Speaker Abstracts

Keynote Lecture (supported by Sigma)

T1 - Stem cells, pluripotency and nuclear reprogramming

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The recent demonstration of in vitro reprogramming using transduction of 4 transcription factors by Yamanaka and colleagues represents a major advance in the field. However, major questions regarding the mechanism of in vitro reprogramming as well as the definition of pluripotent cell states need to be understood and will be one focus of the talk. *a. Mechanisms of direct reprogramming:* Direct reprogramming of somatic cells into induced pluripotent stem cells (iPSCs) can be achieved by over-expression of Oct4, Sox2, Klf4 and c-Myc transcription factors, but only a minority of donor somatic cells can be reprogrammed to pluripotency. We have demonstrated that reprogramming is a continuous stochastic process where almost all donor cells eventually give rise to iPSCs upon continued growth and transcription factor expression. Inhibition of the p53/p21 pathway or over expression of Lin28 increased the cell division rate and resulted in an accelerated kinetics of iPSC formation that was directly proportional to the increase in cell proliferation. These results suggest that the number of cell divisions is a key parameter driving epigenetic reprogramming to pluripotency. In contrast, Nanog over expression accelerated reprogramming in a predominantly cell division rate independent manner. *b. Different states of pluripotency:* Human and mouse embryonic stem cells (ESCs) are derived from blastocyst stage embryos but have very different biological properties, and molecular analyses suggest that the pluripotent state of human ESCs isolated so far corresponds to that of mouse derived epiblast stem cells (EpiSCs). We have rewired the identity of conventional human ESCs into a more immature state that extensively shares defining features with pluripotent mouse ESCs. This was achieved by exogenous induction of Oct4, Klf4 and Klf2 factors combined with LIF and inhibitors of glycogen synthase kinase 3 (GSK3) and mitogen-activated protein kinase (ERK) pathway. In contrast to conventional human ESCs, these epigenetically converted cells have growth properties, X chromosome activation state (aXa), a gene expression profile, and signaling pathway dependence that are highly similar to that of mouse ESCs. The generation of _na_ve_ human ESCs will allow the molecular dissection of a previously undefined pluripotent state in humans, and may open up new opportunities for patient-specific, disease-relevant research. *c. Gene targeting in human ES cells* A major impediment in realizing the potential of ES and iPS cells to study human diseases is the inefficiency of gene targeting. Using Zn finger mediated genome editing we have established efficient protocols to target expressed and silent genes in human ES and iPS cells. Finally, our progress in using iPS cells for therapy and for the study of complex human diseases will be summarized.

The EMBO Lecture

T2 - Lgr5 stem cells in self-renewal and cancer

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The intestinal epithelium is the most rapidly self-renewing tissue in adult mammals. We originally defined *Lgr5* as a Wnt target gene, transcribed in colon cancer cells. Two knock-in alleles revealed exclusive expression of *Lgr5* in cycling, columnar cells at the crypt base. Using an inducible Cre knock-in allele and the Rosa26-*LacZ* reporter strain, lineage tracing experiments were performed in adult mice. The *Lgr5*$^{+ve}$ crypt base columnar cells (CBC) generated all epithelial lineages throughout life, implying that it represents the stem cell of the small intestine and colon. Similar observations were made in hair follicles and stomach epithelium. Single sorted *Lgr5*$^{+ve}$ stem cells can initiate ever-expanding crypt-villus organoids in 3D culture. Tracing experiments indicate that the *Lgr5*$^{+ve}$ stem cell hierarchy is maintained in these organoids. We conclude that intestinal crypt-villus units are self-organizing structures, which can be built from a single stem cell in the absence of a non-epithelial cellular niche. The same technology has now been developed for the *Lgr5*$^{+ve}$ stomach stem cells. Intestinal cancer is initiated by Wnt pathway-activating mutations in genes such as APC. As in most cancers, the cell of origin has remained elusive. Deletion of APC in stem cells, but not in other crypt cells results in progressively growing neoplasia, identifying the stem cell as the cell-of-origin of adenomas. Moreover, a stem cell/progenitor cell hierarchy is maintained in early stem cell-derived adenomas, lending support to the “cancer stem cell”-concept. Fate mapping of individual crypt stem cells using a multicolor Cre-reporter revealed that, as a population, Lgr5 stem cells persist life-long, yet crypts drift toward clonality within a period of 1-6 months. Lgr5 cell divisions occur symmetrically. The cellular dynamics are consistent with a model in which the resident stem cells double their numbers each day and stochastically adopt stem or TA fates after cell division. Lgr5 stem cells are interspersed between terminally differentiated Paneth cells that are known to produce bactericidal products. We find that Paneth cells are CD24+ and express EGF, TGF-a, Wnt3 and the Notch ligand Dll4, all essential signals for stem-cell maintenance in culture. Co-culturing of sorted stem cells with Paneth cells dramatically improves organoid formation. This Paneth cell requirement can be substituted by a pulse of exogenous Wnt. Genetic removal of Paneth cells in vivo results in the concomitant loss of Lgr5 stem cells. In colon crypts, CD24+ cells residing between Lgr5 stem cells may represent the Paneth cell equivalents. We conclude that Lgr5 stem cells compete for essential niche signals provided by a specialized daughter cell, the Paneth cell.

Pluripotency and Epigenetics

T3 - Intergenerational epigenetic control of mammalian early embryonic development

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In mammals, totipotent pre-implantation embryos are formed by fusion of differentiated oocytes and spermatozoa. Acquisition of totipotency concurs with remodeling of chromatin states of both parental genomes, changes in maternally contributed transcriptome and proteome, and zygotic genome activation. Nuclear transfer experiments demonstrated that nuclei of mature germ cells are more proficient in supporting embryonic development than those of somatic cells. It is currently, however, unknown whether the developmental proficiency after natural conception is due to chromatin-encoded epigenetic information inherited from the germline. In my seminar, I will discuss our recent studies addressing the role of Polycomb Group proteins in the inheritance of epigenetic information between generations.

T4 - *Mad2l2* Is essential for epigenetic reprogramming in murine primordial germ cells

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Germ Cells are responsible for the continuity of life by transferring genetic material to the next generation. Following specification from pluripotent epiblast, primordial germ cells (PGCs) reacquire potential pluripotency and undergo genome-wide DNA demethylation, as well as changes in histone modifications (embryonic days E7.5-E9.5). PGC development was studied in mouse mutants lacking the *Mad2l2* gene (Mitotic Arrest Deficient 2-Like 2) gene. They were normally induced and found in the hindgut endoderm at E8.5. However, migratory PGCs were lost from the hindgut and dorsal mesentery by E9.0/9.5. Knock-out PGCs failed to arrest in the G2 phase of the cell cycle. Expression of somatic DNA methylation enzymes was maintained, change of histone 3 methylation was not initiated, and PGC-specific markers were lost. By E10.5 all PGCs had died by apoptosis. Adult females lacked ovaries, and males developed testes depleted of germ cells (Sertoli Cell Only phenotype). A role of Mad2l2 in the G2/M transition was also observed after exposure of *Mad2l2*-deficient embryonic fibroblasts to genotoxic stress. These somatic cells failed to arrest at a G2/M DNA damage checkpoint, encountered _mitotic catastrophe_, and finally died due to accumulation of DNA damage. Together, our results demonstrate a function of Mad2l2 at the G2/M transition, which is essential for PGCs, but not for somatic cells. A model on the function of Mad2l2 in the regulation of the cell cycle will be discussed.

T5 - Development of hematopoietic stem cell: an outline of final scenario

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Hematopoietic stem cell (HSC) is the most extensively studied stem cell, but yet its developmental pathway in mammals has not been fully explained. In this symposium, I will describe what we think would be the final scenario for development of HSC. While some more details need to be worked out, following is our explanation of the process. Upon exfoliation of the mesoderm from the primitive streak, nascent mesoderm expresses both PDGFRα and Flk1. Expression of a transcription factor (TF), Etv2/ER71 in this primitive mesoderm, induces extraembryonic mesoderm (EM), which is distinguished from other mesoderm by the surface phenotype. EM is $Flk1^{+}PDGFRa^{-}$ and give rise to EC and blood cell (BC). This population is completely lost in Etv2 KO. $Flk1^{+}$ EM is fated cell-autonomously to EC, as Etv2 induces an array of molecules that form a self-sustaining TF network required for EC fate. However, its fate is dominantly directed by expression of TFs relating to BC fate. In fact, EM spread over extraembryonic space and diversify into $Runx1^{-}Gata1^{-}$, $Runx1^{+}Gata1^{-}$ and $Runx1^{+}Gata1^{+}$ populations according to their localization. Labeling and tracking experiment demonstrates that $Runx1^{-}Gata^{-}$ population is fated exclusively to EC and contributes to the vascular system of YS and anterior and caudal-dorsal parts of embryo. $Runx1^{+}Gata1^{-}$ cells can differentiate both HSC and EC that contribute to the vascular system of caudal-ventral part of embryo and vitelline connections. All $Gata1^{+}$ population is fated to primitive erythrocytes. We also investigated the routes for each population to enter embryo. The primitive erythrocytes from $Gata1^{+}$ population requires circulation for their entry. $Runx1^{+}Gata1^{-}$ and $Runx1^{-}Gata1^{-}$ populations move directly to embryo by tissue movement before circulation starts. Only $Runx1^{+}Gata1^{-}$ population contributes to the hemogenic EC in the vitelline connection and AGM and differentiate to HSC. Runx1 expression at this stage is essential for priming this population to acquire the potential to HSC. In the symposium, I want to present a part of TF networks characterizing each intermediate stages.

T6 - Gene expression profiling of planarian stem cells suggests deep conservation of molecular mechanisms for pluripotency.

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Planaria possess extraordinary abilities to regenerate due to the presence of pluripotent adult stem cells, called neoblasts. Owing to the development of modern molecular techniques, planaria has emerged as a model system to study stem cell biology and regeneration in vivo. However, a long standing question concerning the evolutionary conservation of pluripotency molecular programs is still unresolved. Thus, in order to uncover the conserved mechanisms underlying stem cell proliferation, maintenance and differentiation, we profiled the transcriptome and proteome of FACS-sorted stem cells from Schmidtea mediterranea, by using high-throughput deep sequencing and shotgun proteomic technologies. Among the hundreds of neoblast specifically expressed genes present in our dataset, all previously identified neoblast markers were recovered. To check gene conservation, we compiled sets of human and mouse genes known to be associated with pluripotency in embryonic stem cells. Strikingly, many of these genes were conserved in planaria and selectively expressed in neoblasts. We further identified conserved germline genes with specific expression in planarian stem cells. Using whole mount in situ hybridization, we could further validate the specific expression of neoblast genes with shared homology to both embryonic stem cell and germ cell expressed genes. Knock down of both types lead in most cases to strong regeneration defects. The overall emerging picture is that planarian stem cells feature molecular hallmarks of embryonic stem cells and of germ cells. Our data suggest that planaria are an informative model system for human stem cell biology, provide a large list of stem cell specific genes that can now be studied in planaria, and further highlight the deep conservation of molecular pluripotency mechanisms during the evolution of animal life.

Signaling in Stem Cells

T7 - The Role of TGFβ signaling in pluripotency and differentiation in human embryonic stem cells

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Together with FGF and Wnt, the TGFβ signaling pathway, is required for maintenance of hESC pluripotency and cell fate determination. Crosstalk between these pathways downstream of receptor activation remains poorly understood. I will first show that TGFβ-activated-kinase-1 (TAK1) acts as a central node integrating TGFβ, MAPK, and BMP signals. Inhibition of TAK1 results in loss of pluripotency and induction of trophoblast fate in a BMP-dependent manner. Phospho-proteomic analysis shows that activation of TAK1 prevents differentiation through the activation of Mek, p38, and JNK and suppression of autocrine BMP signaling. Surprisingly, TAK1 activates Smad 2/3, and is necessary for its receptor-induced phosphorylation. Thus, TAK1 integrates pathway crosstalk to maintain self-renewal and inhibit differentiation. Second, I will show that complete inhibition of Smad1 and 2 signaling, by Smad7 expression, directly converts hESCs to telencephalic neurons. Time-course microarray analysis shows rapid up-regulation of the Notch and Shh pathways, and shift of MAPK and Wnt signaling thresholds. Interestingly, inhibition of FGF signaling at the level of receptors or MEK/ERK transducers does not inhibit but rather accelerates neural conversion. This suggests that contrary to speculation, FGF has no instructive role in human neural induction. Inhibition of Smad signaling has therefore an evolutionary conserved outcome and the principles defined by the "default model" of neural induction in frog cells are applicable to humans. These results highlight a central role for the TGFβ signaling in regulating cell fate choices in hESCs.

T8 - FoxO transcription factors in aging and stem cells

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Aging, long thought to be solely the byproduct of wear and tear, is actually a plastic process, regulated by a combination of genetic and environmental factors. An important question is whether the pool of adult stem cells plays an important role in the plasticity of aging by regenerating tissues in long-lived mammals. In the nervous system, neural stem cells are thought to be critical for learning and memory. Over time, the pool of neural stem cells and their ability to give rise to new neurons decline, raising the possibility that neural stem cell depletion may underlie part of the cognitive dysfunctions during aging. However, the mechanisms that regulate the neural stem cell pool throughout lifespan are largely unknown. Our hypothesis is that genes that regulate lifespan maintain the homeostasis of adult stem cell pools in long-lived species. Transcription factors of the FoxO family play a conserved role in controlling longevity: FoxO orthologs are known to extend lifespan in invertebrates, and single nucleotide polymorphisms in the FoxO3 gene have been shown to be associated with extreme longevity in humans. We have recently found that the transcription factor FoxO3 maintains the neural stem cell pool in adult mice. Genome-wide analysis of genes regulated by FoxO3 in neural stem cells suggests that FoxO3 maintains the adult neural stem cell pool by inducing a program of genes that preserves cellular quiescence and regulates oxygen metabolism. The ability of FoxO3 to trigger a gene program that regulates neural stem cell homeostasis in adult organisms might help counter brain aging in long-lived species, including humans.

T9 - An innovative feeder-free cell culture surface for the expansion of human ES cells

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The expansion and differentiation of stem cells continues to represent a significant challenge in the research setting. Contaminants from animal components combined with batch-to-batch variability of animal derived cell culture matrices have significantly hindered the usage of these types of surfaces for translational and clinical applications. Commercial synthetic matrices can be complex in nature and often cost prohibitive. The Thermo Scientific Nunclon Vita is an energy-treated polymer surface free of animal components or synthetic molecules. It enables culture of human stem cells without the use of feeder layers. Human embryonic stem (ES) cells are grown directly on the polymer surface in conditioned media containing ROCK-inhibitor, and can be sustained for more than ten passages without signs of differentiation. During this presentation, we will present data that demonstrate karyotypic normality, pluripotent status, and induced differentiation to pancreatic endoderm of human ES cells cultured on Nunclon Vita surface.

Pluripotency and Germ Cells

T10 - Mechanism and reconstitution *in vitro* of germ cell specification in mice

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The germ cell lineage ensures the creation of new individuals, thereby perpetuating and diversifying the genetic and epigenetic information across the generations. In mice, the germ cell fate is induced in a subset of pluripotent epiblast cells during early gastrulation. We have been investigating signaling, global transcription and epigenetic dynamics associated with germ cell specification at a single-cell resolution, and our results have led to the concept that germ cell specification involves an integration of three key molecular/cellular events: repression of the somatic program, re-acquisition of potential pluripotency, and an ensuing genome-wide epigenetic reprogramming. Based on these findings, we have recently succeeded in reconstituting the germ-cell specification pathway in mice using pluripotent stem cells *in vitro*. This work will serve as a foundation for the better elucidation of early germ-cell biology, as well as for the reconstitution of the entire germ-cell development process *in vitro*, not only in mice but also in other mammals, including humans.

T11 - Germ line, stem cells and epigenetic reprogramming

Azim Surani[1]

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Primordial germ cells originate from postimplantation epiblast cells in response to BMP4, from cells that have already initiated the process of differentiation towards somatic cell lineages. Evidence shows that this trend is arrested in epiblast cells that are recruited to form the founder population of germ cells through expression of key germ cell determinants: Blimp1/Prdm1, Prdm14, Lin28 and Prmt5. The accompanying epigenetic modifications erase the epigenetic memory of their trajectory towards somatic fate as early germ cells become epigenetically similar to ICM and ES cells. Additional epigenetic modifications, including genome-wide DNA demethylation and histone modifications in early germ cells are important as they progress towards an epigenetic ground state, and for resetting the epigenetic state of germ cells towards the eventual totipotent state. Postimplantation epiblast can also be induced to undergo reversion to ESC-like cells in response to LIF-STAT3 signaling, which results in some similar epigenetic changes as seen during PGC specification, including X reactivation. It is of interest to understand how mechanistically, epiblast cells acquire two distinct phenotypic states. Increasing knowledge of the mammalian germ cell lineage and in vitro epigenetic reprogramming of postimplantation epiblast may provide insights into the mechanisms that regulate their responses to diverse signals, which may induce different epigenetic responses regulating cell fates.

T12 - Induction of pluripotency by Oct4

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The reprogramming of mouse and human somatic cells into pluripotent stem cells, termed induced pluripotent stem (iPS) cells, using fibroblasts (somatic cells) and initially requiring the virally-expressed transcription factor quartet of Oct4, Sox2, c-Myc, and Klf4, was first described in 2006. Later, we reported that Oct4 alone is sufficient to directly reprogram adult mouse and human fetal neural stem cells (NSCs) into iPS cells, indicating that Oct4 plays a crucial role in the reprogramming process. We recently showed that induced epiblast stem cells (iEpiSCs) can be obtained by directly reprogramming somatic cells with the quartet under EpiSC culture conditions. In contrast to somatic cells, primordial germ cells (PGCs) were first induced to pluripotency 20 years ago by the mere modulation of the culture conditions. We converted adult germline stem cells (GSCs) into germline-derived pluripotent stem (gPS) cells. GSCs are unipotent testicular cells capable of not only self-renewing, but also giving rise to sperm. Like embryonic stem (ES) cells, GSCs exhibit significant levels of Oct4 and Klf4, but low levels Sox2 and c-Myc. To better understand the reprogramming process, we sought to identify factors that mediate reprogramming at higher efficiency. We established an assay based on Oct4 reactivation to screen nuclear fractions from extracts of pluripotent cells. BAF chromatin remodeling complexes containing the Brg1 protein enhance the efficiency of quartet-mediated reprogramming of somatic cells to pluripotency. In my presentation, I will discuss new insights into the molecular mechanism underlying the induction of pluripotency. This will largely concern how Oct4 attracts the reprogramming machinery onto DNA.

T13 - Zinc finger nuclease engineering of iPS cells

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No abstract available

Stem Cells in Development: Ectoderm

T14 - Skin stem cells in silence and in action

Elaine Fuchs[1]

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No abstract available

T15 - The opposing transcriptional functions of Sin3A and c-Myc are required to maintain skin homeostasis

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The proto-oncogene c-Myc has emerged as an important stem cell regulator, yet it is largely unknown how c-Myc mechanistically balances self-renewal, proliferation and differentiation processes in adult tissues. Here, we explored the transcriptional roles of c-Myc at the Epidermal Differentiation Complex (EDC), a locus essential for skin maturation. Binding of c-Myc to EDC genes can simultaneously recruit or displace specific sets of differentiation-specific transcriptional regulators, including Klf4 and Ovol-1 or C/EBPA and Sin3A respectively. In this network, we identify Sin3A as a direct repressor of c-Myc activity via de-acetylation of the c-Myc protein. In the absence of Sin3A, genomic recruitment c-Myc to the EDC is enhanced and re-activation of Myc-target genes drive aberrant epidermal proliferation and differentiation. Simultaneous deletion of c-Myc and Sin3A reverts the skin phenotype to normal. In summary, we identify how the balance of two transcriptional key regulators can maintain tissue homeostasis via a negative feedback loop.

T16 - Asymmetric cell division and tumorigenesis in Drosophila and mouse neural stem cell lineages

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Stem cells are characterized by their ability to generate both self-renewing and differentiating daughter cells. We are using Drosophila and mouse neural stem cells as models to understand, how these differences are established and regulated. Drosophila neural stem cells called neuroblasts undergo repeated rounds of asymmetric cell division during which they segregate the cell fate determinants Numb, Prospero and Brat into one of the two daughter cells. In this cell, the determinants prevent self-renewal and induce differentiation. When one or more determinants are missing, all daughter cells continue to proliferate leading to the formation of a stem cell derived brain tumor. How determinants segregate asymmetrically, how they prevent self-renewal and how stem cell tumors are formed are the key questions we are trying to answer. For this, we have used a library of 20,000 transgenic RNAi lines to screen for genes involved in self-renewal control. We have identified over 600 genes required in neuroblasts and have quantified their RNAi phenotypes to assign particular biological functions. Using hierarchical clustering of quantitative phenotypic data and integration with protein interaction data, we have created regulatory networks for neural stem cell self-renewal, differentiation and tumorigenesis and identified a set of transcriptional regulators essential for self-renewal. Analysis of the genes in those networks has revealed a surprising role for duplicated ribosomal proteins in self-renewal control. In addition, our data reveal that alternative splicing of transcriptional regulators, chromatin remodeling and the regulation of transcriptional elongation are important for controlling self-renewal and differentiation and preventing tumor formation in stem cell lineages.

T17 – Neural stem and progenitor cells in developing neocortex – molecular, cellular and evolutionary aspects

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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 progenitors cells and their modes of division. In terms of their cell biology, two principal classes of cortical stem and progenitors cells can be distinguished. One class comprises stem/progenitor cells exhibiting bipolar morphology and apical-basal cell polarity that divide at the ventricular, i.e. apical, surface of the ventricular zone (VZ). These are the neuroepithelial cells and radial glial cells, which are collectively referred to as apical progenitors (APs). The other class comprises stem/progenitor cells dividing in a more basal, abventricular location, notably the subventricular zone (SVZ). These fall into two subclasses (i) radial glia-related progenitors exhibiting monopolar morphology and basal, but not apical, cell polarity, referred to as basal radial glial (bRG) cells (also called outer SVZ (OSVZ) progenitors, outer radial glia or intermediate radial glia); and (ii) progenitors exhibiting nonpolar morphology and lacking overt apical-basal cell polarity, called basal progenitors (BPs) or intermediate progenitor cells.

Stem Cells in Development: Endoderm

T18 - Stem cell derivation and differentiation - learning from developmental biology

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No abstract available

T19 - Production of multipotent anterior definitive endoderm from human pluripotent stem cells

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Human embryonic stem cells (hESCs) as well as induced pluripotent stem cells (IPSCs) represent a unique opportunity to study human development, model diseases in vitro, optimise drug screening platforms and most significantly develop cell replacement therapies for degenerative diseases. One of the main challenges in achieving these clinical promises is the production of populations of progenitor cells that are of high purity, retain their full spectrum of differentiation capacity and are able to expand into numbers relevant for therapeutic needs. Following a normal path of development remains the best approach to generate such cells as well as the development of a universal differentiation protocol applicable to a wide variety of pluripotent cell lines. Anterior Definite Endoderm (ADE) is a derivative of the endoderm germ layer appearing just after gastrulation during mammalian development and which gives rise to the ventral foregut. Importantly, key endoderm organs such as the liver and the ventral pancreas originate from the foregut. Our group has recently described a method to produce foetal hepatocytes and pancreatic progenitors cells from both hESCs and hIPSCs which importantly, respects key developmental stages in vitro including the differentiation of endoderm into ADE and then the differentiation of ADE cells into ventral foregut. The purity of this initial ADE population has very profound effects on the purity and functionality of the downstream differentiated cells types. Therefore, generation of ADE cells represent an essential step of differentiation to generate liver and pancreas cells from hESCs. Here, we demonstrate differentiation of hESCs and hIPSCs into ADE expressing CXCR4 and HHEX using defined culture system devoid of feeders or serum. These cells are multipotent giving rise to hepatocytes, pancreatic cells and lung epithelium. Mature cells types can be produced from this ADE population expressing albumin, α-1-antitrypsin and cytochrome P450 enzymes for hepatocytes as well as many functional properties such as glycogen storage, LDL uptake and inducible cytochrome P450 activity. Insulin producing pancreatic beta cells express PDX1 and HLXB9 are also release insulin in response to glucose challenge. Lung epithelium expresses Nkx2.1, Aquaporin and surfactant protein expression and also undergo branching morphogenesis in 3D culture. Furthermore, our approach allows the production of a near homogenous ADE cells expressing CXCR4 which can then be differentiated into cells expressing albumin (90%) in mature liver cells or PDX1 (90%) in mature pancreatic beta-cells. This presents unique advantages for development of drug screening platforms, disease modelling and transplant therapy applications and also represent a first toward the generation of endodermal cells fully compatible with in vivo applications.

T20 - Establishing developmental competence in endoderm for liver and pancreas

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We are interested in gene regulatory mechanisms that endow multipotent progenitor cells with the competence to induce certain gene programs and not others. Understanding the basis by which different progenitors and stem cells possess particular developmental competencies is crucial for prospectively differentiating stem cells, for directed cellular trans-differentiation, and for controlling metaplasias in disease. As a model system, we investigate the endoderm, one of the early germ layers to arise during gastrulation, and the induction of the liver and pancreas programs. While much has been learned about transcription factors and histone modifications in embryonic stem cells, many of the factors and chromatin states change when the cells differentiate into multipotent germ layer cells, which can develop into certain tissues, but not others. Previous studies in our laboratory identified signaling pathways by which early foregut endoderm cells become committed to liver or pancreas fates. More recently, we discovered that there exists a pre-pattern of chromatin marks in multipotent endoderm cells that distinguish early liver genes from early pancreas genes, even though the genes have not yet been activated. Perturbation of the histone modifying enzymes that make the marks can modulate the balance of liver and pancreas progenitors that are specified. This information is being used to assess the developmental potential of stem cells and to guide their differentiation. We have also found that the endodermal transcription factors FoxA1 and FoxA2 remain bound to a subset of their target genes as cells traverse through mitosis. These “pioneer” transcription factors thus function as epigenetic marks in chromatin and we have found that they play a role in the timely re-activation of tissue-specific genes. Together, the transcription factors and histone modifications help define and maintain the genetic competence of the endoderm and its derivative lineages.

T21 - Neurogenin 3 progenitor cells endogenous to the pancreas contribute to increased beta cell mass

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We previously showed that robust injury by partial duct ligation (PDL) in adult mouse pancreas can activate Neurogenin 3 (Ngn3)+ endocrine progenitor cells and stimulate proliferation of beta cells to increase the beta cell mass. Further dissection of this experimental model now allowed the identification of a subpopulation of beta cells that are prone to replicate and locate mainly in small islets. Following PDL, genetically labeled Ngn3+ cells were highly enriched and prolific in small islets. Beta cell neogenesis was blunted by acute, selective ablation of Ngn3+ cells, independent from pre-existing beta cells. In addition, dilution of genetically labeled pre-existing beta cells occurred first in small, later also in larger islets, showing that non-beta cells contribute to the formation of new beta cells, further supporting the endocrine progenitor concept. Injury-induced neoformation of beta cells in vivo thus directly depends on progenitor cells that need to exceed a critical threshold level of Ngn3 expression to be activated. The present data underscore the importance of Ngn3 gene expression for activation of beta cell progenitors in adult pancreas and provide a platform to explore the mechanism that controls this process.

T22 - miRNA335 controls mesendoderm separation in the mouse

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MicroRNAs (miRNAs) are short RNAs that lead to the degradation of target mRNAs or translational repression of the encoded proteins and regulate processes, such as differentiation, apoptosis, proliferation, and the maintenance of cell and tissue identity. However, the role of miRNAs in the regulation of early lineage decisions is not well understood. In this study, we studied the function of miRNAs in mesoderm and endoderm formation during mouse ES (mES) cell differentiation and gastrulation in the mouse embryo. Using mRNA expression profiling of differentiating mES cells we identified miR335 as a developmentally regulated intronic host gene miRNA. miR335 is encoded in the Mesoderm-specific transcript (Mest) and we identified predicted target recognition sites in the 3`-UTRs of the endoderm-specification factors, Foxa2 and Sox17. We confirmed that the endogenous miR-335 expression levels correlates with its corresponding host gene MEST in mesendodermal progenitor cells and the mesoderm lineage. Studies in mES cells and in mouse gastrulation stage embryos show that the expression of both endoderm-specific transcription factors were significantly down regulated by overexpression of miR335 in the mesendodermal and endodermal lineages. Taken together, these results suggest that mesoderm-specific miR-335 regulates the separation of mesoderm and endoderm by repressing endoderm-specification factors.

T23 - Reconstructing the developmental path of pancreatic insulin-producing beta-cells

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One primary goal in current diabetes research is to devise regenerative or cell replacement therapies for lost insulin-producing beta cells. To accomplish this goal, much effort has been expanded to identify and characterize putative stem or progenitor cells in the adult pancreas or to devise *in vitro* strategies for deriving beta cells from human pluripotent stem cells. I will present research from my laboratory in both areas. We have adapted a human embryonic stem cell (hESC) differentiation protocol that allows for the stepwise generation of pancreatic progenitor cells through their developmental intermediates. Upon transplantation into mice these progenitors differentiate into functional insulin-producing beta cells. However, mature beta cells can still not be derived from hESCs *in vitro*. Employing genome wide next generation sequencing approaches, such as chromatin immunoprecipitation combined with massively parallel sequencing (ChIP-seq) and RNA-seq, on hESC-derived pancreatic cells and their precursors, we have generated an atlas of the changing chromatin landscape associated with pancreatic endocrine cell differentiation. One theme that has emerged from our studies is that terminal differentiation of pancreatic progenitors into functional beta cells is associated with lifting polycomb-mediated gene repression. This derepression is not appropriately initiated *in vitro*. I will discuss how our studies have enabled novel strategies for generating functional beta cells from hESCs *in vitro*.

Pluripotency and Reprogramming

T24 - Dissecting the mechanisms of cellular reprogramming

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My lab is studying the mechanisms of cellular reprogramming using transcription factor-mediated conversion of somatic cells into induced pluripotent stem (iPS) cells. For example, we have identified biomarkers to track and prospectively isolate intermediate cell populations during the reprogramming process and are currently using these to understand the transcriptional, epigenetic and proteomic changes in cells undergoing reprogramming. In addition, we have shown that terminally differentiated beta cells and lymphocytes can be reprogrammed into iPS cells, thus demonstrating that induced pluripotency is not limited to rare adult stem cells as has been suggested. Interestingly, however, we discovered that immature hematopoietic cells give rise to iPS cells more efficiently than any tested mature cell types, suggesting that the differentiation stage of the starting cell can influence the efficiency of reprogramming. We have further identified the p53 and p16/p19 tumor suppressor pathways as roadblocks during the reprogramming process, pointing out similarities between pluripotent cells and cancer cells. One major roadblock for the therapeutic use of iPS cells is the fact that integrating viruses are used to deliver the reprogramming genes to cells, resulting in genetically altered iPS cells. By using adenoviruses expressing the reprogramming factors transiently in cells, we were able to produce iPS cells devoid of any viral elements and thus any genetic manipulation. More recently, we have developed a _reprogrammable mouse_ carrying a single doxycycline-inducible cassette with the four reprogramming genes in all tissues. We are employing this system to perform genetic and chemical screens to identify molecules important during the reprogramming process as well as for comparative studies between iPS cells and embryonic stem cells.

T25 - Oct3/4 activates *Cdx1* to terminate pluripotency at the onset of gastrulation

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Gastrulation marks the onset of germ layer formation from pluripotent embryonic cells maintained by a network including the *Pou5f1* gene, *Oct3/4*. Negative regulation of the pluripotency is a prerequisite to allow germ layer differentiation and subsequent development. We show by Cdx1 knock-down that it functions as a regulator of the onset of gastrulation in agreement with its early expression. In a microarray-based approach to search for Cdx1 targets, we identified the *Xenopus Oct3/4*-like genes, *Oct60*, *Oct25* and *Oct91*, as regulated by Cdx1. All three *Oct3/4*-like genes function as gatekeepers of pluripotency and are expressed in pluripotent cells in the embryo. During late blastula and early gastrula Cdx1 negatively regulates the *Pou5f1* genes, placing Cdx1 as controlling the end of pluripotency and the onset of gastrulation. Of particular interest was the unexpected observation that, prior to gastrulation, the *Xenopus* Oct3/4 factors positively regulate Cdx1 expression through FGF signaling. We further characterized the cross-regulatory network between these genes showing, that with the onset of gastrulation the Oct3/4 factors become negative regulators of *Cdx1* transcription. Then, Oct3/4 initiates its own negative autoregulation through *Cdx1* upregulation to begin the repression of pluripotency in preparation for the onset of gastrulation and germ layer differentiation.

T26 - Role of Polycomb repressors in stem cells, cancer and DNA repair

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Repressive Polycomb-group (Pc-G) protein complexes and the counteracting Trithorax-group (Trx-G) of nucleosome remodeling factors are involved in the dynamic maintenance of proper gene expression patterns during development, acting at the level of chromatin structure. As such, they are important controllers of cell fate. When deregulated, these master switches of gene expression are strongly implicated in formation of a diverse set of cancers. An example is the Pc-G gene Bmi1 which is overexpressed in medulloblastoma, Non small cell lung cancer, hepatocellular carcinoma, prostate cancer, breast cancer and Glioma and is causally implicated in leukemia. We and others have recently implicated Bmi1/Pc-G as a critical regulator of stem cell fate in hemapoietic stem cells, neural stem cells, mammary epithelial precursor cells and ES cells. In addition, we have shown that Bmi1 is regulated by the Shh pathway and that the Ink4a/ARF tumors suppressors are critical Bmi1 target genes in stem cells and in cancer formation. However our recent work on brain cancer (Glioma) and prostate cancer points to important ink4a/ARF-independent Bmi1 targets involved in adhesion and motility. Comprehensive profiling of Polycomb target genes in Drosophila revealed its crucial conserved role in repressing lineage differentiation pathways and morphogens, including Wg, Hh, Delta and Notch. Using genme wide in vivo 4C on larval brains we recently demonstrated that polycomb domains interact in 3D nuclear space and are guided by chromosome architecture. Furthermore, we have characterized in detail an essential E3-ubiquitin ligase activity in the PRC1 Polycomb complex that consists of a functional Ring1B-Bmi1 heterodimer. This E3 ligase activity is required for maintenance of Polycomb repression in normal- and cancer stem cells and hence offers potential novel ways to target cancer stem cells or tumor reforming cells in which the activity of this E3 ligase is hyperactivated. This is further substantiated by a novel way by which the activity of the Ring1B.Bmi1 E3 ligase is controlled and its novel implication in the DNA repair pathway. The implications of these findings for stem cell biology, DNA repair and cancer will be discussed.

Selected references:

Bas Tolhuis, Marleen Blom, Ron Kerkhoven, Ludo Pagie, Hans Teunissen, Marja Nieuwland, Marieke Simonis, Wouter de Laat, Maarten van Lohuizen[*], and Bas van Steensel[*]. Intercations among Polycomb domains are guided by chromosome architecture. PLoS Genet. 2011 in press

Pietersen AM, Evers B, Prasad AA, Tanger E, Cornelissen-Steijger P, Jonkers J, van Lohuizen M. Bmi1 Regulates Stem Cells and Proliferation and Differentiation of Committed Cells in Mammary Epithelium. Curr Biol. 2008 Jul 22; 18(14): 1094-9

Uren A.G[*], Kool J. [*], Matentzoglu K., de Ridde J., Mattison J., van Uitert M., Lagcher L., Sie D. Tanger E., Cox T., Reinders M., Hubbard T.J., Rogers J., Jonkers J., Wessels L. [#], Adams D.J. [#], van Lohuizen M. [#], Berns A[#]. Large scale mutagenesis in p19ARF and p53 deficient mice identifies cancer genes and their collaborative networks. Cell 2008; 133, 727-741

Sophia W.M. Bruggeman, Danielle Hulsman, Ellen Tanger, Tessa Buckle, Marleen Blom, John Zevenhoven, Olaf van Tellingen and Maarten van Lohuizen. Bmi1 controls tumor development in an Ink4a/Arf-independent manner in a mouse model for glioma. Cancer Cell 2007, 12, 328-341

Tolhuis, B., Muijrers, I.[*], de Wit, E.[*], Teunissen, H., Talhout, W., van Steensel, B.[#] and van Lohuizen, M[#]. Genome-wide profiling of PRC1 and PRC2 Polycomb chromatin binding in Drosophila. Nature Genet. 2006; 38, 694-699 ([*]equal contribution; [#]corresponding authors).

T27 - miRNA screening reveals a new miRNA family strongly enhancing the generation of murine induced pluripotent stem cells

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Objectives: Induced pluripotent stem cells (iPSCs) can be generated by ectopic overexpression of the three transcription factors Oct4, Sox2, and Klf4. Recently, several reports highlighted the role of microRNAs (miRNAs), which support induction of pluripotency in somatic cells. Here, conducting a full miRNA library screen during the first days of iPSC generation using an optimized protocol, we investigated if particular miRNAs can accelerate reprogramming and we sought to unravel further molecular pathways involved in the induction of pluripotency. *Methods:* We transduced murine embryonic fibroblasts (MEFs) from OG2 mice (Oct4 promoter-driven GFP expression) with a polycistronic lentiviral construct expressing Oct4, Klf4 and Sox2. One day after transduction, we individually transfected murine miRNAs from a Pre-miR_ miRNA Precursor Library_Mouse V3 containing 379 miRNAs (Ambion) into these MEFs. At day 7 to 10 after transduction, emerging GFP-positive iPSC colonies were counted and further analyzed. *Results:* We could confirm miRNAs previously demonstrated to improve reprogramming, such as members of the mir-290 and mir-302 clusters. In addition, we identified a miRNA family consisting of the miRNAs 130b, 301b and 721, which strongly enhances iPSC generation. Based on in-silico predictions (Targetscan, PicTar, and MicroCosm), followed by target evaluation using western blot and luciferase assays, we were able to identify the homeobox transcription factor Meox2 to be directly downregulated by this miRNA family. *Conclusion:* Applying a full library miRNA screen, we were able to identify a novel miRNA family strongly increasing the induction of pluripotency in MEFs. This newly characterized miRNA family specifically targets the transcription factor Meox2, which was confirmed to play a significant role during iPSC generation. Further studies aim at elucidating other molecular pathways contributing to the enhanced reprogramming activity of these miRNAs.

T28 - A validated mRNA reprogramming protocol for the reproducible generation of integration-free human iPS cells

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To date, the broad implementation of induced pluripotent stem (iPS) cells in regenerative medicine and drug screening applications has been limited by the inability to efficiently derive iPS cell lines that are free from genomic integration. Past publications have highlighted the use of reprogramming technologies that are: 1) directly integrating into the target cell genome, 2) directly integrating into the target cell genome, but can be excised, 3) minimally integrating into the target cell genome. More recent works have utilized recombinant protein or mRNA to derive iPS cell lines that are inherently free from any genomic integration. However, these methods have been plagued by low efficiency and/or the lack of reproducibility. Here, we demonstrate a validated protocol using mRNA for the reproducible reprogramming of human fibroblasts to iPS cells. This process is dependent on a novel, defined media that is essential for complete conversion to iPS. In addition, a highly efficient RNA transfection reagent was developed to ensure that mRNA can be delivered to a range of cell types, including fibroblasts and lymphocytes, with titratable control over expression. This methodology provides a reproducible, non-integrating method for generating iPS cells which has the potential to be expanded to multiple cell types.

Stem Cells in Regeneration

T29 - Permanent silencing of germ line genes upon the differentiation of mouse embryonic stem cells requires the transcriptional repressor E2F6 and CpG methylation

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During early mouse development germ cell specific genes become permanently repressed in somatic cells. Specific re-activation only occurs in primordial germ cells and requires epigenetic reprogramming. How germ cell genes are specifically selected for silencing and how silencing is achieved in the first place is poorly understood. We show that the transcriptional repressor E2F6 is strictly required for the permanent silencing a set of germ cell specific genes. E2F6-dependent gene silencing could be demonstrated to occur upon differentiation of mouse embryonic stem cells. Surprisingly, repressive histone marks were absent from E2F6 target genes but target genes typically contained methylated CpG islands around their transcription start sites. Characterising ES cells lacking E2F6 or DNMT3a/b we could show that both activities were required for target gene repression and epigenetic gene regulation suggesting an E2F6 driven and DNA methylation dependent pathway of germ cell specific gene silencing to be initiated during mouse development as early as pluripotent stem cells go along lineage differentiation.

T30 - Cardiovascular derivatives of embryonic stem cells in cardiac repair and drug discovery

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Derivation of heart and vascular endothelial cells from human pluripotent stem cells (embryonic stem cells or HESCs and induced pluripotency stem cells or hiPS cells) is an area of growing interest both as a route to cell therapy for the heart and as a platform for drug discovery and toxicity. Understanding the underlying developmental mechanisms that control differentiation of pluripotent cells to cardiac progenitors and their derivatives and mimicking these in defined culture conditions in vitro is now essential for moving the field forward. Culture conditions have now been sufficiently refined that cardiomyocyte and vascular differentiation is a fairly efficient and reproducible process. Genetically marked HESCs have been produced in which expression of the green fluorescent protein marker is expressed ubiquitously or driven by specific lineage markers. We have used these tagged lines to trace cardiomyocytes following transplantation into a mouse heart after myocardial infarction and select the progenitors of cardiomyocytes, endothelial cells and smooth muscle cells. Long term survival of the cells and integration into the host heart has been observed and early improvements in cardiac function but these are not sustained. Cardiac repair using stem cell derived cardiomyocytes will likely require more than efficient cardiomyocytes production. More immediate applications of hESC- and hiPSC derived cardiomyocytes and vascular endothelial cells in drug discovery and disease are now close to implementation. Results of these studies, in particular drug responses of hESC-derived cardiomyocytes and an hiPSC model for vascular disease in which Thalidomide has a therapeutic effect, will be shown.

T31 - Mechanisms of pancreatic beta cell regeneration

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Recent studies have shown that insulin-producing pancreatic beta cells have a significant potential for regeneration, suggesting that regenerative therapy for diabetes is feasible. To realize this potential, it is important to elucidate the cellular origins of pancreatic beta cells formed during adult life and the molecular homeostatic mechanisms that regulate beta cell mass. Lineage tracing indicates that new adult beta cells derive mainly by replication of pre-existing beta cells, rather than the differentiation of stem cells. Thus, targeting beta cell replication is an important goal of regenerative biology. We have recently found that the key physiologic driver of beta cell replication is glucose, acting via glycolysis and membrane depolarization. However, glucose can be both mitogenic and toxic to beta cells. I will discuss experiments that aim to understand and uncouple this dual effect of glucose on beta cells in vivo, towards the development of effective pro-regeneration pharmacology.

T32 - miR-125b is an essential regulator of spinal cord injury repair

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The regeneration of complex body structures after injury or ablation, as championed most impressively by salamanders but barely detectable in humans, remains poorly understood at the molecular level. We have attempted to identify the molecular determinants underlying this evolutionary divergence by undertaking a detailed comparative analysis of a regenerative model, the salamander spinal cord, and a corresponding non-regenerative system, rat spinal cord. This approach has identified a small number of highly conserved microRNAs that are differentially regulated in axolotl versus rat. Detailed in vivo studies of one of these microRNAs, miR-125b that is highly expressed in axoltol but low in rat has identified it as a key regulator of the regenerative response in axolotl. Here we will present data showing that tight regulation miR-125b is necessary for regeneration of the ependymal tube and of neurons after spinal cord injury in the axoltol. We have identified SEMA4D as one of the targets of this microRNA in the spinal cord that is necessary for faithful axon regeneration. While another miR-125b target BMF needs to be tightly regulated to control survival of neural stem cells that are essential for regeneration to occur in the axolotl.

Stem Cells in Development: Mesoderm

T33 - Molecular control of cardiovascular regeneration and remodeling

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In principle, heart failure is characterized by impaired contractility. A decline of contractile force is most evident in end-stage heart failure after myocyte degeneration and extensive myocardial remodeling, which is preceded by compensatory hypertrophy and reactivation of the fetal gene program. We found that oncostatin M (OSM), an inflammatory cytokine, is a major mediator of pathological remodeling processes during heart failure. Administration of OSM partially mimick pathological remodeling processes as indicated by activation of the fetal gene program (FGP) and induction of de-differentiation of cardiomyocytes. Conversely, inhibition of the OSM receptor and the MEK/Erk pathway prevents de-differentiation of cardiomyocytes. We postulate that manipulation of OSM signaling represents an attractive therapeutic approach to protect the myocardium and prevent pathological remodeling processes in heart. Activation of distinct cytokine and growth factor signaling pathways are not only instrumental to activate FGP but might also stimulate activation of endogenous cardiac stem cells, which are likely to contribute to cardiac remodeling processes. Numerous studies report the presence of so-called “cardiac stem cells” that can differentiate into different cell types including cardiomyocytes in vitro and in vivo. A conceptual problem in most of these studies is the fact that the markers that are used to identify cardiac stem cells are down-regulated when putative progenitor cells differentiate preventing the detection of stem cell-derived cells in vivo. By utilizing a triple transgenic approach we permanently labeled derivatives of putative cardiac stem cells. Consecutive staining with markers for various lineages allowed identification of the current identity of cells in the adult mouse heart, which have been expressed stem cells markers in the past. The fates of stem cell-derived cells were recorded during normal aging for up to two years revealing a significant contribution to the cardiomyocyte, smooth muscle and endothelial cell lineages. We also investigated the fate of lineage-traced endogenous cardiac stem cells under different pathological conditions leading to heart failure.

T34 - Wnt/β-catenin and Bmp act downstream of Notch signaling in the differentiation of mouse mesodermal cardiac progenitor cells

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How signaling systems cross-talk during formation and differentiation of cardiac progenitors remains intricate and puzzling. Here, we show by the use of mouse genetics that Wnt/β-catenin is an essential downstream effector of Notch/RBPJ signaling in cardiac differentiation. Upregulation of Wnt/β-catenin signaling by mutation of Axin2 enhances Bmp signaling and rescues developmental deficits in RBPJ-mutant hearts. Furthermore, in SHF progenitors isolated by FACS, Axin2 loss in the absence of Notch/RBPJ restores the expression of transcription factors that are crucial for cardiac differentiation. To define the genes whose expression is rescued by upregulated Wnt/β-catenin or Bmp signaling in RBPJ mutants, we analyzed gene expression in mesodermal cardiac progenitor cells of RBPJ single, RBPJ/Axin2 compound and RBPJ/Axin2/Bmp4 triple mutant embryos and in RBPJ/Axin2 compound mutant embryos after ex vivo culture in the absence or presence of Noggin. We were able to subdivide the heart specific transcription factors into a canonical Wnt- and a Bmp-controlled group, *Nkx2-5*, *Isl1* and *Baf60c*, and *Gata4*, *SRF* and *Mef2c*, respectively. Together, our analyses show that Wnt/β-catenin signaling maintains and enhances cardiac differentiation either directly, or indirectly by the activation of Bmp signaling.

T35 - Regulation of entry into the myogenic programme in stem cells in the embryo and in quiescent and activated satellite cells in the adult

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Pax transcription factors play important roles in the regulation of organ and tissue development. In the case of skeletal muscle, Pax3 controls the entry of cells into the myogenic programme in the embryo. Using genetic screens, we have identified Pax3 targets, which regulate different steps in the progression from cell fate choices in multipotent stem cells to entry into the myogenic programme. In the former, mutual repression between *Pax3* and *Foxc2* is associated with the multipotent state; signalling pathways which affect this equilibrium will favour non-myogenic Foxc2-dependent cell fates or the Pax3-dependent myogenic cell fate. Self-renewal versus entry into the myogenic programme is orchestrated by Pax3 regulation of components of the FGF signaling pathway, and subsequent activation of the myogenic determination gene, *Myf5*. Pax3, in the context of skeletal muscle differentiation, is therefore a key upstream regulator of the stem cells that form this tissue. In adult muscle, satellite cells, present under the basal lamina of muscle fibres, are the progenitor cells responsible for regeneration. These cells are marked by the expression of *Pax7* and, in many muscles, also *Pax3*. Unlike the Pax-positive muscle progenitor cells of the embryo, most satellite cells already transcribe the myogenic determination gene, *Myf5*. We show that microRNA-31 regulation of Myf5 mRNA through specific sites in the 3' UTR, together with sequestration of this microRNA with Myf5 mRNA in ribonucleoprotein granules, characterises the quiescent satellite cell. On activation, this post-transcriptional repression is released and the cell rapidly enters the myogenic programme. We therefore propose a model in which post-transcriptional mechanisms hold quiescent stem cells poised to enter a tissue specific differentiation programme.

T36 - The T-box transcription factor Eomesodermin acts upstream of Mesp1 to specify cardiac mesoderm during mouse gastrulation

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Instructive programs guiding cell fate decisions in the developing mouse embryo are controlled by a few so-termed master regulators. Genetic studies demonstrate that the T-box transcription factor Eomesodermin (Eomes) is essential for epithelial to mesenchymal transition (EMT), mesoderm migration and specification of definitive endoderm (DE) during gastrulation. We now report that Eomes expression within the primitive streak marks the earliest cardiac and cranial mesoderm and promotes formation of cardiovascular progenitors by directly activating the bHLH transcription factor Mesp1 upstream of the core cardiac transcriptional machinery. Thus, Eomes conditional mutant embryos fail to form early cardiac progenitors. Additionally, Eomes is indispensible for the generation of DE and cardiomyocytes from ES cells in vitro. In marked contrast to Eomes/Nodal signalling interactions that cooperatively regulate anterior-posterior (A-P) axis patterning and allocation of the DE cell lineage, formation of cardiac progenitors requires only reduces levels of TGFβ/Nodal activity and is accomplished via Foxh1/Smad4 independent mechanisms. Collectively our experiments demonstrate that Eomes governs discrete context dependent transcriptional programmes that sequentially specify cardiac and DE progenitors during gastrulation.

T37 - Pathways regulating self-renewal of hematopoietic stem cells

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Hematopoietic stem cells are used clinically for the rescue of the immune system after high dose chemotherapy for leukemia or lymphoma. HSCs have the ability to self-renew, although the mechanism of this process remains to be understood at a molecular level. Definitive hematopoietic stem cells are derived during embryogenesis and are influenced by distinct signals in the niche of the embryonic aorta. We have demonstrated that the wnt-PGE2 pathway can induce HSCs during embryogenesis. Adult marrow treated with wnt agonists or dmPGE2 leads to increased engraftment in competitive transplantation studies in mice and zebrafish. Human cord blood is a source of HSCs, but few cells are obtained in a typical harvest. In an effort to enhance stem cell engraftment of cord blood, a clinical trial has been undertaken to treat cord blood with dmPGE2, and evaluate engraftment over time. In an effort to understand the targets of the wnt pathway, we have undertaken Chip seq studies of TCF4. Surprisingly, TCF4 binds to cell-specific genes adjacent to the cell-specific transcriptional regulators. This provides a mechanism for how developmental signals such as wnt affect cell differentiation and self-renewal. We also examined the BMP signaling pathway that are also involved in hematopoietic induction during embryogenesis. Chip seq analysis demonstrated that SMAD1, similar to TCF4, binds to cell-specific genes. Our work suggests that signaling transcription factors function together with cell-specific factors to regulate the intrinsic hematopoietic program. These studies may help develop a clinical therapy that could enhance engraftment of HSCs during transplantation.

T38 - Role of αIIb/β3 integrin in the maintenance of hematopoietic stem cells in the mouse embryo aorta

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Objectives: Integrins play multiple roles notably in extracellular matrix-mediated cell adhesion, migration, cytoskeletal organization and cell proliferation/differentiation. The αIIb integrin subunit (CD41) is the first marker indicative of hematopoietic lineage commitment. However, the expression pattern and functional role of integrins on Hematopoietic Stem Cells (HSC) throughout mouse embryonic development remains largely incomplete. Methods: We focused on three major hematopoietic sites where HSCs are generated (aorta) and amplified (placenta, fetal liver) during development. Cells from the three sites were sorted based on CD41 (αIIb), CD61 (β3 subunit of αIIb/β3 integrin) and/or CD51 (αv subunit of αv/β3 integrin) expression and transplanted into irradiated adult recipients to test their long-term HSC potential. Furthermore, we tested the HSC activity in aortas dissected from embryos haploinsufficient or knock-out for αIIb integrins to determine whether this integrin plays a functional role in HSC production. Finally, sections were imaged to locate αIIb/β3 and αv/β3 integrins in the aorta and placenta. Results: We found that only the first generated HSCs of the aorta express both αIIb/β3 and αv/β3 integrins. HSCs from the placenta only express αv/β3 while most fetal liver HSCs do not. We observed a dramatic decrease of HSC activity when mice where transplanted with CD41+/- or CD41-/- aortic cells compared to wild-type cells. We located αIIb/β3 integrins at the contact points between cells that form intra-aortic clusters (where HSCs reside). Conclusion: αIIb/β3 integrin, which is expressed by all HSCs from the aorta, is important to maintain the HSC activity in this site of HSC production. αIIb/β3 integrin might play a major role in the anchorage of HSCs to the aortic endothelium until they are fully competent to further colonize the placenta and fetal liver.

T39 - Dormant blood stem cells and circulating cancer stem cells

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No abstract available

T40 – Stem cell-related features in therapy-induced senescence

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Background: Cellular senescence is a terminal cell-cycle arrest program evoked by cellular stresses such as oncogene activation or DNA-damaging chemotherapy that possesses tumor-suppressive potential. It was recently shown that the senescence machinery limits reprogramming of somatic cells to pluripotency. In turn, senescence mediators such as p16INK4a, p21CIP1 or FoxO transcriptions factors reportedly protect stem cells from uncontrolled division and premature exhaustion. Hence, cellular senescence appears to be intimately linked to stem cell properties ("stemness") by imposing a barrier that preserves self renewal-related capabilities of the cell. We considered here the possibility that therapy-induced senescence (TIS) of tumor cells with inherent self-renewing potential may enforce latent changes of stemness-related functionalities. Methods: We analyzed the transcriptional and functional changes of stem cell-related properties by utilizing Bcl2-overexpressing Eμ-myc transgenic B-cell lymphomas, which robustly enter TIS in response to DNA-damaging anticancer agents such as Adriamycin (ADR) or cyclophosphamide. As conditional models for TIS, we generated Myc-lymphomas in which the function of essential senescence mediators - the histone H3 lyine 9 methyltransferase Suv39h1 and the transcription factors p53 or Klf4 - depends on 4-OH-tamoxifen (4-OHT). Stem cell and other phenotypic markers were measured by flow cytometry. Assays measuring cell proliferation, clonogenicity, and repopulating capacity were performed in vitro and in vivo. Results: Gene expression analyses comparing TIS to non-senescent lymphomas unveiled a massive upregulation of the adult tissue stem cell signature in the senescent group. Moreover, TIS lymphomas became homogeneously positive for the membrane-bound stem cell marker Sca-1, and displayed enhanced aldehyde dehydrogenase and ABC transporter activities as additional stemness-related properties. Interestingly, TIS lymphoma cells also exhibited promiscuity towards the myeloid lineage, further underscoring their transdifferentiation potential. To assess the impact of the latent stemness-related changes on tumor growth, we compared ADR-exposed Eμ-myc lymphomas that had never entered TIS due to lack of functional Suv39h1, p53 or Klf4 to the same lymphomas able to senesce in the presence of 4-OHT. Upon withdrawal of 4-OHT, temporarily senescent lymphomas re-entered the cell-cycle, resumed dividing, and showed an increasingly superior clonogenic potential in serial replatings when compared to their equally ADR-treated but never senescent counterparts. Moreover, in vitro and in vivo competition assays also demonstrated a proliferative advantage of the previously senescent cells, indicative of an increased repopulating capacity, further underscoring a link between senescence and enhanced stem cell functionalities. Conclusions: Our data uncover an unexpected feature of cellular senescence. Considered as an irreversible exit from the cell-cycle in response to potentially harmful stresses, senescence apparently simultaneously permits transcriptional reprogramming into a latent state of qualitatively expanded and quantitatively enhanced stem cell features that adds to the already given self-renewal and tumor-initiating potential every tumor cell in the E_-myc lymphoma model a priori has (Kelly-PN et al., Science, 2007). Hence, senescence promotes a "super-stemness" condition, which exerts its detrimental, highly aggressive growth potential upon removal of the senescence barrier. Although reversal of senescence was experimentally achieved by conditional gene inactivation, senescence-compromising mutations may also be acquired as naturally occurring events, even in a resting cell. These findings raise not only concerns about the long-term benefit of senescence-inducing cancer therapies, but add a new layer to the dynamic complexity and plasticity of the "cancer stem cell" condition.

T41 - Molecular regulation of muscle stem cell quiescence

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We previous demonstrated that activation of the Notch pathway, primarily by Delta-like1 expression in the satellite cell niche activating Notch-1 on satellite cells, is essential for the activation of the satellite cell pool. Expression of the Notch inhibitor Numb leads to the down-regulation of Notch signaling necessary for myogenic differentiation. In order to explore in more detail the molecular regulation of satellite cell function by Notch signaling, we used a conditional gene deletion approach. We crossed a mouse strain in which the key transcriptional regulator in the Notch pathway, RBPj, was flanked by loxP sites with a strain bearing an inducible form of Cre recombinase (CreER) knocked into the Pax7 locus. Administration of Tamoxifen to adult offspring bearing both alleles results in the deletion of RBPj exclusively in satellite cells. Analysis of Notch target genes in purified, quiescent satellite cells from these mice revealed that they were almost all down-regulated compared to cells from control mice. Among the consequences of disruption of Notch signaling in quiescent satellite cells is their gradual depletion without any evidence of apoptosis. Rather, the cells appear to undergo spontaneous activation. Analysis of the kinetics of depletion suggests that active Notch signaling is essential for the maintenance of satellite cell quiescence and that the aberrant activation is associated with premature differentiation and the failure of self-renewal. In a parallel study, we conditionally deleted Dicer in satellite cells using the same Cre driver and a strain with a floxed Dicer allele. Similar to what was seen with RBP-J deletion, deletion of Dicer resulted in spontaneous satellite cell activation, but in this case the activated cells underwent apoptosis, resulting a depletion of satellite cells from the muscle. By miRNA microarray analysis, a group of quiescence-specific miRNAs was identified. Current studies are focusing on the role of individual miRNAs as well as downstream targets of those miRNAs that are essential regulators of satellite cell quiescence and activation.

T42 - Molecular regulation of muscle stem cell function

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No abstract available

T43 - High content analysis with Cytiva™ cardiomyocytes for investigative toxicology

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Cytiva™ Cardiomyocytes derived from human embryonic stem cells on an industrial scale provide an advance towards more clinically predictive assays for assessing cardiotoxicity of new drugs. Data will be presented from multi-parameter high content analysis of Cytiva™ cardiomyocytes in toxicology assays illustrating a powerful complimentary and comprehensive approach to assessing cardiac drug liabilities.

Stem Cells in Development: Ectoderm

T44 - Spatiotemporal regulation of epidermal stem cell behavior by the molecular clock machinery

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The circadian molecular clock allows organisms to coordinate the function of their tissues and organs according to the timing of external entraining cues such as light, temperature and feeding. Clock periodicity is established through multiple interconnected transcriptional and translational networks that impose a rhythmic expression over approximately 10% of the coding genome. Adult stem cells are responsible for tissue maintenance; however, the molecular mechanisms that fine-tune the spatiotemporal behavior of adult stem cells is still poorly understood. Epidermal stem cells ensure that skin homeostasis is maintained. In murine skin, epidermal stem cells cluster at specific niches where, at steady state conditions, they undergo cycles of dormancy and activation. Upon the requirement of cellular replenishment, epidermal stem cells egress the niche and proliferate for a limited number of times to subsequently feed into the differentiated compartment. However, at each round of morphogenesis, only a subset of stem cells becomes active, suggesting that heterogeneous stem cell responsive states coexist within the same niche. Using a circadian clock fluorescent reporter mouse model, we show that the dormant epidermal stem cell niche contains two populations of stem cells at opposite phases of the clock, which are differentially predisposed to respond to niche homeostatic cues. In dormant niches, the core molecular clock circuitry transcriptionally modulates the expression of stem cell regulatory genes to create two coexisting stem cell populations, one predisposed, and one less prone, to activation. Unbalancing this equilibrium of epidermal stem cells, through conditional epidermal deletion of a core clock component, results in a long-term progressive accumulation of non-responsive stem cells, premature impairment of tissue self-renewal, and a significant reduction in the development of squamous cell carcinomas. We propose that the molecular clock machinery finetunes the spatiotemporal behavior of epidermal stem cells within their niche, and that perturbation of this mechanism affects tissue homeostasis and the predisposition to neoplastic transformation.

T45 - Glial cells generating neurons: molecular and cellular mechanisms

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Our understanding of fate determinants endowing glial cells with the capacity of for neurogenesis is still limited. Towards a better understanding of such key regulators I will present data of genomewide expression analysis of neurogenic glial cells, such as radial glia and adult neural stem cells, versus non-neurogenic glia, such as reactive astrocytes. An intriguing finding is that known neurogenic fate determinants are already up-regulated at mRNA, but not protein level in adult neural stem cells, while radial glial cells possess much higher mRNA and protein levels of such neurogenic fate determinants thereby resembling more the neuroblasts in the adult neurogenic zones. I will then present functional data on such neurogenic fate determinants focussing especially on the interaction of neurogenic transcription factors with the chromatin remodelling machinery.

T46 - The cellular response to CNS injury - identification of injury responsive cells that mediate scaring and repair

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The adult central nervous system (CNS) harbors several different stem and progenitor cell populations. However, in the uninjured spinal cord, cellular turnover is restricted to mediate tissue homeostasis. Following traumatic injury, stem and progenitor cells become activated and start to proliferate, mediating wound closure, scar formation and limited tissue repair. We studied several distinct potential stem/progenitor cell populations in their responsiveness to spinal cord injury. Using a cell specific fate mapping strategy utilizing four inducible Cre lines to target astrocytes, oligodendrocyte precursors, ependymal cells and pericytes, we could establish an integrated view of the cellular response to injury. In detail, we found that astrocytes self-duplicate and form the outer compartment of the glial scar while oligodendrocyte precursors generate oligodendrocytes scattered throughout the parenchyma. Ependymal cells generate scar contributing astrocytes and parenchyma oligodendrocytes in response to injury (Barnabé-Heider, Göritz et al., 2010, Cell Stem Cell). In addition to these neural lineage cells we studied pericytes, perivascular cells lining capillaries, which have been suggested to be mesenchymal stem cells. Interestingly, we identified a subpopulation of CNS pericytes as the source of the stromal scar tissue. Following spinal cord injury, pericyte-derived cells outnumber scar-forming astrocytes and differentiate into fibroblast like cells that cluster within the lesion. Inhibition of the pericyte injury response using a genetic system for cell specific proliferation inhibition revealed that pericyte-derived cells are crucial for the re-establishment of tissue integrity (Göritz et al., Science in press).

Stem Cells in Disease

T47 - Modeling bone marrow failure syndromes with iPS Cells

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A major goal of stem cell research is the creation of personalized, patient-specific stem cells for use in disease modeling and as a foundation for gene repair in the context of autologous cell therapy. We have derived induced pluripotent stem cells (iPSC) from several patients with genetic bone marrow failure disorders. Including Dyskeratosis Congenita, Fanconi's anemia, Shwachman-Diamond Syndrome, and Diamond Blackfan Anemia. Hematopoietic differentiation of these lines in vitro recapitulates certain aspects of these diseases and enables novel insights into disease mechanisms, thereby confirming their utility in disease modeling for studies of pathogenesis. Aspects of these studies highlighting advantages and limitations will be presented.

T48 - The polycomb repressive complex and histone 3 lysine 27 trimethylation is essential for cancer stem cell maintenance

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Both hematological and solid tumor formation is driven by a sub-group of cells, termed cancer stem cells (CSCs), that have the properties of self-renewal and differentiation. Several lines of evidence also show that CSCs are resistant to conventional therapies due to increased DNA damage repair mechanisms, increased expression and activity of drug pumps, and their relative quiescence. Therefore, therapies aimed at the majority of cancer cells have, in essence, missed the CSC population, and targeting CSCs may play a pivotal role in the development of cancer therapies. However, studying CSCs presents several challenges to the researcher, and often new techniques and experimental designs are required. To this end, we have established isolation methods, culture techniques and novel assays designed to interrogate these cells in pancreatic, breast and colon cancer. We have developed a novel high throughput screening assay that measures the level of Histone 3 lysine 27 trimethylation, a transcriptionally repressive histone mark catalyzed by EZH2. We have shown that EZH2 is over-expressed and leads to increased levels of trimethylation in CSCs and that knock-down of EZH2 decreases CSCs, measured both by flow cytometry and sphere formation. Moreover, pharmacological inhibition of EZH2 leads to decreased tumor formation. We have also determined by ChIP-on-chip what genes are transcriptionally regulated by H3K27 trimethylation in these model systems. These results translated into a cell-based assay using high content imaging. The use of H3K27 trimethylation to monitor CSCs obviates the need for isolating or enriching the CSCs but, instead, allows the study of effects on CSCs within the context of the normal heterogeneity found within tumors. We have now begun to employ these approaches to screen candidate drugs for effects on CSCs, and have the opportunity to develop novel therapeutics against this important population of cells.

T49 - Towards unification of the cancer stem cell and clonal evolution models of cancer

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The cellular and molecular basis for the heterogeneity that exists within the individual cells that make up a tumour is not well understood. Acquisition of oncogenic mutations combined with clonal evolution is proposed to cause genetic diversity within the cells of a tumor, in turn this will underlie much of the functional heterogeneity within tumours. Often proposed as a mutually exclusive alternate model, the cancer stem cell (CSC) model postulates that heterogeneity arises because of epigenetic differences in gene expression that result in the tumour being organized as a cellular hierarchy sustained by a CSC at the apex. That is, only some cells of the tumor possess stem-like properties that make them more potent in sustaining long-term tumor propagation. Additionally, the clinical relevance of CSC has been challenged by recent reports that some tumours may actually not adhere to a CSC model when the xenograft system is enhanced. As a first step to determine if these two concepts of cancer could be harmonized, we direct addressed whether human leukemia was composed of genetically diverse subclones and whether functionally defined leukemic initiating cells (L-IC) are genetically diverse within an individual sample. We focused on Ph+ ALL because it is considered a single clinical entity where genome-wide DNA copy number alteration (CNA) profiling was recently undertaken. A robust Ph+ B-ALL xenograft system was developed that enabled combined genetic and functional studies of the genetic diversity of functionally defined L-IC derived from diagnostic patient samples. We found that genetic diversity occurs in functionally defined L-IC and that many diagnostic patient samples contain multiple genetically distinct L-IC subclones that are related through a complex evolutionary process. Reconstruction of the genetic events that were present in the subclones combined with assay of the functional properties of each subclone in xenografts enabled us to reconstruct the genetic ancestry of the subclones we detected. Collectively, our data illustrate that leukemic progression can occur in either a linear or branching fashion, with multiple genetic subclones evolving either in succession or independently, respectively. The discovery that specific genetic events influence L-IC frequency and that genetically distinct L-IC evolve through a complex evolutionary process indicates that a close connection must exist between genetic and functional heterogeneity. These findings indicate that there may be more commonalities between clonal evolution and CSC models of cancer than previously thought. Finally, our study points to the need to develop effective therapies to eradicate all genetic subclones in order to prevent further evolution and recurrence.