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ABSTRACT BOOK

TUESDAY 9 September 2014

Mechanistic Principles of Stem cells

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"Centrosomes in Malignant Growth and Impaired Cell Polarity"

Cell fate determinants are unequally segregated to each daughter cell during mitosis in Drosophila larval neuroblasts (NBs). Asymmetry in these cells is not limited to the polarised localisation of cell fate determinants; it also affects the microtubule cytoskeleton and the centrosomes that split soon after cytokinesis giving rise to two single-centriole-containing centrosomes which display significant structural and functional differences regarding pericentriolar material, motility, and fate. Failed asymmetric division of larval NBs impairs neural development and is often tumourigenic. Indeed tumours develop from these when they have either too many or no centrosomes at all. I will present our latest results regarding the identification the molecular machinery that controls the unequal behaviour of mother and daughter centrosomes in larval NBs and the role in terminal differentiation of some of the proteins that we have identified.

Steven Zuryn "Sequential partitioning of histone methylation and demethylation activities determines the robustness of natural transdifferentiation"

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Postmitotic somatic cellular identity is generally a stable feature of multicellular organisms. However, there are naturally occurring instances whereby cells can transdifferentiate into other cell types with distinct functions. Documented examples are rare, but in certain cases, extremely precise and efficient reprogramming outcomes are observed, representing as yet unexploited avenues in which to probe the molecular mechanisms that ensure robust cell conversion. Using unbiased and then targeted genetic screens, we report that a conserved H3K27me3/me2 demethylase, JMJD-3.1, and the H3K4 methyltransferase Set1 complex act to ensure invariant transdifferentiation of post-mitotic Caenorhabditis elegans hindgut cells into motor neurons. At single-cell resolution, we find that perfect conversion, particularly under stressful cellular conditions, requires the precise organization of each histone modifying activity into sequential, discrete phases of conversion. This functional and dynamic partitioning is achieved through a combination of active nuclear degradation of JMJD-3.1 and separable modular interactions between each histone modifier and transcription factors that have conserved roles in cell pluripotency and terminal fate selection. Our results draw parallels between epigenetic mechanisms underlying robust Td in nature and efficient cell reprogramming in vitro.

Cristina Tocchini "TRIMming pluripotency"

Cristina Tocchini[1,2], Jeremy J. Keusch[1], Sarah B. Miller[1], Susanne Finger[1,2], Heinz Gut[1], Michael B. Stadler[1,3], and Rafal Ciosk[1]
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The cytoplasm of oocytes is capable of reprograming somatic nuclei to a pluripotent state, a process known as somatic cell nuclear transfer. Despite this property, oocytes do not undergo embryogenesis without a specific trigger (e.g., fertilization), suggesting that their reprogramming potential is kept at bay by repressive mechanisms. In light of this, we conducted a genetic screen in C. elegans to understand how oocyte reprogramming potential is controlled. As readout, we used a reporter for embryonic genome activation (EGA) to identify premature entrance into a pluripotent state. This screen allowed us to identify LIN-41, a TRIM-NHL protein expressed in the cytoplasm of developing oocytes, as a regulator of pluripotency. In the absence of LIN-41, developing oocytes not only induce EGA but also fully lose their germ cell identity. Rather than completing growth and meiotic arrest, LIN-41 mutants enter the mitotic cell cycle and undergo somatic differentiation, events that normally occur during the very next developmental stage, i.e., embryogenesis. Thus, LIN-41 emerges as a component of the timing mechanism that delays the onset of early embryonic events in oocytes, thereby regulating the transition between generations. Previous studies have shown that LIN-41 is a member of the so-called heterochronic pathway in the soma and its mechanism of action is thought to involve mRNA regulation. To gain a better understanding of LIN-41 function in oocytes, we conducted structure-function experiments on its different domains and could identify and create mutants where the germline function could be uncoupled, at least in part, from the somatic one. Our study is the first to identify a role for any TRIM-NHL protein in the maintenance of germ cell pluripotency. Furthermore this is the first example of a factor that regulates pluripotency specifically in oocytes.

Kevin EGGAN, PhD
Professor of Stem Cell and Regenerative Biology
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"iPS Cells: From Individual Patients To Unifying Pathways Underlying Motor Neuron Degeneration"

ALS is characterized by degeneration of motor neurons, which results in a gradual spreading paralysis and eventually death. Despite intense clinical and basic science research efforts, recent clinical trials for this condition have proven unsuccessful. It is becoming increasingly apparent that motor neuron disease can be caused by any one of dozens of distinct mutations in an ever growing list of genes. However, whether or not any of these diverse mutations converge on a common pathological mechanism remains unknown. I will describe our efforts to use iPS cells to address this question.

Ian CHAMBERS, PhD
Professor of Pluripotent Stem Cell Biology at the MRC Centre for Regenerative Medicine
University of Edinburgh, UK

"Transcription Factor Control of Transitions in Pluripotent Cells"

Embryonic stem (ES) cells are defined by two key characteristics: the ability to differentiate into cells of all three germ layers (pluripotency) and the ability to undergo apparently symmetrical self-renewing cell division, essentially indefinitely. Pluripotent cell identity is governed by the action of a gene regulatory network centred on Oct4, Sox2 and Nanog.
I will discuss findings that shed light on the operation of the central components of this network, including the contribution made to function by target genes and protein interacting partners of the central components. Interestingly, while Oct4 and Sox2 are expressed relatively homogeneously in ES cell culture, Nanog is heterogeneously expressed. We and others have shown that Nanog is under autorepressive control and this autorepression contributes to Nanog heterogeneity. In addition, we have found that ES cells expressing an average of 50% of the wild-type Oct4 levels maintain pluripotency robustly. Significantly, these cells do not require BMP, serum or pharmacological modulators of signalling pathways to propagate the pluripotent state. As these cells express the same level of Oct4 as single cells in a wild-type ES cell population, these findings suggest that robust pluripotency originates from a subset of cells present within wild type cultures and that the full range of parameters present in wild-type cultures enables effective differentiation.

Priyanka Singh "**The centriolar protein Bld10/C ep135 controls centrosome asymmetry in Drosophila neuroblasts"**

Priyanka Singh, Anjana Ramdas Nair and Clemens Cabernard
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Drosophila neuroblasts, the neural precursors of the fly's central nervous system, contain asymmetric centrosomes during interphase, differing in molecular composition, size and age. This asymmetry is required for correct centrosome positioning and spindle orientation during asymmetric cell division. However, very little is known how centrosome asymmetry is molecularly controlled. We found that the centriolar protein Bld10, the fly orthologue of Cep135, controls centrosome asymmetry in Drosophila neuroblasts. We used live imaging to study pericentriolar matrix (PCM) dynamics during the neuroblast cell cycle. Apical wild type centrosomes retain PCM during interphase but basal centrosomes display three distinct phases encompassing PCM shedding, centriole migration and centrosome maturation starting at prophase. Interestingly, we found that bld10 mutant centrosomes never lose PCM, resulting in the premature formation of two active MTOCs. Our in vivo pulse-chase experiments demonstrate that this is due to a lack of PCM shedding as opposed to precocious centrosome maturation. As a consequence, spindle alignment is defective and centrosome segregation is perturbed, resulting in neuroblasts incorrectly retaining the mother centrosome. Since, Cep135 has been implicated in primary microcephaly, a rare neurodevelopmental disorder, so understanding the role of Cep135/bld10 in centrosomal cycle will be instrumental to explain the etiology of microcephaly.

Yanrui Jiang "Genetic analysis of neural stem cell-derived brain tumors"

Yanrui Jiang[1], Elif Eroglu[2], Tetsuo Yasugi[2], Lisa Landskron[2], Juergen Knoblich[2], and Heinrich Reichert[1]
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Neural stem cells are characterized by the ability to self-renew while generating differentiated neuronal progeny through asymmetric cell divisions. The asymmetric divisions of neural stem cells have to be tightly regulated as defects can lead to tumorigenesis. Indeed, increasing evidence suggests neural stem cells are the origin of some brain tumors in human. The central brain of Drosophila consists of about 100 neural stem cells that are called neuroblasts. Mutations in genes that are involved in the asymmetric divisions of neuroblasts, such as brain tumor, numb, or prospero, result in the overproliferation of the neuroblasts and formation of brain tumors. In this study, we use the targeted GAL4/UAS binary expression system, in combination with genomic transgenic RNAi, FACS sorting, and transplantation technique, to study neural stem cell-derived brain tumors. We characterized SWI/SNF chromatin remodeling complex and bHLH transcription factor Daughterless as tumor suppressors in the developing Drosophila brain. Our study has established a Drosophila neuroblast model for analyzing how the balance between self-renewal and proliferation is disrupted in cancer stem cells at the cellular and molecular level, which may be useful for future therapeutic implications both in tumor biology and in stem cell-based regenerative medicine.
(Supported by SNSF & NRP63)

Joerg Betschinger "Controlling exit from pluripotency"

Joerg Betschinger[1], Florian Villegas[1], Jennifer Nichols[2], Patrick Paddison[3], Austin Smith[2]
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Continuous restriction of cellular potency ensures unidirectionality of mammalian development, and is reflected in lineage progression of stem and progenitor cells into mature cell types. While cell type identity programs are fairly well described, transitions between them are less understood. To fundamentally understand mechanisms of cell fate transition we use murine pluripotent embryonic stem cells (ESCs). We performed a large-scale loss of function screen for regulators of ESC fate changeover to identify mechanisms that coordinate exit from pluripotency during differentiation with remodeling of the ESC transcription factor network. Besides others this recovered the tumor suppressor genes Tuberin (Tsc2) and Folliculin (Flcn). Tsc2 and Flcn act in mTOR-signalling and we showed that both genes promote breakdown of the ESC gene regulatory network through inhibition of the bHLH transcription factor Tfe3. Inactivating mutations of Tsc2 and Flcn, and activating translocations of Tfe3 have been identified in kidney cancer patients, suggesting a conserved cell fate function.

Wieland HUTTNER, Prof. Dr. med.
Director
Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany.

"Development and Evolution of the Neocortex – the Cell Biology of Neural Stem and Progenitor Cells"

Our group studies the molecular and cellular mechanisms of neurogenesis in the developing neocortex in the context of mammalian brain evolution, specifically the various types of cortical stem and progenitor cells and their modes of division. With regard to (*i*) the site of mitosis along the apical-basal axis of the cortical wall and (*ii*) the absence or presence of ventricular contact at mitosis, three principal classes of cortical stem/progenitor cells can be distinguished. First, stem/progenitor cells that reside in the ventricular zone (VZ) and that contact the ventricle where they undergo mitosis, i.e. the neuroepithelial cells, apical radial glial cells and apical intermediate progenitor cells, collectively referred to as apical progenitors (APs). Second, stem/progenitor cells that reside in the subventricular zone (VZ) where they typically undergo mitosis and that have delaminated from the ventricle, i.e. the basal (or outer) radial glial cells and basal intermediate progenitor cells, collectively referred to as basal progenitors (BPs). Third, stem/progenitor cells that undergo mitosis in the basal VZ or in the SVZ and that retain ventricular contact, called subapical progenitors.
Our recent progress in studying cortical stem and progenitor cells will be presented.

WEDNESDAY 10 September 2014

Stem Cells in Translation

Markus G. Manz, Prof. Dr. med.
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"Hematopoiesis in Infection and Inflammation"

Hematopoiesis is a paradigmatic somatic stem cell supported organ system. In steady-state homeostasis, there is a flux from hematopoietic stem and progenitor cells to their mature progeny. As mature myeloid cells are mostly post-mitotic and have limited half-lives, they need to be replaced continuously. In case of challenge of the steady-state situation in inflammation or infection, mature myeloid cells are in high demand, execute their effector functions and undergo accelerated consumption. Thus, to ensure host survival, stem and progenitor cells need to enhance their output appropriately. I will review recent evidence on how hematopoietic stem and progenitor cells sense inflammatory and infectious disease products and what are the respective short and long-term consequences of this.

Anna Paczulla "Cdx2 expression enhances human leukemic cells clonogenicity and in vivo repopulation capacity in NSG mice"

Anna Paczulla[1], Sarah Grzywna[2], Matthias Grauer[1], Ursula Kohlhofer[3], Leticia Quintanilla-Martinez[3], Lothar Kanz[2], Claudia Lengerke[1,2]
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Objectives: The caudal-type homeobox (CDX) gene family has been mainly studied during early development for its role in axial elongation and antero-posterior patterning. More recently, CDX genes were shown to interact with the WNT signaling pathway and to regulate embryonic hematopoiesis via downstream HOX genes. The role of CDX genes in adult hematopoiesis is poorly understood and almost no data exists on human cells. Human healthy bone marrow (BM) derived hematopoietic cells express low levels of CDX1 and CDX4 but completely lack CDX2 expression. However, CDX2 expression is found in >80% of human acute myeloid (AML) and lymphoid leukemia (ALL) and its induction in murine BM cells leads to development of myeloid leukemia. Here, we explore the role of CDX2 in human healthy hematopoietic and leukemic cells. Methods: CDX2 expression was modulated via lentiviral treatment in human BM derived CD34+ and SKM-1, EOL-1 and NALM16 leukemic cells. Efficient modulation of CDX2 expression was analyzed by qRT-PCR and immunoblot analyses. CDX2 modified (overexpressing or knockdown) and control cells were subjected to growth, colony forming (CFU), cell cycle, flow cytometry and qRT-PCR assays and analyzed in vivo upon xenotransplantation in NOD/SCID/IL2Rγnull (NSG) mice. Results: shRNA mediated knockdown in leukemic cells strongly reduced their clonogenic capacity while only slightly interfering with growth. Consistently, no influence on proliferation and apoptosis sensitivity was observed by CDX2 expression levels. Importantly, CDX2 knockdown SKM-1 cells transplanted into immunopermissive NSG mice showed severely reduced in vivo leukemogenic properties compared to control cells. Overexpression of CDX2 resulted in a G0/G1 cell cycle arrest in both healthy CD34+ cells and leukemic cells, reducing in vitro growth and CFU formation. Different than published in mice, healthy human stem/progenitor cells transduced to overexpress human

CDX2 were unable to induce leukemia in NSG mice. On the molecular level, CDX2 modified cells differentially expressed KLF4, HOX and WNT-pathway associated genes when compared to control cells. Conclusion: Our data suggest that CDX2 specifically regulates clonogenic capacity in human leukemia cells thereby regulating their tumorigenicity. Molecular mechanisms mediating this effect may include deregulation of HOX, KLF4 and WNT protein expression. CDX2 activation requires cooperative molecular events in order to induce leukemia from human healthy stem/progenitor cells.

Patrick Guye "Engineering a self-organizing liver bud-like hematopoietic niche from pluripotent stem cells"

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Lab for Synthetic Biology, Massachusetts institute of Technology, Cambridge MA, USA.
Human induced pluripotent stem cells (hiPSCs) have tremendous potential for personalized and regenerative medicine, providing us with improved in vitro tissue models and opportunities to address the critical shortage of donor tissues and organs in the long term. While generating hiPSCs has become routine in recent years, most methods of utilizing these promising cells focus on deriving a homogenous population of specialized cells, which has led to modest success in producing stable or complex tissues. In contrast, during embryonic and fetal development, tissues and organs emerge as the product of a heterogenous environment where complex interactions between cells guide developmental processes in a self-contained and stepwise fashion. Here we present a novel approach for generating a complex, organ-like tissue based on an engineered hiPSC population with inducible heterogeneous gene expression. A genetically engineered pulse of GATA6 expression at a wide variety of levels across the population introduces a crucial symmetry-breaking event in hiPSCs. This in turn initiates a gastrulation-like process and rapid emergence, spatial organization and co-differentiation of all three germ layers as a complex function of GATA6 expression levels and tissue context. Within two weeks under standard cell culture conditions, we obtain organized liver-like tissue that contains mesenchymal stem cells, hepatoblasts, cholangiocytes, hemogenic endothelium, pericytes, stellate (-like) cells, definitive hematopoietic progenitors, and spatially separated neuronal progenitors.

Maaike Welling "Dazl is required for TET-mediated reprogramming to a naïve pluripotent state"

Maaike Welling[1], Hsu-Hsin Chen[2,3], Javier Muñoz[4,5], Lennart Kester[1], Jan Philipp Junker[1,6], Lev Silberstein[2,3], Peter Kharchenko[7], Alexander van Oudenaarden[1,6], Albert JR Heck[4,5] and Niels Geijsen[1,8]
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Embryonic stem cell (ESC) cultures exist in a dynamic equilibrium, with heterogeneous expression of several pluripotency genes. The addition of inhibitors of GSK3β and MEK (so-called 2i conditions) has been shown to direct ESC cultures towards a more homogeneous naïve pluripotent state. However, the molecular mechanisms of this naïve transition are not completely understood. We found that Dazl, a RNA-binding protein essential for germ cell development, marks a subpopulation of ESCs that is actively transitioning towards naïve pluripotency. Moreover, Dazl seems to play an essential role in the active reprogramming of cytosine methylation. In Dazl-knockout ESCs, active 5-hydroxymethylation is impaired and in 2i conditions DNA demethylation occurs solely through replication dependent passive mechanisms. Dazl is known to stabilize associated mRNAs and enhance their translation. We found that Tet1 and Tet2 are mRNA targets of Dazl suggesting that Dazl mediates cytosine hydroxymethylation by regulating Tet RNA translation. Our results provide insight in the regulation of the acquisition of naïve pluripotency and demonstrate that Dazl is required for active TET-mediated cytosine hydroxymethylation in ESCs that are reprogramming to a pluripotent ground state.

Zayna Chaker "Neural stem cell management by longevity gene IGF-1"

Zayna Chaker[1,2,3], Hugues Berry[4], and Martin Holzenberger[1,2]
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Production of new neurons in the brain decreases dramatically with age due to progressive depletion of stem and progenitor cell populations (NSCs). Recent studies indicate that circulating factors constitute a systemic aging milieu regulating the birth of new cells. Interestingly, some long-lived mouse strains such as Ames dwarf mutants, with low circulating levels of GH and IGF-1, show increased neurogenesis. Thus, the possibility that genes regulating lifespan and aging also quantitatively modulate stem cells in mammals is more and more explored. IGF-1 plays a pivotal role in aging in different species, and we are asking whether some of the observed longevity effects resulting from down-regulation of this signaling pathway could be explained by local regulation of stem and progenitor cell compartments. To validate this hypothesis, we pursued a dual approach based on biological experiments and mathematical modeling. Using a novel triple transgenic mouse model, we induced specific inactivation of IGF-1 signaling in adult neural stem cells, and followed knockout cell lineages by monitoring a fluorescent reporter. By structuring the study in different time points after KO induction, we could distinguish between short and long-term effects of IGF signaling on cellular regeneration and identify cumulative physiological consequences of a down-regulation of this pathway using behavioral tests. In our mathematical models, the dynamics of all regenerative cell populations are described by a set of differential equations depending on circulating "growth-factor like molecules" (GFs). With this theoretical approach, we are able to conclude on the optimal distribution pattern of GFs over time. Experimentally, we show that long-term inactivation of IGF signaling in NSCs is significantly impacting regenerative cell compartments in all three niches of the adult brain. In the olfactory system, neuroblast maintenance is particularly affected and, as a consequence, the number of newborn neurons integrated in the olfactory bulb is also dramatically changed. Resulting tissue structure modifications have functional consequences on olfactory memory and odor discrimination in aged mutants. Strikingly, old KO animals also display shifted lipid and glucose metabolism, which could be explained by a different olfactory perception. Together, our results show that local modulation of neural cell replacement has dramatic physiological effects on the whole organism, pointing out a novel pathophysiological role for adult neurogenesis.

Chiara Rolando "Drosha-mediated posttranscriptional modifications regulate fate commitment in mouse hippocampal neural stem cells"

Chiara Rolando, Andrea Erni, Dirk Junghans, Verdon Taylor
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Self-renewing and multipotent neural stem cells (NSCs) reside in the dentate gyrus (DG) of the adult mammalian hippocampus. DG NSCs are a heterogeneous cell population fated to become granule neurons. The intrinsic mechanisms that regulate NSC maintenance and fate decisions have not been fully elucidated. The Microprocessor, a multimeric complex of the ribonuclease Drosha and the RNA binding protein DGCR8, binds and cleaves double-stranded hairpins in miRNA primary transcripts in the nucleus to release a precursor miRNA that is exported to the cytoplasm and further processed. The Microprocessor also has miRNA-independent functions, directly targeting and cleaving stem-loop hairpin structures of mRNAs and destabilizing the transcripts. We recently showed that Drosha targets the mRNAs encoding the proneural factor Neurog2, thus preventing NSC differentiation in the embryonic forebrain. We hypothesised that the Microprocessor may regulate NSC activity in the adult nervous system, therefore we deleted Drosha from DG NSCs using the Hes5::CreERT2 allele. Drosha conditional knockout (cKO) reduced proliferation and the number of Sox2+ cells in the DG. Moreover, neurogenesis from Drosha-deficient NSCs was impaired and the number of neuroblasts decreased suggesting exhaustion of the NSC pool. The remaining NSCs in Drosha cKO failed to reactivate after kainate-induced seizures suggesting that they become unresponsive to pathological stimulation. Moreover, three months after Drosha deletion, mitotic NSCs were absent and the remaining radial cells expressed the differentiated parenchymal astrocyte marker S100β. Interestingly, Drosha-deficient NSCs generated oligodendrocytes, a cell type that is normally not produced in the adult DG. In a complementary approach, we performed stereotactic injections of adenovirus GFAP::Cre virus into the adult DG of Droshalox/lox mice which increased oligodendroglial 10-fold compared to controls. Clonal analysis revealed that Drosha-deficient NSCs fail to expand

and confirmed that they differentiate into oligodendrocytes in vitro. We have investigated possible targets of Drosha in the differentiation of NSCs towards oligodendrocytes by Drosha-CLIP (cross-linking and immunoprecipitation). We found that Drosha binds the mRNAs of critical oligodendrocyte and gliogenic transcription factors. Further in silico analysis of hairpin containing mRNAs revealed conserved hairpin loops in these putative Drosha targets. We confirmed the regulation of the key oligodendrocyte and gliogenic transcription factors by conditional ablation of Drosha from NSCs in vitro. Taken together our findings reveal a new miRNA-independent action of the Microprocessor in the maintenance of adult NSCs and control of oligodendrocyte differentiation.

Eline Pecho-Vrieseling "Transneuronal propagation of mutant huntingtin contributes to non-cell autonomous pathology in neurons"

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Huntington's disease (HD) is an autosomal-dominant neurodegenerative disorder caused by an increased number of CAG repeats in the huntingtin gene. Patients typically suffer from a triad of movement, psychiatric and cognitive symptoms. Brain pathology is most prominent in the striatum with a selective and massive degeneration of medium spiny neurons (MSNs). In HD, neurons in different brain regions accumulate abnormal deposits of protein aggregates, a histopathological hallmark that HD shares with other proteinopathies, including prion disease, Alzheimer's disease (AD), Amyotrophic Lateral Sclerosis (ALS), Frontotemporal Lobe Dementia (FTLD) and Parkinson's disease (PD). Evidence has mounted that pathogenic forms of proteins such as α-synuclein (PD), Tau (AD and FTLD) and mutant SOD1 (ALS) can propagate protein aggregate pathology from cell-to-cell in a prion-like fashion thereby causing non-cell autonomous damage. In Huntington's disease (HD) transneuronal spreading of mutant huntingtin (mHTT) and its contribution to non-cell autonomous damage in brain networks is largely unknown. Here we demonstrate transneuronal propagation of mHTT in three different model systems. We show that wildtype (wt) human stem-cell derived neurons (h-neurons) functionally integrate in mouse organotypic brain slices (OTBSs). These neurons acquire mHTT when cultured in OTBSs from HD model mice (R6/2 mice). We provide also compelling evidence, that mHTT propagates from R6/2 cortical neurons to wt MSNs in an ex vivo mixed genotype corticostriatal slice model and that it occurs in the corticostriatal pathway in vivo. mHTT spreading to h-neurons in R6/2 OTBSs is blocked by two different Botulinum neurotoxins (BoNTs) that each inactivate a single critical component of the synaptic vesicle fusion machinery. Moreover, we show that normal h-neurons display non-cell autonomous changes when cultured in R6/2 OTBSs. Some of these changes are more pronounced in h-neurons bearing mHTT aggregates. Altogether, these findings show that transneuronal propagation of mHTT might be an important and underestimated contributor to the pathophysiology in HD.

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"Genetic Stability and Fate Determination in human Pluripotent Stem Cells"

Pluripotent stem cells are characterized by their capacity to self renew or to initiate differentiation leading to the appearance of cells of all three gem layers. Understanding the mechanisms by which these cells make fate decisions is central to their eventual use in a wide range of applications, including regenerative medicine. Recent work has indicated that such pluripotent stem cells may exist in interconvertible substates that exhibit differential propensities for specific fate decisions. At the same time, other work indicates that they may acquire non-random genetic change because such mutations provide variant cells with a selective growth advantage arising from altered patterns of differentiation. We are currently investigating whether these genetic variants influence differentiation by altering the dynamics of interconversion of stem cell substates.

Thomas Michael Marti "Characterizing the DNA damage response in lung cancer tumor initiating cells"

Colin Tièche[1], Renwang Peng[1], Sean Ralph Robert Hall[1], Laurène Froment[1], Patrick Dorn[1], Stephan Christian Schaefer[2], Sabina Berezowska[2], Ralph Alexander Schmid[1] and Thomas Michael Marti[1].
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Background: DNA damage response (DDR) is a complex and multifaceted signaling network, which evolved to maintain genome integrity that is essential for the proper function and survival of all organisms. Lung cancer is the most common cause of cancer-related mortality worldwide. More than 80% of lung tumors are non-small-cell lung cancers (NSCLC). It was postulated that tumor initiation and propagation are mediated by so called tumor initiating cells (TICs), which can self-renew and spawn differentiated progeny. In NSCLC, TICs were identified and subsequent analysis indicated that the DDR and the nucleotide synthesis pathway are deregulated in NSCLC TICs. Methods: The DDR is a highly sophisticated machinery consisting of intrinsically linked molecular mechanisms. Since these mechanisms are linked, they have to be analyzed simultaneously at the single-cell level. In detail, we analyze proliferation, cell cycle distribution, DNA damage marker accumulation and subsequent activation of the signaling pathway, as well as TIC marker status at the single-cell level by multicolor flow cytometry. Results: We identified TICs in the NSCLC cell line A549 and also in primary NSCLC samples. Our preliminary experiments indicate that NSCLC TICs feature enhanced basal activation of the DDR machinery. In addition, we found that DNA damage induction differentially activates the DDR pathway in distinct subpopulations expressing putative TICs markers compared to bulk tumor cells.
Outlook: We plan to characterize the DNA damage response of NSCLC TIC versus bulk cancer cells after treatment with clinically relevant chemotherapeutic agents. Relevance: By identifying key DDR proteins that are dysregulated in NSCLC TICs, we might identify novel targets for pharmacological or genetic intervention to treat lung cancer.

<u>Faye Drawnel</u> "Disease modeling and phenotypic drug screening for diabetic cardiomyopathy using human induced pluripotent stem cells"

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Diabetic cardiomyopathy is a complication of type 2 diabetes. Diabetic cardiomyocytes are structurally disorganized, hypertrophic and prone to oxidative stress and lipotoxicity. These pathologies compromise the contractile properties of cardiac muscle, resulting in a risk of heart failure. Unfortunately, intensive glycemic control does not fully eliminate the risk of cardiac complications in diabetic patients and there are no specific treatments targeting the condition. These life-threatening consequences of diabetes, when combined with an ever-increasing disease burden worldwide, represent a vast unmet medical need. Therefore, in vitro model systems of the condition would be a valuable tool, to increase our understanding of disease biology and for use in drug screening assays. We use human induced pluripotent stem cell-derived cardiomyocytes to develop environmentally-driven and patient-specific in vitro models of diabetic cardiomyopathy. First, we mimic the diabetic extracellular environment to induce a phenotypic surrogate of the condition, which recapitulates key disease biologies. The diabetic cardiomyocytes are hypertrophic, with disorganized sarcomeres, poorly-coordinated calcium transients and irregular cellular contractions. Using this model, we establish a drug screening assay to discover small molecules that prevent development of diabetes-associated cardiomyocyte dysfunction. Positive compounds target cellular calcium signaling and protein processing mechanisms, suggesting pathways and processes that could be future targets for drug development. Next, we consider genetic/epigenetic modifiers of disease progression by deriving cardiomyocytes from diabetic patients with different patterns of disease trajectory. The cardiomyopathic phenotype is recapitulated in the patient-specific models at baseline, to an extent that is dependent on the original patient clinical status. Cardiomyocytes derived from a rapidly, severely progressing diabetic patient display baseline sarcomeric disarray, lipotoxicity and oxidative stress, resulting in unsynchronized cellular contractions. In contrast, cardiomyocytes derived from a slowly progressing diabetic patient retain sarcomeric organization and cellular function. These data demonstrate recapitulation of the in vivo severity of a complex metabolic trait in an in vitro iPS-derived disease model. Finally, we test the small molecules identified in our primary drug screening assay on the patient-specific disease models, identifying subsets of compounds that improve the structure and function of the diseased cells. This work demonstrates how iPS technology can be used to model complex disease biologies and establishes a platform that can be used for the discovery of new targets and treatments for complex conditions that affect millions of people worldwide.

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"Cell Reprogramming to Multi- and Pluripotency by POU Factors"

The pluripotent and multipotent states of mammalian stem cells are governed by the formation of a highly interconnected regulatory network comprising specifically expressed transcription factors organized together with more widely expressed transcription factors. The transcriptional network exhibits a hierarchical structure, with a small number of transcription factors playing an essential role in maintaining cellular potency and regulating the expression and/or function of the numerous auxiliary transcription factors.
POU proteins, eukaryotic transcription factors containing a well-conserved DNA-binding domain, exhibit a wide variety of functions related to the functioning of the neuroendocrine system and development of the organism. The only PouVfI factor Oct4 is expressed in pluripotent stem cells, whereas the PouIII family factors Brn2 and Brn4 are expressed specifically in the neural system.
When the set of master transcription factors comprising Oct4, Sox2, Klf4, and Myc is expressed ectopically in fibroblasts, the transcriptional network supports somatic cell reprogramming to the pluripotent cell state. But when Oct4 is replaced by Brn4, the network mediates the conversion of fibroblasts into multipotent neural stem cells. Oct4 and Brn4 thus appear to play distinct but related roles in remodelling gene expression by influencing the local chromatin status during reprogramming.

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„The Epidermal Stem Cell: a paradigm in regenerative medicine"

Marietta Herrmann "The multilineage potential of pericytes derived from different human tissues"

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Pericytes are constitutive components of microvessels and present in all vascularized tissues. Recently it was suggested that pericytes represent common ancestor cells providing an in vivo source of mesenchymal stem cells (MSCs).[1] This identifies pericytes as a promising cell source for tissue engineering and regenerative medicine. Here, we aimed to compare the plasticity of pericytes isolated from different human tissues. We further evaluated the influence of different in vitro culture conditions on pericyte phenotype and behavior. We purchased human primary retinal pericytes (Cell Systems) and placental derived pericytes (Promocell). Pericytes from human adipose tissue and bone marrow aspirates were isolated as CD45negCD34negCD146pos cells by FACS. The entire plastic adherent population of bone marrow cells (MSCs) and adipose tissue cells (ASCs) were used as positive controls. Cells were analyzed with respect to growth kinetics, cell surface marker expression and multilineage differentiation potential. Our results showed comparable proliferation rates of all tested cell types with population doubling times ranging between 30-50 hours. The expression of putative pericyte markers CD146, NG2 and PDGFRb was highly dependent on the tissue source. Whilst approximately 30% of bone marrow and adipose tissue-derived pericytes were triple positive, retinal pericytes did neither express CD146 nor PDGFRb. In contrast, all pericyte and MSC populations universally co-expressed the MSC markers CD44, CD73, CD90 and CD105 (> 80% positive cells). Interestingly, the culture medium highly influenced the surface marker expression of cells. Assessing the ability of pericytes to differentiate toward osteogenic, adipogenic and chondrogenic lineage revealed that not all pericytes were capable of multilineage differentiation potential. Whilst bone marrow derived cells readily differentiated along all three lineages, retinal pericytes did not differentiate into adipogenic or chondrogenic cells. In conclusion, our results demonstrate that tissue origin and in vitro cell culture conditions both highly affect the cell surface marker expression and plasticity of human pericytes. These findings, along with the accessibility of the different tissues have to be taken into consideration when performing in vitro studies which aim to characterize these cells for clinical use.
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Andrea Banfi "Normalization and accelerated stabilization of VEGF-induced angiogenesis by co-delivery of engineered PDGF-BB and VEGF proteins from fibrin matrices"

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Therapeutic angiogenesis is required both for rapid vascularization of tissue engineered constructs and to treat ischemic conditions. Vascular endothelial growth factor (VEGF) is the master regulator of angiogenesis and its therapeutic potential depends on both its dose and duration of expression. VEGF delivery for at least 4 weeks is required to avoid regression of induced vessels, but sustained and uncontrolled expression can cause angioma growth. Hence, both angioma avoidance and rapid stabilization of new vessels with short-term treatments are required to ensure safety and efficacy. In a gene therapy approach, we previously found that co-expression of the maturation factor Platelet-Derived Growth Factor-BB (PDGF-BB) can prevent aberrant angiogenesis by uncontrolled VEGF levels. Recombinant protein delivery is an attractive approach to overcome safety concerns of VEGF gene transfer, but rapid in vivo clearance prevents the stabilization of induced angiogenesis. Here we hypothesized that delivery of recombinant VEGF and PDGF-BB proteins from a state-of-the-art matrix-bound system, based on the transglutaminase (TG) reaction to bind the modified factor into fibrin hydrogels, can both prevent aberrant angiogenesis by VEGF and ensure rapid stabilization

despite short-term treatment. TG-engineered recombinant VEGF164 and PDGF-BB were cross-linked into fibrin hydrogels and different doses of TG-VEGF, TG-PDGF-BB, both together or no factors (control) were injected in limb muscles of SCID mice. We found that: 1) fibrin gels were completely degraded in 10 days in all conditions; 2) by 2 weeks, co-delivery of TG-PDGF-BB completely normalized aberrant angiogenesis induced by high doses of TG-VEGF, yielding only mature and functionally perfused capillary networks; 3) normalization was achieved with a wide range of PDGF-BB:VEGF ratios (1:3 to 1:20); 4) 10 days of co-delivery of PDGF-BB with both low and high VEGF doses (5 and 50 μg/ml) caused complete stabilization and persistence of induced vessels after 2 months, whereas 50% and 75% regressed with low and high VEGF alone, respectively. Therefore, controlled co-delivery of TG-VEGF and TG-PDGF-BB proteins provides a convenient (off-the-shelf), safe and clinically applicable approach to: 1) expand the therapeutic range of VEGF doses; and 2) rapidly stabilize newly induced vessels with only 10 days' treatment and avoiding genetic modification.

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"Hematopoietic Stem Cell Gene Transfer for the Treatment of Genetic Disease and Cancer"

POSTERS (*alphabetical order, 1st author*)

#1

Tissue-specific cadherin expression is essential for morphogen distribution and cellular responses during mouse gastrulation.

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Cadherin switching from E-cadherin to N-cadherin is a key step of the epithelial-mesenchymal transition (EMT) that occurs during gastrulation. This developmental program is aberrantly activated during epithelial cancer progression at early stages of metastasis formation. The function of the cadherin switch within the complete EMT process is still enigmatic. We previously established a gene replacement approach using genetically modified mice that express N-cadherin under the control of the E-cadherin locus (Ncadki). Upon depletion of E-cadherin (using a second conditional knockout allele) in the mouse epiblast by Sox2-Cre, we induced an ectopic switch in cadherin expression prior to gastrulation. Ncadki embryos died at E8.5. At E7.5 they were identifiable by a smaller epiblast, a rounded shape, enlarged extra-embryonic compartment combined with a massive cell loss from the ectodermal layer. AJs were properly assembled, all three germ layers formed and the epiblast cells contributed to a large variety of tissues in teratomas. Conversely, embryos with epiblast-specific E-cad depletion (Ecadnull), that completely lacked of cell-cell adhesion, showed lethality already at E6.5. Although gastrulation was initiated, each germ-layer in Ncadki embryos was abnormal due to an overall reduction in BMP signaling, indicated by reduced BMPs in the extraembryonic ectoderm and inefficient downstream pSMAD1/5/8 activation. Complete lack of the amniotic cavity, together with an expansion of definitive endoderm (DE) and impaired formation and elongation of the primitive streak at E7.5 resembled the conventional Bmp2 knockout mouse. In vitro data showed that N-cad was not retained at the cell membrane but relocalized to the nucleus in cells derived from the Ncadki epiblast. Furthermore, the N-cadherin cytoplasmic domain interacted with chromatin of transcriptionally active genes independent of β-catenin. TMA of human colorectal cancers showed nuclear N-cad localization correlated with high-tumor grades. We propose a mechanism by which changes in processing and cell localization of N-cadherin is inhibiting BMP signaling and thereby affecting migration and cell fate specification. The N-cadherin nuclear accumulation may be used by the tumor cells as metastatic promoter and inhibitor of BMP signaling, to block its function as tumor suppressor and inducer of differentiation.

#2

Jagged1 as a pivotal regulator of neural stem cell differentiation towards an oligodendrocytic lineage in cells originating from the neurogenic forebrain niche.

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During formation of the brain in mammals, neural stem cells (NSCs) transit through sequential periods of expansion, neurogenesis and gliogenesis. Notch signaling maintains NSCs and blocks transcription of pro-neurogenic factors. Notch ligands are expressed by differentiating progenitors and activate lateral inhibition signals through Notchs on opposing progenitor cells. It has long been proposed that Notch signaling occurs bi-directionally through ligands including Jagged1 (Jag1), which are also type I membrane proteins. However, the molecular mechanisms controlling the transition from stem cell division, where Notch plays a maintenance role, to daughter cell differentiation are poorly understood. To study the role of Jag1, in niche maintenance we transduced NSCs lining the subventricular zone (SVZ) with full length Jag1 (Jag1FL) expressing viruses. Surprisingly, Jag1 induced a fate switch in NSCs to Sox10+ oligodendrocyte precursors. NSCs grown under differentiating conditions in vitro recapitulated this phenotype to some degree. We performed genome-wide RNA-Sequencing analysis to study transcriptome changes in Jag1FL-IRES-GFP transduced NSCs. This screen revealed an up regulation (induction) of key genes involved in oligodendroctye maturation and myelination, which were further confirmed by qRT-PCR. We examined the ability of Jag1FL-IRES-GFP expressing NSCs to remyelinate lesions in mice with focal myelin lesions. Our data indicate a novel role for Jag1 in NSCs maintenance, and displays its potential for regulating fate switch to an oligodendrocytic lineage.

#3

Early phenotypic diversity of sister oligodendrocyte progenitor cells after mitosis and its modulation by aging and extrinsic factors.

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After the completion of postnatal myelination, oligodendrocyte progenitor cells (OPCs) persist in the adult central nervous system where they continuously engage in maturation to sustain myelin turnover and plasticity. It remains obscure how OPCs avoid exhaustion during adulthood. Similar to stem cells, OPCs could self-maintain by undergoing asymmetric divisions generating a progeny either keeping a progenitor phenotype or committed to differentiation. To address this issue in mice, we examined i) the distribution of progenitor and maturational markers in sister OPCs during mitosis and at later time points, and their correlation with early OPC fates; ii) alterations in the distribution of these markers during aging and upon exposure to physiological and pathological stimuli. We found that both in the adult and juvenile (P20) mouse cortex a fraction of dividing OPCs gives rise to sister cells with diverse immunophenotypic profiles and short-term fates. This heterogeneity appears as early as when daughter cells exit cytokinesis, but does not rely on the asymmetric segregation of molecules such as NG2 or PDGFRa expressed in the mother cell. Rather, rapid downregulation of OPC markers after mitosis and upregulation of molecules associated with the progression in the lineage during the late phases of cell division contribute to generate early sister OPC asymmetry. Analysis during aging and upon exposure to physiological (i.e. increased levels of motor activity) and pathological (i.e. trauma or demyelination) stimuli showed that both intrinsic and environmental factors contribute to determine the phenotype of the OPC progeny as soon as cells exit mitosis.

#4

The balance of Id3 and E47 determines BMP-induced adult neural stem cell differentiation into astrocytes after vascular damage.

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Adult neural stem cells (NSCs) of the subventricular zone (SVZ) are an endogenous source for neuronal replacement in CNS disease, but adult neurogenesis is compromised after brain injury in favor of a glial cell fate. The failure of NSCs to differentiate into neurons after brain injury has been attributed to changes in the NSC environment, such as elevated levels of bone morphogenetic proteins (BMPs). However, it is unknown how this unfavorable extracellular environment translates into an intrinsic transcriptional program that regulates NSC differentiation. Our study reveals that the transcriptional regulator inhibitor of DNA binding 3 (Id3) regulates BMP-induced adult NSC differentiation into astrocytes via control of the basic helix-loop-helix transcription factor E47. Genetic depletion of Id3 in mice increases the number of SVZ-derived neurons in the olfactory bulb and decreases the number of astrocytes generated from adult NSCs at the rostral end of the rostral migratory stream and the cortical lesion center after traumatic brain injury. In addition, NSCs isolated from adult Id3-/- mice fail to differentiate into BMP-induced astrocytes, while E47-deficient NSCs readily differentiate into astrocytes in the absence of BMP. Furthermore, ChIP analyses and luciferase assays revealed that E47 represses the expression of astrocyte-specific genes in adult NSCs. These results identify Id3 as the BMP-induced transcriptional regulator, promoting adult NSC differentiation into astrocytes upon CNS injury and reveal a molecular link between environmental changes and NSC differentiation in the CNS after injury.

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#5

Doublecortin in the cerebrospinal fluid after hypoxic-ischemic brain injury in the rat neonate is a biomarker of endogenous neurogenesis.

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Aims: There is an unmet clinical need to monitor neurogenesis in vivo in developmental brain disorders. Biomarkers of neurogenesis may prove valuable for diagnostic, prognostic and therapeutic purposes. Doublecortin (DCX) is considered as a marker for neurogenesis, because it is highly expressed in migrating neuroblasts. Using a rat model of neonatal hypoxia-ischemia (HI), the aims were to (i) quantify DCX in the cerebrospinal fluid (CSF) of neonates prior to and after an HI brain injury, and to (ii) examine whether DCX measured in the CSF reflects neurogenesis in the brain after HI. Methods: Right-sided HI was elicited at postnatal day (P) 7 in Sprague-Dawley rats via ligation of the right common carotid artery and 40 minutes exposure to 8% O2. Control animals received a sham surgery without hypoxic exposure at P7. CSF was collected before (P5) and after (P10) surgery, and DCX was quantified using a specific and highly sensitive immunoassay. BrdU (100 mg/kg) was injected intraperitoneally from P7 to P9. Animals were sacrificed at P10 and their brains were processed for immunohistochemical analysis. Results: In sham-treated neonates, the concentration of DCX in the CSF (DCX-CSF) decreased by 85% between P5 and P10 ($n=11$, $p<0.0001$,

paired t-test). In contrast, DCX-CSF increased by 123% in HI-injured animals (n=11) during the same time interval. Interaction between postnatal day and treatment was significant (two-way ANOVA p=0.0197). In the HI group, a positive correlation between DCX-CSF and stroke severity was observed. DCX immunointensity was increased in the ipsilateral subventricular zone (SVZ) and dentate gyrus from HI-injured animals in comparison to sham animals. The number of BrdU-positive cells was higher in the right SVZ from HI animals versus sham animals (p<0.01). Conclusion: The decline in DCX-CSF in sham neonates between P5 and P10 is in accordance with the well-documented postnatal downregulation of DCX in the brain. The results after HI suggest that DCX-CSF reflects the neurogenic and proliferative responses in the brain 3 days after the insult. Overall, DCX in the CSF appears to be a valid in vivo biomarker of neurogenesis in the rat model of neonatal HI.

#6

Dissecting Epigenetic Mechanisms Involved in Fragile X Syndrome Using Human-Derived Induced Pluripotent Stem Cells.

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Fragile X syndrome (FXS) is a monogenic neurodevelopmental disease and the most common inherited form of mental retardation and autism. FXS is most often caused by a CGG triplet expansion in the 5'UTR of the FMR1 gene, which results in DNA methylation of the FMR1 promoter and its transcriptional silencing. Exceptional healthy individuals have been identified in the families of FX patients. They carry an expanded CGG repeat, but show normal FMR1 epigenetic configuration and expression. In this study we aim to identify the mechanism responsible for the lack of silencing in this unmethylated full mutation (UFM) phenotype. To mimic the time window of the FMR1 silencing during development we derived induced pluripotent stem (IPS) cells from the UFM, FX and healthy individuals. We are analyzing the expression level and epigenetic status of FMR1 in IPS cells and during neuronal differentiation. To better understand the UFM phenotype we are also characterizing the genome wide expression and chromatin status of these cells. Global analysis of DNA methylation revealed very few changes outside of the FMR1 locus, indicating a mechanism specific to FMR1. We believe that identification of the pathway by which UFM escapes epigenetic silencing will provide important insights into the process of FMR1 silencing in FXS and will facilitate identification of targets for drugs with FMR1 re-activating function.

#7

Short-term delivery of fibrin-bound VEGF protein in osteogenic grafts: increased vascularization with efficient bone formation.

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INTRODUCTION: Reconstruction of large bone defects is a major challenge in tissue engineering. Bone marrow mesenchymal stem cells (BMSC) are valuable multipotent progenitors for regene-rative medicine. Spontaneous vascularization of BMSC- loaded osteogenic grafts in vivo is too slow to allow survival of the progenitors in constructs larger than a few millimeters. Stimulation of graft vascularization in vivo is needed for cell survival and efficient bone formation. Vascular endothelial growth factor (VEGF) is the master regulator of angiogenesis. However, we previously found that, while sustained over-expression of VEGF by genetically modified human BMSC was effective to improve vascularization of tissue engineered bone grafts, it also caused an undesired increase in osteoclast recruitment with excessive bone resorption [1]. Here we hypothesized that short- term delivery of VEGF protein bound to fibrin gels may improve graft vascularization without impairing bone formation. METHODS: Primary human BMSC were retrovirally transduced to express VEGF linked to CD8, as a surface marker, or just CD8 [2]. Recombinant VEGF was engineered with a transglutaminase substrate sequence (TG-VEGF) to allow covalent cross-linking into fibrin hydrogels [3-4]. BMSC were seeded on apatite granules in fibrin pellets. Bone formation and vascularization were determined histologically 1, 4 and 8 weeks after ectopic subcutaneous implantation in nude mice. RESULTS: One week after implantation, both the constructs with naive BMSC and fibrin-bound TG- VEGF and those with VEGF-expressing BMSC (VICD8) displayed increased vascularization compared to the controls with naive BMSC only. After 4 weeks fibrin gels were completely degraded in all conditions. After 8 weeks both fibrin-bound TG-VEGF and VEGF-expressing BMSC induced significantly increased vascularization compared to naive BMSC only. However, while bone formation was severely impaired with VEGF-expressing BMSC as expected [1], fibrin-bound recombinant TG-VEGF allowed the formation of bone tissue as efficiently as by naive BMSC alone. DISCUSSION & CONCLUSIONS: These data suggest that VEGF effects on promoting vascularization and bone resorption can be uncoupled by short-term delivery of recombinant VEGF protein, providing an attractive and clinically applicable strategy to ensure both robust

vascularization and bone formation. These data warrant further investigation in critical-size orthotopic models.
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#8

Quantitative multidimensional analysis of bone and bone marrow stem cells in vivo.
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Bone and bone marrow harbor a dual stem cell system where hematopoietic stem cells (HSCs) self-renew and differentiate to produce all blood and immune cells and skeletal or mesenchymal stem cells (MSCs) produce bone, cartilage and the marrow stroma responsible to support hematopoiesis. The molecular phenotype of HSCs is well characterised but the microenvironments or niches where they self-renew and differentiate is less well understood. The cells known as MSCs on the other hand are still poorly characterised and comprise heterogeneous cell subpopulations. Hence, the molecular and cellular architecture of bone marrow is in large part unknown, due primarily to technical difficulties in imaging bone and marrow tissues. We here describe our efforts to develop methods allowing the identification of MSCs in vivo and their lineage hierarchy using multidimensional FACS analysis, bioinformatics tools, 3D multicolor imaging and fate mapping techniques. Flow cytometry on over 20 putative MSC markers combined with clustering algorithms is used to identify skeletal cell subpopulations. These are then mapped in bone sections using up to 11 colors immunofluorescence and deep (700μm) confocal imaging. Genetic labeling and lineage tracing is then used to decipher the lineage hierarchy between these populations. Combined with quantitative 3D image analysis, we also show how our novel imaging technique is useful to analyse the complex microenvironments of the bone marrow niches where HSCs self-renew and their progeny differentiate.

#9

Molecular mechanism of hematopoietic lineage choice instruction.
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Stem and progenitor cell (SPC) fate decisions are regulated by the timed integration of extracellular signals and intracellular molecular states. Through continuous single cell observations we could recently provide evidence for the long disputed instructive effect of the hematopoietic cytokines M- and G-CSF on granulocyte-macrophage progenitor (GMP) lineage choice. M- and G-CSF activate a multitude of signaling pathways that mediate their pleiotropic actions, which include survival, proliferation, and ultimately differentiation. However, the involvement of specific signaling pathways in controlling different cell fates remains poorly understood. Specifically, pathways orchestrating lineage choice instruction remain elusive. The M-CSF receptor (MCSFR) carries eight functional tyrosine residues that transmit M-CSF-evoked signaling. Studies on individual MCSFR tyrosine residue-activated signaling and its concomitant influence on cell fate have mainly relied on myeloid cell lines and/or MCSFR chimeras, often resulting in contradictory conclusions. In this study we established a system allowing the analysis of M-CSF-induced signaling in uncommitted primary progenitor cells. Combining MCSFR loss of function studies to dissect M-CSF-activated signaling pathways with novel bioimaging technologies allowing long-term quantification of single cell behavior, we investigated the molecular mechanism orchestrating M-CSF-instructed lineage differentiation.

#10

Modulation of adult hippocampal neurogenesis in physiological and pathological conditions.
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Adult neural stem cells (NSCs) are found in the subventricular zone (SVZ) and the hippocampal dentate gyrus (DG), are quiescent, express glial markers and depend on Notch signaling (Giachino et al, 2013). Canonical Notch signaling (Hes5) is restricted to NSCs (Lugert et al, 2010). Proliferating NSCs additionally express brain lipid binding protein (BLBP) and are major neurogenic populations in the SVZ (Giachino et al, 2013). It remains to be shown how the brain uses these NSCs under physiological and pathological conditions. We addressed whether cells of the DG exhibit similar levels of heterogeneity as seen in the SVZ. Utilizing cell-type specific fluorescent protein tracing we followed neurogenesis during ageing. We hypothesized that active, BLBP+Hes5+ NSCs are present in the DG and that this active NSC pool, but not the quiescent NSC pool, is lost upon ageing and can be reactivated by kainic acid (KA) induced status epilepticus. Our results illustrate that BLBP+Hes5+ cells are present in the DG as a small population (2.8%) and these cells are mitotically active (85%). The heterogeneous composition of the DG changed significantly during ageing. The aged DG NSC-niche was depleted of active cell components and most prominently the proliferating Hes5+BLBP+ NSCs. Seizure induction within the hippocampal circuitry increased the number of newborn granule cells (Jessberger et al, 2007). We injected aged animals with KA to induce seizures to characterize the behavior of the DG NSC pools. Five days after seizure, active, proliferating NSCs were increased in the DG of aged mice. In light of our current results we propose that a

reactivation of the active NSC pool is possible by seizure induction. We are testing whether these activated NSCs later give rise to DCX+ neuroblasts and mature granule cells. The SNSF and the University of Basel financially support this work.

#11

Drosha post-transcriptionally regulates embryonic neural stem cells.

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During embryonic cortical development neural stem cells (NSCs) lining the ventricles give rise to the different neuronal subtypes in an inside-out sequence (Greig et al. 2013). Deep layer neurons are generated first followed by neurons fated to the upper layers in the developing cerebral cortex. However, it is still unclear how NSC-fate decision is timely controlled, but might be achieved by dynamic transcriptome regulation. Previous results from our lab showed that the RNaseIII Drosha is involved in post-transcriptional gene regulation in NSCs through a miRNA independent mechanism during cortical development (Knuckles et al. 2012). We have shown that Drosha directly binds and cleaves secondary stem-loop structures within the mRNA of the proneural transcription factor Neurogenin 2 thus resulting in destabilization and translation inhibition. Through this mechanism Drosha directly suppresses NSC differentiation in the developing cortex. We hypothesize that Drosha can differentially regulate NSC maintenance during cortical development by targeting distinct proneural factors and downstream fate regulators. To investigate the role of Drosha in vivo we took advantage of the Hes5::CreERT2 allele to conditionally knock-out (cKO) Drosha in NSCs in the developing cortex at different stages of development to follow the consequences of the loss of Drosha. Conditional deletion of Drosha during brain development resulted in a reduction of the NSC pool and an alteration of cortical layer organization, markedly a reduction in the upper cortical layers. We have investigated possible targets of Drosha involved in the precocious differentiation of NSCs by Drosha-CLIP (RNA cross-linking immunoprecipitation). Notably, we found that Drosha binds the mRNA of the critical proneural transcription factor NeuroD6. In line with this observation, in silico analysis of hairpin containing mRNAs revealed conserved hairpin loops in this putative Drosha target. Moreover, we confirmed the regulation of NeuroD6 by conditional ablation of Drosha from embryonic NSCs in vitro. Overall, our results show that Drosha controls proper cortical layering by directly inhibiting proneural genes important for the NSC fate commitment.

Greig L.C. et al. (2013) Molecular logic of neocortical projection neuron specification, development and diversity. Nature Review, 14, 755-769.
Knuckles P. et al. (2012) Drosha regulates neurogenesis by controlling Neurogenin 2 expression independent of microRNAs. Nature Neuroscience, 15,7, 962-969.

#12

Single cell analysis of cytokine-dependent transcription factor dynamics in hematopoietic progenitors.

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Transcription factors act as master regulators of hematopoietic lineage commitment. However cell intrinsic or extrinsic cues controlling these transcription factors remain poorly understood. Considering the importance of cytokines in influencing hematopoietic cell fates, we investigated whether they might directly affect the regulation of the core hematopoietic transcription factor PU.1. Initially we screened for cytokines regulating PU.1 expression. Using time-lapse imaging of cytokine-challenged cells we quantified the expression dynamics of PU.1 protein in vitro continuously in large numbers of individual hematopoietic stem and progenitor cells with highest temporal resolution. We identified several combinations of cytokines that induce elevated PU.1 protein expression. For some cytokines PU.1 induction was detectable within less than 3h after cytokine challenge and peaked prior to the first cell division. Notably this effect was observed already in CD150+CD34-CD48- KSL hematopoietic stem cells. PU.1 induction kinetics were heterogenous among the screened progenitor populations thus in comparison to the single-cell quantification approach, flow cytometric end-point analysis performed only poorly to identify the altered dynamics of PU.1 expression. This illustrates the absolute requirement for continuous single cell quantification to reveal such effects. Our observations have important implications to understand how hematopoietic lineage choice is regulated through transcription factor networks, which are responsive to cytokine signaling.

#13

Long-term expansion of dopaminergic progenitors from human induced pluripotent stem cells (iPSCs) and differentiation into dopaminergic neurons in-vitro.

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Mesencephalic dopaminergic neurons (mDA) are the main source of dopamine in the mammalian central nervous system. The degeneration of mDA cells and the consequent destruction of the nigrostriatal network

cause Parkinson´s disease (PD). Despite numerous advances in our understanding having been made in the last few years, the molecular mechanisms leading to PD are not fully understood. Functional DA neurons derived from human induced pluripotent stem cells (iPSCs) are an important system to improve our knowledge of mDA development and differentiation, and potentially to identify new molecular mechanisms that underlie the disease. Dopaminergic neuron differentiation from human iPS cell lines has been described previously (Kriks et al. Nature, 2011). A combination of different signals, especially FGF8 and SHH, promotes the appearance of ventral mDA progenitors by day 11 in vitro. We have adopted and adapted this system and confirmed the co-expression of early DA markers including Lmx1A and FoxA2 by immunofluorescence and RT-PCR analysis. In order to expand these ventral mid/hindbrain progenitors as a potential source of enriched mDA neurons, we have developed and refined a new protocol. We describe that the Lmx1A and FoxA2 mDA progenitors can be maintained and passaged for more than one month in vitro as a homogeneous population while retaining their marker expression and differentiation potential. Moreover, we developed a protocol that allows us to cryopreserve and thaw these expanded DA progenitors many times. We have evaluated the long-tem cultures and whether the cryopreservation affects mDA differentiation capacity. Using differentiation conditions, expanded DA progenitors generate many TH+ by day 50 and day 80 in vitro which co-express mDA markers including Nurr1, DAT, AADC and VMAT2. Furthermore, we confirmed the generation of mature and functional human iPS-derived mDA neurons by electrophysiology and dopamine release studies. Taken together, our results describe a novel method for long-term expansion of dopaminergic progenitors that retain their differentiation potential to generate mature mDA neurons.

#14

GABAergic synapses on oligodendrocyte precursors: characterization and role of synaptic components in the postnatal cerebellum.

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Neuronal activity influences the timing of oligodendrocyte precursor cells (OPC) differentiation and myelination during early postnatal life. Recent work has suggested that such modulation involves synaptic contacts established between neurons and OPCs. Here, we characterize the repertoire of GABAergic synaptic molecules expressed by OPCs in the postnatal (P7 to P40) cerebellum by in vivo high-resolution morphological techniques and gene expression analysis on cells acutely isolated from the mouse nervous tissue. Postnatal OPCs express high levels of GABA-A receptor subunits, as well as scaffold proteins and cell-adhesion molecules normally associated with inhibitory synapses in neurons. During the first postnatal weeks GABAergic synaptic appositions on OPCs are found in the granule cell layer and the molecular layer of the cerebellar cortex, while the white matter lacks such neuron-to-OPC contacts. Moreover, pharmacological modulation of GABA-A and GABA-B receptors in cerebellar organotypic cultures alters the expression levels of myelin-associated genes, indicating that the level of the GABAergic tone influences OPC maturation and differentiation. Results of this work clarify the molecular composition of GABAergic contacts in OPCs and extend the current knowledge on mechanisms regulating OPC maturation. Moreover, they can provide insights into the pathogenesis of developmental disorders and pathological conditions (e.g. schizophrenia, stroke and multiple sclerosis) where alteration of synaptic inhibition is accompanied by myelination/remyelination defects.

#15

Tribological tissue engineering of cartilage.

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Bone marrow derived mesenchymal stem cells (BMSCs) are frequently used as a source material cell based cartilage repair strategies. Whereas the articulating joint provides a unique, multiaxial load environment, in vitro studies are classically performed under static conditions, or using uniaxial load alone. Using a complex, multiaxial load bioreactor, we have demonstrated that superficial shear, superimposed over uniaxial load, can provide a chondrogenic signal in the absence of exogenous growth factors, namely TGF-β. This response is due to an increase in the production, and activation, of endogenous TGF-β by the mechanically stimulated cells. Using this device, we have also demonstrated that asymmetrical seeding of the construct, with a greater percent of the total cells being deposited in the superficial zone, leads to increased cartilage matrix deposition when using the same number of total cells. Deposition of both glycosaminoglycan and collagen II are increased in asymmetrically seeded scaffolds when compared to homogenously seeded scaffolds. Of note, neither analysis of classical chondrogenic genes using real-time PCR, nor elisa analysis of TGF-β detects any differences between homogenously seeded and asymmetrically seeded constructs. This provides a new insight into mechanically induced chondrogenesis and offers an experimental test bed for clinical therapies. In addition, the therapy in its entirety, including the effect of the rehabilitation protocol, can be investigated using human derived cells. The use of human cells and a more physiologically relevant loading environment, leads to more clinically relevant studies being performed which should increase the potential translation into the clinic.

#16

Engineering of cell-based patch as a controlled VEGF-releasing device for induction of therapeutic angiogenesis.

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Therapeutic angiogenesis induced by exogenous Vascular Endothelial Growth Factor (VEGF) delivery is considered a promising approach to generate new collateral arteries in the ischemic myocardium upon infarction. Nevertheless, uncontrolled release of VEGF at the microenvironmental level might cause the growth of aberrant hemangiomas-like structures. Thanks to a fluorescence-activated cell sorting (FACS)-based technique transduced adipose-derived mesenchymal stromal cells (ASC) that homogeneously express a specific VEGF dose are purified from a heterogeneous primary population. The aim of our study is to generate a VEGF-expressing cardiac patch as a controlled growth factor delivery device for a therapeutic angiogenesis. Human ASC were transduced with retroviral vectors expressing ratVEGF164 linked to a FACS-quantifiable cell surface marker (truncated version of ratCD8). Two primary populations, one expressing safe and specific (SPEC) and the other heterogeneous (ALL) VEGF levels, were FACS-purified and naïve cells were used as control. Cells were cultured for 5 days on a 3mm-thick collagen scaffold in a perfusion-based bioreactor. In order to assess their extrinsic angiogenic potential a 7mm-thick cell-free (empty) scaffold was sutured beneath the cell-based patch and implanted subcutaneously in nude rats for 7 and 28 days. Perfusion-based culture system resulted in high seeding efficiency as well as in a homogeneous spatial cell distribution. Both VEGF-expressing populations caused similar increases in vessel length density compared to control patches (naive ASC), both in the cell-based patch itself (~3.5- and ~4-fold at 7 and 28 days, respectively) and in the empty collagen scaffold (~1.5- and ~6-fold at 7 and 28 days, respectively). The newly induced angiogenesis displayed a morphology of normal capillary networks with vessels covered by pericytes. No aberrant vascular structures were observed in patches expressing heterogeneous VEGF-levels, suggesting that its diffusion in the collagen scaffold may prevent the formation of toxic spots. The survival of implanted cells was also higher in the VEGF-expressing patches as compared to the control. In this study, we showed that tissue-engineered patches generated with both SPEC and ALL VEGF-expressing ASC could induce efficient and normal angiogenesis in critical size (7mm thick) area. Future studies will investigate the VEGF-expressing patch efficacy in a rat model of ischemic myocardium.

#17

The role of β-catenin in the development of neural crest stem cells.

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β-catenin plays a dual role in metazoan organisms. It is a subunit in the cadherin-adhesion complex and is a central mediator in canonical Wnt-signaling. Our objective is to distinguish these roles in neural crest stem cells (NCSC). With a NCSC-specific Cre, we compared conditional knock-out (cKO) of β-catenin (null mutant), cKO of α-catenin (adhesion mutant), and a mouse line in which β-catenin lacks the ability to recruit co-transcription factors, but preserves adhesion and TCF binding (signaling mutant). We analyzed cell cycle progression, and observed that canonical Wnt-signaling regulates lineage specific proliferation temporarily in NCSC during early migration. However, this Wnt dependent proliferation can be divided into two temporally sequential processes, each of which depends on a different function of β-catenin. Furthermore, we analyzed the expression of the transcription factors Krox20, Neurogenin1 and Neurogenin2, which regulate the three waves of neurogenesis in the sensory lineage. Expression of Neurogenin2 and Krox20 are lost in both β-catenin mutants, but not in the adhesion mutant, whereas expression of Neurogenin1 is preserved in the signaling and adhesion mutant, but lost in the null mutant. Our results indicate, that a first wave of proliferation of postmigratory NCSC, and expression of Neurogenin2 and Krox20 depend on Wnt-mediated β-catenin signaling by activation of TCF transcription. However, a second wave of proliferation and expression of Neurogenin1 is regulated by a role of β-catenin independent of signaling and adhesion. Accordingly, we suggest β-catenin acts as a derepressor of the TCF/Groucho repression complex.

#18

Inhibition of Notch signaling releases neural stem cells' tumorigenic potential.

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Gliomas are aggressive brain cancers with limited therapeutic options and poor prognosis for patients. Although it remains controversial, neural stem cells in the postnatal brain are believed to be one origin of gliomas. The Notch signaling pathway is required for neural stem cell maintenance and, accordingly, promotes a self-renewing stem cell-like state in glioma cells. Therefore, Notch signaling is believed to be oncogenic in glioma. Here, we addressed the cell autonomous role of Notch signaling in glioma using conditional genetics in multiple mouse models and discovered an unexpected tumor suppressor function for Notch in the brain. Genetic deletion of core Notch pathway components shows that neither glioma initiation from neural stem cells nor tumor progression depend on Notch signaling activity. In contrast, Notch signaling inhibition accelerates platelet-derived growth factor-driven glioma growth in mice. Notch cooperates with known tumor suppressors to control cell proliferation, and loss of Notch signaling promotes

a premalignant state in adult neural stem cells. We characterized the progression of Notch signaling-deficient cells towards transformation in vivo and performed genome wide analysis of downstream targets of Notch in neural stem cells and glioma cells that may be associated with increased tumor formation. Our findings uncover fundamental differences in the molecular requirements of normal neural stem cells versus glioma stem cells and reveal a novel Notch tumor suppressor function.

#19

The function of the stem cell factor Tlx/Tll in the developing Drosophila visual system.

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During brain formation the path from neural stem cells to differentiated neurons is a step-wise process with many intermediate cellular states. Its correct spatiotemporal regulation ensures proper neuronal identity and connectivity. To identify conserved mechanisms underlying neural stem cell proliferation and neuronal differentiation we are studying neurogenesis of the developing Drosophila visual system. In the optic lobe neuroepithelial cells divide symmetrically to extend the pool of stem cells before switching to asymmetrically dividing neuroblasts. This transition is regulated by a proneural wave that sweeps across the neuroepithelium. The transcription factor Tailless (Tll) reveals high expression in the lateral neuroepithelium and its expression decreases towards the neuroepithelial to neuroblasts transition zone. We find that a 1.5kb tll enhancer fragment can drive restricted expression in the optic lobe. A tightly regulated expression level of Tll is crucial for normal neuroepithelial morphology. Both knockdown of tll expression and tll misexpression targeted to the neuroepithelium leads to extended neuroepithelias with abnormal morphology. Clonal loss- and gain-of tll function leads to apical constrictions of epithelial cells and extrusion. Some of the extruded cells start to express Deadpan (Dpn), a stem cell marker, and aberrantly localize in areas of differentiating neurons. Altogether, our results indicate that Tll has a major role in stem cell identity and neuroepithelial morphology in the optic lobe. Furthermore, through this work we might gain insight into the role of the Tll mammalian homologue Tlx that has also been implicated in regulating neural stem cell maintenance and differentiation.

#20

Enhanced infiltration of mesenchymal-like stromal cells with increased expression of the immunosuppressive ligand PD-L1 and evidence of T cell exhaustion in non-small-cell lung cancer.

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Background: Blockade of the immune checkpoint PD-1/PD-L1 pathway using antibodies has shown durable antitumor responses in non-small cell lung cancer (NSCLC). Despite this initial success, the majority of patients with documented PD-L1 expression fail to respond. There is general agreement that the underlying mesenchymal stromal compartment is critically involved in not only driving tumor phenotype but response to treatment and clinical outcome. However, the immunophenotype and underlying alterations in the mesenchymal stromal cell compartment and triggering events whereby this occurs is not known and whether this occurs via PD-1/PD-L1 pathway remains unexplored in NSCLC. Methods: Fresh NSCLC specimens and matched adjacent nontumor (n=17) were digested to single cell suspensions and analysed using multicolour flow cytometric analysis and/or flow cytometric cell sorting (FACS) for the simultaneous expression of a panel of fluorescently conjugated monoclonal antibodies directed against the following epitopes: CD45-PB, CD31-PB, CD14-PB, CD73-APC, CD90-FITC, EpCAM-PE-Cy7 and PD-L-PE. In parallel, we analysed the quantity and functional orientation of tumor infiltrating lymphocytes (TILs) using a multiparameter flow cytometric approach. FACS sorted tumor-derived mesenchymal stromal cell subsets were activated with human IFN-γ, TNF-α or IFN-γ/TNF-α and the change in membrane expression of PD-1 ligands was examined. Results: We have identified an enhanced infiltration of nonhematopoietic, nonepithelial mesenchymal stromal cell subsets in NSCLC specimens compared to matched uninvolved lung tissue. Specifically, we found an increased infiltration in the Lineage-EpCAM-CD73+CD90+ (n=17, p=0.01) and Lineage-EpCAM-CD73-CD90+ (n=17, p=0.002) but not Lineage-EpCAM-CD73+CD90- subset (n=17, p=0.590) in NSCLC. Further analysis revealed that both Lineage-EpCAM-CD73+CD90+ and Lineage-EPCAM-CD73+CD90- subsets upregulated membranous PD-L1 expression (n=15, p=0.01 and p=0.003, respectively), despite PD-L1 also being expressed in the tumor fraction (Lineage-EpCAM+ fraction). In parallel, both CD4+ and CD8+ TILs show evidence of marked exhaustion via enhanced expression of PD-1 and decreased CD107a (n=15, p=0.0156 and p=0.0156, respectively). Last, membranous expression of the PD-1 ligands, PD-L1 and PD-L2, were upregulated in NSCLC-derived Lineage-EPCAM+CD73+CD90+ cells flowing exposure to interferons. Conclusions: Our observations suggest that an immune reactive tumor microenvironment may fine tune and enhance the immunosuppressive signaling in infiltrating mesenchymal constituent cells via PD-1/PD-L1 pathway in NSCLC. This premise is further strengthened as priming NSCLC infiltrating mesenchymal stromal cells with tumor-derived cytokines modulates their immune status via PD-L1/PD-L2 ligand expression. Further study is required to determine the functional significance of mesenchymal stromal cell compartment on tumor specific T cell immunity and whether they contribute to cancer immune escape in NSCLC via PD-1/PD-L1 pathway.

#21

The molecular control of pluripotency in mouse embryonic stem cells.

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Pluripotent stem cells have the unique ability to both self-renew indefinitely and to differentiate into all somatic lineages and germ cells. The choice between pluripotency and lineage commitment is controlled by a number of extrinsic and intrinsic factors at several molecular regulatory layers. Numerous studies thus far have described a network of transcription factors and epigenetic modifiers centered around Oct4, Sox2 and Nanog which in turn is regulated by extracellular signals and which represents the molecular basis of pluripotency. In addition, it has been suggested that the expression levels of key factors of the pluripotency network are highly heterogeneous at the single cell level in mouse embryonic stem cell (ESCs) cultures. So far, it is still elusive how pluripotency factors regulate each other in single cells and what the significance of the observed heterogeneity is. In this study we aim to show how cells in different states respond to manipulations of the transcription factor network or signaling changes and will link the cellular dynamics to future fate choices. Long-term continuous time-lapse imaging of knock-in reporter cell lines enables us to quantitatively measure endogeneous protein levels of key pluripotency factors in single ESCs over many days. The combined application of microfluidic devices with our imaging platform will allow us to precisely stimulate single cells, observe their immediate responses and track their future fates. With our approach the cellular dynamics of ESC self-renewal and differentiation can be analyzed at an unprecedented resolution. The continuous tracking of single differentiating ES cells together with the analysis of lineage markers will identify potential functionally distinct subpopulations. The high resolution of our data furthermore provides a rich resource for the development of accurate computational models of network dynamics.

#22

A user-friendly method for fast and accurate cell segmentation in long-term microscopy.

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Quantitative long-term cell microscopy provides new insights into numerous biological processes. The large amounts of data generated in such experiments require automated analysis, and many algorithms to detect cells in microscopy images (cell segmentation) have been published. However, existing methods are either not robust enough for long-term microscopy or limited to specific image analysis problems they have been optimized for. We present an algorithm for cell segmentation that can be trained by manually marking cells in an interactive way: training and image segmentation are highly efficient, thus enabling live preview of results. Quantitative comparison against related methods shows that our method achieves comparably high segmentation quality in various long-term imaging experiments involving different cell types and image acquisition modalities.

#23

Developmental engineering of cartilage templates for endochondral ossification using mouse adult mesenchymal stem cells.

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Adult mesenchymal stromal/stem cells (MSC) are able to give rise to various tissue types upon lineage specific differentiation conditions, including cartilage, which is our main focus of research. So far, cartilage generation in vitro is still challenging. All protocols employed for cartilage engineering have failed to obtain articular chondrocytes that maintain their characteristic upon in vivo engraftment. The protocols are difficult to be consistently reproduced and the cartilage display characteristics of growth plate chondrocytes that gets into hypertrophic differentiation program prematurely, consequently leading to the short life of the engrafted tissue in vivo and its differentiation into osteogenic tissue. Part of the problem relies in two aspects: 1-) The mix population of MSC, which harbors a minor and variable fraction of real stem cells and early progenitors. 2-) The protocol of differentiation. Our research goals are: 1-) Characterize in detail the adult endosteum derived MSC (EdMSC) Sca-1+ PDGFR-α+ double positive population as previously described (1), since these cells proved to be the most robust in tri-lineage differentiation assays. 2-) Design a cartilage differentiation protocol based on the molecular program employed by embryonic limb mesenchymal progenitors (LMPs). 3-) Characterize extensively the LMPs to assess their similarities and differences with adult Ed-MSC. Here, we present the identification of four subsets within EdMSC based on the combined analysis of CD73 and CD90 markers. A cell cycle analysis and the expression of CD15 (a marker associated with embryonic and adult stem cells) seem to point out CD90- CD73- cells as the subsets with higher stemness potential. A step wise protocol of cartilage differentiation based on embryonic limb mesenchymal studies (2) proved to be more effective triggering adult EdMSC cartilage differentiation than the standard protocols. Currently, we are working on the characterization of LMPs by flow cytometry and IHC, assessing their performance in cartilage differentiation assays. The protocol of cartilage differentiation is being improved using LMPs and will be tested on the subsets of adult EdMSC. A comparative transcriptome analysis will be carried out to compare the gene expression profile between

LMPs and murine EdMSC CD73- CD90- subset. The use of pure stem/ early progenitor mesenchymal cells combined with step-wise developmental differentiation protocols will allow the generation of engineered cartilage with high endurance in transplantation approaches.

#24

Role of the NSL complex in pluripotency maintenance and transcriptional regulation.

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MOF, a Histone Acetyl Transferase (HAT) is known to acetylate lysine 16 of histone H4 and non-histone substrates such as p53 depending on its association with its interacting partners. MOF resides in two distinct complexes, Male Specific lethal (MSL) and Non-specific lethal (NSL) complex involved in overlapping as well as exclusive functions. In drosophila the NSL complex regulate housekeeping genes and MSL complex is involved in upregulation of male X chromosome. The NSL complex comprises of at least 7 subunits KANSL1, KANSL2, KANSL3, MOF, MCRS1, WDS and MBDR2. However, the function of these complexes in mammalian system is not well defined. Depletion of MOF leads to embryonic lethality in mice; MOF is also known to be misregulated in several cancers. Recent findings have shown that haploinsufficiency of KANSL1 leads to micro-deletion syndrome a genetic disorder with severe phenotype shedding the functional relevance of KANSL complex in the mammalian system beyond regulation of housekeeping genes. In order to understand the function of the KANSL complex in depth we performed systematic analysis in mouse embryonic stem cells (mESCs) by doing the genome wide ChIPseq and transcriptome analysis using RNAseq approach in ESCs depleted of some of the core subunits of the KANSL complex. Depletion of the individual components of the KANSL complex -NSL1, NSL3, MOF and MCRS1 resulted in proliferation defects indicating that they perform non-redundant functions even though they are part of the same complex. We also showed that KANSL complex members are crucial for maintaining the pluripotency of the mESCs, as the depletion of individual subunits resulted in differentiation of the ESCs and also down regulation of some of the pluripotency factors e.g., NANOG, ESRRB and REX1. Members of the KANSL complex were found to regulate transcription by binding the promoters and also the TSS-distal enhancer regions. Correlation of the TSS binding of the KANSL complex members and the RNAseq profiles of the respective knockdowns showed that the transcripts of the TSS bound genes tend to be downregulated upon knockdown of the KANSL components (NSL3 and MOF) indicating that KANSL complex is involved in regulation of active transcription. Furthermore, members of the KANSL complex also bound and regulated the ES cell specific enhancers. Our data provides a valuable source to dissect the functional relevance of NSL complex in pluripotency network and gene regulation.

#25

Molecular control of hematopoietic stem cell behavior by the microenvironment.

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Hematopoietic stem cells (HSCs) are multipotent cells able to produce massive numbers of all mature blood cell types, while self-renewing long-term. Their remarkable potential to regenerate the blood system has long been appreciated and the number of HSC transplantations has increased over the last decades. However, the low frequency of HSCs in the bone marrow and the current inability to maintain them ex vivo without genetic modification pose important challenges towards their improved clinical application. Previous studies revealed that a clonal stroma cell line (AFT024) can maintain the number of HSCs, even upon long co-culture periods. However, the behavior of single HSCs under maintenance conditions and the molecular players involved in the HSC-stroma interaction remain obscure. The purpose of this study is to investigate the underlying mechanism of ex vivo HSC maintenance, by continuous long-term imaging at the single cell level.

#26

A dual role of evi1 during zebrafish developmental hematopoiesis.

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The Evi1 locus was originally identified as a common site of retroviral integration in murine myeloid tumors. Several reports associate Evi1 expression with aggressiveness in myeloid leukemia. Since developmental pathways often reactivate in cancer and more recent studies also suggest involvement of Evi1 in developmental hematopoiesis, we hypothesized that EVI1 also plays critical roles during developmental hematopoiesis and employed the zebrafish (ZF) model to study these aspects. Here, we analyzed zebrafish embryo to under-stand how evi1 modulates early blood development. We found that the zebrafish homologue evi1 co-localizes with several hematopoietic markers (scl, gata1, gata2 and pu.1) during em-

bryogenesis, indicating a role in primitive hematopoiesis. To analyze the functional role of evi1, we performed in vivo loss-of-function studies by injecting control and inhibitory morpho-lino oligonucleotides (MO) suppressing evi1 pre-mRNA splicing, respectively, in zebrafish zygotes. Knockdown of evi1 impairs embryonic myelopoiesis (monitored by pu.1, mpo and l-plastin expression), but no changes were observed in hemangioblasts (scl or lmo2) or primi-tive erythroid progenitor cells (hbae3). Megakaryopoiesis is also impaired as shown by re-duced CD41 expression. Notably, loss of definitive hematopoietic stem cells (HSC) was iden-tified in evi1 morphants in the AGM region, suggesting an additional role in definitive hema-topoiesis. Consistently, no ikaros+ lymphocyte precursor cells or rag1+ T-lymphocytes were observed in evi1 morphants and less circulating globin+, lyz+ and cd41+ cells were detected in transgenic fish analyzed by flow cytometry at 5 dpf. Arterial specific efnb2a and dlc ex-pression instead remained unaltered, indicating that impaired artery identity is not the reason for the HSC phenotype. Previous reports in adult murine hematopoietic cells suggest that Evi1 affects HSC proliferation through regulation of Gata2. Indeed, we could show that co-injection of gata2 mRNA was able to rescue the impaired myeloid as well as the HSC pheno-type in evi1 morphants. However, data generated by TUNEL assays and anti-activated Caspase-3 staining indicates that evi1 may sustain survival of developing AGM-HSCs by enhancing their apoptosis resistance. Interestingly, co-injection of gata2 mRNA seems to be able to restore the apoptotic phenotype mediated by evi1. Taken together, our data suggest that evi1 regulates embryonic myelopoiesis and HSC formation, while leaving primitive eryth-ropoiesis unaltered. The precise molecular basis of evi1-mediated effects on these separate phases of developmental hematopoiesis is currently being explored.

#27

A combined cellular and biomaterial approach for annulus fibrosus rupture repair.

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Introduction: Recurrent intervertebral disc (IVD) herniation and degenerative disc disease have been identified to be the most important factors contributing to increased pain and disability after conventional discectomy. An annulus fibrosus (AF) closure device that provides immediate closure of the AF rupture, restores disc height, reduces further disc degeneration and enhances self-repair capacities is an unmet clinical need. Materials and Methods: In this study, a poly(trimethylene carbonate) (PTMC) scaffold seeded with human bone marrow derived mesenchymal stromal cells (MSCs) and covered with a poly(ester-urethane) (PU) membrane was assessed for AF rupture repair in a bovine organ culture annulotomy model under dynamic load for 14 days. Results: PTMC scaffolds remained within the AF defect and no protrusion of nucleus pulposus (NP) was observed after 14 days. Disc height of PTMC scaffold treated discs was maintained, while disc height significantly decreased after repetitive dynamic load and recovery in the absence of scaffold and membrane repair ($p<0.001$). Type V collagen (potential AF marker) gene expression of implanted MSCs increased after 14 days of culture in discs. Implantation of MSCs up-regulated type II collagen gene expression in the AF tissue next to the PTMC scaffold and in the NP tissue. Furthermore, implantation of MSCs under loaded condition showed a trend towards down-regulation of MMP13 gene expression in the AF tissue next to PTMC scaffold. Discussion: PTMC scaffolds combined with sutured PU membrane restored disc height of annulotomized discs, and prevented protrusion of NP, while MSCs implanted in situ differentiated toward an AF cell like phenotype. Furthermore, results show that a combined cellular and biomaterial approach could positively modulate the adjacent AF and NP cells gene expression and could potentially slow down the IVD degenerative cascade by up-regulating anabolic gene expression and down-regulating catabolic gene expression in the disc tissue. Acknowledgement: This study is funded by the AO Foundation Collaborative Research Program Annulus Fibrosus Rupture Repair.

#28

Asymmetric cell division of hematopoietic stem cells.

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In order to maintain their numbers while constantly replenishing all mature blood cells, hematopoietic stem cells are thought to divide asymmetrically. In this model the future fate of the daughters is determined by the polarized localization of cellular components during division. This subsequently leads to the differentiation of one daughter while the other retains its stem cell capacity. However, this mechanism could never been shown directly in hematopoietic stem cells. It is therefore also possible that these cells divide symmetrically and that later commitment to differentiation is determined by mechanisms not related to division. Previous studies have identified proteins that are asymmetrically segregating during invertebrate and vertebrate progenitor divisions. However, due to technical limitations it was not possible to observe the mitotic segregation of proteins in living hematopoietic cells and link it to future cell fates. We have therefore developed novel bioimaging systems allowing the continuous long-term observation of living hematopoietic stem and progenitor cells at the single cell level. This approach allows us to link the asymmetric segregation of cellular components to the future cell fates of generations of progeny. I am screening for proteins that are asymmetrically segregating during division of murine hematopoietic stem cells. In addition, differentiation marker indicating asymmetric cell fates of the hematopoietic stem cell

progeny are being identified. This approach will enable us to show for the first time asymmetric cell division of hematopoietic stem cells on a molecular and functional level.

#29

Laminin inhibits cardiac progenitor cell proliferation via beta-3-integrin-dependent degradation of YAP.

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Background: The adult heart exhibits limited regenerative potential and responds to injury with extensive remodeling of the extracellular matrix. Adoptive transfer of ex vivo expanded cardiac progenitor cells (CPCs) improves post myocardial infarction remodeling and survival in animals, and first human studies are yielding promising results. However, the efficacy of cell therapy is limited by the lack of proliferation and poor survival of donated CPCs in the infarcted heart. The matrix protein laminin-111 (LN1) is a major contributor to the myocardial niche and to the post-ischemic myocardial microenvironment; however, its role with respect to activation and regulation of CPCs is poorly understood. Methods and Results: C-kit+ rat and Sca1+/CD31- side population mouse CPCs were plated at low density and under low serum (0.1% FCS) conditions on LN1 and fibronectin (FN). Compared to FN, CPCs on LN1 showed impaired cell cycle progression and proliferation. Mechanistically, LN1 led to cytosolic retention and rapid degradation of the downstream Hippo target and transcriptional co-activator Yes-associated protein (YAP) in the absence of canonical Hippo pathway activation. This was mediated by beta3-integrin, as YAP immunoprecipitation showed that YAP interacted with beta3-integrin and beta3- but not beta1-integrin-targeting siRNA partially prevented the LN1-induced YAP degradation. Next generation sequencing and gene ontology enrichment analyses revealed profound changes in expression of YAP-target genes, and genes involved in cell cycle, stimulus response and metabolic processes associated with LN1. Among these, the cell cycle regulator and stress response kinase polo-like kinase 2 (Plk2) was strikingly downregulated by LN1. Conclusion: LN1 is a novel regulator of YAP that acts via a beta3-integrin-dependent non-canonical pathway to downregulate Plk2 and inhibit CPC proliferation. Plk2 may represent a novel target for ex vivo gene therapy to enhance proliferation of adoptively transferred CPCs in the infarcted heart.

#30

Generation of a bone organ in vivo through endochondral ossification by adipose-derived stromal cells.

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INTRODUCTION: Bone marrow derived stromal cells (BMSC) form bone ectopically either through intramembranous ossification, by direct mineralization of a BMSC-laid matrix or through endochondral ossification, by forming a cartilaginous matrix remodelled into bone. Adipose-derived stromal cells (ASC) have yet only been shown to form bone through intramembranous ossification. The goal of this study was to investigate if ASC can form bone through endochondral ossification. METHODS: Stromal vascular fraction (SVF) cells were isolated by collagenase digestion of human adipose tissue from three healthy donors and cultured as monolayer. Expanded ASC were then either centrifuged to create micromass pellets or seeded on 4 mm-diameter, 1 mm-thick collagen-based cylindrical scaffolds (UltrafoamTM). They were then cultured in serum free medium in the presence of TGFβ-3, dexamethasone, ascorbic acid and BMP-6 for 4 weeks (chondrogenic medium). Half of the constructs were further cultured for 2 weeks in serum free medium supplemented with β-glycerophosphate, L-thyroxin and IL1β (hypertrophic medium). All constructs were implanted subcutaneously in nude mice and harvested after 4 and 8 weeks, followed by histological analysis. RESULTS: In vitro, deposition of a cartilaginous matrix and positivity for glycosaminoglycans (GAG), collagen type II and Indian hedgehog (IHH) was shown in pellets and scaffolds. Upon induction of hypertrophy, gene expression analysis showed up-regulation of collagen type X, BSP and MMP13, confirmed at protein level. In vivo, ASC formed bone tissue in pellets and in scaffolds, in chondrogenic or hypertrophic conditions. In pellets, bone was formed after 4 weeks, whereas in scaffolds it was only observed after 8 weeks. All constructs still displayed areas with chondrocytes, GAG and collagen type II. Adjacent areas with bone tissue contained osteocytes, surrounded by a matrix with collagen type X, BSP and MMP13. Osteoclasts were found at the rim of the constructs, indicating matrix remodelling. Bone was vascularized and included bone marrow at 8 weeks. In situ hybridization for human-specific sequences identified osteocytes and osteoblasts derived from the implanted ASC. CONCLUSION: Adipose-derived stromal cells are able to undergo the developmental program of endochondral ossification. Therefore they could be considered possible candidates for treatment of clinical scenarios where osteogenic tissue is needed. The actual endochondral bone regenerative potential of adipose-derived stromal cells needs to be further assessed in an orthotopic model.

#31

Generation of interneurons and astrocytes during cerebellar development.

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The anatomical and functional complexity of the cerebellum is reflected by a remarkable heterogeneity of neuronal and astroglial subsets, both endowed with highly distinctive morphological and spatial features. While mechanisms of neuronal diversification have been partly clarified, astrogliogenesis remains poorly explored. We therefore addressed the genesis of the repertoire of astroglial types. Birthdating approaches demonstrated that astrocytes are generated in accordance to a well-defined outside-in spatio-temporal sequence. These data were further confirmed by transplantation experiments indicating that specific astroglial phenotypes are instructed by temporally defined environmental cues. Clonal analysis further revealed that two progenitor types contribute to the astroglial phenotypic repertoire. One type generates large clones including diverse astroglial types, the other produces exclusively white matter (WM) astroglia. Furthermore, diverse astroglial phenotypes display distinct proliferative rates and developmental potentials after birth. In particular, proliferative astrocytes in the prospective white matter (PWM) are the sole source of interneurons postnatally. Most interestingly, through diverse in vivo fate-mapping approaches and in vitro assays, we showed that a slow-proliferating progenitor with astroglial features resides in the cerebellar PWM and produces both interneurons and WM astrocytes. On the whole, these results reveal that cerebellar astrogliogenesis occurs according to a well-defined spatio-temporal pattern and set the bases to understand heterogeneity in specification and functions of cerebellar astrocytes. Moreover, they unveil a tight lineage relationship between WM astroglia and interneurons generated in the postnatal cerebellum.

#32

Replacement of teratoma formation in immunodeficient mice through bioreactor culture of embryoid bodies for validation of pluripotency of human stem cells.

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The demonstration of pluripotency of newly derived stem cell lines through the formation of teratoma within eight to ten weeks after transplantation into immunodeficient mice has been traditionally the gold standard. Nevertheless, the place of implantation, the different mouse strains as well as the duration of the tested cells to be kept in the mouse are variables in the teratoma formation. In addition, none of the metabolic parameters can be measured in the mouse model. In this study we evaluated the feasibility of in vitro teratoma tissue formation in a shorter time frame by culturing human-derived stem cell lines within collagen scaffolds using a perfusion-based bioreactor device. Embryoid bodies (EBs) derived from two different hESC lines and one iPS line were embedded into a collagen scaffold (Ultrafoam®) and subsequently cultured dynamically in a commercially available bioreactor (Cellec Biotek AG, Basel) or statically in multiwell plates, for up to 5 weeks. EBs were also implanted subcutaneously in immunodeficient mice for 8 weeks and used as controls. Harvested tissues from all the experimental groups were assessed by using both immunohistochemistry (IHC) and qPCR of selected markers for the three germ layers such as alpha-fetoprotein (AFP, endoderm), Brachyury (mesoderm), Nestin and Pax6 (neural ectoderm) and Keratin 5 (non-neural ectoderm). Supernatants of in vitro cultures were collected during each culture medium change and analysed for the glucose consumption of the cells and the production of AFP. Bioreactor-based cultures from both cell lines developed a teratoma-like tissue characterized by the positive expression of three germ layers' markers by IHC, already after 3 weeks of culture. Moreover, AFP expression was detected at both mRNA and protein levels reaching peak values between 3 and 4 weeks, as assessed by qPCR and ELISA respectively. The expression profile of the teratoma built in the bioreactor was also positive for Pax6 and Brachyury on mRNA level. A rising glucose consumption with a peak at around 2 weeks was also observed in bioreactor cultures established from both cell lines. At variance, in static condition neither specific structures nor positive expressions were detected in the formed tissue and supernatants. In conclusion, we present a bioreactor-based method to generate metabolically active teratoma tissues in vitro with similar phenotypic expression of those observed in mice. The method will be further validated to possibly become a surrogate of in vivo teratoma formation tests for novel pluripotent stem cell lines. Future studies will include gene expression profiles of teratoma tissue of the different experimental groups using whole genome expression analysis by Illumina.

#33

Signaling dynamics in mouse embryonic stem cells.

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A crosstalk of signaling pathways regulates the fate mouse embryonic stem cells (mESCs) in terms of self-renewal or differentiation. Whereas the role of the core transcription factors such as Oct4, Klf4, Sox2 and Nanog in maintaining pluripotency has been studied in quite some detail, the upstream signaling

mechanisms involved in cell fate decisions is relatively poorly understood. To investigate signaling dynamics over time in single mESCs in pluripotency and differentiation conditions, we will use a combination of time-lapse imaging, microfluidics, fluorescent signaling reporters and pathway-specific manipulating agents. We will link the activity levels of signaling pathways or components thereof to future cell fate decisions to better understand the regulation of pluripotency and differentiation in mESCs.

#34

Cell cycle reactivation of dormant cochlear stem/progenitor cells in early postnatal FUCCI mice by small molecule compounds.

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Due to the lack of intrinsic regenerative capacity of the mammalian auditory epithelium, hair cell loss results in permanent hearing deficit. Much effort is therefore put into defining novel experimental strategies to prevent sensory cells loss or to regenerate hair cells. Despite the post-mitotic state, a population of dormant tissue resident stem/progenitor cells has been recently described expressing the stem markers Sox2 and Lgr5. In order to identify regulators of cell cycle re-entry capable to trigger stem cell activity, we have established otic sphere assays and organotypic cultures from FUCCI reporter mice. Here, cell cycle re-entry and progression can be promptly and dynamically detected by expression of two fluorescently tagged probes: Geminin-AG, labeling S/G2/M by green fluorescence and Cdt-1-KO2, labeling G0/G1 cells with red fluorescence. Hair cell progenitors have been previously demonstrated by genetic means to be Wnt responsive. In this study, treatment with a selective GSK3 inhibitor (GSK3i), led to an increase of Gem-AG+ cells (from 1.62%+/-0.3 to 11.6%+/-2.8) in primary spheres and a substantial increase of GFP+ cells in Lgr5 GFP mice (from 0.9%+/-0.8 to 10.9%+/-8), as well as an increase of S/G2/M cells in the Lgr5+ population. To test the effect of the GSK3i on undissociated organs, we established organotypic Organ of Corti cultures. Here we detected a significant increase in the fraction of Sox2 proliferating cells upon GSK3i treatment, identified as Sox2+/Gem-AG+ (from 0.7%+/-0.6% to 11.8%+/- 3.12) or Ki67+/Sox2+ (15.6%+/-3.6). Rarely, novel Myo7a+/Edu+ cells were generated in the organotypic culture upon GSK3i treatment. In conclusion, we show for the first time that dormant Sox2/Lgr5 support cells can be triggered to re-enter cell cycle by selective small molecule compounds in postnatal animals. The contribution of these cells to tissue regeneration and in vivo validation of these findings are currently focus of research. In parallel dynamic analysis of cell cycle re-entry by video microscopy using Lgr5 and FUCCI animals is analyzed. The combination of stem cell and cycle reporters utilized provides a robust tool to identify novel regulators of auditory organ regeneration.

#35

AKT kinase governs SOX2 protein expression and cancer stem cell potential in human breast cancer.

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SRY (sex determining region Y)-box 2 (SOX2) is a transcriptional master regulator in early ontogenesis that promotes neuronal and foregut development and confers stemness to embryonic stem cells (ES). In adult cells, activation of the SOX2 locus supports reprogramming of somatic cells into pluripotent stem cells (iPS) and has established tumorigenic potential in various cancer types. In mammary carcinoma, SOX2 expression has been linked to cancer stem cell (CSCs) functions and suggested as predictor of poor clinical outcome. Still, the molecular regulation of SOX2 in breast cancer remains largely unknown. Here, we demonstrate a functional dependence of SOX2 expression on the Ser/Thr-kinase AKT, which regulates localization and stability of the SOX2 protein in human breast cancer cells. We observed a marked up-regulation of SOX2 protein in AKT overexpressing cells, whereas specific AKT kinase inhibition through small molecule inhibitor MK-2206 or lentivirus mediated shRNA knockdown depleted for SOX2 protein. Vice versa, neither overexpression nor knockdown of SOX2 had overt impact on AKT levels or phosphorylation status, suggesting that AKT is an upstream regulator of SOX2 in breast cancer. On a mechanistic level, AKT was shown to influence the stability and/or turnover of SOX2 protein. Breast cancer cells modified to express a mCherry-SOX2 fusion protein showed a pronounced signal intensity decrease in AKT-inhibited over control samples, which was linked to nucleo-cytoplasmic redistribution of SOX2. On a functional level, anti-AKT treatment inhibited sphere formation in primary as well as serial replating tumor spheres assays. This AKT dependent defect could be partially rescued by concomitant induction of SOX2 expression. Our investigations thus unraveled a functional dependence of the stemness factor SOX2 on the Ser/Thr-kinase AKT in mammary carcinoma. These findings suggest that AKT-inhibitors effectively target SOX2-expressing breast cancer (stem) cells, which may be of high clinical significance for the therapy of SOX2 positive tumors.

#36

The vascular-derived blood protein fibrinogen regulates the differentiation of adult neural stem cells into astrocytes after traumatic brain injury.

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The identity of extracellular environmental factors in regulating adult neural stem cell (NSC) fate in CNS disease is a fundamental and unresolved question. However, the effects of blood-derived factors on NSC fate decisions have not been described. Here, we show that the blood-derived factor fibrinogen is deposited in the NSC environment colocalizing with migrating Dcx+ neuroblasts 3 days after cortical brain injury. Strikingly, loss of fibrinogen using fibrinogen knockout mice, revealed a reduction in the total number of NSC-derived astrocytes (BrdU/GFAP-double positive) by 50 % in the body of the lesion center following cortical injury. Application of fibrinogen on primary NSCs increased the percentage of GFAP+ astrocytes by 50%. Furthermore, treatment of SVZ explants with fibrinogen inhibited NSC migration ex vivo. Gene expression microarray analysis of fibrinogen treated NSCs revealed the upregulation of the BMP signaling related genes and the repression of cell cycle related genes. NSC fate and cell cycle progression are dependent on growth factor and integrin signaling. Western Blotting revealed that treatment of NSCs with fibrinogen induced the phosphorylation of Smad1, the transcriptional mediator of BMP signaling and pretreatment of NSCs with a BMP receptor inhibitor partially blocked the fibrinogen-induced NSC differentiation into astrocytes. Furthermore, consistent with the results of our microarray data, Western Blotting revealed that fibrinogen treatment of NSCs resulted in a reduced phosphorylation of FAK and MAPK, both normally phosphorylated upon integrin signaling. In summary, we demonstrate that fibrinogen induces the differentiation of NSCs into astrocytes, potentially mediated by the induction of BMP signaling and the repression of integrin signaling. Our future work will examine the therapeutic potential of fibrinogen inhibition on adult NSC neurogenesis in CNS disease. Supported by the Fazit Foundation Graduate fellowship to SS, the International Graduate Academy fellowship to CB, the European Commission FP7 Grant PIRG08-GA-2010-276989, NEUREX and the German Research Foundation Grant SCHA 1442/3-2 to CS.

#37

Deciphering the role of splicing factors in enhancing the transcription factor-mediated somatic cell reprogramming.

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The use of induced pluripotent stem cells (iPSCs) in clinical setting is limited by the relative poor efficiency and the safety concerns associated with the use of viral constructs in the reprogramming process. A better understanding of the molecular mechanisms underlying somatic cell reprogramming would pave the way towards a broader use of this technology. Much of the current work in the field aims to improve the consistency and efficiency with which iPSCs are obtained from primary somatic cells. Alternative splicing, a post-transcriptional regulatory mechanism responsible for gene regulation and protein diversity, has been recently shown to be an intrinsic part of the reprogramming process. Accordingly, changes in the expression of specific splicing factors have been found to lead to an enhanced efficiency of iPSCs generation1,2. We have carried out a computational analysis of publicly available RNA-seq data obtained from a somatic cell reprogramming system and identified that splicing factors are a class of genes with a striking change in expression between somatic cells and iPSCs. Among these, the epithelial splicing regulatory proteins ESRP1 and ESRP2 that have also been found to play a role in the epithelial-to-mesenchymal transition are strongly up-regulated during reprogramming. In the past year we established that these factors significantly increase the efficiency of iPSC generation in transgenic mouse embryonic fibroblasts that harbor the conventional transcription factors that are required for reprogramming. We are currently pursuing the underlying mechanisms by analyzing transcriptome expression changes along reprogramming time series of mouse embryonic fibroblasts.

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#38

In vitro generation of dynorphin, NARP, and MCH expressing neurons from mouse fibroblasts.

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The sleep disorder narcolepsy is characterized by excessive daytime sleepiness, sleep attacks, cataplexy, hypnagogic hallucinations, and sleep paralysis. Loss of orexin, dynorphin and NARP peptides within the lateral hypothalamus is well established in the brain of narcolepsy patients. In the present project taking advantage of induced pluripotent stem cell techniques we are aiming at producing neurons expressing

lateral hypothalamic markers including NARP, Dynorphin and orexin to test their physiological properties in-vitro. Methods: Neuronal differentiation was carried out using embryonic body (EB) formation method from embryonic mouse fibroblasts. Results: The iPS cells were established and were characterized. There is only one report about differentiation of embryonic stem cells toward hypothalamic progenitor cells (1). Using modified version of this protocol, in preliminary studies, coaxing iPSC lines toward hypothalamic progenitors resulted in expression of hypothalamic genes Rax, Six3, and Vax1. We observed the expression of lhx9 in all SHH treatments and lhx6 gene in 10, 30, 50 ng/ml treatments. The expression of these markers indicates that we have generated cells of the region lateral to PVN and VMH nuclei. Treatment of the EBs with FGF8 and FGF12 resulted in the expression of dynorphin and BMP7 caused the expression of MCH and NARP. Experiments are ongoing to achieve the expression of other markers like orexin and evaluate the transcriptional and physiological properties of these neuronal cells and also to obtain similar hypothalamic cells from human fibroblasts. Conclusion: FGF and BMP signaling are involved in the expression of lateral hypothalamic marker genes.
Ref.: 1-Wataya et al, PNAS, 2008.

#39

Quantitative analysis of mouse ESCs phenotypes using single-cell live imaging.

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Embryonic stem cells (ESCs) have the unique abilities to self-renew and give rise to derivatives of all three primary germ layers. However, even though ESCs consist of a genetically homogeneous cell population, a number of studies have reported heterogeneities in ESCs cultures as a result of the fluctuation of the expression of key regulators responsible for maintaining the embryonic stem cell identity. In addition, even under defined self-renewing conditions, ESCs display a range of different morphologies that are rarely observed or reported in literature, mainly due to technical challenges. Here we combine time-resolved, single-cell live imaging of high spatiotemporal resolution with automated computational approaches to enable the quantitative analysis of the phenotypic characteristics of mouse ESCs in culture. Using our method, we quantify features related to morphology (area, volume, texture, orientation etc.), behavior (motion patterns, speed, etc.) and gene expression (expression of pluripotency and lineage markers). Finally, we use the extracted features to develop computational models for the prediction of morphologically distinct ESCs subpopulations and to analyze the molecular basis of ESCs fate control.

#40

Power-assisted liposuction (PAL) and lipoma-derived stem cells (LMSCs) of multiple symmetric lipomatosis (MSL) – a longitudinal study.

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Background: Lipomatosis is a rare condition leading to grotesque disfigurement. Complete removal is almost impossible and recurrences inevitable. The pathogenesis remains largely unknown. Involvement of adipose-derived stem cells (ASCs) in the development of multiple symmetric lipomatosis (MSL) has been proposed. The aim of this study was to evaluate powered-assisted liposuction (PAL) for the treatment of lipomatosis and to characterize lipoma-derived mesenchymal stem cells (LMSCs). Methods: Magnetic resonance imaging (MRI) was performed in two patients before and after surgery for quality assessment. In order to exclude malignancy incisional biopsies were taken before surgery. Outcome measures included aspiration volumes, duration of surgery, early morbidity, recurrence rates and overall patient satisfaction. Stromal vascular fraction (SVF) was extracted from patients diagnosed with MSL sharing similar phenotypic and clinical characteristics. LMSCs were analyzed by the colony-forming unit-fibroblast (CFU-f) assay and flowcytometry using standard markers (CD105 and CD73 (mesenchymal markers), CD31 and CD34 (endothelial markers) and the pan-haematopoietic marker CD45). Results: Seven male patients aged between 43 and 70 years were identified. The mean liposuction volume equalled 2948±1566ml, the mean surgery time 74±28minutes. One hematoma was observed, whereas one recurrence rate was noticed after a mean follow-up of 14 months. Malignancy was excluded by histology in five patients. Preoperative MRI confirmed the diagnosis with no signs for malignancy and showed an efficient removal of the lesions. After a mean follow-up of 18 months a high patient satisfaction was achieved. Three patients diagnosed with either MSL type I or II could be identified for LMSCs analysis. Molecular analysis of LMSCs showed a phenotype similar to that of stromal cells with high CFU-f frequencies (range 11.9-10.2). Flowcytometric analysis of the LMSCs revealed high levels of CD34 (65±1.8%), CD45 (44±6.7%) and CD73 (48±7.3%), whereas low levels of CD31 (9±2.3%) and CD105 (5.4±0.3%) were detected. Conclusions: PAL can serve as an efficient method for the treatment of MSL. It has a significant effect on tightening of the skin, leading to a high patient satisfaction. LMSCs share similar properties to stromal cells with a strong proliferative capacity. Further molecular studies are needed to regulate their activities at the source of the MSL.

#41

The ALS gene TDP-43 induces p53-mediated apoptosis in Neural stem cells

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The mammalian central nervous system arises from neural stem cells (NSCs). This process is highly regulated and Notch signaling plays a key role in the maintenance and fate of NSCs [1]. TDP-43 is regulated downstream of Notch1 in NSCs. Point mutations in TDP-43 were recently shown to cause Amyotrophic lateral sclerosis (ALS) and affected neurons show cytoplasmic inclusions of TDP-43 protein [2]. Accumulation of TDP-43 in apoptotic neurons is also a characteristic of other neurodegenerative diseases including Alzheimer's disease [3]. Therefore, TDP-43 is suggested to play a role in disease onset or progression but it is not known how it triggers or contributes to the disease phenotypes. We have investigated the function of wild type and mutant TDP-43 protein in NSCs in vivo and in vitro by gain and loss of function approaches. We performed in utero electroporation to both over express and knock down TDP-43 transiently in NSCs in the forebrains of mouse embryos. In our studies we could show, that over expression of TDP-43 and TDP-43 point mutants result in p53-mediated apoptosis of NSCs. In accordance, we were able to rescue the early lethality of hTDP-43-A315T mutant mice by pharmacological inhibition of p53. Furthermore, we show in our studies that TDP-43 binds Cdkn1a mRNA and demonstrate Cdkn1a as being upregulated after TDP-43 expression. This most probably results in altered cell cycle regulation which we observed following TDP-43 and TDP-43-A315T expression.

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#42

Modeling disease severity in an iPSC-based in vitro model of Familial Dysautonomia.

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The goal of this work is to explore the full potential of disease modeling using pluripotent stem cells. The Studer lab has previously shown that the neurodegenerative/neurodevelopmental genetic disease Familial Dysautonomia (FD) can be successfully modeled by the iPSC technology (Lee et al., Nature 2009). However, FD patients present with a range of symptom severity, from severe and mild to the asymptomatic, carrier phenotype. We aim to investigate if this severity range can be reproduced using the iPSC technology and what the mechanism behind this phenomenon may be. We generated iPSCs from FD patients with severe, mild, absent and healthy control patients. We then differentiated them into disease relevant cell types in vitro, including neural crest progenitor cells, peripheral sensory neurons and autonomic neurons. We found that when the cells were differentiated towards neural crest progenitor cells, mild and control patients could generate the cells adequately, while severe lines could only generate certain subtypes. Similarly, mild and control lines could develop into more committed precursors of the autonomic nervous system, while severe lines were not able to generate these cells. Furthermore, mild and control lines could differentiate into peripheral sensory neurons, while severe lines could not. This suggests that mild FD patients can generate the appropriate cell types, thus rendering their disease onset later and less severe. Using long-term survival assays in peripheral sensory neurons, derived from FD-iPSCs, we show evidence that the reason for development of FD symptoms in mild patients is due to degeneration rather than a lack of the relevant cells. This work was supported by a postdoctoral fellowship from the Swiss National Science Foundation.

#43

Ezh2 controls neural progenitor pool size and regional identity in the developing mouse midbrain.

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Precise temporal and spatial control of gene expression is essential for the determination of the size of the developing brain as well as for establishment of correct cell identities in different brain regions. The polycomb group protein Ezh2, enhancer of Zeste homolog 2, is the catalytic subunit of polycomb repressive complex 2 (PCR2) and is primarily responsible for trimethylation of histone H3K27 (H3K27me3). This epigenetic mark contributes to repression of many genes, which are pivotal for neural development. Here we show that Ezh2 is essential for midbrain development in a region-specific manner. After Wnt1-Cre-mediated ablation of Ezh2 in the midbrain we performed whole-genome transcriptome analysis of mutant and control midbrains as well as H3K27me3 ChIP. In the caudal midbrain loss of Ezh2 results in decreased neural progenitor (NP) proliferation due to negative regulation of Wnt/β-catenin signaling and precocious

exit of NP from the cell cycle leading to increased neuronal differentiation. Most intriguingly in the dorsal midbrain Ezh2 ablation not only leads to a loss of midbrain identity markers Pax3 and Pax7 but also to aberrant upregulation of forebrain transcription factors FoxG1 and Pax6 by direct de-repression. Together our data reveal a role of Ezh2 in regulating NP fate decisions and brain area identity by direct and indirect mechanisms.

#44

Yin Yang 1 regulates mammalian cortex development.

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The mammalian neocortex is a highly complex structure which is composed of a broad range of neurons and glial cells. Its organization into six neuronal layers contributes to higher cognitive functions of mammals. The development of the mammalian forebrain is driven by a complex network of signaling pathways and transcriptional regulators. Neural progenitor cell (NPC) proliferation, fate decisions and survival need to be tightly controlled to ensure proper cortex development. The transcriptional regulator Yin Yang 1 (YY1) has been shown to have context-dependent effects on these processes during development and tissue homeostasis of vertebrates. Here, we investigate the role of YY1 during mammalian cortex development. Conditional ablation of YY1 at early stages of cortical development in mice resulted in microencephaly. YY1 mutant mice exhibited decreased cortical size and thickness due to the depletion of the NPC pool at early stages of corticogenesis. YY1-deficiency led to impaired proliferation at embryonic day E12.5. In addition, lack of YY1 induced apoptosis at the onset of neurogenesis but had no effect on survival at later stages of corticogenesis. As suggested by the decreased thickness of the cortex, loss of YY1 decreased the number of deep and upper layer neurons at E18.5. However, the potential to differentiate into distinct neuronal layers was retained. In summary, our results show that the transcriptional regulator YY1 plays a crucial role during a defined time-window in cortical development.

Late abstracts:

#45

Analysis of Ski as a regulator of neural stem cell transcriptome dynamics.

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During cortical development many genes and signaling pathways are active but their functions and interconnections are poorly understood. The protooncogene Ski is a central integrator of signal and transcriptional functions through interactions with different partners. Ski is present in proliferating progenitors cells in the ventricular zone and in subtypes of differentiated neurons of the cortical plate. Deletion of Ski results in precocious differentiation of neuronal stem cells leading to a reduced progenitor pool at early stages of cortical development. In addition, loss of Ski leads to a precocious exit of neural stem cells from the cell cycle disturbing the timing of their differentiation. The aim of the project is to use systems biology to dissect the signaling pathways and transcriptional networks regulated by Ski during early cortical development. To compare the dynamics of various pathways in the presence and absence of Ski, we use in vivo transgenic marking of Ski-deficient neural stem cells (*Hes5::GFP; Ski-/-*) and committed progenitors (*Tbr2::GFP; Ski-/-*) to isolate and analyze the cells by RNA-seq. Using the ISMARA (Integrated System for Motif Activity Response Analysis), we are scanning the changes in the transcriptional profiles of neuronal and committed progenitors comparing Ski wt and KO samples through development.

#46

Systems analysis of mammalian forebrain development.

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The cerebral cortex of mammals is composed of millions of neurons organized into functionally distinct layers. These different types of neurons originate from what has been proposed to be a homogeneous pool of neural stem cells. We have undertaken a systems biology approach to understand development of the mammalian cerebral cortex, to elucidate the mechanisms controlling neuronal fate and differentiation, and to examine the homogeneity of the telencephalic neural stem cell population (NeuroStemX, SystemsX.ch). Through collaborative wet biology and computational modeling approaches we are deciphering the signaling and transcriptional networks that regulate the formation of cerebral cortical neurons. The control of these networks modulates the regimental differentiation and characterization of the neural stem cells, to pattern the complex six-layered structure of the cerebral cortex. Our hypothesis and preliminary data suggest that neural stem cells, rather than being homogeneous, are a heterogeneous population which vary in their transcriptional output over time and this renders them sensitive to extrinsic and intrinsic cues. The integration of the intrinsic and extrinsic signals controls neuron production and fate. A comprehensive understanding of transcriptional regulation and its interplay with an ensemble of upstream factors will pave the way for regeneration of cortical neurons and structures following disease and could have implications for cellular therapy and drug screening.