Fall Meeting 2012

EPIDERMAL CELL BIOLOGY

NAMUR, 13 October, 2012
FUNDP, Pedro Arrupe, Namur, Belgium

Scheduled speakers
Fiona Watt, Cambridge, UK
Wim Declercq, Gent
Patrizia Agostinis, Leuven
Christine Baldeschi, Evry, France
Cédric Blanpain, Bruxelles

Organizers
Yves Poumay
Catherine Lambert
Carine Michiels
Florence Chainiaux

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2012 Autumn meeting of the Belgian Society for Cell and Developmental Biology
October 13, 2012 in Namur, on *EPIDERMAL CELL BIOLOGY*

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MORPHOLOGICAL AND FUNCTIONAL CHARACTERIZATION OF ‘MAS-RELATED GENE RECEPTOR D’ IN THE HUMAN AND MOUSE INTESTINE REVEALS ITS MODULATORY ROLE IN THE INTESTINAL INFLAMMATORY RESPONSE.

Leela Rani Avula (1), Roeland Buckinx (1), Isabel Pintelon (1), Dirk Adriaensen (1), Ligia Craciun (4), Roberto Salgado (4), Joris De Man (3), Benedicte De Winter (3), Luc Van Nassauw (2), and Jean-Pierre Timmermans (1).

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Background: Mas-related gene (Mrg) receptors constitute a family of G-protein-coupled receptors and are mainly expressed in specific sub-populations of nociceptive sensory neurons. Although Mrg receptors have been suggested to be involved in the regulation of inflammatory responses and in neuro-immune communication in peripheral targets, their precise location and role in the gastrointestinal system during (patho)physiological conditions remain largely unexplored.

Aim: To unravel the expression and putative role of an experimentally attractive therapeutic candidate of the Mrg family, MrgD, in the human intestine and in two murine models of intestinal inflammation.

Methods: In order to provide potential clinical relevance, MrgD expression was explored in the human intestine by RT-PCR and immunohistochemistry. The functional role of MrgD was further investigated in two mouse models of intestinal inflammation (intestinal schistosomiasis and TNBS-ileitis), in wild-type and MrgD-/- conditions, using RT-PCR, immunohistochemistry, calcium live cell imaging, and through intestinal motility analysis.

Results: In the human intestine, MrgD expression was detected in neurons and mast cells. In mice, MrgD expression was found de novo in the inflamed ileum of both models, in sensory neurons in particular, as well as in recruited mucosal mast cells (MMCs) in intestinal schistosomiasis. MrgD-deletion led to increased MMC recruitment (25-35% increase in MMC density, P<0.01) in intestinal schistosomiasis, and to an altered neuropeptide expression (10-12% increase in calcitonin gene-related peptide expression, P<0.01) influencing mainly the intrinsic component of intestinal innervation in both models, but did not cause any effect on intestinal motility. Beta-alanine, a ligand of MrgD, evoked Ca2+ influx and also modulated MrgD-mediated ATP-induced Ca2+ influx in a subset of in vitro model of MMCs, i.e. in a subset of bone marrow-derived mast cells.

Conclusions: MrgD is expressed in neurons and mast cells in the human and mouse intestine. In mice, MrgD is involved in the regulation of MMC recruitment in intestinal schistosomiasis, and in sensory neuromodulation in both models, but does not play a significant role in gut motility. MrgD is activated by its ligand beta-alanine and possibly by ATP in MMCs in vitro. Our findings provide the basis for considering MrgD as a novel experimental therapeutic target in intestinal inflammatory pathologies.
THE INTRACELLULAR TRAFFICKING OF BSRP-A (BRAIN SPECIFIC RECEPTOR PROTEIN A) IS MEDIATED BY TWO TYROSINE-BASED MOTIFS LOCATED IN ITS C-TERMINAL TAIL.

Marielle Boonen and Michel Jadot.

URPHYM-Laboratoire de Chimie Physiologique, University of Namur.

Brain Specific Receptor Protein A (BSRP-A) is a type I transmembrane protein that is predominantly expressed in neurons. While the role of BSRP-A in these cells is still unknown, it has been reported that the knock-down in mice of the three members that comprises the BSRP family (BSRP-A, -B, and –C) impairs the synaptic maturation and results in motor dysfunction. In order to gain insight into the function of BSRP-A, we expressed BSRP-A fused to a myc tag into HeLa cells and studied its subcellular localization and trafficking by a combination of morphological and biochemical approaches. This revealed that a small fraction of the BSRP-A proteins is detected in vesicles that are positive for the lysosomal marker LAMP-2, and that BSRP-A partially localizes to the plasma membrane. Next, we investigated the putative role of the YXXPhi and NPXY-type motifs located in the C-terminal tail of BSRP-A, which are consensus sequences known to mediate the endo/lysosomal sorting of many membrane proteins. The mutation of the critical tyrosine residues of these motifs resulted in an increased proportion of BSRP-A at the plasma membrane, demonstrating the importance of these signals for the normal trafficking of the protein. Moreover, using 125-I-labeled anti-BSRP-A antibody to study the internalization’s rate of BSRP-A (and of our different mutants), we revealed that BSRP-A is endocytosed by the cells and that the NPXY motif is required for this process. Finally, we investigated the biosynthesis of BSRP-A in transfected HeLa cells and in the neuron cell line N1E-115 by metabolic labeling.
THE HUMAN AXILLARY ENVIRONMENT HARBORS 2 MICROBIOME ECOTYPES.

Chris Callewaert (1), Frederiek-Maarten Kerckhof (1), Michael S. Granitsiotis (2), Mireille Van Gele (3), Tom Van de Wiele (1) and Nico Boon (1).

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The human axillary vault is an ideal environment for a large number of microorganisms, which are of particular interest in the formation of body odour. Previous research performed on the axillary microbiome was mainly based on culture-dependent techniques. Here, a molecular-based study was conducted on 50 healthy subjects in order to describe the interpersonal, intrapersonal and temporal diversity of the axillary microbial community by means of pyrosequencing and denaturing gradient gel electrophoresis. Both techniques clearly designated the existence of two distinct armpit types, in which 65% of the cases Staphylococcus species, and 35% of the cases Corynebacterium species were the abundant microorganisms. Particularly the Corynebacterium species have been shown to contribute to axillary malodour. The clustering did not seem to depend on nationality or age. The gender had a substantial influence with the female subjects predominantly clustering in the Staphylococcus group. The deodorant usage had a considerable impact, with a linear increasing microbial diversity and richness, when a higher frequency of deodorant was applied. The intrapersonal diversity, when observing the left and right axillary community of one person, was generally low, with still the half of the total analysed axillary samples showing differences in band pattern, suggesting left-right asymmetry. When assessing the temporal diversity, a relatively stable pattern was perceptible, indicating a robust core axillary microbiome. Only 1 out of 27 followed-up subjects experienced a bacterial community switch, going from the Corynebacterium to the Staphylococcus armpit type. The enhanced understanding of the axillary microbiome is necessary to gain further insight into the malodour generation.
THE SCIN SIMULATION: A NOVEL IN VITRO TECHNIQUE TO STUDY THE ODOUR GENERATION OF MICROBIAL COLONIZATION ON THE SKIN.

Chris Callewaert (1), Benjamin Buysschaert (1), Mireille Van Gele (2), Tom Van de Wiele (1) and Nico Boon (1).

1: Ghent University, Ghent; 2: Ghent University Hospital, Ghent.

Bromhidrosis is a condition in which abnormal offensive body odour emanates from the skin, caused by bacterial breakdown of apocrine sweat secretions. Due to the poor understanding of the problem and the lack of a decisive diagnosis, research on this topic is minimal. In this research, an in vitro SCIN simulation (Skin Community INteraction simulation) was set up to simulate the microbial colonization on the skin in order to generate its typical odour. The focus was set on the axillary region, in which a representative solution of amino acids, salts and fatty acids was supplied, resembling the human axillary sweat. The fatty acids were obtained from acid hydrolysis of human subcutaneous skin fat, obtained from plastic surgery operation, with added squalene and corrected pH. The microbial communities were grown on an ex vivo human skin explant, obtained from plastic surgery operation, or on an agar surface. Denaturing gradient gel electrophoresis (DGGE) monitoring after 10, 15, 20 or 25 days resulted in an eminent resemblance of the different bacterial communities, normally present in vivo. A remarkable improvement was noted when comparing the SCIN artificial sweat with other artificial sweat solutions from ISO, EN and AATCC. To study the odour development, the headspace fraction was trapped with Tenax tubes or SPME fibers and analyzed on GC/MS. The investigations are still ongoing but seem promising to study the human body odour development.
AXILLARY BACTERIOTHERAPY AS A SUCCESSFUL TECHNIQUE TOWARDS BROMHIDROSIS.

Chris Callewaert (1), Tess Plaquet (1), Jessica Bostoen (2), Tom Van de Wiele (1) and Nico Boon (1).

1: Ghent University, Ghent;
2: Ghent University Hospital, Ghent.

Fecal transplantation or bacteriotherapy has been successfully applied in the human gut for the treatment of Clostridium difficile. In this research, we report a successful bacteriotherapy of the axillary microbiota to treat bromhidrosis. Bromhidrosis is a condition in which abnormal offensive body odour emanates from the skin, caused by bacterial breakdown of sweat secretions. A case study was performed on a monozygotic (identical) male twin who did not co-habit, in which one twin had a significant body odour and the other did not, as determined by a trained odour panel. Molecular analysis on their axillary samples showed significant differences, in which one twin had mostly Corynebacteria as dominant bacteria, while the other twin had mostly Staphylococci as dominant species in the axillae. Corynebacteria are linked to the generation of body odour as they possess the enzymatic capacity to convert long-chain fatty acids to typical odorous short-chain fatty acids, while Staphylococci do not possess these enzymes. Axillary bacteriotherapy was applied on one axilla of the odorous twin, using an axillary sample of the non-odorous twin. The other axilla of the odorous twin was not treated and used as a reference. Significant differences were seen for the hedonic value, the intensity and several odour characteristics on a short time scale after the treatment. Research is still ongoing to determine the effect on a longer time scale. Axillary bacteriotherapy seems a promising technique to treat bromhidrosis.
DETERMINATION OF THE MOLECULAR PLAYERS OF ADAPTATION TO ANTI-ANGIOGENIC THERAPY IN BREAST CANCER BY QUANTITATIVE PROTEOMIC AND HIGH MOLECULAR MALDI IMAGING.

Cimino J. (1,2), Sounni NE. (1), Calligaris D. (2), Debois D. (2), De Pauw E. (2) and Noel A. (1).

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2: Mass Spectrometry Laboratory, GIGA-R, Department of Chemistry, University of Liege, 4000 Liege.

Breast carcinoma is the most common and second leading cause of cancer mortality in women. The recognition of the “angiogenic switch” as a rate-limiting secondary step in tumorigenesis led to extensive pre-clinical researches on angiogenesis and finally the approval of VEGF-neutralizing antibodies (bevacizumab) and VEGF receptor tyrosine kinase inhibitors (RTKs:Sunitinib). The Sunitinib has been used clinically in patients with breast cancer refractory to other therapeutic agents. Unfortunately, like the cytotoxic therapies, these drugs do not produce lasting effects and resistance to treatment appeared clinically. Questions have emerged about the failure of anti-angiogenic therapy in clinic and the limitations of predictive preclinical models, and also about the molecular assessment of all stages of tumor adaptation and metastatic disease. To this end, we applied quantitative proteomics and imaging mass spectrometry tools to visualize and study the profiles of proteins and small molecules associated with tumor treated or not with Sunitinib using a novel preclinical model of breast carcinoma cells.

In this project, we first developed a reproducible model of resistance to Sunitinib of human triple negative breast cancer MDA-MB-231 cells expressing luciferase gene. Cells were subcutaneously injected into mice RAG1-/- and divided into four experimental groups including, control mice treated with vehicle or Sunitinib for 30 days and sacrificed 1 days after treatment withdrawal or when tumor reached a volume of 300 mm3. In the second step. Tumors were analyzed using a nanoAcquity UPLC Synapt TM HDMS TM G1 (Waters, Manchester,UK) and Mass Spectrometry Imaging. For quantitative proteomic analyses of tumors, a bioinformatics analysis was used with the Protein lynx global server 2.2.5 software. Imaging mass spectrometry was performed on tissue sections of tumors and organs subsequently colonized by metastases. Matrix sublimation was used to coat tumor sections (14 µm-tick) with 1.5 Diaminonaphthalene for lipids analysis and Sinapinic acid for entire proteins analysis. Ion cartographies were recorded with a Solarix 9.4T FTMS instrument for lipids and with an Ultraflex II TOF-TOF instrument for entire proteins (Bruker Daltonics, Germany) with a spatial resolution of 100 µm.

Global proteomic revealed different protein profiles between tumor treated or not with Sunitinib. The Mass Spectrometry Imaging detected differences in intensity and location of some proteins and lipids are also associated with some histological features including inflammatory, necrotic and angiogenic areas. Bioinformatics analysis will be applied to ensure the integration of all data in order to provide the basis for identifying molecular pathways activated during the acquisition of refractoriness to drug treatments.
RIPK4 IS REQUIRED TO PREVENT CADHERIN-MEDIATED EPITHELIAL FUSIONS CHARACTERISTIC FOR POPLITEAL PTERYGIUM SYNDROMES.

Philippe De Groote (1,2), Hong Thi Tran (2), Mathias Fransen (2), Corinne Rösselet (1,2), Bram De Craene (1,2), Giel Tanghe (1,2), Kirsten Leurs (1,2), Barbara Gilbert (1,2), Riet De Rycke (1,2), Christopher J. Guérin (1,2), Pamela Holland (3), Marek Haftek (4), Geert Berx (1,2), Peter Vandenabeele (1,2), Saskia Lippens (1,2), Kris Vleminckx (2) and Wim Declercq (1,2).

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Receptor interacting kinase 4 (RIPK4) is a member of the RIP kinase family that has been linked to epidermal differentiation because RIPK4-/- mice show aberrant epidermal differentiation associated with fusion of all external orifices. These fusions are similar to popliteal pterygium syndromes in humans, for which a genetic link with RIPK4 was recently reported. However, the mechanism underlying these phenotypes is unclear. We show that absence of RIPK4 results in fusion of several epithelia during development, leading to palatal clefting and skin webbing and found that RIPK4 prevents these fusions through its function in periderm maturation and by regulating E-cadherin membrane expression. Likewise, overexpression of RIPK4 in HaCaT keratinocytes results in cell scattering, characterized by E-cadherin internalization and cytoskeletal changes. In addition, RIPK4-/- mice display loss of cell polarity in the granular layer and show a severe skin barrier defect, as both the outside-in and the inside-out barriers are affected. In Xenopus, depleting zygotic RIPK4 results in a phenotype partially reminiscent to what is observed in mice and humans, while depletion of both maternal and zygotic RIPK4 causes defective gastrulation. The latter defect is also observed upon expression of dominant-negative IRF-6, a transcription factor also genetically linked to human popliteal pterygium syndromes. We found that IRF-6 controls RIPK4 expression and that wild-type, but not kinase-dead, RIPK4 can complement the gastrulation defect in Xenopus caused by IRF-6 malfunctioning. We provide a novel molecular link between IRF-6 and RIPK4 that unifies the different popliteal pterygium syndromes to a common molecular pathway.
THE EMT TRANSCRIPTION FACTOR ZEB2 PLAYS A MAJOR ROLE IN MELANOGENESIS AND METASTATIC MELANOMA.

Geertrui Denecker (1,2), Niels Vandamme (1,2), Joachim Taminau (1,2), Kelly Lemeire (2), Alexander Gheldof (1,2), Bram De Craene (1,2), Mireille Van Gele (3), Lieve Brochez (3), Mairin Rafferty (4,5), Girish Malya Udupi (4), Balazs Balint (5), William Gallagher (4,5), Lionel Larue (6), Jody Haigh (2,7), Jean-Christophe Marine (8,9) and Geert Berx (1,2).

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5: OncoMark Limited, NovaUCD, Belfield Innovation Park, University College Dublin, Belfield, Dublin 4, Ireland;
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7: Vascular Cell Biology Unit, Department for Molecular Biomedical Research, VIB, Ghent;
8: Center for the Biology of Disease, Laboratory for Molecular Cancer Biology, VIB, Leuven;
9: Center for Human Genetics, Katholieke Universiteit (KU) Leuven, Leuven.

The ZEB family of transcription factors are crucial for migration of neural crest cells and formation of their derivative structures during subsequent embryonic development. Detachment of single cells and invasion into the surrounding tissue are necessary for these processes which are also considered to be the driving forces in malignant tumor progression. To investigate the importance of ZEB2 during both melanocyte homeostasis and melanoma progression, we used the melanocyte specific Tyr-Cre mouse line to conditionally delete or overexpress ZEB2 in the melanocyte lineage. Our data clearly show that ZEB2 plays a crucial role in melanogenesis as melanocyte specific deletion of ZEB2 (ZEB2MC-KO) causes congenital loss of hair pigmentation. Interestingly, although melanoblast migration was severely affected by the absence of ZEB2, melanocytes were still able to reach the bulge and bulb area of the hair follicles, where they remain undifferentiated. In order to assess the contribution of ZEB2 in melanoma, we used the Tyr::N-RasQ61K preclinical mouse melanoma model in which we modulated ZEB2 expression in the melanocytes. Our results clearly indicate that the status of ZEB2 is strongly related with the formation of highly malignant melanoma which metastasizes to the lymph nodes, liver, lungs and the peritoneum. Knock down of ZEB2 in primary melanocytes results in an upregulation of ZEB1 and concomitant downregulation of melanocyte lineage markers, which reveals an intricate balance between these two ZEB-family members in the transcriptional regulation of melanocyte differentiation. Our data are also relevant for human melanomagenesis as the ZEB2 expression status is associated with melanoma ulceration and patient survival. In conclusion, we demonstrate that ZEB2 has a crucial role in the differentiation status and migratory behavior of both melanocytes and melanoma cells.
TMEM45A GENE : A NEW EPIDERMAL DIFFERENTIATION MARKER ?

Aurelie Hayez (1), Aleksandar Sekulic (2), Marie Reynier (3), Catherine Lambert de Rouvroit (1), Carine Michiels (4) and Yves Poumay (1).

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TMEM45A gene expression has been reported to favor chemoresistance of cancer cells (MDA-MB231 and HepG2 cells) in hypoxic conditions. To date, this gene and the protein it encodes have been poorly studied in normal or cancer cells regarding expression levels, localization and potential functions. Because differentiated keratinocytes are resistant to chemotherapy, we studied TMEM45A expression in normal human epidermal keratinocytes. With increasing densities of autocrine monolayers, TMEM45A mRNA expression level was found upregulated after confluence and concomitant with expression of markers of epidermal differentiation such as keratin 10 and involucrin. The concomitance with cell density was also independently revealed by a transcriptomic approach. In addition, upregulation of transmembrane protein 45A expression during terminal differentiation was noticed by immunohistochemical and Western blotting analysis. When keratinocytes at very low cell density were cultured in high calcium concentration that induces expression of differentiation markers, an increase in TMEM45A mRNA level was observed, suggesting that the gene expression is related to differentiation instead of cell density. In a culture model of Reconstructed Human Epidermis (RHE), TMEM45A mRNA expression is stable during tissue morphogenesis and transmembrane protein 45A is localized in the superficial layers of the RHE. In normal human skin, the protein is preferentially located in granular layer. Moreover, TMEM45A mRNA level is higher in granular keratinocytes-enriched fraction than in basal keratinocytes-enriched fraction. Therefore, in regard of its regulation, we conclude that TMEM45A expression is concomitant with differentiation and postulate that TMEM45A gene product is involved during late differentiation of normal human epidermal keratinocytes.
VALIDATION OF POTENTIAL PROTEIN INTERACTORS BY FLUORESCENT TAGGING AND CO-IMMUNOPRECIPITATION.

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Syntenin-2 is a protein containing 2 PDZ domains. Proteins containing PDZ domains function as molecular scaffolds in the formation and location of multiprotein complexes, such as large signaling complexes (see [1]). The diverse biological functions of these proteins include establishment and maintenance of cell polarity, control of cell adhesion, the regulation of cell growth, development, and differentiation [1,2]. The functions are thought to result from the high number of interacting partners, which associate with PDZ proteins through direct interaction with their PDZ domains. Syntenin-2 is a nuclear PDZ protein known to interact with phosphoinositides [3, 4]. However, the interacting protein partners of syntenin-2 in the nucleus are unknown. Using a TAP-tag based strategy, we could only isolate a few syntenin-2 interactors [5] suggesting that most complexes do not survive the extensive washing steps used in this two-step purification strategy. Therefore, the isolation of the complexes was performed based on a single tag. Using Protein A-tagged forms of syntenin-2 stably expressed in MCF-7 cells, we have identified by MALDI TOF/TOF mass spectrometry 109 potential interacting proteins after isolation of the syntenin-2 protein complexes by IgG sepharose. The potential interacting proteins could be classified in 8 known metabolic pathways. However, as single tag isolation procedures effectively increases the number of false positives, validation steps are essential. The large set of potential interacting proteins makes it difficult to rely on co-immunoprecipitation methods using specific antibodies against each individual interactor. Therefore, fluorescent fusions of the potential interacting proteins were co-expressed with syntenin-2 in cells allowing to test for colocalization in microscopy. The presence of syntenin-2 and of the interactors was also evaluated by Western blotting and the complexes were co-precipitated using antibodies against the fluorescent label. This method allows rapid validation of potential interacting proteins.

Acknowledgements

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References

OPPOSITE REGULATION OF HAS1 AND HAS3 MRNA EXPRESSION DURING NORMAL HUMAN KERATINOCYTE DIFFERENTIATION AND IN LESIONAL EPIDERMIS FROM ATOPIC DERMATITIS.

J. Malaisse (1), A. Nikkels (2), C. Lambert de Rouvroit (1), B. Flamion (1) and Y. Pournay (1).

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Hyaluronan (HA), a major component of extracellular matrix, is synthesized by three HA synthases (HAS1, 2, and 3). In the epidermis, most of the available data suggest that HA is mainly produced by HAS3. However, studies showing a simultaneous increase of HA synthesis and HAS3 mRNA expression were performed on keratinocytes treated with various drugs affecting proliferation and differentiation. In order to investigate HAS functions in the epidermis in untreated conditions, we used two models of spontaneous keratinocyte differentiation: confluent monolayers and organotypic cultures of human keratinocytes. On both models, we have measured HAS mRNA expression and HA production in the culture medium. During keratinocyte differentiation, an increased HA concentration in the extracellular medium was observed in both models, concomitant with increased HAS1 mRNA expression in monolayers and stable HAS1 mRNA expression in organotypic cultures. In contrast, HAS2 and HAS3 mRNA expression were decreased in both models during the differentiation process. To compare with a pathological condition where HA production is altered, we studied the tissue distribution of HA and the expression level of HAS mRNA in healthy skin biopsies versus atopic dermatitis (AD) lesion biopsies. In AD lesional biopsies, we observed an increase in HA staining accompanied by increased HAS2-HAS3 and decreased HAS1 mRNA expression. Altogether, our data suggest that HAS1 is involved in the production of extracellular HA by keratinocytes during normal differentiation. In contrast, HAS2 and HAS3 are expressed in response of keratinocytes to stress or in pathological conditions, indicating distinct functions for HAS2-3 in regard of HAS1.
ROLE OF OXIDATIVE DNA DAMAGE IN POST-SENESCENCE NEOPLASTIC EMERGENCE.

Joe Nassour, Sébastien Martien, Emeric Deruy, Peter Ostoich, Laure Sabatier and Corinne Abbadie.

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Senescence is a state of growth arrest elicited by numerous stresses, including telomere shortening, non-telomeric DNA damage, oxidative stress or oncogenic activation. It occurs in vivo with aging and is resumed in vitro with time and doublings. It is assumed that senescence prevents transmission of age-associated damages to daughter cells, including DNA damages, hence functioning as a tumor suppressor mechanism. Here, we examine the mechanisms that could enable senescence evasion to give rise to transformed cells. We took advantage of the comparison of normal human dermal fibroblasts (NHDFs) and normal human epidermal keratinocytes (NHEKs). Indeed, NHDFs display a classical irreversible senescence plateau resulting mainly from telomere erosion. In contrast, senescence in NHEKs is telomere-independent and reversible, with some senescent keratinocytes which spontaneously resume mitosis and generate clone of cells that we have characterized as transformed and tumorigenic. We have shown that oxidative stress is the motor of this post-senescence neoplastic emergence (PSNE) (Gosselin et al, Cancer Research, 2009, 69, 7917). We investigate here whether oxidative stress operates via the generation of mutagenic DNA damages.

We have analyzed the accumulation of DNA double-strand (DSB) and single-strand (SSB) breaks. Comet assays and immunofluorescent analyses data show that senescent NHDFs and NHEKs differentially accumulate these two types of breaks, with NHDFs preferentially accumulating DSBs and DDR foci, and NHEKs preferentially accumulating SSBs and SSBR foci. In consequence of their DSBs, NHDFs efficiently accumulate and activate p53, and are arrested at the G1 phase of the cell-cycle. In contrast, p53 activation is not detected in NHEKs which appear arrested at all phases of the cell-cycle.

Our results support the view that the outcome of senescent cells might differ according to the nature of the dominant DNA damage they encounter: DSB might be responsible of a robust and irreversible cell-cycle arrest, whereas SSB might be a cause of emergence of pretumoral cells through their mutagenicity.
Abstract 13: Poster

13: Piret Jean-Pascal
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CYTOTOXICITY OF MULTI-WALLED CARBON NANOTUBES IN THREE SKIN CELLULAR MODELS: EFFECTS OF SONICATION, DISPERSIVE AGENTS AND CORNEOUS LAYER OF IN VITRO RECONSTRUCTED HUMAN EPIDERMIS.

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Carbon nanotubes (CNT) are produced industrially worldwide because of their impressive array of physical and chemical properties, making them suitable for multiple applications in advanced technologies and medicine. However, many questions are raised about CNT adverse effects on human health because of their high surface area and bonding with redox-cycling compounds such as catalytic metals. In this study, the potential toxic effects of multi-walled carbon nanotubes (MWCNT) were investigated on human N-hTERT telomerase-immortalized keratinocytes, on human SZ95 SV-40 immortalized sebocytes and on in vitro reconstructed human epidermises. MWCNT were subjected to several dispersion protocols leading to different agglomeration states from raw and agglomerated particles to isolated entities obtained by sonication in the presence or not of dispersive agents (hydroxypropylcellulose and Pluronic F108). Results showed that: (i) Water suspended MWCNT, as micrometric agglomerates, were not harmful to skin cells, except minor effects in keratinocytes, (ii) mild sonication slightly decreased nanotube agglomeration but increased cytotoxicity on keratinocytes, (iii) addition of hydroxypropylcellulose or Pluronic F108, which improved MWCNT dispersion, masked the harmful effects of sonicated MWCNT. Altogether, these results indicate that MWCNT induced cytotoxicity in human keratinocytes after a short exposure (24–48 h), particularly when they were sonicated before cell incubations. However, the cytotoxic effects of raw and sonicated MWCNT could be prevented in presence of dispersive agents. No cytotoxic effects were observed in SZ95 sebocytes or in in vitro reconstructed epidermises underlining the importance to compare results between different in vitro models.
The incidence of carcinoma greatly increases with ageing, but the cellular and molecular mechanisms underlying this correlation are only partly known. It is well established that senescent fibroblasts promote the malignant progression of already transformed cells through the secretion of inflammatory mediators.

We investigated here whether the senescent fibroblast secretome has an impact on the very initial stages of carcinogenesis. We used an in vitro culture model of normal primary human epidermal keratinocytes in which cells with transformed and tumorigenic properties systematically and spontaneously emerge from the senescence plateau.

We show that in the presence of conditioned media from autologous dermal senescent fibroblasts, the frequency of post-senescence emergence is increased, and the epithelial-mesenchymal transition and migratory properties of the post-senescent emergent cells are exacerbated. Using pharmacological inhibitors, siRNA transfections, and blocking antibodies, we demonstrate that MMP-1 and MMP-2 matrix metalloproteinases, known to participate in late stages of cancer invasion and metastasis, are responsible for this enhancement of early migratory capacities. In addition, we show that MMPs act through the activation of the protease-activated receptor, PAR-1, whose expression is specifically increased in post-senescence emergent keratinocytes.

The physiopathological relevance of these results was evidenced by showing that the expression of both MMPs and PAR-1 is increased in skins from aged people versus young ones. Collectively, our results suggest that this MMP-PAR-1 axis could participate in the initiation of non-melanoma skin cancers, through the co-activation of both the dermal and epidermal compartments during skin aging.
A NEW ACTOR OF BONE RESORPTION?

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The cells that resorb the bone matrix, the osteoclasts, are giant multinucleated cells generated by the fusion of cells of the monocyte lineage. When in contact with bone, osteoclasts polarize and form a ruffled border where their secretory lysosomes fuse with the apical plasma membrane. An actin ring seals the osteoclast to the bone surface, thereby generating an extracellular compartment, referred to as the resorption lacuna. This lacuna is often compared to an “extracellular lysosome” as it is acidified by the lysosomal vATPase, which generates the optimal pH conditions to allow the secreted lysosomal acid hydrolases to actively degrade the bone (i.e. cathepsin K). One of the characteristics of the lysosomal proteins that are known to be essential for the bone resorbing function of the osteoclasts (vATPase, cathepsin K, TRAP, etc.) is the up-regulation of their expression upon the differentiation of monocytes into osteoclasts, by contrast to more ubiquitously expressed acid hydrolases (cathepsin D for example). Interestingly, using a microarray approach and RT-PCR, we found that among the selected acid hydrolases that increase during the process of differentiation is an acidic glycosidase whose lysosomal localization remains an unresolved question. We confirmed this increase of expression by western blotting and by an enzymatic assay, and started a study of its subcellular localization of this protein in the osteoclasts. Next, to investigate its importance in the bone remodeling process in general, we measured the effect of its knock-down in mice on the bone density by pQCT (peripheral Quantitative Computed Tomography).
Towards the interactome between skin microbial communities and the skin epidermal cells.

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Interaction between the skin epidermis and the microbial communities plays an important role in the human health and disease status. The present research is focused on the development of an empirical interactome framework of the global human skin cells with the skin microbial communities. Using high-throughput next-generation sequencing, aimed functional analysis and validated skin models, a framework will be created to link the metabolic interactions of the epidermal cells and skin microbial communities. The discovery of the specific connections between skin cells and microbial populations may lead to the development of new methods to predict human skin physiology states as well as protection against potential infectious diseases.
IDENTIFICATION OF MANNOSE 6-PHOSPHATE SIGNAL-INDEPENDENT PATHWAY(S) FOR THE TARGETING OF ACID HYDROLASES TO LYSOSOMES.

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During their passage through the Golgi, newly synthesized lysosomal acid hydrolases are modified by two enzymes (GlcNAc-1-phosphotransferase and “Uncovering enzyme”) to expose mannose-6-phosphate (M6P) signals on their oligosaccharidic chains. These signals are recognized by two receptors (CD-MPR and CI-MPR) at the trans-Golgi network, which initiates the packaging of the hydrolases in clathrin-coated vesicles and their transport to lysosomes. Mucolipidosis type II (i.e. GlcNAc-1-phosphotransferase deficiency) is a pathology characterized by the loss of the M6P signal on acid hydrolases, which results in their hypersecretion by the cells. Consequently, undegraded material accumulates inside the lysosomes thus depleted of their acid hydrolases. However, it has been observed that not all cell types of mucolipidosis II patients mis-sort their hydrolases, suggesting that alternative pathway(s) to the lysosome exist(s). To study these unknown trafficking routes, we started a search for putative M6P-independent sorting receptor(s) among the binding partners of one of the main lysosomal acid hydrolase, cathepsin D. To isolate these partners, we fractionnated a membrane fraction prepared from mouse kidney (a tissue that retain normal acid hydrolase levels despite the loss of the M6P signal) over a cathepsin D affinity column. Proteins that eluted at lysosomal pH (4.5) were sent for mass spectrometry analysis. One interesting candidate was thus identified, a protein with six transmembrane domains containing several putative lysosomal sorting signals in its sequence. To investigate how our candidate could be involved in acid hydrolase trafficking, we first tested if this protein is enriched in clathrin coated vesicles, similarly to M6P receptors. Then we tested wether an overexpression of our candidate in Hek293 cells can affect the subcellular trafficking of cathepsin D, using western blotting and pulse chase labelling techniques.
ANOCTAMIN 1, A SELECTIVE MARKER FOR INTERSTITIAL CELLS OF CAJAL IN THE ZEBRAFISH INTESTINE.

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Over the last decade, the zebrafish has emerged as a leading model organism to study vertebrate development. Molecular pathways, organ systems and physiology are well-conserved between zebrafish and mammals. Most major organ systems are present in the larvae within 5 days post fertilization (dpf). In addition to studies of vertebrate development, the zebrafish is also used to investigate human disease conditions including functional gastrointestinal (GI) disorders. Interstitial cells of Cajal (ICC) are specialized cells that generate electrical slow waves initiating GI motility. In mammals, ICC are predominantly identified by the expression of the receptor tyrosine kinase, kit. Presence, distribution patterns and expression features of these ICC are known to be affected in various GI motility disorders. Anoctamin 1 (Ano1, also known as DOG1), a Ca2+-activated Cl--channel, has recently been shown to be a specific ICC marker in mice, primates and humans. It is also shown that Ano1 is essential in the generation of slow waves and regulates ICC proliferation. This study aimed at testing the validity of Ano1 as ICC-marker in the zebrafish intestine.

Using multiple immunofluorescent staining methods, sections and whole mounts of adult zebrafish intestine as well as isolated intestines of zebrafish embryos and larvae (3 to 6 dpf) were analyzed for the expression of Ano1, along with the general marker acetylated tubulin (a-tub).

In adult zebrafish intestine, Ano1-immunoreactive (IR) particles revealed ICC-like cells in two distinct layers forming a 3-dimensional network. A loose layer of bipolar ICC-like cells, resembling intramuscular ICC (ICC-IM) were interposed between smooth muscle cells in the circular muscular layer. A layer of multipolar Ano1-IR cells, resembling myenteric plexus ICC (ICC-MY), were intertwined with the myenteric plexus. Both layers were interconnected. Close associations between a-tub-IR fibers and the Ano1-IR network were observed.

In zebrafish embryos a few cells expressing a faint granular Ano1 immunoreactivity, scattered throughout the intestinal wall, were observed at 3 dpf. At 4 dpf, cells containing Ano1-positive granules were appearing especially in the distal intestine. By 5-6 dpf, a clear 3-dimensional network of cells expressing Ano1 began to form throughout the intestine.

The present study demonstrates that Ano1, just as in mammals, is a selective marker for ICC-like cells in the zebrafish intestine. The first Ano1-IR cells have appeared at 3 dpf in the embryonic intestine, indicating that proliferation of ICC-like cells begins at this time point. Furthermore, it is hypothesized that ICC-like cells organize the spontaneous regular activity of the embryonic intestine, because the first appearance of ICC-like cells in the embryonic intestine occurs nearly at the same time that the first spontaneous regular contractile waves are observed in the embryonic intestine.
REPLICATIVE SENESCENCE IN HUMAN FIBROBLASTS CULTIVATED UNDER 5% OR 21% OF OXYGEN.


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Replicative senescence is characterized by an irreversible growth arrest of proliferative cell types. Senescent cells display many other senescence-associated biomarkers as senescence-associated β-galactosidase (SA β-gal). Here we studied the impact of different oxygen pressures on the appearance of senescence. Cell cultures of human dermal (FS) or lung (WI-38) fibroblasts were performed in parallel at physiological (5%) versus atmospheric (21%) oxygen pressure. Every three passages, cells were lysed in order to perform protein analyses (2D-DIGE gels, oxyblots and western blots), RNA analyses (high density DNA array and semi-quantitative real time PCR) and study of senescence biomarkers (cell counting and SA β-gal). In the current study, we identified proteins which abundance is modified in young fibroblasts compared to senescent fibroblasts either at 5% O2 or 21% O2. We detected a delayed appearance of senescence when cells were cultivated at 5%.
TAMOXIFEN INHIBITS GROWTH AND DEVELOPMENT OF VARIOUS MOUSE TISSUES INCLUDING SKIN EPITHELIUM.

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Aim: Tamoxifen (TAM)-inducible CreERT recombinase is widely used for conditional (in)activation of specific gene expression in transgenic mice. One important application is genetic cell lineage tracing to decipher origin and extent of tissue formation during development or after tissue injury. However, off-target effects of the chemical inducer TAM are rarely taken into account in the analysis of data obtained in abovementioned experimental models. In present study we investigate whether TAM affects growth and development of a variety of tissues. The hair follicle undergoes cycles of growth, regression, and rest, driven by stem cell quiescence or activation. Hepatocytes and pancreatic endocrine or exocrine cells present a basal growth rate that can be increased by organ injury, while duodenal crypt cells have a high basal turnover. We investigated whether TAM affected the proliferation of these different cell types and the differentiation cycle of hair follicles.

Method: The effect of subcutaneous TAM or vehicle (control) was studied in Balb/c mice. Hair follicle development, and hepatocyte or duodenal cell cycle were studied in normal Balb/c mice. Pancreas regeneration was studied in Balb/c mice that had undergone partial pancreatectomy (PPx) or pancreatic duct ligation (PDL). Skin, pancreas, liver and duodenum were dissected post-mortem and analyzed by histology. Proliferation was studied by antibody detection of the (i) cell cycle marker Ki67 or (ii) DNA-incorporated thymidine analog 5-iodo-2'-deoxy-uridine that was administered via the drinking water.

Results: In adult mice, TAM inhibited the basal proliferation of hepatocytes and bile duct cells in liver, and of duct, acinar, and beta cells in pancreas, but not of duodenal crypt cells or sinusoidal liver cells. TAM also inhibited proliferation of the endocrine beta cells following pancreas injury. In addition, TAM prevented transition from telogen- to- anagen phase in the developmental cycle of hair follicles.

Conclusion: TAM significantly inhibits the proliferation of various cell types both in liver and pancreas. It also inhibits normal differentiation of hair follicle cells in skin epithelium. The effects of TAM on proliferation and development appear cell specific since duodenal crypt and sinusoidal liver cells were not affected. While TAM induces reporter gene activation in lineage tracing studies, it may also affect cellular growth and tissue composition. In addition, our results suggest that hair follicle differentiation in skin epithelium requires estrogen receptor activity.