A COST Action CA15214 WG1-WG2-WG3-WG4
Networking Meeting

Mechanobiology of cells and tissues in health and disease

8th - 9th November 2018,

Ljubljana, Slovenia

Host:

- Mirjana Liović, Faculty of Medicine, University of Ljubljana, Slovenia

Scientific Committee:

- Pavle Andjus, Faculty of Biology, University of Belgrade, Serbia
- Andreja Ambriović-Ristov, Rudjer Bošković Institute, Croatia
- Rui Travasso, Center for Computational Physics, University of Coimbra, Portugal
- Nuno Saraiva, Health Sciences and Technology School , Universidade Lusófona, Lisbon, Portugal
- Mirjana Liović, Faculty of Medicine, University of Ljubljana, Slovenia

Technical help, organization and logistics:

- AdriaBio doo, Postojna, Slovenia

Venue:

- City Hotel, Dalmatinova ulica 15, SI-1000 Ljubljana, Slovenia

Sponsor:

- Cytoskeleton Inc. , https://www.cytoskeleton.com/
- Slovenian Biochemical Society, http://www.sbd.si
- VWR, https://www.vwr.com/
- AdriaBio doo, https://adriabio.com

Meeting Program

- ***Thursday, 8th November***

Biophysics of cell and tissue structure I *(all presentations are 30 minutes long followed by a 15 minute discussion, Q&A)*

10:30am -10:45am – Welcome Introduction

10:45am – 11:30am – **Rui Travasso**, University of Coimbra, Portugal

"*MATHEMATICAL MODELING OF CELL MOVEMENT AND ANGIOGENESIS*"

11:30am – 12:15am – **Špela Zemljič-Jokhadar**, University of Ljubljana, Slovenia

"*CYTOMECHANICS MEASURED BY AFM AND OPTICAL TWEEZERS AFTER F-ACTIN DISRUPTION*"

12:30pm – 2pm - ***Buffet lunch***

2pm - 3 pm - Invited Lecture, **Duško Ilić**, Kings College London, United Kingdom

"*FULL THICKNESS SKIN MODELS FROM HUMAN PLURIPOTENT STEM CELLS FOR IDENTIFICATION AND TESTING EFFECTIVENESS OF PERSONALIZED THERAPIES IN ATOPIC DERMATITIS*"

Biophysics of cell and tissue structure II

3pm – 3:45 pm – **Juan Carlos Rodriguez-Manzaneque**, University of Granada, Spain

"*ELUCIDATING THE ACTIONS OF ADAMTS EXTRACELLULAR PROTEASES IN HETEROGENEOUS EX-VIVO AND IN-VITRO CULTURE MODELS"*

3:45pm – 4:30 pm – **Mara Grube**, University of Latvia, Latvia

"FTIR SPECTROSCOPY FOR EVALUATION OF THE CELLS METABOLIC STRESS"

4:30pm – 4:45pm – ***Coffee break***

4:45pm -5:30 pm – **Reet Kurg**, University of Tartu, Estonia

"CANCER-TESTIS ANTIGENS MAGE-A PROTEINS EXPRESSION IN EXTRACELLULAR VESICLES RELEASED BY CELLS"

5:30pm – 6:15pm – **Pavle Andjus**, Univeristy of Belgrade, Serbia

"THE EXTRACELLULAR MATRIX GLYCOPROTEIN TENASCIN-C AND MATRIX METALLOPROTEINASES MODULATE CEREBELLAR STRUCTURAL AND FUNCTIONAL PLASTICITY"

*7pm – **Networking dinner***

- ***Friday, 9th November***

New methodologies to study mechanobiology of cells and tissues

9am – 9:45am – **Nuno Saraiva**, University of Lisbon, Portugal

"IMPACT OF A MANGANESE(III) PORPHYRIN IN CANCER CELL MIGRATION"

9:45am – 10:30am – **Ramunas Valiokas**, Center for Physical Sciences and Technology, Lithuania

"NANOPATTERNED HYDROGEL SURFACES FOR TISSUE FORMATION STUDIES AND ENGINEERING"

*10:30am – 10:45am – **Coffee break***

10:45am – 11:30am – **Andreja Ambriović – Ristov**, Rudjer Bošković Institute, Croatia

"CHARACTERISATION OF THE INTEGRIN αV-DEPENDENT ADHESOME IN MDA-MB-435S MELANOMA CELLS"

11:30am – 12:15pm – **Polonca Ferk**, University of ljubljana, Slovenia

"UV-INDUCED GENTIC AND EPIGENETIC CHANGES IN HUMAN SKIN CELLS IN VITRO"

*12:30 – 2pm - **Buffet lunch***

Mechanobiological principles of rare and common diseases

2pm – 2:45pm – **Beata Čunderlikova**, Comenius University, Slovakia

"EXTRACELLULAR MATRIX AS AN IMPORTANT COMPONENT OF EXPERIMENTAL IN VITRO MODELS OF DISEASES"

2:45pm – 3:30pm – **Melek Özturk**, Istanbul University, Turkey

"OKADAIC ACID-INDUCED TAUPATHY MODEL AND PEPTIDYL-PROLYL CIS-TRANS ISOMERASE EXPRESSION IN PRIMARY CORTICAL NEURONS"

*3:30pm – 3:45pm – **Coffee break***

3:45pm – 4:30pm – **Serap Arbak**, Acibadem University, Turkey

"MICROSCOPICAL INVESTIGATIONS ON THE PUTATIVE EFFECTS OF NANOPARTICLES ON TESTIS TISSUE"

4:30pm – 5:15 pm – **Mirjana Liović**, University of Ljubljana, Slovenia

"IPSC CELLS DERIVED FROM EPIDERMOLYSIS BULLOSA PATIENTS AS DISEASE MODEL SYSTEMS"

Structural analysis of biomolecules involved in mechanobiology

5:15pm – 6:00pm – **Sergei Strelkov**, University of Leuven, Belgium

"KERATIN IF STRUCTURE, REVISITED"

6:00pm – 8pm - ***Meeting wrap-up and networking dinner***

- ***Saturday, 10th November***

Breakfast and departure

ABSTRACTS

INVITED LECTURE

FULL THICKNESS SKIN MODELS FROM HUMAN PLURIPOTENT STEM CELLS FOR IDENTIFICATION AND TESTING EFFECTIVENESS OF PERSONALIZED THERAPIES IN ATOPIC DERMATITIS (WORK IN PROGRESS)

Duško Ilić

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I will present a current project that we are working on, which is supported by the LEO Foundation in Denmark. The project aims to utilize the latest advances in stem cell science, gene-editing and tissue engineering to develop and fully validate innovative 3D in vitro models of skin, similar to the native skin in atopic dermatitis (eczema) patients.

Dusko Ilic received his MD degree and BSci in Molecular Biology at the University of Belgrade, Serbia. After obtaining a PhD degree at Tokyo University, Japan, he did postdoctoral work at the University of California in San Francisco. He held the position of Adjunct Associate Professor at the University of California San Francisco, Consultant at the Veteran Affairs Medical Center, San Francisco, and Director of R&D in StemLifeLine, a California-licensed,state-of-the-art cell culture facility that followed cGTP for derivation and the long-term cryopreservation of human embryonic stem cell lines (hESC). Since 2009, he is at the Assisted Conception Unit at Guy's Hospital, King's College London, where he derived the first clinical grade hESC under xeno-free conditions. Focus of his work is translational stem cell research.

CHARACTERISATION OF THE INTEGRIN αV-DEPENDENT ADHESOME IN MDA-MB-435S MELANOMA CELLS

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Integrins are heterodimeric glycoproteins consisted of α and β subunits that binds cells to extracellular matrix proteins. Upon integrin clustering, multimolecular integrin adhesion complexes (IACs) form that facilitate the linkage between integrins and the actin cytoskeleton and permit bidirectional signalling. The αV integrin is expressed in most tumour cells where it regulates a diverse array of cellular functions and plays a role in anti-tumour drug resistance. The aim of our work was to assess αV-dependent changes in IAC composition in MDA-MB-435S melanoma cells in order to better understand the increased sensitivity to paclitaxel and vincristine upon integrin αV knockdown. Integrin αV-specific shRNA was cloned into pSUPER.puro, transfected into MDA-MB-435S cells using Lipofectamine, and cell clones were selected using puromycin. The sensitivity of cells to antitumor drugs was determined using an MTT assay. Cell migration was monitored using a Transwell assay. IACs were isolated following crosslinking and their molecular composition analysed using mass spectrometry (MS)–based proteomics. Flow cytometry, western blot, confocal microscopy and Interference Reflection Microscopy (IRM) were done using standard protocols. In two MDA-MB-435S-derived cell clones with decreased expression of integrin αV, expressing 15% (2αV) or 5% (3αV) of the control cells amount, increased sensitivity to paclitaxel and vincristine, decreased sensitivity to cisplatin, and decreased migration were observed in line with previous results obtained following transient transfection with integrin αV siRNA. In cell clones 2αV and 3αV, which were smaller than control cells and lacked stress fibres, the number of focal adhesions was shown to be significantly lower as observed by IRM and immunofluorescence detection of phospho-paxillin, phosphoFAK and phospho-Src. MS analysis of isolated IACs from control MDA-MB-435S, 2αV and 3αV cells identified 282 proteins, including 36 out of 60 consensus adhesome proteins. As expected, in clones 2αV and 3αV, integrins αV, β3 and β5 were detected at much lower levels compared with control cells. In addition, lower levels of talin-1 and 2, vinculin, α-actinin-4, tensin-3, filamin-A and -B, liprin β1 and plectin were detected. These data will enable follow-up analyses of the mechanisms of signalling by integrins αVβ3/β5 and therefore, represent a valuable resource to improve our understanding of the mechanisms involved in adhesion control of cell sensitivity to antitumor drugs and metastatic potential.

THE EXTRACELLULAR MATRIX GLYCOPROTEIN TENASCIN-C AND MATRIX METALLOPROTEINASES MODULATE CEREBELLAR STRUCTURAL AND FUNCTIONAL PLASTICITY

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The study focuses on the role of TnC in the modulation of the enrichhed environment (EE)-induced structural plasticity in the cerebellum by applying imaging to monitor the distribution of perineuronal nets (PNN), changes in the presynaptic terminals, and the activities of the major enzymes that degrade ECM. For this purpose, mice with a deficiency of TnC (TnC - / -) or MMP-9 (MMP-9 - / -) aged 3 weeks were exposed to standard breeding conditions (SC) or EE 4 or 8 weeks.
We showed that the exposure to EE for 8 weeks leads to a decrease in the intensity of staining of PNN, as well as a decrease in the size of GABAergic and an increase in the number and size of glutamatergic synaptic terminals in control mice. In contrast, TnC - / - mice showed a reduced intensity of PNN staining compared with control animals grown in SC, while their exposure to the EE did not lead to a decrease, but to a slight increase in intensity of PNN. In addition, the EE did not affect the density of the two types of synaptic terminals in TnC - / - mice, while the size of inhibitory, albeit not excitatory, synaptic terminals was increased. It was also shown that MMP-9 affects the rearrangement of PNN and synaptic plasticity in the cerebellum between 4th and 8th week of EE exposure. These findings are further confirmed by the results obtained on MMP-9 - / - mice.
The strutural plasticity findings were confirmed on the behaivoural level on TnC - / - mice that show spontaneous nocturnal hyperactivity, as well as poor sensomotor coordination and low swimming speed. These changes were partly abrogated after exposure to EE. Coversely, lack of TnC diminished the positive effects of the EE on the ability to learn and memorize, and its the anxiolytic effects.
Thus, the interactions between TnC and MMP-9 are crucial for the regulation of structural plasticity in the cerebellum. The effect of the genetic basis on the behavioral responses can be altered by the exposure of animals to a highly stimulating environment.

MICROSCOPICAL INVESTIGATIONS ON THE PUTATIVE EFFECTS OF NANOPARTICLES ON TESTIS TISSUE

Serkan Deveci[1], Merve Acikel Elmas[2], Deniz Yucel[2], M. Selcuk Keskin[3], Sibel Demirci Delipinar[4], Ismail Seckin[4], Ahmet Sahin, [3]Serap Arbak[2]

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The use of nanobiotechnology in human health has been increased in recent years. Drug carrier nanoparticles with their wide range of uses and advantages are promising approaches for the treatment of many diseases. The aim of this study is to investigate the effects of oral and local treatment of the silica based nanoparticles on testis structure to be used clinically in the field of urology. Nanoparticles (NanoXact^{TM} Silica, from NanoComposix, Inc) with a diameter of 80 nm were used in this study. Solution of nanoparticles (10 mg/mL, in Milli-Q water) was sonicated for 20 min in water bath sonicator before application. In oral administration group, nanoparticles were applied to animals via gavage as 20 mg/kg. On the other hand, nanoparticles were applied topically to the penis in coconut oil as 2mg/cm^2 for local application. Both local and oral applications were done every three days for 35 days, and the animals were sacrificed on day 35. Testes samples were fixed with 10% neutral buffered formalin solution. After fixation, testes tissues were prepared for paraffin sections. Sections were stained with Haematoxylin–Eosin (H&E) and Periodic acid–Schiff (PAS) reaction for light microscopical examination. H&E and PAS- stained sections were evaluated for histopathological scoring. Histopathological scoring was performed by the modification of Hess's data and number of normal, regressive, degenerative or atrophic tubules were determined (Hess, Linder, Strader, & Perreault, 1988). In order to determine the localization and expression of occludin, an integral tight junction membrane protein, in seminiferous epithelium, samples were examined using semiquantitative and statistical analysis of occludin immunreactivity. For transmission electron microscopical examination, testes tissue samples were fixed in 2.5% glutaraldehyde and processed for epoxy resin embedding. Normal morphology of seminiferous tubules were observed in control group. In both gavage and local groups, the number of normal tubules decreased while the number of regressive tubules has been increased. It was observed that the number of degenerative and atrophic tubules was higher in oral administration group compared to local application group. The immunohistochemical results showed that occluding immunoreactivity was higher in local group comparing to the oral administration group. Ultrastructural examinations demonstrated germinal epithelial damage, which was less in local group compared to oral administration group. The results revealed that toxicity of silica nanoparticle on the male reproductive system is low in both applications, particularly in local application. Therefore, these silica nanoparticles would be used as a drug delivery system in the treatment of male reproductive system disorders for the further studies.

EXTRACELLULAR MATRIX AS AN IMPORTANT COMPONENT OF EXPERIMENTAL IN VITRO MODELS OF DISEASES

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The proper interaction between the cells and surrounding extracellular matrix (ECM) is crucial in physiology and is disrupted in many pathologies. In order to identify effects of ECM on cellular processes that are active under clinically relevant conditions, in vitro models enabling studies under defined experimental conditions with possibility to modify cellular and noncellular composition and increase complexity are helpful. Three-dimensional (3D) cell cultures based on ECM offer this possibility.
Application of 3D cultures based on ECM components relevant to carcinogenesis has shown that ECM affects different aspects of cell behaviour from morphogenesis, differentiation, production of extracellular vesicles, metabolic activity as well as response to treatment intervention in the form of photodynamic inactivation, in cell-type and ECM-type dependent manner. Vimentin expressing cell line was influenced by surrounding ECM at most, especially with regard to morphogenesis, differentiation, and response to photodynamic inactivation. Biophysical properties of the ECM surrounding the cells, namely collagen type I fiber density, have shown the most considerable modifications in the course of multicellular cluster formation in the case of vimentin expressing cell line. Mechanobiological measurements have suggested more distinct accompanying changes in the stiffness of collagen type I matrix around clusters of vimentin expressing cells than around clusters of cells negative for this differentiation marker.
Results of our research indicate that presence of the proper ECM composition in experimental in vitro models is crucial for understanding mechanisms behind pathophysiology of cancer and other diseases.

Acknowledgement: This work was supported by VEGA 1/0070/16 and STSM Grant from COST Action CA15214.

UV-INDUCED GENTIC AND EPIGENETIC CHANGES IN HUMAN SKIN CELLS *IN VITRO*

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Sun exposure with sunburn is established as a major environmental risk factor for melanoma, the main cause of death from skin cancers. Our studies on UV-induced primary human melanocytes showed that crucial events on transcriptomic level (nanostring mRNA analyses) happen mainly in MAPK signalling. The key switch in PanCancer® signalling pathways was observed after UVA irradiation. The investigated transcriptomic changes showed wavelength and dose dependency. In addition to genetic changes that contribute to the initiation and propagation of melanoma, epigenetic alterations, i.e. lower expression levels of 5-hydroxymethylcytosine (5-hmC) have been reported. Our results show the 5-hmC loss might be UV-driven in a dose-dependent manner. These findings need further evaluation for their biological significance in determining potential melanoma precursors.

FTIR SPECTROSCOPY FOR EVALUATION OF THE CELLS METABOLIC STRESS

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Fourier transform infrared (FTIR) spectroscopy is more and more frequently applied as an approved technique in natural and life sciences. An infrared spectrum provides specific data for identification and evaluation of the overall molecular composition, chemical bonding and structure, also enabling qualitative and quantitative analyses of samples incl. microbial cells, mesenchymal stem cells, cancer cells, etc. Minimal requirements for sample pretreatment, significantly lower sample amounts to compare with biochemical or chromatography methods, and the fact that all analyzed components are represented together in a single spectrum, are just some of FTIR advantages. Therefore, FTIR spectroscopy methods are frequently used in biomedical science as a label-free method providing objective and reliable diagnostic information in a relatively non-invasive manner. FTIR spectroscopy is applied to evaluate, characterize, classify, or distinguish the cell composition based on the data analyses of specific absorption bands. The response of cells to various growth factors *via* the biochemical composition is well known and has been studied by FT-IR spectroscopy. A novel method for FTIR-microscopy of small quantities of biosamples as a hydrated film on a diamond anvil cell will be presented. This method allows to speed up the FTIR spectroscopic analysis of various biosamples, particularly in those cases, where the available amount of biomaterial is a limiting factor.

To our experience it is appropriate to use FTIR spectroscopy to estimate the cell metabolic responses induced by various growth environment-induced stress factors. Incl., mechanical and thermal stress during bioprinting.

We suggest that FTIR spectroscopy analyses along with other modern cell biology and biochemistry methods could contribute to multidisciplinary collaboration studies applying for appropriate Co-ERANET or ERANET grant programs or other relevant national/international funding's.

CANCER-TESTIS ANTIGENS MAGE-A PROTEINS EXPRESSION IN EXTRACELLULAR VESICLES RELEASED BY CELLS

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Melanoma antigens (MAGE-A) represent a unique class of tumor antigens which are expressed in a wide variety of malignant tumors, while their expression in healthy normal tissues is restricted to germ cells of testis, fetal ovary and placenta. Their restricted expression and immunogenicity make them ideal targets for immunotherapy in human cancers. MAGE-A expression is observed mainly in cancers that have acquired malignant phenotypes, invasiveness or metastasis, and the expression of MAGE-A family proteins has been linked to a poor prognosis in cancer patients.

We have previously shown that MAGEA4 and MAGEA10 proteins are expressed on the surface of retrovirus virus-like particles (VLP-s) induced by over-expression of MLV Gag-protein. In the current study, we have analyzed the expression of MAGE-A proteins in naturally occurring extracellular vesicles (EVs) released by mammalian cells. We show that ectopically expressed MAGE-A proteins are incorporated into extracellular vesicles using different mammalian cell lines. MAGE-A proteins are expressed on the surface of EVs and are resistant to the treatment with salt and non-ionic detergents. MAGE-A proteins can also be used to guide recombinant proteins, e.g. EGFP and Cherry, onto the surface of EVs allowing to follow the behavior of EVs in real time. Our study shows that some MAGE-A proteins, locating both in the nucleus and cytoplasm of the cell, are directed to the surface of EVs released by cells. However, the mechanism of this phenomena and its biological

IPSC CELLS DERIVED FROM EPIDERMOLYSIS BULLOSA PATIENTS AS DISEASE MODEL SYSTEMS

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We have engineered iPSC cells from primary keratinocytes derived from skin biopsies of an epidermolysis bullosa simplex Dowling-Meara phenotype patient with a known mutation in keratin 5 (K5 E475G), and a control sample obtained from a healthy donor. The reprogramming process included non-integrating Sendai virus vector cell reprogramming, selection, cell line expansion, cell line characterisation (CGH, STR, differentiation markers, teratoma assay) and skin equivalent development. The two resulting iPSC cell lines have now been also registered with the Pluripotent Stem Cell Registry (https://hpscreg.eu/), as MLi002-A (iEBS) and MLi003-A (iWT). We have set up protocols for differentiation of iPSCs cells into different epithelial cell types, keratinocytes and fibroblasts, which will be then used to assemble a new 3D full skin equivalent for studying and testing wound healing and the effects of drug repurposing (this part of work is included in the EU project 4D-HEALING). In this context we are also going to prepare iPS cells and 3D skin equivalents for Dystrophic EB, a very severe type of EB that is due to mutations in vimentin. We are also working on protocols for cell differentiation into other cell types to increase the complexity of our future 3D model systems.

OKADAIC ACID-INDUCED TAUPATHY MODEL AND PEPTIDYL-PROLYL CIS-TRANS ISOMERASE EXPRESSION IN PRIMARY CORTICAL NEURONS

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The microtubule associated protein tau plays important roles in microtubule stability, neurite development and axonal transport. The accumulation of intracellular neurofibrillary tangles (NFTs) are the major pathological hallmarks of the neurodegeneration in Alzheimer's disease (AD). NFTs are mainly composed of hyperphosphorylated tau. In pathological conditions, tau hyperphosphorylation inhibits the binding of tau to microtubules, so causing destabilization of microtubules and leading to neurodegeneration. Tau protein hyperphosphorylation is also associated with the abnormal activities of kinases and phosphatases that are regulated by Pin1 protein. Pin-1 (peptidyl-prolyl cis-trans isomerase) plays important roles in various cellular processes such as cancer, aging and neurodegenerative diseases. Pin-1 has a neuroprotective role in AD, and co-localizes with NFTs, mediates tau dephosphorylation by PP2A. Pin1 regulates the phosphorylation of Ser/Thr sites of tau protein, and promotes microtubule assembly. The activation and action mechanisms of Pin1 are still unclear in AD. It is suggested that Pin-1 acts on tau conformation and function. We focused on consequences of tau hyperphosphorylation on Pin-1 expression and its subcellular localization in hippocampal pimary cortical neurons. Given the relation between Pin1 expression and tau hyperphosphorylation we hypothesized that the tau pathology may also involve Pin1 dysregulation. Okadaic acid (OKA) that is used for experimental model for tauopathy and AD studies inhibits PP2A activity. OKA suppresses PP2A and causes tau hyperphosphorylation. 25 nM OKA at 8h is an efficient dose for tau hyperphosphorylation in cultured neurons. In our studies we observed the high levels of cytotoxicity in the OKA-treated groups. It might be indicate that OKA-dependent neuron damage could also implicate tau hyperphosphorylation. However, we reported 25 nM OKA treatment induced neuronal damage but not significant cell loss. We observed that OKA-induced tau hyperphosphorylation resulted in impairment and retraction of neurites. Our results indicated that tauopathies might be associated with the defects in neurite structure. Both the observed neuronal damage and hyperphosphorylation of tau were consistent with the use of OKA treatment as a suggested model of neurodegenerative tauopathy. We demonstrate that OKA induced tau hyperphosphorylation and OKA induced Pin1 downregulation might be two different mechanisms where the alteration in Pin1 expression is expected to result in further tau phosphorylation. With further studies, the relationship between PP2A and Pin1-mediated neuronal death through the inhibition of tau hyperphosphorylation will help to explain the molecular mechanisms underlying AD pathology and possible therapeutic use of Pin1 in AD in the future.

ELUCIDATING THE ACTIONS OF ADAMTS EXTRACELLULAR PROTEASES IN HETEROGENEOUS EX-VIVO AND IN-VITRO CULTURE MODELS

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Recent studies on tumor heterogeneity are including the dynamism of extracellular components due to proteolytic remodeling. The high number of proteases and the specific characteristics of each biological setting make necessary a deeper knowledge of these processes. In our laboratory we are studying the extracellular protease ADAMTS1 for many years. This molecule was first reported as an anti-angiogenic factor, and likewise more recent works supported this activity. However, both pro-tumorigenic and pro-metastatic properties have also been described in distinct tumor models, highlighting its microenvironment-dependent actions.

While our work supports a protumorigenic contribution of stroma ADAMTS1 in a syngeneic tumor model with B16F1 murine melanoma cells, we observed a dysfunctional vasculature correlating with hypoxia and an altered infiltration of macrophage and immune-related cells. In an attempt to understand these phenotypic changes, we approached ex-vivo aortic assays that confirmed a deficient sprouting activity in the absence of the protease. We are also investigating the properties of endothelial and tumour cells when they are co-cultured under various conditions. Importantly, we observed that the characteristic capillary-like phenotype of endothelial cells and some plastic tumor cells was clearly compromised by other tumor cells that do not express ADAMTS1, and we are interested to evaluate effects on the polarization of macrophages. In line with ongoing work, we would pursue a deep analysis of the constituents of the ECM, including ADAMTSs substrates, to unveil the significance of the extracellular microenvironment.

IMPACT OF A MANGANESE(III) PORPHYRIN IN CANCER CELL MIGRATION

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Manganese(III) porphyrins (MnPs) mimic superoxide dismutase, scavenge different reactive species (RS) and modulate redox signaling. MnPs are currently in clinical trials in patients submitted to chemo- or radiotherapy, due to their ability to boost anticancer treatments while protecting off-target tissues. Although RS are implicated in the metastatic process, only scarce studies have addressed the impact of MnPs in metastases. Herein we characterized the impact of non-cytotoxic levels of an MnP ($MnTnHex\text{-}2\text{-}PyP^{5+}$) in metastases-related processes. In renal cancer cells 786-O, MnP (0.25 μM) decreased chemotaxis. This MnP (5 μM) was also studied in MCF7 and MDA-MB-231 breast cancer cells alone and in combination with doxorubicin (dox; 0.1 μM). The co-treatment decreased the collective motility of MCF7, the chemotactic migration of both cell lines, and the proteolytic invasion of MDA-MB-231 cells. MnP also counteracted the increase in random MDA-MB-231 cell migration induced by dox. To explore the underlying mechanisms, the effects in cell spread/area, focal adhesions, intracellular RS levels, and NF-kB activity were studied. Our results suggest that MnP may have a beneficial impact in reducing cancer cells migration and warrant further studies regarding MnP-based anticancer approaches.

Keratin IF structure, revisited

Sergei Strelkov

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Historically keratins were among the first intermediate filament (IF) proteins to be studied structurally. Such studies are clearly indispensable towards the understanding of normal keratin IF functioning as well as the pathological mechanism of keratin mutations leading to disease. More recently there has been a substantial progress, through both crystallography and *in silico* structure prediction, towards elucidating the atomic structure of the elementary IF dimer and the soluble tetramer. However, experimental data on higher-order architecture of keratin IFs are still lacking sufficient detail. Application of modern methods, such as cryoelectron microscopy and chemical crosslinking coupled to MS analysis, are a key to further progress in the keratin structure field.

MATHEMATICAL MODELING OF CELL MOVEMENT AND ANGIOGENESIS

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Biochemical processes are often tightly coupled with physical mechanisms. For example, the way cells organize in a tissue is dependent on their epigenetic states but also on the properties of the adhesion forces between the cells and with the extracellular matrix. Vessel formation and remodeling depend on blood flow, vessel mechanics, tissue mechanics, growth factor diffusion, and matrix mechanical properties and degradation. In this talk I will present several examples of computational models that simulate cell migration and vessel formation taking into account the mechanical interplay between the cells and their microenvironment. I will focus on the ability for these models to suggest testable hypothesis and to provide new insights into the mechanisms that drive the dynamics in these biological systems.

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NANOPATTERNED HYDROGEL SURFACES FOR TISSUE FORMATION STUDIES AND ENGINEERING

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We present applications of unconventional nanofabrication techniques for studying functional modifications of biocompatible hydrogels. Chemically crosslinked collagen hydrogels have been successfully tested as materials for the next generation of tissue scaffolds and implants. However, little is known about the influence of their surface composition and topography on interactions with adherent cells. For example, Islam et al. observed a surface pattern-dependent enhancement of the proliferation rate on recombinant human collagen type III hydrogels, however the origin of this effect has not been analyzed (1). The excellent elastomechanical properties (Young's modulus close to 1 MPa) and optical transparency of these hydrogels make them suitable for studying the related mechanisms on the molecular level. Therefore, we have developed a platform for probing the influence of nanoscopic ECM protein patterns on the cytoskeletal organization of single cells, e.g. human foreskin dermal fibroblasts (HDFs). Also, we have structured the surface of crosslinked porcine collagen (PC) and 2-methacryloyloxyethyl phosphorylcholine (MPC) hydrogel by creating regular patterns of nanoscopic topographies: nanoholes and nanospikes. We have found that the surface nanostructures promote adhesion of HDFs, despite the studied material itself displays strong interaction with this type of cells. Interestingly, the cells conformed to the topographies on the hydrogel surface, precisely repeating the surface nanostructures in their intra-cellular organization. Morphometrical analysis showed that the axial ratio (AR) of the cells, which were growing on flat vs. nanostructured PC-MPC hydrogel, decreased and they became more multipolar. We also observed vinculin conglomerations in cells cultivated onto the nanohole topograhpies. Thus, we have demonstrated that the developed toolbox is suitable for precise control of the inner architecture on the single-cell level as well as for systematic dissection and studies of different mechanisms related to cell-ECM interactions and tissue formation.

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CYTOMECHANICS MEASURED BY AFM AND OPTICAL TWEEZERS AFTER F-ACTIN DISRUPTION

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The sub-membrane actin cortex in animal cells undergoes a continuous turnover and enables a quick mechanical response to stimuli. The majority of the cortical F-actin (which represents roughly 50 % of total cellular F-actin) is generated only by two nucleators: formin Diaph 1 and Arp 2/3 complexes. To analyze the potential influence of Arp2/3 on the cellular mechanics we measured cell stiffness of endothelial cells before and after the treatment with CK-869, which is a known suppressor of Arp2/3 activity. Complementarily, cells were also treated with F-actin disrupting drugs Cytochalasin-D (an inhibitor of actin polymerization) and Jasplakinolide (an actin polymerization promoter) for reference purposes. The cellular stiffness was measured in parallel by means of two different tools that operate in different force regimes and with probes of different geometrical shapes. Atomic force microscopy (AFM) induces large deformations with a pyramidal typ and probes bulk cellular stiffness, and optical tweezers (OT), which induce shallower deformations with a micro-sphere. In agreement with previous studies, the results with both methods showed a reduction in cell stiffness for Cytochalasin-D treated cells, and no significant difference for Jasplakinolide. Importantly, the CK-869 treatment caused a significant reduction in cell stiffness when probed with both methods. Hence, the combined employment of AFM and OT techniques appears as an excellent tool for a more complete, level wise, characterization of cell stiffness.