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Advances in Biomedical Research IV

Mediterranean Institute for Life Sciences, Split, Croatia
October 4th - 09th, 2021.

Book of Abstracts

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Programme

October 4. (Monday) 2021.

17:00 - 18:00	Arrival & Registration
18:15 - 18:20	**INTRODUCTION of the Speaker** *by Igor Stagljar*
18:20 - 19:00	**"Instability of the epigenome" - *The EMBO Keynote Lecture*** *René Medema, Netherlands Cancer Institute, Amsterdam, The Netherlands*
19:00 - 21:00	**Songs, Mediterranean food and wine**

October 5. (Tuesday) 2021.

09:00 - 12:10	**Session #1**
SESSION CHAIR:	**Eric Brown, McMaster University, Hamilton, Canada**
09:00 - 09:35	**"Optogenetic control of mitotic spindle proteins"** *Iva Tolic, Institute Rudjer Boskovic, Zagreb, Croatia*
09:35 - 10:10	**"Lymphatics and cancer"** *Mihaela Skobe, Icahn School of Medicine, Mount Sinai Hospital, New York, USA*
10:15 - 11:00	*Coffee break*
11:00 - 11:35	**"Current questions in prion science"** {VIRTUAL TALK} *Adriano Aguzzi, University of Zurich, Switzerland*
11:35- 12:10	**"Viewing the Metabolic Syndrome from Kidney Stones"** *Orson Moe, University of Texas Southwestern Medical Center, Dallas, USA*
12:10 - 13:00	***1st WORKSHOP – WOMEN IN SCIENCE*** *Moderator: Igor Štagljar & Mladen Merćep* *Panel Members: Iva Tolic, Mihaela Skobe, Katarina Trajkovic, Ivana Carev*
13:00 - 14:00	*Lunch*
14:00 - 17:30	*Free activities*

17:30 – 19:45	**Session #2**
SESSION CHAIR:	**Bernd Wollscheid, ETH Zurich, Switzerland**
17:30 - 18:05	**“Novel live cell-based screening technologies for identification of precision drugs against non-small cell lung cancer”** *Igor Stagljar, University of Toronto, Canada*
18:05 - 18:40	**“Strategies to overcome adaptive resistance to targeted cancer therapies”** *Poulikos Poulikakos, Icahn School of Medicine, Mount Sinai Hospital, New York, USA*
18:40 - 19:15	**"Sigma 1 receptor - role in neuronal signaling and in neurodegeneration"** *Ilya Bezprozvanny, University of Texas Southwestern Medical Center, Dallas, USA*
19:15 - 19:45	**"Cellular Plasticity in Cancer"** {VIRTUAL TALK} *Lynne Postovit, Queens University, Kingston, Canada*
20:00 - 21:00	*Dinner*

October 6. (Wednesday) 2021.

09:00 – 12:25	**Session #3**
SESSION CHAIR:	**Iva Tolić, Institute Rudjer Boskovic, Zagreb, Croatia**
09:00 - 09:35	**"Oncogene-mediated signaling in transgenic mouse models of human breast cancer"** *William Muller, McGill University, Montreal, Canada*
09:35 - 10:10	**"Game Changer: cell free DNA"?** *Bernhard Zimmermann, Natera Inc, San Carlos, USA*
10:10 - 10:45	**“Association with proteasome determines pathogenic threshold of polyglutamine expansion diseases”** *Meewhi Kim, University of Texas Southwestern Medical Center, Dallas, USA*
10:45 - 11:15	*Coffee break*
11:15 - 11:50	**"Metabolic choice in an unfolding world"** *Anita Krisko, University Medical Center, Göttingen, Germany*
11:50 - 12:25	**“Antibody-Conjugates as Protein Switches for Therapeutics and Diagnostics”** *Shawn Owen, University of Utah, Salk Lake City, USA*
13:00 - 14:00	*Lunch*
14:00 - 18:00	Free activities
18:00 - 22:00	City Tour and dinner in town (optional)

October 7. (Thursday) 2021.

09:00 – 11:20	**Session #4**
SESSION CHAIR	**Katarina Trajkovic, MedILS, Split, Croatia**
09:00 - 09:35	**"Light-mediated discovery of surfaceome nanoscale organization and intercellular receptor interaction networks"** *Bernd Wollscheid, ETH Zurich, Switzerland* ***Lecture sponsored by the Journal of Molecular Biology***
09:35 - 10:10	**" A non-coding single nucleotide polymorphism at 8q24 drives IDH1-mutant glioma formation"** *Daniel Schramek, Mount Sinai Hospital, Toronto, Canada*
10:10 - 10:45	**"Mitochondrial Biology and Cancer: Contributions and Consequences"** *Jerry Chipuk, Icahn School of Medicine, Mount Sinai Hospital, New York, USA*
10:45 - 11:20	**"Detection and characterization of carbonylated proteins"** *Mladen Merćep MedILS, Split/ Srebrnjak children's hospital, Zagreb, Croatia*
11:20 - 13:00	**Poster session I** / *Coffee break*
13:00 - 14:00	L*unch*
14:00 - 17:30	*Free activities*

17:30 - 19:50	**Session #4**
SESSION CHAIR:	**Anita Krisko, University Medical Center, Göttingen, Germany**
17:30 - 18:05	**"Bugs, drugs and cell systems"** *Eric Brown, McMaster University, Hamilton, Canada*
18:05 - 18:40	**"TBA"** *Miroslav Radman, MedILS, Split, Croatia*
18:40 - 19:15	**"Regulation of signaling in inflammatory diseases by RIP kinases"** {VIRTUAL TALK} *Domagoj Vučić, Genentech, South San Francisco, CA, USA*
19:15 - 19:50	**"Translational and metabolic perturbations in neoplasia"** {VIRTUAL TALK} *Ivan Topisirovic, McGill Univesrity, Montreal, Canada*
20:00 - 21:00	*Dinner*

October 8. (Friday) 2021.

09:00 – 11:45	**Session #5**
SESSION CHAIR:	**Bernhard Zimmermann, Natera Inc, San Carlos, USA**
09:00 – 09:35	**Neuroinflammation and brain pathology following congenital cytomegalovirus infection“** *Ilija Brizić, University of Rijeka, Croatia*
09:35 – 10:10	**"Cellular parabiosis in aging"** *Katarina Trajkovic, MedILS, Split, Croatia*
10:10 – 10:45	**“Exploring the epigenomic context of mutational processes"** {VIRTUAL TALK} *Rosa Karlic, Faculty of Science, University of Zagreb, Croatia*
10:45 – 11:00	**Real-time analysis of cellular metabolism with Agilent's Seahorse XF technology** Svetoslav Kalaydijev, Agilent Tecnologies
11:00 – 11:05	**Closing Lecture** **INTRODUCTION of the Speaker** *by Igor Stagljar/Miroslav Radman*
11:05 – 11:45	**"Structural basis of SARS-CoV-2 translational shutdown and programmed ribosomal frameshifting”** *Nenad Ban, ETH Zurich, Switzerland*
13:00 - 14:30	*Lunch*
14:30 - 19:00	*Free activities*
19:00 - 23:00	*Conference Banquet*

October 9. (Saturday) 2021.

Departure

Abstracts

Perturbations in 3D genome organization can promote acquired drug resistance

Anna G Manjón[1,4], Daniel Peric-Hupkes[2,4], Ning Qing Liu[2,4], Anoek Friskes[1,4], Stacey Joosten[3,4], Hans Teunissen[2,4], Marleen Aarts[1,4], Stefan Prekovic[3,4], Wilbert Zwart[3,4], Elzo de Wit[2,4], Bas van Steensel[2,4], René H Medema[1,4]

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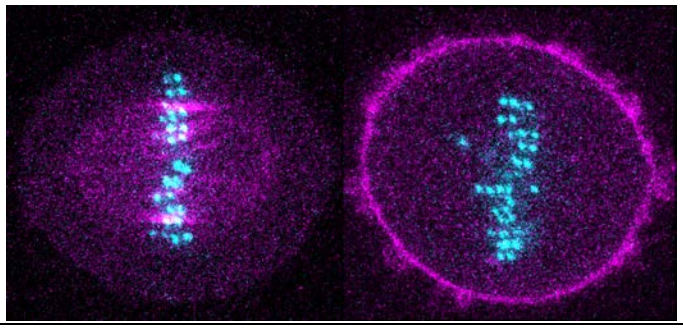
[2] *Division of Gene Regulation, NKI*
[3] *Division of Oncogenomics, NKI*
[4] *Oncode Institute*

Acquired drug resistance is a major problem in the treatment of cancer. Cells can acquire resistance to Taxol by derepressing the ABCB1 gene, encoding for the multidrug transporter P-gP. We investigated the mechanisms underlying such ABCB1 derepression. We show that activation of the ABCB1 gene is associated with specific epigenetic modifications of the ABCB1 promoter, and also with a striking change in the localization of the ABCB1 locus within the nucleus. We propose a model in which deregulation of the 3D genome topology could play an important role in tumor evolution and the acquisition of drug resistance.

Optogenetic control of mitotic spindle proteins

Mihaela Jagrić[1], Patrik Risteski[1], Jelena Martinčić[1], Iva M. Tolić[1*] *Division of Molecular Biology, Ruđer Bošković Institute, Bijenička cesta 54, 10000 Zagreb, Croatia*

In recent decades, RNA interference has been a major tool for studying the role of individual proteins in cell biology. However, the effects of this method are evident only after a long time period, leading to difficulties in the interpretation of phenotypes. Optogenetics is a novel technology that enables fast, reversible, and precise control of protein activity by light. In this talk, I will show the optogenetic method that we developed to acutely remove proteins from the mitotic spindle to the cell membrane by using blue light. To study the mechanism of chromosome alignment at the spindle center, which is crucial for proper chromosome segregation, we removed the microtubule crosslinker PRC1 from the spindle. This resulted in partial disassembly of bridging fibers, which link sister kinetochore fibers, and chromosome misalignment. Tracking of the microtubule plus-end protein EB3 revealed longer antiparallel overlaps of the remaining bridging microtubules upon PRC1 removal, accompanied by misaligned and lagging kinetochores. Kif4A/kinesin-4 and Kif18A/kinesin-8 were found within the bridging fiber and were lost upon PRC1 removal, suggesting that these proteins regulate the overlap length of bridging microtubules. We propose that PRC1-mediated crosslinking of bridging microtubules and recruitment of kinesins to the bridging fiber promote chromosome alignment by overlap length-dependent forces transmitted to the associated kinetochore fibers.



Lymphatics and cancer

Michaela Skobe
Department of Oncological Sciences and Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Lymphatic vessels play multifaceted roles in cancer, inflammation, and immunity that are context dependent and need to be better defined. In cancer, lymphatics are best known for their role in facilitating cancer metastasis, but recently, they have emerged as a new player that contributes to shaping of the tumor immune microenvironment. Here, we investigated the role of lymphangiogenesis in oncolytic viral immunotherapy. Oncolytic viruses hold great promise for cancer immunotherapy because of their ability to directly kill tumor cells, promote immunogenic tumor cell death and induce inflammation in the tumor. We used an avian paramyxovirus, commonly known as Newcastle disease virus (NDV), to treat B16F10 melanomas in mice. To investigate how lymphangiogenesis influences the outcome of viral immunotherapy, tumor cells were transfected with Vascular Endothelial Growth Factor-C (VEGF-C), a key lymphangiogenesis factor, and NDV treatment was administered intratumorally. In presence of VEGF-C, NDV treatment led to eradication of tumors and mice remained tumor-free in 90% of the cases, for more than a year. When rechallenged with new tumor injections, mice were protected and did not develop new tumors, indicating induction of long-term immunological memory. To gain insight into the underlying mechanisms of tumor rejection, we performed high-dimensional immunophenotyping using Aurora Spectral flow cytometry, that allows simultaneous detection of ~ 30 fluorophores. Immunophenotyping of tumors treated with NDV, VEGF-C or both, revealed changes in T-cells and NK cells composition and activation unique for tumors expressing VEGF-C and treated with NDV. These data demonstrate that lymphangiogenesis stimulated by VEGF-C alters tumor immune cell composition and activation, what enhances anti-tumor effects of NDV oncolytic virus and leads to tumor eradication. Thus, VEGF-C holds potential for therapeutic use in combination with cancer immunotherapie

Current questions in prion science

Adriano Aguzzi *Institute of Neuropathology, University Hospital Zurich, Switzerland*

Transmissible spongiform encephalopathies (TSEs) are neurodegenerative diseases of humans and many animal species caused by prions. The main constituent of prions is PrP^{Sc}, an aggregated moiety of the host-derived membrane glycolipoprotein PrP^{C}. Prions were found to encipher many phenotypic, genetically stable TSE variants. The latter is very surprising, since PrP^{C} is encoded by the host genome and all prion strains share the same amino acid sequence. Here I will review what is known about the infectivity, the neurotoxicity, and the neuroinvasiveness of prions. Also, I will explain why I regard the prion strain question as a fascinating challenge – with implications that go well beyond prion science. Finally, I will report some recent results obtained in my laboratory, which is attempting to address the strain question and some other basic issues of prion biology with a “systems” approach that utilizes organic chemistry, photophysics, proteomics, and mouse transgenesis.

Viewing the Metabolic Syndrome from Kidney Stones

Orson Moe, University of Texas Southwestern Medical Center, Dallas, USA

Kidney stones (urolithiasis) is increasing in incidence and prevalence globally and in particular stones comprise of uric acid. The increase in uric acid stones parallels that of the epidemic in the metabolic syndrome, obesity, and type 2 diabetes mellitus. When an obese or diabetic patient gets a kidney stone, it is 3 times more likely to be a uric acid stone. Uric acid stones form not because of high urine uric acid but because of low urine pH h=which drives the formation of the sparingly soluble uric acid from the more soluble urate anion. Thus, the underlying pathophysiology of uric acid urolithiasis is aciduria and alkali therapy prevents formation of uric acid stones. The origin of the low urine pH is multifactorial that can be traced to the gut microbiome, hepatic dysfunction from steatosis, and impaired formation of urinary buffer due to renal steatosis and lipotoxicity. The renal mechanisms of impaired buffering of acid are complex and is partially due to impairment of the local renal adiponectin system. Alleviation of hepatic lipotoxicity and activation of the renal adiponectin system are possible therapeutic interventions in addition to alkali therapy. Uric acid stones can be considered an integral part of the metabolic syndrome.

On the hunt for precision drugs against the EGFR mutants involved in non-small cell lung cancer

Igor Stagljar
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Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada
Department of Biochemistry, University of Toronto, Toronto, ON, Canada
Mediterranean Institute for Life Sciences, Meštrovićevo Šetalište 45, Split, Croatia
School of Medicine, University of Split, Split, Croatia

Lung cancer, with a 5-year survival rate of 15%, is a major cause of cancer mortality. Roughly 85% of cases are non-small cell lung cancer (NSCLC), 15-50% of which involve mutant forms of Epidermal Growth Factor Receptor (EGFR). EGFR is thus an important therapeutic target, and a number of small-molecule and antibody therapies have been approved to date. Effectiveness has been limited, however, by off-target effects and the rapid development of drug resistant EGFR mutants during treatment. Additionally, there are a variety of EGFR mutants for which no effective targeted treatments are available. As such, development of new therapeutics is essential.

Our lab recently created a new live-cell drug screening assay called MaMTH-DS (1), based on our Mammalian Membrane Two-Hybrid (2-4). MaMTH-DS allows for sensitive, large-scale identification of small molecules targeting specific disease-associated interactions via a more diverse range of mechanisms than can be detected by traditional approaches. Using MaMTH-DS we identified a novel compound (called EMI1), that specifically inhibits an oncogenic triple mutant of EGFR (L858R-T790M-C797S) resistant to current therapeutics (1).

During my talk, I will report on our recent endeavours to identify derivatives of EMI1 via classical medicinal chemistry (5) and Artificial Intelligence (AI)-based approaches as well as how we characterized characterized compound mechanism of action and therapeutic potential against NSCLC.

We believe that the integration of our experimental technologies with advanced artificial intelligence, coupled with strong collaborative academic and pharmaceutical support, will allow us to rapidly develop improved, targeted treatments for patients suffering from serious EGFR-triple mutant- as well as other RTK-associated cancers.

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Petschnigg, J. et al. The mammalian-membrane two-hybrid assay (MaMTH) for probing membrane-protein interactions in human cells. Nature Methods **11**, 585–92 (2014).

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Strategies to overcome adaptive resistance to targeted cancer therapies

Poulikos I. Poulikakos, PhD Associate Professor Icahn School of Medicine at Mount Sinai, New York, NY USA

Targeting oncogenic signaling using small molecule inhibitors has shown clinical activity in cancer treatment, but it is frequently limited by adaptive drug resistance that prevents complete inhibition of signaling in the tumor by the drug. Recently, better mechanistic understanding of adaptive resistance and the development of inhibitors targeting components of the adaptive machinery (such as selective inhibitors of Receptor Tyrosine Kinases, SHP2, SOS, dimeric RAF and others) enable the design of more effective combinatorial therapeutic approaches. We will discuss insight and approaches based on elucidating and exploiting allosteric properties of small-molecule inhibitors for effective targeting of components of oncogenic signaling in their native conformations in cells. For most tumors, a "3-drug" combinatorial strategy will potentially be most effective: one drug targeting oncogenic signaling directly, such as a CDK4/6 or MEK inhibitor ("pathway" inhibitor), one drug targeting the mutated oncoprotein in the tumor, such as a BRAF(V600E) or RAS(G12C) inhibitor ("therapeutic index" inhibitor) and one drug targeting components of the feedback loop that is responsible for adaptive resistance, such as a SOS or SHP2 inhibitor ("feedback" inhibitor). This strategy is aimed at achieving potent oncogenic signaling inhibition in the tumor while retaining a high therapeutic index, and enables the development of a roadmap for the treatment of cancer using drug combinations tailored to the specific driver oncoprotein.

Sigma-1 receptor – a role in neuronal signaling and neurodegeneration

Ilya Bezprozvanny[1,2]

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The sigma 1 receptor (S1R) is a 223 amino acid-long transmembrane endoplasmic reticulum (ER) protein. Agonists of S1R demonstrated neuroprotective effects in variety of preclinical models and there are several on-going clinical trials of S1R agonists in neurodegenerative disorders. However, signaling functions of S1R are poorly understood. In our recent studies we tested the hypothesis that biological activity of S1R in cells can be explained by its ability to interact with cholesterol. By performing experiments in reduced reconstitution systems, we demonstrate direct effects of cholesterol on S1R clustering. We identify a novel cholesterol-binding motif in the transmembrane region of human S1R. Mutations of this motif impair association of recombinant S1R with cholesterol beads, affect S1R clustering in vitro and disrupt S1R subcellular localization. Further, we found that S1R agonists cause disruption of S1R clusters. Based on these results we propose that S1R-cholesterol interactions enable the formation of cholesterol-enriched microdomains in the ER membrane. We hypothesize that a number of secreted and signaling proteins are recruited and retained in these microdomains. This hypothesis is consistent with the results of an unbiased screen for S1R-interacting partners which we performed using the APEX technology. We further propose that S1R agonists enable the disassembly of these cholesterol-enriched microdomains and the release of accumulated proteins such as ion channels, signaling receptors, and trophic factors from the ER. We also propose that these cholesterol-enriched microdomains form the basis for formation of membrane contact sites between ER and other subcellular organells such as mitochondria and plasma membrane

Literature

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HER2Δ16 Integrates A Network of Immunomodulators to Promote an Immune Cold Microenvironment in Breast Cancer

Sherif Samer Attalla[1,2], Jonathan Boucher*[3], Hailey Dall-Proud[1,2] Dongmei Zuo[2], Virginie Sanguin-Gendreau[2], Tarek Taifour[2,4], Gabriella Johnson[1,2], Vasilios Papavasilou[2], Mark Barok[4], Heikki Joensuu[4], Philippe Roux[3] and William J. Muller[1,2,3]*

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Key words: breast cancer, mouse models, HER2/Neu, immunology, immune microenvironment
**Contributed equally to this work*
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Despite the importance of the tumor immune microenvironment in the therapeutic response to immunotherapies targeting either the driving oncogene or immune checkpoints, the underlying mechanisms governing sensitivity to these agents remain to be fully explored. HER2 and its oncogenic splice variant HER2D16 have previously been implicated in breast cancers and other tumor types as important oncogenic drivers that can modulate the tumor microenvironment. Using a duplex RNA in situ hybridization approach we show that expression of HER2Δ16 is associated with worse patient outcome independent of therapeutic regimen. To further explore the molecular basis for the ability of HER2 variants to modulate the tumor microenvironment, we generated transgenic mouse models expressing either HER2 or HER2D16 in the mammary epithelium in a doxycycline-inducible fashion. Although mammary epithelial expression of HER2 or HER2D16 induced mammary tumors with similar onset, analyses of the microenvironment in these tumors revealed profound differences in their immune, vascular and mammary epithelial components, with HER2D16 tumors exhibiting an invasive pathology with low immune infiltration. Consistent with the “immune-cold” phenotype observed in HER2D16-derived tumors, transcriptomic analysis revealed suppression of Type I and II interferon signaling relative to HER2-derived tumours. Furthermore, we identified an array of cytokines preferentially inhibited in HER2Δ16 tumors and noted upregulation of CHI3L1, a prominent immunosuppressive cytokine. Moreover, using cell surface proteomic approach we identified ENPP1 as a functional regulator of the cytokine expression and immune exclusion. Taken together, these observations argue that the HER2D16 splice variant suppresses inflammatory cytokines and upregulates immunosuppressive cytokines to generate an immune-cold tumor microenvironment that supports tumor progression.

Cellular Plasticity in Cancer

Michael Jewer, Laura Lee, Mackenzie Coatham, Gilles Lajoie, Cheng-Han Lee, Ola Larsson, Ivan Topisirovic and Lynne-Marie Postovit

Cellular plasticity, concomitant with the ability of a cancer cell to alter its phenotype, is integral to therapy resistance and metastatic spread. Although plasticity is increasingly recognized as a hallmark of aggressive tumors, molecular underpinnings governing the acquisition and maintenance of a plastic state are poorly understood. We have determined that stresses such as hypoxia and therapy induce plasticity in cancer cells via the coordinated reprogramming of the epigenome and the translational machinery. Moreover, the loss of key epigenomic regulators, such as SMARCA4, seem to also facilitate the acquisition of a dedifferentiated plastic phenotype. In this lecture, we will review studies showing how the integrated stress response can promote breast cancer plasticity by enabling the selective translation of reprogramming factors, such as NODAL; and will discuss the mechanisms by which SMARCA4 loss induces dedifferentiation in endometrial cancers. These studies illuminate putative targets for the prevention of cellular plasticity in cancer.

Oncogene-mediated signaling in transgenic mouse models of human breast cancer

(HER2∆16 Integrates A Network of Immunomodulators to Promote an Immune Cold Microenvironment in Breast Cancer)

Sherif Samer Attalla[1,2], Jonathan Boucher*[3], Hailey Dall-Proud[1,2] Dongmei Zuo[2], Virginie Sanguin-Gendreau[2], Tarek Taifour[2,4], Gabriella Johnson[1,2], Vasilios Papavasilou[2], Mark Barok[4], Heikki Joensuu[4], Philippe Roux[3] and William J. Muller[1,2,3]*

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[5]*Helsinki University Hospital and University of Helsinki, Helsinki FIN-00290, Finland*
Key words: breast cancer, mouse models, HER2/Neu, immunology, immune microenvironment
**Contributed equally to this work*
Corresponding author: William Muller, william.muller@mcgill.ca

Despite the importance of the tumor immune microenvironment in the therapeutic response to immunotherapies targeting either the driving oncogene or immune checkpoints, the underlying mechanisms governing sensitivity to these agents remain to be fully explored. HER2 and its oncogenic splice variant HER2D16 have previously been implicated in breast cancers and other tumor types as important oncogenic drivers that can modulate the tumor microenvironment. Using a duplex RNA in situ hybridization approach we show that expression of HER2∆16 is associated with worse patient outcome independent of therapeutic regimen. To further explore the molecular basis for the ability of HER2 variants to modulate the tumor microenvironment, we generated transgenic mouse models expressing either HER2 or HER2D16 in the mammary epithelium in a doxycycline-inducible fashion. Although mammary epithelial expression of HER2 or HER2D16 induced mammary tumors with similar onset, analyses of the microenvironment in these tumors revealed profound differences in their immune, vascular and mammary epithelial components, with HER2D16 tumors exhibiting an invasive pathology with low immune infiltration. Consistent with the “immune-cold” phenotype observed in HER2D16-derived tumors, transcriptomic analysis revealed suppression of Type I and II interferon signaling relative to HER2-derived tumours. Furthermore, we identified an array of cytokines preferentially inhibited in HER2∆16 tumors and noted upregulation of CHI3L1, a prominent immunosuppressive cytokine. Moreover, using cell surface proteomic approach we identified ENPP1 as a functional regulator of the cytokine expression and immune exclusion. Taken together, these observations argue that the HER2D16 splice variant suppresses inflammatory cytokines and upregulates immunosuppressive cytokines to generate an immune-cold tumor microenvironment that supports tumor progression.

Game Changer Cell-Free DNA

Bernhard Zimmermann
R&D, Natera Inc. San Carlos, CA, USA

With Cell-free DNA circulating in the blood a new player has entered the arena in diagnostics.
I will report on how clinical testing is changing medical practice (the game) of the management of multiple disease areas: in Prenatal Care (NIPT, non-invasive prenatal testing), in Transplant Organ Health monitoring and with a main focus on Oncology. The latter focusing on Minimal Residual Disease detection, Treatment Monitoring and how ctDNA testing will impact Clinical Trial Design.

Association with proteasome determines pathogenic threshold of polyglutamine expansion diseases

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Expansion of glutamine residue track (polyQ) within soluble protein is responsible for eight autosomal-dominant genetic neurodegenerative disorders. These disorders affect cerebellum, striatum, basal ganglia and other brain regions. Each disease develops when polyQ expansion exceeds a pathogenic threshold (Qth). A pathogenic threshold is unique for each disease but the reasons for variability in Qth within this family of proteins are poorly understood. In the previous publication we proposed that polarity of the regions flanking polyQ track in each protein plays a key role in defining Qth value. To explain the correlation between the polarity of the flanking sequences and Qth we performed quantitative analysis of interactions between polyQ-expanded proteins and proteasome. Based on structural and theoretical modeling, we predict that Qth value is determined by the energy of polar interaction of the flanking regions with the polyQ and proteasome. More polar flanking regions facilitate unfolding of α-helical polyQ conformation adopted inside the proteasome and as a result, increase Qth. Predictions of our model are consistent with Qth values observed in clinic for each of the eight polyQ-expansion disorders. Our results suggest that the agents that can destabilize polyQ α-helical structure may have a beneficial therapeutic effect for treatment of polyQ-expansion disorders.

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Metabolic choice in an unfolding world

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Protein homeostasis (proteostasis) encompasses the equilibrium between synthesis, conformational maintenance, and degradation of damaged proteins. While protein synthesis has a critical role in making the proteome and promoting cell growth, failure to eliminate misfolded proteins can lead to inactivation of functional proteins as well as cell degeneration and death.

Cells undergoing stress exhibit changes at different levels of their functioning, including transcriptional regulation, new protein synthesis, metabolic reprogramming, and even dramatic changes in subcellular organization. Sequestering misfolded proteins into insoluble aggregates is a part of the cellular attempts to remain functional even in the conditions of proteotoxic stress. Their formation is proposed to be beneficial during heat shock for multiple reasons, the dominant one being the removal of toxic protein conformers from the soluble phase. In budding yeast *Saccharomyces cerevisiae*, three major sequestration sites for misfolded proteins exist: IPOD (insoluble protein deposit), INQ (intranuclear quality control compartment), and cytosolic Q-bodies. Moreover, stress granules (SGs) typically form during various stress conditions through interactions of proteins and RNA by phase separation in the cytosol.

Despite the indisputable importance of cellular proteostasis on multiple levels, the relationships between proteostasis and other cellular pathways remain poorly understood. In this context, our project seeks the understanding of the interplay between RNA and protein homeostasis and the cellular metabolic activity, and the role it plays in cellular longevity.

We have found that metabolic stress is a trigger of protein sequestration into distinct compartments. We put forward compartment dissolution as the critical process that determines the steady-state aggregate size, rather than their assembly by protein deposition. Depositing proteins into aggregates and their timely dissolution during recovery from the stress provide a significant fitness advantage during glucose deprivation, which offers new perspectives on the role of protein sequestration into isolated deposits in cell adaptation to stress. Our findings also reveal that SGs undergo a fission event, essential for their complete complete clearance. Moreover, we describe a prominent new role of Hsp42 in SG dynamics: the chaperone is required for SG fusion, fission and colocalization with its markers and the disaggregation machinery.

Understanding the interplay between protein disaggregation and metabolic activity of the cell is of universal importance due to the association of protein aggregation with aging and many medical conditions.

Antibody-Conjugates as Protein Switches for Therapeutics and Diagnostics

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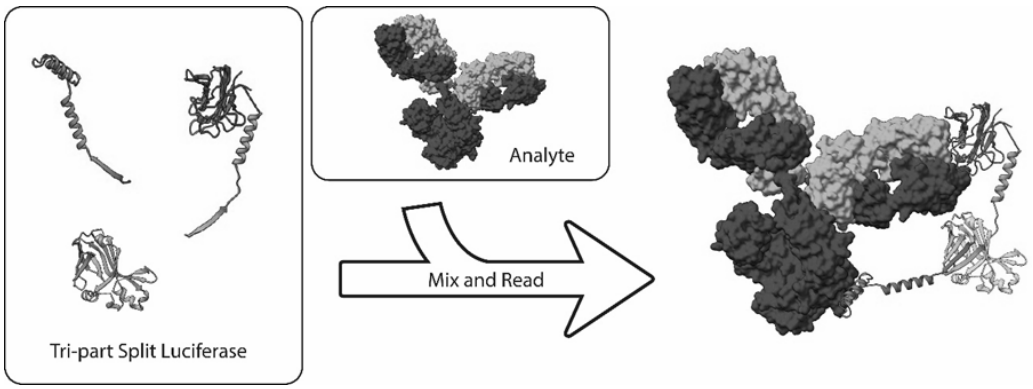
Antibodies are important biological scaffolds used in biotherapeutics and diagnostics. The utility of antibodies can be expanded by coupling them with small-molecule drugs or proteins. We are utilizing protein engineering of split-enzymes to construct a new class of antibody-mediated therapeutics and diagnostics. In our approach, an enzyme is split into inactive components and fragments of the split-enzyme are fused to individual antibodies or binding proteins. The binding of the antibodies places the split-enzyme fragments in proximity where they reform the active enzyme. For diagnostics, we engineered the luciferase NanoLuc® into three different fragments and fused two of these to binders.[a] Because luciferase activity is dependent on binding to the antigen, these split-luciferase platforms are 'wash-free' homogenous immunoassays which are as sensitive and more rapid than current technologies, without cumbersome processing steps. We have used our approach for several different applications, including quantifying the cancer biomarker HER2 and the HER2-EGFR protein-protein interaction to improve cancer treatment[a], assays to monitor therapeutic drug levels of Humira® (adalimumab) and Remicade® (infliximab)[b], and rapid diagnostic to assess COVID-19 antibody levels in patients.[c]



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Light-mediated discovery of surfaceome nanoscale organization and intercellular receptor interaction networks

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Delineating the molecular nanoscale organization of the surfaceome is pre-requisite for understanding cellular signaling. Technologies for mapping the spatial relationships of cell surface receptors and their extracellular signaling synapses would open up diagnostic, and therapeutic opportunities with the possibility to engineer extracellular signaling. Here, we develop an optoproteomic technology termed LUX-MS that exploits singlet oxygen generators (SOG) for the light-triggered identification of acute protein interactions on living cells. Using SOG-coupled antibodies, small molecule-drugs, biologics and intact viral particles, we show that not only ligand-receptor interactions can be decoded across organisms, but also the surfaceome receptor nanoscale organization ligands engage in with direct implications for drug action. Furthermore, investigation of functional immunosynapses reveals that intercellular signaling between antigen-presenting cells and CD8+ T cells can be mapped providing molecular insights into T cell activation with spatiotemporal resolution. LUX-MS based decoding of surfaceome signaling architectures thereby provides a molecular framework for the rational development of theranostic strategies.

A non-coding single nucleotide polymorphism at 8q24 drives IDH1-mutant glioma formation

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Establishing causal links between genetic polymorphisms and increased heritable risk of developing cancer is a major challenge and directly relates to the 2018 National Cancer Institute Provocative Question PQ3: Do genetic interactions between germline variations and somatic mutations contribute to differences in tumor evolution?

To answer this PQ, we focused on the single nucleotide polymorphism rs55705857 (A>G), which is associated with a ~6-fold increased risk to develop *IDH*-mutant low-grade glioma (LGG). This makes the non-coding rs55705857 one of the highest genetic associations with cancer, comparable with inherited *BRCA1* gene mutations and the risk of developing breast cancer or other familial glioma genes such as NF1/2, CDKN2A or p53. Like most cancer-related risk SNPs identified by genome-wide association studies (GWAS), rs55705857 is located in non-coding regulatory regions on 8q24. These GWAS tag-SNPs are usually themselves not the causal variant but are in linkage disequilibrium with one or more causative variants, which generally remain unknown. How such non-coding germline variants interact with acquired somatic mutations to facilitate cancer development is even less well understood.

By fine-mapping the locus, we reveal that rs55705857 itself is the causal variant and is associated with molecular pathways that drive human LGG. To functionally test rs55705857, we generated the first IDH1^{R132H}-driven LGG mouse model faithfully recapitulating all truncal LGG mutations using direct *in vivo* somatic CRISPR/Cas9 gene editing. Of note, mutating the highly conserved, orthologous mouse rs55705857 locus in addition to the truncal LGG mutations dramatically accelerated tumor development from 463 to 172 days and increased penetrance from 30% to 75%, demonstrating the SNP's oncogenicity

Mechanistically, we show that rs55705857 resides within a brain-specific enhancer, which is hyperactivated upon mutant IDH1-epigenome re-programming. In addition, we found that the risk allele disrupts OCT2/4 binding, resulting in increased interaction with the Myc promoter and increased Myc expression. The hyperactive chromatin status combined with the tissue specificity of this enhancer explains the cooperativity between mutant IDH and rs55705857 and why rs55705857 is associated specifically with *IDH*-mutant glioma, but not any other cancers. Overall, our work generates new LGG models and reveals mechanisms of the heritable predisposition to lethal glioma in ~40% of LGG-patients that carry the rs55705857 allele.

Mitochondrial Division and Cancer: Causes, Consequences, and Coincidence

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Mitochondrial division is essential for mitosis and metazoan development, and the impact of mitochondrial division in cancer has recently become apparent. Here, we examine the direct effects of oncogenic MAPK mediated cellular transformation on the mitochondrial dynamics machinery and observe a positive selection for dynamin related protein 1 (DRP1), a protein required for mitochondrial network division. Loss of DRP1 prevents oncogene-induced mitochondrial dysfunction, and renders cells resistant to transformation. Conversely, in human tumor cell lines with activating MAPK mutations, inhibition of these signals leads to robust mitochondrial network reprogramming initiated by DRP1 loss resulting in mitochondrial hyper-fusion and increased mitochondrial metabolism. These phenotypes are mechanistically linked by ERK1/2 phosphorylation of DRP1 serine 616; $DRP1^{S616}$ phosphorylation is sufficient to phenocopy transformation-induced mitochondrial dysfunction, and $DRP1^{S616}$ phosphorylation status dichotomizes $BRAF^{Wt}$ from $BRAF^{V600E}$ positive lesions and informs which patients should be monitored more frequently for melanomagenesis. At present, we are investigating the implications of chronic mitochondrial division in oncogene-induced senescence, the mitochondrial unfolded protein response, and the immunobiology of melanoma *in situ*.

Detection and characterization of carbonylated proteins

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We are investigating the impact of oxidative disbalance in the context of the mechanisms of aging and age-related disorders. Studying oxidative damages of proteins, the most abundant and the effectors macromolecules of all cell functions, is essential to understand the cellular responses to oxidative stress. The protein carbonyls groups are markers of oxidative protein damage, and they are present in low concentrations but can increase in vivo from 2 to 8 times. The carbonyl groups can react with hydrazide or aminooxy probes to create hydrazone or oxime bonds, respectively. The methods using 2,4-dinitrophenylhydrazine were introduced by Earl R. Stadtman and Rodney L. Levine in the early ’90s but were not improved since.

Therefore, we undertook to improve:

- detection of carbonylated proteins by western blotting,
- detection and quantification of carbonylated proteins by two-dimensional differential gel electrophoresis of oxidized proteins (2-D OxiDIGE), and

to enable identification of carbonylated amino acids in the specific protein by mass spectrometry.

By optimizing the reaction of the aminooxy-biotin and protein carbonyl groups, followed by their detection with the streptavidin fluorescent probes, we developed a new blotting method for detecting carbonylated proteins transferred to the membrane and named it the oxime-blot method. This method represents a significant improvement in detecting carbonylated proteins in complex biological matrices (e.g., whole-cell lysates, plasma proteins, and immunoprecipitated proteins). It allows quantification of the protein carbonylation and its expression simultaneously.

The 2D-OxiDIGE is a method derived from 2D Difference Gel Electrophoresis and allows the detection and quantification of carbonylated proteins.

The LC-MS method allows the detection and the validation of protein carbonyls groups using a reporter ion produced by the fragmentation of an innovative aminooxy-probe.

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Stjepan Orhanović - Faculty of Science Split
Gordan Džamonja, Ana Marija Babić, Matea Jelavić, Irena Drmić Hofman - University Hospital of Split

Bugs, drugs and cell systems

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Antibiotic drug resistance has reached crisis proportions, owing to a dearth of new antibiotics. In fact, the last antibiotic of new chemical class and mechanism – approved for human use – was discovered more than 30 years ago. This is despite a renaissance in antibiotic discovery in recent decades that has used modern target-based drug discovery methods. In the Brown Laboratory, we are increasingly turning to cell-based chemical and systems biology approaches to overcome the shortcomings of reductionist target-focused paradigms that have dominated and failed. In this lecture, I will describe our efforts to chart chemical and genetic interactions in bacteria on a genome-scale in order to better understand the complexity of bacterial survival strategies and to reveal fresh approaches for antibiotic drug discovery.

TBA

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Regulation of signaling in inflammatory diseases by RIP kinases

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Proper maintenance of organismal and cellular homeostasis requires careful regulation of signaling pathways. Modulation of signaling can be achieved on many levels including posttranslational protein modifications, which can influence protein's stability, subcellular localization, biological activity and many other functional and structural properties. The most common and best studied modifications are phosphorylation and ubiquitination of proteins on distinct amino acid residues. Given the need for antibodies that can detect posttranslational modification of a substrate with a particular ubiquitin chains, we developed a new class of bispecific antibodies which allow simultaneous recognition of RIP1 or RIP2 and select ubiquitin linkages. Bispecific RIP1-K63 or RIP1-linear (Lin) linkage ubiquitin chain antibodies can reveal specific RIP1 ubiquitination and show cellular localization of ubiquitinated RIP1. Similarly, RIP2-K63 and RIP2-Lin bispecific antibodies can recognize selective RIP2 ubiquitination with K63 or linear linkages. Furthermore, using RIP2-K63 and RIP2-Lin bispecific antibodies we examined IBD patient samples and found prominent K63-linked and linear RIP2 ubiquitination in ulcerative colitis and Crohn's disease patient samples. In addition, we generated a K63-Lin bispecific antibody for simultaneous recognition of K63-linked and linear ubiquitination in diverse signaling pathways. In summary, these bispecific antibodies represent a major conceptual advancement for future development of inflammatory biomarkers.

Translational and metabolic perturbations in neoplasia

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Alterations in mRNA translation and perturbations in energy metabolism play a pivotal role in homeostatic stress responses. Moreover, dysregulation of these processes is thought to be a prominent factor in tumorigenesis and tumor progression across a broad spectrum of cancers. Notwithstanding that mRNA translation represents one of the most energy consuming processes, the mechanisms that orchestrate translational and metabolic programs in mammalian cells remain incompletely understood. Our work suggests that one such mechanisms that is engaged in mammalian cells may be centered on the mechanistic target of rapamycin complex 1 (mTORC1). To this end, we demonstrated that mTORC1 coordinates mitochondrial ATP production and energy consumption by the translational machinery.

Furthermore, malignant cells must overcome energy stress caused by frequent limitations in nutrient and oxygen supply in their microenvironment while providing sufficient energy and building blocks for processes that drive neoplastic growth including mRNA translation. Emerging evidence suggests that instead of being “addicted” to a limited number of metabolic pathways, cancer cells exhibit metabolic plasticity whereby they possess the ability to rapidly engage alternative metabolic pathways to adapt to constant fluctuations in nutrient and oxygen supply. It appears that metabolic plasticity of cancer cells also plays major roles in overcoming therapeutic insults and facilitating metastatic spread of the disease. We have recently provided evidence that alterations in mRNA translation mediated by the mTORC1/eukaryotic translation initiation factor 4E (eIF4E)-binding protein (4E-BP)/eIF4F axis may act as a major factor underlying metabolic plasticity of a variety of cancer types (e.g., breast and renal cancers and melanoma). These mTORC1/4E-BP/eIF4E-directed translational programs are seemingly coordinated with hypoxia-inducible factor 1 (HIF1)-dependent transcriptional programs. Importantly, when engaged, these mechanisms dramatically reduce efficacy of clinically used inhibitors of oncogenic kinases.

Altogether, these findings highlight cellular networks that may govern orchestration of translational and bioenergetic programs in homeostatic and pathological states in the variety of mammalian cells and systems. These results will be discussed in conjunction with our more recent efforts that are focused on the elucidation of molecular underpinnings of metabolic plasticity and their role(s) in the adaptation to stress, tumor initiation and progression.

Mechanisms of neuroinflammation and brain pathology in congenital cytomegalovirus infection

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Congenital cytomegalovirus (cCMV) infection is a leading viral cause of mental retardation and sensorineural hearing loss in infants and children. Since human CMV is strictly species-specific, mechanistic studies of CMV infection rely heavily on animal models. We have previously shown that infection of newborn mice with mouse CMV recapitulates major hallmarks of human cCMV infection: virus dissemination to the brain parenchyma, neuroinflammation, altered brain development and sensorineural hearing loss. Early innate immune mediators, including NK/ILC1 cells, IFNγ and TNFα, drive neurodevelopmental pathology, while adaptive T cells establish virus control and clearance of infectious virus[1]. Since latent CMV can reactivate at any time, the proinflammatory environment in the brain is basically maintained for life. While previous studies have provided several important insights into the pathogenesis of cCMV infection, the mechanisms and determinants that govern brain inflammation and pathology are largely unknown, limiting potential intervention strategies against sequelae of cCMV infection. On the basis of our preliminary results, we postulate that microglia play a central role in the pathogenesis of cCMV infection, including virus control, regulation of inflammation and recruitment of peripheral immune cells.

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Protein carbonylation and cellular parabiosis in aging and age-related diseases

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Living organisms accumulate oxidative damage as they age. Protein carbonylation, a form of oxidative damage to proteins, is a biomarker of aging with a possible role in inducing age-related phenotypes and diseases. We study an intrinsic susceptibility of proteins to carbonylation with the aim to identify protein features responsible for the relative resistance of some proteins to carbonylation and cellular functions that evolved to be intrinsically resistant to carbonylation. Moreover, we aim to decipher the molecular mechanisms through which carbonylated protein aggregates lead to age-related cell death. On the tissue level, cells acquire various kinds of damage in a stochastic manner, yet their phenotypes and consequently the diseases might appear only after years or decades of latency. We study the non-cell autonomous mechanisms that regulate this latency in the mixed populations of healthy and aberrant cells.

Exploring the epigenomic context of mutational processes

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One of the main challenges in cancer biology is the identification of the cell-of-origin (COO) in cancer. We previously demonstrated that the landscape of somatic mutations in cancer is associated with the chromatin marks of the cell-of-origin and developed a novel approach, based on machine learning methods, that can successfully identify COO by utilizing different epigenetic features of the cell-of-origin and somatic mutations found in the cancer genome (Polak *et al.*, 2015). We further extended our analysis and showed that this principle is generalizable to common cancer types and subtypes (Kübler *et al.*, 2019). The prediction accuracy of models used to predict the COO varies across cancer types, potentially caused by the fact that somatic mutations in human cancers can be generated by various underlying mutational processes. Different mutational processes give rise to characteristic mutational patterns, called mutational signatures, which can be identified using various mathematical approaches. It was recently shown that mutations generated by distinct mutational processes are differentially enriched in active/inactive chromatin domains (Akdemir *et al.*, 2020), implying that they are differentially related to the epigenomic context of the cell-of-origin and that the inclusion of mutational signature information might positively impact the accuracy of predicting the COO.
We tested this hypothesis by exploring the impact of genomic regions with high APOBEC mutational signature on the outcome of the models for predicting the cell-of-origin in different subtypes of breast cancer, frequently enriched in APOBEC-mediated somatic mutations. We were able to show that the inclusion/exclusion of APOBEC mutations does influence the prediction accuracy of our models, highlighting the crucial role of the specific cell type of origin and mutational process in shaping the mutational landscape and early tumor evolution.

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Structural basis of SARS-CoV-2 translational shutdown and programmed ribosomal frameshifting

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We are investigating bacterial and eukaryotic ribosomes and their functional complexes to obtain insights into the process of protein synthesis. Building on our studies aimed at revealing the structures of eukaryotic cytosolic and mitochondrial ribosomes (Kummer et al. 2018), we are now investigating eukaryotic translation initiation, targeting of proteins to membranes, regulation of protein synthesis, and how viruses reprogram host translation. Previously, we studied how Hepatitis C virus genomic RNA can bind mammalian ribosomes to achieve translation of viral mRNAs in the absence of some canonical cellular translation initiation factors (Quade et al. 2015). With our recent research activities we contributed to the understanding of how SARS-CoV-2, the virus that is responsible for the COVID-19 pandemic, shuts off host translation to prevent cellular defence mechanisms against the virus (Schubert et al. 2020). Furthermore, using a combination of cryo-electron microsocpy and biochemical assays we also investigated the mechanism of programmed ribosomal frameshifting, one of the key events during translation of the SARS-CoV-2 RNA genome that leads to synthesis of the viral RNA-dependent RNA polymerase and downstream viral proteins (Bhat et al. 2020).

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Posters

Unravelling the mechanisms of aging: aggregates formed by oxidatively damaged, carbonylated proteins lead to age-related cellular dysfunction

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Aging is a physiological process characterized by a time-dependent deterioration of cellular functions and as such it represents a major risk factor for age-related pathological conditions including neurodegeneration, cancer, and cardiovascular diseases. Impaired proteostasis, and in particular oxidative damage to proteins and accumulation of aggregates are common occurrences in age-related pathological conditions. Moreover, oligomers and aggregates formed by the specific proteins such as a-synuclein [1], Ab [2], and SOD1 [3] have been associated with neurodegenerative diseases - Parkinson's and Alzheimer's diseases, as well as the Amyotrophic lateral sclerosis (ALS), respectively. These oligomers can expose their hydrophobic residues which then interact with lipids and disrupt or damage the cellular membranes, possibly by forming pores. Interestingly, a recent study shows that widespread protein aggregation contributes to aging process in *C. elegans* tissues [4], suggesting a more general role of protein aggregation in aging. However, the exact mechanism of age-related protein aggregate toxicity is still unclear.

The aim of this project is investigation of the mechanism through which aggregates formed by oxidatively damaged, carbonylated proteins lead to age-related cellular dysfunction. In particular, we study the interactions of the oligomers and aggregates formed by carbonylated proteins with the cellular membranes, which may lead to the membrane permeabilization and consequently to the loss of cell integrity. To address this issue, we characterize oligomers and aggregates of carbonylated proteins and examine their cellular distribution. Membrane damage is assessed upon induction of protein carbonylation in the living cells and upon exposure of the naïve cells to aggregates that were pre-formed in the cells exposed to oxidative stress.

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Functionalized Au15 nanoclusters as luminescent probes for protein carbonylation detection

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Protein carbonylation is a form of oxidative damage to proteins and as such represents a biomarker of aging. Several methods using fluorescent dyes coupled with carbonyl-specific hydrazide or aminooxy moiety are currently used to measure the level of protein carbonyls *in vitro*. Nevertheless, these dyes display limited biocompatibility, solubility or photostability. Hence, we sought to develop a novel method for protein carbonyl detection with a better potential for *in vivo* application. To that end we took advantage of the ligand-protected gold nanoclusters (AuNCs) which have drawn considerable attention as contrast agents for bioimaging and biosensing applications in the past decade. In this proof-of-principle study we establish a strategy to tailor AuNCs for the precise detection of protein carbonylation. We produce $Au_{15}SG_{13}$ (SG for glutathione) with atomic precision and functionalize it with a thiolated aminooxy moiety to impart protein carbonyl-binding properties. Mass spectrometry and molecular modelling reveal the key structural features of $Au_{15}SG_{12}$-Aminooxy and its reactivity towards carbonyls. Finally, we demonstrate that $Au_{15}SG_{12}$-Aminooxy detects protein carbonylation in gel-based 1D electrophoresis by one- and two-photon excited fluorescence. Importantly, to our knowledge, this is the first application of an AuNC that detects a post-translational modification as a nonlinear optical probe. The significance of protein carbonylation in the aging field and more broadly of post-translational modifications in life sciences may open avenues for the use of $Au_{15}SG_{13}$ and other nanoclusters as contrast agents with tailored surface functionalization and optical properties.

Combes, G.F., Fakhouri, H., Moulin, C. et al. Functionalized Au15 nanoclusters as luminescent probes for protein carbonylation detection. Commun Chem 4, 69 (2021). https://doi.org/10.1038/s42004-021-00497-z

Targeted metabolomics approach for investigation of the metabolome in dogs infected with *Babesia canis*

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Canine babesiosis is an important worldwide tick-borne disease caused by the intra-erythrocyte protozoa of different *Babesia* species [1]. Although the disease process primarily affects erythrocytes, it may also have multisystemic consequences [2]. The emergence of innovative -omics technology, such as metabolomics, has led to the development of strategies aimed at identifying specific and sensitive metabolites among thousands of small molecules present in biological fluids and tissues [3]. Metabolomics methodologies have been divided into two distinct groups: untargeted metabolomics and targeted metabolomics [4]. Targeted metabolomics identifies and quantifies the abundance of defined groups of known, chemically characterized and biochemically annotated metabolites. Blood and urine contains a multitude of unstudied and unknown biomarkers that may reflect physiological and pathological states of tissues and organs. The goal of the study was to obtain serum metabolom of dogs naturally infected with *Babesia canis* and healthy dogs using targeted metabolomics approach on the liquid chromatography coupled to mass spectrometry (LC-MS) platform. Metabolites were analyzed using the Absolute IDQ p400 kit (Biocrates Life Science AG, Innsbruck, Austria), for the targeted metabolomic analysis of up to 408 metabolites divided into 11 metabolite classes. According to the manufacturer instructions provided with the kit, metabolites were extracted from dog serum on the specific 96-well plate system for protein-removal, internal standard normalization and derivatization. Analyses were made according to the manufacturer's protocol using the Biocrates Met*I*DQ software (Biocrates Life Science AG, Innsbruck, Austria) for data processing, quality assessment, data export and mapping of measurements with chemical and biochemical background information. Targeted metabolomics analysis of serum samples was performed for 24 dogs through combined LC-MS/MS and FIA-MS/MS analysis. Univariate analysis resulted in detection of 68 significant metabolites in healthy dogs versus dogs with babesiosis. The most significant metabolites are serotonin, methionine sulfoxide, citrulline, proline and others. Biological functions of differently abundant metabolites indicate the involvement of various pathways in canine babesiosis including glutathione metabolism, alanine, aspartate and glutamate metabolism, glyoxylate and dicarboxylate metabolism and others pathways. In conclusion, the research of serum samples of dogs infected with *B. canis* demonstrated potential metabolites and pathways significantly changed in dogs with canine babesiosis.The targeted LC-MS metabolomic's approach profiled the metabolic change in serum of dogs infected with *B.canis.*

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Cellular parabiosis – the role of cell-to-cell communication in phenotypic suppression

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The current research efforts to delay aging and age-related diseases mostly target cell autonomous mechanisms such as oxidative stress and DNA damage. In the recent years, non-cell autonomous mechanisms regulating health and longevity are emerging as promising alternatives.[1] This inspired us to develop a novel concept of cellular parabiosis – the phenotypic suppression of aberrant phenotypes in individual cells by the adjacent healthy cells through the molecular traffic[2].
In this study we investigated the effects of the healthy cells on the phenotypes of cells irradiated with UVC/UVB which mimic the age-related oxidative damage. Irradiated fluorescently labelled cells were seeded on a feeder layer composed of either intact cells or equally irradiated cells and phenotypes of the labelled cells, such as the cell number and nuclear morphology, were monitored over time. Our data show that the survival of the irradiated cells was diminished when they were growing on an intact feeder layer as compared to the irradiated feeder layer. We conclude that the healthy cells may promote elimination of damaged cells from the cellular populations. This corresponds to a phenomenon of "assisted suicide" previously described for DNA damage-induced germ cell apoptosis in *Caenorhabditis elegans*[3]. Such mechanism could protect cells from the toxic effects of their dying neighbours, ultimately preserving the fitness of the tissue.

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Serum proteome profiling in canine babesiosis using a label-basedquantitative proteomics approach

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Introduction: Babesiosis is a disease of significant veterinary importance and emerging vector-borne zoonosis with worldwide distribution. It is caused by the intra-erythrocyte protozoal parasites, with *Babesia rossi* being the most severe of all the large Babesia parasites infecting dogs. The disease can be clinically classified into uncomplicated and complicated forms with a wide range of clinical presentations from a mild, subclinical illness to complicated forms and death. The aim of this study was to assess serum proteomic profile from dogs with babesiosis and healthy dogs using label-based proteomics approach.
Materials and methods: In this study 32 dogs naturally infected with *B. rossi* and 20 healthy dogs were included. Diseased dogs were subdivided into 18 uncomplicated cases and 14 complicated cases of babesiosis. Serum samples were processed using Tandem Mass Tag (TMT) label-based quantitative approach. High resolution LC-MS/MS analysis was carried out using nanoLC system coupled to Q Exactive Plus mass spectrometer. Protein identification and quantification were performed using Proteome Discoverer. Statistical and bioinformatic analysis were done using R and Cytoscape.
Results: Altogether, 311 quantifiable proteins were identified (2 unique peptides, 5% FDR). There were 75 master serum proteins with statistically different abundance between the dogs with babesiosis and healthy dogs, including retinol binding protein 4, alpha-1-acid glycoprotein 1-like, vitamin D binding protein, apolipoprotein E, transferrin receptor, paraoxonase and others.**Conclusion:** Shotgun TMT-based high-resolution proteomic profiling allowed identification of potential serum biomarkers for differentiation of disease severity in canine babesiosis caused by *B. rossi*. These findings may be applicable to the understanding and study of haemoprotozoal diseases.

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