

The transcription factor NF-kappaB is critical for induction of cancer, including adult T-cell leukemia, which is linked to infection by human T-cell leukemia virus type 1 (HTLV-1) and expression of its regulatory protein Tax. HTLV-1-mediated immortalization of T lymphocytes, a basic event for subsequent cell transformation, results mainly from the ability of Tax to trigger T-cell proliferation through various mechanisms including activation of specific cellular genes via the NF-kappaB pathway, promotion of cell cycle progression and deregulation of apoptosis.

Methods:

A series of Tax mutants with substitutions of serine or lysine residues were analyzed by two dimensional gel electrophoresis for the identification of the various modified forms of Tax. The intracellular localization of each mutant and its colocalization with cellular factors such as the RelA subunit of NF-kappaB and the p300 transcriptional coactivator were analyzed by laser scanning confocal microscopy. The ability of these mutants to activate gene expression via the NF-kappaB pathway was also analyzed by dual luciferase reporter assay.

Results:

We observed that Tax was modified by ubiquitination, sumoylation and acetylation in a phosphorylation-dependent manner and that these post-translational modifications were instrumental to Tax-mediated activation of the NF-kappaB pathway. In the cytoplasm, ubiquitinated Tax molecules activated the IkappaB kinase complexes leading to the translocation of the RelA subunit of NF-kappaB into the nucleus. Phosphorylation-dependent translocation of Tax into the nucleus led to Tax sumoylation, a modification critical for the assembly of Tax-containing nuclear bodies and for the recruitment of the RelA subunit of NF-kappaB and the transcriptional coactivator p300 in these nuclear structures. These nuclear bodies also included a form of Tax, which was acetylated by p300 on a lysine located in its carboxy-terminal domain. Our results indicate that the four modifications act in concert to implement sequential steps of the Tax-mediated-kappaB activation cascade.

Conclusion:

We conclude that a hierarchical sequence of post-translational modifications create populations of Tax molecules having different intracellular localizations and interacting partners and exhibiting different functions in the transcriptional properties of this viral oncoprotein.

5: Brosson Sébastien
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PROTEOMIC ANALYSIS OF THE ENDOCYTIC PATHWAY OF TRYPANOSOMA CRUZI

Sébastien Brosson (1), Didier Salmon (2) and Sabrina Bousbata (3)

1: Proteomic Platform-Parasitology, IBMM ULB, B-6041 Charleroi
2: Parasitology - Proteomic Platform, IBMM ULB, B-6041 Charleroi
3: Proteomic Platform-Parasitology, IBMM ULB, B-6041 Charleroi

Endocytosis is the basic mechanism by which macromolecules are internalized by most eukaryotic cells, including pathogenic protozoa. Trypanosoma cruzi, the responsable agent of Chagas disease, undergoes a complex life cycle. Epimastigotes are proliferative forms that live inside the insect vector and subsequently differentiate into metacyclic trypamastigotes capable to infect mammalian hosts. After invasion trypamastigotes differentiate into amastigotes, the proliferative intracellular forms. Epimastigotes take up their nutrients by avidly endocytosing macromolecules from the extracellular medium however, the nutrition mechanisms of amastigotes and trypomastigotes are poorly understood.

Studies of T. cruzi have shown that this protozoan exhibits certain peculiarities in its endocytic pathway that distinguish it from other cells especially, epimastigotes having highly polarized cells and two sites of macromolecule ingestion: the flagellar pocket and a highly specialized structure known as the cytostome. The cargo of the endocytic vesicles is delivered to unusual structures called reservosomes, which are localized at the posterior region of the protozoan. The combination of electron microscopy studies and cytochemical data indicate that the membrane lining the cytostome is rich in glycoconjugates. To understand the role of these glycoproteins in the endocytosis, we followed the endocytic pathway using tomatolectin (TL) and GSLII, two lectins known to recognize short and long PNAL-glycoproteins, respectively. Following uptake of Alexa594-conjugated transferrin, which marks the endocytic pathway, the trypanosomes were then subjected to direct fluorescence microscopy using FITC-TL or Alexa488-GSLII. TL followed the same endocytic pathway as transferring suggesting the implication of PNAL proteins in endocytosis. In addition, proteins enriched using both lectins were identified by LC-Q-TOF resulting in the characterization of an already known PNAL glycoprotein (cruzipaïn) of the endocytic pathway but also in other unknown proteins that are still under investigation. These preliminary results indicate the implication of PNAL proteins in T.cruzi endocytosis. Further characterization of these proteins can help to better understand the nutrition mechanisms of amastigotes, the human virulent forms.

6: Brysse Anne
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ZONULA OCCLUDENS-1 (ZO-1) CONTRIBUTES TO THE METASTATIC PROGRESSION OF BREAST TUMOR CELLS

Anne Brysse (1), Myriam Polette (2), Sandrine Bindels (1), Arnaud Bonnomet (1), Mélanie Mestdagt (1), Agnès Noël (1), Philippe Birembaut (2), Jean-Michel Foidart (1) and Christine Gilles (1)

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Increasing data suggest that the acquisition of migratory and invasive properties by tumor cells is associated with epithelial-to-mesenchymal transition (EMT) processes. At the molecular level, EMT phenomena involve, among other mechanisms, a reorganisation of cell-cell adhesion complexes as well as an increased expression of proteolytic enzymes including matrix metalloproteases (MMPs).

ZO-1 is classically known as a submembranous cytoplasmic molecule contributing to the structural organization of tight junctions (TJs). A concept has recently emerged that, in addition to its structural role in TJ organization, ZO-1 could be involved in signalling pathways favouring tumor progression once delocalized from TJs.

Here, we first analyzed the subcellular localization of ZO-1 in relationship with the invasive potential of tumor cells. By comparing different cell lines in vitro by immunostaining, we found ZO-1 mainly localized at the membrane of non-invasive cell lines (MCF-7), whereas a predominantly diffuse cytoplasmic staining was observed in invasive tumor cells (BT-549). We also examined ZO-1 distribution in breast tumor biopsies. In in situ carcinomas, ZO-1 was expressed mostly at the cell membrane whereas a cytoplasmic/nuclear staining was observed in invasive breast cancers.

These data prompted us to examine the potential pro-metastatic role of ZO-1. We thus employed shRNAs to downregulate ZO-1 in human breast tumor cell lines: MDA-MB-231 LUC D3H2LN and MDA-MB-435 LUC, largely described as highly metastatic cell lines once injected subcutaneously in mice. Using an in vivo imaging system (IVIS 200, Xenogen) allowing the visualisation of luciferase expressing cells in living animals, we obtained preliminary results showing that transfection of ZO-1 shRNA can inhibit the invasive properties of this cells. So far, our data suggest a pro-metastatic of ZO-1. Experiments are ongoing to determine the potential target genes of ZO-1 signalling in our models

7: Charron Sébastien
presenting author ; e-mail : sebastien.charron@umh.ac.be

WNT AND FOXO SIGNALING PATHWAYS IN FSHD

Sébastien Charron, Céline Vanderplanck, Alexandra Belayew and Frédérique Coppée

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Facioscapulohumeral muscular dystrophy (FSHD) is a dominant disorder with a prevalence of 1 in 20,000 birth. It is characterized by muscle degeneration and atrophy progressing asymmetrically in an antero-posterior gradient, affecting first the face, followed by the scapulae and the foot dorsiflexors. At the age of 45, 20% of patients are wheelchair-dependent. Primary myoblasts derived from FSHD muscle biopsies are more sensitive to an induced oxidative stress than control myoblasts and present a differentiation defect.

The genetic defect is linked to contractions of a 3.3-Kb repeated element named D4Z4 in the 4q35 subtelomeric region. In non affected individuals there are at least 11 copies of the D4Z4 unit while patients only have 1 to 10 copies left. The molecular mechanism of FSHD is complex and involves a change in chromatin structure that upregulates the expression of several proximal genes. Our group has identified the DUX4 gene within each D4Z4 element and the homologous DUX4c gene proximal to the repeat array. DUX4 is a transcription factor that targets a large set of genes in primary myoblasts, among which PITX1 expressing another transcription factor that is specifically induced in FSHD, and MYOD that is inhibited, leading to differentiation defects.

Several targets of the WNT and FOXO intracellular signaling pathways are found among the hundreds of genes dysregulated in FSHD. We want to study their contribution to the FSHD phenotype and their link with DUX4/DUX4c/PITX1 expression. We first evaluated the WNT pathway status with a luciferase reporter vector. This vector contains TCF/LEF transcriptional response elements linked to a firefly luciferase reporter gene. TCF/LEF is directly activated by beta-catenin, the key element of an acitvated WNT pathway. Upon transfection of C2C12 mouse myoblasts with a DUX4 expression vector, we observed a 30-fold increase of luciferase activity as compared to controls.

In a second experiment, we analyzed the expression of Atrogin 1 (MAFbx) and MuRF1, two E3 ubiquitin ligases specifically induced by FOXO during muscle atrophy. Their expression was increased both in FSHD myoblasts and in control myoblasts transfected with a DUX4 expression vector as compared to controls.

In conclusion this study showed an activation of the WNT pathway in FSHD myoblasts and suggested a role for DUX4 in this process. In addition we showed that DUX4 could activate genes involved in muscle atrophy.

8: Close Pierre
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PURIFICATION OF NUCLEAR RNPS, CHARACTERIZATION OF THEIR CONTENT AND FUNCTION IN TRANSCRIPTION

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The production of functional messenger RNA is a complex process that comprises of not only RNA synthesis by RNA polymerase II but also multiple RNA processing steps, such as capping, splicing and polyadenylation. Accumulating evidence suggests that many of these latter events occur co-transcriptionally and that processing factors are often recruited to genes as they are transcribed. Further, it is becoming clear that the nascent RNA itself recruits factors involved in its own transcription and processing. In this work, we have developed a protocol to purify nuclear nascent mRNP particles by tagging the most abundant hnRNP protein, hnRNP A1, and maintaining RNA integrity throughout. Interestingly, we found that most of the 3' end processing and termination factors co-purified with nuclear mRNPs and that disrupting mRNP formation may lead to an excess of RNAPII pausing at the 3' end of human genes. Furthermore, we identified a new uncharacterized protein included in early nuclear mRNPs that also interacts with RNAPII. We propose that this newly described protein may link mRNP formation to RNAPII transcription.

9: Delval Stéphanie
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ON THE MODE OF ACTION OF THE HOXA1 TRANSCRIPTION FACTOR IN MAMMARY CARCINOGENESIS

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Hoxa1 is a transcription factor of the homeotic (Hox) family of homeodomain proteins involved in patterning embryonic territories and governing organogenetic processes. In addition, Hoxa1 has been shown to display oncogenic activity and to be overexpressed in the mammary gland in response to a deregulation of the autocrine growth hormone (GH). It has therefore been suggested that Hoxa1 plays a pivotal role in mammary carcinogenesis under those conditions of GH misregulation. While the functions of Hoxa1 have been well investigated, its mode of action under normal and pathologic situations remains poorly documented.

The Hoxa1 protein interacts with Pbx transcription factors by the required intermediate of a hexapeptide motif. Replacement of conserved WM amino acids by AA in this hexapeptide (Hoxa1WM-AA mutant) results in a loss of Hoxa1 function during embryonic development. This suggests that the normal embryonic activity of Hoxa1 relies on its partnership with Pbx. We now assess the importance of this partnership for the oncogenic activity of Hoxa1.

Expression vectors for Hoxa1WM-AA and wild type Hoxa1 have been stably transfected in human breast MCF7 cells. MCF7 cells are transformed cells which keep several epitheloid characters so they define a widely used model to study key processes of oncogenesis in vitro. Cellular clones for Hoxa1 or Hoxa1WM-AA expression have been obtained. They have been validated for Hoxa1 and Hoxa1WM-AA expression, as well as for the proper nucleus localization of the proteins. The different clones are now characterized for proliferation, resistance to apoptosis, anchorage-independent growth and contact inhibition,… Transcriptional activity assays were also performed in cellular clones with a EphA2 enhancer-luciferase reporter gene which revealed that the wild type Hoxa1 protein is transcriptionaly active in the clones, in combination with Pbx1a and Prep1, whereas Hoxa1WM-AA is not.

Results show that while the wild type Hoxa1 protein induces an enhancement of cell proliferation, Hoxa1WM-AA does not. Quantification of the anti-apoptotic gene expression Bcl2 seems to show a higher expression in cells expressing Hoxa1 compared to Hoxa1WM-AA. Our in vitro data therefore support the hypothesis that the interaction between Hoxa1 and Pbx is critical for the Hoxa1 mediated mammary carcinogenesis. Clones tumorigenicity will soon be assessed in vivo by subcutaneously inoculating cells in nude mice.

10: Deroanne Christophe
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RhoC, BUT NOT RhoA, IS INVOLVED IN PROSTATE CANCER CELLS TUMORIGENESIS THROUGH GSK3beta-DEPENDENT REGULATION OF NAG-1

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The small GTPases of the Rho family are key elements in signal transduction pathways. Activated RhoGTPases interact with effectors that trigger a wide variety of cellular responses. Within the RhoA-related sub-class, both RhoA and RhoC, in contrast to RhoB, are reported to contribute to cancer progression. To gain insight into the individual role of RhoC and RhoA in prostate cancer cells (PC-3) phenotype, we used siRNA to specifically knock-down each of them. The silencing of RhoC, and not that of RhoA, significantly decreased the anchorage-independent growth of PC-3 and increased the expression of several genes encoding tumor suppressors such as NAG-1, p21Cip1 and p8. This transcriptomic analysis also highlighted the differential regulation of SPARC, a matricellular protein, by RhoA and RhoC. Interestingly, the transfection of RhoC-silenced PC-3 with a siRNA targeting NAG-1 reversed most of these gene regulations and restored the anchorage-independent growth of PC-3. Tumor growth of PC-3 in nude mice was significantly delayed by intratumoral injection of siRNA targeting RhoC, an inhibitory effect relieved by co-injecting siRNA targeting NAG-1. The up-regulation of NAG-1 following RhoC silencing requires p38MAPK and GSK3beta but not Erk1/2. These results suggest that RhoC contributes to tumorigenesis of PC-3 through the repression of NAG-1 expression.

11: Desmet Déborah
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EFFECT OF INTERMITTENT HYPOXIA ON NRF2 AND NF-kB ACTIVITY IN ENDOTHELIAL CELLS

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Intermittent hypoxia (IH) occurs in solid tumors due to transient blood flow. It has been shown to induce oxidative stress in cancer cells, as well as in endothelial cells. In this work, we investigated the specific effects of IH, compared to continuous hypoxia (CH), on Nrf2 and NF-kappaB, two transcription factors involved in the response of cells to oxidative stress, in endothelial cells. EAhy926 endothelial cells were incubated under IH (4 cycles of 1 hour hypoxia followed by 30 minutes reoxygenation), or under CH for 6 hours. We showed that in EAhy926 endothelial cells, IH, to a higher extent than CH, induced Nrf2 nuclear translocation while they had no effect on tBHQ induced nuclear translocation. Nevertheless, Nrf2 DNA binding activity and transcriptional activity were reduced under IH and CH, when stimulated by tBHQ or SIN-1. NF-kappaB nuclear abundance was increased under CH, but not under IH. When NF-kappaB was activated by TNFalpha, CH and IH increased nuclear translocation, DNA binding activity and transcriptional activity of the transcription factor. The secretion of IL-8 cytokine was increased in the same conditions. These results suggest than IH could influence Nrf2 and NF-kappaB activity. It seems that oxidative stress induced by IH is high enough to induce inflammatory response via NF-kappaB up-regulation, while Nrf2 is not sufficient to counter it. A pro-inflammatory environnement could favour the propensity of endothelial cells to angiogenesis, leading to tumour growth and metastasis.

12: Detilleux Julien
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INFLUENCE OF MEMBRANE TYPE 1 MATRIX METALLOPROTEASE EXPRESSION ON TGF-BETA SIGNALING IN HUMAN BREAST ADENOCARCINOMA CELLS

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Introduction:

Matrix metalloproteases (MMPs) constitute a family of proteolytic enzymes that are involved in tumor and metastatic progression. MMPs are divided into two groups: secreted MMPs and MMPs linked to the cell membrane (MT-MMPs). Among MT-MMPs, MT1-MMP is the most overexpressed MMP in human tumours. Moreover, the expression of MT1-MMP by the cancer cells promotes tumoral growth, metastatic dissemination as well as the acquisition of an angiogenic phenotype. These observations show that MT1-MMP is a potential target for the development of new anti-tumoral therapies. However, the exact mechanisms by which MT1-MMP affects tumor progression remain poorly defined. To better characterize the involvement of MT1-MMP in the tumor progression, our laboratory has compared, by using DNA microarrays, the transcriptomes of human mammary adenocarcinoma cells (MCF7), expressing (MT1) or not (VEC) the human MT1-MMP.

Results:

Interestingly, the results show a modulation of several components of TGF-beta signaling. TGF-beta signaling is involved in diverse cellular processes such as proliferation, adhesion, apoptosis, motility and differentiation. MT1-MMP expression decreases the expression of signaling components such as THBS1 (an activator of latent TGF-beta), TGF-beta 2, DAB2 (that allows the connection between SMADs and TGF-beta receptor) and SMAD 3 and increases the expression of inhibitors such as Inhibins (that prevent the connection of TGF-beta to its receptor) and KLF4 (that prevents the interaction between SMAD2 and SMAD4). Globally, MT1-MMP expression decreases TGF-beta signaling as confirmed by a TGF-beta reporter gene assay. In breast cancer cells, a decreased TGF-beta signaling induces a more epitheloid morphology. This morphology is characterized by less elongated cells and more compact colonies. Video-microscopy analyses demonstrate that the elongated cells are migrating. This more cohesive morphology induced by MT1-MMP expression is also observed in MCF-7 cells transfected with a mutated MT1-MMP lacking its intra-cytoplasmic domain (Delta-CD) but not in cells transfected with a catalytically inactive mutant (Inactive). Furthermore the treatment of MT1 cells with a synthetic inhibitor of MMPs (BB-94) reverses the cohesive morphology. In conclusion, the more cohesive morphology is dependent of the catalytic activity but not of the intra-cytoplasmic domain. In addition, blocking TGF-beta signaling in control cells by using a specific inhibitor of TGF-beta receptor kinase (SB-431542) is sufficient to recapitulate the more cohesive morphology induced by MT1-MMP, demonstrating the implication of this signaling pathway in the MT1-MMP dependent morphological alterations.

13: El Hour Mehdi
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HIGHER SENSITIVITY OF ADAMTS-12-DEFICIENT MICE TO TUMOR GROWTH AND ANGIOGENESIS

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ADAMTS (A Disintegrin And metalloproteinase domain with ThromboSpondin motifs) constitute a family of endopeptidases related to matrix metalloproteinases (MMPs). These proteases have been largely implicated in tissue remodelling and angiogenesis associated with physiological and pathological processes. To elucidate the in vivo functions of ADAMTS-12, we have generated a knockout mouse strain (Adamts12-/-) in which Adamts12 gene was deleted. The mutant mice had normal gestations and no apparent defects in growth, life span and fertility. By applying three different in vivo models of angiogenesis (malignant keratinocyte transplantation, Matrigel plug and aortic ring assays) to Adamts12-/- mice, we provide 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 host tissue were increased. Opposing results were obtained by using medium conditioned by cells overexpressing human ADAMTS-12 which inhibited vessel outgrowth in the aortic ring assay. This angio-inhibitory effect of ADAMTS-12 was independent of its enzymatic activity since a mutated inactive form of the enzyme was similarly efficient in inhibiting endothelial cell sprouting in the aortic ring assay than the wild type form. Altogether, our results demonstrate that ADAMTS-12 displays anti-angiogenic properties and protect the host towards tumor progression.

14: Gengoux Emily
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BASAL AND INDUCED RELEASE OF sVEGFR-2 BY ENDOTHELIAL CELLS

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Angiogenesis is essential for successful placentation and for tumor growth. Among the various angiogenic factors, we are particularly interested in the VEGF family and their receptors. They are key players for normal endothelial function. The two main VEGF receptors implicated in angiogenesis are VEGFR1 and VEGFR2. A variant transcript of VEGFR-1 lead to a soluble form: sVEGFR-1. It is detected in plasma of pre-eclamptic women, during ischemia and in some cancer cases. A soluble form of VEGFR-2, named sVEGFR-2, is detected in plasma of healthy people, in leukaemia and in systemic lupus erythematosus cases. However, this form remains uncharacterized and its physiological or pathological role is still unknown.

The aim of this study was to characterize the mechanisms leading to sVEGFR-2 release by endothelial cells (EC). In that view, we first characterized the cellular and soluble forms of VEGFR-2 in basal and phorbol 12-myristate 13-acetate (PMA)-induced conditions. Our results show that, at the cellular level, EC produce not only a mature (230kDa) form and an immature (210 kDa) form of VEGFR-2, but also a smaller N-terminal intracellular protein of 160 kDa. EC also release sVEGFR-2 (160 kDa) in their supernatant. PMA stimulates the release of sVEGFR-2 and concomitantly, it increases the expression and production of the different forms of VEGFR-2 (230kDa, 210kDa and intracellular 160kDa).

There are three main described mechanisms that could be involved in the generation of soluble receptors: 1. the shedding of the ectodomain of the membrane-associated receptor, 2. the alternative splicing, and 3. the release of exosome-like vesicles. Recent data of Swendeman et al. described the implication of the ADAM-17 in the ectodomain shedding of sVEGFR-2. In our experimental conditions, we have evaluated the effect of BB94 (a synthetically inhibitor of a broad spectrum of MMPs and ADAMs) and of TIMP3 (a natural inhibitor of ADAM-12, -17 and -19) on the basal and PMA-induced secretion of sVEGFR-2. BB94 and TIMP3 only partially inhibit the secretion of sVEGFR-2, both in basal and in PMA-stimulated conditions. These results show that MMPs and ADAMs contribute at least in part to the secretion of sVEGFR-2. However the production of sVEGFR-2 cannot be completely explained by the activity of ADAM17. Furthermore, PMA is known to induce a signal transduction pathway involving the protein kinase c (PKC). The PKC inhibitor, GF109203x, doesn't affect the basal release of sVEGFR-2, however, it inhibits the PMA-induced one. These results indicate that the basal release of sVEGFR-2 is not PKC-dependant.

In conclusion, our results strongly suggest that the release of sVEGFR-2 by EC relies on different cellular mechanisms and cannot be completely explained by the shedding activity of ADAM17. Particularly, the implication of the N-terminal intracellular form of VEGFR-2 of 160 kDa remains a matter of investigation.

15: Gillard Magali
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DEFECTIVE CELL MIGRATION IN MELANOMA-DERIVED CELL LINES DEFICIENT FOR THE ALPHA-TUBULIN ACETYLASE COMPLEX ELONGATOR

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IKAP/hElp1 protein (human Elp1) is the largest subunit of Elongator and is required for the functional integrity of this complex. The histone acetyl transferase activity of Elongator helps the transcriptional machinery to move on the template of particular genes to transcribe. Recent work in our laboratory demonstrated that IKAP/hElp1 deficient cells have defects in cell migration. We have also established a link between a lack of tubulin acetylation and the loss of the Elongator integrity.

To characterize the molecular mechanisms that link Elongator to cell migration, we have first generated a cellular loss of function model of Elongator by targeting the transcript of the IKAP/Elp1 through RNA interference in a melanoma (B16-Fl)-derived cell line. In vitro, we observed that cells deficient for IKAP/hElp1 have delayed migration. We also observed a decrease in the level of acetylated alpha-tubulin in those cells. Experiments of cell growth in soft agar showed that cells deficient for Elongator proliferate slower than control cells. We are developing a three dimensions culture system to characterize the role of Elongator in cell-matrix interactions. We are now crossing conditionally invalidated mice for Elp3 through homologous recombination with Tyr-CRE transgenic mice in order to assess the biology of the melanocytes deficient for Elp3 in vivo. Tumour formation and progression will also be investigated in Elp3-deficient mice that express an oncogenic Ras mutant in melanocytes.

New substrates of Elongator will be characterized in control or transformed melanocytes deficient for Elp3 in order to define the ones whose acetylation is required for the invasive and metastatic potential of melanomas.

16: Haase Günther
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THE CELLPROLIFERATION AND APOPTOSIS IN THE PRESENCE OF AMINO-ACIDS IN ORGANOTYPIC CULTURE OF TISSUES OF DIFFERENT AGE

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The regulation of repair processes in tissues of the organism through the stimulation of cellproliferation or it´s inhibition in apoptosis is accomplished by regulatory peptides. An information on different effects of amino-acids on some cellular processes is currently appearing. The most adequate method for quantitive determination of the effect of preparations being examined is the organotypic culturing of tissues, changes in the amount of cells in explant growth zone serving as a criterion of the screening evaluation of the biological activity of substances. The effect of 20 L-amino-acids on the dynamics of the development of spleen-, liver- and braincortex explants from 1 and 21 days old rats on organotypic tissue cultures were studied. The area index (AI) was calculated in arbitrary units as ratio of the area of the whole explant to the area of the central zone. Asparagine, lysine, arginine and glutamic acid have the oppositely directed effects, that depend on the degree of the tissue maturity on these processes. At concentrations of 0.05ng/ml these amino-acids produce an inhibitory effect on the cell proliferation in immature tissues (1 day old rats) by 28-35% compared with the controll. The same four amino-acids induced an opposite effect on the explants of mature tisues. They stimulated cell proliferation. AI was greater by 32-40% compared with the controll. An immunohistochemical assay of the expression of proapoptotic protein p53 in the zone of these explants from 21 days old rats revealed that the introduction into the culture media with these special amino-acids did not affect the expresion of p53. In 1 day old rats a sharp increase in the area of expression of p53 was observed by 52-120% compared with the control. One can suppose that 4 out of 20 amino-acids encoded by DNA can regulate cell proliferation and apoptosis in these special tissues at different stages of the organisms development. These fine results were then tested on patients with different chronic infections, tumors and also Tchernobyl victims in different hospitals in St.Petersburg, Kiew and Orenburg/Sibiria with extraordinary good results. This led to a patent.

17: Johnen Nicolas
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SPATIO-TEMPORAL LOCALIZATION OF INTERMEDIATE FILAMENTS IN THE ORGAN OF CORTI BETWEEN THE EMBRYONIC DAY 18 (E18) AND THE POST-NATAL DAY 15 (P15) IN RAT

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The mammalian auditory organ, the organ of Corti (OC), is composed of mechanosensory hair cells and nonsensory supporting cell types. based on their morphology and physiology, a least four types of supporting cells can be identified in the OC: inner pillar cell, outer pillar cell, phalangeal cell and Deiter's cells. The structure of this organ is well reported in adult but its development is still little known.

Using antibodies directed against different proteins of intermediate filaments cytoskeleton, we studied the spatial-temporal localization of cytokeratins (typical of epithelial cells) and vimentin (typical of mesenchymal cells) during the differentiation of the OC in rat from the embryonic day 18 (E18) to the postnatal day (P15).

Whatever the antibody used, we observed an obvious labelling over the supporting cells after the birth. In particular, an intense labelling is observed in the pillar cells and in the Deiters' cells at P8 and at P10.

These results suggest that the epithelial-mesenchymal transition might be implicated in the opening of Corti's tunnel between the pillar cells and the formation of the Nuel's spaces between the Deiters' cell and their outer hair cells, at P8 and at P10 respectively.

18: Kahr Irene
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FUNCTIONAL ANALYSIS OF THE CANDIDATE TUMOR SUPPRESSOR PROTEIN PROTOCADHERIN-10

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Protocadherins constitute the largest subgroup in the cadherin superfamily of adhesion molecules. They have six or seven cadherin motifs tandemly repeated in their extracellular domain, but their cytoplasmic regions show no similarity at all to those of classical cadherins and are also very distinct from each other, suggesting a potential for interaction with various signaling pathways and/or cytoskeletal elements.

It has been shown that protocadherin-10 (Pcdh10) mediates homophilic cell adhesion in vitro as well as cell sorting in cell culture and in vivo (Hirano et al., 1999). However, Pcdh10-mediated adhesion should be weaker than that by classical cadherins, Pcdh10 has been implicated in cancer, as multiple human carcinoma and lymphoma cell lines as well as tumors show frequent promoter methylation of PCDH10. Ectopic expression of PCDH10 in tumor cells with methylated PCDH10 promoter strongly suppressed tumor cell growth, migration and invasion (Ying et al., 2006 and 2007).

We are currently generating conditional Pcdh10 KO mouse models to delete Pcdh10 in a tissue- and time-specific manner. On the one hand, a model in which all isoforms of Pcdh10 can be knocked out will be established. This mouse will then be crossed with different Cre mice as well as various tumor mouse models to elucidate the role of Pcdh10 in normal physiology as well as tumor formation and progression. Additionally, a second mouse model is generated, in which only the long isoforms are conditionally knocked out, what includes the ablation of two conserved cytoplasmic domains, CM1 and CM2. The latter mouse model will be used to explore the role of these conserved domains in various intracellular signaling pathways.

To identify new cytoplasmic interaction partners of PCDH10, we applied an innovative technology called MAmmalian Protein-Protein Interaction Trap (MAPPIT) (Eyckerman et al., 2001, Lievens et al., 2004). The first MAPPIT screen for interaction partners of the cytoplasmic domain of PCDH10 has meanwhile been completed and the results were analyzed in cooperation with the group of Prof. Jan Tavernier, Ghent University. In addition, Pcdh10 was used in a commercial Yeast 2-Hybrid (Y2H) screen (Hybrigenics). Unfortunately, until now both assays yielded only a few weak interactors. More recently, a Y2H screen was performed using a GAL4-based two-hybrid system. The positive clones from that screen are currently investigated and validated in more detail.

19: Kersse Kristof
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DISSECTING THE CONTRIBUTION OF CASPASE-1 TO INFLAMMATION

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Caspase-1 contributes to the inflammatory response via two independent signaling pathways. On the one hand it mediates the maturation of the pro-inflammatory cytokines pro-interleukin (IL)-1beta and pro-IL-18 through its proteolytic activity, while on the other the N-terminal caspase recruitment domain (CARD) is necessary and sufficient as activator of the nuclear factor of the kappa-enhancer in B-cells (NF-kappaB). The latter property is dependent on the interaction with Rip2, a CARD-containing serine/threonine kinase involved in specific NF-kappaB activating pathways. In the present study, we describe the identification and characterization of two residues, D27 and R45, contained respectively in the acidic and the basic patch of the caspase-1 CARD domain that contribute crucially in caspase-1-mediated NF-kappaB signaling. Although the basic patch represents the interaction interface for Rip2 binding and caspase-1 homodimerization, the exact contribution of the acidic patch in this signaling cascade remains elusive. However, the acidic patch appears to be only one important for the proteolytic activation of caspase-1 by the inflammasomes, a group of large, multimeric protein platforms whose assembly is triggered by a plethora of stimuli. In addition, analysis of the inhibiting properties of the caspase-1 CARD mutants per se revealed that the caspase-1 CARD D27 mutant can be used as a general caspase-1 inhibitor, blocking both NF-kappaB activation as well as pro-IL-1beta maturation, while the caspase-1 CARD R45 mutant specifically blocks caspase-1 mediated NF-kappaB activation. Hence, both represent useful tools for caspase-1 research.

20: Keutgens Aurore
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THE REPRESSING FUNCTION OF THE ONCOPROTEIN BCL-3 REQUIRES CtBP WHILE ITS DEGRADATION INVOLVES PSMB1 AND THE E3 LIGASE TBLR1

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The oncogenic protein BCL-3 activates or represses gene transcription through binding with the NF-kB proteins p50 and p52 and is degraded through a phospho- and GSK3-dependent pathway. However, the mechanisms underlying both its degradation and its ability to regulate gene transcription remain poorly understood. Parallel screenings involving both yeast-two-hybrid analysis and biochemical purification led to the identification of the proteasome subunit PSMB1 as a key molecule required for BCL-3 degradation. Those screenings also defined not only CtBP as a molecule required for the ability of BCL-3 to repress gene transcription but also TBLR1, an E3 ligase that is critical for BCL-3 degradation through a GSK3-independent pathway. Importantly, all interactions require unique motifs within the N-terminal part of BCL-3. Thus, our data defined a mechanism by which the LSD1/CtBP complex is required for the repressing ability of an IkB oncogenic protein and established a functional link between the E3 ligase TBLR1 and NF-kB.

21: Lecomte Julie
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CHARACTERIZATION OF BONE MARROW-DERIVED CELLS RECRUITED INTO MOUSE TUMORS

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During tumor evolution, the invasion of cancer cells into surrounding tissue is associated with considerable tissue remodeling called “cancer induced stromal reaction” or “desmoplastic reaction”. The main constituents of cancer stroma are inflammatory cells, small blood and lymphatic vessels, fibroblastic and myofibroblastic cells, and extracellular matrix components. Although roles of inflammatory cells and endothelial cells have been reported to be involved in tumor immunity and neoangiogenesis, those of fibroblasts which participate in cancer progression have not been fully elucidated yet.

Fibroblasts, which are widely distributed and play a key role in tissue fibrosis, represent a main source of interstitial collagens. This cell type is heterogeneous with respect to a number of phenotypic and functional features. This heterogeneity may arise not only from the cellular activation and differentiation processes but also from their different cellular origins. Fibroblasts and myofibroblasts, i.e. activated fibroblasts expressing alpha-smooth muscle actin, which produce collagen and extracellular matrix proteins constitute the “desmoplastic reaction” and have been suggested to represent an important player in tumor invasion.

In the present study, we investigated in vivo the putative contribution of bone marrow-derived cells into two different cancer types: malignant murine keratinocytes (PDVA and BDVII). Mice were engrafted with bone marrow isolated from transgenic mice expressing green fluorescent protein (GFP), and the different types of cancer cells were subcutaneous injected. Bone marrow-derived cells positive for GFP were detected in each type of tumor xenografts. This was confirmed by immunohistochemistry stained against GFP. There is no doubt that bone marrow-derived cells (BMDC) are recruited into tumors and participate in cancer progression. The mechanisms through which they contribute to tumor development are numerous: inflammation, angiogenesis and also stromal reaction. Interestingly, in the two types of malignant murine keratinocytes (PDVA and BDVII), bone-marrow derived cells were mostly localized in connective tissue bundles. These cells were fusiforms with a fibroblast-like morphology and were specifically associated with collagen deposition. The GFP+ bone marrow-derived cells express different fibroblastic/mesenchymal markers, such as alpha-SMA, Thy1, NG2, and FSP1.

Our results suggested that bone marrow-derived cells (GFP+) are efficiently recruited into tumor, expressed several fibroblastic markers in vivo, and actively take part in the desmoplastic reaction. We showed that alpha-SMA-expressing myofibroblasts can produce MMP13 and can participate in tissue remodelling.

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UNRAVELLING RIP4 SIGNALLING IN THE SKIN

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RIP4 belongs to a family of S/T kinases, the Receptor Interacting Protein (RIP) kinases, generally involved in activation of the transcription factors NF-kappaB and AP-1. RIP4 consists of an N-terminal kinase domain, an intermediate domain and a C-terminus containing 11 ankyrin repeats.

Overexpression of different RIP4 domains shows that the kinase domain is sufficient and necessary to activate NF-kappaB and AP-1. Furthermore, in contrast to other RIP kinases the RIP4 kinase activity is required for activation of these transcription factors. We could demonstrate that active RIP4 is phosphorylated and ubiquitinated. It is not clear which kinase is responsible for RIP4 phosphorylation. We could show that unphosphorylated RIP4 can homodimerise and autophosphorylate. In addition, our data suggest that RIP4 phosphorylation is a prerequisite for its poly-ubiquitination. By mutation analysis we could correlate RIP4 modification to its capacity to activate NF-kappaB and AP-1. The S residues of the T-loop, formerly claimed to be crucial for RIP4 activation, are not required for activation of NF-kappaB or AP-1.

Both NF-kappaB and AP-1 signalling are required for skin homeostasis and epidermal barrier function. NF-kappaB is also a key modulator of skin inflammation. RIP4-deficient mice are characterized by perinatal lethality due to suffocation because all external orifices are fused. Furthermore, they are born with a strong skin phenotype that includes hyperplasia, absence of stratum corneum, parakeratosis and abnormal development of hair follicles and sebaceous glands (Holland et al., Curr. Biol. 2002). In analogy with IKKalpa-deficient mice (Hu et al., 1999, Takeda et al., 1999), RIP4-deficient mice display a severe skin barrier defect, probably by lack of proper tight junction formation. However, the defects in skeletal development found in IKKalpha knockouts are not occurring in RIP4 deficient mice. In vitro, RIP4 overexpression in keratinocytes results in induction of terminal differentiation. This event is also dependent on RIP4 kinase activity. In view of these observations we are investigating which RIP4-dependent molecular pathway is involved.

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DISTRIBUTION, DIFFERENTIATION AND SURVIVAL OF INTRAVENOUSLY ADMINISTERED NEURAL STEM CELLS IN A RAT MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Transplantation of neural stem cells (NSC) is a challenging therapeutic strategy for treatment of neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS). In order to get insight into the potential of intravenous delivery of NSC in the central nervous system (CNS) affected by ALS, rat NSC marked with green fluorescent protein were injected in ALS rat model and their wild type counterparts. The injected cell fates were followed 1, 3 and 7 days after transplantation. The highest efficiency of cell delivery to CNS was found in symptomatic ALS (up to 15%), moderate in presymptomatic ALS (up to 10%), and the lowest in wild type animals (up to 2%). NSC injected in ALS animals preferentially colonized the motor cortex, the hippocampus, and the spinal cord, and their differentiation was characterized by decrease of nestin expression and the appearance of Map2, GFAP, O4, and CD68 positive cells. Moreover, in ALS rat model differentiation occurred earlier than in wt animals. Tumor necrosis factor (Tnf) administration increased CNS delivery of transplanted cells in wild type and presymptomatic, but not in ALS symptomatic animals. Moreover, Tnf-related increase in NSC differentiation and survival has been detected.

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IMPAIRED BLOOD-BRAIN AND BLOOD-SPINAL CORD BARRIERS IN MUTANT SOD1-LINKED ALS RAT

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Blood-Brain Barrier (BBB) and Blood-Spinal Cord Barrier (BSCB) impairment is an additional accident occurring during the amyotrophic lateral sclerosis (ALS) progression. In this work we aimed to decipher if BBB/BSCB leakage appeared before critical detrimental events and could serve as a marker preceding clinical symptoms. Three different BBB leakage markers: Evans Blue, IgG and hemosiderin, were used to look at the SOD1-linked ALS rat model at presymptomatic and symptomatic stages. Although IgG and hemosiderin could be detected at presymptomatic stage, Evans Blue extravasation which fits best with BBB/BSCB impairment could only be seen at symptomatic stages. BBB/BSCB impairment was further substantiate by showing at symptomatic stages decreased mRNA expression of ZO-1 and occludin as well as agrin, a basal membrane constituent. Electron microscopic data substantiate a toxic environment around endothelial cell and peri-vascular swollen astrocyte end-feet showing oedema-linked BBB opening.

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IDENTIFICATION AND CHARACTERIZATION OF DIRECT TARGET GENES OF THE MURINE HOXA2 TRANSCRIPTION FACTOR

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Hox genes code for well studied transcription factors involved in many developmental processes, including nervous system patterning. Paradoxically, Hox proteins remain poorly characterized regarding their mechanisms of action. The aim of our project is to study the mode of action of the murine Hoxa2 by identifying direct target genes and characterizing how Hoxa2 regulates them.

In a previous work, 32 target genes have been identified in a cellular model. With the aim to discriminate between direct and indirect Hoxa2 target genes within this set, two complementary strategies were established.

Firstly, an expression vector for an inducible Hoxa2 protein has been designed based on the removal of an “intein” excisable protein intron (Paulus, 2007). The goal is to control the Hoxa2 transcriptional activity under conditions of translation inhibition. Upon induction of the Hoxa2 activity, the addition of a translation inhibitor (e.g. cycloheximide) will prevent expression of secondary target genes elicited by potential transcription factor primarily activated by Hoxa2. As revealed by western blot analysis, the Hoxa2-intein fusion protein was correctly expressed in cellular models. In addition, after induction of intein excision by 4-hydroxytamoxifen, Hoxa2 showed nuclear localization. Unfortunately, transcription activation assays failed to prove that the spliced Hoxa2 was active. Additional work is under progress to figure out why the matured Hoxa2 cannot activate target genes.

Secondly, to regulate direct target genes, Hoxa2 needs to bind cognate DNA sequences. Chromatin immunoprecipitation (ChIP) allows identifying such sequences, which in turn reveals the target genes directly controlled by Hoxa2. Hoxa2-targeted ChIP was developed and adapted for COS7 simian cells and murine teratocarcinoma P19 cells differentiated along the neurectodermal lineage by retinoic-acid treatment. Although known Hoxa2 target enhancers are recovered by ChIP, we still need to optimize the method to decrease background immunoprecipitation of control non-target DNA.

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SOLUBLE FORMS OF VEGF RECEPTOR-1 AND -2 CAN PROMOTE VASCULAR MATURATION

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In the early steps of angiogenesis, endothelial cell (EC) proliferation leads to nascent vessel formation. These vessels are then stabilized by coverage with mural cells. Members of the VEGF family constitute key regulators of angiogenesis. sVEGFR-1 and sVEGFR-2 are soluble forms of the membrane-bound receptors of VEGF. They are overproduced in some pathology and they exhibit in vivo anti-angiogenic properties.

Development of anti-angiogenic therapies is an important challenge for the treatment of cancer and ocular or skin disorders characterized by excessive angiogenesis and abnormal vasculature. Besides triggering immature vessel pruning, anti-VEGF therapies are characterized by the induction of vessel normalization, particularly by increasing their pericyte coverage.

In this context, the aim of this study is to understand and characterize the role of sVEGFR-1 and sVEGFR-2 by identifying their potential implication in interactions between EC and mural cells. Our results show that sVEGFR-1 and sVEGFR-2 are not only acting as VEGF-trappers, but are involved in a dialogue between EC and mural cells that leads to mural cell migration. Our data provide new insights into mechanisms regulating physiological and pathological angiogenesis and vessel normalization observed under VEGF-targeted therapies.

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NEW TARGETS ISSUED FROM 2D-DIGE PROTEOME STUDIES IN MOUSE MODELS OF ASTHMA

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A complex network of interactions between inflammatory cells, peptidic mediators, extracellular matrix components and proteases is thought to be involved in the control of asthma-related inflammation and remodeling. To date, new mediators of proteic nature displaying significant activity in the physiopathology of asthma are still to be unveiled.

The main objective of this study was to uncover potential target proteins by using Two-Dimensional Differential In-Gel Electrophoresis (2D-DIGE) from placebo or allergen-exposed mice in airway inflammation and remodeling.

Gels were imaged with the Typhoon Variable Mode Imager and processed using the DeCyder Differential Analysis Software. Then, statistical analyses (Student's t-test) were applied to validate only proteins that demonstrate a significant change in abundance between control and treated samples. For mass spectrometry analysis, a preparative gel was run and a spot-picking list was generated. Finally, spots with significant variation have been identified by MALDI-TOF-TOF.

When studying proteins from placebo or allergen-exposed mice in 2D-DIGE fluorescence images, we identified a group of proteins that were modulated in the lungs from allergen-exposed animals in the acute inflammation protocol. Among those, we point out the protein, GRP78 (BIP) as being overexpressed in asthma. We also demonstrate that this protein is upregulated in allergen-induced inflammation by western blot and immunohistochemisry. The HSP 70 family comprises four highly conserved proteins, HSP 70, HSC 70, GRP 75 and GRP 78, which serve a variety of roles. They act as molecular chaperones facilitating the assembly of multi-protein complexes, participate in the translocation of polypeptides across cell membranes and to the nucleus, and aid in the proper folding of nascent polypeptide chains.

PDIA6, another protein identified, was underexpressed in mice exposed to allergen from airway inflammation model and this result was confirmed by western blot. PDIA6, a member of the PDI family, has two conserved thioredoxin domains (CXXC) that catalyze the formation, breakdown, and isomerization of disulfide bond between two cysteines. The precise in vivo functions of GRP78 and PDIA6 in airway inflammation and remodelling models are still unknown.

We conclude that determinins of the differential proteomic profile of lungs from control and allergen exposed mice using 2D-DIGE/MS turns out to be an excellent approach to identify new key biomarkers of lung inflammation.

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EFFECTS OF X-RAYS AND ALPHA PARTICLE IRRADIATIONS ON GENE EXPRESSION PATTERN IN HUMAN LUNG CARCINOMA A549 CELLS

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Radiotherapy has been in constant progress for the past century but few studies have been performed concerning hadrontherapy. In this work, we compared the effects of irradiations using X-rays or heavy charged particles (alpha particles) on gene expression in a human lung carcinoma cell line (A549). The X-rays radiations were carried out using a cobalt irradiator (250 kV) whereas a particle accelerator was used to produce the alpha particle beam (100 keV/_6,m) necessary for the hadrontherapy. For the latter, cells were irradiated vertically in an irradiation chamber with a monoenergetic beam homogeneous over a ~0.5cm² surface. Quantitative reverse transcription-polymerase chain reaction results showed that mRNA overexpression of proinflammatory cytokines, such as Tumor Necrosis Factor, Interleukin-6 and Interleukin-8, was higher when A549 were irradiated with alpha particles than with X-rays. This is consistent with a Relative Biological Efficiency (RBE) of alpha particles that can be 5 fold higher than X-rays RBE. Interestingly, p21 mRNA was dose dependently overexpressed by both types of irradiations. The overexpression was higher at short time (2h30) after X-rays irradiations compared to alpha particle irradiations while this profile was reversed 24h after irradiation. Further studies will be performed to complete these expression patterns as well as to obtain survival fraction curves.

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OCULAR ANTERIOR SEGMENT DYSGENESIS FOLLOWING DELETION OF P120CTN IN NEURAL-CREST STEM CELLS

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The development of the ocular anterior segment depends substantially on the periocular mesenchyme cells, which are derived predominantly from neural crest cells. Specific and differential cell adhesion is expected to be instrumental in induction, migration and differentiation of neural crest cells. Here we report that one of the cell adhesion components, p120ctn, plays an important role during the development of anterior segment structure. Wnt1-Cre-mediated deletion of floxed p120ctn alleles in neural crest cells results in serious ocular anterior segment dysgenesis (ASD), such as iridocorneal angle closure, complete anterior chamber obliteration, iris and ciliary body hypoplasia, and cornea malformation and opacity. A complete penetrate phenotype is visible about three weeks after birth, but histological defects can be seen from the E18.5 stage. To investigate the underlying molecular mechanism, we performed gene expression analysis in the affected eyes. The results show that N-cadherin expression was significantly decreased in the iridocorneal angle cells and corneal endothelium, suggesting that abnormal cell sorting following N-cadherin downregulation is at the basis of the ocular ASD. These data indicate that by maintaining the stability of the cell adhesion complex, p120 is critical for ocular mesenchyme development. In view of these findings, p120ctn abnormalities might have a role in the pathophysiology of mammalian eye development

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EFFECTS OF A MUTATION IN LESSEN/TRAP100 ON THE EXPRESSION OF SEROTONIN AND NEURONAL NITRIC OXIDE SYNTHASE IN THE ENTERIC NERVOUS SYSTEM

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The gastrointestinal tract exhibits a variety of movements that depend on the region studied and the timing and composition of the last meal. Primary control of these movements is exerted by the enteric nervous system. Different neuromodulators regulating excitatory and inhibitory reflexes, are involved. Nitric oxide, produced by neuronal nitric oxide synthase (nNOS), has been identified as an important inhibitory neurotransmitter causing relaxation, while the proposed role of serotonin (5HT) in the gut is associated with increased gastrointestinal motility. In this study, we have examined the expression of nNOS and 5HT in the enteric nervous system of zebrafish lessen/trap100 (lsn) mutants that exhibit some of the characteristics seen in patients with Hirschsprung's disease. Hirschsprung's disease is a congenital disease characterized by the absence of ganglia in the enteric nervous system of the distal part of the gastrointestinal tract, and an impaired motility of the intestine.

Using a double immunolabelling method, wild-type (WT) and lsn zebrafish embryos at 96 hours post fertilization were quantitatively analysed over the three functional regions of the zebrafish intestine: the proximal (PI), middle (MI) and distal intestine (DI).

In the whole intestine of mutant zebrafish, a significant reduction in the total amount of neurons (labelled with the panneuronal marker Hu) was observed (±40%). This reduction was most pronounced in the DI (±80%). In contrast, there was no significant reduction of neurons in the PI. Serotonergic neurons were found in significantly lower numbers in the PI and MI and were absent in the DI of mutant fish. The proportion of serotonergic neurons was significantly decreased in mutants in all parts (WT vs lsn: ±25% vs ±15% in the PI and ±20% vs ±3% in the MI). The overall amount of nitrergic neurons was reduced (±40%), but the proportional expression in the PI and MI remained similar (±40%).

These results reveal that the effect of the mutation in lsn on the amount of neurons is more pronounced in the DI, corresponding with what is observed in Hirschsprung's disease. While the proportion of serotonergic neurons is affected in lsn mutants, the proportion of the nitrergic population is not. Previous studies have shown that 5HT is involved in the initiation of motility. Although the large reduction of neurons in the DI will have the most prominent influence on the intestinal motility, it is hypothesised that the reduced proportion of serotonergic neurons in lsn mutants affects the development of motility patterns in the PI and MI by onset of oral feeding. Further studies are necessary to support this hypothesis.

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GENE METHYLATION PROFILING FOR BREAST CANCER SUTYPE DISCRIMINATION USING ILLUMINA'S INFINIUM TECHNOLOGY

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In this study, we sought to determine whether inflammatory breast cancer (IBC) can be distinguished from non-IBC based on gene-specific patterns of aberrant methylation. A total of 72 DNA samples were analysed using the HumanMethylation27 DNA analysis BeadChips, which interrogate 27,578 highly informative CpG sites spanning more than 14,000 genes. Samples were derived from 19 IBC, 43 non-IBC and 10 normal breast tissues from healthy women. Beta-values, representing the ratio of the intensity of the methylated bead type to the combined locus intensity, were recorded for each CpG site via the BeadStudio software. Unsupervised hierarchical clustering analysis of the 1,000 beta-values with greatest standard deviation in the total sample population revealed that the 10 samples from normal breast tissues clustered together on terminal branches. A t-test with FDR<0.1 identified 15,833 CpG sites to have different beta-values in normal and cancerous samples. For 74% of these CpG sites, beta-values were increased in breast cancer. For each CpG site, we calculated the median beta-value in the control population and this value was used to normalise beta-values in the cancer population. Unsupervised hierarchical clustering of the 1,000 normalised beta-values with the greatest standard variation in the cancer population identified 4 robust clusters (average silhouette width of 0.085, P<0.05). A Fisher's exact test demonstrated that sample clustering was associated with M status (P=0.026), patient age (P=0.049) and tumour grade (P=0.057). No associations were detected between sample clustering and ER, PR, HER2 status, tumour subtype (IBC or non-IBC) or stage. The mean methylation index (# of normalised beta-values >0 / total # of CpG sites) in the tumour samples was 0.67. One-way Anova analysis revealed significant differences in methylation indices between sample clusters (P=0.002). After filtering out CpG sites for which absolute normalised beta-values were larger than 0.2 in 25% of tumour samples, a t-test identified 515 CpG sites to be differentially methylated between IBC and non-IBC and 516 CpG sites to be differentially methylated between M+ and M0 samples. This is the first genome-wide approach to identify gene-specific alterations in IBC. Using hierarchical clustering, methylation patterns could not classify IBC. However, we did find about 500 CpG sites to be differentially methylated between IBC and non-IBC. Further analysis is necessary to unravel the biological importance of this observation.

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GENE EXPRESSION ANALYSIS OF MAS-RELATED GENE (Mrg) RECEPTORS IN THE NORMAL AND INFLAMED MURINE ILEUM

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Mas-related gene (Mrg) receptors are a complex family of G protein-coupled orphan receptors exhibiting considerable interspecies differences. In mice, approximately 50 Mrg receptors are reported of which 20 are sequenced. A subset of these Mrg receptors is expressed in sensory neurons.

Due to the lack of detailed data on the gastrointestinal expression of Mrg receptors, we aimed to investigate their presence and distribution in the murine ileum and their involvement in neuroimmune interaction during intestinal inflammation. We used two animal models of intestinal inflammation, namely intestinal schistosomiasis and 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced ileitis. To unravel which Mrg receptors are differentially expressed in the ileum and to obtain a more extensive view on the affected molecular pathways in the control versus inflamed animals, we performed a gene expression analysis of the full transcriptome using the Agilent Whole-Mouse Genome Oligo Microarrays, which consisted of about 44,000 probes. Additionally, the relative expression levels of the 20 Mrg receptors were quantitatively analysed using Real-Time PCR. Furthermore, immunohistochemical analyses with the available commercial antibodies directed against MrgE and MrgF, were performed on cryosections and whole-mounts.

Microarray analyses resulted in 281 and 332 significantly dysregulated genes in the acute phases of intestinal schistosomiasis and TNBS-induced ileitis respectively. Cytokine-cytokine receptor interaction and neuroactive ligand interaction pathways were highly affected in both models. No altered gene expression levels of any of the Mrg receptors were detected, whereas Real-Time PCR revealed significant increased expression of MrgA4, A7, B1, B2 and D during intestinal schistosomiasis and of MrgA2, A4, A5 and A7 during TNBS-induced ileitis. The mRNA levels of MrgE and F were significantly reduced during TNBS-induced ileitis. The reason for not being able to detect differential gene expression of Mrg receptors using microarray analysis was probably due to low copy numbers of their mRNAs. Immunohistochemical staining showed that both MrgE and MrgF were expressed in a subpopulation of enteric neurons.

In conclusion, the present data indicate that, in accordance to what has been proposed previously, some Mrg receptors appear to be involved in inflammatory responses in both inflammation models we examined.

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IDENTIFICATION OF DIFFERENTIALLY EXPRESSED PHOSPHORYLATED PROTEINS IN AN ANIMAL MODEL OF MULTIPLE SCLEROSIS (MS)

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Introduction:

Multiple sclerosis (MS) is an inflammatory autoimmune disease of the central nervous system. The underlying molecular processes remain poorly understood, but are crucial in the search for new therapeutic options. Protein phosphorylation may be involved in the pathology of MS and experimental autoimmune encephalomyelitis (EAE), an animal model of MS. The aim of this study is to identify differentially expressed phosphorylated brain proteins in a quantitative way during EAE disease course.

Methods:

To analyze differentially expressed phosphorylated proteins, protein extracts from 'blood-free' brain stems of control and EAE Lewis rats were separated by 2D-gel electrophoresis. In order to detect phosphorylated proteins, these gels were stained with a fluorescent dye, Pro-Q diamond phosphoprotein gel stain (Invitrogen). The Ettan DIGE Imager (GE Healthcare) was used for scanning. Mass spectrometry was used for identification of phosphorylated proteins.

Results:

Post-gel total protein staining is used as quality control and thus normalization of the phosphoprotein signals. Moreover total protein staining is useful for matching different gels. Furthermore, we are able to study the expression profile of the identified phosphoproteins throughout the disease course through matching of the phospho-specific 2D-spotmaps with the spotmaps of a previous quantitative 2D-DIGE experiment (control, onset, top and recovery), in which 65 differentially expressed proteins were identified (significance: ANOVA smaller than 0.01 and at least 1.3 fold regulated). Thirty-seven of these proteins were matched to this experiment, of which 8 are probably phosphorylated.

The identification of a panel of differentially expressed phosphorylated proteins in the disease course, could provide information about the global disease processes in EAE and MS. Protein phosphorylation is of great importance to the protein function. This global overview of phosphorylation during disease invites new studies to unravel the complicated molecular biological processes in the pathology of MS.

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DECREASING CIAP1 PREFERENTIALLY SENSITIZES NECROPTOSIS INDUCED BY TNF

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Necrosis has for a long time been considered as an accidental and uncontrolled cell death type as a consequence of physico-chemical stress. Recently, it has become clear that necrotic cell death is as well controlled and programmed as caspase-dependent apoptosis, and that it may be an important cell death mode with important pathological and physiological relevance, and therefore the process has been renamed as necroptosis. Crucial players during TNF-induced necroptosis are the kinases RIP1 and RIP3. Several studies illustrated that, if RIP1 ubiquitination is impaired, RIP1 functions as a proapoptotic molecule. To address the role of the E3 ligases of RIP1, c-IAP1 and c-IAP2, and RIP1 ubiquitination we targeted these E3 ligases via chemical inhibition and RNA interference. Interfering with expression of c-IAP1 protein, strongly increased sensitivity to TNF-induced necroptosis both in mouse fibrosarcoma L929 cells and in the human T cell lymphoma cell line Jurkat deficient for FADD. In addition, this enhanced cell death was inhibited by knocking down RIP1 and RIP3. Necrosis induced by other death stimuli, such as hydrogen peroxide, anti-Fas plus zVAD-fmk and dsRNA (Poly(I:C) plus IFN-beta), were not affected in c-IAP1 lowered conditions, indicating that decreasing c-IAP1 protein preferentially sensitizes necroptosis induced by TNF.

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STRUCTURE-FUNCTION ANALYSIS OF THE UBIQUITIN-EDITING AND NF-KAPPAB INHIBITORY FUNCTIONS OF A20

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A20 is a negative feedback regulator of NF-kappaB activation in response to TNF, IL-1 and Toll-like receptors. A20-deficient mice demonstrate spontaneous inflammation, cachexia and premature death, due to persistent activation of NF-kappaB. Interestingly, A20 has two ubiquitin-editing domains mediating its NF-kappaB inhibitory function. The N-terminal ovarian tumor protein (OTU) domain shows deubiquitinating activity towards specific NF-kappaB signaling proteins (RIP1, TRAF6 and NEMO), whereas the C-terminal domain has ubiquitin ligase activity towards RIP1 and is characterized by seven zinc fingers (ZFs). These ZFs are known to mediate A20 dimerization and the binding of several other proteins such as ABINs, 14-3-3, NEMO, RIP1 and TAX1BP1. To further characterize the role and regulation of these ubiquitin-editing domains in the NF-kappaB inhibitory function of A20, we have performed detailed structure-function studies.

Expression of the A20 OTU domain as such was not sufficient to inhibit NF-kappaB activation in response to TNF, IL-1, poly(I:C) or LPS. Therefore, we hypothesized a role for dimerization of the OTU domain. However, inducible dimerization of the OTU domain by fusion to Gyrase B does not lead to NF-kappaB inhibition nor affects A20's deubiquitinating activity on NEMO. Interestingly, overexpression of the ZF containing domain of A20 on its own is sufficient to inhibit NF-kappaB activation. Moreover, we could demonstrate that both ZF4 and ZF7 contribute to the NF-kappaB inhibitory potential and ubiquitin ligase activity of A20. Further studies are concentrating on the mechanisms regulating the activity of ZF7, such as binding to ubiquitin and subcellular localization, as well as the effect of A20 ZFs on IRF activation.

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ABIN-3, TAX1BP1 AND A20, COOPERATING INHIBITORS OF NF-KAPPAB SIGNALING

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Stimulation of TLRs leads to the initiation of several signaling cascades including the NF-kappaB pathway. As NF-kappaB dependent gene expression mediates chronic inflammation, a predisposing factor for the development of pathologies such as neoplasia, cardiomyopathies, autoimmune disorders or cancers, a tight regulation of the NF-kappaB signaling pathway is crucial. Ubiquitination of signaling intermediates plays a key role in both positive and negative regulation of the NF-kappaB pathway. We recently showed that TAX1BP1 recruits the ubiquitin-editing protein A20 to both TRAF6 and RIP1, leading to their de-ubiquitination. Consistent with these observations, TAX1BP1-/- MEF cells demonstrate increased NF-kappaB activation in response to LPS, IL-1 and TNF. Interestingly, we found that TAX1BP1 also interacts with ABIN-3, an A20-binding inhibitor of NF-kappaB. ABIN-3/TAX1BP1 binding depends on the first ubiquitin-binding zinc finger (UBZ1) of TAX1BP1 and the ABIN homology domain (AHD) 3 in ABIN-3. Deletion of AHD3 or mutation of UBZ1 partially disrupts the ability of respectively ABIN-3 or TAX1BP1 to inhibit NF-kappaB activation. In addition, we show that depletion of TAX1BP1 (via RNAi or in knockout cells) impairs the NF-kappaB inhibitory potential of both ABIN-1 and ABIN-3. These data suggest that the potential of ABIN-3 and TAX1BP1 to inhibit NF-kappaB activation in response to TNF, IL-1 or LPS is dependent on their mutual interaction.