**Session I: BASIC BIOLOGY OF PLURIPOTENCY & REPROGRAMMING**

## MOLECULAR AND SIGNALING FOUNDATIONS OF HUMAN NAÏVE PLURIPOTENCY

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Different conditions have been recently devised to isolate MEK/ERK signalling independent human naïve pluripotent stem cells (PSCs) that are distinct from conventional primed PSCs and better correspond to pre-implantation developmental stages. While all the different naïve conditions described thus far endow human PSCs with different extents of naivety features, capturing human pluripotent cells that retain all characteristics of ground state pluripotency captured in rodents and while maintaining genomic and differentiation integrity remains a major challenge. Here we engineer reporter systems that allow multistep functional screening for conditions that can endow both the molecular and functional features expected from human naive pluripotency. We establish that simultaneous inhibition of three defined signaling pathways is essential for enabling expansion of teratoma competent fully naïve human PSCs in defined and xeno-free conditions. Divergent signaling and transcriptional requirements for maintaining naïve pluripotency were found between mouse and human. Finally, we establish alternative naïve conditions in which MEK/ERK inhibition is substituted with inhibition for other unique signaling pathways, that allow obtaining human naïve PSCs with diminished risk for loss of imprinting and excessive global DNA hypomethylation. Our findings set a framework for the signaling foundations of human naïve pluripotency and may advance its utilization in future translational and clinical applications.

## HAPLOID HUMAN EMBRYONIC STEM CELLS: FROM DEVELOPMENTAL BIOLOGY TO GENETIC SCREENINGS

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We have recently generated haploid human embryonic stem (ES) cells from unfertilized human oocytes. The haploid human ES cells exhibited typical pluripotent stem cell characteristics, such as self-renewal capacity and a pluripotency-specific molecular signature. Although haploid human ES cells resembled their diploid counterparts by several aspects, they also displayed distinct properties including differential regulation of X-chromosome inactivation and genes involved in oxidative phosphorylation, alongside reduction in absolute gene expression levels and cell size. Interestingly, we found that a haploid human genome is compatible not only with the undifferentiated pluripotent state, but also with differentiated somatic fates representing all three embryonic germ layers both in vitro and in vivo. Furthermore, we demonstrated the utility of haploid human ES cells for loss-of-function genetic screening by analyzing a haploid gene-trap mutant library. To define the es sentialome of human pluripotent stem cells we generated a genome-wide loss-of-function library in haploid human ES cells utilizing the CRISPR/Cas9 technology using about 180,000 guide RNAs, targeting virtually all coding genes. Using this library, we characterized the essential genes in human pluripotent stem cells, showed the relative role of each cellular compartment in promoting or restricting cell growth, and categorized human genetic disorders according to their role in early embryogenesis. Thus, haploid human ES cells hold a great potential for biomedically-relevant functional genomics to unravel genotype-phenotype interactions in the context of human development and disease.

**A CHEMICAL SCREEN IDENTIFIES BROMODOMAIN INHIBITORS OF CBP/EP300 AS FACILITATORS OF HUMAN CELLULAR REPROGRAMMING**

**Tamer Onder**
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Silencing of the somatic cell-type specific genes is a critical yet poorly understood step in reprogramming. To uncover pathways that maintain cell identity, we performed a reprogramming screen using inhibitors of chromatin factors. Here we identify acetyl-lysine competitive inhibitors targeting the bromodomains of coactivators CBP and EP300 as potent enhancers of reprogramming. These inhibitors accelerate reprogramming, are critical during its early stages and, when combined with DOT1L inhibition, enable efficient derivation of human iPSCs with OCT4 and SOX2. In contrast, catalytic inhibition of CBP/EP300 prevents iPSC formation, suggesting distinct functions for different co-activator domains in reprogramming. CBP/EP300 bromodomain inhibition decreases somatic-specific gene expression, histone H3 lysine 27 acetylation (H3K27Ac) and chromatin accessibility at target promoters and enhancers. The master mesenchymal transcription factor PRRX1 is one such functionally important target of CBP/EP300 bromodomain inhibition. Collectively, these results show that CBP/EP300 bromodomains sustain cell type specific gene expression and maintain cell identity.

**COLOR-CODING THE EARLY MOUSE EMBRYO TO REVEAL ITS SPATIAL ARCHITECTURE**

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Early embryonic development in mammals is implemented through the robust acquisition of specialized cellular properties by individual cells that specify the basic embryonic lineages (gastrulation) and later form the first organs (organogenesis). Recent breakthroughs in transcriptional profiling produce maps of embryonic cell lineages with single-cell resolution, allowing unprecedented insight into cell-lineage diversification and developmental dynamics. However, such datasets lack information regarding the original spatial position of individual cells. Specifically, the rapid nature of transcriptional changes and the morphological characteristic of the post-implantation embryo poses unique technical challenges that still preclude full understanding of these key events.

To overcome these obstacles, we developed a novel method that uses fluorescent dyes to “index” spatial information in the early embryo. Screening for candidate dyes using zebrafish embryos and embryonic stem cells, identified ideal molecules that are both tissue-permeable and retained following single-cell dissociation. We further established suitable techniques for delivering the dyes into live embryos, which were subsequently imaged and subjected to single-cell transcriptional analysis. Initial analysis of single-cell data obtained from E7.5 embryos enabled us to associate specific cell populations to their predicted spatial position. To this end, we are developing a probabilistic framework for embedding spatially tagged single cells into a physical model of the developing embryo. Briefly, we infer probability distributions for the localization of each transcriptional cell types by integrating prior knowledge and experimental information from registered dyes intensities. Using the resulting profiles, facilitates mapping cell lineages to multiple sub-compartments in the developing embryo and determining how initially identical lineages diverge as a function of spatial position. In combination with perturbation experiments, our approach will allow disentangling the relative roles of intrinsic cellular processes from extrinsic effects of the localized environment upon cell-fate choices.

**THE PROCESS OF MANUFACTURING HUMAN INDUCED PLURIPOTENT STEM CELLS FOR CLINICAL APPLICATIONS**

Ejona Rusha[1], Polyxeni Nteli[1], Anna Pertek[1], **Friederike Matheus[1]**, Torsten Tonn[2,3], Martin Hildebrandt[4], Sebastian Knoebel[5], Sebastian Diecke[6], Thure Adler[7], Micha Drukker[1]
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The manufacturing of human induced pluripotent stem (iPS) cell lines according to clinical standards is fundamentally important for autologous and allogeneic regenerative therapies globally. This sector is growing rapidly and received an investment of over one billion US dollars over recent months for creating biotech companies that specialize in cell therapies. Despite this, there is little information regarding the suitability of iPS cell lines that were, and are, being manufactured in GMP facilities for receiving the necessary authorizations to be used as a "starting material" in process of production of clinical grade differentiated cells.
We have created a roadmap, prototyped and received regulatory approvals to manufacture autologous and allogeneic iPS cells as starting materials for manufacturing of differentiated cells for clinical applications. The unique features of our manufacturing process are 1. the use of fibroblasts as a starting material, as apposed to blood mononuclear cells by other programs, meaning that our cells will likely fit better neuronal differentiation; 2. the use of mRNAs to transiently express reprogramming factors, as apposed to use of viruses by other programs, which create high risk; and 3. we selected several donors that are collectively immunologically matched to dozens of mullions of individuals from European ancestry.
During the lecture, we will explain the principles of risk assessment that guide the qualification of reagents, instruments, and processes for the manufacturing of clinical grade human iPS cells under GMP standards. We will furthermore illustrate the criteria that underlie healthy donor selection and tissue procurement for the generation of human iPS cells that can be used as starting material in cellular therapies. Finally, we will explain critical regulatory considerations in the manufacturing of iPS cells and their differentiation for creating clinical grade cell products.

**Session 2: STEM CELLS AND PATHOPHYSIOLOGY**

## IDENTIFICATION OF A LATENT RESIDENT PROGENITOR POPULATION IN THE ADULT TENDON

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Tendons connect and transfer force between the muscles and bones of the body, making them highly prone to injury. These injuries have imperfect healing, resulting in scarring and reduced mobility. Progress in the tendon field has been limited by a lack of markers to identify tendon progenitor cells in vivo. To impact regenerative medicine approaches to treat tendon injury and degeneration, it is essential to identify the cell types and pathways responsible for tendon repair mechanisms. In our study, we first sought to determine the cell turnover rates during growth, homeostasis and injury using the inducible TetO-H2B-GFP system and EdU/BrdU labeling. We found higher proliferation rates during postnatal stages prior to 1 month of age, and significantly lower turnover rates (>5%) after 1 month of age, which indicate a transition from cell growth to physiological homeostasis in the limb tendons. By combining the TetO-H2B-GFP pulse-chase method with Axin2-CreErt2 labeling, we found that 10% of the adult tendon cells were labeled by Axin2 and that this distinct subset were latent slowly cycling cells. Axin2+ cells in culture were enrichmed for tendon genes and had higher proliferation rates compared with Axin2- cells. Lineage tracing experiments in an Achilles tendon injury model showed that Axin2+ cells were the predominant cell population at the healed site, which proliferated and expressed Scleraxis (Scx)-GFP, a marker of tendon fates. Deletion of Porcupine, a factor necessary for Wnt secretion, in Scx-lineage cells resulted in impaired healing. Surprisingly, Axin2-CreERt2 deletion of Porcupine resulted in a similarly disrupted healing response with significantly decreased proliferation, gene expression, and Axin2+ cell infiltration. These data suggest the possibility that Axin2+ cells initiate their own healing response through autocrine signaling mechanisms. Our work identifies Axin2+ cells as a unique progenitor cell type in the adult tendon that are central mediators of tendon healing.

## SUBEPITHELIAL TELOCYTES CONSTITUTE THE INTESTINAL STEM CELL NICHE

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Stem cell niches provide essential signals and growth factors to sustain proliferation and self-renewal of stem cells in continuously self-renewing organs such as the intestine. We identify large mesenchymal cells expressing the winged-helix transcription factor forkhead box l1 (FOXL1) and the surface platelet derived growth factor a (PDGFRa). These cells are telocytes which have a unique cell structure with long processes that extends hundreds of micrometers . FOXL1+ telocytes cover the entire gut epithelium from crypt base into the villus tips and are expressing key signaling pathway molecules such as members of the Wnt, BMP, Shh, FGF and TGFb gene families in a localized fashion. Inhibition of Wnt proteins secretion from FOXL1+ telocytes causes loss of proliferating cells in the crypt compartment, rapid crypt collapse, and death of the mutant mice within a few days. Thus, FOXL1+ telocytes constitute the intestinal stem cell niche which is absolutely required for stem cell function.

**Session 3: STEM CELL DIFFERENTIATION AND DEVELOPMENT**

## TRANSLATING HUMAN DEVELOPMENT TO NEW THERAPIES WITH HUMAN PLURIPOTENT STEM CELLS

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Human pluripotent stem cells (hPSCs) provide an unlimited source of cells for modeling human cardiovascular development and disease in vitro and for developing new therapies to replace heart tissue damaged by age or disease. To be able to successfully study and treat diseases that affect specific regions of the heart, it is essential to be able to generate the appropriate target population from hPSCs in vitro. We have used a developmental biology-guided approach to identify the key signaling pathways that promote the efficient differentiation of ventricular and atrial cardiomyocytes in vitro. In these studies, we found that retinoic acid (RA) signaling at the mesoderm stage of development is essential for specifying the human atrial lineage. Additionally, we show that atrial and ventricular cardiomyocytes develop from distinct mesodermal subpopulations that can be identified by the expression of retinaldehyde dehydrogenase 2 (RALDH2), an enzyme involved in RA synthesis and glycophorin A (CD235a), respectively. These mesoderm populations are induced by different concentrations of BMP and Activin/Nodal signaling. The RALDH2+ atrial mesoderm, but not the CD235a+ ventricular mesoderm responds to retinol (RA precursor) to generate atrial cardiomyocytes indicating that this lineage is specified through an autocrine signaling loop during human heart development. Further molecular and functional analyses have shown that the generation of optimal atrial and ventricular cardiomyocyte populations depends on appropriate mesoderm specification. Taken together, these findings provide strong evidence that the human atrial and ventricular lineages segregate early at the mesoderm stage of development. With these developmental insights, we are now able to generate highly enriched atrial or ventricular cardiomyocyte populations for disease modeling and cell therapy applications.

## DIFFERENTIATING EMBRYONIC STEM CELLS TOWARDS THE SOMATIC CELL TYPES OF THE GONADS

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Sex determination is the decision as to whether an organism will develop as male or female. During embryonic development in mammals, the bipotential gonad can follow either a testicular (in XY) or an ovarian (in XX) cell fate, depending on the expression (and repression) of key transcription factors.
Disorders of Sex Development (DSD), which affect 1: 2500-4000 newborns, are common birth defects in human.
As of today, there is no system that allows the study of mouse sex determination or human DSD pathologies *in vitro*. Therefore, developing an *in vitro* system that closely resembles the *in vivo* state, for both human and mice, will allow us to better study the process of sex determination as well as explore in a human-related context the mechanisms behind mutations leading to DSD pathologies. Here, we developed a robust and gradual differentiation protocol that mimics the *in vivo* process of gonadal development. Mouse Embryonic Stem Cells (mESCs) are first differentiated to Epiblast stem cells (EpiSC) then to

early mesoderm, followed by intermediate mesoderm (IM- the lineage from which the gonad develop) and finally, into early gonadal progenitors. This protocol relies solely on modifying culture conditions and growth factors. Conversion of this protocol to the use with human Induced Pluripotent Stem Cells (iPSC) from health XY and XX individuals as well as 46, XY female DSD patient indicate that this system can be used to model DSD pathologies *in vitro*.

**Session 4: NEUROSTEM CELLS**

## PLURIPOTENT STEM CELL DERIVED MODELS OF NEUROLOGICAL DISEASES REVEAL EARLY TRANSCRIPTIONAL HETEROGENEITY

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Many neurodegenerative diseases (NDs) develop only later in life, when cells in the nervous system lose their structure or function. In genetic forms of NDs, this late onset phenomenon remains largely unexplained. Here we used juvenile forms (72Q; 180Q) of Huntington's disease (HD) iPSCs, differentiated them into neuronal progenitors, and obtained single cell expression profiles. We show a global increase in gene expression variability in HD. Autophagy genes become more stable, while energy and actin-related genes become more variable in the mutant cells. Knocking-down several differentially-variable genes resulted in increased aggregate formation, a pathology associated with HD. We further validated the increased transcriptional heterogeneity in CHD8+/- cells, a model for autism spectrum disorder. Overall, our results suggest that although NDs develop over time, transcriptional regulation imbalance is present already at very early developmental stages. Therefore, an intervention aimed at this early phenotype may be of high diagnostic value.

## ENHANCEMENT OF CARDIOVASCULAR REGENERATION BY DIRECT CARDIAC REPROGRAMING OF NON-CARDIOMYOCYTES INTO CARDIOMYOCYTES-LIKE CELLS USING COMBINATORIAL MODIFIED MRNAS APPROACH

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**Background:** Ischemic heart disease remains a major cause of morbidity and mortality in the western world causing a significant societal and economic burden. The fundamental problem is the massive loss of cardiomyocytes (CMs) post-myocardial infarction (MI) that gets replaced by cardiac fibroblasts and scarring. Reprogramming of cardiac fibroblasts into CM-like cells in vivo represents a promising strategy for cardiac regeneration; however, the traditional use of viral delivery method hampered its application into clinical settings due to the uncontrolled and low efficiency of the delivered genes.
**Methods:** We used a modified mRNA (modRNA) gene delivery platform to deliver cardiac reprogramming genes together with reprogramming supportive genes at different ratios, in vitro and in vivo.
**Results:** We found that a combination of cardiac reprogramming and supportive genes (7G) modRNA, delivered to sorted, non-CMs cells, every 3 days for 14 days, doubled cardiac reprogramming efficiency (57%) in comparison to the traditional known reprogramming genes (27% for Gata4, Mef2c and Tbx5) and further 28 days of repeated transfection resulted in few beating CMs in vitro. Moreover, one time delivery of 7G at the time of MI in lineage tracing mouse model indicated that 35% of the non-CMs in the scar area were reprogramed to CM-like cells, 28 days post-MI. In addition, delivery of 7G in mice with MI showed significant improvement in cardiac function and capillary density, while scar size was reduced. Mechanistically, we showed that 7G leads to significant upregulation of 16 key

angiogenic factors (including VEGF-A, HGF and Angiopoietin1) 28 days post-MI. Interestingly, the increased ratio of one of the reprogramming genes further enhanced angiogenic gene expression in MI mice compared with those exposed to 7G modRNA cocktail. **Conclusions:** We showed modRNA cocktails leads to non-CMs reprogramming into CM-like cells by promoting cardiovascular regeneration post-MI.

**IN VIVO VISUALIZING THE FATE OF INTRA-ARTICULAR INJECTED MESENCHYMAL STEM CELLS DURING TREATMENT OF SUPRASPINATUS TENDON TEARS**

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Supraspinatus tendon (ST) tear is a widespread disorder characterized by functional deficits in the shoulder such as weakness, decreased range of motion, and debilitating pain. Mesenchymal stem cell (MSC) therapy provides a unique opportunity for redirecting the healing process away from scar formation and toward the regeneration of a fibrocartilaginous tendon-bone insertion (enthesis). However, MSC therapy remains underutilized and perhaps underrated due to the limited evidence of dynamic visualization of cellular behavior in vivo. Here, second near-infrared fluorescence imaging with biocompatible PbS quantum dots (QDs) provides a cellular migration map and information on the biodistribution and clearance processes of three densities of intra-articularly injected, labeled MSCs to treat supraspinatus tendon tear in mice. Intra-articular injection avoids entrapment of MSCs by filter organs and reduces the QD-induced organ toxicity. Notably, the MSCs share a similar migration direction, but the moderate density group is somewhat more efficient, showing the longest residence time and highest cell retention rate around the footprint during the repair stage. Furthermore, quantitative kinetic investigation demonstrates that labeled MSCs are cleared by feces and urine. Histomorphometric analysis demonstrates that the moderate density group achieves maximum therapeutic effect and labeled MSCs do not induce any injury or inflammation to major organs, which suggests that administration of too many or few MSCs may decrease their effectiveness. Such an imaging approach provides spatiotemporal evidence for response to MSC therapy in vivo, facilitating the optimization of MSC therapy

**Session 5: HEMATOPOEIESIS & EPIGENETICS**

## DAILY LIGHT AND DARKNESS ONSET METABOLICALLY REGULATE BLOOD AND BONE FORMING STEM CELLS: THE ROLE OF BM NOREPINEPHRINE, TNF AND MELATONIN

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Hematopoietic stem and progenitor cells (HSPC) are essential for daily mature blood cell production, host immunity and osteoclast mediated bone turnover. The timing at which stem cells give rise to mature blood and immune cells while maintaining the bone marrow (BM) reservoir of undifferentiated HSPC and how these opposite tasks are synchronized is poorly understood. Previous studies revealed that daily light onset activates norepinephrine (NE) induced BM CXCL12 downregulation, followed by CXCR4+ HSPC release to the circulation. Recently, we reported that daily light onset induces transient elevations of BM NE and TNF, which metabolically program BM HSPC differentiation and recruitment to replenish the blood. In contrast, darkness onset induces lower elevations of BM NE and TNF, activating melatonin production, which metabolically reprogram HSPC, increasing their short and long-term repopulation potential, and BM maintenance. How the functions of BM retained HSPC are influenced by daily light and darkness cycles and their clinical potential are further discussed.

## EVOLUTIONARY ORIGIN OF THE MAMMALIAN HEMATOPOIETIC AND IMMUNE SYSTEMS FOUND IN A COLONIAL CHORDATE

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Hematopoiesis is an essential process that evolved in multicellular animals. At the heart of this process are hematopoietic stem cells (HSCs), which are multipotent, self-renewing and generate the entire repertoire of blood and immune cells throughout life. Here we studied the hematopoietic system of *Botryllus schlosseri*, a colonial tunicate that has vasculature, circulating blood cells, and interesting characteristics of stem cell biology and immunity. Self-recognition between genetically compatible *B. schlosseri* colonies leads to the formation of natural parabionts with shared circulation, whereas incompatible colonies reject each other. By means of flow-cytometry in combination with screened antibodies by Cytof, lectins, and fluorescent enzymatic reagents, we isolated 34 *B. schlosseri* cell populations. Using whole-transcriptome sequencing of defined cell populations, and diverse functional assays, we identified HSCs, progenitors, immune-effector cells, and the HSC niche. Our study implies that the HSC and myeloid lineages emerged in a common ancestor of tunicates and vertebrates and suggests that hematopoietic bone marrow and the *B. schlosseri* endostyle niche evolved from the same origin. Furthermore, we identified a *B. schlosseri* cytotoxic cell population originating from large granular lymphocyte-like cells and demonstrated that self-recognition inhibits cytotoxic reaction.

**EPIGENETIC PROGRAMMING OF MUSCLE STEM CELLS**

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One of the most obvious manifestations of body homeostasis is that many tissue types are capable of undergoing renewal following local injury as occurs with wound healing, muscle replacement or liver regeneration following partial hepatectomy. Despite extensive work characterizing these molecular biological and physiological events, little is known about the epigenetic changes that take place during stem cell activation. We have used whole-genome analysis to examine the methylation changes that take place in satellite cells following muscle injury. The results suggest that these stem cells undergo massive programming to generate a new methylation pattern. These stem cells then retain this epigenetic signature for a long time period, even after they have returned to their quiescent expression phenotype. This new underlying structure, however, is probably what allows them to produce terminal muscle cells in a more rapid and efficient manner when responding to a second bout of injury.

**DELETION OF CTG EXPANSION IN MYOTONIC DYSTROPHY TYPE 1 (DM1) REVERSES ABERRANT METHYLATION IN HUMAN EMBRYONIC STEM CELLS BUT NOT AFFECTED MYOBLASTS**

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Myotonic dystrophy type 1 [DM1, (OMIM 160900)] is an autosomal dominant form of muscular dystrophy that affects a wide range of body systems. It results from a CTG repeat expansion (50 – >3,000 triplets) in the 3'-UTR of the dystrophia myotonica protein kinase gene (DMPK). When the CTGs extensively expand, it results in DMPK aberrant methylation, reduction in mRNA of a downstream gene neighbor, SIX5, and the development of the congenital and most severe form of the disease (CDM).

To explore whether hypermethylation could be reversed in DM1 hESCs and patient myoblasts, we monitored methylation levels following deletion of the CTGs by gene editing using dual CRISPR/Cas9 approach. We show the excision of the repeats in undifferentiated hESCs (CTG2000) resets the locus by abolishing abnormal methylation and H3K9me3 enrichments, and restoring SIX5 transcription. This is in striking contrast to affected myoblasts, where methylation levels (100%) remain unchanged following deletion of a large repeat expansion (CTG2600).

Altogether, we provide evidence for a shift from a reversible to an irreversible heterochromatin state by large enough expansions as a consequence of cell differentiation; hence, the correction of the mutation in cells of patients may not be enough as a therapeutic approach in the event of CDM, as originally thought. Furthermore, our data imply that mutant hESCs may not be an appropriate platform for drug screening and development to target the undesired epigenetic modifications that are elicited by the mutation. More generally, the findings of this study may have much wider implications as they may apply to all other disease-causing mutations that correspond with a change in DNA methylation, including other pathologic noncoding repeat expansions, parental imprinting defects and various chromatin modifying enzymes.

**DISSECTING THE FUNCTIONAL ROLES OF PARENT-SPECIFIC DNA METHYLATION DYNAMICS DURING EARLY EMBRYONIC DEVELOPMENT**

**Ariella Weinberg-Shukron**[1], Raz Ben-Yair[1], Alon Shtrikman[1], Ayelet-Hashahar Orenbuch[1], Nozomi Takahashi[2], Anne Ferguson-Smith[2,3], Yonatan Stelzer[1]
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Fertilization in mammals initiates extensive remodeling of the epigenome that includes genome-wide removal of DNA methylation. In a notable exception to this global trend, several parent-specific differentially methylation regions (DMRs) escape demethylation, thus establishing epigenetic memory (parental imprinting) that will persist to the adult soma. As global methylation patterns are regained in a cell-dependent manner in the post-implantation embryo, these germline-derived DMRs serve as *cis*-acting foci to establish ''secondary'' DMRs at the promoters of imprinted genes. While parental imprinting is essential for mammalian development, the functional significance of secondary DMRs and their hierarchical interactions with primary DMRs remain to be elucidated. Here, we set out to systematically dissect the functional roles of germline and secondary DMRs in regulating imprinting *in vivo*. For this purpose, we established a novel conditional mouse that allows temporal excision of an intergenic germline DMR that regulates imprinting at the Dlk1-Dio3 locus. Deleting the germline DMR in the sperm caused a paternal-to-maternal switch in methylation and gene expression and resulted in significantly smaller embryos with early postnatal lethality. Unexpectedly, we further identified variable methylation levels at the secondary DMR which positively correlated with embryo size and postnatal survival, suggesting a crucial role for the secondary DMR in post-implantation development. Strikingly, paternal deletion of the germline DMR in E5.5 embryos caused a much more severer phenotype, compared with germline deletion of the same region, resulting in early embryonic lethality. Taken together these results suggest a crucial time-dependent hierarchical interaction between the germline and somatic DMRs peaking at E5.5 when the secondary DMR is established. Our results shed light on the functional roles of parent-specific methylation during development and expand our understanding of imprinting dynamics and parental epigenetic memory.

**FIGHTING AGAINST PROMOTER DNA HYPER-METHYLATION: PROTECTIVE HISTONE MODIFICATION PROFILES OF STRESS-RESISTANT INTESTINAL STEM CELLS**

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Nowadays stem cell research demonstrates stem cell heterogeneity in many tissues with subpopulations being particular resistant to genomic stress. As an example, stress-sensitive intestinal stem cell (ISCs) have been shown to become replaced by more resistant populations following moderate irradiation. Here, we asked for specific epigenetic profiles of such subpopulations.
Performing ChIP-seq, we observed histone modification changes in the intestine weeks after irradiation and/or following Msh2 loss. Among the common changes, we identified H3K4me3 recruitment to the promoter of H3K27me3 target genes. By RNA-seq, we demonstrate that this recruitment surprisingly occurs without changes of the average transcription of affected genes.
Applying a mathematical model of epigenetic regulation of transcription, we show: i) that H3K4me3 recruitment can be explained by stronger DNA binding of H3K4me3 and H3K27me3

histone methyl-transferases as a consequence of lower DNA methylation and ii) that the recruitment is capable of protecting the genes' promoter against hyper-methylation during DNA repair. The scenario also implicates stable transcription despite of H3K4me3 recruitment, in agreement with our RNA-seq experiments.

Recently, we have suggested that promoter DNA hyper-methylation originates in DNA repair and that even successful DNA repair might confer this kind of epigenetic long-term changes. Our present results suggest that stress-resistant ISCs are largely protected against promoter hyper-methylation of H3K27me3 target genes.

**Session 6: CLINICAL TRANSLATION OF STEM CELLS**

**EPITHELIAL STEM CELLS IN CELL AND GENE THERAPY**

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LAMB3-dependent generalized Junctional Epidermolysis Bullosa (JEB)was targeted by transplantation of epidermal cultures originated from transgenic epidermal stem cells.Wereport life-saving regeneration of the entire epidermis on a seven-year-old JEB child suffering from a devastating form of JEB.The regenerated transgenic epidermis remained stable throughout the entire follow-up period and did not form blisters, even upon shear force. The proviral integration pattern was maintained in vivo and epidermal renewal did not cause any clonal selection. Clonal tracing showed that the human epidermis is sustained by a limited number of long-lived stem cells, detected as holoclones, that can extensively self-renew and produce short-lived progenitors that replenish terminally differentiated keratinocytes.
In studying the different behaviour of JEB and COL7A1-dependent generalized Dystrophic EB (RDEB) cultures we discovered a pivotal role of YAP in sustaining human epidermal stem cells, which explains the progressive stem cell loss observed in JEB. Epidermal stem cell depletion of primary JEB keratinocytes is due to perturbation of the YAP/TAZ pathway. YAP/TAZ expression is significantly decreased in JEB keratinocytes, which do not contain nuclear YAP but only phosphorylated, inactive YAP. The JEB phenotype is recapitulaled by Laminin 5 ablation and consequent YAP/TAZ down-regulation in normal cells. Restoration of adhesion properties by Laminin 5-gene therapy rescues normal nuclear levels of YAP/TAZ and clonogenic potential. Enforced YAP recapitulates Laminin 5-gene therapy in JEB cells, thus uncoupling adhesion from proliferation in epidermal stem cells. This work has important clinical implication for an efficient ex vivo gene therapy of JEB.

**TISSUE-LEVEL MECHANOSENSITIVITY: PREDICTING AND CONTROLLING THE ORIENTATION OF 3D VASCULAR NETWORKS**

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Understanding the mechanosensitivity of tissues is a fundamentally important problem having far-reaching implications for tissue engineering. Here we study vascular networks formed by a co-culture of fibroblasts and endothelial cells embedded in three-dimensional biomaterials experiencing external, physiologically-relevant forces.
We show that cyclic stretching of the biomaterial orients the newly formed network perpendicularly to the stretching direction, independently of the geometric aspect ratio. A two-dimensional theory explains this observation in terms of the network's stored elastic energy if the cell-embedded biomaterial features a vanishing effective Poisson's ratio, which we directly verify. We further show that under static stretch vascular networks orient parallel to the stretching direction due to force-induced anisotropy of the biomaterial polymer network. Moreover, static stretching followed by cyclic stretching reveals a competition between the two mechanosensitive mechanisms.
Furthermore, The two cell types show distinctly different sensitivities to mechanical stimulation. The fibroblasts, sense the stress directly and respond by increased alignment,

proliferation, differentiation, and migration, facilitated by YAP translocation into the nucleus. In contrast, the endothelial cells form aligned vessels by tracking fibroblast alignment.
These results demonstrate tissue-level mechanosensitivity and constitute an essential step toward developing enhanced tissue repair capabilities using well-oriented vascular networks.

**A SWITCH IN NUTRIENT SENSING TRIGGERS MATURATION OF BETA CELLS**

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A drastic transition at birth, from constant maternal nutrient supply in utero to intermittent postnatal feeding, requires functional changes in the metabolic system. Despite their central role in metabolic homeostasis, little is known about how pancreatic beta cells adjust to the new nutritional program. Here, we describe a role of nutrient-sensing in controlling beta cell maturation. Our experiments show that after birth beta-cells undergo a functional shift from amino acid- to glucose-stimulated insulin secretion that correlates with the nutritional environment of the animal. This metabolic adaptation is mediated by a switch in nutrient sensitivity of the mTORC1 pathway, which leads to intermittent mTORC1 signaling activity after birth. Disrupting nutrient sensitivity of mTORC1 in mature beta cells, via deletion of upstream nutrient-responsive inhibitors, affects insulin secretion and reverts them to an immature functional state. Finally, we show that manipulating the nutrient sensitivity of mTORC1 in stem cell-derived beta cells in vitro strongly enhances their glucose-responsive insulin secretion. These results reveal an important mechanism for the application of stem cell-derived tissues in regenerative medicine by which nutrient sensing and mTORC1 signaling regulate functional maturation, thereby enabling a metabolic adaptation to a mature environment.

**SINGLE CELL MECHANICAL ANALYSIS OF HUMAN PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES FOR DRUG TESTING AND DISEASE MODELING**

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**Background:** The advent of human pluripotent stem cell–derived cardiomyocytes (hPSC-CMs) provided exciting tools for cardiovascular physiological studies, disease modeling and drug testing applications. Current platforms for studying the mechanical properties of human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) as single-cells do not measure forces directly, require numerous assumptions, and cannot study cell mechanics at different loading conditions.
**Objective:** To establish a novel platform to assess the active and passive mechanical properties of single-cell hPSC-CMs at different loading conditions and to demonstrate the potential of this approach for drug testing and disease modeling applications.
**Methods and Results:** To allow morphological maturation, hPSC-CMs were treated with Tri-iodo-thyronine hormone, Insulin-like growth factor-1 and dexamethasone. The hPSC-CM were then lifted and attached to a highly sensitive optical-force transducer and a piezoelectric length controller and electrically-stimulated. The attached hPSC-CM remained intact and contractile allowing evaluation of their passive and active mechanical properties. Utilizing this technique, single-cell hPSC-CMs exhibited positive length-tension (Frank-Starling) relationships, and appropriate inotropic, klinotropic, and lusitropic changes in response to treatment with isoproterenol. The unique potential of the approach for drug testing and disease modeling was exemplified by treating the cells with doxorubicin (a potential

cardiotoxic anti-cancer agent) and omecamtiv mecarbil (a positive ionotropic drug currently in stage 3 clinical trial). The results of these studies recapitulated the drugs' known actions to suppress (doxorubicin) and augment (omecamtiv mecarbil at low dose) cardiomyocyte contractility. Finally, novel insights were gained regarding the cellular effects of these drugs as doxorubicin treatment led to cellular mechanical alternans and high doses of omecamtiv mecarbil suppressed contractility and worsened the cellular diastolic properties.
**Conclusion:** A novel method that allows direct active and passive force measurements from single hPSC-CMs at different loading conditions for the first time was established and validated. Our results highlight the potential implications of this novel approach for pharmacological studies and disease modeling studies.

**MODELING OF ANIRIDIA-RELATED KERATOPATHY AND IDENTIFICATION OF NOVEL DRUGS FOR POTENTIAL TREATMENT**

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Haploinsufficiency of PAX6 in humans is the main cause of congenital aniridia, a rare eye disease characterized by iris hypoplasia and reduced visual acuity. Patients have also progressive disorders including cataract, glaucoma and corneal abnormalities making their condition very challenging to manage. Aniridia-related keratopathy (ARK), caused by a combination of factors including limbal stem-cell deficiency, impaired healing response, abnormal differentiation, and infiltration of conjunctival cells onto the corneal surface, affects up to 95% of patients. It usually begins in the first decade of life resulting in recurrent corneal erosions, sub-epithelial fibrosis with corneal decompensation and opacification. Unfortunately, current treatment options for aniridia patients are currently limited. Although animal models partially recapitulate this disease, there is no in vitro cellular model of AKT needed for drug/therapeutic tools screening and validation. We used genome editing to introduce a nonsense mutation into one allele of the PAX6 gene in limbal cells, a reservoir of stem cells able to differentiate into corneal cells. The mutated cells displayed reduced cell proliferation and cell migration but enhanced cell adhesion. Known PAX6 targets expression was also altered. By High Throughput Screening of a FDA-approved library, we identified two small compounds, already used as anti-psychotic in medicine, that rescued both PAX6 endogenous protein level, PAX6-target gene expression and altered cell migration of the mutant limbal stem cells. In vivo validation is under way. Since these two compounds are already used in psychiatric medicine, their repurposed use to treat topically aniridia eye could become realistic as soon as validated in vivo for efficacy and safety on aniridia-like mice.

**GENERATING A HUMAN DERIVED ESOPHAGEAL RAFT CULTURES FOR THE STUDY OF DEVELOPMENT AND DISEASE**

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The esophagus is composed of squamous epithelium enclosed in layers of mesenchyme, including muscles, blood vessels and innervating enteric neurons, that is required to transport food from the pharynx to the stomach.

Perturbation of the esophagus epithelium is hallmark of esophageal diseases, with or without mesenchymal or neural involvement. Our knowledge regarding these conditions and our effort in finding therapeutic solutions are limited due to the differences between mouse and human esophagus and the absence of good human derived esophageal cultures.

Recently, two protocols for the directed differentiation of human pluripotent stem cells (PSC) into 3D human esophageal organoids (HEO) and raft cultures were published (Trisno et al. 2018, Zhang et al. 2018). While these cultures have been excellent systems to study esophageal epithelium, they lack any contribution of mesenchyme thus limiting the study of epithelium-mesenchyme interaction during esophagus development and esophageal diseases such as Epidermolysis Bullosa and Esophagus Squamous Cell Carcinoma.

We are developing several approaches to include mesenchyme in human esophageal epithelial cultures. In one approach we are generating mesenchyme separately and incorporating it into esophageal organoids. In another approach we have modified human PSC differentiation conditions to generate esophageal raft cultures that contain a layer of epithelium and mesenchyme. We will use these more physiologic systems to study development and diseases of the esophagus and for developing esophageal transplantation models.

**Session 7: ONGOING CLINICAL TRIALS WITH CELL THERAPY**

## STEM CELL THERAPY IN CHILDREN WITH DUCHENNE MUSCULAR DYSTROPHY. PHASE I–II PEDIATRIC CLINICAL TRIAL

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**Purpose:** Duchenne muscular dystrophy (DMD) is an X-linked recessive disorder that ultimately leads to progressive muscle degeneration. The main purpose of this study was to investigate the effects of allogeneic Wharton jelly-derived mesenchymal stem cells therapy in children with DMD

**Patients and methods:** Four ambulatory and five nonambulatory male patients were included in this study. Real time PCR and immunohistochemical analysis were used to analyze quantitaive dystrophin gene expression. All patients were treated with 2 × 106 cells/kg dose of allogeneic Wharton jelly-derived mesenchymal stem cells via intra-arterial and intramuscular multiple local administration. Before and after treatment, all patients followup tests, including respiratory function tests, cardiac measurements with ejection fraction, clinical scale tests, MR images of the muscles and quantitaive muscle strength measurement were done

**Results:** Increased myoblastic signal intensity was noted in T1 weighted MRI of the vastus intermedius muscle in one patient. Chimerism effects were detected in 6.7 % and fused cells were noted in 3% in all muscle biopsies by FISH analysis. Dystrophin gene expressions were noted in post-treatment biopsies by using real time qPCR and immunoctychemistry staining. Significant corroletions were noted between improved pulmonary function test and dystrophin gene expression in post-treatment muscle biopsies During clinical trial, one of the pediatric patients developed cardiac insufficiency and he was treated with allogenic stem cell by using intracoronal microcatheter angiography

**Conclusion:** In our pediatric clinical trial, phase I / II study, we have been able to show quantitative increased dystrophin protein level in muscle bıopsies after stem cell therapy, by using real time PCR method. Our immunocytochemistry staining also support these findings as well. In this clinical study, we have important steps achieved first time in pediatric patents with DMD who were treated with stem cell. These are the first in pediatric based clinical trials.

**Session 8: STEM CELLS AND CANCER**

## CANCER-RELATED MUTATIONS IDENTIFIED IN PRIMED AND NAIVE HUMAN PLURIPOTENT STEM CELLS

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Human pluripotent stem cells (hPSCs) are known to harbor chromosomal aberrations that might affect their tumorigenic potential. More recently, point mutations in the gene coding for the p53 tumor suppressor (*TP53*) have been found in hPSCs. These mutations gradually take over the culture, suggesting they provide a growth advantage in vitro. However, it remains unclear whether other cancer-related genes acquire recurrent mutations during hPSC propagation. Here we established a strategy to identify such mutations by comparison of genomic data from early and late passage hPSCs. Analysis of over 170 samples (from 46 studies) of the two most commonly used hPSC lines revealed mutations in over 20 verified cancer-related genes other than *TP53*. Similar mutations were found in analysis of over 400 induced pluripotent stem cell samples (from 24 studies). Importantly, naive hPSCs were found to harbor four-times more cancer-related mutations on average than their primed counterparts. These mutated genes corresponded to the mechanisms of action of the chemical inhibitors inducing a naive state, suggesting that selective pressures imposed by them resulted in increased cancer-associated mutational burden. Together, our results suggest that prolonged culturing and pluripotent cell state transition enhance hPSC cancer-related mutagenesis. These mutations should be taken into consideration in future applications, especially in clinical contexts.
(This work has been accepted for publication in *Cell Stem Cell*)

## MOLECULAR SIGNATURES OF CHORDATE DEVELOPMENT: TWO DISPARATE PATHWAYS, ONE CHORDATE

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Sexual development in chordates is well-described by embryogenesis. Other developmental pathways including asexual reproduction and whole body or tissue regeneration differ in origin but establish the same body axes, morphogenetic patterning and organ formation. Although studies have identified conserved aspects of embryogenesis across and within phyla, this pathway has not been linked to the other developmental pathways. In particular, it is

unknown whether and how organogenesis differs during sexual, asexual and regenerative processes, how the stem cells that mediate them differ and if convergent morphology implies convergent molecular mechanisms. Colonial tunicates provide a key to answering these questions, they are unique amongst chordates in possessing two disparate developmental pathways that produce the adult body, either sexually through embryogenesis, or through a stem cell mediated asexual renewal termed, blastogenesis. Using the model organism Botryllus schlosseri we have combined transcriptome sequencing of major embryonic and blastogenic stages and multiple tissues and stem cell populations with confocal, two-photon and electron microscopy to characterize the molecular and morphological signatures along both developmental pathways. We identify de novo periods of transcriptional transition and shared molecular characteristics including stem cell associated transcription factors. We also identified the developmental origin of hematopoiesis, germ cells and central nervous system organogenesis timeline. This study generated the most complete gene profile database produced to date on the entire embryogenesis process and the first to describe asexual development to a similar resolution. By combining microscopy with transcriptome sequencing, it demonstrates the extent to which convergent morphology implies convergent molecular mechanisms and reveals the basic principles and evolutionary conserved elements of chordate development. It also uncovered the exact time when tissue specific precursor cells emerge in both developmental pathways, suggesting a link between embryonic and adult tissue specific stem cells.

**A UNIQUE CROSSTALK BETWEEN TUMOR CELLS AND HEMATOPOIETIC STEM CELLS REVEALS A MYELOID SIGNATURE CONTRIBUTING TO METASTASIS**

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Metastasis is the major cause of death in cancer patients. Recent studies have demonstrated that the crosstalk between different host and tumor cells in the tumor microenvironment regulates tumor progression and metastasis. Specifically, immune cell myeloid skewing is a prominent promoter of metastasis. While previous studies have demonstrated that the recruitment of myeloid cells to tumors is a critical step in dictating tumor fate, the reservoir of these cells in the bone marrow (BM) compartment and their differentiation pattern has not been explored.

Here we utilized a unique model system consisting of tumor cell clones with low and high metastatic potential (met-low and met-high, respectively) derived from melanoma and breast carcinoma cell lines. Hematopoietic stem cells (HSCs) and their early progenitor subset were defined as Lin-/Sca1+/CD117+, representing LSK cells. BM transplantation experiments using GFP-positive LSK cells derived from met-low and met-high tumor bearing mice were carried out to study lineage differentiation. The genetic signatures of LSK cells were analyzed by single cell RNA-sequencing (scRNA-seq). This analysis included unbiased automated annotation of individual cell types by correlating single-cell gene expression with reference transcriptomic data sets (SingleR algorithm) in order to evaluate the proportions of cell types in BM and reveal cell type-specific differentially expressed genes. Expression patterns of proteins originated from tumor cells were analyzed using a range of multi-omics techniques including nanostring, protein array, and mass spectrometry analysis. Tumor proteomic data was integrated with differential receptor expression patterns in BM cell types to reveal novel crosstalk between tumor cells and HSCs in the BM compartment.

Mice bearing met-high tumors exhibited a significant increase in the percentage of LSK cells in the BM in comparison to tumor-free mice or mice bearing met-low tumors. These results were confirmed by functional CFU assays of peripheral blood of met-high compared to met-

low tumor bearing mice. In addition, mice that underwent BM transplantation with GFP-positive LSK cells obtained from met-high inoculated donors exhibited an increased percentage of circulating GFP-positive myeloid cells in comparison to counterpart mice transplanted with LSK cells from met-low inoculated donors. Moreover, scRNA-seq analysis of LSK cells obtained from the BM of met-low and met-high tumor bearing mice revealed that met-high tumors induce the enrichment of monocyte-dendritic progenitor population (MDP), confirmed also by flow cytometry. To uncover the possible factors involved in myeloid programming of LSK cells, we performed a proteomic screen of tumor conditioned medium and integrated the results with the scRNA-seq data analysis. This analysis revealed that the IL-6-IL-6R axis is highly active in LSK-derived MDP cells from mice bearing met-high tumors. An adoptive transfer experiment using MDP-GFP+ cells obtained from BM of met-high tumor bearing mice demonstrated that met-high tumors directly dictate HSC fate decision towards myeloid bias, resulting in increased metastasis. Evidently, blocking IL-6 in mice bearing met-high tumors reduced the number of MDP cells, and consequently decreased metastasis.

Our study reveals a unique crosstalk between tumor cells and HSCs. It provides new insight into the mechanism by which tumors contribute to the presence of supporting stroma. Specifically, tumors secreting IL-6 dictate a specific genetic signature in HSCs that programs them towards myeloid differentiation, thereby inducing a metastatic switch.