Please note that the selected abstracts were chosen by the editors and this publication does not include all abstracts presented at the congress.

Oral Presentations

Angiogenesis and the tumor microenvironment as targets for cancer prevention

Adriana Albini

Dallaglio K1, Rossi T1, Bruno A2, Noonan DM2,3

1IRCCS “Tecnologie Avanzate e Modelli Assistenziali in Oncologia” – Arcispedale S. Maria Nuova - Reggio Emilia, Italy
2 Polo Scientifico e Tecnologico, Fondazione MultiMedica Onlus, Milano, Italy
3 Department of Biotechnology and Life Sciences, University of Insubria, Varese, Italy

Tumors are tissues where several cellular and molecular “players” orchestrate cancer fate. This complex scenario, termed the tumor microenvironment (TUMIC), several cellular components, a part from the transformed cells, participate and cooperate, including inflammatory cells, tumor associated fibroblasts, endothelial cells, pericytes and stromal components. The link between inflammation and angiogenesis in cancer has been extensively investigated, suggesting that these components represent crucial targets for prevention approaches. Preventative strategies must be suitable for healthy individuals, with little or no toxicity, and must target host components. Anti-angiogenic therapy has been validated as an effective anti-cancer strategy for an increasing number of cancer types and more than 120 novel anti-angiogenic agents are currently in clinical trials. Furthermore, a growing body of preclinical, clinical and epidemiological data demonstrates that angiogenesis inhibition can be applied to cancer prevention, giving rise to the field of angioprevention. Angioprevention often uses natural compounds and derivatives that prevent and reduce tumor progression. The ability of some dietary components, like terpenoids (CDDO-Me, CDOO-Im), resveratrol, green tea catechins, quercetin, curcumin, to inhibit tumor progression and angiogenesis, both in vitro and in vivo, have been substantially documented. Many of these compounds exert anti-oxidant, anti-angiogenic, and anti-inflammatory effects on host cells and show anti-proliferative and pro-apoptotic effects on several tumors, including leukemia, melanoma, prostate, breast, colon, brain, and pancreatic cancers. These compounds are well tolerated and often found in food products that can be added to diet. Furthermore, most phytochemicals could be taken on a long-term basis to either prevent primary tumor formation or tumor recurrence. We demonstrated the ability of phytochemicals to efficiently target several component of the TME, including the inflammatory component (neutrophils, Natural Killer cells), the endothelium and interestingly also the “dormant” component represented by the cancer stem cells. Finally, we also demonstrated that Metformin and Phenformin, two bugianides commonly employed in treatment of patients with type-2-diabetes, showed promising chemo and angiopreventive activity in vitro and in vivo that is associated with epidemiological evidence of prevention of several cancers. Our experience confirms that angiogenesis and inflammation are excellent targets for tumor prevention, and increased efforts in angioprevention approaches to prevent carcinogenesis and tumor progression are needed.

Towards an integral diagnostic system for acute kidney injury

Francisco J. Lopez-Hernandez

Co-authors: Sandra M. Sancho-Martínez, Laura Vicente-Vicente, Laura Prieto, Víctor Blanco-Gozalo, Marta Prieto, Yaremi Quiros, Miguel Fontecha, Alfredo G. Casanova, Moisés Pescador, Carlos Martínez-Salgado, Ana I. Morales, José M. López-Novo.

Instituto de Investigación Biomédica de Salamanca (IBSAL) - Universidad de Salamanca, Salamanca, Spain

http://dx.doi.org/10.1016/j.nhtm.2015.12.001
2307-5023/© 2015 European Society for Translational Medicine. Published by Elsevier Ltd. All rights reserved.
Acute renal failure (ARF) is an old term coined in the 1950s referring to a clinical syndrome whereby the patient’s kidney excretory function is suddenly reduced to the extent of needing dialysis to preserve life. One decade ago, the term ARF was superseded by the term acute kidney injury (AKI), coinciding with the first international consensus on ARF functional definition and classification. The new term (AKI) was adopted to comprise also those casescouring with acute increments in plasma creatinine, which are not severe enough to need dialysis. The concept behind AKI applies to a number of etiologically different, pathological circumstances characterized by an abrupt insult resulting in functional or structural alterations in the kidneys ranging from mild dysfunction with no underlying renal tissue damage (such as in purely pre-renal AKI), to functional failure with severe parenchymal damage needing dialysis. AKI diagnosis based on plasma creatinine increments has important conceptual shortcomings and practical (i.e. clinical) limitations. Late sensitivity of plasma creatinine to AKI detection calls into question the concept of AKI itself. Evidently, a large renal damage or functional alteration has occurred when plasma creatinine increases, which invokes a new clinical concept which needs to be addressed, classified, and introduced in the conceptual and functional definitions of AKI. As a result, a new horizon of diagnostic demands is opened up by the new concepts. Among these new concepts are (i) a correct and specific etiological and pathophysiological diagnosis of AKI to enable correct patient handling; (ii) a diagnostic capability to detect the increased predisposition of AKI before an episode occurs (or even before a potential cause of AKI be used or applied), to stratify patients preemptively according to their individual risk; and (iii) diagnostic monitoring of renal tissue repair and subclinical renal function evolution, or renal sensibility to AKI remains after an episode of AKI is resolved, and plasma creatinine has returned to normal values. In this latter case, surveillance of a potential connection between AKI and chronic kidney disease poses a clear, potential application. Consequently, an integral diagnostic tool must be developed to comply with these requirements, with the ultimate objective of significantly improving AKI diagnosis; and thus, AKI prevention, clinical handling and patient prognosis, both in the short and the long term.

**Development of translatable region-specific molecular signatures for sub-acute drug induced kidney injury**

Sara Snelling

Co-authors: S. Dremier, P. De Ron, S. Glineur, A. Nogueira da Costa

1. Faculty of Biological Sciences, University of Leeds, UK
2. Non-Clinical Development, Investigative Toxicology, UCB Biopharma Sprl, Belgium

Drug attrition due to drug-induced kidney injury (DIKI) is a cause of drug failure in pre-clinical studies. While FDA qualified pre-clinical urinary DIKI biomarkers allow for early detection of acute nephrotoxicity, a higher level of kidney lesion specificity is still warranted. Furthermore, the preclinical–clinical translation of molecular DIKI biomarkers requires further investigation using sub-acute pre-clinical models. This work aims at developing kidney region-specific molecular biomarker signatures in the context of sub-acute nephrotoxicity. A 28 day toxicity study was conducted in Wistar rats. Nephrotoxicants affecting different kidney regions— cisplatin (proximal tubule), puromycin (glomerulus) and N-phenylanthranlylic acid (NPAA) (collection ducts) were administered individually to independent groups (n=24) and animals were sacrificed at days 7, 14, 21 and 28. Histopathology, urinary protein biomarkers, toxicogenomic (mRNA) analyses and miRNA detection are currently being performed. Histopathology findings confirmed region specificity of each administered drug. Cisplatin induced lesions consisted of minimal to moderate tubular degeneration/necrosis by day 7, followed by subsequent recovery. Puromycin induced a clear increase in glomerular vacuolation as of day 7, maintaining both severity and incidence throughout the study. Glomerulosclerosis was also observed, appearing only at day 28. Administration of NPAA induced renal papillary degeneration from day 7 to 28. Treatment-specific urinary biomarker profiles were observed, even beyond the day 7 study end-point of classical acute DIKI studies. In cisplatin treated rats, clusterin levels showed a 14-fold increase by day 3, with a subsequent decrease to near baseline level by day 7, confirming its usefulness as an acute tubular injury biomarker. For puromycin treated rats, fold changes of up to 40× were observed for clusterin levels from day 3, and were maintained until day 28, providing evidence for it being a strong potential indicator of glomerular injury in a sub-acute setting. Toxicogenomic analysis at days 7, 14, 21 and 28 (0.5 × < fold change ≥ 2 × , p < 0.05) identified changes in mRNA expression profiles that are associated with apoptotic, inflammatory and xenobiotic metabolism pathways. Treatment-specific mRNA expression profiles were observed, with a single mRNA being regulated at all-time points in cisplatin and another in NPAA treated rats. Puromycin induced fold changes above 10× (p < 0.05) by day 14 for 36 genes. A panel of 68 urinary miRNAs associated with kidney injury, including miR-155, miR-21 and miR-30, are currently being assessed in all treatment groups, aiming at increasing the robustness of region-specific molecular biomarker signatures. Overall, our findings confirm potential tubular, glomerular and collection duct specific molecular profiles of a more sub-acute nature than previously reported in pre-clinical studies. On-going validation and integration efforts will further reinforce these findings with a potential preclinical–clinical translatability in a clinical DIKI context.

**Advanced light microscopy: recent developments and applicative potential for translational medicine**

Jörg Gotzmann

Max F. Perutz Laboratories, Vienna, Austria

2014 was somehow the year of Light Microscopy by awarding the noble prize for the development of superresolution microscopy techniques. 2015 is the UNESCO “International Year of the Light”. Developments, which underscore the increasing impact of light microscopy for all facets of biomedical research. The currently available portfolio of imaging technologies provides a perfect platform for translational projects bridging the gap between basic and applied science. In recent years we observed fast and revolutionary development of new biooptical technologies, which opened new routes of investigative potential. In my talk I will try to highlight a selection of emerging light microscopy techniques with a special focus on their applicative potential for “bed-to-benchside” research.

**Therapeutic exploitation of the cancer glycose; a drug discovery study of the therapeutic potential of the glycane-binding protein galectin-9 for colorectal k-Ras mutant cancer**

Edwin Bremer

University of Groningen, University Medical Center Groningen (UMCG), Department of Surgery, Translational Surgical Oncology, Groningen, The Netherlands
Exeter University Medical School, Devon, United Kingdom

Glycosylation is an intricately controlled posttranslational modification process of proteins and lipids that is important for a variety of cellular functions. Aberrant glycosylation is one of the hallmarks of cancer and represents a major and largely under-explored target for cancer therapy. Here, I will discuss results of our early drug discovery program for the glycan-binding protein galectin-9 as an example of the potential promise of targeting aberrant glycosylation for cancer therapy. Galectin-9 (Gal-9) is an important regulator in T-cell immunity, but also regulates endocytosis and polarity of epithelial cells. In many tumor types, Gal-9 expression is lost during tumor progression, and is thought to have a tumor suppressor function. In our studies we uncovered that treatment of human colon cancer with Gal-9 induces prominent vacuole formation and subsequent cell death in k-Ras mutant, but not in b-Raf mutant cells. Further, Gal-9 inhibited tumor growth and prolonged the survival of tumor-bearing mice without signs of toxicity toward normal cells. Upon Gal-9 treatment, exogenously added Gal-9 accumulated into lysosomes and activated an aberrant form of autophagy that was halted at the stage of autophagosome/lysosome fusion. Further, the antitumor activity of Gal-9 directly correlated with basal autophagic flux, showing highest sensitivity in colon cancer cells with highest flux. Thus the glycan-binding protein Gal-9 has potent antitumor activity towards refractory k-Ras mutant colon carcinoma cells by inducing fatal frustrated autophagy. Since mutated k-Ras is present in 30–45% of colorectal cancers and is associated with poor prognosis and resistance to chemotherapy, targeting of the Gal-9 regulatory axis may have clinical potential for the treatment of patients with this type of cancer.

Correlation between genetic modifications and inflammation in uveal melanoma

Martine J. Jager

Department of Ophthalmology, LUMC, Leiden, The Netherlands

Purpose: Uveal melanoma is a rare disease, which frequently leads to metastases. While monosomy of chromosome 3 is a well-known prognostic parameter in uveal melanoma, loss of expression of BAP1 (a gene located on chromosome 3) has recently been added as a determinant for prognosis. Although loss of BAP1 is correlated to chromosome 3 loss, the two are not the same (Koopmans 2014). Additionally, we know that the presence of inflammation is correlated to a bad survival. We wondered whether inflammation occurs only in BAP1-negative/monosomy 3 tumors.

Methods: We studied expression of BAP1 by immunohistochemistry (IHC) in the presence of different leukocytes by IHC and RNA expression in 54 tumors, and chromosome status with SNPs. The clinical status of the patient was also determined.

Results: Loss of BAP1 expression was a frequent phenomenon: 30 of the 54 tumors had a loss of BAP1 expression. These tumors showed an increased infiltration with CD3, CD4, CD8 T lymphocytes, CD68 macrophages and a higher vessel density. However, also tumors that had BAP1 expression but no monosomy 3 loss, and tumors with monosomy 3 but no BAP1 loss had more infiltrating cells than UM with BAP1 and disomy 3. The BAP1 +/disomy 3 tumors showed the best survival.

Conclusion: Loss of BAP1 expression but also loss of one chromosome 3 are associated with an increased leukocytic infiltrate in UM, and carry a worse prognosis than those with BAP1 expression/disomy 3. It remains to be determined which mechanism attracts leukocytes to BAP1-negative/monosomy 3 tumors.

CT-1 regulates renal fibrosis induced by obstructive nephropathy

Carlos Martinez-Salgado

Co-authors: Nuria Perretta-Tejedor, José M. Muñoz-Félix, Cristina Cuesta, Isabel Fuentes-Calvo, Nélida Eleno, José M. López-Novoa

Institute of Biomedical Research of Salamanca (IBSAL)-IECSCYL, Salamanca, Spain

Chronic kidney disease (CKD) is characterized by a progressive decrease in glomerular filtration rate that eventually leads to renal failure. Tubulointerstitial fibrosis, a major determinant of CKD, leads to an accumulation of extracellular matrix (ECM) proteins, due to an imbalance between synthesis and degradation of ECM. Cardiotrophin-1 (CT-1) is a member of the interleukin-6 (IL-6) family of cytokines, originally identified as a protein capable of inducing hypertrophy in neonatal ventricular cardiomyocytes. CT-1 has a variety of functions that sometimes have opposite effects in different contexts. It can promote cell survival but also can cause tissue damage. We have previously shown that CT-1 is an early biomarker of hypertension and diabetes-induced target organ damage. Although chronic exposure to high doses of CT-1 in experimental animals is associated with cardiac, vascular and renal fibrosis, the role of CT-1 in renal fibrosis is not well known. Thus, we studied the severity of renal tubule-interstitial damage induced by unilateral ureteral obstruction (UUO), an experimental model of tubulointerstitial-fibrosis, in animals lacking CT-1 (CT-1-/-). We also analysed the CT-1 induced effects on ECM protein synthesis in renal myofibroblasts obtained from obstructed kidneys and the intracellular mechanisms involved. UUO was performed in CT-1-/- mice and their respective controls (WT) during 3 days – to analyse inflammatory effects and initial tubule-interstitial damage – and during 15 days – to evaluate renal tubule-interstitial fibrosis. Cultured myofibroblasts were stimulated with 40 ng/ml CT-1. After 3 days of UUO, obstructed (O) kidneys from CT-1-/- mice showed higher expression of ICAM-1 and COX-2 than O kidneys from WT mice. After 15 days of UUO, O kidneys from CT-1-/- mice show higher expression of collagen I than O kidneys from WT. In addition, Sirius red staining showed increased tubulointerstitial fibrosis in O kidneys from CT-1-/- mice than in their respective O controls. CT-1 treatment (100 μg/kg/day) reduced the increased ICAM-1 expression observed after 3 days UUO; the same treatment reduced the increases in collagen I, CTGF and the extension of Red Sirius-stained area in O kidneys after 15 days UUO. On the other hand, the in vitro studies showed that renal myofibroblasts expressed CT-1. Stimulation with CT-1 increased collagen I expression in CT-1-/- and WT myofibroblasts as well as fibronectin in WT cells. This study shows that endogenous CT-1 regulates renal fibrosis induced by obstructive nephropathy, possible due to its regulation of the inflammatory process. Although the effects appeared to be opposite in vivo and in vitro, this may be due to different regulation of extracellular matrix degradation in both experimental scenarios. This study suggests a potential therapeutic property of CT-1 in CKD.

Autophagy-dependent regulation of tubule formation in the brain endothelial cell line bEND.5

Mauro Cataldi

Co-authors: Valeriana Sblendorio, Chiara Vigliotti, Lucio Annunziato
Background and aims: The formation of new blood vessels in the brain takes place both as a part of reparative responses, for instance after stroke, or as part of serious diseases such as tumor growth. Conditions that promote autophagy such as oxygen deprivation also markedly promote angiogenesis in the brain. Therefore, in the present work, we explored the hypothesis that autophagy could have a role in blood vessel formation by brain endothelial cells. To this aim we evaluated the effect of drugs acting at different steps of the autophagy pathway on tube formation in vitro by bEND5 cells, an endothelial cell line originally established from the brain of transgenic mice expressing middle T polyoma oncogene. These cells retain many of the characteristics of differentiated brain endothelial cells and form capillary-like tubes on matrigel-coated plastic.

Methods: bEND.5 cells were seeded into 96 wells plastic dishes precoated with matrigel. Tube formation was quantified 22 hours after seeding by calculating cumulative areas of vessel lumens using the Image J software. VEGF was assayed with ELISA whereas VEGFR-I, VEGFR-II, beclin and LC3 were assessed by Western blot analysis. Cell viability was evaluated with MTT assay.

Results: bEND.5 cells released measurable VEGF concentrations in culture media and expressed both VEGFR-I and VEGFR-II. The anti VEGF monoclonal antibody Bevacizumab (0.2–1.8 μM) did not prevent in vitro tubulogenesis suggesting that in these cells, tube formation does not depend on autocrine VEGF release. Moreover, tube formation was not modified by combined glucose-oxygen deprivation, a strong VEGF-inducing condition. Metformin (3 mM), an autophagy inducer acting on the early autophagy initiation step through AMP-K activation, increased beclin protein levels at Western blot analysis but it did not significantly modify cumulative tube area. Conversely, 3-methyl-adenine (10 μM) and LY-294002 (100 μM), two PI-3 kinase inhibitors that block autophagy elongation step, significantly decreased normalized tubule area and reduced 15 kDa/18 kDa LC-III ratio. Tube formation was also significantly prevented by two drugs acting on the autophagosome degradation step, the lysosomotropic agent chloroquine (30 μM), that prevents endosomal acidification, and the V-ATPase inhibitor pantoprazole (1 nM). None of the tested drugs decreased cell viability at MTT.

Conclusion: Collectively, these data suggest that in brain endothelial cells cultured in vitro, new blood vessel formation may take place through VEGF-independent, autophagy related mechanisms. These mechanisms could be potentially relevant in drug resistance to anti VEGF-therapies.

The role of miR-122 in the hepatocellular carcinogenesis

Zhaohua Zhong1

Co-author: Wenran Zhao2

1 Department of Microbiology, Harbin Medical University, Harbin, China
2 Department of Cell Biology, Harbin Medical University, Harbin, China

miR-122 is highly abundant in liver and a hepato-specific microRNA. There is evidence that miR-122 expression is down-regulated in human hepatocellular carcinomas (HCC). It is unknown whether miR-122 affects the cellular behavior of hepatoma cells. In this study, the effects of miR-122 on the viability and apoptosis of hepatoma cells were investigated. The viabilities of Huh-7 and HepG2 cells treated with miR-122 or anti-miR-122 antisense RNA (anti-miR-122) were analyzed by ATP-based luminescent assay and scratch assay. The apoptosis of Huh-7 and HepG2 cells treated with miR-122 or anti-miR-122 were analyzed by annexin V-based flow cytometry and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) detection. The miR-122-coding genes in both cell lines were sequenced. Though two putative promoter sequences for miR-122 gene at 18q21.31 were detected, the miR-122-coding sequence was missing in HepG2 cells. This might be the cause which leads to the absence of miR-122 expression in HepG2 cells. There was no significant difference between the viabilities of HepG2 cells transfected with miR-122 and mock HepG2 cells. However, the viability of Huh-7 transfected with anti-miR-122 was significantly elevated at 24, 36, and 48 hours posttransfection compared to that of mock Huh-7 cells. Scratch assay showed similar result. Both flow cytometry and TUNEL assay showed the apoptotic level of Huh-7 transfected with anti-miR-122 significantly decreased at 48 hours posttransfection. miR-122 overexpression did affect the cell cycle of HepG2 cells, but the knockdown of miR-122 expression caused the population of Huh-7 cells in G0–G1 phase significantly increased. Our data demonstrate that miR-122 downregulated the viability but upregulated the apoptosis of hepatoma cell Huh-7. The absence of miR-122 expression in HepG2 cells was due to the loss of miR-122-coding sequence in chromosome 18. These results implied that aberrant expression of miR-122 may contribute to hepatocarcinogenesis. The mechanism of miR-122 in the HCC cells has been further studied.

This work was supported by the Natural Science Foundation of China (NSFC) (81271825 to Z. Zhong and 31270198 to W. Zhao).

Oxidative stress in hostile environments: a translational omics-based approach to personalized assessment and countermeasures development

Michael A. Schmidt

Research Innovation Center, Colorado State University, USA
The environment of space is perhaps one of the most hostile imaginable providing many challenges to the human physiology, therefore to extended habitation and exploration. Many sources of Oxidative Stress and Damage (OxSAD) result from the human body’s adaptation to reduced gravity and higher radiation levels. Earth based Omics technologies and analyses may hold solutions, along with innovative approaches in translational medicine, to partially resolving or reversing the adverse conditions experienced in space, while simultaneously translating those approaches back to the Earth’s medical community. One concern is assuring the stability and integrity of an individual’s DNA prior to and during the rigors of space. With modern scientific tools such as telemedicine and advances in genomics, proteomics, and metabolomics (“omics”), the means exist to address syndromes (similar to advanced aging in some cases), at the systemic level by enlisting a personalized approach to the assessment of the spaceflight physiology and to countermeasure development. Other areas of spaceflight related dysregulation are paramount areas for the assessment and implementation of translational methodologies: (1) cardiovascular fluid shifts, intracranial hypertension and neuro-ocular impairment, (2) immune insufficiency and suppression/viral re-expression, (3) bone loss and fragility (osteopenia/osteoporosis) and muscle wasting. We will discuss a global strategy to ensure healthy humans in flight through “Omics”.

**Big data mining for the hospital patients of cardiovascular diseases**

Kunlun He

Department of Translational Medicine, Chinese PLA General Hospital, Beijing, China

Cardiovascular diseases are major health problems worldwide with high incidence and mortality rate, so the earlier diagnosis and prevention were more important. Based on the current data resources from our hospital, we proposed to set up big data mining platform of cardiovascular diseases to integrate the longitudinal data from the hospital’s electronic medical record (clinical information for the past 15 years merged with clinical examination, medical image and pathological diagnosis). It involved 200,000 patients from northern areas of China and some genomic data of heart failure patients have also been included. The assessment models of cardiovascular diseases and prediction models of heart attack and heart failure by multi-dimensional data analysis such as Support Vector Machines, Artificial Neural Network have been explored. Then the models have been validated and modified by another hospital patients. Our research will be benefitted on the clinical management of cardiovascular diseases and improve the understanding of the process of these diseases.

**Analyzing large data-sets. How to organize large sample data-sets and to retrieve additional information**

Antonio Facchiano, Daniela D’Arcangelo

Istituto Dermopatico dell’Immacolata, IDI-IRCCS, Rome, Italy

**Introduction**

The strong technological improvement of the last few decades led to the accumulation of an overwhelming and impressive amount of data in all fields of investigations. This is the good news.

However, the bad news is a somehow unexpected drawback, i.e., retrieving relevant data from the existing archives may often represent a stressful and frustrating task; nowadays, making literature searches and data interpretation like looking for a needle in a haystack.

Tons of new data are collected everyday with the daily publication of many new Google pages, in virtually any field. As an example within medicine-related fields, publication of new articles with “cancer” keyword is constantly increasing, reaching the astronomical amount of 3160557 articles with “cancer” keyword, published in PubMed up to December 31, 2014.

Handling such enormous data-sets is a multidisciplinary effort, requiring statistical, epidemiological, mathematical, bioinformatics, linguistic and bio-medical expertise. In fact, handling enormous data-sets is not just a matter of storage-space or retrieving techniques; rather it represents a higher-complexity level problem in data-interpretation, opening a new universe of analytical approaches.

In the current presentation we will show two examples of novel approaches to handle large biological data-sets and to retrieve novel aggregated relevant information.

**Results**

We report here the systematic investigation of the role each of the 117 chemical elements is known to play in melanoma, as compared to other cancers. As we have previously shown (Facchiano et al., “The role of chemical elements in melanoma”, *New Horizons in Translational Medicine*, 2015, 2, 73–80), 954 PubMed abstracts were analyzed, having “melanoma” in the title and any of the known chemical element. As a result of this approach, we now have a detailed occurrence-map of each chemical element in different cancers, such as melanoma and others, with potential applications in cancer pathogenesis or diagnosis.

We also report here how we handled 5934 PubMed abstracts, having “melanoma” as a main keyword; the aim of this study was to identify the most frequently used keywords associated to melanoma and to investigate how melanoma-associated fields have changed, as function of time in the last 15 years. The analysis allowed us to identify, for instance, the “autophagy” field as one of the most relevant fields in melanoma investigation as compared to other cancers such as breast-, colon- and prostate cancer (D’Arcangelo et al. “BAMM: a preliminary Bibliometric Analysis on Melanoma Manuscripts”, *Pigment Cell. Melanoma Res. 26*, 415–417, 2013).

**Conclusions**

Developing novel approaches to handle large data-sets available from the literature as well as from omics experiments, will allow to more efficiently extract novel information from existing data.

**Increased tissue load of FeO₂-containing PLGA-NPs coupled with anti Coll IV antibodies in normal and metastatic liver by focused microwave-induced hyperthermia**

Chiara Castellani

Co-authors: Rekha Cappellini1, Vlad Porumb2, Mona Abdel-Mottalib5, Chiara Fedeli1, Regina Tavano1, Ionut Tudorancea2, Gabriel Dimofte2, Alf Lamprecht3,4, Annalisa Angelini2, Emanuele Papini1, Marny Fedrig03

1 Department of Biomedical Sciences, University of Padua, Italy
2 University of Medicine and Pharmacy “Gr. T. Popa” Iasi, Romania
3 University of France-Comè, Besançon, School of Medicine
4 Pharmacy and Department of Pharmaceutics, Institute of Pharmacy, University of Bonn, Germany
5 Department of Cardiac, Thoracic and Vascular Sciences, University of Padua, Italy

**Background:** Nanoparticles, due to their loading versatility and targeting possibilities, are promising cancer therapeutics.
However, their efficacy is often compromised by the limited tumor-accumulation. Combined hyperthermal-chemotherapy has been demonstrated useful for local tumor control.

**Purpose:** We tested if local hyperthermia (42°C), induced by focused MW irradiation ameliorates the diffusion of PLGA-NPs coupled with anti-coll IV antibodies in normal and in metastatic liver tissues. Magnetic iron nanoparticles were embedded in the PLGA matrix to allow a second hyperthermic effect mediated by previously administered tissue-entrapped NPs upon MW further irradiation.

**Methods:** Adults male Wistar WAG/Rij rats were divided into groups: (1) controls (ctr); (2) healthy rats with nanoparticles (PLGA) injection; (3) healthy rats with PLGAs injection and heated; (4) rats with liver tumor-induced by colon-carcinoma 531 cells injection with/or not PLGA injection ad heated. Injection of PLGA nanoparticles containing oxide iron and QDs (Lumidot 590) was through ileo-colic vein after MW heating the isolated middle liver lobe. Livers were collected for histology. PLGAs identification, localization and distribution were performed by fluorescence immunohistochemistry and confocal microscopy for cytokertatin-20, pan-cytokeratin, vWf, CD68 (macrophages) and alpha-collagen IV.

**Results:** In both healthy and tumor-induced liver rats PLGAs injection resulted in a good localization. PLGAs were detected also after 48 h after injection. MV heated livers showed a better retention of PLGAs. After hyperthermia of liver metastases, PLGA-NPs also accumulated more efficiently in peri-tumor area, in the cytoplasm and nucleus of hepatocytes, endothelial cells, and macrophages, and within the tumor mass, apparently following collagen IV fibers distribution.

**Conclusions:** Localized hyperthermia via focused MW irradiation, enhances the selective tumor and peri-tumor accumulation of magnetic PLGA-NPs, a step potentially improving drug delivery to tumor metastasis. The secondary activation of NPs trapped iron oxide by MW may ensure an even more selective hyperthermic effect, possibly synergizing with the chemotherapeutics.

**CheTherDel project (ERA-Net Euronanomed JTC-3 2011) financing the study.**

**Role of bile extracellular vesicles in differentiating malignant from nonmalignant biliary stenosis**

**Valeria Severino**

Co-authors: Yohann Couté2, Jean-Marc Dumonceau3, Myriam Delhaye4, Solange Moll5, Jean-Louis Frossard1, 6, Annarita Farina1, 7

1Department of Internal Medicine Specialties, Geneva University, Geneva, Switzerland
2U1038, CEA IRTSV, Biologie à Grande Echelle, INSERM, Grenoble, France
3Gedyt Endoscopy Center, Buenos Aires, Argentina
4Department of Gastroenterology, Hepatopancreatology and Digestive Oncology, Erasme University Hospital, Université Libre de Bruxelles, Bruxelles, Belgium
5Department of Pathology, University Hospital Geneva, Geneva, Switzerland
6Division of Gastroenterology and Hepatology, University Hospital Geneva, Switzerland
7Department of Human Protein Science, Geneva University, Geneva, Switzerland

When biliary stenosis occurs, the inner lumen of the bile duct is reduced and bile stagnates above the stenosis. Such an abnormal condition makes bile able to collect and concentrate components released by tissues surrounding the stenosis thus providing an enlarged depiction of their extracellular environment. Among intercellular mediators emitted by cells, extracellular vesicles (EVs) have gained considerable attention as potential sources of new cancer biomarkers. EVs are cell-derived membrane structures, ranging from 30 nm to 2000 nm, whose composition in DNA, microRNA, mRNA, lipids, proteins and peptides is strictly related to their cellular origin. Tumor cells exhibit an intensified tendency to produce EVs showing the unique characteristic of horizontally transferring oncogenic material. In this context, the amplification effect of bile collected upstream the stenosis offer a powerful diagnostic opportunity to identify novel EVs signatures able to distinguish between malignant and nonmalignant biliary stenosis. Here we analysed bile samples from 40 patients presenting with biliary stenosis of malignant (pancreatic cancer, cholangiocarcinoma) and nonmalignant (chronic pancreatitis, biliary stones) origin. The study was approved by Ethics Committee of Geneva and Erasme Hospitals, and written informed consent was obtained for each patient. EVs were isolated by using differential centrifugation and characterized for shape and size by Electron Microscopy and Nanoparticle Tracking Analysis. The enrichment of bile EVs populations was then confirmed by immunoblot evidence for principal EVs markers (i.e., TSG101, ALIX, HSP70.1 and CD9). EVs proteins were subsequently extracted from pooled bile samples, subjected to proteomic analysis by GeLC–MS/MS and identified against the SwissProt database. This allowed the identification of 519 proteins with at least 2 unique peptides (FDR < 1%). Among them, all the most known EVs markers stood out. A number of cancer-associated proteins were also identified and the overexpression of 4 of them (CD133, c-SRC, CEAM6 and NGAL) was verified in malignant samples. The diagnostic performances of bile EVs were finally evaluated, allowing the identification of a new cancer-associated signature able to discriminate between malignant and nonmalignant biliary stenosis with 96.0% sensitivity, 100.0% specificity, 100.0% positive predictive value and 95.2% negative predictive value. Compared to conventional serum tumor marker CA19-9, bile EVs proved significantly more accurate (p-value=0.0081), with an overall area under the receiver operating characteristic (ROC) curve of 0.996 (95% CI: 0.986–1.006) vs. 0.808 (95% CI: 0.670–0.946). In conclusion, our results highlight the potential role of bile EVs in the differential diagnosis of biliary stenosis and support the concrete possibility of translating cancer-derived EVs signatures in clinical settings.

This work was supported by the “Fondation Ernst et Lucie Schmidheiny” and the “Ligue Genevoise Contre le Cancer”. The authors report no financial conflict of interest relevant to the subject of this study.

**Susceptibility to breast cancer: From genetics to prevention**

**Ulrich Pfeffer**

IRCCS AOI San Martino – Istituto Nazionale per la Ricerca sul Cancro, Genova, Italy

Susceptibility to breast cancer is determined by environmental (life style, diet, reproductive history) and genetic factors. Genetic determination is strong for carriers of mutations in BRCA1 and BRCA2, two high penetrance and low incidence tumor suppressor genes whose mutations determine an over 80% lifetime risk of breast cancer. These two genes only explain approximately 40% of all breast cancers with pronounced familiarity but no additional “strong” breast cancer genes have been identified. Multigenic determination and “private” mutations have been proposed for non BRCA1 and 2 mutated familiar cases. The genetic background
is believed to also contribute to the cancer risk of the 90% of breast cancers that occur sporadically since women with comparable environmental risk factors show a highly variable breast cancer incidence. Genome wide association studies have identified dozens of breast cancer risk associated polymorphisms (SNPs) and solid evidence has accumulated for some of these SNPs in validation studies. Genetic risk assessment can be translated into clinical applications through the inclusion of genetic information in lifestyle interventions and through the selection of subjects with increased risk for more intense screening protocols. Given that the risk associated with single SNPs rarely exceeds a hazard ratio of 1.3 (30% increased risk) the translational relevance of single SNP analyses is, however, limited. Multigenic models must be developed in order to guide preventative measures. There is also evidence that genetic risk associations differ for different breast cancer subtypes. Since the risk for estrogen receptor positive breast cancer appears to be influenced by the lifetime exposure to estrogens (early menarche, late menopause, reproductive history) women with an increased genetic risk for ER+ breast cancer could pay particular attention to estrogen related risk factors including diet. Prevention strategies can thus be targeted to specific genetic and environmental risk factors. We present the association analysis of genetic variants with somatic breast cancer mutations performed on exome and whole genome sequencing data. We show for the first time that carriers of a specific risk associated allele are at increased risk to develop breast cancer with a specific somatic mutation. In addition to increase our understanding of cancer biology and the complex genetic interactions in carcinogenesis our finding delivers evidence for the feasibility of prevention strategies where the effects of a specific mutation for which the subject is at risk could be prevented.

**Bridging the gap between translational research and precision medicine clinical workflow**

Wanmei Ou
Oracle Health Sciences, Boston, USA

Advances in genome diagnostics are starting to make an impact on patient care. The key challenge is how to enable a multidisciplinary care team to collaborate using genomic data from an individual patient. We describe an architecture which enables treating physicians, genetic counselors, researchers, molecular and pathologists to interact with the data using role-based interfaces which are designed for their task in the clinical workflow. Such a system can speed up the discovery of actionable biomarkers and transform the routine clinical care with the latest and the greatest findings, accelerating the evolution of human care.

**Complex tissue models that mimic the tumour microenvironment, heterogeneity, invasion, drug sensitivity and progression of cancers**

Matthias Nees

Co-authors: Malin Akerfelt, Johanna Björk, Mervi Toriseva, Sean Robinson, Vidal Fey, Ilmari Ahonen, Hannu-Pekka Schukov

University of Turku, Faculty of Medicine, Department of Bio-medicine & Anatomy, Turku, Finland

The efficacy of Drug Discovery in Oncology suffers from many shortcomings, and the majority of clinical studies still fail due to the lack of efficacy and/or unexpected toxicology. This process would generally benefit from biologically more relevant and complex model systems used in pre-clinical research. These models should more faithfully recapitulate the biology, histology, heterogeneity, and dynamic interactions of cancer cells, at least with the most relevant cell types present in the Tumour Microenvironment (TME). In particular, this should capture the interactions between tumour and stromal cells (cancer-associated fibroblasts, CAFs), and other cell types such as endothelial and immune cells. All of these processes are likely to affect drug sensitivity and/or resistance of tumours. Furthermore, also the choice of biologically relevant Extracellular Matrix (ECM) and other environmental factors must be considered carefully. To allow automated, robust and scalable high content screening (HCS) campaigns at reasonable cost and significant experimental throughput, cell- and tissue-based model systems must be thoroughly miniaturised and standardised, despite their biological complexity. In addition, scalability enables variable scopes of screening modes aiming at target validation, drug discovery, or lead discovery/lead development.

This talk will demonstrate how these demands can be addressed by a combination of cell-based assays, microscopic imaging, and automated image analysis (AMIDA software). This pipeline allows us to monitor and quantitate a large spectrum of diverse morphometric features formed in (cancer) microtissues. As a comprehensive, real-time and live cell platform for phenotypic drug discovery, it can be combined with specific and targeted readout as well as endpoint biomarker analyses. We demonstrate the use of siRNAs and biologicals for functional validation of putative biomarkers and targets. The living tissues generated display a multicellular architecture that resembles the histology of clinical biopsies. Multiple dynamic aspects inherent to tumour biology, such as invasion/motility, drug sensitivity versus formation of resistance, and heterogeneity, can be quantitatively captured. Bioinformatic, statistical and mathematical approaches are utilised to customise informative assays for specific applications. The clonal assay format further allows the use of very limiting numbers of rare cells, e.g. from primary patient-derived tissue cultures.

**Animal models characterization: from the bench to the bedside**

Elizabeth K. Leffel

Leffel Consulting Group, LLC, Washington, DC, USA

Bringing a new drug or vaccine to market can take 12–16 years and $2–8 billion, with a failure rate exceeding 95%. In the last five years, the field of ‘translational medicine’ has grown exponentially and will continue to expand as we strive to improve the success rate of drug development, while decreasing the time and cost to bring effective treatments to patients. The design, characterization and selection of appropriate animal models is a key to the success of drug development. It is perhaps the largest contribution area for translational research, requiring an in-depth understanding of systems biology and the disease pathogenesis enabling the identification of efficacy biomarkers. With vaccine development, the “correlate of protection” links preclinical immunological endpoints to humans. In therapeutics, biological endpoints or “biomarkers” are required to demonstrate efficacy in the animal models and in humans. In many cases (e.g., Ebola vaccine), efficacy testing cannot be completed in a human population because the disease incidence is too small to properly design a statistically powered trial. Reliance on preclinical models and appropriate biomarkers to bridge data can only be done using translational research strategies. These approaches must be integrated at every stage of vaccine development. At the Research and Development (R&D) stage, molecular tools are now available to screen likely candidates and discreetly discern the mechanism of action. In the preclinical stage, characterization of the animal model should include:
Development of tailored antimicrobial treatment regimens and novel host-pathogen insights for respiratory tract infections and sepsis. (TAILORED-Treatment)

John P. Hays
Co-authors: The TAILORED-Treatment Consortium
Erasmus University Medical Centre, Rotterdam, The Netherlands

Despite their immense contribution to global healthcare, antibiotics are currently recognized as the most misused drugs in the world with global overuse estimated at 40–70%. Antibiotic misuse often causes preventable adverse events that impact on patient care. Importantly, misuse also leads to the emergence of antibiotic resistance, one of the major threats to global health today. Unfortunately, current diagnostic tools for facilitating the appropriate use of antibiotics are often inadequate as antibiotic consumption and the spread of antibiotic resistance are continually increasing. TAILORED-Treatment (www.tailored-treatment.eu) is an EU FP7 funded project consortium comprising 7 European partners. Our main goal is to establish a broad-based strategy (not limited to a particular antibiotic group) that can be implemented on a broad scale to increase the effectiveness of antibiotic and antifungal therapy, reduce adverse events, and help limit the emergence of antimicrobial resistance in children and adults. The TAILORED-Treatment project is designed to maximize impact on patients and physicians while integrating and synergising with current EU funded research strategies. At the heart of the TAILORED-Treatment project is a prospective clinical study in which we will recruit 1200 patients (> 2000 patient samples) presenting with respiratory tract infections and/or sepsis. Patient cohorts will include equal representation of genders, children and adults. State-of-the-art molecular and biochemical technologies (transcriptomics, proteomics, genomics, microbiota analysis) will be developed and applied to characterize the host-pathogen interaction at the genomic, transcriptomic, proteomic, and clinical level. The data collected will be added together in a large-scale unique multi-dimensional dataset which will be stored in a publically available database, accessible to the EU scientific and clinical community. The consortium partners will also develop and apply new computational tools to interrogate the data, in order to provide new insights into personalized host-pathogen interactions, including the discovery of novel biomarkers for patient diagnosis and disease monitoring. In this respect, we will construct a predictive personalized treatment algorithm that will lead to informed and personalized antibacterial, antifungal and antimicrobial treatment regimens (indication, dosage, and duration) that are tailored to the needs (type of infection, presence of novel biomarkers etc.) of children and adults presenting with respiratory infections and sepsis. Finally, the algorithm and large-scale unique multi-dimensional dataset will be built into an easily navigable web-based, free-to-use, decision support system, ready for use by physicians to explore, test and assist in patient-tailored antimicrobial treatment decisions. The result will be a large-scale unique multi-dimensional dataset stored in a publically available database, which is accessible to the EU scientific and clinical community, as well as an easily navigable web-based, free-to-use, decision support system ready for use by physicians to explore, test and assist in patient-tailored antimicrobial treatment decisions.

Prenatal exposure to Trichostatin A: Epigenetic factors and animal models of autism

Tomasz Schneider
Co-authors: Brown Lawrence, Peirson Stuart, Bannerman David
Durham University School of Medicine, Pharmacy & Health, Stockton-on-Tees, United Kingdom

Prenatal exposure to valproic acid (VPA) in rodents is one of the best characterized models of autism with a strong face, construct, and predictive validity. Due to a double mechanism of action of VPA (GABAergic vs. histone deacetylase (HDAC) inhibition), it was suggested that epigenetic mechanisms might be responsible for inducing both behavioural and morphological autistic-like aberrations in VPA model (Kataoka et al., 2011). Recent paper by Moldrich and colleagues (2013) confirmed that autistic-like aberrations can be reproduced in animals exposed to Trichostatin A (TSA, HDAC inhibitor) at the critical prenatal developmental stage based on the VPA model. The new model shows a set of behavioural aberration, including decreased social reciprocal interaction, described previously in VPA model and autism, but cognitive and stress-related behaviour and circadian rhythms have not been studied in the TSA model. Cognitive decline, increased anxiety, and sleep problems are a prominent part of autistic phenotype, and predict severity of the core symptoms.

Materials and methods: C57BL mice were mated and the day when the plug was found was designated as the 1st day of gestation. On gestational day 12 pregnant females were injected with 1mg/kg of Trichostatin A dissolved in 1% DMSO solution. Control females received 1% DMSO solution only. No difference in pregnancy duration, maternal behaviour, litters’ size, or number of offspring was found. Both male and female offspring were used. Experiments were conducted in adolescence (PND 30–45) and adult (> 2 months) animals. A broad battery of tests was used including the elevated plus maze, light/dark box, spontaneous alternation task, Y maze spatial novelty, reciprocal social interaction, object recognition test, prepulse inhibition, swimming Y maze, 3-chamber sociability test, and circadian rhythms including light phase shifting and entrainment.

Results: TSA mice showed (1) increased anxiety in the light/dark box, but not in the elevated plus maze; (2) increased perseverative behaviour; (3) no difference in an object recognition test or Y maze spatial novelty test, but a deficit in associative learning in a swimming Y maze; (4) no difference in sociability ad social novelty preference in the 3-chamber apparatus; (5) no difference in sensorimotor gating; (6) no difference in circadian activity and responses to light phase shifting and entrainment. In summary, prenatal exposure to TSA led to increased anxiety, cognitive aberrations, and increased perseveration, which are important aspects of autistic phenotype, but had no impact on sociability measured in the 3-chamber apparatus.
Stromal exosomes convey increased proliferation and chemoresistance to colorectal cancer cells via ERK and AKT activation respectively

Rahul Bhome

Co-authors: Rebecca Goh, John Primrose, Emre Sayan, Alex Mirnezami

Cancer Sciences, Southampton University, Southampton General Hospital, Southampton, UK

Introduction: Exosomes are extracellular vesicles which are transferred between stromal and epithelial cells in the tumour microenvironment. Their cargo consists of nucleic acids and proteins which can be shuttled from one cell to another. Stromal exosome transfer influences cancer progression in breast cancer models but the mechanisms are not well defined in colorectal cancer. We aimed to investigate the functional effects of stromal exosomes on colorectal cancer cells.

Method: Exosomes were isolated from MRC5 fibroblasts grown in exosome-deplete fetal calf serum by serial ultracentrifugation. The isolate was validated by transmission electron microscopy, western blotting/immunogold staining and confocal microscopy. Changes in cellular protein expression were measured by western blotting. Cell proliferation was measured on days 1–4 by nuclear staining and cell counting. Chemoresistance was measured after 24-hour exposure to oxaliplatin (300 nM) by flow cytometry for mitochondrial depolarisation.

Results: Fibroblast exosomes were shown to be correctly sized (40–100 nm) with a lipid bilayer structure, demonstrated CD63 and TSG101 proteins by western blot/immunogold staining and showed uniform size and DNA content by flow cytometry with propidium iodide staining. Fibroblast exosome transfer to colorectal cancer cells was clearly shown by fluorescence and confocal microscopy. After exosome transfer, colorectal cancer cells demonstrated increased ERK1/2 phosphorylation, increased AKT phosphorylation and increased Bad phosphorylation. This corresponded with increased proliferation and chemoresistance respectively.

Conclusion: Genetic information within fibroblast exosomes is translated into altered protein expression in colorectal cancer cells when transfer occurs. Increased ERK activity corresponds with increased cell proliferation. Increased AKT phosphorylation leads to increased Bad phosphorylation, which is a known anti-apoptotic switch. This corresponds with chemoresistance to oxaliplatin.

Deregulated stromal and epithelial microRNAs: Role in predicting recurrence in Stage II colorectal cancer

Alex Mirnezami


University of Southampton Cancer Sciences Division, Somers Cancer Research Building, Southampton University Hospital NHS Trust, Southampton, UK

Background: MicroRNAs (miRNAs) are a class of small highly conserved RNAs that provide widespread expression control through the translational repression of mRNA. MiRNAs have fundamental roles in the regulation of intracellular processes, and their importance during malignant transformation and metastasis is becoming increasingly well recognized. In recent years, deregulated miRNAs have also been identified in cancer associated stromal cells during disease progression.

Aim: In this study, we aimed to separately characterise patterns of deregulated miRNA expression in colorectal epithelium and stroma, and examine their clinical utility as early markers of metastasis.

Experimental design: Using high-through screening and high-sensitivity quantitation techniques, coupled with laser microdissection (LMD) we identified epithelial and stromal miRNAs deregulated in 10 paired CRC and normal colonic tissue samples. Findings were validated and further profiling conducted, in a second cohort of 50 patients with stage-II disease, with and without metastasis at 5 years.

Results: In the stroma, deregulated miRNAs distinguished tumour from paired normal tissue and early (Duke’s A) from late stage (Duke’s C) CRC specimens. Furthermore, miRNA expression profiles in tumour epithelium and stroma were almost entirely distinct: Upregulation of known oncogenes miR-21 (stromal) and miR-106a (epithelial) occur in different tumour strata in CRC. MiRNA candidates, alone and in combination predicted short DFS in stage-II CRC. Stomal miR-21 (HR = 2.68, p = 0.015); stromal miR-556 (HR = 2.60, p = 0.018); epithelial miR-106a (HR = 2.91, p = 0.008); combined (All High vs. All Low: HR = 5.83, p = 0.002).

Conclusions: Stromal and epithelial miRNA profiles may be used to identify patients at high risk of recurrence in stage-II disease. Our data supports the notion that stromal as well as epithelial miRNAs play important roles during disease progression, and that separate miRNA profiling of the tumour compartments may be a valuable aid to therapeutic decision making.

Translational bioinformatics in action: Re-defining the current diseases taxonomy

Erfan Younesi

Fraunhofer Institute for Algorithms & Scientific Computing SCAI, Sankt Augustin, Germany

Currently, diagnosis of diseases is based on old criteria that have not witnessed major changes over the past century. Current disease classification systems such as ICD (international classification of disease) are based on old schemas dating back to 1885 and make use of symptoms measured clinically or standard laboratory and imaging techniques to establish major types and subtypes of diseases. Nowadays, advanced technologies have enabled us to produce a deluge of molecular and clinical data. However, the challenge for translational research is to link these data across multiple biological scales in the context of human diseases. Indeed, incorporating molecular and genomic information into diagnosis of complex diseases could transform drug development and medicine. In contrast to the established disease classification systems, a “mechanism-based taxonomy” is based upon the knowledge about the biological pathways involved in the etiology of a disease to guide the classification of disease classes and subclasses. Translational bioinformatics plays a key role in redefining the current disease taxonomy by bridging the gap between the molecular and clinical realms. The talk will cover...
Infectious hepatitis viral diseases are major health problems in both developing and developed countries, and are transmitted via parenteral and faeco-oral routes. Hepatitis E virus (HEV) is an important cause of severe hepatitis in humans and is responsible for unusually high rates of mortality in pregnant women because of the development of fulminating liver disease. In our work, we have demonstrated that the HEV capsid comprises capsomeres of a homodimeric structural capsid protein (E2) that forms a partially enclosed shell. Using information garnered from the crystal structure, we verified the mechanism by which the E2s domain protrudes from the viral surface to engage with host cells to initiate infection. Following this, we determined the crystal structure of the HEV E2s (I) domain in complex with the 8C11 Fab, which specifically neutralizes antibody, at 1.9 Å resolution to identify the 8C11 epitope(s) involved in the binding. Through mutational analysis and cell model assays, we identified the most crucial residue for HEV interaction and the genotype I preferred neutralization by 8C11. Very recently we have studied the broad neutralization of all genotypes of HEV by a cross genotype mAb 8G12. The antibody:antigen complex structure, mutational studies and cell model assays showed that 8G12 equally recognizes all 4 genotypes of HEV and neutralizes the infection. Overall, these findings provided data that was instrumental in the creation of a vaccine for HEV, and will lead to the development of antibody-based specific drugs for the treatment against HEV.

Identification of a new cell population endowed with a healing capacity and constitutively circulating in healthy conditions

Robert Tasso

Co-authors: C. Lo Sicco1, D. Reverberi2, M. Cilli2, U. Pfeffer2, R. Cancetta1

1 Department of Experimental Medicine (DIMES), University of Genova & IRCCS A.O.U. San Martino-IST, National Cancer Research Institute, Genova, Italy
2 IRCCS A.O.U. San Martino-IST, National Cancer Research Institute, Genova, Italy

Stem and progenitor cells are the critical units for tissue maintenance, regeneration, and repair. The activation of regenerative events in response to tissue injury has been correlated with mobilization of tissue-resident progenitor cells, which is functional to the wound healing process. However, until now there has been no evidence for the presence of cells with a healing capacity circulating in healthy conditions. Here, we identified a rare population of cells present in the peripheral blood of healthy mice that actively participates in tissue repair. These Circulating Healing (CH) cells were identified by an innovative flow cytometry strategy as small cells not expressing CD45 and lineage markers. The analysis of their global transcriptome revealed their uniqueness when compared to other cells characterized by varying stemness degree, including Hematopoietic Stem cells (HSCs), Mesenchymal Stem Cells (MSCs), and Very Small Embryonic-Like (VSEL) Stem Cells. Moreover, CH cells presented a high expression of key pluripotency-associated genes and positive selective markers of the epiblast developmental stage. CH-labeled cells derived from healthy Red Fluorescent Protein (RFP)-transgenic mice and systemically injected into syngeneic fractured wild-type mice effectively migrated and engrafted in wounded tissues, and ultimately differentiated into tissue-specific cells. Accordingly, the number of CH cells in the peripheral blood rapidly decreased following femoral fracture. These findings uncover the existence of constitutively circulating cell populations that may represent novel, accessible, rapid and very versatile effectors of therapeutic tissue regeneration.

Identification of genes specifically expressed in stem cells using an unbiased data-driven approach

Frank Staubli

Co-authors: Gaelle Messerli, Philip Zimmermann

Nebion AG, Zürich, Switzerland

Stem cells promise a precious source for the treatment of various degenerative diseases due to their capacity for self-renewal and ability to differentiate along multiple cell lineages. Gene expression analysis, both genome-wide and targeted at specific gene subsets, has played a key role in improving our understanding of the genetic attributes of stem cells through identification of molecular signatures that characterize normal stem cell function. In this study, we searched for genes most specifically expressed in stem cells as compared to over 1400 other tissues, cell types and cell lines. We used the “Genevestigator” curated gene expression data platform to screen a compendium of 26,982 profiled samples on the Affymetrix Human 133 Plus2 chip. We initially selected all embryonic stem cell lines as target, and all other cell lines and normal tissues (including primary cells) as base in order to identify the top 50 genes. Removing redundant transcripts yielded a list of 46 unique transcripts, of which several are well known in stem cell research (e.g. ESRG) but about half of the genes identified have not yet been associated with stem cell development or pluripotency in the literature, and about one fourth has not yet been characterized at all. From the 3032 experimental conditions tested, less than 100 caused significant changes of expression in any of the stem cell specific genes. To further identify patterns of co-regulation, we created a new data matrix containing only conditions that significantly regulate the 46 genes and ran various bi-clustering and hierarchical clustering analyses. Interestingly, genes from one cluster identified are non-responsive to almost all 3032 conditions, except for the stem cell differentiation studies where they are strongly down-regulated. Most of the transcripts from this cluster are uncharacterized and thus represent potential novel targets. Moreover, we were able to identify clusters of potentially co-regulated genes. To explore the space of transcripts beyond this list, we performed a co-expression analysis of one example gene, ESRG, across conditions relevant for this gene and retrieved the top-100 transcripts. Three main clusters turned up, one largely overlapping with the cluster having the...
unknown transcripts described before, one having immune-responsive and some other genes and a third one revealing four POU class homeobox genes. Thus, “Genevestigator” allowed us to easily exploit over 25,000 profiled samples and to efficiently identify genes highly specific for a cell type and to study the regulation of these genes in response to perturbations. In fact, while all stem cell specific genes possessed the same profile by tissue and cell type, subsets of genes had very distinct patterns of regulation across perturbations.

In vivo/in vitro translation of a molecular signature in the context of preclinical drug-induced kidney injury

Caroline Bertea

Co-authors: S. Snelling, P. De Ron, S. Dremier, B. Massant, G. Toussaint, J.P. Valentin, A. Nogueira da Costa

1 Faculty of Pharmacy, University of Lorraine, France
2 Faculty of Biological Sciences, University of Leeds, UK
3 Non Clinical Development, UCB Biopharma SPRRL, Belgium

Drug-induced kidney injury (DIKI) is one of the contributors to drug attrition seen at pre-clinical and clinical settings during drug development. The development of tools that would allow for an earlier, more reliable and sensitive detection of acute renal toxicity during drug development are warranted. This study aims at developing a molecular biomarker signature evaluating the in vivo/in vitro translatability of renal toxicity in the context of DIKI. The in vivo work comprised of a 7-day toxicity study conducted in male Wistar rats. Rats were administrated daily (by gavage) with UCB compound X (vehicle, 10, 100 mg/kg/day) known to induce DIKI in the pre-clinical setting. Post-compound gavage, tissue and urinary samples were collected for histopathology, protein biomarker and toxicogenomic analysis. Histopathological findings were identified at 100mg/kg on day 1, with degeneration and single cell necrosis in proximal tubules being evident at day 3. These findings correlated with increased urinary levels of clusterin, KIM-1 and albumin (3.9, 5.7 and 3.9-fold respectively at day 1, 100 mg/kg vs. control). By whole genome expression analysis, we identified a panel of 16 genes of interest (0.5 < fold-change > 2, p < 0.05), involved in Wnt and TNF signalling pathways and extracellular matrix and focal adhesion pathways. In order to determine in vivo/in vitro translatability, rat (NRK-52E), canine (MDCK) and human (HK-2, RPTEC/TERT1) kidney cells and primary rat, canine and human renal tubular epithelial cells were exposed to UCB compound X and reference nephrotoxicans (cisplatin, cyclosporin-A and ochratoxin-A). Cell viability was measured over 72 hours after compound exposure by Real Time Cell Analysis. After a 24 h, loss of cell viability was observed in MDCK (48%) and NRK-52E (30%) cells with UCB compound X at 100 μM. RNA was extracted at 6, 24, 48 and 72 h after compound exposure and the expression profiles of the genes of interest, previously validated in the in vivo study, was evaluated by qRT-PCR. In NRK-52E cells, UCB compound X (100 μM) induced deregulation of 4 genes identified in vivo, two of which were also deregulated by cisplatin (100 μM), cyclosporin-A (10 μM) and ochratoxin-A (2 μM). Further validation in MDCK, HK-2 and primary kidney cells is on-going. In the preclinical setting, our results show the potential in vivo/in vitro translatability of a molecular biomarker signature in the context of DIKI. The evaluation of a potential preclinical-clinical translatability will be further assessed in the context of acute kidney injury patients.

Development of a decision-making biomarker for CRTH2 antagonism in clinical studies

Daniel Strasser

Co-authors: Hervé Farine, Martin Holdener, Jochen Zisowsky, René Roscher, Julie Hoerner, Martine Gehin, Patricia N. Sidharta, Jasper Dingemanse, Peter M.A. Groenen

Actelion Pharmaceuticals Ltd, Basel, Switzerland

Biomarkers have shown to improve success rates in the development of novel drugs, providing essential information in the early phases of clinical development for decision-making. Chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2) is pursued as a drug target for a number of inflammatory diseases. CRTH2 antagonists block the activation and migration of key inflammatory cells such as eosinophils, basophils, and Th2 cells. The mechanism of action of CRTH2 antagonists was established in cells isolated from human blood. Biomarkers derived from these experiments were included in clinical studies to investigate the mechanism of action and potency of CRTH2 antagonists in human. For clinical phase I studies with the CRTH2 antagonist ACT-453859, a follow-up molecule of setipiprant, inclusion of the most precise and robust pharmacodynamic (PD) biomarker with a clinically relevant target effect was desired to aid phase II dose selection. Candidate biomarkers such as IL-13 secretion from Th2 cells and CRTH2, CD11b and CD203 modulation on basophils and eosinophils in whole blood were compared in terms of signal intensity and variability. Blockade of CRTH2 receptor internalization was finally chosen as PD biomarker and rigorously tested in a feasibility study. The assay showed excellent robustness, an intra-assay precision of 5% and inter-subject variability smaller than 15%. Based on phase II clinical study results with setipiprant, 90% CRTH2 receptor blockade was defined as clinically relevant PD effect. This target PD effect provides the means to take decisions based on the data generated in the phase I clinical studies with ACT-453859.

Molecular biomarkers in neurosciences: Two-way translational strategies supporting drug development and early disease detection

André Nogueira da Costa


1 UCB Biopharma Sprl, Belgium
2 Department of Neurodegenerative Diseases and Hertie Institute for Clinical Brain Research, University of Tübingen, Tübingen, Germany
3 German Center for Neurodegenerative Diseases (DZNE), Tübingen, Germany

Discovery and development of new drugs in the neuroscience field has faced several challenges. For these to be overcome, understanding disease mechanisms and the associated value of translational biomarkers is key. The latter has been the focus of growing interest due to recent developments in the field of Alzheimer’s and Parkinson’s disease (PD) where breakthrough discoveries were done on the pathophysiology which have led to the identification of novel clinical molecular biomarkers. In the preclinical setting, the discovery, development and validation of translational biomarkers has to overcome limitations such as limited number of predictive animal
models, lack of clinical-to-preclinical validation of biomarkers, low-sample volume of biofluids and reduced assay robustness. Translatability is key for the successful implementation of molecular biomarkers in the preclinical and clinical settings. We have developed two-way translational biomarker strategies, in the context of neurodegeneration, focusing on multi-biomarker approaches evaluating disease pathology progression. Our strategies are based on (1) the identification of mechanistically relevant, translatable biomarkers; (2) the assessment of changes to mRNA, miRNA and protein biomarkers in CSF and plasma with a range of technological platforms; (3) a translatable bioanalytical approach and (4) the use of relevant preclinical models and clinical cross-sectional and longitudinal cohorts. In the preclinical arena, we explored a ‘reverse-translational’ strategy to investigate the performance of pT181 as a cerebrospinal fluid (CSF) molecular biomarker in a transgenic mouse model expressing human Tau with frontotemporal dementia mutation. Our results show distinguishing profiles in non-transgenic and transgenic mice (90% sensitivity) associated with a robust bioanalytical approach allowing the analysis of pT181 in 1 μl of CSF. In the clinical arena, we are evaluating a panel of molecular biomarkers (Tau, pT181, total α-Syn, p-α-Syn, ApoA1, among others) in the plasma and CSF of healthy individuals and early PD patients. These data are being integrated with genome wide association studies (GWAS) and clinical endpoints to define an algorithm that would support early diagnosis, and eventually early detection, of PD. These findings will then be ‘reverse-translated’ to the preclinical setting in line with the proposed strategies. Our two-way translational molecular biomarker strategies (1) have shown the potential applicability of pT181 in both clinical and preclinical settings and (2) are focusing on the development of an extended panel of molecular biomarkers to better characterize neurodegeneration and neuroinflammation in the clinical setting with the potential to be applicable in the preclinical setting.

The challenges and opportunities for diabetes preventative services in family medicine

Josie Messina and Rebecca Morris

Co-authors: Sanders and Campbell
University of Manchester, Centre for Primary Care, Manchester, UK

Background: Family practice is seeing an alarming rise in diabetes cases which could potentially be prevented through lifestyle interventions. Clinical trials indicate that diabetes risks can be cut by as much as 60% through lifestyle interventions; however very little is known about the practicalities of providing such services in family medicine.

Objectives: To explore how physicians and nurses approach diabetes prevention for at risk-patients in routine appointments in family medicine to understand how health professionals personalize care for diabetes prevention.

Methods: Four medical centres serving mixed urban city populations in the UK recruited 32 at-risk patients for appointment observations. Follow-up interviews with 30 patients, and 20 professions were completed. Thematic analysis uncovered themes in the data.

Results: Patients and health professionals placed a high value on preventative services in family medicine, although competing interests, lack of time, and motivation to change proved to be barriers. Professionals made note of the challenges of working in urban practices where socio-economic status and health literacy varied considerably. Opportunities for change arose from a new diagnosis of pre-diabetes or a patient’s family history. This was a health professionals chance to engage the patient in a tailored lifestyle change plan. For instance, professionals provided lifestyle advice depending on a patient’s level of risk, health literacy, and lifestyle. This approach was useful for patients and they valued Family Medicine Clinics as a venue for preventative services.

Conclusion: Very little is known about diabetes prevention outside of clinical trials, and this study demonstrated that Family Medicine can play a useful role in promoting healthy lifestyles for diabetes prevention despite challenges.

Space flight omics and their relation to translational medicine on earth

Michael A. Schmidt

Research Innovation Center, Colorado State University, USA

Space flight is one of the most extreme conditions encountered by humans. Advances in Omics methodologies (genomics, epigenomics, transcriptomics, proteomics, and metabolomics) have revealed that unique differences exist between individuals. These differences can be amplified in extreme conditions, such as space flight. In this session, we explore the role of targeted and untargeted omics in describing how converging variables can aggregate across molecular networks to affect astronaut health, safety, and performance on space exploration missions. We further explore how integrated omics can be used to accelerate discovery of space-based countermeasures for missions with short timelines, such as the first human mission to Mars in 2022. This includes an examination of how such personalized measures are relevant before, during, and after space missions. We finally explore how these findings may be translated to earth-based discovery and medical practice.

The role of biomedical technologies in modern drug discovery and early clinical development

Araz A. Raoof

Janssen Pharmaceutical, Belgium

The pharmaceutical industry faces a major challenge to improve R&D innovation and productivity. Current approaches to drug discovery are not sustainable due to high clinical attrition rates primarily. Recent advances in biomedical technologies (namely Biospecimens, single cell analysis, multi-parametric readouts and predictive modelling) using patient derived materials offer a great opportunity to unravel the biological pathways involved in human diseases and therefore, a whole new approach to drug discovery. In this presentation we highlight the essentials of this new model and explain how it could close the translational gap between bed and bench and thereby significantly improve R&D productivity.

Specific PIWI-interacting RNA (piRNA) expression patterns in human cancer

Alessandro Weisz

Co-authors: Francesca Rizzo, Antonio Rinaldi, Giovanna Marchese, Angela Cordella, Giovanni Nassa, Maria Ravo, Roberta Tarallo
Laboratory of Molecular Medicine and Genomics, University of Salerno, Baronissi (SA), Italy

PIWI-interacting RNAs (piRNAs) represent a newly discovered class of small noncoding RNAs abundantly expressed in germ cells, where they control genome stability via transposon silencing. Recent studies showed that piRNAs are expressed also in somatic cells, including stem and differentiated ones, where they control gene expression at transcriptional and post-transcriptional level, by modulating DNA methylation and RNA stability. By small RNA-Seq we detected specific patterns of piRNA expression in cancer cell lines and neoplastic and pre-neoplastic lesions of the breast, endometrium, liver and colon that are significantly different from those in paired normal tissue of the same organs and correlating with the clinical phenotype of the disease and, in some cases, with responsiveness to therapy. The cellular levels of specific piRNAs were found responsive to growth conditions, mitogens and transcription factors in cancer cell lines ‘in vitro’. Western blotting and/or Q-PCR revealed in all cases the presence of PIWIL (PIWI Like) subfamily proteins and most other components of the piRNA biogenesis and effector pathways, suggesting that these are indeed functional in cancer cells. The putative mRNA targets of deregulated piRNAs, identified by computational analysis, belong to molecular pathways known to be active and of importance in cancer cells, suggesting how these small noncoding RNAs could exert a dominant control on the activity of key functions in transformed cells. When combined, these results reveal a potential role of somatic piRNAs in carcinogenesis and maintenance of the cancer cell phenotype.

RESEARCH SUPPORTED BY: AIRC (Grant IG-13176), MIUR (PRIN 2010LC747T_002) and CNR (InterOmics Flagship Project).

miR181b is induced by the chemopreventive polyphenol curcumin and inhibits breast cancer metastasis via down-regulation of the inflammatory cytokines CXCL1 and -2

Beatrice Bachmeier

Co-authors: Emanuel Kronski, Micel E, Fiori, Ottavia Barbieri, Simonetta Astigiano, Valentina Mirisola, Peter H. Killian, Antonino Bruno, Arianna Paganin, Francesca Rovera, Ulrich Pfeffer, Christian P. Sommerhoff, Douglas M. Noonan, Andreas G. Nerlich, Laura Fontana

Department of Surgical and Morphological Sciences, University of Insubria, Varese, Italy
Department of Biotechnologies and Life Sciences, University of Insubria, Varese, Italy
Department of Pathology, Academic Hospital Munich-Bogenhausen, Munich, Germany

Chronic inflammation is a major risk factor for the development and metastatic progression of cancer. We have previously reported that the chemopreventive polyphenol Curcumin inhibits the expression of the proinflammatory cytokines CXCL1 and -2 leading to diminished formation of breast and prostate cancer metastases. In the present study, we have analyzed the effects of Curcumin on miRNA expression and its correlation to the antitumorigenic properties of this naturally occurring polyphenol. Using microarray miRNA expression analyses, we show here that Curcumin modulates the expression of a series of miRNAs, including miR181b, in metastatic breast cancer cells. Interestingly, we found that miR181b down-modulates CXCL1 and -2 through a direct binding to their 3′-UTR. Overexpression or inhibition of miR181b in metastatic breast cancer cells has a significant impact on CXCL1 and -2 and is required for the effect of Curcumin on these two cytokines. miR181b also mediates the effects of Curcumin on inhibition of proliferation and invasion as well as induction of apoptosis. Importantly, overexpression of miR181b in metastatic breast cancer cells inhibits metastasis formation in vivo in immunodeficient mice. Finally, we demonstrated that Curcumin up-regulates miR181b and down-regulates CXCL1 and -2 in cells isolated from several primary human breast cancers.

Epigenetic regulation of gene transcription and cell cycle progression by Ikaros in high-risk leukemia

Sinisa Dovat

Co-authors: Chunhua Song, Yali Ding, Chandrika Gowda, Sunil Muthusami, Kimberly J. Payne

1Pennsylvania State University College of Medicine, PA, USA
2Department of Pediatrics, PA, USA

The development of high-risk pediatric B-cell acute lymphoblastic leukemia (B-ALL) is associated with deletions or inactivating mutations in a single allele of the Ikaros (IKZF1) tumor suppressor. Ikaros encodes a DNA-binding zinc finger protein that has been shown to associate with chromatin remodeling complexes and to regulate transcription of its target genes via chromatin remodeling. However, the mechanisms through which Ikaros exerts its tumor suppressor effects in leukemia are unknown. Here, we used chromatin immunoprecipitation coupled with next generation sequencing (ChIP-seq) to identify Ikaros target genes and functional experiments to determine mechanisms that regulate Ikaros activity in leukemia. ChIP-seq experiments performed in human B-ALL cell lines and in primary B-ALL cells with wildtype Ikaros alleles, revealed that Ikaros binds to the promoter regions of a large set of genes that are essential for cell cycle progression. Ikaros gain-of-function and loss-of-function experiments show that Ikaros negatively regulates transcription of these genes and suggest that regulation of cell cycle progression is one mechanism of Ikaros tumor suppression in leukemia. Serial quantitative chromatin immunoprecipitation (qChIP) experiments spanning the promoters of Ikaros target genes suggests that Ikaros represses transcription of its target genes by inducing heterochromatin, as evidenced by the presence of H3K9me3 and H3K27me3 and the loss of H3K9ac histone modifications. Next, we analyzed Ikaros binding to promoters of its target genes in primary high-risk B-ALL that is characterized by the deletion of one Ikaros allele resulting in Ikaros haploinsufficiency. qChIP analysis of Ikaros DNA binding in primary high-risk B-ALL cells from 3 different patients revealed that Ikaros DNA-binding affinity is severely reduced. Impaired Ikaros binding to the promoter regions of its target genes in high-risk leukemia was associated with the loss of H3K9me3 and the strong presence of H3K9ac by qChIP. This indicates that impaired Ikaros function in high-risk leukemia results in the loss of heterochromatin around promoters of Ikaros target genes and the
formation of open chromatin that favors transcription. In conclusion, we present evidence that the Ikaros tumor suppressor protein regulates cell cycle progression by repressing the transcription of genes that promote cell cycle progression via chromatin remodeling in leukemia. In high-risk leukemia with Ikaros haploinsufficiency, Ikaros DNA-binding affinity is impaired resulting in the loss of Ikaros-mediated repression of its target genes. Our results identify epigenetic regulation of cell cycle gene expression as a novel mechanism of Ikaros tumor suppressor activity in leukemia.

Experiences and challenges in running a Bioinformatics Core facility

Puthen Veettil Jithesh

Sidra Medical and Research Center, Doha, Qatar

Bioinformatics is an essential component driving translational research. The collection, storage, analysis and interpretation of large volumes of data generated by next generation sequencing (NGS) technologies require appropriate infrastructure and expertise. A Bioinformatics Core provides an ideal facility that caters to the needs of a variety of research projects in an institution, relieving the investigators from recruiting and training Bioinformatics specialists for their projects. However, setting up and running a Bioinformatics Core Facility is far from trivial due to the diverse nature of projects and data types, expectations and timelines for delivery. Bioinformatics Core Facility at Sidra provides collaborative Bioinformatics support to researchers from within the institution and several other organizations in Qatar. Projects that are handled by the Core range from rare diseases with a few samples to data from large scale population genomics projects. I will discuss how the challenges associated with dealing such diverse projects are managed in our Core Facility giving examples of projects and resources.

Poster Abstracts

Human iron status: Hepcidin as novel clinical biomarker compared to conventional diagnostic biomarkers

Dietmar Enko, Helga Wagner, Gernot Kriegshäuser, Robert Stolba, Gabriele Halwachs-Baumann

Institute of Laboratory Medicine, General Hospital Steyr, Steyr, Austria

Background: Hepcidin is considered as a key regulator of human iron homeostasis, lowering the serum iron in the blood circulation by binding and down regulating the iron exporter protein ferroportin in the basolateral site of duodenal enterocytes and reticuloendothelial macrophages. High hepcidin serum-levels decrease the iron transport out of the enterocytes as well as the ability of macrophages to export recycled iron from senescent enterocytes. The aim of the present study was to investigate, whether hepcidin is a useful additional clinical biomarker to reflect the actual human iron status, especially functional iron deficiency (ID), as a state of inadequate iron supply to erythropoietic precursor cells in the bone marrow despite the presence of storage iron in the hepatocytes/macrophages.

Methods: All in all, 233 consecutive hospitalized adult patients with suspected ID, were included. In total, 33.91% (n=79) were male and 66.09% (n=154) were female. The median age was 69 (range: 20–93) years. All subjects were investigated for hepcidin, reticulocyte hemoglobin content (Chr), soluble transferrin receptor (sTfR)/log ferritin ratio (i.e. Thomas plot), sTfR, ferritin, transferrin saturation (TSAT), C-reactive protein (CRP) and for complete blood cell count. The hepcidin measurements were performed with the recently launched European Community (CE)-marked enzyme-linked immunosorbent assay (ELISA) (Hepcidin-25 bioactive ELISA; DRG Instruments GmbH, Marburg, Germany). Functional ID was defined as a Chr < 28 pg. Separate logistic regression models were calculated with all potential biomarkers to evaluate and compare the predictive performance with respect to functional ID in patients without (CRP ≤ 0.5 mg/dL) and with (CRP > 0.5 mg/dL) acute-phase reaction, respectively.

Results: The hepcidin measurements correlated with parameters of iron metabolism. There was a positive correlation with serum ferritin (p < 0.0001, Pearson correlation coefficient 0.2608) and TSAT (p = 0.0349, Pearson correlation coefficient 0.1383), and a negative correlation with transferrin (p < 0.0001, Pearson correlation coefficient −0.6021), sTfR (p < 0.0001, Pearson correlation coefficient −0.3736), and iron (p = 0.1617, Pearson correlation coefficient −0.0920). There was also a positive correlation between CRP and hepcidin (p < 0.0001, Pearson correlation coefficient 0.2581). One hundred seventeen patients with CRP > 0.5 mg/dL showed a distinctly higher hepcidin median value (35.60 [range: 4.27 – 80.03] ng/mL) as compared to 116 patients with CRP ≤ 0.5 mg/dL (18.55 [range: 3.77 – 73.01] ng/mL). With respect to functional ID, sTfR/log ferritin ratio and sTfR were of better positive predictive value (PPV) (sTfR/log ferritin ratio: 58.33 and 70.83%; sTfR: 60.00 and 60.00%) than when compared to hepcidin (PPV: 37.74 and 42.86%) and ferritin (PPV: 27.54 and 46.15%) in both subgroups.

Conclusions: The sTfR/log ferritin ratio, as well as sTfR, were better predictors of functional ID in patients with and without acute-phase reaction as compared to hepcidin-25 and ferritin.

The OTR antagonist, OBE001, inhibits both spontaneous and OT-induced contractions of human pregnant myometrium in vitro

Oliver Pohl, Shankari Arulkumaran, Sung Hye Kim, Andre Chollet, Phillip R. Bennett, Vasso Terzidou

ObsEva SA, Geneva, Switzerland

Currently the only drug licensed in Europe for inhibition of preterm contractions is the intravenous oxytocin (OT)/arginine vasopressin receptor antagonist Atosiban. OBE001 is a more selective OT receptor antagonist than Atosiban and may be administered orally. Here we compare the effects of Atosiban and OBE001 on spontaneous and OT-induced contractions of human pregnant myometrium in vitro. Experiments were performed using a DMT Myograph 800MS in oxygenated Krebs solution, with ADI Powerlab software allowing simultaneous measurements of eight muscle preparations. Once regular contractions had been established for 20+ minutes baseline measurement of contraction frequency, contraction peak, contraction duration, work per contraction (area under curve) and total work (area under all contractions) were made. The inhibitor compound was then added and the effects upon contractility measured in the next ten minutes. The effect of the inhibitor upon agonist (OT) was then measured by adding increasing concentrations of OT (1, 10, 100 nM) at 10 min intervals. Atosiban was studied at 6, 60 and 600 nM. Atosiban had no effect upon spontaneous contractions (and may have agonist effects upon the rate of contractions at low...
concentration). Atosiban antagonized the effects of oxytocin upon the rate of contractions and peak tension with a dose dependent effect, although at lowest concentrations the effects were partly agonist. OBE001 was studied at 6, 60 and 600 nM. OBE001 inhibited spontaneous contractions in a dose dependent way, affecting rate, contraction peak tension, and contraction duration and therefore having an overall effect upon total work. OBE001 antagonized the effect of oxytocin in a dose dependent way, affecting all parameters and leading, at high concentrations, to a complete abolishment of contractility. At equimolar concentrations there was little difference between Atosiban and OBE001 at 6nM, but OBE001 was superior to Atosiban at 60nM and 600nM, and unlike Atosiban, totally inhibited contractility. OBE001 appears to be a promising candidate tocolytic with a better tocolytic profile than Atosiban.

**Identification of new, non-invasive biomarkers of cisplatin cytotoxicity: Potential application on the prevention of nephrotoxicity**

S.M. Sancho-Martínez1,2, L. Prieto García1,2, F. Sánchez Juanes3, J.M. López Novoa1,2, C. Martínez-Salgado1,2,3, F.J. López Hernández1,2,3

1 Unidad de Fisiopatología Renal y Cardiovascular, Instituto “Reina Sofía” de Investigación Nefrológica, Departamento de Fisiología y Farmacología, Universidad de Salamanca, Salamanca, Spain
2 Instituto de Investigación Biomédica de Salamanca (IBSAL), Salamanca, Spain
3 Instituto de Estudios de Ciencias de la Salud de Castilla y León (IECSYL), Unidad de Investigación, Hospital Universitario de Salamanca, Salamanca, Spain

Cisplatin is a chemotherapeutic cytostatic drug widely used in the treatment of many types of cancer. Nephrotoxicity is, however, a dose-limiting side effect that significantly reduces its therapeutic effectiveness. In vivo, cisplatin induces necrosis of the proximal tubule, and apoptosis of the distal segment epithelial cells. It has been observed that, in cultured cells, cisplatin induces both apoptosis (at low doses) and necrosis (at higher ones). Identification of new markers will allow us to better monitoring the pharmaco-toxicological evolution of drug treatments in a theranostic, personalized manner. These markers might serve to differentiate, in a non-invasive manner, the cytotoxic scenarios caused by cisplatin and the level of damage inflicted by the drug. Mouse cortical tubule (MCT) cells were treated with cisplatin (0–100 μM) for 18 h in serum-depleted medium. Cell viability/proliferation was assessed by the MTT method. The cell death mode was determined by LDH activity in the culture medium, and by caspase activity and DNA fragmentation in cell extracts. A differential proteomics study was carried out with the culture medium after 18 h of cisplatin exposure in order to find secreted proteins associated to different modes of cisplatin-induced cell death. Selected proteins were also studied by western blot. cisplatin provoked a clear concentration-dependent apoptotic phenotype (in the range 3–30 μM), and a mixture of cells with necrotic phenotype and cells with apoptotic phenotype with the highest concentration used (100 μM); and the presence of both necrotic (LDH) and apoptotic parameters. The proteomic analysis of cell culture supernatants revealed that several proteins, including chaperones related to (1) the endosomal-lysosomal system, (2) cytoskeletal structure, secreted proteins related to protein transport in serum, proteases and alpha-enameolase, a cytosolic glycolytic enzyme. This in vitro approach might be useful for identifying novel urinary markers associated to drug nephrotoxicity, which originate in particular cell types subject to the influence of a determined drug under variable scenarios of toxicity, some of which might be related to early stages of nephrotoxicity. This system also offers ethical advantages that might help to reduce the use of experimental animals.

**Comparison of effects among glutamine, vitamin, and amino acids solution on growth and viability of hybridoma cells**

Hak-Zoo Kim, Youn-je Park, Soo-Hyun Kim

Kongju National University, Yonsei University College of Medicine, Chungnam and Seoul, Korea

Hybridoma cells have been engineered to produce a desired antibody against a specific antigen. These monoclonal antibodies produced in hybridoma cells have a particular value in the field of medical diagnostics for diseases, therefore it is important to obtain monoclonal antibodies in large amounts. Hybridoma cell cultured in basic culture media was rapidly faced on death phase in three days with the depletion of nutrients in the environment. To obtain a large amount of monoclonal antibody from hybridoma cell cultivation, various components are added in basic culture media for optimization of media compositions. In this study, the effects of various media components such as glutamine, amino acids and vitamin solution were investigated on growth and viability of hybridoma cell, and the productivity of monoclonal antibody. As a result, glutamine had a greater effect than other nutrients on viable cell density, viability and productivity of monoclonal antibody. The addition of 4 mM glutamine (total 8 mM) increased the viable cell density, viability and productivity of monoclonal antibody by approximately 2.3-fold and 4.5-fold more than control (4 mM), respectively. This result suggests that glutamine play a crucial role in cell growth and production of monoclonal antibody in hybridoma cell.

**BMP9 induces extracellular matrix protein synthesis through ALK1 and ALK5 receptors**

Carlos Martinez Salgado, José M. Muñoz-Félix, Nuria Perretta-Tejedor, Cristina Cuesta, Isabel Fuentes-Calvo, Nélida Eleno, José M. López-Novoa

Institute of Biomedical Research of Salamanca (IBSAL)-IECSYL, Salamanca, Spain

Tissue fibrosis normally occurs in chronic diseases in kidney, liver, heart, lungs and skin. Myofibroblast activation and extracellular matrix protein (ECM) buildup are the most important features of tissue fibrosis. Several molecules are involved in this phenomenon, being TGF-β1 the most important and studied profibrotic cytokine. Two TGF-β1 type 1 receptors have been described: ALK1 that phosphorylates Smad1/5/8 proteins, and ALK5 that phosphorylates Smad2/3 proteins. Our research group has previously described that ALK1 regulates ECM protein synthesis in mouse embryo fibroblasts and regulates renal fibrosis in experimental models. Bone morphogenetic protein 9 (BMP9) was described as the natural ligand for ALK1 in endothelial cells. The goal of this study was to analyze the putative role of BMP9 in the regulation of ECM protein synthesis. We stimulated mouse embryo fibroblasts (MEFs) with BMP9 under different approaches to inhibit ALK1 and ALK5 receptors. We inhibited ALK5 with SB431542 and BMP receptors (ALK1,2,3,6) with dorsomorphin-1. Moreover we used cells with lower expression of ALK1 such as ALK1 heterozygous MEFs (ALK1+/−) and we performed ALK1 knockdown with siRNA in MEFs. Stimulation with 20 ng/ml BMP9 leads to an increase in collagen I, fibronectin and connective tissue.
growth factor (CTGF/CCN2) synthesis. Stimulation with BMP9 at the same concentration phosphorylates Smad2/3 and Smad1/5/8 proteins. Inhibition with SB431542 and dorsomorphin-1 blocked BMP9-induced ECM protein synthesis. BMP9 effect was abolished in cells pre-stimulated with the ALK5 inhibitor SB431542 or the ALK1,2,3 inhibitor dorsomorphin-1, suggesting that ALK5 and a BMP type I receptor was involved in BMP9 function. Moreover, BMP9-induced phosphorylation of Smad2/3 was blocked in cells pre-treated with SB431542, whereas BMP9-induced phosphorylation of Smad1/5/8 and also Smad2/3 proteins were blocked after dorsomorphin-1 treatment. BMP9-induced Smad2/3 and Smad1/5/8 phosphorylation were lower in ALK1 heterozygous MEFs and after ALK1 knockdown with siRNA than in their respective controls, indicating the relevance of the ALK1 receptor in BMP9-induced responses. Summarizing, this is the first study that identifies BMP9 as a profibrotic factor that promotes ECM protein synthesis through ALK1 and ALK5 activation in fibroblasts.

Diabetic conditions downregulate the expression of R-spondin-1 in pancreatic islet and upregulate in peri-islet regions

Ho Seon Park, So Hyun Park, Min Yeong Choi, Shinae Kang, Hak Zoo Kim

Yonsei University College of Medicine, Seoul, Republic of Korea

Diabetes mellitus and pancreatic cancer have long been considered to be clinically related to each other. Despite the tremendous epidemiological evidences indicating relevance between two major human diseases, the precise mechanism to link two diseases is still unclear. We compared the expression patterns of β-catenin in pancreatic islet and peri-islet regions from diabetic mice and their normal litters. The expression levels of β-catenin within the islet regions were lower in diabetic mice, whereas those of peri-islet regions were higher compared to normal mice. R-spondin-1, a secreted agonist of Wnt signaling pathway, was previously identified as a β-cell growth factor. We have found similar expression patterns of R-spondin-1 and β-catenin in the islet region. The expression of R-spondin-1 in the islet region decreased, whereas the expression increased in theperi-islet region in diabetic mouse, compared to control mouse. We assumed that pancreatic cancer formation or progression may be influenced by adjacent pathological diabetic islet condition. We further investigate the expression levels of R-spondin-1 from pancreatic β cell (Min6) and pancreatic cancer cell lines (AsPc-1 and Panc-1) in vitro diabetic condition. The expression level of R-spondin-1 was down-regulated in Min6 cells, whereas up-regulated in AsPc-1 and Panc-1 cultured in high glucose conditions, which correlates with in vivo R-spondin-1 expression study. These results provide new insights about the R-spondin-1 as a molecular linker of diabetes and pancreatic cancer.

Pathological changes of coronary artery imaging related blood biomarker profile changes

Yaping Tian, Xingwang Jia

Chinese PLA General Hospital, Beijing, China

There were enough reports confirmed that the metabolic disorder of lipid and inflammatory factor in blood could lead to the damages of endangium, and progress to atherosclerosis gradually. The purpose of this study was to evaluate the risk degree of cardiovascular disease by combined Dual source CT results with blood biomarkers and lipids analysis. The study had enrolled 205 person and the condition of coronary artery was examined by Dual source CT and the patients were divided into group A (control group without plaque), group B (calcification group) and group C (none calcification group, and combination group). The gene expression of IL-β, IL-6, IL-8, IFN-γ, MCP-1, VWF, MTHFR, L-Selectin, TNFα, Ubiquitin, MCSF, ICAM-1, ID2, HMOX-1 and LDL-R in peripheral blood had been analyzed by using GeXP technology. Ten cytokines had been detected by liquid chip method including IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IFN-γ, MCP-1, TNFα and GM-CSF. The variations of blood lipid and hsCRP level were evaluated on Hitachi automatic analyzer. The results showed that the level of systolic blood pressure, GLU, TH, TG, APOB, APOC2 and hsCRP increased significantly in group C and the AUC was 0.720 in discriminating group C patients from control group with sensitivity of 60.5% and specificity of 76.8%. There was no significant different item between calcification group and control group. The expressions of IL-1β, IL-6, IL-8, MCP1 were significantly different between group C (without diabetes and control group). Blood cytokine levels showed that IL-6 of group C increased significantly compared with control group and AUC was 0.592 in discriminating group C patients from control group. A diagnostic model had been developed by using IL-6 combined with biochemical items, which yield an AUC of 0.746 in discriminating group C patients from control group with sensitivity of 78.0% specificity of 65.1%. In conclusion, the pathology process of coronary artery atherosclerosis was closely related with blood gene expressions, cytokines level and abnormal metabolizing of lipids. Multi-biomarker diagnostic model could provide more diagnosis information to evaluate the risk degree of cardiovascular disease.

Determination of the association between polymorphisms of miRNA genes that target DNA methyltransferases and methyl binding proteins and lung cancer

Cansu Ozbayer, Irfan Degirmenci, Derya Ustuner, Guntulu Ak, Faruk Saydam, Ertugrul Colak, Hasan Veyes Gunes, Muzaffer Metintas

Eskisehir Osmangazi University Faculty of Medicine, Eskisehir, Turkey

Recep Tayyip Erdogan University Faculty of Medicine, Rize, Turkey

Lung cancer (LC) is a disease that develops by uncontrolled cell growth in tissues of the lung and the most common cause of cancer-related death. Most significant risk factors for LC are tobacco smoke, genetic factors, radon gas and asbestos. Micro-RNAs are highly conserved, non-protein-coding, 18-24 nucleotides length, small RNAs. miRNAs bind target mRNAs which are complementary to their sequences and post-transcriptionally regulate gene expression through translational repression or mRNA degradation. Several observations link dysregulation of miRNA expression to the development and progression of tumors and miRNAs can act as oncogenes or tumor suppressors. The hypermethylation of CpG-rich DNA in the promoter of genes and downregulation of tumor suppressors are important for tumorogenesis. It has been reported that being a type of epigenetic modifier, miRNAs may regulate epigenetic mechanism including abnormal methylation of the promoter regions or histone modifications. In our study, we used current data bases and determined miRNAs that targets DNA methyltransferases (DNMTs) and Methyl Binding Proteins (MBDs) involved in DNA methylation. In addition, genetic variants of this mRNA sequences were scanned and 37-SNPs on 22-miRNA genes that targets DNMTs and MBDs were determined. The aim of our study was to determine the association between these variants of mRNA genes and
LC. Accordingly, 90-controls and 90-patients were included in our study. DNAs were isolated from blood samples and these DNA samples were genotyped by Sequenom MassARRAY System. The demographic characteristics of patients and types of genetic variation were evaluated by appropriate statistical methods. The association between rs188912830 gene variant of mir3202 targeting MeCP2 protein and LC has been indicated by both subtypes. The presence of C allele in patients with LC and its subtypes was significantly lower and the absence of C allele was determined to be hazardous 7429-times compared to presence. Significant association between rs318039 gene variant of mir1274 targeting DNA methyltransferase 3b and subtypes of LC were determined. When allele distributions were compared, the number of individuals with C allele was significantly lower in the subgroups. No significant association was found for rs72563729 variant of Mir200b gene targeting DNA methyltransferase 3a and rs145416750 variant of Mir513c-gene targets TRDMT1 in terms of genotype and alleledistribution when compared to subtypes of LC. All individuals were found to be ancestral genotypes regarding other 33-variations. Consequently rs188912830 and rs318039 variations have been determined to be associated with subtypes of LC. Our research is important due to being the first study to indicate the functional characterization of gene variants of miRNA genes targeting DNA methylation.

MirRNA-122/155 maybe a new therapeutic option in persistent virus infection diseases

Wuqi Song, Aimei Li, Aixia Zhai, Jun Qian, Lanlan Wei, Zhaohua Zhong, Fengmin Zhang

Department of Microbiology, Harbin Medical University Key Lab for Infection and Immunity of Heilongjiang Province Key Lab for Pathogenic Biology of Heilongjiang Province Education Bureau, Harbin, China

MirRNA-122/155 have been reported as interferon related microRNAs, and can induce the type I IFN expression. Our studies already show that Mir-122/155 can directly band to some virus genes such as BDV P and N genes, HPV16 E6 and E7 genes. On the other hand, we also found Mir-122/155 can inhibit SOCS1/3 expression by banding on the mRNA coding region or 3′-Utr. Persistent infection with the virus is often accompanied by interferon expression suppression; high level SOCS expression plays an important role in these processes. In a recent study we found type I IFN has been induced by exogenous MirRNA-122/155 with inhibition of SOCS1/3 in virus persistent infected cells including OL/BDV, SiHa, Huh7. The virus replication even has been inhibited follow the enhanced IFN. Comprehensive above founds, we believe that MirRNA-122/155 maybe a new therapeutic option in persistent virus infection diseases.

The differentiation potential of human cord blood unrestricted somatic stem cells into hepatocyte-like cells on 3-dimensional nanofibers and its effect on CCl4 model of liver cirrhosis

Faten Salah Mahmoud, Zeinab Demerdash, Manal Kamel, Hanan El Baz, Taghreed Gaafar, Dina Abdelhady, Olfat Hammam, Salwa Mohamad, Heba Khalil, Shimaa Atta, Tamer Taha

1 Immunology Department, TBRI, Egypt
2 Clinical and Chemical Pathology Department, Faculty of Medicine Cairo University, Egypt
3 Pathology Department, TBRI, Cairo, Egypt
4 Gynecology and Obstetrics Department, NRC, Egypt

Background: Unrestricted somatic stem cells (USSCs), earlier progenitors of cord blood mesenchymal stem cells, hold great promise for liver tissue engineering. Scaffolds are three-dimensional (3D) matrices that provide the initial structural support for cells to attach, proliferate, and differentiate, facilitating the formation of an extracellular matrix (ECM). In vitro culture of USSCs on Nano fibrous scaffold could initiate and enhance their differentiation potential into hepatocytes-like cells.

Aim of the work: In this study we induced the differentiation of USSCs into hepatocytes in two- and three-dimensional (2D and 3D) culture systems. The effect of Nano fibrous scaffold (3D) on their differentiation potentiality was evaluated. The differentiated cells were injected into a murine model of liver cirrhosis to compare the effect of both 2D and 3D culture systems on the condition of liver illness. The more promising culture system is to be adopted in the in vitro-hepatic differentiation of USSCs, to improve their future application in cell-based therapies in patients with chronic liver diseases.

Materials and methods: USSCs were isolated from human cord blood, propagated and characterized. Hepatogenic differentiation of USSCs was performed on both 2D and 3D culture systems. Differentiation potentiality of both systems was evaluated using, indocyanin green (ICG) cellular uptake method, periodic acid Schiff (PAS) staining method, immunohistochemistry analysis for albumin and alpha fetoprotein (AFP), scanning electron microscopy (SEM), and ELISA for albumin and alpha 1 antitrypsin secretion. The differentiated cells on 2D or 3D culture systems were injected intravenously in a murine model of CCl4 induced liver cirrhosis. 90 days after injection, animals were sacrificed and livers were examined histopathologically and stained with immunoperoxidase staining for diagnosis and evaluation. Sera were collected and tested for ALT, AST and albumin. Normal and pathological control groups were involved in the experiment.

Results: SEM showed adherence of cells in the nanofiber scaffold during hepatic differentiation. Differentiated hepatocyte-like cells were more abundant, more mature, and hexagonal in shape in the 3D culture system. Both systems were positive for ICG uptake, PAS, albumin and AFP staining. Albumin secretion was significantly higher in 3D culture system while alpha1antitrypsin secretion increased equally in both 2D and 3D cultures system. Histopathological results showed that both groups had evidence of improvement of the stage of fibrosis when compared to the control group with best improvement appeared in the group treated with differentiated cells on 3D system. Immunoperoxidase staining showed evidence of successful engraftment and differentiation in both groups with higher percentage of AFP and Albumin positive cells in the group treated with cells differentiated on 3D culture system. Liver functions of tested groups showed improvement of ALT, AST and Albumin levels in both groups when compared to the control with best results observed in the group treated with cells differentiated on 3D culture system.

Conclusion: In vitro differentiation of USSCs on Nano fibrous scaffold could be superior to conventional 2D cultures. Moreover, cells cultured on 3D culture system have a better therapeutic potential as proved by the evaluation of the pathological and chemical state of the experimental models.

This work is extracted from the project 1410 supported by the Science and Technology Development Funds (STDF), Cairo, Egypt.
Correlating behaviour and molecular endpoints in the dopaminergic system after modafinil administration in a preclinical model

Poppy Winlow1, Andre Nogueira da Costa2, Pjerrrette de Ron2, Sarah Dremer2, Andrew Jenkins2, Etienne Hanon2, Peter Theill2

1 Faculty of Biological Sciences, University of Leeds, UK
2 Non-clinical Development, UCB Biopharma Sprl, Belgium

Modafinil (2-diphenylmethyl-sulphonyl-2-acetamide), a drug marketed to treat narcolepsy, is known to bind to the dopamine transporter directly. However, its mechanism of action continues to be poorly characterised and its potential for abuse remains controverted. The aim of this study was to further elucidate the mechanism of action of modafinil at a behavioural and molecular level utilising a preclinical model. A conditioned place preference (CPP) paradigm was implemented in mice (n = 15) to investigate the rewarding properties of modafinil. Whole genome expression analysis was performed on the ventral tegmental area (VTA), nucleus accumbens (NAC) and prefrontal cortex (PFC) brain samples taken after the CPP study to identify differentially regulated transcripts. The behavioural study showed that modafinil administration (65 mg/kg i.p.) induced a significant conditioned place preference with a 16% increase in the change of preference for the drug-paired side after conditioning when compared to the vehicle group (Student’s t-test, p = 0.0029). Locomotor activity showed a 176% increase during each thirty minute session after modafinil administration, consistent with its psychostimulant properties. At a molecular level, modafinil induced changes (0.5 < fold-change > 2; p < 0.05) to mRNA expression profiles in the VTA (120 mRNAs), NAC (23 mRNAs) and PFC (19 mRNAs). Furthermore, a molecular signature consisting of twelve up-regulated mRNAs (Tgtp1, Igtp1, Ifi47, Igtp, Gbp2, Gbp3, Gbp7, Gbp10, Igmn2, Cd274, Ly6a, and Xdh) was identified as common to the three brain regions and subsequently validated by qRT-PCR. A potential association with interferon-γ (IFN-γ) was proposed based on the deregulation of p47 family and Gbp family of IFN-γ induced GTases. There was also a weak up-regulation (fold-change < 2) of Stat1 and Ifi1 transcription factors in the three brain regions, supporting their potential involvement as upstream regulators of the common mRNA signature. Principal component analysis of the expression profiles allowed us to discriminate the findings according to treatment and brain region. When evaluating the association of the behavioural phenotype to the molecular profile, multiple linear correlation analysis showed a strong correlation (R² > 0.75) between the behavioural and molecular endpoints in the three brain region. Our findings propose an effect of modafinil at a behavior and molecular levels which may be associated with induction of IFN-γ associated immune response. The latter may be implicated in modafinil treatment of fatigue in the clinic and have a subsequent effect on various neurotransmitters such as dopamine. These findings suggest a link between the behavioural and molecular events in the context of modafinil administration.

Quantification and detection of mutant mitochondrial DNA in peripheral blood cells in type 2 diabetes

Je Hoon Lee, Dong Wook Jekarl, Hae-il Park, Yeong Sic Kim, Yonggoo Kim

Department of Laboratory Medicine, School of Medicine, The Catholic University of Korea, Seoul, Republic of Korea

Background: Mitochondrial dysfunction has reported in several diseases including diabetes, cancer, skeletal muscle disorders, and several assays have been developed to quantify the level of deleted mitochondrial DNA (mtDNA) molecules in single cells. We investigated the mtDNA content and mutant forms in type 2 diabetic patients.

Materials and methods: mtDNA content from peripheral blood cells (WBCs and platelets) was measured by real-time PCR in 50 type 2 diabetes patients and 10 control group. Mutant A3243G mtDNA was assessed by RCR-RFLP and sequencing method. Several biochemical markers (fasting glucose, HbA1c, 1,5-AG, glycated albumin) were analyzed.

Results: mtDNA content was decreased in DM patients (0.75 ± 0.19) compared with normal control (1.11 ± 0.44, P < 0.015) in both WBCs and platelets. A3243G mutation in patient group was not found, but a few polymorphism were identified (A3537G and T3552A).

Conclusion: mtDNA content could be useful marker for assessment of DM patient, which suggests that mtDNA is involved in the pathogenesis of diabetes.

Translational research in stem cell therapies

F. Ruiz-Navarro, G. Kobinia

Austrian Society for Regenerative Medicine, Vienna, Austria

The ancient scientific Aristotelic scripts in which certain animals could achieve the regeneration of tissues and function was probably the seed of one of the most intense areas in the Regenerative medicine field, stem cells.

The term cellular therapies is wide from definition through application, a matter of fact is that the recent discoveries about the potency of stem cells opened already a wide door to treat several diseases that until now are untreatable, such as cerebral palsy, dilated cardiomyopathy, limb ischemia or type 1 diabetes, etc.

Since the discoveries of Martin, Evans and Kaufman in 1981 basic research groups started a marathon race searching for the best cell extract, cultivate, modified and apply. However, this race has been running, in most of the cases, far from the bed of the patients and very close to the bench. The discovery of new cells made in laboratories, by biochemistry modifications, cultures, genetic inductions, etc., had turned the field in a seeking of custom cells that can be sold later, dropping out the main necessities in the clinics as they are having effective treatments reachable for every patient and institution. This situation had made an enormous gap between clinicians and basic scientists that limit the application of cellular therapies in the daily practice.

A clear example, is that the adult stem cells, proved in several clinical studies as safe, feasible and with encouraging outcomes do not have any special place among basic scientists. These therapies, with more intense basic research guided towards a better clinical applications, could now already be available in the regular practice. In several situations the autologous use of stem cells had been used in pilot cases or as unproven medical treatment in the clinical practice, due to the fast growing use of stem cells without complete understanding of the mechanism of action urge translational activity and every clinical application should be at any circumstance bond by strong rational proof of concept and safety. Turning the face into feasible solutions of cell therapies that are actually realistic to introduce in the clinical practice.

The Austrian Society for Regenerative Medicine (ASRM), recently founded, is developing a program of stem cells translational research intended to form a bridge between clinicians and basic scientists in the field of regenerative medicine by giving basic
discovering a fast track to clinical trials and then to the common practice. We encourage the participation of universities and scientific groups around the country to contact the ASRM to discuss and develop a plan of translation.

Comparative analysis of quinolone resistance in clinical isolates of Klebsiella pneumoniae and Escherichia coli from Chinese children and adults

Ying Huang1,2, James O. Ogutu1, Jiarui Gu1, Fengshu Ding3, Yuhong You4, Yan Huo1, Hong Zhao1, Wenjing Li1, Zhiwei Zhang1, Wenli Zhang1, Xiaobei Chen2, Yingmei Fu1,2,2,2, Fengmin Zhang1,2,2

1Department of Microbiology, Pathogenic Biology, Harbin Medical University, Harbin, China
2Heilongjiang Provincial Key Laboratory of Immunity and Infection, Harbin, Heilongjiang, China
3Harbin Children’s Hospital, Harbin, Heilongjiang, China
4Department of Laboratory Diagnosis, Heilongjiang Provincial Hospital, Harbin, Heilongjiang, China

The objective of this study was to compare quinolone resistance patterns and gyrA mutations in clinical isolates of Klebsiella pneumoniae and Escherichia coli from Chinese adults who had used quinolone in the preceding month, and children without any known history of quinolone administration. The antimicrobial susceptibilities of 61 isolates from children and 79 isolates from adults were determined, and the mutations in the quinolone resistance-determining regions in gyrA gene were detected by PCR and DNA sequencing. The ciprofloxacin resistance and types of gyrA mutations in isolates from children and adults were compared and statistically analyzed. 62.3% of K. pneumoniae and E. coli isolates from the children were ciprofloxacin-resistant, while 70.9% of the isolates from adults carried ciprofloxacin-resistant (p = 0.033). The double mutation Ser83→Leu + Asp87→Asn in the quinolone resistant isolates occurred in 73.7% isolates from the children and 67.9% from the adults, respectively (p = 0.0479). Chinese children without history of quinolone administration were found to carry fluoroquinolone-resistant Enterobacteriaceae isolates. The occurrence of ciprofloxacin resistance and the major types of gyrA mutations in the isolates from the children were similar to those from adults. The results indicate that precautions should be paid on environmental issues resulting from widespread transmission of quinolone-resistance to control antibiotic resistance.

Exploration of health sciences students’ dietary habits in a Technological Educational Institution in Greece*

Deltsidou Anna1, Mastrogiannis Demos2, Kapreli Eleni3, Mpourdas Dimitris4, Papageorgiou Elpiniki5, Raftopoulos Vasili6, Noulia Maria7, Lampani Maria8, Lykeridou Aikaterini10

1. Associate Professor, Midwifery Department, TEI of Athens, Greece
2. Lecturer, Nursing Department, TEI of Central Greece, Greece
3. Associate Professor, Physiotherapy Department, TEI of Central Greece, Greece
4. Ergophysiology Lab, PESS of Athens, Greece
5. Assistant Professor, Electrical Engineering Department, TEI of Central Greece, Greece
6. Assistant Professor, Mechanical Engineering Department, TEI of Central Greece
7. Associate Professor, Nursing Department, Technological University of Cyprus
8. Associate Professor, Nursing Course, University of Nicosia, Cyprus
9. Midwife, MSc
10. Professor, Midwifery Department, TEI of Athens

Introduction: Healthy nutritional habits are established in early stages of life and constitute the cornerstone of maintaining a satisfactory health status both with regard to prevention of diseases as well as in rehabilitation.1,2 It would be anticipated from health sciences students undergoing training that their engagement in courses dealing with the importance of healthy diet, would lead them to implement a healthy pattern of food intake.

Aim: The aim of the study was to explore nutritional habits of health sciences students.

Methodology: In the present cross-sectional study, 200 randomly selected students were invited to participate. Data were collected by using a semi-quantified food frequency questionnaire (FFQ) which has originated from a nationwide survey (Trichopoulos et al., 1990) which has been validated for its test–retest reliability (Tsoutoupolou, 2010). Participants also filled in socio-demographic data. Data analysis was conducted by using the Statistical Package for Social Sciences (SPSS) v.20.

Results: Overall, 178 students were enrolled in the study (94 females and 84 males). Males had a mean Body Mass Index (BMI) value of 24.49 while women had a mean BMI value of 23.25 (U = 2883.0, p = 0.002, r = −0.23). There was no significant difference in smoking habits. Participants stated that they were consuming vegetables at least once or twice per week (88.6%), legumes (68.5%) and small sized fishes (22.6%). Many students (60.8%) stated that they usually order fast food at least twice a week, consumed mayonnaise (64.8%) and refreshments (38.8%). It was noted that men were consuming more quantity of the visible fat in all kinds of meat in relation to women (x2 = 10.231, p = 0.017, V = 0.242, p = 0.017, OR = 3.6) as well as consumed white bread more often (x2 = 15.182, p = 0.01, V = 0.294, p = 0.01, OR = 3.7). Also, smokers tended to consume snacks more often in relation to non-smokers (x2 = 15.654, p < 0.001, V = 0.300, p < 0.001, OR = 6.6); however, non-smokers reported that they consumed more meals on a daily basis (x2 = 8.539, p = 0.014, V = 0.223, p = 0.014, OR = 2.56).

Conclusion: The fact that students in the current study consume healthy as well as less healthy foods leads to the conclusion that there is a need for intervention from the corresponding faculty aiming at enhancing and establishing a healthier dietary pattern. Health promotion interventions should start at the community level, in primary and secondary schools, encouraging local and national active involvement.

References

*The research project "Non invasive measurement of the haemodynamic parameters and of the advanced end glycation products
Exploration of students’ views regarding the effectiveness of health care professionals’ interventions for smoking cessation

Deltisidou Anna1, Mastrogiannis Demos2, Kapreli Eleni3, Mpourdas Dimitris4, Zarikas Vasilios5, Papageorgiou Elpiniki6, Raftopoulos Vasilios7, Noola Maria8, Lampadiari Maria9, Lykeridou Aiakaterini10
1. Associate Professor, Midwifery Department, TEI of Athens, Greece
2. Lecturer, Nursing Department, TEI of Central Greece, Greece
3. Associate Professor, Physiotherapy Department, TEI of Central Greece, Greece
4. Ergophysiology Lab, PESS of Athens, Greece
5. Assistant Professor, Electrical Engineering Department, TEI of Central Greece, Greece
6. Assistant Professor, Mechanical Engineering Department, TEI of Central Greece, Greece
7. Associate Professor, Nursing Department, Cyprus University Technological, Cyprus
8. Associate Professor, Nursing Course, University of Nicosia, Cyprus
9. Midwife, MSc
10. Professor, Midwifery Department, TEI of Athens, Greece

Introduction: It has been well established in the international literature that smoking is a risk factor for cardiovascular diseases and malignant transformations.1-6 One of the most important roles of health care professionals is the one associated with effective and adequate interventions towards diseases prevention. Therefore, health care professionals should consider smoking cessation as one of their primary concerns.

Aim: The aim of this study was to explore health sciences students’ views with regard to the role and the effectiveness of health care professionals’ interventions as a potential role model as well as their potential adequacy in smoking cessation capabilities.

Methodology: In the current cross-sectional study, 200 randomly selected students were invited to participate. Data were collected by using the Global Health Professions Students Survey (GHPSS) and participants also filled in an anonymous questionnaire regarding sociodemographic information. Data analysis was conducted using the Statistical Package for Social Sciences (SPSS) v.20.

Results: Two hundred questionnaires were initially administered. The final sample consisted of 178 students (89%). 42.1% of the respondents do not perceive health care professional’s role when he comes to provide advice to patients and to the public about quitting smoking, while 74.7% do not think that health care professionals adequately advise smokers to quit smoking, a notion more often embraced by women rather than men ($\chi^2=7.24$, $p=0.007$, phi = 0.220, $p=0.003$, OR = 2.9) and non-smokers rather than smokers ($\chi^2=6.141$, $p=0.013$, phi = −0.187, $p=0.013$, OR = 2.3). However, men more frequently believe that health care professionals should not regularly advise patients who smoke cigarettes ($r_s=0.167$, $p=0.026$) or other tobacco products ($r_s=0.220$) to quit.

Conclusion: According to our findings, it appears that students tend to question the existing adequacy and effectiveness of health care professionals with regard to their projected overall image and role in society in the field of interventions for smoking cessation. Nurses, physicians and other health care professionals involved in the effort to advise people to quit smoking should look into the present results and reconsider their approach in order to reverse this impression, in case it should be accepted.

Keywords: Role model; Efficiency; Health care professionals; Students; Smoking cessation

References

“The research project “Non invasive measurement of the haemodynamic parameters and of the advanced end glycation products (AGEs) level in students smokers and in students who intake caffeine” is materialized in the context of the Operational Programme “Education and Lifelong Learning” (Managing Authority: Ministry of Education & Religious Affairs) and is co-financed by Greece and the European Union (European Social Fund).