PhD ZOOMinars !!! 30 JUNE 2020

9:00 Main Zoom Hall

- *Video tour: Our Amazing PhDs!*
- Opening Remarks
- *Video tour: What's in Sessions 1 and 2?*

Session 1 Chair: Yael Segev

9:20 **1 May Meltzer**: Directed Evolution to Engineer GPCRs With Improved Structural Stability *(Stas Engel & Niv Papo)*

9:45 **2 Ayelet Shagal**: Antibody Immune History Profiles of Influenza in Vaccinated and Unvaccinated Individuals *(Tomer Hertz)*

Session 2 Chair: Tomer Cooks

3 Hadar Klapper Goldstein: An implantable system for long-term assessment of atrial fibrillation substrate in freely moving rats exposed to underlying pathological conditions *(Yoram Etzion)*

4 Ohad Wormser: Deciphering the molecular basis of hereditary neuro-ophthalmological disorders, focusing on SCAPER *(Ohad Birk)*

10:15 Main Zoom Hall

- *Video tour: Our Amazing Research Environment!*
- *Live! Session chairs discuss the freshly completed sessions*
- *Researchers discuss: How to overcome your fear of writing a paper!*
- *Video tour: What's in Sessions 3, 4, 5?*

Session 3 Chair: Roi Gazit

10:35 **5 Lee Admoni-Elisha**: The role of SETD6-mediated methylation of TWIST1 in Glioblastoma multiforme *(Dan Levy)*

11:00 **6 Arik Shvartsman**: Mechanisms of persistent Na+ current generation in soma and processes of Layer 5 pyramidal neurons *(Ilya Fleidervish)*

Session 4 Chair: Tomer Hertz

7 Aviram Trachtenberg: Mechanistic and therapeutic assessment of synergistically acting combinations of phenolic compounds in acute myeloid leukemia models *(Michael Danilenko)*

8 Inbar Bandach: Excess interleukin 1 (IL1) worsens and IL1 inhibition improves renal damage and anemia in a mouse model of chronic kidney disease *(Yael Segev)*

Session 5 Chair: Shira Knafo

9 Yuval Yogev: The genetic and molecular basis of monogenic neuromuscular and neurological diseases, with focus on Autism *(Ohad Birk)*

10 Hananel Elul: Protection of the mouse testis tissue and sperm production from X-ray induced damages by pharmaceutical compounds that increase telomerase *(Esther Priel)*

11:30 Main Zoom Hall

- *Video tour: So Much Food and Alcohol!*
- *Live! Session chairs discuss the freshly completed sessions*
- *Researchers discuss: Tips for writing your thesis!*
- *Video tour: What's in Sessions 6, 7, 8, 9?*

Session 6
Chair: David Stepensky

11:50 **11 Yulia Michailov**: Effect of Cancer (Leukemia) and Chemotherapy Treatment on Spermatogenesis Development and Sperm Quality in Mouse System *(Mahmoud Huleihel)*

12:15 **12 Vitic Zagorka**: Neuroprotective effects of BMP5/7 against α-synuclein-induced neurotoxicity in a Parkinson's disease mouse model *(Claude Brodski)*

Session 7
Chair: Alex Braiman

13 Moumita Chakraborty: ZnR/GPR39 modulates KCC activity in Estrogen Negative Breast Cancer Cells *(Michal Hershfinkel)*

14 Milica Markovic: Drug targeting strategy for the treatment of inflammatory bowel disease: a novel phospholipid-based prodrug approach *(Arik Dahan)*

Session 8
Chair: Moshe Elkabets

15 Max Drabkin: Unraveling the molecular basis of common human diseases through studies of unique monogenic kindreds *(Ohad Birk)*

16 Ofek Oren: Protein Engineering in Mammalian Cells - Developing novel inhibitors for amyloids aggregation *(Ran Taube & Niv Papo)*

Session 9
Chair: Ayelet David

17 Simona Krasnopolsky: The role of the cellular transcription elongation machinery in controlling HIV transcription and promoting viral latency *(Ran Taube)*

18 Roy Moscona: Novel Insights Into the Impact of BORIS/CTCFL on Chromatin Remodeling, Transcriptome and Their Clinical Implications in Cancer *(Eitan Rubin)*

12:45 Main Zoom Hall

- *Video tour: Kids! So Many Kids!*
- *Live! Session chairs discuss the freshly completed sessions*
- *Researchers discuss: Coping with stress in your thesis track!*
- *Video tour: What's in Sessions 10, 11, 12, 13?*

Session 10
Chair: Claude Brodski

13:05 **19 Daniel Halperin**: Deciphering the molecular basis of neurological disorders in isolated populations *(Ohad Birk)*

13:30 **20 Boško Mitrović**: Role of Enteropathogenic Escherichia coli EscV protein in the assembly and the functioning of the Type III secretion system *(Neta Sal-Man)*

Session 11
Chair: Ehud Ohana

21 Ohad Stoler: Changes in mitochondrial calcium levels during evoked action potentials *(Ilya Fleidervish)*

22 Hen Popilski: Influence of doxorubicin-loaded liposome surface groups on the systemic drug disposition, and the balance of its pharmacological effects *(David Stepensky)*

Session 12
Chair: Mahmoud Huleihel

23 Muhammad Yousef: The Intracrine role of Interleukin-1alpha *(Alex Braiman)*

24 Manu Prasad: Inhibition of MEK1/2 by trametinib sensitizes MAPK driven head and neck cancers to anti-PD-1 immunotherapy *(Moshe Elkabets)*

Session 13
Chair: Ran Taube

25 Nenad Milošević: Drug-free polymer conjugates for treating chronic inflammatory diseases *(Ayelet David)*

26 Aner Ottolenghi: Life-Extended Glycosylated IL-2 Promotes Treg Induction and Suppression of Autoimmunity *(Angel Porgador)*

14:00 Main Zoom Hall ... we finish at 14:30

- *Video tour: Life Outside the Lab!*
- *Live! Session chairs discuss the freshly completed sessions*
- *Our Amazing PhDs give you Tips for Your Track!*
- *Researchers describe how they searched and found their own postdoc mentors!*
- Biomedicine Awards
- Concluding remarks

PhD student seminars 30 June 2020

Abstracts

Session 1 at 9:20

1

Directed Evolution to Engineer GPCRs With Improved Structural Stability

May Meltzer[1], Niv Papo Avram[2] and Stas Engel[1]

[1]Department of clinical biochemistry and pharmacology, Faculty of Health Sciences, Ben-Gurion University of the Negev and [2]Department of Biotechnology Engineering and the National Institute for Biotechnology

G-protein-coupled receptors (GPCRs) mediate most of our physiological responses to different stimulants, and have extensive potential as therapeutic targets for a broad spectrum of diseases. GPCRs are considered potential drug targets and around 40% of approved drugs act as GPCR agonists or antagonists. The rate of GPCR-targeting drugs entering the market has been reduced and pharmaceutical companies are seeking for new information that relay in structural and biochemical studies of GPCR function. The most efficient approach in drug discovery remains a structure-based (SB) approach, in which design of new drugs is guided by the available structure of protein-ligand complexes. SB approach in GPCR, however, is hampered by the tremendous difficulties associated with the expression, isolation and crystallization of these proteins. GPCRs are extracted from membrane by detergents. For crystallization purposes, short chain (< C9) detergents such as n-Octyl-β-D-glucoside (OG) are particularly useful, but they are poor mimics of the lipid bilayer and most GPCRs are unstable in the detergent-solubilized form. To overcome this problem, mutagenesis is employed to improve structural stability of GPCRs in the presence of short-chain detergents. The current methods of GPCR engineering to achieve structural stabilization, however, have limited efficiency, they are cumbersome and low-throughput; in addition, the scope of GPCRs that could be handled by these techniques is limited. The main goal of this study is to develop a high-throughput yeast-based methodology for a directed evolution of structurally stable GPCRs amenable to crystallization and structural analysis in the presence of short-chain detergents. This methodology takes advantage of the yeast's eukaryotic protein synthesis machinery and the presence of the detergent-resistant cell wall to link the receptor's phenotype (resistance to detergent) to its genotype (mutation(s) responsible for this phenotype) and enable a 'directed evolution' of structurally stable GPCR variants. Human adenosine A2a-Receptor (A2aR) is the model protein for these studies. The proposed methodology would dramatically facilitate GPCR structure-based drug discovery by simplifying the generation of GPCR variants amenable to crystallization and structural analysis. Besides practical importance, this study will enable mapping of the structural determinants of GPCR stability ('stability landscape'). The proposed methodology is not exclusive to GPCRs, but could be used to generate stable variants of other membrane proteins, including ion channels, which are important drug targets as well and extremely difficult to crystallize. The availability of such technique is expected to boost the field of drug discovery, which is currently in a deep stagnation.

2

Antibody Immune History Profiles of Influenza in Vaccinated and Unvaccinated Individuals

Ayelet Shagal[1], Lilach M. Friedman[1], Joshua Petrie[2], Emily Martin[2], Arnold Monto[2] and Tomer Hertz[1,3*]

[1]Department of Microbiology, Immunology and Genetics, and the National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev, Beer-Sheva, Israel; [2]Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor Michigan, USA; [3]Vaccine and Infectious Disease Division, Fred Hutch Cancer Research Center, Seattle, WA, USA

Introduction: Vaccinations, the most cost-effective public health interventions, prevent spreading of infectious diseases. They trigger the immune system to generate protective memory responses. Protection induced by vaccinations is complex and multifaceted, involving innate, humoral, and cell-mediated immunity. Antibodies production has a key role in vaccine-induced protection. Substantial differences between individuals in vaccine-induced immune response have been observed. Many factors contribute to this high heterogeneity in immune responses, including age, gender, and the individual's 'immune history' – the preexisting antibody repertoire of previously encountered pathogens and vaccines. **Aim:** To study the role of the immune history of pre-existing anti-influenza antibodies in protection from influenza infection, and to assess novel serology biomarkers as correlates of risk and protection in vaccinated and unvaccinated individuals. We used serum samples from FluVacs – a randomized double-blind placebo-controlled influenza vaccine efficacy trial comparing the inactivated (TIV) and live-attenuated (LAIV) influenza vaccines in 165 adults aged 18-65, conducted in 2007-2008. **Results:** We developed a novel influenza antigen microarray spotted with whole-inactivated influenza viruses, recombinant surface glycoproteins and their respective overlapping peptides. IgG and IgA binding levels to the vaccine strains exhibited a significantly higher rise in TIV group, compared to the LAIV vaccinated group. The levels of antibodies to historical influenza strains in the participants sera were highly heterogeneous at baseline. This trial included vaccine failure cases, in which vaccinated participants were infected with the influenza H3N2 strain post-vaccination. We compared the baseline and post-vaccination antibody profiles of all subjects who subsequently became infected to those who did not. For this purpose, we defined a Baseline Immune History (BIH) score, calculated as the baseline magnitude of antibodies to all H3N2 strains spotted in the arrays, and ranked the individuals to high, medium, and low BIH groups. A significant reduction in infection rate was observed for the high-BIH group, both in vaccinated and placebo-vaccinated subjects. This correlation was stronger for IgA levels to recombinant HA proteins of the H3N2 strains. **Conclusions:** Our novel influenza antigen microarray platform offers a multidimensional method to quantify antibodies for hundreds of antigens simultaneously. The association between the pre-vaccination level of anti-influenza systemic IgA antibodies and post-vaccination infection rate may indicate serum IgA as a novel correlate of protection. Our results highlight the role of immune-history as a baseline measurement that may be predictive of vaccine-induced immune responses and protection. A better understanding of the effect of preexisting antibodies on the vaccination response may pave the way to improved vaccine immunogenicity and efficacy.

Session 2 at 9:20

3

An implantable system for long-term assessment of atrial fibrillation substrate in freely-moving rats exposed to underlying pathological conditions

Hadar klapper-goldstein[1,2] Murninkas Michael[1,2], Gillis Roni[1,2], Eran Levanon[1,2], Elyagon Sigal [1,2], Wesam Mulla[1,2] and Etzion Yoram[1, 2]

[1]Cardiac Arrhythmia Research Laboratory, Department of Physiology and Cell Biology, Faculty of Health Sciences, and [2]Regenerative Medicine and Stem Cell (RMSC) Research Center, Ben-Gurion University of the Negev, Beer-Sheva, Israel

Background: Atrial fibrillation (AF) is a growing epidemic responsible for substantial economic costs, morbidity and mortality. Common pathological conditions such as heart failure, metabolic syndrome and increased activation on the renin-angiotensin-aldosterone system, converge to contribute to electrical and structural changes in the atrial tissue and promote AF by mechanisms that remain poorly understood. Drugs aimed to target atrial remodeling are attractive new options to prevent AF perpetuation. However, early pre-clinical testing of such drugs is currently difficult due to the absence of reliable, and affordable, animal models. In the present work we developed and validated a chronically implantable device, enabling serial evaluations of AF substrate in rats exposed to pathological insults. **Methods:** Rats implanted with our atrial pacing & recording devices were divided to three groups: a. Sham (n=10) subjected to solvent only and serving as a control, b. Aldo (n=9) subjected to excessive aldosterone levels c. MI (n=10) exposed to anterior wall myocardial infarction. AF substrate was evaluated two- and four-weeks post implantation using a standard AF induction protocol. Results were also compared to a previous Base group (n=12) in which AF substrate was studied one week following implantation only. All animals passed echocardiography. At the endpoint, left atrial histology was evaluated for fibrosis and serum samples were analyzed for inflammatory markers (IL-6, TNF-alpha). **Results:** Rats exposed to ALDO or MI demonstrated a progressive increase in atrial fibrosis and concomitant increase of AF substrate compared to the Base group. In the MI group, AF duration was correlated with infarct sized and inversely correlated with ejection fraction. Unexpectedly, the Sham group also developed marked AF substrate over time. Further analysis indicated that the later phenomenon was not related to atrial fibrosis or systemic inflammation. An additional experiment in which implanted rats could interact through a barrier also excluded chronic-social isolation as the cause for the AF substrate supporting the notion that local effects of the implanted electrode probably mediate this outcome. **Conclusions:** We demonstrate the first system for repetitive AF substrate evaluation in freely moving rats exposed to underlying pathological conditions. This system should greatly improve pathophysiology studying and reliable testing of new therapies.

4

Deciphering the molecular basis of hereditary neuro-ophthalmological disorders, focusing on SCAPER

Ohad Wormser and Ohad Birk

The Shraga Segal Department of Immunology, Microbiology and Genetics, Faculty of Health Sciences, Ben Gurion university of the Negev, Beer sheva, Israel

Introduction: Inbred communities, such as the Bedouin population of southern Israel and several Jewish diasporas, are affected by autosomal recessive diseases due to founder mutations. In my Ph.D, I focused on identifying the molecular basis of monogenic neuro-ophthalmological disorders through studies of these unique cohorts. **Aim**: Identifying novel disease-causing genes underlying monogenic neuro-ophthalmological disorders, deciphering their downstream molecular mechanisms. In particular, investigating the molecular basis of a novel Bardet-Biedl syndrome (BBS) phenotype. **Results**: We identified 25 human mutations causing hereditary eye disorders. Of the mutations identified, 15 were novel. Association of novel mutations to the disease phenotypes was demonstrated through segregation analysis, qRT-PCR, or computer modeling. Of the novel mutations, three were in genes not previously known to be causing those diseases. In the presentation, I will focus on one of these three diseases: an autosomal recessive BBS phenotype, with intellectual-disability, RP, obesity, brachydactyly and male infertility. By combining exome sequencing and homozygosity mapping (LOD score 3.88), we demonstrated that a truncation mutation in SCAPER caused the disease. The translated protein was ~50kDa smaller than WT-SCAPER. As BBS phenotypes are mostly ciliopathies, we focused on a possible role of SCAPER in cilia. In transfected cells, the aberrant protein remained sequestered to the primary cilia, mostly at their tip, while the WT was rarely localized along the ciliary axoneme and basal body. Over-expression of both WT and mutant SCAPER resulted in formation of microtubule-bundles. Live-cell imaging recapitulated SCAPER localization to primary cilia, affecting microtubules during mitosis. SEM studies did not demonstrate structural differences in primary cilia of patient-derived fibroblasts vs controls. However, significantly longer cilia were demonstrated in cells transfected with mutant-SCAPER vs. WT, as well as in human affected fibroblasts vs. controls. Interestingly, we demonstrated that the azoospermia in affected males was due to an early germ cell maturation defect not related to cilia. Moreover, null mutations in Drosophila SCAPER homolog- ssp3 did not affect viability and female fertility but specifically disrupted male fertility. Cytological analysis revealed that in ssp3 mutant testis, microtubule behavior was highly abnormal, leading to severe meiotic defects in most dividing spermatocytes. **Conclusions**: We identified 25 human mutations causing neuro-ophthalmological disorders, including 15 novel mutations, three of which were in novel disease-causing genes. Specifically, in-depth studies of one of these diseases demonstrated that a BBS ciliopathy syndrome can be caused by a mutation in SCAPER, and that SCAPER plays a role in ciliary dynamics, affecting microtubule-related mitotic progression and cilia length. We also demonstrated an essential role of SCAPER/Ssp3 in spermatogenesis in both humans and flies, suggesting that this protein regulates microtubule behavior during both human and Drosophila meiosis. Aside from their scientific significance, our findings are being widely implemented in the clinical arena, enabling carrier testing and disease prevention in wide communities in southern Israel and worldwide.

Session 3 at 10:35

5

The role of SETD6-mediated methylation of TWIST1 in Glioblastoma multiforme

Lee Admoni-Elisha and Dan Levy

Department of Microbiology, Immunology and Genetics, BGU

Introduction: Lysine methylation is involved in the regulation of broad spectrum of cellular processes with direct and indirect effects on human pathologies. While extensive studies were done on lysine methylations of histone proteins, increasing evidence reveals that lysine methylation occurs also on non-histone proteins. SETD6 is a member of the lysine methyltransferase family and was found to participates in several cellular processes, such as the NF-κB pathway, Wnt/β-catenin pathway and oxidative stress response. Glioblastoma multiforme (GBM) is the most common and aggressive primary brain tumor in adults. It is characterized by high invasion and migration abilities which makes it very difficult to treat. One of the processes that contribute to the highly aggressive phenotype of GBM is the Epithelial to Mesenchymal Transition (EMT). EMT is induced by four main transcription factors known as TWIST1, ZEB1, Snail and Slug, which suppress epithelial genes and activate mesenchymal genes. Our preliminary data suggest that SETD6 is downregulated in the highly invasive mesenchymal subtype of GBM, which raised the hypothesis that it regulates one of the transcription factors involved in EMT. **Aim:** To elucidate the molecular mechanism and cellular effect of TWIST1 methylation by SETD6 in GBM. **Results:** We found that SETD6 methylates TWIST1 at K33 (K33me) and that they physically interacts at chromatin. To test if TWIST1 methylation affects its transcriptional

activity, we performed a RNA-seq experiments. The analysis of these experiments revealed that a subset of EMT related target genes which control cell adhesion, ECM, migration and collagen organization are differentially expressed in the K33R TWIST1 mutant cells, suggesting that TWIST1 methylation is important for the regulation of these genes. Moreover, we found that TWIST1 methylation regulates the expression of LINC-PINT, a long-non-coding RNA, previously found to regulate adhesion and migration genes in several types of cancer. These alterations in gene expression led to inhibition of adhesion, migration and drug resistance of the cells. **Conclusions:** Our results suggest that TWIST1 methylation by SETD6 regulates cell adhesion, migration and drug resistance in GBM by activation of LINC-PINT.

6

Mechanisms of persistent Na^+ current generation in soma and processes of Layer 5 pyramidal neurons

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Background: In addition to the well-described, fast-inactivating component of the Na^+ current, neocortical neurons also exhibit a slowly inactivating, persistent Na^+ current (I_{NaP}), that plays a role in determining AP threshold and in synaptic integration. Here, we used high-speed fluorescence imaging of the Na^+ indicator, SBFI, to quantitatively describe the I_{NaP} kinetics in distinct compartments of Layer 5 pyramidal neurons. **Results:** Two-second-long voltage ramps from −70 to 0 mV elicited Na^+ signals that were clearly detectable in soma, basal and proximal apical dendrites. The signals in axon initial segment (AIS) were larger, began at more negative voltages and reached plateau at ~ -30 mV, whereas signals in soma and dendrites were smaller and grew throughout the ramp. Computer simulations showed that the unique shape of the AIS Na^+ transients reflects simultaneous influx and diffusional outflow of Na^+ ions from the AIS to soma and internode. In order to quantify Na^+ influx separately, we delivered voltage ramps of progressively larger amplitude and duration, keeping their speed constant. Rate of SBFI fluorescence change was measured at the end of each ramp, where it is determined by both influx and diffusion, and immediately after the voltage jump back to the holding voltage of -70 mV that is expected to deactivate I_{NaP}-mediated Na^+ influx within few milliseconds. By subtracting these rates, we obtained parameter that is strictly proportional to the I_{NaP} mediated Na^+ influx at the end-voltage of the ramp. Based on these measurements, we found (1) that in both axonal and somatodendritic membrane I_{NaP} is primarily generated by "modal gating" mechanism, with little contribution of "window" current, (2) that Na^+ channels in these compartments have similar propensity to generate I_{NaP}, and (3) that I_{NaP} has leftward shifted voltage dependence in the axon compared with the other compartments. This research was supported by the Israel Science Foundation (grant No. 1302/14)

Session 4 at 10:35

7

Mechanistic and therapeutic assessment of synergistically acting combinations of phenolic compounds in acute myeloid leukemia models

Aviram Trachtenberg[1], Somaya Alterate[1], Katarzyna sidoryk[2], Boris Polyak[3] and Michael Danilenko[1]

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Background: Acute myeloid leukemia (AML) is a devastating cancer of the blood characterized by uncontrolled proliferation of immature myeloid blasts. Despite initial responses to chemotherapy, prognosis remains poor, especially for elderly patients who cannot tolerate aggressive treatment. We have previously shown that the polyphenols curcumin (CUR) from *Curcuma longa* and carnosic acid (CA) from *Rosmarinus officinalis*, applied at non-cytotoxic concentrations of each agent, synergistically induce massive apoptosis in human AML cells both *in vitro* and *in vivo*. Still, the mechanism of the anti-leukemic activity of CUR+CA is not fully understood, and the low bioavailability of CUR limits its potential of clinical application. **Aims:** To investigate the role of the PI3K/Akt/mTOR, Nrf2/antioxidant response element (Nrf2/ARE), and NFκB cytoprotective pathways in the cytotoxic effects of CUR+CA on AML cells. To address the low bioavailability issue of CUR in two ways: (1) by determining the potential of nanoparticles encapsulating CUR+CA (CUR+CA-NPs) for the treatment of leukemia in mouse models of AML and (2) by searching for a more bioavailable low-molecular weight CUR-like compound capable of cooperating with CA against AML cells. **Results:** We found that the effects of CUR+CA on the phosphorylation of Akt and the levels of Nrf2 and its downstream effectors are cell-type dependent. For instance, U937 cells demonstrated activation of the Nrf2 pathway and deactivation of Akt, and the opposite effects were observed in KG-1a and HL60 cells. In HL60 and KG-1a cells inhibition of Akt accelerated CUR+CA-induced apoptosis. In contrast, NFκB activity was not affected by CUR+CA in any cell line tested. In cell culture experiments, CUR+CA-NPs cytotoxicity was comparable to that of free CUR+CA. However, using the MLL-AF9 mouse model of AML *in vivo,* we found no beneficial effect of CUR+CA-NPs. Screening of thirteen structurally similar hydroxycinnamic acid derivatives (HCADs) synthesized by a Polish collaborator revealed that only methyl 4-hydroxycinnamate (MHC) and methyl ferulate (MF) mimicked CUR in the ability to synergistically cooperate with CA in inducing selective killing of AML cells. Comparatively, the apoptotic effect of MHC+CA was stronger and faster than that of MF+CA. Furthermore, MHC+CA was more permeable across Caco-2 cell monolayers than MF+CA or CUR+CA-NPs, as determined by a functional cytotoxicity assay. However, like CUR+CA, the MHC+CA combination was ineffective in the MLL-AF9 mouse model. **Conclusions:** The results suggest that the Nrf2 and Akt pathways protect AML cells against CUR+CA and that the protection by Nrf2 is superior to that by Akt, as seen by the slower apoptotic effect in cells showing Nrf2 activation. Murine AML cells carrying the MLL-AF9 mutation may be insensitive to our combinations. Therefore, testing of other *in vivo* AML models is currently in progress. Similar to CUR, MHC and MF cooperate with CA to specifically kill AML cells and this effect strongly depends on both the position of the hydroxyl group on the aromatic ring and the modification of the carbonyl group in HCAD molecules. Collectively, the synergistically acting combinations studied here, particularly MHC+CA, may represent a prototype novel treatment of AML, especially in elderly or unfit patients.

8

Excess interleukin 1 (IL1) worsens and IL1 inhibition improves renal damage and anemia in a mouse model of chronic kidney disease

Inbar Bandach[1], Yael Segev[1] and Daniel Landau[2,3]

[1]Shraga Segal Department of Microbiology and Immunology, Faculty of Health Sciences, Ben Gurion University of the Negev, Beer Sheva; [2]Institute of Nephrology, Schneider Children's Medical Center of Israel, Petach Tikva and [3]Sackler School of Medicine, Tel Aviv University, Israel

Background: Inflammation in chronic kidney disease (CKD) is mostly due to activation of the innate immune system, in

which Interleukin (IL-1) is a key player. Anemia of CKD, originally thought to be due to impaired renal erythropoietin (EPO) synthesis and disturbed iron metabolism, may also be due to EPO resistance, clinically associated with inflammation. IL1 receptor antagonist knockout (RaKO) mice show arthritis and excessive inflammation. Inhibition of IL-1 was shown to be beneficial in many low-grade inflammatory conditions, but its role in CKD and anemia is unknown. **Methods**: Wild-type (WT) and RaKO mice were fed with 0.2% adenine or control diets, leading to 4 groups: WT, WT-CKD, RaKO, RaKO-CKD. In a second experiment using the same CKD model, we treated CKD mice with P2D7KK, a novel anti-mouse IL1 antibody. **Results**: enhanced inflammation in uremic RaKO mice with adenine-induced CKD provoked both higher degrees of renal insufficiency and anemia in comparison to wild-type CKD, in association with downregulation of renal hypoxia-inducible factor -2 (HIF2), as well as decreased bone marrow EPO-receptor (EPOR) and transferrin receptor (TFR) (controlled by EPO), in addition to hepcidin-mediated hypoferremia. In contrast, administration of P2D7KK, an anti-IL1β monoclonal antibody, to CKD mice resulted in a lower grade of systemic inflammation, better renal function and blunted anemia. The latter was associated with upregulation of renal HIF-2α and bone marrow EPO-R and TFR. **Conclusions**: this supports the distinct role of inflammation and IL-1 particularly, in CKD progression as well as anemia. Novel treatments to reduce inflammation through this and other pathways may improve renal function, attenuate the anemic state or increase the response to exogenous EPO.

Session 5 at 10:35

9

The genetic and molecular basis of monogenic neuromuscular and neurological diseases, with focus on Autism

Yuval Yogev[1] and Ohad S. Birk[1,2]

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Introduction: Delineating the pathological process underlying genetic diseases could prove a difficult task. High genetic variation in the general population, as well as environmental impact on gene expression and complex and multifactorial inheritance patterns impose a great challenge when trying to detect culprit variations. However, detecting and unraveling of these mechanisms can be of great scientific value. Our approach to the problem is in the study of disease in isolated populations, or in pedigrees with apparent Mendelian inheritance. **Aim:** To elucidate the genetic and molecular basis underlying monogenic neurological and neuro-muscular disorders. For this, we used next generation sequencing, SNP-based genotyping and segregation analysis to identify a genetic basis for these diseases, followed by functional studies for molecular characterization of novel disease-causing mutations. **Results:** In the first project, we described a novel syndrome of spastic paraparesis with kyphoscoliosis and demonstrated that it is caused by a mutation in *KY*. we showed that the disease cannot be attributed to the protein's sarcomere stabilizing function alone, and suggested it also has an important function in the neuromuscular junction. In a different project, we has discovered that a severe form of limb girdle muscle dystrophy with involvement of respiratory muscles is caused by a missense mutation in a gene relevant to statin myopathy. we have demonstrated through detailed biochemical studies the disease mechanism and generated cellular models for metabolic and metabolomic analysis. We then synthesized and purified large amounts of the direct downstream metabolite lacking in the patients, as well as obtained permission for and initiated a clinical study/compassionate treatment of patients with this compound, never given to humans before. In studying monogenic cases of Autism spectrum disorder, we managed to find 3 novel mutations, delineating molecular mechanisms of autism. We have generated CRISPR-KI cell models of these variants and are en-route to show a possible converging mechanism in several unrelated cases of autism. Outside the scope of my PhD thesis I have several projects relating to congenital malformations, drug discovery and cancer, such as phocomelia, glioblastoma and Kaposi sarcoma. Lastly, in addition to 'wet' biological research, I also conduct several bioinformatics studies, with interesting insights to be published in the coming year. **Conclusion:** The study of monogenic diseases can have major impact in two major fields. In clinical medical practice, identifying the cause of a genetic disease allows for prenatal screening and carrier identification, and might also hold therapeutic promise in the studied population. In addition, identification of a disease-associated variation can aid in diagnosing patients with similar phenotypes, not only from the studied population, that bear genetic variations in the same gene or metabolic pathway. Moreover, identifying monogenic disease-causing variants holds great scientific interest, as it may illuminate biological processes that were yet to be studied, in both normal and disease state. Studies of monogenic disease in homogenous populations may also contribute to the study of sporadic forms of similar phenotypes, by implying a common cause or converging pathophysiological processes.

10

Protection of the mouse testis tissue and sperm production from X-ray induced damages by pharmaceutical compounds that increase telomerase

Elul H and Priel E

The Shraga Segal Dept. of Microbiology, Immunology & Genetics, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel

Introduction: The telomerase reverse transcriptase (TERT) is expressed and active in the testes and is important for the spermatogenesis process. Short telomeres were identified in men with oligozoospermia and idiopathic infertility. Shortened telomeres in spermatozoa are markers for abnormal spermatogenesis. In addition to its role in the re-elongation of telomeres, TERT possesses non-canonical functions: protects cells from apoptosis, participates in the repair of DNA strand breaks and regulates the expression of genes. We synthesized novel compounds that transiently increased TERT expression and activity in various human and mouse cells and tissues. The compounds protected cells from damages induced by oxidative stress. **Aim:** To investigate the effect of increasing telomerase expression and activity in mouse testis, by telomerase activators (AGS), on the spermatogenesis process before and after their exposure to X-ray radiation. **Results**: TERT expression and activity in the mouse testis, increased following a single AGS dose. The expression of spermatogenesis markers (VASA and CREM) increased followed by a significant enhancement in the sperm count. Exposure of mice to X-ray radiation (2.5 Gy) damaged the testis tissue and decreased sperm count and sperm parameters (motility, normal morphology). Treatment of the X-ray irradiated mice with a single dose of AGS, significantly restored testis tissue morphology, the expression of spermatogenesis markers and decreased the number of seminiferous tubules that exhibited DNA strand breaks. AGS treatment of irradiated mice restored the sperm count and sperm motility and reduced the percentage of sperm cells with altered morphology. A higher sperm count and an increase in testis tissue regeneration, following X-ray radiation, was also detected in AGS treated mice 21 and 30 days after irradiation and treatment. **Conclusion**: This study suggests the ability of telomerase increasing compounds to restore the normal tissue morphology, the spermatogenesis process and the sperm count in damaged testes. Therefore, these compounds may be used as possible therapy in conditions that reduced male fertility.

Session 6 at 11:50

11

Effect of Cancer (Leukemia) and Chemotherapy Treatment on Spermatogenesis Development and Sperm Quality in Mouse System

Yulia Michailov and Mahmoud Huleihel

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Introduction: It was known that some types of cancers including leukemia can impair semen parameters and hormones and affect male fertility even before anti-cancer treatment. The mechanisms behind these effects have not fully been studied. **Aims**: To examine the effect of acute myeloid leukemia (AML) and Cytarabine chemotherapy on mouse semen parameters, spermatogenesis process and on male fertility. **Results:** We showed that AML and Cytarabine impaired semen parameters and fertility capacity of the mice. Both of the treatments adversed the histology of the testis and increased apoptosis of cells in the seminiferous tubules and apoptotic signals. Also, they effected the percentages of cells of different stages of spermatogenesis, and the activity of cells of the testicular microenvironment that supports spermatogenesis. AML induced inflammatory factors in the testes. We used GCSF in order to abolish/decrease/tolerate the effect of AML and Cytarabine on spermatogenesis and sperm parameters. **Conclusions:** This is the first study shows that AML affects not only semen quality but also spermatogenesis process. We show that AML causes testicular hormonal and autocrine/paracrine imbalance and cellular microenvironment, including increasing of different inflammatory factors. The combination of AML and chemotherapy increased the adverse effects of chemotherapy alone. In addition, we succeeded to decrease/tolerate the damage effect of AML and chemotherapy by GCSF. Thus, our study deepens our understanding part of the mechanisms involved in male infertility following AML and chemotherapy treatment, and the possibility to tolerate this effect effects by GCSF. Our results may open new therapeutic strategies for male infertility treatments in cancer patients.

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Neuroprotective effects of BMP5/7 against α-synuclein-induced neurotoxicity in a Parkinson's disease mouse model

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Introduction: Bone morphogenetic proteins (BMPs) are members of the TGF-beta protein family and use SMADs as intracellular signal transduction proteins. BMP5/7 and SMAD1 support the survival of dopaminergic (DA) neurons *in vitro*, and as recently shown, also promote neurogenesis of DA neurons *in vivo* and differentiation in human induced pluripotent and neural stem cells. Notably BMP7 overexpression protects DA neurons in different toxin-induced Parkinson's disease (PD) models. However, there is no data on the role of BMPs in maintaining normal alpha-synuclein levels and their neuroprotective effects on DA neurons in alpha-synuclein PD animal models. **Aim:** To determine whether BMP5/7 can prevent α-synuclein associated loss of DA neurons *in vivo*. **Results:** We report that BMP5/7 prevent α-synuclein induced loss of DA neurons, motor impairments and associated gliosis. Moreover, we demonstrate that BMP5/7 treatment significantly reduce α-synuclein accumulation. Complementarily, loss of BMP/SMAD signaling pathway leads to the accumulation of α-synuclein. **Conclusion:** In Parkinson's disease animal model, based on the viral overexpression of alpha-synuclein in the substantia nigra, BMP5/7 can prevent the loss of DA neurons and associated motor impairments. Further experiments will provide the mechanisms by which BMPs mediate their therapeutic effects.

Session 7 at 11:50

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ZnR/GPR39 modulates KCC activity in Estrogen Negative Breast Cancer Cells

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Introduction: Breast cancer malignant cells exhibit deviant signaling patterns and changes in Zn^{2+} accumulation and distribution, but the link between these pathways is not well understood. ZnR/GPR39 is a Gq coupled receptor that is selectively activated by Zn^{2+} and triggers intracellular Ca^{2+} release and subsequent activation of signaling pathways closely linked to cancer progression. In addition, ZnR/GPR39 activates members of the K^+/Cl^- cotransporter family (KCC) that has also been associated with cancer. Yet, the link between Zn^{2+}, ZnR/GPR39 and the pathways leading to cancer progression have not been elucidated. **Aim:** To identify the mechanistic link between ZnR/GPR39 and KCC activity and its influence on breast cancer growth and metastasis. **Results:** We show here by fluorescent imaging that in estrogen receptor (ER) negative breast cancer BT20 cells, ZnR/GPR39 enhances transport activity mediated by KCC, monitored as the rate of NH_4^+ transport used as a surrogate to K^+. Using a scratch assay paradigm on BT20 cells we show that ZnR/GPR39 activation of KCC enhances cell proliferation and migration. Using the XTT proliferation assay and the transwell cell invasion assay, we show that extracellular Zn^{2+} triggers ZnR/GPR39 dependent activation of KCC3 isoform and increases proliferation and migration rates in both cell lines. Moreover, Zn^{2+} activation of ZnR/GPR39 and KCC3 promotes formation of F-actin stress fibers in both BT20 and MDA-MB-453 cells. Finally, Zn^{2+} treatment induces ZnR/GPR39 and KCC3 dependent MMP-2/9 activity, as seen by gelatin zymography, and this activity is required for stress fibers formation. **Conclusions:** Our study identifies a pathway that links Zn^{2+} dyshomeostasis and breast cancer progression, via activation of ZnR/GPR39 and KCC3 that regulate key modulators of cell migration and invasion, MMP2/9 secretion, and stress fiber formation.

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Drug targeting strategy for the treatment of inflammatory bowel disease: a novel phospholipid-based prodrug approach

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Introduction: To date, drug delivery strategies in inflammatory bowel disease (IBD) target a general intestinal region (*i.e.* colon) regardless of where the inflammation is located. An orally delivered compound with activity specifically at inflamed sites in the gastrointestinal tract (GIT) and limited systemic absorption would be a major advance in the therapeutic approach of IBD. For this purpose, we propose a prodrug, linking active drug to phospholipid (PL), the substrate of phospholipase A_2 (PLA_2). PLA_2

hydrolyses the *sn*-2 bond within PL, liberating fatty-acid (FA) and lysophospholipid. In our PL-based prodrug, the *sn*-2-positioned FA is replaced with cyclosporine, so PLA_2 is to be used as the prodrug-activating enzyme, releasing the free drug from the PL-complex. The expression/activity of PLA_2 is significantly elevated in the inflamed intestinal tissues of IBD patients, hence, our prodrug design may target the free cyclosporine specifically to the inflamed sites throughout the GIT. **Aim:** The overall goal of this research is to study and develop a novel drug targeting approach for treating IBD, based on PL-based prodrugs. To achieve this overall goal, the following specific aims were pursued: (1) to design and synthesize a series of PL-cyclosporine prodrugs, differing in their linker length between the PL and the drug moiety; (2) to study the *in-vitro* PLA_2-mediated activation of the different prodrugs; (3) to use powerful modern *in-silico* modeling tools to simulate PLA_2-mediated activation and reveal mechanistic insights concerning the optimal PL-cyclosporine design; (4) to investigate the PL-cyclosporine in 2 animal models, colonic brush border membrane vesicles (cBBMV) and intestinal perfusion, in diseased *vs*. healthy intestine. **Results:** Newly synthesized PL-cyclosporine prodrugs with 6, 8, 10 and 12 -CH_2 linkers demonstrated significant difference in PLA_2-mediated activation. Rapid (5min) hydrolysis of the entire dose was obtained for PL-C12-cyclosporine; PL-C10-cyclosporine showed similar high activation, PL-C8-cyclosporine less, and PL-C6-cyclosporine was no different than the control group. Activation was also studied in the newly developed enzymatically enriched cBBMV model which showed 3.4-fold higher PLA_2 in diseased *vs.* healthy animals. Quick and complete activation of the entire dose was obtained for the PL-C12-cyclosporine, less and slower activation was obtained for the 6/8-CH_2 in diseased vesicles; incubation with cBBMV from healthy colons demonstrated marginal activation. MD simulations demonstrated the origin of this different hydrolysis rates, which undoubtedly showed the difference in the mechanism of binding to the enzyme active site between PL-C12-cyclosporine and PL-C6-cyclosporine. In the single-pass intestinal perfusion method, the highest activation of the PL-cyclosporine conjugate (10-CH_2) was observed in the colon of rats with DSS-induced colitis, with ~60% of the initial prodrug dose converted into the free drug, whereas the DSS-induced colitis group perfused with the PLA_2 inhibitors showed very little activation (~20%). **Conclusions:** This study demonstrates excellent agreement of *in-silico/in-vitro/ in-vivo* studies of PL-cyclosporine prodrugs in the presence of PLA_2.The potential to target sites of inflammation in any gut region together with an extended therapeutic index, and decreased drug levels in non-diseased tissues makes this orally delivered prodrug approach an exciting new therapeutic strategy for the treatment of IBD.

Session 8 at 11:50

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Unraveling the molecular basis of common human diseases through studies of unique monogenic kindreds

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Introduction: Common multifactorial human diseases are mostly caused by combined polygenic heredity and environmental factors. As many determinants are involved in the pathogenesis of such diseases, elucidation of their molecular pathophysiology is challenging. Unique kindreds in which such diseases are caused by monogenic mutations enable insights to the molecular / biochemical / developmental pathophysiology of those diseases. Studies of such kindreds through linkage analysis combined with whole exome sequencing enables identification of genomic loci shared between all affected individuals; follow up studies focusing on genomic variants within these loci pinpoint the precise mutation underlying each disease. Downstream effects of these mutations can be investigated using in-vitro and in-vivo studies, unraveling the molecular pathophysiological process of the disease. In my PhD I focused on three common diseases: Atrial fibrillation, Gout and Otosclerosis. **Aim**: To study monogenic variants of three common genetically inherited diseases, identifying the genetic mutation underlying each disease, studying the molecular effects of each mutation in-vitro, and unraveling the pathophysiological process of each disease through generation and studies of genetically engineered animal models. **Results**: In project I, I studied a family affected with autosomal dominant early-onset nocturnal atrial fibrillation. I identified a novel heterozygous mutation in a gene encoding a cardiac potassium channel not previously linked to a human disease. Using two-electrode voltage clamp technique and an expression system in Xenopus oocytes, I demonstrated a gain-of-function effect in the mutant channel and proposed a mechanism linking the mutation to the disease phenotype. I also suggested a novel explanation for the nocturnal onset of symptoms in our patients due to circadian variation in the expression of the channel which is markedly elevated nighttime. I then generated CRISPR/Cas9 mice harboring the human mutation in the mouse ortholog of the human gene and studied their phenotype. In project II, I showed that a homozygous inactivating mutation in the mitochondrial enzyme D-lactate dehydrogenase causes Gout, a classical disease whose etiology remains mostly unknown. Through metabolomics analyses of patients' samples and in-vivo experiments in mice, I demonstrated that the disease occurs due to accumulation of D-lactate, whose renal clearance is coupled to reabsorption of uric acid, the culprit metabolite causing Gout. I then generated CRISPR/Cas9 mice harboring the human mutation in the mouse ortholog of the human gene and delineated their phenotype. In my third project, I identified the first gene associated with otosclerosis, a common cause of progressive hearing loss in adults. I generated mice harboring the human mutation using CRISPR/Cas9 and was able to demonstrate hearing loss in the mutant mice using two separate hearing assays. Next, I showed signs of otosclerosis in isolated middle ear samples dissected from mutant mice. **Conclusion**: I identified three novel mutations in genes causing three common diseases, and through in-vitro and in-vivo experiments unraveled the molecular basis and pathophysiology of these diseases. Thus, identification of novel disease-causing monogenic mutations provides insights as to the molecular basis of common diseases.

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Protein Engineering in Mammalian Cells - Developing novel inhibitors for amyloids aggregation

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Aggregation and accumulation of the 42-residue amyloid β peptide (Aβ42) in the extracellular matrix of neurons and within these cells is considered a major cause of neuronal cell toxicity in Alzheimer's disease (AD) patients. It is hypothesized that extra- and intracellular Aβ42 oligomers and fibrils interacts and disrupt the plasma and mitochondrial membranes fluidity, permeate these membranes and form pores enabling dysregulated calcium signaling and amyloid toxicity in AD, eventually leading the neuronal cells into apoptosis. Therefore, molecules that bind to Aβ42 and prevent its aggregation are therapeutically promising as AD treatment. Here, we show that a non-self-aggregating Aβ42 variant carrying two surface mutations, F19S and L34P ($Aβ42_{DM}$), strongly binds Aβ42 and modifies both extra- and intracellular Aβ42-aggregation pathway resulting in low order

Aβ42 oligomers with reduced neuronal cell uptake and toxicity. We also showed that Aβ42$_{DM}$ protects neuronal cells from Aβ42-induced accumulation of intracellular toxic levels of calcium and apoptosis. Aβ42$_{DM}$ also inhibits Aβ42- induced mitochondrial membrane potential depolarization in intact neuronal cells and abolishes Aβ42 mediated decrease in cytochrome C oxidase activity in isolated mitochondria. Currently, we aim to establish Aβ42$_{DM}$ protective activity towards Aβ42 aggregation and activity in AD *in-vivo* models to further develop Aβ42$_{DM}$ as potential drug for AD treatment. Overall, we present a new approach for inhibiting Aβ42 aggregation and toxicity both within and outside cells and provide here a mechanistic explanation for the Aβ42$_{DM}$ inhibitory activity against Aβ42. Accordingly, we strongly believe that Aβ42$_{DM}$ can be potentially used as a therapeutic lead for treating AD.

Session 9 at 11:50

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The role of the cellular transcription elongation machinery in controlling HIV transcription and promoting viral latency

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Despite the introduction of effective anti-retroviral therapy (ART), the Human Immunodeficiency Virus (HIV) persists in an infected cell reservoir that harbors a transcriptionally repressed yet replication competent virus, thus still remains an obstacle towards complete eradication. As transcription silencing is key in establishing the HIV reservoir, significant efforts are being made to understand the mechanism that regulate HIV gene transcription, and identify host factors that promote viral latency. In this study we performed a whole genome CRISPR-based knockout screen in T cells to identify genes that are potentially involved in regulating HIV latency. Our screen identified several top candidates, where their depletion in infected cells led to activation of HIV gene transcription. Of these genes, the KARAB-containing Zing Finger Protein, ZNF304, was identified as a top hit that silenced HIV gene transcription. Expression levels of ZNF304 were low at transcriptionally active HIV infected cells, and increased upon their entry into latency, where the virus was transcriptionally silenced. ZNF304 occupancy on the viral promoter followed protein expression levels, and were high in cells where HIV gene expression was repressed. ZNF304-mediated HIV gene silencing was initiated via recruitment to the viral promoter of a co-repressor complex that included both Polycomb Repression Complex (PRC) and the GLP methyltransferase. Indeed, depletion of ZNF304 expression led to a decrease in H3K27me3, H2AK119Ub and to a lesser extent H3K9me2 histone repression marks on the HIV promoter. Importantly, ZNF304 depletion delayed the re-entry of HIV infected cells into latency. We conclude that ZNF304 silences the HIV promoter by recruiting transcriptional repressive complexes KAP1-GLP/PRC thus promoting viral latency.

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Novel Insights Into the Impact of BORIS/CTCFL on Chromatin Remodeling, Transcriptome and Their Clinical Implications in Cancer

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Introduction: Brother of Regulator of Imprinted Sites (BORIS) is a DNA binding protein, also known as CCCTC binding factor-like (CTCFL). BORIS is expressed in the testis and becomes aberrantly expressed in different types of cancer, thus the classification of BORIS as a cancer-testis antigen. Aberrant BORIS expression in cancer is linked to alterations in gene expression and epigenetic modifications that impact crucial biological processes. In melanoma, BORIS expression is higher in metastasis compared to primary tumors, indicating a role for BORIS in melanoma progression. Currently, BORIS functions in melanoma remain to be elucidated. **Aim:** Here, we set out to investigate the impact of BORIS on melanoma progression, its effect on chromatin accessibility and gene expression. We sought out to identify the unique pattern of BORIS targets pathways and processes; it effects in melanoma cells. Thus, enabling us to identify a signature of BORIS activity that will be validated in clinical data. **Methods:** To study the influence of BORIS in Melanoma, MM057 cell-line was used. Cells were transfected with a vector that either carried the target gene BORIS (BOR), under a Doxycycline (DOX) activated promoter, or an Empty-Vector (EV). Cells culture extracts were sequenced with RNA Sequencing (RNA-Seq) and Assay for Transposase Accessible Chromatin using Sequencing (ATAC-Seq). A bioinformatics pipeline was used to carry out the analyses of the raw sequencing data and integrating the different data sources to meaningful insights, using Bioconductor packages in costume R scripts and available computational tools. **Results:** We demonstrate that ectopic BORIS expression in melanoma cells promotes phenotype switching from a proliferative to invasive state at the transcriptional and phenotypic level. Using in silico analyses, aimed at superimposing gene expression and chromatin accessibility data, we identified potential genes and transcriptional regulators that contribute to this switch. Differential gene and chromatin analyses revealed novel transcription factors (TF) and regulatory regions that drive tumor progression as a consequence of BORIS overexpression. **Conclusions:** BORIS acts as an epigenetic tumor driver in melanoma, with an influence on gene expression and chromatin accessibility. The BORIS affected genes and TFs are going to be validated with clinical data in order to define BORIS signature pattern of transcriptome manipulation in melanoma. The ability of this signature to predict the outcome will be tested using data mining algorithms.

Session 10 at 13:05

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Deciphering the molecular basis of neurological disorders in isolated populations

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Introduction: Genetically isolated populations have proved to be conducive to mapping and identifying mendelian diseases, as studying of rare recessive disorders within consanguineous populations requires only a handful of affected individuals. **Aim**: To identify causative mutations and molecular mechanisms underlying rare monogenic and multifactorial neurological diseases within isolated populations. Specifically, we focused on three different diseases: 1) A congenital syndrome of severe neurological defects with microcephaly and global psychomotor retardation culminating in premature death; 2) Severe familial Attention-Deficit/Hyperactive Disorder (ADHD) and 3) A congenital syndrome of intellectual disability, hypotonia, dysmorphism and thinning of the corpus callosum. **Results**: In the first project, we discovered a disease-causing mutation in *SEC31A*. We demonstrate that *SEC31A* is ubiquitously expressed and that the mutation triggers nonsense-mediated decay of its transcript, comprising a practical null mutation. Recapitulating the human phenotype, knockdown *SEC31A* Drosophila flies had defective brains and early lethality. Moreover, in line with *SEC31A* encoding one of the two coating layers comprising the canonical COP-II complex, CRISPR/Cas9-mediated *SEC31A* mutant cells

demonstrated reduced viability through upregulation of ER-stress pathways. In the second project, we show that familial ADHD can be caused by a missense mutation in ---, encoding the ---, known to play a significant role in synaptogenesis. In line with the human phenotype, CRISPR/Cas9-mutated knock-in mice harboring the human mutation, recapitulated core features of hyperactivity with impairment of presynaptic vesicle clustering, attenuated synaptic release and reduction in tyrosine hydroxylase distribution within ventral midbrain. Furthermore, specific downstream molecular pathways were affected in both the ventral midbrain and prefrontal cortex of mutated mice. In the third project, we delineate the clinical synopsis of a novel syndrome mapped to a ~4Mbp locus on chromosome 7, currently seeking for distinct regulatory variations within this region, potentially underlying the syndrome. In addition, several other novel mutations in known genes have been identified and published, broadening the phenotypic spectrum of Leigh syndrome, Charcot-Marie-Tooth disease type 4B3 and 4B1 and other neuropathies. **Conclusions**: We demonstrate that a novel neurological syndrome is caused by a null mutation in *SEC31A*, assigning the fifth human phenotype to germline mutations in components of the COP-II complex. Further, we delineate the role of aberrant ---in ADHD pathophysiology; the first monogenic non-syndromic familial ADHD described to date. Lastly, we describe a novel disease-associated locus mapped to a ~4Mbp locus on chromosome 7.

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Role of Enteropathogenic Escherichia coli EscV protein in the assembly and the functioning of the Type III secretion system

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Type III secretion system (T3SS) is highly sophisticated nanomachine that consists of approximately 20 different proteins that assemble to a syringe-like structure and facilitates delivery of many bacterial effectors directly from the cytoplasm of gram negative bacteria into the host cells, thereby crossing the bacterial inner membrane, peptidoglycan and outer membrane, as well as the host plasma membrane (Diepold & Wagner, 2014). Pathogens like enteropathogenic *Escherichia coli* (EPEC), *Salmonella*, *Shigella*, and *Yersinia* are attenuated and often rendered completely avirulent by a lack of their T3SSs, demonstrating the importance of the system in pathogenicity (Hueck, 1998). The T3SS secretes in a hierarchy manner three groups of substrates: early, intermediate (also known as translocators) and late substrates (or effectors). It has been previously reported that a high degree of diversity among translocated effectors reflects the specific lifestyle of bacterium and the unique requirements of each, symbiotic or pathogenic, interaction with a particular eukaryotic host (Tampakaki et al., 2004; Heijden & Finlay, 2012; Mota et al., 2005; Galan, 2009). On the contrary, the export machinery itself (often referred as injectisome) is conserved among many bacterial species (Cornelis, 2006; Galan & Wolf-Watz, 2006). EscV protein is one of the integral membrane proteins essential for the assembly and the function of T3SS. Focus of our research involves investigating the structure and role of seven predicted EscV transmembrane domains (TMDs) in homo- and hetro-oligomerization with specific emphasis on the structure-function relationship of the protein and other T3SS components, likewise its general impact on the T3SS assembly and function.

Session 11 at 13:05

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Activity-dependent mitochondrial calcium elevations in soma and processes of layer 5 pyramidal neurons

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Introduction. Although it is widely recognized that neuronal activity elicits an increase in glucose and oxygen consumption, the mechanisms underlying this activity-metabolic feedback are elusive. One possible mechanism is that, in active neuron, cytosolic Ca^{2+} elevations propagate to the mitochondrial matrix where they enhance ATP synthesis by activating enzymes of the Krebs cycle. Here, we explored the spike-evoked mitochondrial Ca^{2+} elevations in soma and processes of Layer 5 pyramidal neurons by measuring fluorescence of genetically encoded mitochondria-targeted Ca^{2+} indicator using two-photon microscopy. **Results.** Modified Ca^{2+} indicator, mitoGCaMP6m, containing neuron-specific promoter (hSyn) and a mitochondrial targeting sequence was delivered using adeno-associated viral vector AAV9 by in-vivo intracortical injection. In infected cortical neurons, mitoGCaMP6m exclusively labeled rod-type discrete intracellular structures and co-localized with mitochondria-specific markers. Whole cell recordings were obtained from weakly mitoGCaMP6m expressing neurons. The pipette solution was supplemented with Ca^{2+} dye, in order to compare the mitochondrial and cytosolic Ca^{2+} transients. Trains of 1-100 action potentials triggered by brief current steps elicited cytosolic and mitochondrial Ca^{2+} transients in somas, dendrites and axons of Layer 5 pyramidal neurons. The mitochondrial Ca^{2+} transients were significantly larger in soma and apical dendrites, as compared with thin basal dendrites and proximal axon. In contrary, synaptic stimuli elicited largest mitochondrial Ca^{2+} transients at basal dendrites. When presynaptic glutamate release coincided with postsynaptic action potential, causing a "Hebbian" potentiation of the affected synapses, the Ca^{2+} elevation in the adjacent mitochondria was dramatically enhanced. Bath application of APV (selective NMDA receptor antagonist) or intracellular application of QX-314 (voltage gated sodium channel blocker) almost abolished the mitochondrial Ca^{2+} transients in response to synaptic stimulation. In all neuronal compartments, the amplitude of mitochondrial Ca^{2+} elevations were found to be steeply dependent on firing frequency, with spikes at higher frequency (>50 Hz) eliciting 3-4 times larger signals, whereas cytosolic Ca^{2+} elevations were almost insensitive to firing frequency. **Conclusion.** The significant dependence of mitochondrial Ca^{2+} elevation on neuronal firing frequency may play a major role in shaping the enigmatic activity-metabolic feedback in cortical neurons. Spike-time dependent, Hebbian plasticity of cortical synapses, most probably, requires mitochondrial Ca^{2+} elevation.

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Influence of doxorubicin-loaded liposome surface groups on the systemic drug disposition and the balance of its pharmacological effects

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Introduction. Liposomal anti-cancer formulations were developed to enhance the accumulation of the encapsulated anti-cancer drugs in the tumor through the enhanced permeability and retention (EPR) effect. However, the extent of this EPR effect has been overestimated, and only 0.7% of the systemically-administered nano-formulation accumulates in the solid tumor in clinical settings. Enhanced intra-tumoral distribution of the liposomes is expected to increase the exposure of the

"deep" parts of the tumor to the drug and its anti-cancer activity. Such improved intra-tumoral distribution can be attained via their decoration with the cell penetration enhancers (CPEs). CPEs are natural short peptides or synthetic compounds enriched in positively-charged amino acids, that can cross efficiently the cell membranes. We focused on the arginine-based dendritic CPE, tetraArg-[G-1]-distearoyl glycerol (DAG-Arg_4), anchored to the liposome bilayer using a hydrophobic residue. DAG-Arg_4 is a synthetic non-peptidic CPE, with higher charge density than the natural linear polypeptides, high *in vivo* stability and resistance to proteases, nontoxic and bio-compatible. **Aims.** To investigate the effect of the CPE surface groups and their potential masking by the PEG groups (PEG_{5k} -DSPE) on the intra-tumoral vs. systemic disposition of doxorubicin-loaded liposomes, and on the balance of their desired/undesired pharmacological effects. We used doxorubicin-loaded liposomes decorated with these groups and investigated their disposition in mice with an orthotropic model of breast cancer using several analytical approaches. **Results.** Liposomes decorated with DAG-Arg_4, with or without PEG_{5K}, undergo efficient endocytosis by the 4T1-Luc cells *in vitro*, and delivered doxorubicin to the cell nuclei. Liposomes decorated with DAG-Arg_4-PEG_{5k} and with PEG_{5k} induced more substantial anti-cancer effect in *in vivo* 4T1-Luc orthotopic breast cancer model in mice, as compared to that of the non-decorated liposomes. The DAG-Arg_4-PEG_{5k}-decorated liposomes accumulated to a substantial extent in the lungs and in the liver, and their accumulation in the tumor and in the spleen was lower, as compared to the PEG_{5k}-decorated liposomes. **Conclusions**. The *in vivo* disposition of the studied liposomal formulations did not correlate closely with their *in vitro* endocytosis data. Decoration with the DAG-Arg_4 residues substantially affected the distribution and tissue accumulation of the liposomes. Some of these changes were beneficial (e.g., reduced accumulation in the spleen), but others were undesired (reduced accumulation in the tumor, enhanced accumulation in the lungs). Overall, it appears that the decoration with the DAG-Arg_4 negatively affects the tissue distribution of the liposomes. More efficient incorporation of these residues into the liposomal formulation can be potentially attained using a "smart" delivery system that masks the surface CPE residues in the systemic circulation, and exposes them when the liposome reach the tumor.

Session 12 at 13:05

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The Intracrine role of Interleukin-1alpha

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Introduction: Breast cancer is the most common malignancy in women and the second leading cause of female cancer-related death worldwide. The basal form of Triple Negative Breast Cancer (TNBC) is especially malignant, as the tumor cells lack expression of cell surface receptors for Estrogen (ER) and Human Epidermal factor Receptor-2 (HER2), which are targeted during treatment of other types of breast cancer. Primary tumors contain heterogeneous assemblies of cells that participate in constant interplay between malignant cells and a dynamic local microenvironment, continuously changing and adapting during tumor progression. Interleukin-1 (IL-1) is a pleiotropic cytokine family that primarily affects inflammatory and immune responses, it also regulates other homeostatic functions of the body. IL-1 is considered as a primary 'alarm' pro-inflammatory cytokine, which is generated initially in the inflammatory response and amplifies and sustains it. The main IL-1 agonistic molecules are IL-1α and IL-1β, which differ in their producing cells and their sub cellular localization. IL-1α belongs to a unique group of cytokines, termed dual-function cytokines. IL-1α is retained intracellularly in many cell types and forms of IL-1α are capable of triggering different regulatory mechanisms beyond the realm of immunology. **Aims:** Comparative and quantitative study of the effects produced by intracellular IL-1α in 4T1 breast cancer cells *ex vivo* and the effect of intracellular IL-1α on breast cancer development *in vivo*. **Results:** We suppressed the endogenous IL-1α production in 4T1 breast cancer cells by using CRISPR/Cas9 system. Additionally, we induced IL-1α expression in controlled manner by using Tet- on lentiviral system. We found that overexpression of IL-1α protein slows down proliferation of 4T1 cells. While assessing viability using annexin-7aad commercial kit to evaluate the effect of the IL-1α expression on viability in WT/KO, we have found that IL-1α expression increases cell death in apoptotic way. Exposing cells to various stress conditions (reduced serum, glucose starvation, stimulation of hypoxia) has shown that IL-1α KO cells are less sensitive to stress induced by reduced serum or glucose starvation. Re-expression of either the IL-1α precursor or the N-terminal IL-1α propiece in the KO cells restored the WT phenotype. Functionality of the IL-1α Nuclear Localization Sequence proved to be critical for this effect. The *In vivo* experiments have demonstrated that KO cell are substantially less tumorigenic than the WT 4T1 cells. **Conclusions:** The results provide new insight into the functional role of the intracellular IL-1α during the breast cancer tumor development and on the level of the malignant cells *ex vivo* and *in vivo*. KO of IL-1α in triple negative breast cancer cell line (4T1) increase the resistance to certain types of stressful conditions while reduces tumorigenicity of the cell *in vivo*.

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Inhibition of MEK1/2 by trametinib sensitizes MAPK driven head and neck cancers to anti-PD-1 immunotherapy

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Introduction: Immune checkpoint blockers that are targeting the programmed cell death protein-1 (PD-1) have become the standard of care for the therapy of patients with recurrent or metastatic head and neck cancer (HNC). However, anti-PD-1 therapies induce durable clinical responses only in a fraction (15–20%) of HNC patients, while most patients exhibit innate resistance. Thus, there exists an urgent need to enhance anti-PD-1 efficacy by exploring therapeutic combination to convert anti-PD-1 resistant tumors into sensitive ones**.** Emerging evidence suggests that tumor immune microenvironment imparts resistance to immunotherapy, and conventional treatment strategy, which has an immunomodulatory action, can be combined to enhance anti-PD1 efficacy. **Aim:** In this work, we aimed to use anti-PD-1 resistant-murine HNC models to uncover innate resistance mechanism to anti-PD-1, and to investigate rationale-based therapeutic combination to transform anti-PD-1 resistant tumors into sensitive ones. **Results:** We have developed two murine cell line models using 4NQO carcinogen-induced tumors from the tongue and lip of immunocompetent mice to study anti-PD1 resistance. Immunotherapy studies using these models demonstrated that these lines are resistant to anti-PD1 therapy. Genomic analysis of these 4NQO lines implicated that they possess KRAS mutation (MAPK pathway-dependent) and several other mutations which are well documented to provide immunotherapy resistance in multiple type of cancers. On further analysis, it was found that in these KRAS mutant cell lines shown to over-expression colony stimulating factor-1 (CSF-1) and this aberrant CSF1 signaling in the stromal compartment increases myeloid responses and reprograms the tumor microenvironment to diminish T cell-mediated anti-tumor immunity. Earlier reports states that KRAS mutation up regulate CSF1 through MAPK pathway. Our HNC mice model is also MAPK (ERK1/2) pathway-dependent and inhibiting this pathway can considerably

change the immune microenvironment favorable for immunotherapy. Hence, we combine MAPK inhibition using a MEK inhibitor, trametinib with anti-PD-1 therapy. Our result showed a promising anti-tumor response on combinatorial therapy. Most tumors were eliminated during combination treatment, and the cured mice were shown to possess immune memory on subsequent re-challenging with tumors. The tumor elimination observed during combinational therapy is through the infiltration of cytotoxic $CD8_+$ T cells. **Conclusions:** The use of the MEK inhibitor trametinib in combination with anti-PD-1 antibodies has significantly increased CD8 T cell infiltration and showed better therapeutic effects than individual treatments in pre-clinical HNC models provide the first progressive indication to use this effective combination for the treatment of HNC cancers possessing KRAS/MAPK mutations. Based on our findings we propose that the combination of a MEK inhibitor and an anti-PD-1 monoclonal antibody would be therapeutically beneficial for HNC patients (18%) with MAPK pathway mutations.

Session 13 at 13:05

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Drug-free polymer conjugates for treating chronic inflammatory diseases

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Endothelial cell adhesion molecules (CAMs) facilitate a multistep process of transendothelial migration of inflammatory cells from the peripheral blood into the inflamed tissues. Although the presence of these inflammatory cells (leukocytes) at the sites of inflammation is crucial in acute tissue damage, it can significantly contribute to the progression of chronic inflammatory diseases. Modulating these vascular-leukocyte interactions has been proposed as a promising strategy to treat many chronic diseases including cancer. We developed a series of vascular targeted polymer-peptide conjugates based on N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer for targeting CAMs (E-selectin, P-selectin, vascular cell adhesion molecule 1 (VCAM-1)) and disrupting their function in inflammatory cells infiltration. HPMA copolymer with multiple copies of high-affinity E-selectin binding peptide (P-Esbp, contains on average 8 copies of peptide per polymer chain) demonstrated strong adherence to E-selectin, (IC_{50} value of 6 nM), and inhibited the adhesion of HL-60 cells to E-selectin. P-Esbp was shown effective in targeting E-selectin on tumor vascular endothelium and inflamed atherosclerotic lesions in mice models. Considering the crucial role of E-selectin in pathogenesis of chronic liver and renal diseases, I further investigated the ability of P-Esbp to inhibit neutrophil recruitment and inflammation in mouse model of alcohol-induced liver injury and renal injury. Pre-treatment of mice with P-Esbp prior to alcohol binge attenuated alcohol-induced serum transaminase (ALT, AST) elevation, reduced pro-inflammatory cytokines (TNFα and IL-1ß) and chemokines (MIP-2/CXCL2 and MCP-1/CCL2). Also, the up-regulation of neutrophil marker Ly6G and the number of MPO positive cells in the injured tissue was significantly reduced by the treatment, indicating diminished neutrophil infiltration. Moreover, as a result of P-Esbp treatment, E-selectin expression in the liver (mRNA and protein level) was downregulated, suggesting a potential to decrease ongoing local inflammatory response[6]. The control polymer with a scrambled version of E-selectin binding peptide, P-(EsbpScrm), did not affect the biochemical, histological and inflammatory markers, confirming the attenuation of alcohol-induced liver inflammation by blocking E-selectin-mediated activity and the potential to treat liver disease associated with inflammation. The anti-inflammatory activity of P-selectin and VCAM-1 binding copolymers (P-Psbp and P-Vcbp) is currently being investigated, alongside P-Esbp, in murine models of kidney inflammation.

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Life-Extended Glycosylated IL-2 Promotes Treg Induction and Suppression of Autoimmunity

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IL-2 is the master-regulator cytokine for T cell dependent responses and is crucial for proliferation and survival of T cells. However, IL-2-based treatments remained marginal, in part due to short half-life. Thus, we aimed to extend IL-2 half-life by flanking the IL-2 core with sequences derived from the extensively glycosylated hinge region of the NCR2 receptor. We termed this modified IL-2: "S2A". Importantly, S2A blood half-life was extended 14-fold compared to the clinical grade IL-2, Proleukin. Low doses inoculation of S2A significantly enhanced induction of Tregs ($CD4_+$ Regulatory T cells) *in vivo*, as compared to Proleukin, while both S2A and Proleukin induced low levels of $CD8_+$ T cells. In a B16 metastatic melanoma model, S2A treatment was unable to reduce the metastatic capacity of B16 melanoma, while enhancing induction and recruitment of Tregs, compared to Proleukin. Conversely, in two autoimmune models, rheumatoid arthritis and DSS-induced colitis, S2A treatment significantly reduced the progression of disease compared to Proleukin. Our results suggest new avenues for generating long-acting IL-2 for long-standing treatment and a new technique for manipulating short-life proteins for clinical and research uses.