New Horizons in Translational Medicine 4 (2017) 48–98

Contents lists available at ScienceDirect

New Horizons in Translational Medicine

journal homepage: www.elsevier.com/locate/nhtm

Abstracts: 5th Annual Congress of the European Society for Translational Medicine (EUSTM-2017), 20–22 October 2017, Berlin, Germany

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Oral Abstracts

Engineering Solutions for Repairing the Heart: A 2017 View

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Regenerative medicine is a promising field with the potential to overcome the increasing need for donor organs either by stopping disease progression (e.g. with cells, genes or biologics) or by providing novel organ options. Furthermore, regenerative medicine strategies are unlike other treatments in that they are meant to persevere and treat the underlying injury rather than symptoms. This requires a level of persistence and safety and long term efficacy not always previously required for new therapies. In the past decade, clinicians have been able to utilize cell and gene therapies in unprecedented numbers, but with mixed results. At the same time, scientists have engineered organs (bladder, esophagus and blood vessels) that are considered simple structurally and functionally. However, regenerative medicine is yet to fully succeed with cells or genes or to fabricate fully functional solid organs such as kidneys, livers, lungs, and hearts. Yet, development of organs in the laboratory is proceeding both via 3D printing and use of decellularized scaffolds.[1,2]

I will discuss how the development of organogenesis methodologies are providing new insights into current regenerative medicine therapies, such as cardiac cell therapy[3], providing glimpses into heretofore unexplained cardiac disease states such as heart failure with preserved ejection fraction, and describe the state of the art for cardiac “patches” and building of whole human sized hearts.

I will also describe how studies on cardiac extracellular matrix (ECM) secondary to building cardiac patches or whole heart have provided unexpected insights into the major contribution of the ECM in conditions as disparate as heart failure[4], postpartum cardiomyopathy, positive versus neutral cell therapy outcomes and congenital heart disease. Finally, I will describe pre-clinical large animal studies that suggest partial in-vivo recellularization may be possible and open the door for next generation organ building strategies.

Suggested Reading: [1] D.A. Taylor, L.C. Sampaio, A. Gobin, Building new hearts: a review of trends in cardiac tissue engineering, American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 14(11) (2014) 2448-59.

[2] D.A. Taylor, R.B. Parikh, L.C. Sampaio, Bioengineering Hearts: Simple yet Complex, Curr Stem Cell Rep 3(1) (2017) 35-44.

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

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https://doi.org/10.1016/j.nhtm.2017.11.001

Available online 08 November 2017

Biomimetic strategies in regenerative medicine

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All regenerative processes in living tissues draw on reservoirs of stem cells, which boast the unique skill of generating committed phenotypes able to progress along maturation. Stem cells are intricately connected with their niche and receive from it constant chemical, structural and mechanical inputs, which drive their commitment and specification. Due to their great plasticity and immunosuppressive potential, stem cells-based therapy represents a powerful tool to re-activate the regenerative processes in damaged tissues by stimulating resident cells. Despite their great potential, however, their use in clinic is limited by the lack of systems able to control their fate and ensure a safe manipulation in vitro before their transplant in vivo.

Endogenous cell stimulation can be also achieved by developing smart biomimetic materials able to guide tissue regeneration at multiple levels by recapitulating the extracellular matrix. The understanding of the fundamental steps occurring during tissue homeostasis and wound healing is the starting point to develop materials that can efficiently provide:

i) tissue structure and mechanical properties,
ii) bioactive cues to the residing cells for migration, attachment, proliferation, and differentiation,
iii) the regeneration-permissive environment able to initiate the natural body's healing potential, thus avoiding robust and often chronic immunologic response by host tissue that is currently a pervasive threat for infectious or functional complications.

The translation of such approaches to the clinic provides an alternative solution to the therapies and surgical procedures currently used to rescue tissues' loss of function.

Secretome released from amniotic derived progenitor cells: Therapeutic implication in veterinary medicine

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Objectives: The unfavourable microenvironment of injured or degenerating tissues may result in the death or apoptosis of a large proportion of implanted mesenchymal stem cells (MSCs) in the short period immediately post-transplantation. Recently, new therapeutic approaches based on the use of stem cell derivatives (conditioned medium (CM), and microvesicles (MVs)) to regenerate tissues and improve their functions were proposed, suggesting that paracrine mechanisms are involved in the regeneration process. Aims of this study were to investigate the presence and type of MVs secreted by equine amniotic mesenchymal cells (AMCs), and their preliminary in vitro effects on equine pathologies as tendon lesion, endometritis and severe equine asthma that is a lung-inflammatory disorder similar to human asthma.

Method: MVs were obtained by ultracentrifugation at 100.000g for 1h at 4°C of the CM derived from culture of cells of three different amnion membranes. MVs size was evaluated by NanoSight technology and transmission electron microscopy (TEM). Equine tendon and endometrial cells, and alveolar macrophages (AMs) were used as target cells to evaluate the uptake of MVs labeled by PKH-26. Then, all cell lines were stressed by lipopolysaccharide (LPS) and qRT-PCR expression of pro-inflammatory genes, such as tumor necrosis factor-α (TNF-α), interleukin (IL)-6, IL-1β, metallopeptidase (MMP) 1 and 13, and of an anti-inflammatory gene, such as transforming growth factor-ß (TGF-ß), was evaluated in the in vitro LPS stressed cells. The release of TNF-α, IL-6, and TGF-β1 was measured using commercial available ELISA kits.

Results: NanoSight showed that AMCs secrete MVs in the range of 100-200 nm. Results, also confirmed by TEM that revealed budding of AMC membrane, showed that AMCs secrete MVs with size ranging between 100 and 1,000 nm, with a predominance of vesicles between 100 and 200 nm. The vesicles were roughly spherical. Because of size and morphological characteristics, the vesicles observed were mainly shedding vesicles. The MV uptake in all cells lines was gradual over time but an inverse correlation between concentration and time was found in MV uptake equally by tendon and endometrial cells. Optimal concentration of MV-uptake in all cell lines was of 40x 106 MVs/ml at 24 h. This dose was used for experiments with LPS. A significantly higher expression of pro-inflammatory genes was observed in cells after exposure of LPS, compared to control cells. The presence of MVs induced a significant ($P < 0.05$) down-regulation of TNF-α, IL-6, IL-1β, MMP1 and MMP13 expression in all cellular lines after in vitro LPS stress. Tendon cells constitutively express TGFb that decreased after LPS treatment, but it is restored from MVs. Data by ELISA were overlapping those obtained by qRT-PCR.

Conclusions: AMCs are the focus of great interest in veterinary regenerative medicine for their in vitro multilineage differentiation potential, their great in vitro expansion, and for their potential therapeutic applications in vivo in spontaneous equine tendon lesions, where they were used for the first time. Their paracrine effects have been demonstrated in in vitro and in vivo studies (Lange-Consiglio et al. 2016) using CM, suggesting that soluble factors are implicated in the AMC effects and cell-cell contact is not necessary for their action. Cell-derived MVs are described as a new mechanism for cell-to-cell communication, and to understand whether or not these MVs might be involved in the regeneration of tendon injuries previously treated in vivo with CM, we recapitulated in vitro the inflammatory process by stimulating tendon cells with LPS. In addition, we investigated the effect of MV also on endometrial cells and AMs. Our data suggest that MVs could contribute to modulate pro- and anti-inflammatory genes possibly useful in tendon, endometrium and lung regenerative medicine.

Reference: Lange-Consiglio A, D Rossi, S Tassan, R Perego, F Cremonesi and O Parolini. (2013). Conditioned medium from horse amniotic membrane-derived multipotent progenitor cells: immunomodulatory activity in vitro and first clinical application in tendon and ligament injuries in vivo. Stem Cell Dev 22:3015–3024.

Biomaterials design for Translational Medicine

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Objectives: Creating biomaterials for tissue healing has been a daunting challenge in the past twenty years. During this time frame, many efforts for developing new devices to support different clinical practices failed to complete the translational steps from “bench to bedside”. Successful treatments will require advances in areas ranging from basic cell biology to material synthesis. One of the main reasons why the biomaterials field did not capitalize on the past promising advances is due to the lack of a complete investigation of the physiological and molecular mechanisms involved in the biomaterial/tissue interactions. This justification should not be considered an oversight but rather a new perspective on facing the biomaterial development in order to accelerate the process of clinical translation.

Method: We described at the molecular (RT-PCR), cellular (flow cytometry) and tissue level (histology), the physiological response to a functionalized biomimetic materials using different animal models. Depending on the final application we established different protocols to validate the safety and efficacy of these materials in order to start the pathway toward the FDA approval.

Results: An overview of our past and present working areas focusing on the important steps of biomaterials design toward the FDA approval and the final translation in clinical practice will be presented.

Conclusions: Our results confirmed that we are able to engineer the composition of biomaterials in order to achieve the desired final aim. What we described here is a collection of our platforms that summarize a new attractive way of investigation about biomaterial development for tissue engineering.

Development and clinical applications of in vivo fluorescence imaging in digestive surgery

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Objectives: The aim of this study is to demonstrate current status of translational research for clinical application of intraoperative fluorescence imaging. The authors have developed intraoperative fluorescence imaging using indocyanine green (ICG) for real-time visualization of the biliary tracts (fluorescence cholangiography), hepatic cancers, and hepatic segments. Recently, a novel fluorophore activated by pancreatic chymotrypsin has also been developed for prevention of postoperative pancreatic fistula by visualization of pancreatic leak during surgery.

Method and Results: 1) Fluorescence cholangiography: fluorescence images of the extrahepatic bile ducts can be obtained by intrabiliary injection of ICG solution (0.025 mg/mL) or preoperative intravenous injection of ICG (2.5 mg). The latter technique begins to be used worldwide for confirmation of the bile duct anatomy during laparoscopic cholecystectomy.

2) Identification of hepatic malignancies: Following preoperative intravenous injection of ICG (0.5 mg/kg), fluorescence imaging enables identification of subcapsular hepatic tumors during hepatectomy, through visualization of ICG retaining in hepatocellular carcinoma tissues and in non-cancerous hepatic parenchyma around liver metastasis. This technique helps surgeons confirm location and surgical margins of hepatic tumors to be removed especially during laparoscopic surgery, where palpation of the hepatic surfaces is limited.

3) Identification of hepatic segments: ICG solution (0.25 mg/5 mL) is injected into a tumor-bearing portal branch under ultrasound guidance. ICG can also be administered intravenously following closure of a portal pedicle. Boundaries of the hepatic segments are clearly identified by fluorescence imaging throughout hepatectomy procedures.

4) Visualization of pancreatic leak: A novel fluorophore, “chymotrypsin probe (glutaryl-phenylalanine hydroxymethyl rhodamine green with added trypsin)” has been designed. In our series of pancreatectomy, leaking pancreatic juice was clearly visualized by fluorescence imaging following topical administration of chymotrypsin probe on the filter paper that had been attached to the pancreatic stump. In a swine pancreatectomy model, direct administration of chymotrypsin probe on the pancreatic stump visualized pancreatic leak in real time, enabling surgeons to suture close the leakage sites. Currently, safety trial of chymotrypsin probe has been completed for clinical trials to evaluate safety and efficacy of the present technique in reducing incidence of postoperative pancreatic fistula.

Conclusions: Intraoperative fluorescence imaging using ICG is becoming a standard navigation tool for intraoperative visualization of biological structures, which may enhance accuracy of hepatobiliary surgery. Fluorescence imaging of pancreatic juice using chymotrypsin probe has a potential to reduce a risk of critical complications after pancreatic surgery.

A Library-based Approach to Realize Intraoperative Rapid Imaging of Tiny Tumors by Novel Fluorogenic Probes for Aminopeptidases

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Objectives: Fluorescence imaging is one of the most powerful techniques currently available for continuous observation in cellulo and in vivo. Suitable fluorescence probes are naturally of critical importance for fluorescence imaging, but only a very limited imaging can be done because of the lack of flexible design strategies for small molecule-based fluorescence probes. Here, we established new rational design strategies of small molecule-based fluorogenic probes, developed novel probes, and succeeded some medical applications such as rapid fluorescence imaging of tiny tumors during operations.

Method and Results: We have succeeded to develop novel fluorogenic probes for various aminopeptidases based on our newly established rational design strategy with intramolecular spirocyclization. For example, gGlu-HMRG, a novel fluorogenic probe for gamma-glutamyltranspeptidase (GGT), which is well-known to be upregulated in various cancer cells, was developed. By applying gGlu-HMRG to various cancerous cell lines whose GGT activity is upregulated, fast enzymatic reaction of gGlu-HMRG with GGT occurs on the plasma membrane to yield highly fluorescent product HMRG, which enabled us a novel, sensitive and fast-responding fluorescence imaging of tiny tumors in vivo. In mouse models of disseminated human peritoneal ovarian cancer, activation of gGlu-HMRG occurred within 1 min of topically spraying onto tissue surfaces that are suspected of harboring tumors, with high tumor-to-background contrast.

Encouraged by the promising results described above, we next examined the feasibility of clinical application of gGlu-HMRG during surgical procedures. gGlu-HMRG was topically applied to freshly excised human breast specimens containing various lesions together with normal tissues. We found that tumorous lesions exhibited a time-dependent increase of green fluorescence originated from the cleavage product of gGlu-HMRG, and were clearly distinguished from surrounding mammary gland and fat. Breast tumors, even those smaller than 1 mm in size, could be easily discriminated from normal mammary gland tissues with 92% sensitivity and 94% specificity, within 5-15 min after probe application.

Furthermore, we recently succeeded in preparing a library of activatable fluorescence probes composed of more than 300 probes for various aminopeptidases. We applied these probes on biopsy samples or resected specimens from esophageal cancer patients, and tried to find out tumor-specific aminopeptidase activities. As a result, we could find out the enzymatic activity of dipeptidyl peptidase-4 (DPPIV) were upregulated in tumor-positive biopsy samples, but not with tumor-negative biopsy samples. Further, we could also detect cancer region in the resected human fresh specimens by topically spraying DPPIV-activatable fluorescence probes within 10 min. These results clearly demonstrate that probes for DPPIV are practical for clinical application to detect human esophageal cancer during endoscopic or surgical procedures, because of its rapid and strong activation upon reaction with DPPIV on the surface of cancer cells.

Conclusions: The use of fluorogenic probes targeted to tumor-specific molecules could provide a paradigm shift in surgical or endoscopic procedures, improving the outcome for cancer patients by providing a powerful new imaging technique for rapidly and specifically detecting cancerous lesions. A major advantage over current imaging modalities is that an intraoperative fluorescence imaging system offers a large field of view for inspection. Based on above achievements, we believe this rapid, low-cost, and easy method of spraying activatable fluorescence probes represents a breakthrough in intraoperative margin assessment and alternative image guidance during treatment. Considering the potential clinical benefits of intraoperative fluorescence imaging of tumor sites, preclinical/clinical trials of these promising probes should be advanced urgently.

Real-time Quantitative Fluorescence Imaging for Surgery

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There is a pressing clinical need to provide image guidance during surgery. Currently, assessment of tissue that needs to be resected or avoided is performed subjectively leading to a large number of failures, patient morbidity and increased healthcare cost. Because near-infrared (NIR) light propagates deeply within living tissues and interacts with molecular constituents, it offers unparalleled capabilities for objectively identifying healthy and diseased tissue intraoperatively. These capabilities are well illustrated through the ongoing clinical translation of fluorescence imaging during oncologic surgery. In this presentation, we will review our efforts to provide real-time & wide-field image-guidance during surgery using NIR diffuse optical imaging. We will present our latest results in fluorescence and endogenous imaging towards real-time monitoring and image-guided surgical intervention.

Roadmap for clinical application of novel fluorescence probes enabling intraoperative cancer diagnosis

Sven Mieog[1]

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Objectives: Our aim was to assess the tolerability and feasibility of intraoperative fluorescence tumor imaging using SGM-101 in patients undergoing a surgical exploration for pancreatic cancer (PC). Intraoperative differentiation between tumor and healthy tissue is often difficult in PC and this can lead to incomplete tumor removal. Near-infrared (NIR) fluorescence is a promising novel imaging technique that has the potential to improve intraoperative demarcation of PC and radical resection rates. We studied SGM-101, fluorescent-labelled antibody that targets carcinoembryonic antigen (CEA), and bevazucimab-800CW, a fluorescent-labelled antibody that targets vascular endothelial growth factor (VEGF) which is abundantly expressed in PC.

Method: In this tolerability and feasibility study, patients were injected intravenously in a dose-escalating schedule with SGM-101 and bevazucimab-800CW at varying time points before surgery (2-4 days). Tolerability assessment was performed at regular intervals after dosing. The surgical field was imaged using the Quest NIR imaging system and the SurgVision, respectively. Concordance between fluorescence and tumor presence on histopathology was studied.

Results: SGM-101 specifically accumulated in CEA-expressing primary tumors and peritoneal and liver metastases, allowing real-time intraoperative fluorescence imaging. The mean tumor-to-background ratio (TBR) was 1.6 in primary tumors and 1.7 in metastatic lesions. Out of 21 lesions, one false positive lesion was detected (CEA-expressing intraductal papillary mucinous neoplasm) and two false negative lesions were detected. The data of the bevacuzimab-800CW study will be presented at the conference.

Conclusions: The use of fluorescent-labelled antibodies was safe and feasible for the intraoperative detection of both primary PC and metastases. The current technique should be improved further to maximize TBR and sensitivity.

Easy and Rapid Analysis of Arsenic by Using QCM Biosensor

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Objectives: Arsenic is one of the hazardous polluters of soil and potable water. Especially, the rapid detection and quantification of arsenic would take an important place in drinking water management. Current analysis methods require well-armed laboratories, well-educated operators and expensive instruments. These techniques are not applicable for the field use.

Method: The gold surface of Quartz Crystal Microbalance (QCM) has been activated with thiols by benefiting from the hydrolysis and condensation of (3-Mercaptopropyl)trimethoxysilane (MPS) at higher pH in aqueous ethanol solution. 0.05% Phenylarsine oxide (PAO) dissolved in 99.78 % deionized water and 0.17% Sodium Hydroxide (NaOH) has been prepared as stock solution. The sensor surface with thiol groups has been exposed to PAO solutions diluted at various ratios.

Results: 1 nM, 10^2 nM, 10^3 nM and 10^4 nM of PAO solutions have been applied on surface, and thiol groups on the surface have captured Arsenic-Phenyl complexes by covalent binding, and for those samples 3 Hz, 22 Hz, 35 Hz, and 47 Hz of resonance frequency shifts in 30 minutes have been recorded as the mean of three serial experiments, respectively.

Conclusions: It has been understood that the arsenic level of DI water can be determined by using thiol-active QCM biosensor quantitatively. When the water and soil pollution effect of arsenic and the importance on public health are taken into account, the novel, easy and rapid analysis technique in this study could take a remarkable place in the screening of drinking water pollution due to arsenic. Also, this study exposes a promising procedure to readily detect the other heavy metals causing soil and drinking water pollution.

Effect of attachment of trileucine on the biological activity and accumulation of cholesterol-siRNA conjugates

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Objectives: Small interfering RNAs (siRNAs) are 21-23 bp RNA duplexes, capable of inducing homologous mRNA cleavage. They are considered as drugs candidates for treatment of diseases associated with deregulation of gene expression. Two main problems - delivery to the cells and intracellular localization - limit their biomedical applications. The covalent attachment of siRNA to the biogenic molecules naturally performing the corresponding functions could be used to solve this problem. Within this study, we investigated effect trileucine fragment attached to the sense strand of siRNA on the biological activity of cholesterol-siRNA conjugates targeted to *MDR1* mRNA.

Method: siRNAs were synthesized on an automatic ASM-800 synthesizer using solid-phase phosphoramidite synthesis. Modified polymer prepared using cholesteryl chloroformate was synthetized to introduce a cholesterol residue onto the 3'-end of the oligonucleotide. Trileucine residue was attached at the 5'-end via commercially available 5'-Carboxy-Modifier C10. The study of the biological activity of RNA was performed on KB-3-1 carcinoma cell line expressing *GFP-MDR1* chimeric gene. Lipofectamine 2000 was used in certain experiments as a carrier. Accumulation of siRNA conjugated was evaluated by siRNA specific stem-loop RT-qPCR assay. Gene silencing activity was determined by reducing GFP fluorescence measured by flow cytometry analysis.

Results: Recently, we have shown that cholesterol-siRNA conjugates in which cholesterol residue is attached to the 5'-end of sense strand are not toxic, penetrate into tumor cells, suppress P-glycoprotein synthesis and restores the sensitivity of a drug resistant cell line to cytostatics. Comparison of the biological activity of siRNAs bearing cholesterol at 5'-end of sense strand (5'-conjugate) with those bearing cholesterol at the 3'-end (3'-conjugate) showed that both conjugates exhibited similar efficacy of suppressing the target gene when delivered using Lipofectamine and 3'-conjugate were twofold less active in comparison with 5'-conjugate upon delivery in a carrier-free manner. Probably, the 5' and 3' differences in the length and structure of linker, connecting cholesterol and siRNA in the conjugates, affects their biological activity. To check the possibility to increase bioperformance of 3'-conjugates novel conjugates of 3'-Chol-siRNA were synthesized in which trileucine attached to 5'-end of sense strand directly or via C10 linker to stimulate endosomal escape of siRNA. We showed the both trileucine containg 3'-Chol conjugates display biological activity similar to 3'-conjugate. We supposed that endosomolytic activity of trileucine is not manifestation due to low density of trileucine residues or its insuffi

Determination of the Effect of Saffron on Liver Regeneration After Partial Hepatectomy via PI3K/AKT/mTOR Signal Pathway

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Objectives: The liver is continuously exposed to diverse insults, which may culminate in pathological processes causing liver disease. An effective therapeutic strategy for chronic liver disease should control the causal factors of the disease and stimulate functional liver regeneration. Preclinical studies have shown that interventions aimed at maintaining PI3K/Akt/mTOR activity in a dysfunctional liver meet most of the criteria. After 2/3 partial hepatectomy, PI3K/Akt/mTOR is activated and allows the growth of hepatocytes. Complementary medicine is used to treat various diseases and can be obtained from a large number of plants that are found in nature. *Crocus sativus* L. (saffron) has many biological effects such as antioxidant property. The present study investigated the regenerative and protective effects the aqueous saffron extract on liver regeneration.

Method: In this study, *Wistar albino* rats were divided into 8 groups with 5 rats in each group. First four groups were the control groups. Distilled water was given single dose (10 ml/kg) to the control groups by gavage method for 15 days. The other four groups were the saffron groups. The aqueous saffron extract were given single dose (100 mg/kg solved in 10 ml/kg distilled water) to these groups by gavage method for 15 days. We were performed 2/3 partial hepatectomy (PHx) on the rats all of the groups and analyzed proliferation using PCNA immunochemistry. After PHx, a laparotomy was performed on the groups at either 0 h, 6h, 12h and 24h. Using RT-PCR method and Western blot analysis; IGF-1, IRS-1, PI3K, PDK1, PTEN, AKT1, TSC1, RHEB, mTORC1, P70S6K1, 4EBP1, ERK genes expression and p-mTOR protein expression levels were observed 0h, 6h, 12h, 24h after PHx.

Results: The data showed that the aqueous saffron extract had a significant role in increasing the process of liver regeneration when given with gavage method, and it protected the liver as assessed by PCNA and the p-mTOR levels. After saffron treatment, IGF-1 ($p < 0.001$), IRS-1($p < 0.01$), PI3K ($p < 0.05$), TSC1 ($p < 0.01$), RHEB ($p < 0.01$), mTORC1 ($p < 0.05$) genes expression levels significantly increased after 6 hours laparotomy in comparison to controls. When we were compared with control, AKT1 ($p < 0.001$), ERK ($p < 0.01$), P70S6K1 ($p < 0.05$) gene expression levels and PCNA results importantly increased after 24 hours laparotomy.

Conclusions: Our results demonstrated that saffron has protective and proliferative effects on liver regeneration.

Human African trypanosomiasis (Sleeping Sickness)

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Human African trypanosomiasis (HAT), also known as sleeping sickness, is one of the world's neglected diseases. It puts 70 million people at risk in 36 countries of sub-Saharan Africa where it greatly reduces the quality of human health. Caused by protozoan parasites of the genus *Trypanosoma* and transmitted by the bite of the tsetse fly, the disease is invariably fatal if untreated or inadequately treated. HAT has two forms, the more frequent and chronic *T.b. gambiense* and the less frequent and more acute *T.b rhodesiense* which have differing clinical phenotypes. Following the early hemolymphatic stage, the parasites cross the blood-brain barrier (BBB) to enter the CNS to cause the encephalitic stage characterised by a constellation of neurologic features. Diagnostic staging to distinguish the early from the late stage relies on CSF analysis but is controversial, unreliable and highly problematic since the intravenous drugs for treating CNS disease are very toxic. Most importantly, the arsenical drug melarsoprol, the only available effective drug for treating *rhodesiense* HAT, kills or maims up to 10% of all patients receiving it due to the development of a severe post-treatment reactive encephalopathy which is fatal in half the cases. The neuropathogenesis of CNS HAT is complex and probably multifactorial, but there appears to be a critical balance between pro-and counter-inflammatory cytokines that determines, at least in part, the disease outcome. A better knowledge of how trypanosomes cross the BBB is crucial to a better understanding of CNS disease, and a highly reproducible mouse model of HAT has enhanced our knowledge of disease neuropathogenesis significantly and has identified several potential therapeutic targets. Very recently some new potential drugs have emerged including melarsoprol-cyclodextrin inclusion complexes which are orally effective and non-toxic in the mouse model and which will soon be tested in man in a proposed phase 2 clinical trial. The translation of these highly encouraging laboratory findings with this fusion drug to the patient in the African field is now an exciting and realistic possibility.

Salivary Calcium Detection for Early Diagnosis and Screening Diseases by Using QCM Biosensor

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Objectives: Saliva is a promising biomarker source because calcium, potassium, magnesium level, pH are directly related to state of health. Calcium concentration of saliva is a remarkable biomarker to diagnose and monitor important diseases and health conditions such as osteoporosis, cystic fibrosis, asthma, diabetes, post-menopausal health, pregnancy health, etc. Besides, the collection of saliva is a patient-friendly and comfortable method because of its non-invasive nature. The early, rapid and quantitative determination of salivary calcium level could be useful for point of care diagnosis and treatment. In this study, the aim is to rapidly detect and easily quantitate salivary calcium level.

Method: The gold surface of Quartz Crystal Microbalance (QCM) has been activated with thiols by benefiting from the hydrolysis and condensation of (3-Mercaptopropyl)trimethoxysilane (MPS) at higher pH in aqueous ethanol solution. Thiols on sensor surface have been become amine-reactive NHS esters via Sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (Sulfo-SMCC) in Phosphate Buffered Saline (PBS). Then, surface has been saturated 5% Bovine Serum Albumin (BSA) suspansion in PBS to prevent possible non-specific bindings due to saliva content and capture the Ca^{2+} chelator Ethylenediaminetetraacetic acid (EDTA) functionalized with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and N-hydroxysulfosuccinimide (Sulfo-NHS). Eventually, Ca^{2+} in saliva sample has been detected by EDTA on the surface.

Results: 50 ml of saliva sample have been collected from 33 years old, male, healthy subject on an empty stomach at once. The calcium concentration of sample has been measured to be 5.9 mg/dl by using Back Scattering Interferometry (BSI). 2 ml of saliva from the same sample have been analyzed with QCM in 30 minutes, and 19 Hz of resonance frequency shift has been recorded as the mean of three experiments. Afterward, the calcium concentration of saliva sample has been increased by 1.25 times, 1.5 times, and 2 times, and 32 Hz, 41 Hz and 46 Hz of resonance frequency shifts have been recorded as the mean of three experiments for each, respectively.

Conclusions: Results clearly demonstrate that the calcium level of saliva has been determined by using QCM biosensor quantitatively. On the other hand, since this detection protocol is applicable to simpler and more portable biosensor platforms such as electrochemical biosensors, early detection of important diseases, point of care diagnosis and appropriate treatments could be performed easily and rapidly.

Identifying Critically Ill Children on the Pediatric Intensive Care Unit at High Risk of Acute Kidney Injury

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Objectives: Acute Kidney Injury (AKI) is a serious complication in critically ill children, associated with increased morbidity and mortality. Neutrophil Gelatinase Associated Lipocalin (NGAL) has been shown to be a promising biomarker for the prediction of AKI, but no study to date has evaluated both urine and plasma NGAL to determine their diagnostic accuracy in a large cohort of critically ill children. This study aimed to evaluate the diagnostic and prognostic value of initial and serial urine (uNGAL) and plasma NGAL (pNGAL), urea and creatinine concentrations in critically ill children to predict AKI.

Method: Children admitted to a tertiary paediatric intensive care unit (PICU) were enrolled in the study. Plasma urea and creatinine and uNGAL and pNGAL (pNGAL was only measured on 295 children) were determined daily. Children were categorised as AKI or no AKI (KIDIGO stage 2). Chi- squared tests were used to analyse categorical data, Mann Whitney test for continuous variables, and area under the curves (AUC) for biomarker performance. Logistic regression Cox proportional-hazards models were fitted to the outcome RRT, using the independent variables where an association was found in the univariate analysis.

Results: 657 children were enrolled on the study with a median age at admission of 1.01 years (IQR, 0.30, 5.01). The most common reason for admission was cardiac surgery (n = 350, 53.3%). 104 (15.83%) children developed AKI within 72 hours of admission. uNGAL and serum creatinine concentrations where significantly higher in those patients who developed AKI over all 7 days after admission whereas pNGAL where only significantly higher on the first 3 days. The maximal AUC for uNGAL was 0.749 (CI, 0.694 - 0.805; $p < 0.0001$) on day 1 with an optimal cut off value 61.15 ng/ml. The maximum AUC for pNGAL was 0.684 (CI, 0.602 - 0.765; $p < 0.0001$) on day 2. The AUC for creatinine was 0.890 (CI, 0.851 - 0.928; $p < 0.0001$) on day 2, with an optimal cut off value of 49.5umol/L.

Higher PELOD severity score (OR 1.16, 95% CI 1.10-1.24), longer bypass time (OR 1.01, 95% CI 1.00-1.02), and higher blood lactate (OR1.26, 95% CI 1.07-1.48) and procalcitonin (OR 1.01, 95% CI 1.00-1.01) were associated with increased likelihood of receiving RRT. Older age was associated with a decreased likelihood of receiving RRT (OR 0.87, 95% CI 0.75-0.97). The risk of RRT was higher in children who had cardiac surgery.

Conclusions: Urine and plasma NGAL increase significantly in critically ill children who develop AKI. The risk of RRT increases with higher values of urea and creatinine, and in children who have had cardiac surgery. This model could be used to plan optimal timing of pre-emptive RRT, and also provide thresholds for randomised trials of pre-emptive RRT.

This work was funded by the NIHR Biomedical Research Centre in Microbial Diseases, Liverpool and the Alder Hey Charity.

Extracting relevant information from large expression datasets. Examples in melanoma field and vascular angiogenesis.

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The scientific progress of the last few years led to the development of "omics" (genomics, proteomics, metabolomics, integrated omics) and led to the construction of very large collections of molecular and biological data, derived from hundreds or thousands patients. Accessing such databases requires computers with increasing computing powers. Researchers in the field of astrophysics are generally more confident to manage such large data, while biology and medicine researchers are still implementing appropriate strategies for the correct management and for the correct interpretation. Specific bioinformatics, statistics and maths complemented by biochemistry and molecular biology expertise are required. We present here some examples of published and ongoing studies in the field of melanoma investigation, aimed at the identification of relevant molecular mechanisms and specific biomarkers.

Identification of new therapeutic proteins from animal venom glands for oncology.

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Objectives: Our objective is to identify new therapeutic genes from venom glands of *Bothrops jararaca* snake and *Ectatoma* ants.

Method: We performed a deep transcriptome analyses of venom glands from B. jararaca and Ectatoma opaciventre. De novo transcriptome assembly was done with Velvet software. Full-length transcript from interesting therapeutic genes were cloned into expression vectors.

Results: So far, we have identified 1,547 full-length transcripts from *B. jararaca venom gland, from which we selected and cloned* two phospholipases A2. Moreover, we identified 410 full-length transcripts from *E. opaciventre* venom gland transcriptome.

Conclusions: Cancer is one of the greatest health problem in the world, affecting millions of people every year. Cancer treatment usually combines surgery, chemo- and radiotherapy, with limited success, encouraging studies and developments of more efficient pharmacological agents against this disease. Animal-origin proteins are underexplored potential reservoirs of molecules with potential pharmacological effect. For example, more than 90% of snake venom dry weight is composed of enzymes, such as phospholipases A2, proteases, L-amino acid oxidases, esterases and another non-enzymatic proteins and peptides, with potential pharmacological properties. Analysis of new natural sources of therapeutic proteins may represent novel therapeutic possibilities for cancer treatment.

DNA-targeted molecular radiotherapy for ovarian cancer

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Every year almost 240,000 women are diagnosed with ovarian cancer worldwide, and 152,000 women die of this disease. In the USA, 22,440 new ovarian cancer cases are expected in 2017 and 14,080 deaths. The ovarian cancer death rate of 10 per 100,000 women remains unchanged since 1950s. It is apparent that we need to identify new therapies for ovarian cancer to transform this decades-long status quo.

We report on the outcome of the DNA-targeted molecular radiotherapy in one patient diagnosed concurrently with two primary tumors: pseudomyxoma peritonei of the ovary and the breast cancer. Five months after the cytoreductive surgery, patient relapsed. Four months later, intensifying and unremitting symptoms of abdominal pain necessitated additional tumor debulking and resection of the distal small bowel and the right colon. DNA analyses of frozen tumor specimen indicated 9.8% cells in the S phase. The high S phase fraction suggested that the DNA-targeted treatment may be beneficial. Patient was treated IP with 185 MBq ^{123}IUdR and a total of ~2,035 MBq ^{125}IUdR in six fractionated doses - under the FDA- and IRB-approved compassionate use protocols. Complete blood counts, liver, kidney and thyroid function values were within normal range throughout the treatment and at the end of all treatments. Approximately 20% radioactivity remained in the peritoneal cavity 24 h post-treatment. Any ^{125}IUdR entering the systemic circulation was rapidly catabolized and excreted predominantly through the renal clearance pathway. The primary metabolite present in circulation was free ^{125}I. The second-look surgery performed 16 weeks after the final dose of ^{125}IUdR revealed no evidence of cancer in areas well perfused with ^{125}IUdR. The ^{125}IUdR therapy alleviated symptoms and produced durable response (stable disease reported at 24 months after the 1st dose) with no apparent radiation-associated side effects. Given that the first recurrence was apparent at 5 months after the extensive debulking surgery and required debulking and resection of parts of the bowel four months later, the recurrence delay of ~2 years and overall positive outcome of this experimental therapy is promising.

Based on the result of this clinical study, we designed and tested a series of receptor-targeted and DNA-co-targeted theranostics for a nontoxic curative molecular radiotherapy of advanced ovarian cancer. The drugs were designed specifically to improve the *in vivo* properties of the parent drug for the intraperitoneal treatment of ovarian cancer. The target is butyrylcholinesterase [BChE], a cancer cell-associated biomarker recently established in our laboratories as a good target in ovarian cancer. *BChE* gene is amplified in ovarian carcinoma and is associated with poor outcome. BChE protein is overexpressed in human ovarian cancer cells. *Cyclo*Saligenyl monophosphates of 5-radioiodo-2'-deoxyuridine specifically target BChE and co-target DNA. When radiolabeled with Auger-electron emitters, lethal doses of radiation are delivered directly into the DNA of cancer cells while sparing normal tissues. The unique future of these drugs is their ability to recognize BChE with high specificity, be translocated into the cytoplasm and after intracellular processing produce one radioactive metabolite that is efficiently incorporated into the DNA of proliferating ovarian cancer cells, i.e., these cells that signal aggressive disease. Moreover, the structure of these drugs include groups allowing for the lock-in mechanism, which traps the radioactivity within the cancer cells assuring their sustained availability throughout the cell cycle of the unsynchronized and heterogeneous tumor cell population. The site-of-decay-dependent radiotoxicity assures that cells of normal tissues will be spared and only targeted ovarian cancer cells will be killed. Therapy studies conducted in mice xenografted with chemo- and radioresistant OVCAR-3 tumors indicate that new drugs can be developed into a powerful treatment for ovarian cancer. Moreover, these drugs when labeled with diagnostic radiohalides can be used for imaging affording a novel individualization of treatment. Each patient's disease can be imaged and analyzed using diagnostic radionuclide to determine if the targeted Auger electron therapy is warranted.

Genetic characterization, BAP1 expression and clinical outcome in a set of Uveal Melanomas.

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Objectives: Uveal melanoma (UM) exhibits recurring chromosomal abnormalities and specific gene mutations, which are related to tumor evolution/progression. The most common chromosome abnormality in UM is monosomy 3. Other chromosomal imbalances of UM involve chromosomes 1p, 6 and 8. Mutations in GNAQ and GNA11 are early events promoting cell proliferation. BAP1 mutations are associated with rapid metastasis and EIF1AX mutations with prolonged metastasis-free survival. SF3B1 mutations have an intermediate risk and are associated with late metastasis. Knowledge of the presence of recurring chromosomal abnormalities in combination with mutational profiling may give valuable predictive information on the clinical outcome of UM patients.

Method: Sixty-two uveal melanoma samples were collected from patients who underwent enucleation. DNA was extracted and analysed for the presence of losses/gains in chromosomes 1p, 3, 6 and 8 using multiplex ligation-dependent probe amplification (MLPA). Chromosome 3 status was additionally studied by microsatellite analysis (MSA), to detect the presence of isodisomy 3. The presence of mutations in GNAQ, GNA11, BAP1, EIF1AX and SF3B1 genes was investigated by Sanger sequencing. BAP1 protein expression was determined by immunohistochemistry (IHC) with a mouse monoclonal antibody raised against amino acids 430-729 of human BAP1.

Results: The results were analyzed and compared with the metastatic tumor evolution in the patients. Altogether, our results confirm the already established associations between presence of specific chromosome/gene alterations and metastatic risk. Nevertheless three cases assigned to good-prognosis group (low metastatic risk) on the basis of their features (disomy 3, BAP1 wt and BAP1 with nuclear positive immunostaining) were instead metastatic. Concerning the association between mutation status and BAP1 IHC staining, we found that 10 negative samples for BAP1 IHC did not show any BAP1 mutation (all the samples were monosomic, and 6 out of 10 were metastatic, follow-up 6 months-2 years). The analysis of BAP1 promoter in three discordant cases did not show any difference in CpG island methylation. On the other side, the 44 BAP1 mutated cases correspond in all but one case to the loss of BAP1 immunostaining in the nucleus of the cells. The positive nuclear BAP1 immunostaining in a BAP1 mutated sample, characterized by a missense BAP1 mutation in the ubiquitin carboxy-terminal hydrolase (UCH) domain, therefore consisted in a false-negative prediction regardless to the protein functionality.

Conclusions: Considering IHC low cost and simplicity, toghether with the fact that, until now, BAP1 mutations were not detected in tumors with positive nuclear BAP1 immunostaining, BAP1 IHC was suggested as the first choice in prognostic testing of UM patients. Our results, showing the presence of 1 case with a missense mutation in UCH domain and a positive BAP1 immunostaing signal, suggest that also for IHC (just as for BAP1 Sanger sequencing) caution should be taken when using this technique as unique test for BAP1 functionality.

Rabeprazole is effective for bile reflux esophagitis after total gastrectomy in a rat model.

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Aim: To evaluate the effect of PPI (Rabeprazole) on esophageal bile reflux in esophagitis after total gastrectomy.

Methods: Twenty-one 8 week old male Wistar rats were studied. They were performed esophagoduodenostomy of total gastrectomy to induce esophageal reflux of biliary and pancreatic juice. Five rats were performed the sham operation (Sham) (n = 8). On the postoperative day 7, they were treated with saline (Control) (n = 8) or PPI (Rabeprazole 30mg/kg per day, ip) (n = 8) for 2 weeks. On the postoperative 21 days, all rats were sacrificed and each esophagus was evaluated histologically. Esophageal injury was evaluated by macroscopic and microscopic findings as well as the expression of COX2. We measured bile acid in the esophageal lumen and common bile duct.

Results: At 3 week after surgery, a histological study analysis revealed an increase in the thickness of the epithelium, elongation of the lamina propria and basal cell hyperplasia in the esophageal mucosa. The macroscopic ulcer score and microscopic ulcer length of the control group were significantly higher compared to those of the rabeprazole treated group. The expression of COX2 was significantly increased according to the immunostaining in the control group compared to rabeprazole treated group. Although there was no difference between the control and PPI groups in the total bile acids in the common bile duct, the bile acid in the esophageal lumen was significantly decreased in the rabeprazole treated group due to augmentation of the duodenal motor complex.

Conclusion: With this model, rabeprazole is good effect for reflux esophagitis after total gastrectomy from bile reflux. Bile acid is an important factor in the mucosal lesion induced by duodenal reflux.

Polypharmacy management in older adults in Italy: Results from the FRIENDD pilot study

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Objectives: Polypharmacy is among the most important determinants of poor medication adherence in older adults. In addition, it increases the risk of hospitalization, worsens the prognosis of older patients and is economically inefficient because it increases the costs of medical care. The health system in Italy is still largely centered on hospitals and there is no governmental direction for polypharmacy management in the older adults. In the context of EIP on AHA we are performing a pilot study named FRIENDD, with the aim of better understanding how the fact that older patients are often admitted to the hospital impact on their polypharmacy.

Methods: To establish how drug therapy of individual patients is modified by hospital team at the time of hospital admission we compared drug therapies at the time of admission with those at the time of discharge from the medical records of patients admitted to the Geriatrics and Cardiology Wards of the Federico II University Hospital during the last five years.

To evaluate how the medical prescription given at the discharge from the hospital is modified when the patient goes back to the community, we are performing a systematic follow-up of the patients discharged from the aforementioned hospital wards by phone calls.

Results: At the time of writing, 708 medical records have been examined. 299 patients were females and the remaining 409 were males. 359 patients were older than 65. In the whole population, the average number (95% CI) of drugs at the time of hospital admission and of discharge was 5.0 (0.254) and 6 (0.265) respectively, and this difference was statistically significant. When only older patients were considered, 6 (0.348) and 7(0.365) drugs were taken at the admission and discharge, respectively. These values were significantly different from those obtained in younger patients i.e 3 (0.328) and 5(0.347) at admission and discharge, respectively. On average there were 3 (0.516) and 4 (0.549) potential drug interactions per patients in the therapies at admission and at discharge. In older adults we observed a higher number of potential drug interactions both at admission-4(0.767) and at discharge- 5(0.876)- but these values were not significantly different from those observed in patients younger than 65 (3 (0.700) and 4 (0.704), at admission and discharge, respectively). The first follow-up time point at 15 days from discharge has been completed in 48 patients. Four patients reported that the drug therapy was modified from what prescribed at the time of discharge.

Conclusions: Admission/readmission to the hospital significantly increases polytherapy and drug interactions in older adults. A significant proportion of drug prescriptions are modified after discharge from the hospital.

Pentacyclic triterpenoids as a platform for new antiviral drugs discovery

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Objectives: Triterpenoids are a wide-spread group of natural products and a polycyclic secondary metabolite in many terrestrial plants. Their anti-viral activity and action as immunomodulators has been demonstrated in many studies. Because of various advantages, triterpenoids are used as a platform for drug development. Earlier, we showed that Soloxolone methyl (methyl 2-cyano-3,12-dioxo-18βH-olean-95 9(11),1(2)-dien-30-oate, SM) a semisynthetic derivative, obtained by direct modification of the A and C-rings of glycyrrhetinic acid, possesses high antiproliferative and pro-apoptotic activities with respect to different cancer cell lines. Also, we showed that SM displays anti-inflammatory activity. Here, we studied the anti-influenza virus A (IVA) activity of SM.

Method: MDCK and A549 cells were used for in vitro studies. Anti -influenza effect was estimated by focus forming assay. The mechanism of action of SM was assessed using indirect immunofluorescence assay, time-of-addition assay, virus binding and penetration assays. A mechanistic study was performed using molecular docking and Western blotting. The inhibition of virus-induced cytokine release was studied with ELISA. To evaluate the efficacy of SM against IVA in vivo, Balb/c mice were used and histopathological analysis was performed. All experiments were performed on the influenza A/WSN/33 (H1N1) virus.

Results: The study has revealed that SM possesses anti-influenza activity. This activity was observed both *in vitro* and *in vivo*. *In vitro*, SM was shown to inhibit virus replication in a dose-dependent manner. Obtained data agree with the observed reduction in amount of NP and M2 proteins in SM-treated IVA-infected cells. The time course assay indicated that SM exerts its antiviral activity at the early stage of viral replication caused impaired virus binding to and uptake into cells. A mouse study was performed to determine the *in vivo* potential of SM against IVA. SM administration significantly reduced the levels of lung viral titers compared to those of untreated mice. Moreover, intranasal administration of SM before and after infection prevented the development of inflammatory changes in the lung tissue caused by the virus. We found that SM significantly suppressed the production of IL-6 and TNF-α *in vitro*, key cytokines in the pathogenesis of influenza infection. Also, SM treatment of IVA mice significantly suppressed lung pathology, improve the factors of nonspecific resistance and reduce the level of pro-inflammatory cytokines in the lung tissue. A mechanistic study showed that SM efficiently inhibited influenza virus-induced activation of NF-κB pathway and EGFR.

Conclusions: The obtained data clearly demonstrate that SM exhibits anti-viral effects against influenza virus infection, leading to the suppression of IVA production. SM causes impaired virus binding to and uptake into cells; a decrease in virus titers in cultured cells and the lung tissue of infected mice; a reduction in lung lesions and attenuation of the cytokine cascade after viral infection. Thus, SM can be applied to combat viral infection and to prevent severe complications in the lungs caused both by the virus itself and by cytokine storm.

This research was supported by the Russian Science Foundation (Grant № 17-75-20120).

Calculating Stress – From The Theoretical Concept to Clinical Practice

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Objectives: To date, contemporary science still lacks a satisfactory tool for the objective expression of stress, especially on a long-term scale. Recently, we introduced a new–thermodynamically derived–approach to stress measurement, based on entropy production in time and independent of the quality or modality of stressors. Simply, we proposed a novel model of stress response measurement based on thermodynamic modelling of entropy production, both in the tissues/organs and in regulatory feedbacks.

Results: Using entropy as a departure point, we have arrived at a holistic thermodynamic model of health and disease, whose universal character incidentally corresponds to Selye's general theory of stress but doesn't contradict the current understanding of stress. We proposed that the resulting calculation of stress entropic load, the mathematical expression of stress-related increase in entropy production, could become an entirely new tool for predicting adverse health outcomes in specific settings. However, this concept has been entirely theoretical so far and evaluation of pilot data is a necessary next step in evaluation of the utility of the entire concept.

Conclusions: In this particular study, we compare the utility of stress entropic load in interpretation of various subsets of data obtained from the moderately stressed subjects. We also discuss in detail the implementation of our concept into clinical practice, as the precise measurement of stress has not been specifically optimized in the existing literature so far.

The study was supported from the project: CETOCOEN PLUS (CZ.02.1.01/0.0/0.0/15_003/0000469).

Extracellular vesicles of murine dendritic cells efficiently activate antitumor cytotoxic T-lymphocytes targeted to melanoma B16 cells

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Objectives: Extracellular vesicles of late endosomal origin are produced by the most cells and take part in paracrine intercellular communication. Immunostimulating antitumor potential of extracellular vesicles of dendritic cells (DCs) are insufficiently studied, therefore work in this direction is actual and has great potential to develop highly efficient methods of antitumor immunotherapy. The study was performed to evaluate the ability of extracellular vesicles derived from DCs to prime T-lymphocytes with proliferation fully functional antitumor cytotoxic T-killers.

Method: Murine (C57Bl/6 mice) DCs were generated from bone marrow DC progenitors by culturing in the presence of IL-4 and GM-CSF for 6 days. DCs were loaded with complexes of total melanoma RNA/mannosylated liposomes. Transfected DCs were incubated for 24 h in the medium containing exosome-free serum (10%) and extracellular vesicles of DCs were isolated by sequential centrifugation. Splenocytes of C57Bl/6 mice were primed by (a) extracellular vesicles of transfected DCs, (b) transfected DCs, (c) conditioned medium from transfected DCs for 4 days. Primed splenocytes were cocultured with melanoma B16 cells for 60 h to evaluate antitumor cytotoxic potential of splenocytes.

Results: Mannosylated cationic liposomes targeted to mannose receptors on the cellular surface of DCs were used to deliver total tumor RNA into murine DCs. Mannosylated liposomes contained mannosylated lipoconjugate 3-[6-(α-D- mannopyranosyloxy)hexyl]amino-4-{6-[*rac*-2,3-di(tetradecyloxy)prop-1- yloxycarbonylamino]hexyl}aminocyclobut-3-en-1,2-dione), polycationic lipid 2X3 (1,26-bis(cholest-5-en-3β-yloxycarbonylamino)-7,11,16,20-tetraazahexacosane tetrahydrochloride) and helper-lipid DOPE (1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine) at a molar ratio 1:3:6. Physicochemical and biochemical characterization of extracellular vesicles produced by transfected DCs was performed. The main estimated parameters included size and surface charge of extracellular vesicles, intravesicular total content of protein and RNA, expression of surface markers (CD9, CD63, CD81, MHC II, CD80, CD86) and morphology of vesicles by flow cytometry and electronic microscopy, respectively. It was demonstrated that extracellular vesicles of DCs transfected with melanoma RNA were able to activate efficient antitumor cytotoxic T-lymphocytes (CTL) that lysed melanoma B16 cells with almost the same efficiency as CTLs primed with transfected DCs (1.8 and 2.1-fold decrease in melanoma cell number comparing to control, respectively). Splenocytes activated by conditioning medium from transfected DCs were less efficient (1.4-fold decrease in melanoma cell number comparing to control) and lysed melanoma cells as unprimed splenocytes (nonspecific control).

Conclusions: The ability of extracellular vesicles of DCs to prime efficient antitumor cytotoxic T-lymphocytes was demonstrated *ex vivo*. Obtained data provide the basis for the development of novel immunotherapeutic approach allowing to proceed from dendritic cell-based antitumor vaccines to non-cellular personalized vaccines that can be easily standardized, characterized, and storage for a long period without significant loss of immunotherapeutic activity.

This work was supported by Russian Foundation for Basic Research (grant #17-04-00999), Russian Science Foundation (grant #17-74-10144).

miR-30c-5p regulates macrophage-mediated inflammation and pro-atherosclerosis pathways

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Objectives: Atherosclerosis is an inflammatory disease wherein cholesterol-loaded macrophages play a major role. MicroRNAs and microparticles can transduce and propagate inflammatory pathways and are involved in cardiovascular disease. We aimed at screening and validating circulating microRNAs correlated with atherosclerosis development in humans and to dissect the molecular mechanisms associated with the atherosclerotic process using in vitro and in vivo experimental approaches.

Method: A panel of 179 secreted miRNAs was screened in plasma samples of patients with and without atherosclerosis, and validated cross-sectionally and prospectively in patients followed for up to 11 years. Atherosclerotic vascular lesions were evaluated with High-Resolution B-mode ultrasonography of carotid arteries. miRNAs validation was performed by qPCR. Target genes for miRNA were identified and compared using a bioinformatic tool with prediction algorithm. Human macrophage was used in vitro gene silencing strategies. Microparticles and apoptosis assessment were performed by flow cytometry. A systemic miRNA knockdown and carotid injury in mice were conducted in accordance with Principles of Animal Care.

Results: Seven candidate miRNAs selected by the unbiased analysis were validated in n = 99 participants and we found that miR-30c-5p showed significant differential expression in the same direction as in the discovery set, displayed the best discriminatory capacity of patients with carotid plaques and was inversely correlated with total and LDL cholesterol, carotid IMT, presence and future development of plaques. Therefore, reduction of plasma miR-30c-5p is associated with prevalent atherosclerosis and precedes plaque development by up to 11 years. In vitro miR-30c-5p was downregulated by oxidized LDL via the scavenger receptor CD36 and inhibition of pri-miR processing by Dicer. All candidate targets of miR-30c-5p were assessed in macrophages treated with oxLDL and other stimuli and we found that Caspase-3 was the target gene most strongly affected by oxLDL. In turn, miR-30c-5p down-regulation was responsible for the effects of oxidized LDL on macrophage: IL-1β release, caspase-3 expression and apoptosis. miR-30c-5p loaded into microparticles was uptaken by macrophages and regulated target genes, like caspase-3, at transcriptional level. To establish the relevance of this pathway on endothelial damage as the earliest step of atherogenesis, we show that systemic miR-30c-5p knockdown induced caspase-3 and impaired endothelial healing after carotid injury in mice.

Conclusions: We identified the miR-30c-5p as a potential modulator of the atherosclerotic pathway by an unbiased screening of circulating miRNAs associated with atherosclerosis in humans and we provide, for the first time, data in support of an autocrine/paracrine activity of miR-30c-5p loaded into microparticles. As inflammation and apoptosis are well known contributors to the atherosclerotic process, we hypothesized that these pathways may be relevant to atherosclerosis development. Therefore reduction of miR-30c-5p promotes early atherosclerosis, by conveying pro-inflammatory pro-apoptotic signals and impairing endothelial healing, mediated by changes in plasma lipids. This adds to the lipotoxic pathways potentially leading to atherosclerosis. Our data suggest that preserving miR-30c-5p levels may result in vascular protection against the early damage of atherosclerosis and stimulation of miR-30c-5p is a candidate direct anti-atherosclerotic therapy. Intriguingly, we speculate that the anti-atherosclerotic and anti-inflammatory effects of miR-30c-5p may be carried simultaneously at a systemic level by circulating microparticles. Furthermore, we argue that the therapeutic role of miR-30c-5p goes well beyond its role on lipoprotein synthesis, involving anti-inflammatory pathways critical for plaque development, thereby making this epigenetic regulator a top-ranked target for innovative therapies.

Intermittent prolonged fasting modulates cardiometabolic risk factors and body composition including visceral fat tissue in healthy subjects

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Objectives: Intermittent prolonged fasting (IPF) and caloric restriction have been shown to ameliorate cardiometabolic problems, extend life expectancy, alleviate inflammatory and degenerative diseases, and reduce cancer promotion in both animal models and human observational studies. The current research was designed to investigate whether IPF could modulate cardiometabolic risk factors such as adipocytokines (Interleukin [IL]-10, leptin, adiponectin, resistin, visfatin, and apelin), BMI, body fat percent, visceral fat tissue (VAT) surface area, insulin, insulin-like growth factor (IGF-1), and inducible nitric oxide synthase (iNOS) in healthy subjects.

Method: A prospective cross-sectional study was conducted on 60 healthy subjects. All subjects had abdominal T2-MRI scan pre- and post-28 days IPF to determine changes in VAT surface area. Blood samples were also taken from all subjects on the same MRI scanning days. Body weight, BMI, fat and fat-free mass were measured pre-and-post IPF using TANITA body composition analyzer. Subjects were not restricted to any caloric or dietary regimen during their IPF period. Pre- and post-changes on the selected cardiometabolic risk factors, VAT surface area, insulin and IGF-1, and iNOS were compared using a Wilcoxon matched pairs test

Results: Sixty (37 men and 23 women) participants aged from 18-58 years (35.46 ± 12.57 years) were recruited. Daily complete fasting duration ranged from 15:13 to 15:18 (hours: minutes) for a period of 28 days. Post-IPF body weight (86.18 ± 16.82 Kg), BMI (29.92 ± 6.03 kg/m2), fat mass (26.15 ± 9.85 Kg), fat-free mass (60.025 ± 10.55 Kg), and waist circumference (92.83 ± 13.41 cm) were significantly lowered ($P < 0.05$) compared to pre-IPF stage (87.54 ± 16.76, 30.28 ± 5.85, 26.90 ± 9.60, 60.64 ± 10.68 and 94.68 ± 13.01, respectively). Likewise, post-IPF adiponectin (19.10 ± 9.48 ng/ml), and IGF-1 (0.4 ± 0.91 ng/ml) were also significantly reduced ($P < 0.05$) compared to preIPF (23.378 ± 9.84, and 1.02 ± 0.98; respectively). However, post-IPF leptin (3.96 ± 32.01 ng/ml) and apelin (1.67 ± 1.07ng/ml) were significantly increased ($P < 0.05$) compared to the pre-IPF (2.07 ± 19.51 and 1.46 ± 0.92; respectively). Other parameters were not changed significantly at the end of IPF. The average mean ± SD of VAT surface area for all the subjects analyzed were significantly lowered in post-IPF (100.4 ± 71.7) compared to the pre-IPF (107.5 ± 74.6 cm2) ($P < 0.05$).

Conclusions: Intermittent fasting could in fact modulate several cardiometabolic risk factors, body weight and BMI, VAT surface area, fat mass and circulatory adiponectin and IGF-1. A caloric non-restricted intermittent prolonged fasting proven to induce some positive health implications that may help reduce cardiometabolic risk factors in healthy subjects.

The current research was funded by the Vice Chancellor Office for Scientific Research Graduate Studies, Grant no. (VC/GRS/SR. 83/2015), University of Sharjah, Sharjah, UAE.

Early effects of cyano-enone-bearing triterpenoid Soloxolone methyl on human cervical carcinoma cells: a gene network-based microarray analysis

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Objectives: Pentacyclic triterpenoids are a large class of bioactive natural isoprenoids. To date, a huge number of semisynthetic derivatives have been synthesized on the basis of triterpenoid scaffold among which triterpenoids bearing cyano-enone functionality in ring A were identified as the most bioactive molecules. Although extensive investigation of antitumor activity of cyano-enone-bearing triterpenoids their early effects on cells has not yet been well studied. Earlier, we showed that new glycyrrhetinic acid derivative methyl-2-cyano-3,12-dioxo-11-deoxo-18βH-glycyrrhet-1(2),9(11)-dieneoate (Soloxolone methyl, SM) induce tumor cell death by triggering of apoptosis. To analyze early effect of SM on tumor cells we investigated its effect on gene expression profile.

Method: Human carcinoma KB-3-1 cells were treated by 1 μM of SM for 1-10 h and then total cellular RNA was extracted and subjected to reverse transcription. cDNA probes were analyzed on HumanHT-12 v4 BeadChip (Illumina, USA). Differentially expressed genes (DEG) were detected using geneXplain 3.0 (geneXplain, Germany). Bioinformatic analysis of DEGs was performed by Cytoscape 3.5.1. Hierarchical agglomerative clustering of DEGs was carried out using ClusterMaker2 1.1.0 plugin. Protein-protein interaction (PPI) network of DEGs was constructed using BioGRID human database 3.4.129. Search of modules into PPI network was performed by MCODE 1.4.2 plugin. Functional annotation of DEGs was performed using ClueGO 2.3.3 plugin or ToppFun resource.

Results: Treatment of KB-3-1 cells by SM resulted in alteration of expression of 1246 and 265 genes by 1.5- ($DEG^{(1.5)}$) and 2-fold ($DEG^{(2)}$), respectively ($p < 0.001$). The most representative cluster of $DEGs^{(2)}$ (75.3% of $DEGs^{(2)}$) included genes associated with response to oxidative and endoplasmic reticulum (ER) stress, glucose starvation and apoptosis ($p < 0.05$). Early (1-4 h) and late (6, 10 h) $DEGs^{(1.5)}$ were identified: 368 and 1013 genes, respectively. Early $DEGs^{(1.5)}$ were divided into two sets – 231 unique genes that altered expression only at 1-4 h and 137 general genes with changed expression during all treatment time. Functional annotation of unique early $DEGs^{(1.5)}$ revealed that these genes are associated with metabolic processes, regulation of cellular architecture, inhibition of cell migration and TGF-β and PI3K-Akt signaling pathways. In general, early $DEGs^{(1.5)}$ are associated with ER stress, activation of Nrf2, amino acid metabolism and TNF, Nrf2, AP-1, interleukin-4, 10, 13 signaling. PPI network of early $DEGs^{(1.5)}$ was constructed. This network consisted of 177 nodes and 284 edges, among which APP, RPS6KB2, HSPA, HNRNPK, PSMA3, HNRNP4 and CDKN1A were identified as key nodes. MCODE clustering of obtained PPI network revealed five modules of interconnected proteins. Data of microarray analysis were validated by RT-PCR and western blotting.

Conclusions: The obtained data revealed that SM-induced tumor cells death can be associated with ER and oxidative stress and metabolic perturbation. Besides triggering of apoptotic signaling, treatment with SM was found to switches on compensatory mechanism in tumor cells, counteracting the cell death (Nrf2 and AP-1 survival signaling). Early response of tumor cells on SM action was shown to include: (a) inhibition of glycolysis, sterol metabolism (1 h), clathrin-dependent endocytosis (2 h), cholesterol biosynthesis (2-4 h) and actin filament polymerization (3 h); (b) activation of transcription from RNA polymerase II promoter in response to stress (1 h), response to unfolded proteins (3 h), ER and oxidative stress (4 h). SM was found to modulate a wide range of intracellular signaling pathways. Revealed key nodes can be considered as probable master regulators of SM effect on tumor cells.

This research was supported by Russian Science Foundation grant 17-75-20120.

Cutaneous and uveal melanoma as models for different types of tumor evolution

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Recent insight into the structure of genomic alterations occurring during tumor progression has dramatically changed our understanding of the molecular evolution of human cancers. In addition, whole genome and whole exome sequencing has allowed for the identification of mutation signatures which in part are related to the action of known carcinogens.

Cutaneous melanoma (CM) is the cancer with the highest frequency of mutations most of which consist in C > T transitions induced by UV light. However, the main driver mutations in the genes BRAF and NRAS are most likely not UV-induced indicating a diversity between initiating and promoting mutations. Therapy of CM has recently been dramatically changed through the introduction of targeted therapy (TT) for BRAF V600 mutated cases and immune checkpoint blockers (ICB) for all cases. Targeted therapy shows activity in most cases in which the target is mutated but resistance overcomes the effect frequently within less than a year. ICBs show a lower response rate but induce long lasting responses with cases of complete and durable remission of metastatic melanoma patients. Intrinsic and acquired resistance remain, however, a challenge.

These striking achievements are not matched by uveal melanomas (UM) that are on the other end of the scale of mutation frequencies. UMs are most likely not induced by UV-light that is almost entirely absorbed by the lens and the corpus vitreous. Main drivers of carcinogenesis are mutations in one of two G-proteins, GNAQ and GNA11 or, more rarely, in the G-protein coupled receptor, CYSLTR2 or the downstream effector phospholipase Cβ4. Different from what observed for CM, UM does not respond to any targeted therapy and only in few instances to ICBs. Given the low mutation frequency also matched by very few but characteristic chromosomal alterations, the molecular evolution of UM is well delineated whereas relatively few is known for CM. This leads to a very precise prognostication for UM with the apparent paradox of the absolute absence of efficacious therapies.

In this presentation I will discuss how these two malignancies can be used to further our understanding of the molecular evolution of cancer with relevance for its therapy.

Exogenous nucleases as tools for searching therapeutic targets/ oncomarkers among tumor-associated nucleic acids

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Objectives: The most important feature of tumor progression is the overexpression of tumor-associated regulatory and coding RNAs, and the activation of signaling cascades targeted to maintaining the survival of tumor cells and transformation. Tumor carries out its dissemination due to: reprogramming of healthy cells by transfection of tumor-derived exDNA (Trejo-Becerril et al, 2012), including fragments of retrotransposons (Helman et al, 2014); paracrine regulation with miRNA (Dalmay et al, 2006); and the strengthening of neutrophil extracellular traps by tumor-specific exDNA (Wen et al, 2013). Therefore nucleases with antitumor activity are considered as perspective tools for searching therapeutic targets among tumor-associated nucleic acids.

Method: Mice C57Bl/6J with i.m. transplanted LLC were i.m. treated with RNase A (0.7 μg/kg)or DNase I (0.12 mg/kg,). Libraries of low- and high molecular weight RNAs from blood serum and tumor tissue, and libraries of exDNA from blood serum were prepared by the SOLiD™ kits (Applied Biosystems) and were sequencing using the SOLiD™ V5.5 protocols. Analysis of differential expression was performed with Cufflinks software v.2.0 and Bioscope software 1.3. Sequencing data have been submitted to the GEO (GSE63758) and NCBI BioProject (PRJNA313482). KEGG database was used for pathway analysis and Gene Card database was used for functional gene analysis.

Results: Among the miRNAs affected by RNase A in tumor tissue and blood of mice with LLC we detected miR-29b, miR-21, miR-10b, miR-451a, miR-17, miR-18a, miR145, and miR-31, associated with LLC progression. Among genes downregulated by RNase A and associated with the decrease of LLC invasion potential that can be considered as possible targets we detected: Smoc2 promoting proliferation and migration; Hipk3 and Bcl2l2 encoding negative apoptosis regulators; Lin28a, Zcchc6, and Zcchc11, which act as suppressors of miRNA biogenesis; Trib3 which regulates the activation of MAP kinases in MAPK pathway and blocks Akt kinases promoting cell proliferation and survival; Map2k4 associated with tumor progression; Lrrfip2 which is an activator of the canonical Wnt signaling pathway. The level of fragments encoding Hmga2, Myc, Jun, and Fos genes were elevated in the blood of mice with LLC in comparison with healthy mice. At LLC progression we also observed the elevated levels of GC-poor sequences and fragments encoding tandem repeats of retrotransposon family. Following DNase I treatment we observed: the decrease of the level of GC-poor sequences; the decrease of levels of sequences corresponding to tumour-associated genes Hmga2, Myc and Jun and the decrease of 224 types of tandem repeats.

Conclusions: We detected eight miRNAs and several coding RNAs responsible for adhesion disruption and progression of murine Lewis lung carcinoma having homology to non-small cell cancer of human that can be used as therapeutic targets for downregulation in tumor cells. Among fragments in exDNA those encoding tumor-associated genes Jun- and Fos can be proposed as possible markers of malignant diseases, particularly for detection of lung cancer. We revealed that treatment with DNase I led to a decrease in the content of tandem repeats that refer to mobile genetic elements such as retrotransposons. Among retrotransposon-specific fragments Lx repeats could serve as markers of carcinogenesis, particularly for tumors of epithelial origin. The most important result obtained in the work is that DNase I decreased the level of B-subfamily repeats having homology to human ALU repeats, known as markers of carcinogenesis, to the level of healthy animals, and therefore B-subfamily repeats can be used for cancer detection.

This research was supported by RAS programmes "Molecular and Cellular Biology" (grant 6.1), by the Russian Science Foundation (Grant No. 14-44-00068), and SS-7623.2016.4.

Hepatitis B virus genotypes and variants: Molecular characterization and clinical implications

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Objectives: There is increasing evidence that viral genotype influences the clinical course of disease and response to therapy. The objectives of this study were to identify hepatitis B virus (HBV) genotypes circulating in the Philippines; and investigate the presence of surface gene variants across the "a" determinant region, which is the most important target for diagnosis and prophylaxis. It is well recognized that HBV variants can destroy the antigenicity or immunogenicity of the S gene which may result to false negative assay or vaccine escape.

Method: Viral DNA was amplified by nested PCR and HBV genotypes were identified through restriction fragment length polymorphism (RFLP). HBV isolates which produce atypical restriction patterns or untypable genotypes were further characterized by sequence and phylogenetic analysis of the S gene. Single or multiple variants that lie between amino acid positions 100 to 160 were analyzed by Sanger sequencing. The hydrophobicity profile was performed using bioinformatics tools.

Results: RFLP analysis showed that 76%, 10% and 14% of the isolates belonged to HBV genotypes A, B and C. Genotypes D to J were not found. Ninety one percent of the isolates produced a fragment characteristic for genotypes A, B or C after enzyme digestion with *Sty* I, *Dpn* I, *Hpa* II, *Eae* I and *Bsr* I. The remaining 9% were untypable. Sequence and phylogenetic analysis of the S gene revealed that the untypable isolates belonged to genotypes A (67%); B (11%) and C (22%). Meanwhile, fifty five percent of the isolates showed single or multiple variations which led to amino acid changes.

Conclusions: HBV A is the most prevalent genotype circulating in the country. The identification of an untypable genotype can be resolved by direct sequencing of the S gene, and this approach can also be used to detect surface gene variants. Taken together, our findings underscore the importance of sequencing to accurately identify viral genotypes, detect HBV variants and thus, aid physicians in decision making for optimal clinical management.

This work was supported by St. Luke's Medical Center through the Research and Biotechnology Group, with the collaboration of the St. Luke's Liver Disease and Transplant Center.

Normalization of renal function after AKI: is it a real reflection of a healthy renal state?

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Objectives: AKI is characterized by the loss of renal function in hours or a few days. Although most patients recover renal function when adequate treatment is carried out, sometimes kidney structure is not completely repaired, predisposing patients to suffer new renal events. Subclinical damage after AKI is not detected due to the lack of available technology. We hypothesized that renal structures may be altered after AKI although renal function seems recovered. We evaluated the presence of subclinical damage after an AKI episode and whether this hidden structural alteration may predispose kidneys to suffer a new episode of AKI.

Method: Male Wistar rats were treated with one dose of cisplatin (5mg/kg) (AKI group) or saline (control group). Urine, blood and kidney samples were collected in: D4 (AKI development, serum creatinine (sCr) > 2); R0 (renal function normalized (sCr < 0.7) after AKI); R1 (one week after renal function normalized). To analyze the functional risk after AKI, rats were treated during 6 days with a subtoxic dose of gentamicin (50 mg/Kg/day) in R0 and R1 points and sacrificed in day 7. Renal function was analyzed by creatinine clearance, blood urea nitrogen (BUN) and proteinuria using colorimetric methods. Renal histological studies were performed after hematoxylin-eosin staining.

Results: Rats receiving cisplatin developed AKI (with increased sCr, BUN and proteinuria and decreased creatinine clearance). 8 days after cisplatin administration, rats recovered renal function. Histological studies showed relevant structural alterations in AKI group. It was noteworthy that kidneys from rats with normalized renal function (in R0 and R1) continued having morphological alterations although in a smaller degree. Rats receiving gentamicin at subtoxic doses after renal function normalization (in R0 an R1) developed AKI again between the 4^{th} and 6^{th} day of gentamicin administration (evaluated by renal function and hematoxylin-eosin staining). AKI was not developed in rats treated only with a subtoxic doses of gentamicin (control rats).

Conclusions: Summarizing, there are structural alterations when renal function is normalized after an episode of AKI, which entails a functional risk that may drive to a new AKI episode when kidneys are exposed to subtoxic doses of a drug.

This work was supported by grants from Instituto de Salud Carlos III: PI15/01055, DTS15/00166 and Kidney Research Network REDINREN RD016/0009/0025, co-funded by FEDER).

Chronic Fatigue Syndrome: From altered [NADH/NAD+] ratio to persistent ankyrin-mediated disease.

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Objectives: Chronic Fatigue Syndrome/Myalgic Encephalomyelitis (CFS/ME) remains a subject of research and clinical debate. The disease mechanism is still unknown and a huge diversity of potantial triggers (viruses, metals, endotoxins, altered microbiome, undefined environmental factors) makes it almost unlikely to select a single cause and cure to stop this debilitating condition. The objective of our study is to find one common principle triggering this medical condition.

Method: Measurement of urine redox capacity and chemiluminometric analysis of extraceullar NADH-induced reactive oxygen species generation by plasmamembrane associated NADH oxidase of peripheral white blood cells (PBMC) obtained from patients fulfilling the ICC criteria for ME/CFS (n=162) versus luminescence kinetics (CPM) observed with peripheral white blood cells obtained from healthy controls (n=82).

Results: We show that hypoxia-mediated shifts of dioxygenase actvities between indoleamine 2,3 dioxygenase and 2-oxoglutarate-dependent dioxygenases in response to undefined triggers of disease are key to the full understanding of the unexplained symptoms of CFS/ME. Based on the outcome of the NADH induced chemiluminescence which is a measure of the [NADH/NAD+]cyt in the cytosol and in the matrix of the mitochondria [NADH/NAD+] a chemiluminescence stimulation index SI (maximal reponse stimulated by Verapamil/basal response) is defined. Whereas the range of SI of healthy controls is 12+/-2, the range observed with 185 CFS/ME patients is 6+/-4. A lower stimulation index corresponds to an altered [NADH/NAD+] ratio in the cytosol and the mitochondria and a lower efflux of hydrogen peroxide out of the mitochondria. Mitochondrial hydrogen peroxide efflux controls the activity of the inhibitor of hypoxia-inducible factor (FIH). FIH catalyzes the hydroxylation of HIF-1 and ankyrin repeat domains (ARD).

Conclusions: A low SI of the PBMC as described corresponds to less hydrogen peroxide efflux out of the mitochondria of the cells under study and implies a higher activity of FIH and hydroxylation of ARD domains in crucial protein interactions such as Myosine phosphatase D, RNase-L, Notch 2, IkBa, TRPV, Ank G and the like of causes the complex symptomatology of CFS/ME. The common factor in the complex symptomatology of CFS/ME is an over-acting FIH due to an altered [NADH/NAD+] ratio.

Naturally occurring disease models to facilitate clinical and translational medicine.

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Objectives: At the expense of clinical discomfort/disappointment, numerous experimental animals, and enormous costs translation of fundamental findings into clinical practice often fails. The predictive value of inbred mice strains seems limited. The implementation of clinical investigations in pets (e.g. dogs with their specific population structure) is a possible valuable and cost-effective tool to bridge the gap between bench-and-bedside. The objectives are to select specific naturally occurring diseases in pets to facilitate clinical and translational medicine.

Method: The Dutch government sponsored a project to initiate a DNA-biobank of all newborn pure-breed dog puppies. Together with an electronic database that allows for rapid epidemiological studies, and a huge clinical patient load, all ingredients are available to design valuable human pre-clinical screens. The specific population structure of dogs works as a genetic magnifier to discovery of causative and modifier genes, which is crucial in preventive medicine.

Results: Some examples of the value of pets in human clinical research include the holmium-166-based microspheres in various cancers in man, dogs, and cats (F Nijsen, SA van Nimwegen), local delivery of anti-inflammatory controlled-release drugs in orthopedics (MA Tryfonidou, BP Meij), miRNAs as biomarkers for hepatic diseases and the development of liver stem cell transplantations (B Spee, LC Penning). Other research topics include various tumors (pituitary, adrenal, lymphoma), hepatic copper accumulation (Wilsons disease). Often rare diseases in humans do present in larger numbers in dogs, e.g. Cushing disease, Wilsons disease.

Conclusions: The examples of the holmium research, the unique model of dogs with hepatic copper accumulation (Wilsons disease) and the slow drug-release studies to alleviate pain and improve intervertebral disc regeneration in dogs shown that the objectives can be met.

The very deep sequencing of the canine genome has been instrumental for the development of modern molecular tools to study and modify gene expression in clinical material, cell lines and in expanding and differentiating 3D-stemcell-organoids. The most recent revolution in bioprinting further allows for improved disease modelling, drug screens and novel stemcell-based regenerative medicine approaches. In summary, naturally occurring disease models are a not yet fully exploited valuable and cost-effective tool to facilitate clinical and translational medicine.

Dutch Research Council (NWO ZON/MW 116004121) supported this work.

Multi-technological development of a clinical decision support system for acute kidney injury prevention in the context of hypertension and pain treatment

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Objectives: Some of the most prescribed drugs worldwide may cause pre-renal Acute Kidney lnjury (pre-renal AKI), an abrupt reduction in glomerular filtration rate caused by altered systemic and renal haemodynamics. Pre-renal AKI-inducing drugs are diuretics, ACEIs and ARAs for the treatment of hypertension, and NSAIDs for the treatment of pain. Double and triple therapies are common. Incidence of pre-renal AKI increases with multi-therapy (up to 30%). Additional factors are thus key determinants of whether treatments cause AKI or not. ldentifying these determinants and developing a system to evaluate them preemptively, is essential to predict outcome and personalise treatment.

Method: We have generated an international consortium (Disease and Theranostic Modelling, DisMOD) to integrate experimental data from animal models and patients into a systems biology-driven mathematical-computational model. When fed with specific analytic data from each patient, the model will describe the status of the hemodynamic regulatory network in that specific patient and, according to it, will make predictions on the effects caused by drugs on blood pressure and glomerular filtration. Experimental pathophysiology and pharmacology, pharmacogenomics, systems biology, computational and data-based modelling, clinical studies and software-developing technologies are engaged in DisMOD.

Results: A systems biology map of the blood pressure and systemic and renal hemodynamics regulation has been developed to identify the key players, whose level of activation will serve as the "descriptors" of the system in the computational model. A friendly computer interface integrating the predictive model will be developed into a clinical decision support system (CDSS) that will help physicians to stratify patients, and optimize antihypertensive and analgesic drug treatments, handling and resources with 4P (predictive, preventive, personalized, participative) and cost-effectiveness criteria. This first-in-class CDSS is designed to help improve the clinical handling of hypertensive patients suffering (or not) of occasional or chronic painful conditions, by choosing the right drugs and adjusting posology in an individual basis with the goal of optimising therapy and minimising risks.

Conclusions: Overall, this is an open and ongoing project that:

1. Provides personalized treatment for hypertension and pain based on disease mechanisms knowledge.
2. Integrates patient's multidimensional and longitudinal data.
3. Uses proteomics and pharmacogenomics, systems biomedicine, network analysis and computational modelling.
4. Performs pre-clinical and clinical studies.
5. Addresses sex and gender differences.
6. Involves patient associations.
7. Consider regulatory aspects.
8. Develop a dissemination and commercialisation plan.
9. Focuses on a complex disease, hypertension, with high prevalence and economic impact.

Longitudinal biomarkers in critically ill children to predict serious bacterial infection and prolonged intensive care stay.

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Objectives: The differentiation of the systemic inflammatory response (such as can occur post-operatively) from serious bacterial infection (SBI) in children on intensive care, is difficult. Clinicians require a reliable marker that changes early in the course of bacterial infection, and correlates with clinical progression to enable real–time monitoring. We aimed to determine the discriminative ability of Procalcitonin (PCT), Resistin, C-reactive protein (CRP) and neutrophil gelatinase associated lipocalin (NGAL to diagnose SBI in children admitted to the Paediatric Intensive Care Unit (PICU). As a secondary outcome measure, we investigated these biomarkers as risk factors for death and prolonged PICU stay.

Method: Children admitted to a tertiary PICU were enrolled in the study. Plasma PCT, Resistin, CRP and NGAL were determined daily. Children were categorised as having SBI or no SBI. Chi- squared tests were used to analyse categorical data, Mann Whitney test for continuous variables, and logistic regression Cox proportional-hazards models were fitted to the outcome prolonged PICU stay (PICU stay above median).

Results: A total of 657 children were enrolled on the study with a median age at admission of 1.01 years (IQR, 0.30, 5.01). PCT: for the SBI group, the median drop from Day 1 to Day 5 was 71% ($p < 0.0001$). NGAL: for the SBI group, the median drop from Day 1 to Day 4 was 42% ($p = 0.0099$). Resistin: for the SBI group, and the median drop from Day 1 to Day 2 was 30%. CRP: for the SBI group, and the median drop from Day 2 to Day 5 was 72% ($p < 0.0001$). No significant changes were observed in the no SBI group. PCT and CRP were significantly higher in children with prolonged PICU stay ($p < 0.005$). For prolonged PICU stay, the model including the maximum values as opposed to the initial values, was a better fit (AUC of 78.62% compared to 68.05%). The odds of having prolonged PICU stay was 1.5 times more if their initial NGAL value was doubled.

Conclusions: We demonstrate clear biomarker profiles distinguishing children with SBI at admission and those without, and rapidly falling measurements in response to antimicrobial therapy. PCT appears to show the biggest and most sustained falls, suggesting serial measurements could be used to guide antimicrobial stewardship.

This work was funded by the NIHR Biomedical Research Centre in Microbial Diseases, Liverpool and the Alder Hey Charity.

Absolute quantification of biomarkers using Raman spectroscopy

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Raman spectroscopy has been developed for the rapid analysis of key drugs and metabolites in human biofluids and so is a highly useful tool for precision medicine. Raman spectroscopy is a non-destructive vibrational spectroscopy technique and when coupled with metal nanoparticles can detect very low levels of target biomolecules. In the first half of this presentation we will demonstrate this for the quantification of uric acid in urine during pregnancy, and by developing chromatographic separation prior to Raman for the assessment of methotrexate and its natural and unnatural metabolites. Finally, as Raman when coupled with microscopy has a spatial resolution of ca. 1 um it can be used for imaging of biological systems and this will be demonstrated for drug detection in eukaryotic cells.

Key words:
Raman, SERS, quantification, imaging

Computational prediction of personalized therapies in onco-hematological malignancies

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Objectives: Management and treatment of malignancies is still not a satisfactorily accomplished task for the majority of the patients, despite the continuous development of novel, targeted therapies. The heterogeneity of the disease makes it difficult to choose the best medication, even if we know the genetic background of the tumour.

Method: To help an advance in this problem, we developed an efficient bioinformatics toolkit called Turbine. The system is capable of the dynamic simulation of cellular signalling events, and can also differentiate between cellular phenotypes (e.g.: proliferation, apoptosis). The signalling network can be "personalized" to a given cell type or patient's tumour building in the respective genomic and transcriptomic data to the general network skeleton of human, cancer-related signalling interactions. Turbine can also utilise information about further external conditions (e.g.: hypoxia, nutrient amount, etc.). It is also possible to simulate the effects of drugs with given pharmacologic ligands.

Results: We have built and advanced the aforementioned signalling network – which currently consists of the ~3200 interactions of ~1300 proteins and microRNAs - by manually curating publicly available articles and databases. We modelled the inner workings of multiple cell lines (e.g. SUDHL4, KARPAS-422, etc.) by adding their corresponding transcriptomic and genomic data to the simulations, thus creating our "Simulated Cell". Then we could examine, which signalling pathways are activated, and which drugs are the most effective in triggering cell death. With the changes in apoptotic and proliferation rate in different simulated drug concentrations, we were able to determine a virtual concentration for each drug which is lethal to the fifty per cent of a cell type in silico (LD50). To verify the accountability of our predictions, we compared them to in vitro measurements, and we found that in the case of the 21 tested drugs 34% of the in silico LD50 values were within one order of magnitude of the experimental ones. This percentage was higher than 70 in the case of particular drugs (e.g.: Masitinib, Temsirolimus).

Conclusions: Based on these results, our system can aid the development of therapeutic agents in cancer with streamlining the development of new medications, and extending the range of the already available ones.

Daniel Veres and Ivan Fekete are founders of the company Turbine Ltd.

Who to follow? Recommendation systems used to improve metabolic profiling of Hepatic Encephalopathy.

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Objectives: List of discriminating identified metabolites (according to the studied disease between two groups), known as "metabolic profiles", are the observable outcomes of metabolic modulations. Those lists of compounds are of great value to better understand the underlying biochemical shifts induced by a disease. Nevertheless, those lists are incomplete mainly due to the nature of LC/MS and the ability to identify compounds. Moreover, the analysis of human biofluids only represents the modulations of metabolites exchanged between the tissue and its environment, overshadowing potential metabolites of interest which are involved in intracellular metabolic processes and not released nor uptaken by the tissue.

Method: We propose an approach combining metabolic networks (union of all metabolic reactions) and medical text mining to propose metabolites which may expand the biological interpretation by "filling the gaps" of metabolic profiles. The network strategy is inspired from social network recommendation engines such as the ones used by twitter or media broadcasters. It allows finding upstream and downstream metabolites biochemically related to the ones in the profile. The text mining approach consists in automatically mining the literature to retrieve metabolites that are significantly associated with the perturbation under study.

Results: The approach had been successfully applied to high resolution LC/MS metabolomics data obtained on Cerebrospinal fluid (CSF) of patients affected by hepatic encephalopathy. The proposed methodology combined with interactions with analysts allowed increasing the metabolic profile size by 40%. Some of the metabolites suggested by our recommendation system were confirmed using standards. Other propositions were confirmed as metabolites of interests when analyzing patient clinical data.

Conclusions: Combining metabolic profiling and algorithmic solutions used in recommendation systems proved as a powerful new approach to better decipher the metabolic modulations induced by hepatic encephalopathy. Focus had been made on compounds that were not necessarily monitored or considered in first instance. This approach the power of holistic approaches in undertaking the study of complex diseases such as hepatic encephalopathy.

This work was supported by the French Ministry of Research and National Research Agency as part of the French MetaboHUB (Grant ANR-INBS-0010), by the Wellcome Trust grant 105614/Z/14/Z and by PhenoMeNal project, European Commission's Horizon 2020 programme, grant agreement number 654241.

Poster Abstracts

Anthocyanin for hypertension: A systematic review and meta-analysis

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Objectives: To assess systematically the evidence and quality of current research on the AC for the management of HT.

Method: PubMed, Scopus, the Cochrane Library and MEDLINE were searched from 2000 to February 2016 for relevant randomised controlled trials (RCTs). While the quality of the selected trials was assessed using the Cochrane Risk of Bias Assessment Tool, the result was analysed using Cochrane software RevMan 5.3. Outcomes examined were SBP and DBP.

Results: A total of seven studies involving 503 subjects was included in the inclusion criteria. In these studies, variations in terms of the types of interventions, comparators used, and duration of trials were noted. As the comparisons comprise of placebo (capsule and beverages) and medical treatment (captopril or lisinopril), subgroup analyses were performed. The subgroup analysis of five studies using placebo as control showed no significant lowering of effect of AC on SBP [MD: -2.99 mmHg (95% CI: -3.66, -2.33) with a low heterogeneity (I2 = 42%, p = 0.14). However, the effects of AC was significant on DBP [MD: -1.93 mmHg (95% CI: -2.45, -1.41) with low heterogeneity (I^2 = 66%, p = 0.02) when compared to placebo. Subgroup analysis of two studies using drugs (captopril and lisinopril) as controls showed no lowering effects of AC on both SBP and DBP, however the results showed be interpreted with cautious as only two studies were used. Overall, AC was found to be well tolerated for short-term use.

Conclusions: The effectiveness of AC may, in part, be mediated for management of HT specifically on lowering DBP. Further rigorously designed trials with larger sample sizes are warranted to confirm the AC for management of HT.

Immunomodulatory properties of amniotic stem cells obtained through a newly established isolation protocol

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Objectives: Human amniotic mesenchymal stem cells (hAMSCs) have a therapeutic potential in tissue repair because of their capacity for multipotent differentiation and their ability to modulate the immune response through their paracrine activity. The aim of this study was to optimize protocols for the isolation of a homogenous hAMSCs population and to investigate their immunomodulatory properties in view of their potential use in cell therapy. The ability of hAMSCs to induce differentiation of human monocytes into macrophages was evaluated and their influence in driving important macrophage functions assessed.

Method: For cell isolation the amniotic membranes, collected from 21 healthy pregnant women gently provided by Children's Hospital Salesi (Ancona), were stripped from the underlying chorionic membrane followed by extensive washing and subsequent cutting of the membrane into small pieces. The resulting pieces were submitted to an enzymatic digestion treatment including Collagenase type IV and Dnase I. The efficacy of isolation protocol, gradually improved by introducing some modifications, was also evaluated. Growth curves and colony forming unit-fibroblastic (CFU-F) assay were performed at passage 1 and cells at passage 4 were analysed by qualitative PCR for the expression of pluripotent (Oct-4 and Nanog), mesenchymal (CD44, CD90, CD73, CD105), and hematopoietic (CD34) associated markers. For immunomodualtory and immunosuppressive potential assessment, PMA (phorbol 12-myristate 13-acetate) was used to stimulate the differentiation of monocytes into macrophages and co-colture (in cell-cell contact or in a transwell system) with hAMSCs was performed in the early stages of macrophage differentiation. Macrophages phenotype was then confirmed by evaluating pro- and anti-inflammatory markers.

Results: The cellular yield from term amnion was up to $3x10^6$ hAMSCs per 9 cm^2 of starting material following the optimized protocol. Cells obtained show a reasonable proliferation rate. Qualitative PCR revealed the expression of pluripotent (Nanog and Oct4), mesenchymal (CD73, CD90, CD44, CD105) associated markers and the absence of the hematopoietic marker CD34. The cell-surface antigen profiles of isolated cells analyzed by flow cytometry showed the isolated cells are strongly positive for MSC-specific surface markers, such as CD44, CD73, and CD90. hAMSCs, stimulated with pro-inflammatory cytokines, expressed high levels of PGE-2, COX-2, and TGF-β that are genes responsible for the production of soluble factors- mediated immunosuppression. Moreover, in an inflammatory environment the co-culture of hAMSCs with PMA-stimulated monocytes resulted in hAMSCs capability of reducing the switch of macrophages to pro-inflammatory M1-phenotype and inducing an anti-inflammatory potential by shifting the differentiation of macrophages into M2-phenotype at both time points, 48 and 72h.

Conclusions: Taken together our data support the hypothesis that hAMSCs are effective therapeutic agents enhancing the repair of injured tissues playing a key role in inhibition of the pro-inflammatory immune response. Moreover, they hold the potential to modulate immune cells function by reacting to the stimuli provided following an injury and releasing bioactive molecules able to activate a pro-regenerative cellular and molecular cascade. Based on these evidences, hAMSCs are good candidates for the development of a cell-based therapy that can target macrophages for the resolution of inflammation. Further studies are still required to fully elucidate the mechanims by which hAMSCs exert such effect on macrophages and to identify specific molecules that are involved in their beneficial potential.

Expression of circulating Muscarinic Receptors in infants with severe idiopathic life-threatening events: From rabbits to humans.

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Objectives: An overexpression of Muscarinic 2 Receptors (M_2R) was found in heart and blood of rabbits presenting a vagal hyperreactivity. M_2R overexpression was also described in heart of cases of Sudden Infant Death Syndrome (SIDS). The main objective of this study was to analyze the expression level of M_2R in blood of infants presenting idiopathic Apparent Life-Threatening Events (iALTE) compared to control infants and to cases of ALTE with identified etiology. The secondary objectives were: to compare the acetylcholinesterase (AchE) expression in the blood of these groups of infants, to compare the ratios of M_2R/AchE expression in blood cells.

Method: 26 infants less than one year old were enrolled, including 17 who had a first episode of severe ALTE defined as "an episode that is frightening to the observer and that is characterized by some combination of apnea, color change, marked change in muscle tone, choking or gagging". The severity was defined as direct admission to an intensive care unit. A blood sample was collected at admission for RT-PCR assessments of mRNA expression (M2R, AchE). Clinical and biological investigations were performed to determine the etiology of ALTE during the hospitalization. Cases with no specific diagnosis were classified as idiopathic (iALTE).

Results: The diagnostic procedure was performed for all 17 infants. An etiology was identified (e.g. epilepsy, pertussis infection, gastro-esophageal reflux) in 12 cases of ALTEs and 5 cases remain unexplained after these investigations (iALTE). A third group consisted of 9 healthy control infants who had no family history of ALTEs or SIDS. The median M_2R expression was not significantly different between the 9 healthy control infants (0.19 [range, 0.02-1.30]) and the 12 infants who experienced ALTEs with a known cause (0.13 [range, 0.01-0.65]). In contrast, M_2R expression was significantly higher in the 5 infants who experienced iALTEs compared with other 2 groups of infants (11.73 [range, 3.32-16.35]) ($p < 0.001$). The same results were found for the AchE expression: significantly difference between iALTEs and ALTEs ($p < 0.01$) and between iALTEs and controls ($p < 0.05$). The ratio of M_2R/AchE expressions was significantly higher in iALTE group compared to ALTEs ($p < 00.1$) and to controls ($p < 0.05$). As for M2R expression, there was no significant difference between ALTEs and controls in AchE expression. The ratio M_2R/AchE was also unchanged.

Conclusions: The present data, together with our previous findings, suggest that parasympathetic hyperreactivity may be a common vulnerability between SIDS and severe iALTEs. In this study, the average M_2R expression in the 5 infants who experienced iALTEs was 20 to 50 times higher than the other 2 groups of infants with no overlapping. It seems unlikely that the receptor upregulation is driven by certain diseases or treatments because all the values of M_2R expression in the ALTE group were not different from healthy control infants. These preliminary observations imply that iALTEs and ALTEs of known etiology may have different underlying mechanistic pathways. Moreover, the increase of the expression of AchE is interpreted as a compensatory mechanism. However, this compensation seems to be unsufficient to prevent the occurrence of iALTEs, as suggested by the comparison of ratios of M_2R/AchE expressions.

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National Institute for Health and Medical Research (INSERM), French Society of Cardiology, Medical Research Foundation (FRM), French Ministry of Health, University Hospital of Strasbourg supported the work.

Malignancies after kidney transplantation are associated with an increased risk of graft loss but not of chronic rejection.

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Objectives: Studies on the role of malignancies on kidney graft survival are lacking: we retrospectively analyzed the impact of non-cutaneous malignancies (NCM) on death-censored graft survival with a time-dependent multivariable Cox model, adjusted by known prognostic factors. The aim of this study was to evaluate the association between cancer occurrence and risk of graft failure in a registry of kidney transplants.

Method: From 1998 to 2013, 672 adults receiving their first kidney transplant from a deceased donor, with at least six months of follow-up (male: 61.9%; median age: 53), were included in the registry. To illustrate the effect of NCM incidence on graft failure risk, a modified Kaplan–Meier method was used to estimated cumulative hazard rates of kidney failure by the presence of tumor: the assignment to the tumor group was updated at the time of diagnosis. To quantify the tumor effect as hazard ratio, multivariable adjusted Cox models were fitted considering the diagnosis of NCM as a time-dependent covariate.

Results: During a median follow-up of 4.65 years, 59 graft failed and 40 patients developed a NCM (5-years incidence: 5.6%). Graft failure risk increased significantly after NCM occurrence (HR 3.31; $p = 0.004$): this result was confirmed by the multivariable model (HR: 3.27; 95%CI = 1.44-7.44, $p = 0.005$) adjusted by gender, donor age, underlying nephropathy, acute rejection, and baseline renal function. When comparing HRs of NCM on the cause-specific graft failure, for chronic rejection (HR = 0.55; 95% CI: 0.07–4.08) versus other causes (HR 15.59, 95%CI 5.43–44.76), they were significantly different ($p = 0.002$). Graft loss rates in patients with NCM were 5.3/100pt-yrs if immunosuppressive therapy (IS) was reduced and 6.8/100pt-yrs if not: the interaction between IS reduction and NCM diagnosis was not significant (Poisson regression; $p = 0.11$).

Conclusions: This is the first study evaluating post-transplant malignancies as a time-dependent covariate for graft survival: patients with a NCM are at increased risk of graft failure, and more efforts should be addressed to improve graft outcomes acting on malignancy-associated nephropathies.

Acupuncture for chronic non-specific low back pain: a systematic review and meta-analysis.

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Objectives: To assess the effectiveness of acupuncture for chronic non-specific LBP (CNLBP).

Method: We searched CENTRAL, MEDLINE, Embase, Chinese databases, and trial register databases up to April 2017. We only included RCTs. Population: CNLBP. Intervention: acupuncture, acupuncture + treatment. Comparator: sham intervention, no treatment, usual care, other active therapies, another style of acupuncture. Outcome: pain, back-specific function status, quality of life, pain disability, global assessment, side effects.

Two authors independently screened the studies, assessed the risk of bias and extracted the data. Two acupuncturist authors assessed the appropriateness of acupuncture treatment respectively. Qualified studies were meta-analyzed using random-effects model in Review Manager 5.3. GRADE approach was adopted to assess the quality of evidence.

Results: We included 38 RCTs (7340 participants). A few trials were assessed as inadequacy on acupuncture due to not using commonly treated BL23 and BL25 acupoints, using unrelated Guanyuan acupoint, < 6 sessions, < 15-min duration, and insufficient training hours. Compared to sham intervention, acupuncture alleviated pain more (MD -8.50, 95% CI -13.27 to -3.74) but not in back-specific function at immediate term (SMD -0.07, 95% CI -0.24 to 0.10). For quality of life at short term, little difference was found (SMD 0.24, 95% CI 0.03 to 0.45). Acupuncture was better than no treatment on pain (MD -2.22, 95% CI -2.85 to -1.58) and function (SMD -0.42, 95% CI -0.49 to -0.34) at immediate term. Acupuncture showed more pain relief (MD -10.26, 95% CI -17.11 to -3.40) and greater function (SMD -0.47, 95% CI -0.77 to -0.17) than usual care at immediate term. Acupuncture was superior to usual care in physical quality of life (MD of 4.20, 95% CI 2.82 to 5.58) but not in mental quality of life clinically (MD of 1.90, 95% CI 0.25 to 3.55) at short term. Similar incidence of adverse events was estimated between acupuncture and all comparators, pain and dizziness reported as common events.

Conclusions: There was low to moderate quality of evidence that compared with sham intervention, acupuncture may slightly alleviate pain immediately after the treatment because the magnitude did not meet the clinically significant threshold of 15 points or 30% change. Acupuncture did not improve the function at immediate term, neither did it improve the quality of life at short term. There was moderate-quality evidence that compared with no treatment acupuncture was able to improve pain and function at immediate term, but there was no evidence for the quality of life outcomes. When acupuncture was compared with usual care, there was low-quality evidence that acupuncture seemed to slightly relieve pain and improve function at immediate term. For the quality of life at short term, acupuncture was superior to usual care in physical quality of life but not in mental quality of life clinically. Acupuncture procedure is generally safe. There was some limited evidence on acupuncture comparing with other active therapies, another style of acupuncture, and acupuncture added to the intervention comparing to the intervention alone. However, future high-quality studies are required to improve the confidence in those estimates, and to add more useful evidence in the three comparisons.

Utility of new biomarkers to identify the risk of developing AKI in patients treated with cisplatin

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Objectives: Cisplatin and carboplatin are metal complexes considered one of the most effective anti-neoplastic drugs available at the moment, commonly used in children and adults to treat solid cancers. Despite its known effectiveness, cisplatin has a limited use in clinical practice because of its nephrotoxicity. Over 25-35% of adult patients develop AKI after the administration of a single dose of cisplatin. By other side, carboplatin despite having a lower nephrotoxic potential, requires higher doses to achieve an adequate therapeutic effect, which may compromise renal function in a manner analogous to cisplatin. Preclinical studies performed in our laboratory have identified urinary biomarkers capable of predicting the risk of developing AKI following the administration of cisplatin and other drugs. The aim of the present study was to evaluate the usefulness of those biomarkers in oncological patients undergoing chemotherapy with cisplatin or carboplatin.

Method: Urine from 60 unselected patients treated with cisplatin or carboplatin, was obtained from volunteers from the Oncology Service of the Hospital Universitario de Salamanca (Spain). The urine was collected at 0h (before the antineoplastic administration) and 72h (time of maximum damage) after each chemotherapy cycle. To evaluate renal function, plasma creatinine and urea levels, calcemia and magnesemia were obtained from blood tests performed on the same days the urine samples were collected. Patients were divided in two groups: (i) Cases (patients who suffered alteration of at least two of the four blood parameters evaluated at some point in their treatment) and (ii) Control (patients who did not suffer those alterations after three cycles of chemotherapy). Basal urine and urine previous to the moment of greater alteration of the parameters considered was selected. The following biomarkers were quantified: Proteinuria and N-acetyl-β-D-glucosaminidase (NAG) activity by colorimetric methods; and Neutrophil gelatinase-associated lipocalin (NGAL) and Albumin by ELISA.

Results: Proteinuria, NAG and Albumin levels in the Cases group showed statistically significant differences with respect to the Control group (both at baseline and before renal damage). NGAL, on the other hand, only showed significant differences at basal time.

Conclusions: It can be concluded that Proteinuria, NAG, Albumin and NGAL might be useful biomarkers to identify cancer patients at risk of developing AKI before it happens. Although the number of patients has to be increased in order to confirm this issue, it seems that these patients at higher risk could be identified even before they receive their first chemotherapy cycle. In that case, the oncologist could perform various strategies to minimize it or avoid the use of other nephrotoxic drugs during the period of time that the patient is receiving chemotherapy.

Kurlavirus, a novel Marseilleviridae isolated from Mumbai, India, exhibits remarkable genomic relatedness with geographically distant Noumeavirus

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Objectives: We isolated large DNA virus from sewage samples in Mumbai, which successfully infected *Acanthamoeba castellanii.* The objective of the study was to identify, characterize and classify the new virus particle, using comparative genomics. Using the genomic data we developed a computational pipeline for high throughput Big Data analytical framework for screening environmental metagenomes for novel viruses and phages.

Method: Kurlavirus BKC-1 was isolated from the sewage water sample collected in Mumbai, India (geolocation 19.070875 N, 72.871775 E). Virus enrichment from the sample was performed by successive rounds of centrifugation, followed by infecting *Acanthamoeba castellanii* and final recovery from lysate. Infection with supernatant resulted in near-complete lysis 5 days postinfection. Transmission electron micrograph of the purified preparation showed the presence of virus particles of about 150 nm in diameter.

Results: Post adapter trimming and read filtering, *de novo* assembly using in-house pipeline yielded 1 contig of 361,368 bp and a median coverage of 1533x with 43% GC. Open reading frame (ORFs) prediction with GeneMarkS yielded 386 ORFs, which is 71 ORFs lesser than *Marseillevirus* (457 ORFs). The Blastp annotation of Kurlavirus yielded only 64 genes with known function . Characteristic of *Marseilleviridae*, Kurlavirus encodes 3 histone subunits (H2A, H2B/H2A fusion and H3 like), the catalytic subunit of B-family DNA polymerase, D5-like helicase-primase, A32-like packaging ATPase, and the major capsid protein (MCP). Concurrent with previous findings, and unlike other families of Megavirales, no tRNA was found to be encoded by Kurlavirus. Phylogenetic analysis using concatenated amino acid sequences of the aforementioned core genes showed Kurlavirus to be closely related to the recently described Noumeavirus, which is classified with Lausannevirus and Port-miou virus as *Marseilleviridae* Lineage B. Whole genome alignment of Kurlavirus with Noumeavirus, Lausannevirus and Marseillevirus shows greater synteny within the Lineage as compared to Marseillevirus, which is reflective of the phylogeny. However, even within the lineage, we observe large scale genomic rearrangements. An 82% average nucleotide identity between Kurlavirus and Lausannevirus confirms the low genome level sequence similarity. Concurrently, 6 ORFs were found to have no homologs with other *Marseilleviridae.* The genomic data from *Kurlavirus,* and other large viruses discovered from Mumbai to generate metagenomic control material for standardization of big data metagenomics analytical framework. Using varying proportion of large viral reads, we were able to retrieve and build whole genomes from the metagenomes.

Conclusions: Discovery of closely related *Marseilleviridae* from different geographies indicates their widespread presence and a probable common ancestor. The genomic description of more NCLDVs will augment better classification and provide novel insights into the evolution of these viruses. Currently we have performed whole genome shotgun metagenomics of environmental samples, which are being screened using the big data analytical framework.

Research in our lab is supported by grants from BRNS (2012/37B/26/BRNS/1062), DST (SR/SO/BB-0031/2012) and DBT (BT/PR4808/BRB/10/1029/2012). AC is supported by IIT Bombay Post-Doctoral Fellowship.

Clinical and functional implications of MicroRNA-182 in RET gene mutation-induced progression of medullary thyroid cancer

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Objectives: Dominant-activating mutations in the RET proto-oncogene, a receptor tyrosine kinase, are responsible for the development of medullary thyroid carcinoma (MTC) and causative for multiple endocrine neoplasia (MEN) type 2A and 2B. These tumors are highly aggressive with a high propensity for early metastasis and chemoresistance. This attribute makes this neoplasia an excellent model for probing mechanisms underlying cancer progression.

Method: The expression level of miR-182 was measured in MTC tumor specimens and in TT cells by real-time RT-PCR. TT cells and modified NThy-ori 3.1 that stably express RETM918T were used to investigate RET-dependent regulation of miR-182. Identification and validation of miR-182 targets and pathways was accomplished with luciferase assays, qRT-PCR, Western blotting and immunofluorescence. In vitro, overexpression and knockdown experiments were carried out to examine the impact of miR-182 and HES1 on invasion and migration.

Results: We found that miR-182 expression is significantly upregulated in MTC patient samples and tumor-derived cell lines harboring mutated RET. Inhibition of RET oncogenic signaling through a dominant-negative RETΔTK mutant in TT cells reduces miR-182, whereas overexpression of RETM918T in NThy-ori 3.1 cells increases miR-182 levels. We further show that overexpression of this miRNA in NThy.miR-182 cells promotes the invasive and migratory properties without affecting cell proliferation. MiR-182 is upregulated after RET induced NF-κB translocation into the nucleus via binding of NF-κB to the miR-182 promoter. Database analysis revealed that HES1, a repressor of the Notch pathway, is a target of miR-182, whose upregulation correlates with loss of HES1 transcription in MTC tissue samples and mutant RET cell lines. Moreover, we demonstrated that the 3'UTR of the HES1 mRNA bearing the targeting sequence for miR-182 clearly reduced luciferase reporter activity in cells expressing miR-182. Decreased expression of HES1 promotes migration by upregulating Notch1 inhibitor Deltex1 and consequently, repression of Notch1.

Conclusions: We demonstrate a novel mechanism for MTC aggressiveness in which mutated RET/NF-κB-driven expression of miR-182 impedes HES1 activation in a negative feedback loop. This observation might open new possibilities to treat RET oncogene associated metastatic cancer.

Work related to this paper was supported by the FORUN program (grant 889011) from Rostock University Medical Center.

E2F1 signaling promotes chemoresistance and lymphogenic metastasis in penile cancer revealing new prognostic biomarkers

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Objectives: For penile cancer (PC) there are no known molecular predictors of lymphatic spread and/or chemoresistance. Studies of the molecular pathology of this cancer and potential biomarkers have been hampered by the lack of PC-derived cell lines and, therefore, had to rely mainly on histological methods. With the recent establishment of cell lines from patients´ primary tumor tissues and metastases, this has changed, allowing us for the first time molecular and functional analyses in penile cancer.

Method: To search for functional biomarkers that can predict malignant progression and treatment responsiveness, we used four patient-derived PC cell lines and measured invasion and capillary tube formation, responsiveness to genotoxic drugs, and mRNA and protein expression. Data were further validated in E2F1 transcription factor knockdown and overexpression experiments. We quantified E2F1 transcript levels in a set of nonmetastatic tumours (NM), metastasised primary tumours (PT), and lymph node metastases (M) from 24 patients. E2F1 immunohistochemistry was performed in another set of 13 PC biopsies. Relationships between different parameters were analysed using Student t tests. Transcript levels in patient samples were compared using Mann-Whitney U tests. Significance was set at $p < 0.05$.

Results: In cell lines established from lymph node metastases, E2F1 was more abundantly expressed, pRB was inactivated, and CDK2, CDK4, and cyclins D and E were elevated in comparison to cells from primary PC. High expression of E2F1 enhanced the migratory and invasive capacity of cells, while its depletion reduced invasiveness. In addition, E2F1 rendered metastatic cells resistant to chemotherapy and increased lymphatic endothelial tubule formation. We showed that VEGFR-3 and VEGF-C are upregulated in response to high E2F1 as well as mesenchymal markers, such as N-cadherin, vimentin, and slug. E2F1 was clearly upregulated in infiltrative and metastatic primary tumours and metastases (NM vs PT, $p < 0.05$; NM vs M, $p < 0.0005$). E2F1 Quick scores increased from grade I to grade III tumours. The results demonstrate that the transcription factor E2F1 is a key activator of a pro-lymphangiogenic phenotype in penile cancer and promotes chemoresistance. As decisive E2F1-related biomarkers, we identified critical EMT factors and VEGFR-3/VEGFC to be required for early nodal metastasis that might assist in stratifying PC patients for different treatment regimens.

Conclusions: The availability of penile cancer cell lines allows molecular research on the mechanisms underlying metastasis and chemotherapy. A critical pathway involved in both features has been identified and may lead to better patient stratification and improvement of therapeutic options.

This study was supported by FORUN grant 889009 from Rostock University Medical Centre and the German Federal Ministry of Education and Research (BMBF) as part of the project eBio:SysMet (0316171 to B.M.P.).

Cardiac M2 macrophages in wound healing following myocardial infarction

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Objectives: Myocardial infarction (MI) remains the leading cause of mortality all over the world. One of the most ambitious goals in modern cardiology is to regenerate the injured myocardium. Alternatively activated M2 macrophages perform decrease inflammatory response and regulate fibrosis and angiogenesis. However the role of M2 macrophages in myocardial regeneration is less clear. In this study we determined temporal dynamics of M2 cardiac macrophages in infarct, peri-infarct and non-infarct zone in wound healing after MI.

Method: The study included 41 patients with fatal MI type 1 and 9 persons without cardiovascular abnormalities who died instantly due to fatal trauma (control group). All patients with fatal MI were divided into 4 groups depending on the timeline of MI pathology. In addition to histopathological analysis macrophages infiltration was assessed by immunohistochemistry with antibodies against common macrophage marker CD68 and M2 markers CD163, stabilin-1. Statistical analyses were performed with Statistica for Windows 10.0.

Results: In comparison with normal myocardium the common number of macrophages (CD68+) and number of CD 163+ M2 macrophages in the infarct and peri-infarct area increased from the very first day and peaked in the reparative phase. In the late stage of MI their number was not decreased to normal range. The quantity of stabilin-1+ macrophages in the infarct and peri-infarct zones during the first 3 days of MI was less than in the normal myocardium. It increased on 4^{th} -10^{th} days of MI and later 10^{th} days none decreased. Consequently temporal dynamic of CD 163+ and stabilin-1+ in the two described areas was different. In the non-infarct area the common number of macrophages increased in the first 3 days, peaked during the reparative phase and was not significantly decreased in the late phase. The quantity of CD163+ M2 in the non-infarct zone was not significantly changed during wound healing after myocardial infarction. However in comparison with normal ranges their number was significantly increased on the first day and 4^{th} -10^{th} days. In contrast with normal myocardium we observed decrease of number stabilin-1+ M2 macrophages on 1-3^{th} days and return their number to normal range in the reparative phase.

Conclusions: In our study we observed biphasic cardiac macrophage response following acute MI reminded a murine model. The difference was in a prolonged high-grade CD68+, CD163+ and stabilin-1+ macrophages infiltration after 10^{th} day of MI, whereas in murine model the number of reparative monocytes decreased towards days 9-10 of MI. We have detected increase of number CD68+ and stabilin-1+ macrophages in non-infarct area on days 4-10 after MI. Furthermore, we observed differences in temporal dynamic of CD163+ and stabilin-1+ M2 macrophages in all three areas. We have put forward stabilin-1 as a diagnostic M2 macrophage biomarker in wound healing following acute MI. Results demonstrate the perspectives for providing of macrophages properties to clinical conditions.

This work was supported by the Russian Foundation for Basic Research, grant №16-04-01268, 2016-2018.

Prevalence and Species Distribution of Oral Candida with their Antifungal Susceptibility Profile among Patients with Diabetes Mellitus in Murtala Specialist Hospital, Kano, Nigeria.

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Objectives: Disease conditions such as diabetes mellitus have shown to have potentiality in causing species of Candida which are commensal in the oral cavity especially *Candida albicans* to become pathogenic and causing lesions in the mucosa of the oral cavity. This necessitate the need to conduct this study to increase awareness on the need to routinely investigate this important disease condition for better understanding to the clinicians and for better management of the patients. The study was aimed at determine the prevalence and species diversity of oral candidal pathogens, antifungal susceptibility profile, and relationship between glycemic control and oral Candida colonization.

Method: A hospital based descriptive cross sectional study was carried from November, 2016 to January, 2017 where 49 samples of saliva from type 2 Diabetic patients were collected with equal number of 49 samples from the control group of healthy volunteers who were age and sex matched, without history of diabetes. *Candida species* were isolated and identified using Sabouraud's Dextrose Agar (SDA), gram staining, germ tube test and Analytical Profile Index (API) 20 Aux C Kit. An in vitro Antifungal Susceptibility Testing was conducted.

Results: Out of the 49 samples analyzed from patients with diabetes mellitus, 24(49%) *Candida spp* were isolated, and 11(22.4%) among 49 samples from Non diabetic subjects with an overall prevalence of 36 %. *C. albicans* is the most frequently isolated specie in both diabetics and Non diabetics with prevalence of 54.2% and 73% respectively. The non *albican* species (NAC) were encountered more in the diabetics than Non diabetics with prevalence of 45.8% and 27% respectively. *C. tropicalis* is the most prevalent among the NAC specie, *C. famata* the least while *C. glabrata, C.tropicalis, C.krusei* and *C. dublinensis* carrying equal prevalence. Susceptibility profile of azoles in the diabetics showed *C. albicans* isolates to be 80% susceptible to all the azoles, *C. glabrata* 50% susceptible, *C.tropicalis* 75% susceptible, *C.krusei* 67 % *C.duliniensis* 33% susceptible and *C. famata* 100% susceptible to all the azoles. Overall *C. famata* is more sensitive to the azoles, than *C.albicans* with *C. dubliniensis* with the least susceptibility.In the Non diabetics, C. albicans isolates are 63% susceptible to azoles with *C.tropicalis*, *C. famata* and *C. gulliermondii* 100% sensitive to all the azoles.

Conclusions: While it is certain that even in healthy individuals to a varying percentages *candida spp* forms part of the normal flora of the oral cavity, it is also true that, diabetes mellitus patients do not only harbor these organisms in a much higher prevalence but also in increasing density much higher than the healthy subjects. More so the NCAC species thought to be more virulent and pathogenic are in increasing frequency in these individuals. Significant resistance was found by species isolated from the diabetics and *C. dubliniensis* has shown the least susceptibility to routinely prescribed fluconazole. Although prevalence and density of oral colonization by *candida spp* is much higher in diabetics than healthy subjects, no relationship was found between fasting blood glucose and prevalence of *candida spp* so also the density of oral candida colonization in the diabetic group.

Effect of composition consisting of short double-stranded immunostimulatory RNA and liposomal delivery vector 2X3-DOPE in murine melanoma model.

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Objectives: Immunotherapy is widely used for treatment of different tumor types. Melanoma is an immunogenic tumor, so strategies to enhance the immune response to the tumor have been developed. Short dsRNAs, depending on their sequence, structure and delivery system, can stimulate innate and adaptive immunity. Earlier 19-bp dsRNA with 3'- trinucleotide overhangs (isRNA) was shown to display immunostimulatory activity (Kabilova et al., 2016). The aim of this study was to investigate toxicity, immunostimulating and antitumor effect of composition consisting of isRNA and 2X3-DOPE in C57Bl/6 mice with melanoma B16.

Method: C57Bl/6 mice with subcutaneously transplanted melanoma B16 were injected on days 9 and 13 of tumor growth with complexes of isRNA/2X3-DOPE (P/N, 1/4). On day 14 of tumor growth mice were euthanized, and tumor nodes, spleens, thymuses and livers were collected for further histological and immunohistochemical analysis. Immunostimulation was evaluated by diameters of lymphoid follicles, volume densities of white and red pulp in spleen, cortex and medulla in thymus and cytokine levels. Toxicity was determined by volume densities of dystrophy and necrosis in liver. Antitumor activity was estimated on the base of tumor size and CD4/CD8-lymphocyte infiltration in tumor.

Results: Treatment of melanoma B16 with isRNA/2X3-DOPE complexes displayed no toxicity, inhibited tumor growth and increased survival of tumor-bearing animals. isRNA/2X3-DOPE was found to activate the antitumor immune response expressed in 2-fold increase in the number and diameter of lymphoid follicles of spleen, formation of large germinal centers and their fusion with each other, 4-fold increase in the volume density of spleen white pulp, and 2.5-fold increase in the volume density of thymus cortex in comparison with untreated animals with melanoma B16. Immunohistochemical study revealed 1.7-fold increase in the volume density of CD8-lymphocyte infiltration of melanoma B16 tissue at the border of normal tumor tissue and necrosis after isRNA/2X3-DOPE treatment. The number of CD4-lymphocytes remained at the untreated level. Significant increase of interferon-alpha level was observed in the serum of mice with melanoma B16 after isRNA/2X3-DOPE injection in comparison with untreated animals. TNF-alpha and IL-6 serum levels did not change after treatment.

Histological study of livers of mice with melanoma B16 without treatment revealed pronounced destructive changes in the liver tissue occupied about 50% of the whole liver parenchyma. Administration of isRNA/2X3-DOPE did not cause an increase in destructive changes in the liver of tumor-bearing animals.

Conclusions: At the present time, compounds with immunostimulating activity are widely used for treatment of different histological types of tumors, for example, melanoma, glioma, lung and prostate cancer. The use of these drugs can increase the effectiveness and reduce the toxicity of standard regimens for the treatment of tumors, in particular hepatotoxicity, since most antitumor drugs undergo biotransformation in the liver with the formation of toxic metabolites. It was demonstrated that systemic administration of isRNA/2X3-DOPE into mice with melanoma B16 induces specific and non-specific activation of immune system, and as a result has a pronounced antitumor effect for this tumor type. It should be noted, that isRNA/2X3-DOPE does not cause systemic and local inflammation and has no pronounced toxicity to the liver and to the whole organism in comparison with another immunostimulating agents (for example, Poly I:C). Thus, composition consisting of 19-bp dsRNA with 3'- trinucleotide overhangs (isRNA) and cationic liposomes 2X3-DOPE can be used as adjuvant immunostimulating therapeutic in antitumor treatment.

This work was supported by Russian Science Foundation (project 16-15-10105).

Prolonged cigarette smoke alters mitochondrial metabolism, cellular polarity distribution in human oral epithelial cells

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Objectives: Cigarette smoke is one of the major contributors in oral cancer development. It is well known to induce oxidative stress and enhance the reactive oxygen spices (ROS) formation. Excessive ROS and associated oxidative stress are considered to be a driving force in alteration in mitochondrial metabolism as well as in cancer progression. Additionally ROS also acts as secondary signaling intermediates which control cellular phenotype and polarity distribution. In present study we intend to explore the effect of cigarette smoke on oral epithelial cells in terms of ROS production and a correlation amongst the mitochondrial heme-metabolism, cellular polarity distribution and epithelial to mesenchymal transition will also be established.

Method: Total 115 subjects were categorized into four study groups: nonsmoker (NS), cigarette smoker (CS), oral leukoplakia (OLK) and oral squamous cell carcinoma (OSCC). Smokers were further categorized into three sub-groups cording to pack-year (P-Y). Label-free differential interference contrast (DIC) microscopy was performed for cellular morphological assessment. Fluorescence microscopy and spectroscopy was performed to measure the inter-cellular metabolites (like NADH, FAD, porphyrin etc.) and the cellular redox ratio was calculated. Cellular iron content and production of reactive oxygen species were also measured. Cytoskeleton distribution, cellular polarity and heme metabolism were observed to corroborate the effect of cigarette smoking with cancer progression and alteration in cellular metabolism.

Results: Cytomorphometry revealed that cytoplasmic parameters such as cytoplasmic area (CA), cytoplasmic diameter (CD) were gradually decreased from control (NS) to smoker group with low P-Y (< 10 yrs) to high P-Y (> 20 yrs) as well as in patients with OSCC. Unlike cellular parameters, nuclear parameters such as nuclear area (NA), nuclear diameter (ND), and nucleus to cytoplasmic ratio (N:C) were increased from NS to OSCC. Mean autofluorescence intensities for blue (ex350 nm; em450nm) and red (ex408 nm; em630 nm) emission were increased with higher P-Y and in OSCC. Atomic absorption spectroscopy (AAS) analysis revealed the increasing trend in accumulation of iron in the epithelial cells from nonsmoker to OSCC. Drastic changes in distribution of actin filament in oral epithelial cells were observed among the study groups. Loss of Protease-activated receptor (PAR) protein with high P-Y value indicated loss of cell polarity and progression of carcinogenesis. Similar high expression was also observed in OLK and OSCC which clearly indicates cigarette smoking enhances the chances of oral cancer progression. Gene expression analysis showed upregulation of delta-aminolevulinate synthase 1 (ALAS1) and heme oxygenase-1 (HO-1) genes and down regulation of ferrochelatase (FECH) and ATP-binding cassette sub-family G member 2 (ABCG2) genes of mitochondrial heme metabolic pathway with increased P-Y.

Conclusions: Oral epithelial cells from smoker groups exhibited drastic changes in cellular morphology like CA, CD, NA, ND and N:C with increase in duration of smoking exposure. Changes in autofluorescence intensities of related intercellular metabolites as well as redox differences clearly indicated the alteration in cellular metabolic pathways. Cytokeratine distribution and change in cellular impedance in different study groups are the evident of cancer progression. Oral epithelial cells exhibit apical basal polarity, which enable to carry out special function. Therefore, loss of cellular polarity causes the transition from normal to malignant epithelium due to the exposure of cigarette smoke. The expression of markers related to heme metabolism signifies the accumulation of protoporphyrin IX, toxic byproduct of heme metabolic pathway, which may lead to the progression carcinogenesis in habitual smokers. In present study, we have correlated the possible factors that may cause of cancer progression with prolonged exposure of smoking. However the in-depth molecular pathway is yet to be studied.

Fellowship for Ripon Sarkar was supported by UGC, Govt. of India.

Neurochemical Monitoring in the Injure Brain

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Objectives: Monitoring of the neurochemical changes, participating in the neuronal injury, constitutes the essential groundwork for the development of an effective treatment and drug development. Our data manifested a difference for the pathophysiological processes in early stages of ischemic injury from those relevant for adult subjects. Unfortunately, these biological phenomenal specificities are rather neglected in the perinatal stage, particularly in the field of the development of novel drugs and therapies.

Method: The microdialysis is a technique permitting continuous sampling of the interstitial fluid chemistry of tissues and organs. It is a minimally invasive and simple method to be used in the clinical set-up. The microdialysis catheter is used to collect samples of all small molecular substances contained in the interstitial fluid and is detected and quantified by appropriate analytical techniques. Nowadays, the combination of high performance liquid chromatography (HPLC) and mass spectrometry (MS), or HPLC and nuclear magnetic resonance (NMR), allows a facile separation and parallel detection of even very low analyte concentrations contained in microdialysates.

Results: Clinical trials with microdialysis on adults gradually expand. It is widely used on patients in neurointensive cares. Combination with other technique, as Doppler flowmetry, PET or EEG allows to multimodal monitoring of the patient. There are several clinical trials with microdialysis in neonates, but almost all are based on inserting microdialysis probe subcutaneously or intraperitoneally. However, data about non gastric diseases can be obtained mainly from statistical mapping of patients. From ethical point of view clinical trials of diseases in neonates are hardly realisable. Current knowledge of tissue biochemistry (basal levels of several essential compounds) in immature brain is from tissues sampled postmortem or by biopsies which do not fully respond to physiological conditions in tissue. Thus, animal models represent great opportunity for study of immature brain physiology and pathologies. In neurobiology is used in behavioral studies, studies of neurological disorders (Alzheimer's, Huntington's or Parkinson's disease, brain trauma, epilepsy, ischemia, spinal cord injury), studies of neuropsychiatric diseases (depression, schizophrenia), pain research, studies of BBB (disruption and transport through the barrier) and many others. This innovative and less invasive technique has a great advantage of possibility for measurements on living animal in anaesthesia or freely moving.

Conclusions: From our point of view the already-mentioned combination of both methods (MD and HPLC/MS) have unique possibility for the monitoring of tissue biochemistry and metabolomic mapping in immature brain in almost real-time condition. Using MD and following HPLC/MS analysis we successfully mapped levels of amino acids and other low molecular weight molecules in discrete regions of immature brain. Moreover, due to high sensitivity of HPLC/MS method can be applied for determination of D and L stereoisomers of amino acids, which are important not only for a normal brain development but also play a role in some pathologies. A potential of proposed method can found a place in clinical practice and for biomarkers monitoring of various CNS diseases. For example, we proved efficiency of both techniques in the study of neurochemical correlates of focal cerebral ischemia development and ischemia outcome in immature brain. However the potential of this methodology is not fully explored, we believe that it might be extremely effective in the drug delivery to the discrete regions (reverse microdialysis), that can help in a selective and considerate treatment. In addition, this methodology might serve as a great base for the development of age and condition specific “fast-detection” kits/chips, etc.

AZV -17-31852A a LO1611