Stem cells, stroke and inflammation

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Stem cells can be used to generate neurons and glia cells that are lost in neurodegenerative diseases. Besides cell replacement, stem cell-based approaches improve function in animal models by remyelination, trophic actions, and modulating inflammation. Endogenous neural stem cells are novel therapeutic targets because they produce neurons and glia in response to injury and could be affected by the degenerative process. During this process of maturation and functionally integration of new neurons derived from grafted stem cells or endogenous sources numerous choices are made, such as proliferation or quiescence, cell survival or death, migration or establishment, growth or retraction of processes, synaptic integration, or tuning of synaptic transmission. Microglia are located within the neurogenic niches and as well as in the are where newly form cells migrate. In addition, there is infiltration of immune cells from the blood towards the damaged tissue and formation of macrophages. Activated microglia and monocyte-derived macrophages became interesting candidates for modulating neurogenesis in injured brain. Most studies report an acute decrease in the survival of new neurons caused by molecules released from classically activated microglia. However, microglial activation and effect of macrophages are more heterogeneous and the transformation from a pro- to an anti-inflammatory cytokine profile and the deactivation of microglia is not well defined. Significant hurdles remain before these findings can be responsibly translated to novel therapies for patients with neurodegenerative disorders. In particular, we need to learn how to control stem cell proliferation, survival, and migration in the pathological environment. Before clinical trials with stem cell-based approaches are initiated, we need to know to a much greater extent how to control stem cell differentiation into specific phenotypes, induce their integration into existing neural and synaptic circuits, and optimize the functional recovery in animal models of neurodegenerative disorders.

Cartilage tissue engineering: from the nose to the knee

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In embryonic transplantation models, mesenchymal cells derived from the neural crest ('mesectoderm') have a unique regenerative capacity and developmental plasticity, associated with a 'Hox-negative' profile. In adult mice, mesectoderm- but not mesoderm-derived skeletal *stem* cells can adopt the Hox-positive status of heterotopic transplantation sites, leading to robust tissue repair. However, it remains unknown whether developmental origin and Hox-negativity are associated with regenerative capacity and plasticity also in *differentiated* cells from developed individuals. Here it is shown that adult human mesectoderm-derived nasal chondrocytes (NC) can be constitutively distinguished from mesoderm-derived articular chondrocytes (AC) by the lack of expression of specific HOX genes (e.g., HOXC4, HOXD8). In contrast to AC, NC can be extensively cultured and serially cloned while conserving the ability to form cartilage tissue. NC can also stably adopt a Hox-positive profile typical of AC upon implantation into articular cartilage defects and directly contribute to their repair. Hence, HOX-negative *differentiated* mesectoderm cells in adult individuals retain a previously unrecognized self-renewal capacity and degree of plasticity, typical of embryonic- or stem-cell systems.

The study highlights the relevance of NC as a possible cell source for cell- or tissue-therapy in regenerative medicine, also in view of their easy availability in an autologous setting. In the field of cartilage tissue engineering, the results reinforce previous findings on the more reproducible re-differentiation and cartilage forming capacity of human NC, not exposed to degenerative processes, as compared to AC from age-matched donors or in an isogenic setting. The proof of principle that autologous NC can participate in the repair of articular cartilage defects in a goat animal model, combined with the previous demonstration that NC favourably respond to mechanical forces typical of joint loading and can recover from inflammatory processes, supports their clinical test for articular cartilage repair. Indeed, also based on the here presented findings, the Basel University Hospital has recently started a first-in-man phase I clinical study to treat traumatic knee joint defects with cartilaginous constructs generated from autologous NC (http://clinicaltrials.gov Identifier: NCT01605201).

Control of lineage specification by the notch pathway in human cardiac precursor cells

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The regenerative potential of the heart relies on a pool of cardiac precursor cells (CPCs). However, the pathways that are required for the mobilization, expansion and commitment of CPCs have not been identified. In the present study, we evaluated whether the Notch pathway was implicated in the commitment and differentiation of human fetal and adult CPCs. Fetal CPCs were obtained from ventricles at 12 to 14 weeks of gestation whereas adult CPCs from right atrial appendages of cardiac patients undergoing surgery. Isolated fetal and adult CPCs demonstrated identical cell surface phenotype, characterized by no expression of hematopoietic markers (CD45) and endothelial stem cell markers (CD34 and CD31), and expression of mesenchymal stem cell markers (CD73, CD90 and CD105). Furthermore, both CPCs expressed early cardiac markers such as Nkx2.5, GATA4 and Mef2c but no late cardiac markers expressed in functional cardiomyocytes. The two populations differ in their cardiogenic potential. In vitro, a large percentage of fetal CPCs differentiate into functional cardiomyocytes and to a lesser extent into smooth muscle cells whereas adult CPCs gave rise essentially to smooth muscle cells. To test their cardiogenic potential in vivo, CPCs were injected into SCID neonates via the temporal vein. Both fetal and adult human cells were detected in the murine heart, and appeared to differentiate into cardiomyocytes. This indicates that, if transferred into the appropriate environment, adult CPCs revealed their cardiogenic potential. The Notch pathway plays crucial roles in the development of cardiovascular system and is still highly activated during the first weeks of age. In order to test whether the Notch pathway could be used to force adult CPCs into the cardiogenic lineage, CPCs were stimulated in vitro using immobilized Notch ligands, i.e. either Jagged 1 or Delta-like-1. In fetal CPCs, both Jagged1 and Delta-like-1-mediated Notch activation increased proliferation and early cardiac gene expression (NKx2.5, GATA4 and Mef2C). In adult CPCs, activation of the Notch pathway using Jagged1 increased the level of Nkx2.5 expression (but not GATA4 or Mef2c) without stimulating proliferation. In contrast, Delta-like1 stimulated proliferation as well as GATA4 and Mef2c expression (but not Nkx2.5). To determine whether the increase in cardiac transcription factor expression reflected a concomitant increase in the number of CPCs, cells were switched to a differentiation medium following stimulation by either Jagged1 or Delta-Like1. Indeed, data indicated that the net production of differentiated cardiomyocytes was increased after transient Notch activation. Altogether, these results indicate that the Notch pathway could be used to reactivate the cardiogenic potential of human adult CPCs.

Melanoma biology: lessons from stem cells

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Patients with giant congenital nevi are at high risk for developing melanoma, the most aggressive skin cancer. The precise molecular and cellular mechanisms of this malignancy remain to be further characterized. We have recently identified the crucial role of the neural crest transcriptional factor Sox10 in melanoma formation and demonstrated that interfering with Sox10 levels counteracts *NrasQ61K*-driven melanoma in vitro and in vivo. Microarray analysis of the putative Sox10 target genes revealed that another member of the Sox gene family, Sox9, was upregulated upon Sox10 loss, suggesting a potential role of Sox9 in preventing melanoma development. To address this, we have conditionally inactivated simultaneously both Sox10 and Sox9 in the melanocytic lineage of *Tyr::Nras*^{Q61K} mice and observed the restoration of the characteristic hyperpigmentation phenotype in the *Tyr::Nras*^{Q61K} mice even in the absence of Sox10. Furthermore, in the vast majority of human melanoma samples analyzed expression of Sox10 and Sox9 was mutually exclusive. Taken together these findings establish the importance of an antagonistic action of Sox10 and Sox9 as functional regulators of melanoma development.

Dopaminergic differentiation of human neural stem cells: Effect of levodopa

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Idiopathic Parkinson's disease (PD) is characterized by progressive degeneration of melanin containing dopaminergic neurons in *substantia nigra pars compacta* in the upper brain stem. The loss of dopaminergic neurons results in a gradual decrease of striatal dopamine levels, typically leading to rigidity, tremor, hypokinesia and postural instability.
Although direct dopamine receptor agonists are frequently used in treatment of PD, the most effective drug to elicit an improvement of the motor symptoms remains levodopa (3,4-dihydroxyphenylalanine).
One of the prospects for a curative treatment for PD is to replace the lost midbrain dopaminergic neurons. Preclinical and clinical trials have demonstrated that fetal dopaminergic neurons have the potential to markedly improve motor function in animal models and patients. However, this source of cells will never be sufficient to use as a widespread therapy. Over the last 20 years, scientists have been searching for other reliable sources of dopamine neurons, and stem cells are strong candidates.
Our research is focussed on -1) experimental *in vivo* and *in vitro* studies of midbrain dopaminergic neuron development, -2) regulation of neural stem cell proliferation, migration and dopaminergic differentiation, -3) neurotrophic factors and neuroplasticity in the nigrostriatal system, and -4) cell replacement strategies for treatment of PD.
My presentation will cover our recent and ongoing stem cell research with particular focus on dopaminergic differentiation strategies and the effect of levodopa.

Age-dependent neuronal regeneration of mouse retina

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In adult mammalian vertebrates spontaneous retina regeneration is absent and in species like fish and birds Müller glia cells de-differentiate into progenitor-like cells, which regenerate up to all types and numbers of retinal neurons. Recent studies showed that in rodent retina a regenerative program could be reactivated in and ex vivo. We investigated the age-dependence of Müller glia reprogramming into an adult stem cell state as well as their potential to regenerate neuronal progeny. After retinogenesis is complete the still young Müller glia de-differentiate and re-enter the cell cycle at higher numbers compared to the adult retina. Within a few days the Müller glia adult stem cell competence is reduced to levels comparable to previously published results in the adult mouse retina in vivo. Our data suggest that a defined developmental program restrict the Müller glia regenerative capacity within a week after end of retinogenesis. Interestingly, conditional transgene expression of SV40-large t-antigen in mouse retina ex vivo overcomes some of the restriction of Müller glia proliferation. We are currently investigating the underlying mechanisms that age-dependently restrict retina regeneration.

Cell transplantation for spinal cord injury: From bench to bedside

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Pre-clinical studies have demonstrated successful stem cell transplantation in experimental spinal cord injury models. Using human central nervous system derived neural stem cells cellular integration with regenerative capacity has been shown. Long term survival of transplanted cells at 16 weeks with significant motor function improvement was described. These studies have laid the foundation for an international Phase I clinical stem cell transplantation trial in patients with thoracic spinal cord injury. In this talk I will review part of the preclinical data, describe the trial design and present some oft the 6 months clinical outcome results.

Notch 1 maintains corneal epithelial identity by attenuating AP-1 mediated inflammation

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The cornea is an avascular tissue consisting of a self-renewing, stratified epithelium with an underlying stroma containing keratocyte fibroblast cells and parallel arrays of collagen fibres. In response to injury, the corneal epithelium can be repaired by resident corneal epithelial stem cells, which in humans are enriched at the limbus. However, in circumstances where corneal epithelial stem cells are depleted, such as in patients with severe bi-lateral burns, this regenerative capacity is lost and vision is impaired. Thus, strategies aimed at generating corneal epithelial cells from other types of epithelial tissue are of clinical relevance. In a recent study, the role of Notch1 in the corneal epithelium was addressed by conditionally deleting Notch1 in stratified epithelial tissues including the cornea. In the absence of Notch1, the homeostatic maintenance of the corneal epithelium is normal. However, during wound repair, the corneal epithelium undergoes a fate switch to epidermis. Interestingly, this squamous cell metaplasia occurs in a non-cell autonomous manner and is associated with changes in the underlying stroma that subsequently induce the corneal to epidermal fate switch. Identification of the cellular and molecular factors which induce both the stromal remodeling and the epidermal fate conversion will reveal novel insights into the role of Notch in stratified epithelial tissues and raises the possibility of inducing reciprocal fate switches in stratified epithelial cells from other tissues. Here, we demonstrate that the absence of Notch1 in the corneal epithelium results in a chronic inflammatory response during wound repair due to unattenuated expression of pro-inflammatory mediators such as c-jun/AP-1. As a secondary effect of chronic inflammation, specific signaling pathways, including wnt/□-catenin, are elevated, resulting in fate conversion to epidermis. Collectively, these data identify Notch as a key regulator of inflammation in stratified epithelia and suggest that elevated wnt signaling imposes epidermal identity on corneal epithelial progenitor/stem cells.

Modeling Early Heart Development in Down Syndrome using Sibling hESC Lines

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Human embryonic stem cells (hESC) carrying known diseases provide excellent models for examining the cellular consequences of a disease from the earliest time in development. Due to differing genetic backgrounds, however, hESC lines are known to display intrinsic differences in their differentiation capacities and epigenetic patterns. These genetic differences result in significant differences upon differentiation, thus making it difficult to detect small variations between diseased and control lines. Using a hESC sibling model of disease provides a more sensitive approach to detecting small variations due to greater genetic similarity. In this study, we have isolated and characterized a trisomy 21 (T21) hESC model of Down syndrome (DS) using sibling hESC lines as controls. As congenital heart defects (CHD) are the leading cause of morbidity DS, we examined the genetic pathways associated with cardiogenesis to ascertain perturbations in development which may lead to CHD. Upon differentiation, T21-hESC show many significant differences in expression of genes associated with both mesodermal and cardiac development, which is particularly evident with genes associated to the secondary heart field (SHF).

Additionally, genes of the T-box transcription factor family were found to be significantly overexpressed in T21-hESC. Some of these perturbations also coincide with known causative genes for CHD observed in the general population.

Furthermore, we identified at least one gene located on chromosome 21 which may account for some of these perturbations. Therefore, our work shows for the first time, that T21-hESC and their sibling control lines are a useful model facilitating the identification of differentially expressed genes associated with early cardio genesis, which may underlie the cause of CHD observed in DS.

Predicting stem cell fate changes by differential cell cycle progression patterns

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Stem cell self-renewal, commitment and reprogramming rely on a poorly understood coordination of cell cycle progression and execution of cell fate choices. Using existing experimental paradigms it has not been possible to probe this relationship systematically in live stem cells *in vitro* or *in vivo*. Alterations in stem cell cycle kinetics probably occur long before changes in phenotypic markers are apparent and could be used as predictive parameters to reveal changes in stem cell fate. To explore this intriguing concept, we developed a single-cell tracking approach that enables automatic detection of cell cycle phases in live (stem) cells expressing fluorescent ubiquitylation-based cell-cycle indicator (FUCCI) probes. Using this tool, we have identified distinctive changes in lengths and fluorescence intensities of G1 (red fluorescence) and S/G2-M (green) that are associated with self-renewal and differentiation of single murine neural stem/progenitor cells (NSCs) and embryonic stem cells (ESCs). We further exploited these distinctive features using fluorescence-activated cell sorting to select for desired stem cell fates in two challenging cell culture settings. First, as G1 length was found to nearly double during NSC differentiation, resulting in progressively increasing red fluorescence intensity, we successfully purified stem cells from heterogeneous cell populations by their lower fluorescence. Second, as ESCs are almost exclusively marked by the green (S/G2-M) FUCCI probe due to their very short G1, we substantially augmented the proportion of reprogramming cells by sorting green cells early on during reprogramming from a NSC to an induced pluripotent stem cell state.
Taken together, our studies begin to shed light on the crucial relationship between cell cycle progression and fate choice, and we are convinced that the presented approach can be exploited to predict and manipulate cell fate in a wealth of other mammalian cell systems.

Intercellular adhesion balances quiescence versus activation in skin stem cells

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The outmost layer of the skin, the epidermis and its appendages are renewed throughout life-time. This process requires cyclic proliferation of epidermal progenitor or stem cells (SC) which reside in compartments such as the basal epidermis and distinct areas of the hair follicle (HF). Adhesion disrupting autoantibodies against the desmosomal cadherin Dsg3, such as produced in the autoimmune blistering disease Pemphigus vulgaris (PV), were observed to preferentially home to these SC compartments. There, they impair cell-cell adhesion and increase proliferation, consistently observed in PV mouse models as well as human PV patients.

Using these models, our studies let us to identify a number of remodeling mechanisms involved in this process. In the epidermis and/or epidermally-derived keratinocytes, c-Myc is upregulated via a process involving depletion of the transcriptionally active armadillo protein plakoglobin followed by a more rapid outward migration and evacuation of proliferating keratinocytes. In the multipotent HFSC niche, the antibody-triggered SC activation resulted in a transient loss of label retaining cells and downregulation of the bulge “stemness signature”, indicating profound alterations in epidermal SC homeostasis. However HFSC were not permanently lost due to a mechanism precluding de novo HF induction.

In summary, our data demonstrate that loss of Dsg3 adhesion results in progenitor and SC activation followed by epidermal remodeling. These findings identify PV as an excellent model to study protective measures of the injured epidermal compartments to preserve the size of the SC population.

Instructing the epigenome in stem and differentiated cells

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Chromatin and DNA modifications have emerged as a critical component for gene regulation in higher eukaryotes. Yet how these epigenetic variables are targeted to specific sites of the genome and how they influence cellular potential and identity is still poorly understood.

We have generated global maps of DNA methylation, histone modifications and replication in higher eukaryotes using stem cell differentiation as a dynamic cellular model for pluripotency, lineage commitment and terminal differentiation.

This analysis allowed us to identify genomic sites that change their epigenetic status cell-state specific. Based on the resulting datasets we generate models how these epigenetic variables are targeted, which we test by genetic perturbation of involved modifiers and mutation of putative recruiting elements.

Our results suggest that the actual DNA sequence of regulatory regions is a key determinant of their DNA methylation state, a finding, which will be discussed in the light of current models of the function of epigenetic restriction during development.