

## **ARTD1 mediated activation of FGF4 transcription is crucial for the initiation of reprogramming**

Fabienne A. Weber<sup>1</sup>, Giody Bartolomei<sup>2</sup>, Michael O. Hottiger<sup>2</sup> and Paolo Cinelli<sup>1,3</sup>

*1Institute of Laboratory Animal Science, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich*

*2Institute of Veterinary Biochemistry and Molecular Biology, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich*

*3Division of Trauma Surgery, Center for Clinical Research, University Hospital Zurich, Sternwartstrasse 14, CH-8091 Zurich*

Pluripotent stem cells have the potential to differentiate into any cell type of the body and therefore represent a valuable tool for future therapeutic applications. The recent discovery of Takahashi and Yamanaka that it is possible to establish induced pluripotent stem cells (iPSCs) by reprogramming differentiated somatic cells has opened new perspectives in the field of regenerative medicine. Despite many groups worked in the last few years on the refinement of reprogramming in order to optimize this technology for the use of iPSCs in clinical applications the mechanisms underlying the process of reprogramming are still largely unknown. Originally reprogramming of mouse and human fibroblasts was performed by the retroviral-mediated introduction of the four transcription factors, Oct4, Sox2, Klf4 and c-Myc (the so called Yamanaka factors). How these factors directly drive the process of reprogramming and which additional components are involved still needs to be carefully analyzed. One of the Yamanaka factors, the transcription factor Sox2 (SRY (sex determining region Y)-box 2) is a main player in maintaining pluripotency in embryonic stem cells (ESCs). Therefore, it is most likely that the regulation of Sox2 is critical for the generation of iPSCs. One enzyme that has been demonstrated to post-transcriptionally regulate Sox2 is ADP-ribosyltransferase diphtheria toxin-like 1 (ARTD1, formally called poly-ADP-ribose polymerase 1). ADP-ribosylation plays an important role in numerous biological processes, such as maintenance of genomic stability, cell differentiation, cell death, replication and transcriptional regulation. Different roles of ARTD1 in the regulation and maintenance of pluripotency have also previously been described.

In this study, we aimed at analyzing the role of ARTD1 during reprogramming, paying special attention at its role in the first days upon transduction with the Yamanaka factors. We found that PARylation of Sox2 by ARTD1 plays an important role in the generation of iPSCs. Fibroblasts deficient for ARTD1 show a strongly decreased reprogramming efficiency after retroviral-mediated transduction of the Yamanaka factors. The same could be observed through the inhibition of the enzymatic activity of ARTD1 in wildtype fibroblasts. Our data further show that ARTD1 mediated PARylation of Sox2 is involved in the regulation of the expression of FGF4, which activation plays a crucial role during the first steps of reprogramming.

## Predicting stem cell fate changes by differential cell cycle progression patterns

Marta Roccio<sup>1</sup>, Daniel Schmitter<sup>1,2</sup>, Marlen Knobloch<sup>3</sup>, Yuya Okawa<sup>1</sup>, Daniel Sage<sup>2</sup> and Matthias P. Lutolf<sup>1,\*</sup>

*University of Bern Department of Clinical Research, Murtenstrasse 50, CH-3010 Bern*

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.

## **Effects of stress exposure on heart regeneration in zebrafish**

Pauline Sallin and Anna Jazwinska,

*University of Fribourg, Dept. of Biology, Unit of Zoology, Fribourg, Switzerland*

In contrast to mammals, the zebrafish heart can fully regenerate after myocardial infarction. Recent studies have characterized different molecular and cellular mechanisms regulating cardiac restoration. However, the impact of environmental and psychological factors on this reconstructive process has not yet been addressed. In this study, we investigate the role of stress on heart regeneration after cryoinjury-induced myocardial infarction. We tested several different stressors on adult zebrafish, such as heat shock, crowding and caffeine exposure, and measured their effects on the stress-hormone levels using cortisol assays. These factors markedly elevated cortisol secretion and affected fish behavior in comparison to control animals. We found that a daily stress after cryoinjury severely impaired cardiac regeneration. In all three stress-models, the injured zebrafish heart failed to resolve the scar tissue and to replace it with a new myocardium. Our data demonstrate that chronic stress affects the efficiency of heart regeneration in zebrafish. Understanding how psychological and environmental factors influence the regenerative processes may lead to new therapeutic approaches in human.

## **Inflammation alone is sufficient to switch cardiomyocyte proliferation in the adult zebrafish heart**

Felix Baier, Pauline Sallin and Anna Jazwinska

*University of Fribourg, Dept. of Biology, Fribourg, Switzerland*

In contrast to mammals, adult zebrafish have the innate ability to fully regenerate the heart muscle after traumatic injury. This capacity depends on cardiomyocyte proliferation within the intact part of the ventricle, and their expansion into the damaged area. The molecular mechanisms that stimulate the cell-cycle entry in the heart muscle are poorly understood. In our study, we identified that cardiomyocyte proliferation is activated by the immune response. Shortly after infarction, fibrin deposition attracts fibroblasts and inflammatory cells that promote wound healing. During this reparative phase, we detected elevated indices of cardiomyocyte proliferation. Such an activation of the myocardium was unexpectedly identified also in uninjured hearts only in the presence of inflammation, which was induced either by chest skin incision or by microinjection of the immunogenic particles into the peritoneal cavity. We show that a systemic inflammation leads to the accumulation of leukocytes and macrophages on the intact heart surface, and to the elevation of the pro-inflammatory cytokines. This environment correlates with the enhanced cardiac cell division that is confined to the outer layer of the myocardium. Subsequently, we will investigate the effect of immunosuppression on the number of dividing cardiac cells upon inflammation. Our results indicate that inflammation alone is sufficient to trigger cardiomyocyte proliferation, which is a key mechanism of heart regeneration in zebrafish. This finding reveals an unexpected positive link between the early injury signal and the subsequent regenerative response.

## Functional characterization of stem cell-derived cardiomyocytes

Irene C. Marcu<sup>1</sup>, Pernilla Hoffmann<sup>2</sup>, Marisa Jaconi<sup>2</sup>, Nina D. Ullrich<sup>1</sup>

*<sup>1</sup>Department of Physiology, University of Bern, Switzerland;*

*<sup>2</sup>Department of Pathology and Immunology, University of Geneva, Switzerland.*

The prospect of using stem cell-derived (SC-) cardiomyocytes in cardiac regenerative medicine provides new hope for the treatment of myocardial infarction (MI) and the prevention of heart failure. Despite of recent studies using SC-cardiomyocytes for preclinical and clinical trials, the functional properties of these newly developed cells have not yet been sufficiently described to ensure successful integration into the damaged myocardium. In this study, we characterized the electrophysiological properties of single SC-cardiomyocytes and signal transmission between cells. The aim of this project was to evaluate the functional properties of SC-cardiomyocytes and compare them with native neonatal and cultured cardiac HL-1 cells. Using the “hanging drop” method, murine embryonic stem cells (mESCs) were directed into the cardiogenic lineage and formed spontaneously beating embryoid bodies. Beating areas were enzymatically dissected to obtain single and small groups of cells. Using the whole-cell patch clamp technique, cells were voltage-clamped and examined for cardiac ion channels. In the current-clamp mode, action potentials were measured. Simultaneously, Ca<sup>2+</sup> signals were recorded using fluo-3 and confocal laser-scanning microscopy. The gap junction permeant fluorescent dye Calcein was used to study intercellular communication between neighbored myocytes, and expression of gap junction proteins was confirmed in immunocytochemical assays. Our results show that two types of cardiac cells can be distinguished, spontaneously active and silent but excitable SC-cells, as reflected in the shape of their action potentials. SC-cardiomyocytes functionally express the cardiac voltage-dependent Na<sup>+</sup>, L- and T-type Ca<sup>2+</sup> channels and the pacemaking HCN channel in spontaneously active cells. Current properties and pharmacology are comparable with native cardiomyocytes. Immunocytological examination revealed strong expression of the major cardiac gap junction protein connexin-43. Functional tests of intercellular coupling confirmed electrical coupling between SC-cardiomyocytes. Permeation kinetics of calcein dye transfer in SC-cardiomyocytes were similar to HL-1 cells, but significantly reduced in comparison with neonatal cells. In conclusion, we provide a clear functional profile of single and coupled SC-cells and their similarities with native cardiomyocytes and HL-1 cells, which makes SC-cardiomyocytes realistic candidates for cell therapy.

## Identification of epithelial stem cells in rodent incisors

Granchi Z., Graf D., Alexiou M., Barrandon Y., Rochat A., Claudinot S., Stolf D., Mitsiadis T.A.

*ZZM, University of Zürich, Institut of Oral Biology, CH-8032 Zürich*

Continuous growth of rodent incisors requires a high regenerative potential. In these particular teeth, specialized cells secreting enamel and dentin, the tooth-specific mineralized tissues, are dying and replaced by newly differentiated cells. This constant turnover strongly indicates the presence of one or more adult stem cell populations in the incisors of rodents.

Many attempts have been done during recent years to identify a stem cell niche in the epithelium of the rat or mouse incisors. Most of the results support the idea that this niche is localized at the Cervical Loop (CL) area of incisors.

In order to characterize this or other putative stem cells niches, we tested the contribution of dental epithelial cells from various locations during incisor development and regeneration. For that purpose we study the movement of dental epithelial cells after Dil staining and their final contribution to the various epithelial cell layers of the incisor. Furthermore, we realized an *in vitro* recombination assay using GFP-labelled and unlabeled epithelium to create chimeric incisors and detect the movement of GFP-positive epithelial cells.

Our results show that putative dental epithelial stem cells from the CL area are able to contribute to the epithelial cell layers of the incisor. These cells participate in both in incisor's homeostasis and repair.

## **An *in vitro* expansion score for tissue engineering applications with human bone marrow derived mesenchymal stem cells**

Alessandro Bertolo<sup>1,2</sup>, Marco Mehr<sup>1</sup>, Tiziana Janner-Jametti<sup>1</sup>, Ursula Graumann<sup>1</sup>, Niklaus Aebli<sup>3,4</sup>, Martin Baur<sup>5</sup>, Stephen J. Ferguson<sup>6</sup>, Jivko Stoyanov<sup>1,2\*</sup>

*1Swiss Paraplegic Research, Nottwil, Switzerland; 2Institute for Surgical Technology and Biomechanics, University of Bern, Switzerland; 3Swiss Paraplegic Centre, Nottwil, Switzerland; 4School of Medicine, Griffith University, Brisbane, Queensland, Australia; 5Cantonal Hospital of Lucerne, Lucerne, Switzerland; 6Institute for Biomechanics, ETH Zurich, Switzerland.*

Human bone marrow-derived mesenchymal stem cells (MSCs) have limited growth potential *in vitro* and cease to divide due to replicative senescence, which from a tissue engineering perspective has practical implications, such as defining the correct starting points for differentiation and transplantation. Patient variability poses a problem for cell expansion, as the times spent in culture before the loss of the required differentiation potential are not the same. This study aimed to develop a score set which can be used to quantify the senescent state of MSCs and predict if cells preserve their ability to differentiate to osteogenic, adipogenic and chondrogenic phenotypes, based on colony forming units (CFU) assay, population doubling time (PDT), senescence-associated  $\beta$ -galactosidase (SA- $\beta$ -Gal) activity, cell size, telomere length and gene expression of MSCs cultured *in vitro* over eleven passages. This set of morphological, physiological, and genetic senescence markers was correlated to the ability of MSCs to differentiate. Differentiation efficiency was assessed by marker genes and protein expression. CFU decreased with increasing passage number, whereas SA- $\beta$ -Gal activity and PDT increased, however the correlation with MSCs' differentiation potential was sometimes unexpected. The expression of genes related to senescence was higher in late-passage cells than in early-passage cells. Early-passage cells underwent efficient osteogenic differentiation, with mid-passage cells performing the best in chondrogenic differentiation. Late-passage cells preserve only adipogenic differentiation potential. Based on this marker set, we propose a senescence score where combined markers give a reliable quality control of MSCs, depending not only on mechanistic passage number.

## The role of Nogo-A in orofacial development and regeneration

Pagella Pierfrancesco<sup>1,2</sup>, Winkler Kristian<sup>1</sup>, Alexiou Maria<sup>1</sup>, Graf Daniel<sup>1</sup>, Schwab Martin<sup>3</sup>, Mitsiadis Thimios<sup>1</sup>

*1. Institute of Oral Biology, Centre of Dental Medicine, University of Zurich, Switzerland; 2. Molecular Life Sciences PhD Program, University of Zurich and ETH Zurich; 3. Brain Research Institute, University of Zurich and ETH Zurich, Switzerland.*

The importance of innervation in the development and the regeneration of the craniofacial complex is not yet clearly established. Accumulating evidence is proving that innervation plays a key role in the development of some craniofacial organs, such as taste buds (Oakley and Witt 2004) and salivary glands (Knox, Lombaert et al. 2010). However, such a role has not been adequately investigated yet for other craniofacial organs; among these, teeth.

Tooth development results from a complex set of interactions between the oral epithelium and the cephalic neural crest derived-mesenchyme. The role of innervation in tooth initiation and development has been the subject of intense investigation at the level of experimental embryology. Previous studies on mouse embryos indicated that tooth initiation is independent of innervation. However, other studies in a polyphyodont teleost have demonstrated that the participation of nerve fibres is a prerequisite for tooth formation. The tooth is a well-defined peripheral target organ for nerve fibres derived from the trigeminal ganglion (TG). Only limited and controversial information is available regarding pioneer axon guidance to the developing oral epithelium and more specifically to the tooth target field (Stainier and Gilbert 1990) in the period preceding tooth initiation (E8.5-E9.5). However, during development nerves do not enter the dental papilla until crown formation starts. This innervation dynamics clearly suggests that dental tissues secrete neurite growth inhibitory molecules during development.

Nogo proteins, and particularly Nogo-A, were discovered and extensively studied in the context of injury and repair of nerve fibres in the central nervous system (Schwab 2010). In this context, Nogo-A emerged as an important regulator of axon growth and regeneration, cell motility and growth. However, much less is known about the physiological role of Nogo-A in development and in intact adult organisms (Schwab 2010). For its role in nerve fibres growth and regeneration, Nogo-A may play an important role also in craniofacial innervation. Related to this function, Nogo-A may also be involved in other key processes of development, related to innervation, such as blood vessels patterning (Carmeliet 2003). Moreover, Nogo-A may play innervation-independent roles in different organs development, including teeth and other craniofacial structures.

The aim of this work is to determine the role of Nogo-A in craniofacial development, with a particular focus on tooth development. This is being accomplished by studying the role of Nogo-A in craniofacial innervation, and therefore studying the role of innervation in target organs development; at the same time, we are investigating possible innervation-independent roles of Nogo-A during development.

## **FGF signaling promotes actinotrichia formation during zebrafish fin regeneration**

Chassot Bérénice, Page Lionel, Jazwinska Anna

*University of Fribourg, Zoology, Chemin du Musée 10, 1700 Fribourg*

In contrast to non-regenerative repair or scarring of an amputated limb in mammals, zebrafish can fully regenerate their appendages lost due to injury. This unique ability depends on the creation of a blastema that constitutes an apical growth zone underneath the wound epidermis. In this study, we set out to identify genes that are transcriptionally upregulated during blastema formation. Accordingly, two related genes encoding a novel protein Actinodin were identified. Both genes were weakly expressed exclusively at the distal margin of the uninjured fins, but they were hundred-folds induced during fin regeneration. Immunofluorescent analysis demonstrated that Actinodin is a secreted protein that colocalizes with the actinotrichia, the collagenous skeletal structures at the tip of the appendage. In the fin regenerate, Actinodin assembles a scaffold-like branched tubular structure between the wound epidermis and the blastema. Pharmacological and genetic inhibition of FGF signalling resulted in a rapid degradation of the Actinodin network. Such an effect was not observed after inhibition of other regeneration signals, such as IGF, or TGF-beta. We will investigate the role of Actinodin for fin regeneration using morpholino technology. Our study indicates that the proteins of the distal fin represent an early regeneration markers that are triggered by FGF signalling during appendage regeneration.

## Wild-type ALK and both ALK-R1275Q and ALK-F1174L activating mutations display oncogenic activity in murine neural crest progenitor cells

Annick Mühlethaler-Mottet<sup>1</sup>, Gisèle Montavon<sup>1</sup>, Aurélie Coulon<sup>1</sup>, Marjorie Flahaut<sup>1</sup>, Katia Balmas Bourlout<sup>1</sup>, Katya Nardou<sup>1</sup>, Nicolas Jauquier<sup>2</sup>, Jean-Marc Joseph<sup>2</sup>, Pu Yan<sup>3</sup>, Olivier Delattre<sup>4</sup>, Lukas Sommer<sup>5</sup>, Isabelle Janoueix-Lerosey<sup>4</sup>, and Nicole Gross<sup>1</sup>

<sup>1</sup>Paediatric Oncology Research, Paediatric Department, University Hospital CHUV, CH-1011 Lausanne. Switzerland

<sup>2</sup>Paediatric Surgery, Paediatric Department, University Hospital CHUV, CH-1011 Lausanne. Switzerland

<sup>3</sup>Pathology Institute, University Hospital CHUV, CH-1011 Lausanne. Switzerland

<sup>4</sup>INSERM U830 Laboratoire de Génétique et Biologie des Cancers, Institut Curie, Paris Cedex 05, France

<sup>5</sup>Cell and Developmental Biology, Anatomy Institute, University of Zurich, 8057 Zurich, Switzerland

Neuroblastoma (NB), a neural crest-derived embryonal tumour, is a heterogeneous childhood malignancy that often presents as an incurable disease. The anaplastic lymphoma receptor tyrosine kinase gene (*ALK*) was shown to be overexpressed, mutated or amplified in the majority of NB. The most common mutation, ALK-F1174L displayed an efficient transforming activity *in vivo*, and was shown to drive NB formation in transgenic mouse models. We aimed at comparing the oncogenic potential of ALK-wt, with the two most frequent activating mutations ALK-F1174L (only present in familial NB), and ALK-R1275Q (present in familial and sporadic NB). For this purpose, ALK-wt and mutated sequences were stably expressed in the murine neural crest progenitor cells JoMa1.

ALK-F1174L only conferred tumorigenic potential to JoMa1 cells *in vitro* as measured by clonogenic assays. In contrast, ALK-wt, or ALK-R1275Q expressed in JoMa1 cells were capable to drive tumour formation *in vivo*, after subcutaneous or orthotopic (adrenal gland) implantations. Interestingly, JoMa1-ALK-F1174L displayed an increased *in vivo* tumorigenic potential compared to ALK-wt and ALK-R1275Q, as ALK-F1174L derived tumours grew much faster. Orthotopic tumours and tumour-derived cell lines strongly upregulated c-myc expression, however, they remained dependent on the ALK initiating oncogene, as specific ALK inhibition reduced their viability and completely inhibited their clonogenic capacity *in vitro*. Interestingly, subcutaneous tumours displayed a NB-like phenotype, while orthotopic tumours are under characterisation.

This is the first demonstration of an oncogenic activity of ALK-wt and ALK-R1275Q mutation *in vivo*. Thus, in addition to ALK-F1174L, ALK-wt and ALK-R1275Q mutation may be instrumental in driving NB development.

## **Modeling Early Heart Development in Down Syndrome using Sibling hESC Lines**

Bosman Alexis, Letourneau Audrey, Ronzoni Flavio, Wirth Mélanie, Hovatta Outi, Antonarakis Stelyanos, Jaconi Marisa

*Geneva University, Dept Pathology and Immunology, 1 rue Michel Servet, 1211 Geneva*

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 over-expressed 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.

## **Influence of ALDH activity in the stem cell properties of Neuroblastoma cells**

Marjorie Flahaut<sup>1</sup>, Nicolas Jauquier<sup>2</sup>, Annick Mühlethaler-Mottet<sup>1</sup>, Jean-Marc Joseph<sup>2</sup> and Nicole Gross<sup>1</sup>

*<sup>1</sup>Paediatric Oncology Research Unit, Paediatric Department, University Hospital, CHUV, CH-1011 Lausanne, Switzerland,*

*<sup>2</sup>Paediatric Surgery, Paediatric Department, University Hospital, CHUV, CH-1011 Lausanne. Switzerland*

**Background:** Neuroblastoma (NB) is the most aggressive extracranial childhood tumour. The existence of a cell hierarchy with a minor sub-population of so-called cancer stem cells (CSCs) may be at the origin of the typical heterogeneity of this tumour. However this CSC population has not yet been formally identified in NB.

**Methods:** A microarray time course analysis of serial NB spheres passages allowed us to specifically "profile" the NB stem cell-like phenotype and identify, among others, ALDH1A2 as a potential tumour-initiating cells (TIC) marker for NB.

**Results:** Three ALDH1 isoforms involved in development and in the synthesis of retinoic acid have been already identified as functional stem cell markers in leukaemia and breast cancers. By using the Aldefluor flow cytometry-based assay, we confirmed that increased ALDH activity was observed in a restricted number of cells upon NB sphere passages, suggesting that a pre-existing ALDH<sup>high</sup> cell subpopulation was selected during the self-renewal process. Conversely, specific inhibition of ALDH activity resulted in significant reduction in the self-renewal capacity and in the clonogenic potential of NB cells. Moreover, NB cells resistance to various drugs was partially abolished by inhibition of ALDH activity, however in a cell line- and drug-dependent fashion.

**Perspectives:** ALDH activity-based cell sorting is now used to investigate the functional behaviour of ALDH<sup>high</sup> and ALDH<sup>low</sup> cell subpopulations. ALDH<sup>high</sup> active cells express higher amount of MDR1 and/or CD133, two NB-TICs profile markers. Whether ALDH activity, combined with TICs-profile markers, identifies a tumour-initiating cell population involved in the maintenance of the tumour is currently explored.

## **Tooth abnormalities in FGFR1/FGFR2 double mutants**

Anna Filatova, Lucia Jimenez Rojo, Thimios Mitsiadis

In collaboration with Sabine Werner

*Institut of Oral Biology, University of Zürich, Zürich*

The fibroblast growth factor (FGF) and the FGF receptors (FGFR) have been shown to play an important role in tooth formation and regeneration (Kettunen et al., 2000; Harada et al. 2002; Klein et al., 2008). Concerning the FGFRs, it has been shown that FGFR1 mutants die at the age of E7.5-9.5, but the conditional knockouts (K14-Cre; *Fgfr1<sup>fl/fl</sup>*) survive and present problems with ameloblast differentiation and adherence, which results in a defected enamel formation (Yamaguchi et al., 1994; Takamori et al., 2008). FGFR2 mutants die soon after birth, that is why an rtTA transactivator/tetracycline promoter approach that allows inducible and reversible attenuation of FGFR2b signalling was used to investigate the role of FGFR2 (rtTA; *tet(O)sFgfr2b*). These mice exhibit an abnormality at the posterior end of the incisors where the cervical loop is located. Incisors have impaired growth and fail to form enamel (Parsa et al., 2010; Yongshun Lin et al., 2011).

To date the roles of FGFR1 and FGFR2 have been investigated only separately. For this reason, we study teeth in the FGFR1/FGFR2 double mutants using the Keratin5-Cre driver.

Our results show enamel defects but also defects in the root and surrounding alveolar bone. The enamel is rough and dimpled irregularly. Incisors are not closed on their lingual side. We have shown that expression of dentin sialophosphoprotein is higher in teeth of these mutants. Moreover we have observed defects in tooth crown morphology, indicating a more general role of FGFs in tooth formation.

# **Non-viral Gene Transfer of Growth and Differentiation Factor 5 (GDF-5) to Primary human Mesenchymal Stem Cells – A path to Gene Therapy for Degenerative Disc Disease?**

Christian Bucher<sup>1,2</sup>, Amiq Gazdhar<sup>2</sup>, Lorin M Benneker<sup>3</sup>, Thomas Geiser<sup>3</sup> & Benjamin Gantenbein-Ritter<sup>1</sup>

*1 Tissue & Organ Mechanobiology, Institute of Surgical Navigation & Biomechanics, University of Bern*

*2 Department of Pulmonary Medicine, University Hospital, Bern, Switzerland*

*3 Orthopedic Department, Insel University Hospital, Bern, Switzerland*

## **Introduction**

Nucleofection allows a straightforward and efficient gene transfer to cells of interest. With transfection of mesenchymal stem cells (MSC) the high proliferation capacity and their ability to differentiate into different cell types of the mesenchyme can be enhanced. In order to reveal their potential in cell- or gene-based therapies it is of great interest to enhance some of their features through gene delivery strategies. Transfection with a full ORF clone plasmid of growth and differentiation factor 5 (GDF-5) leads to increased production of endogenous GDF-5. We hypothesized that upregulation of genes by the produced GDF-5 will differentiate the MSCs towards IVD cells without the need of exogenous growth factor (=discogenesis). In addition 3D culture system with alginate beads enhances the differentiation.

## **Material and Methods**

Isolated MSCs were cultured and expanded for two weeks (up to passage 3) and then transfected with a GFP-tagged ORF clone of the GDF-5 gene by nucleofection using the nucleofector from Amaxa™ (Lonza, Basel, Switzerland). Fluorescence was monitored after 7 and 14 days and was detected up to 28 days after transfection.

## **Results**

GDF-5 is upregulated by a 9 mean fold. KRT19 (a marker for discogenesis) is 4 fold upregulated when transfected MSCs are encapsulated in 1,2% alginate versus same encapsulation of untransfected cells. Extracellular matrix genes like collagen type 1 (col1) and type 2 (Col2A1) were also upregulated. SOX9 a major transcription factor for induction of chondrogenesis was found to be upregulated. mRNA of GDF-5 was found upregulated by about three fold relative to untransfected control.

## **Conclusions**

Non-viral gene transfer of hMSCs provides an option to new therapeutic strategies. With nucleofection a stable transfection of passage 0 and 1 MSCs was obtained. Nucleofection is a straightforward method to insert genes of interest into target cells without the need of silenced viruses. GDF-5 pushes MSCs towards chondrocyte differentiation and especially due to the KRT19 upregulation also towards discogenesis. The transfected MSCs will be used for inorgan injection. The survival rate of transfected MSCs should be higher than sham MSCs. The hypoxic milieu of the intervertebral disc should differentiate the MSCs to useful cells for tissue regeneration.

## **Promotion of angiogenesis of brain endothelial cells by conditioned medium treatment critically involves the PI3-kinase pathway**

Anna Lena Fuchs, Jennifer Staudigl, Stefanie Seiler, Nicole Porz, Hans R. Widmer, Stefano Di Santo

*Department of Neurosurgery Research Laboratory and Regenerative Neuroscience Cluster, University of Bern and University Hospital Bern*

**Background:** Recent reports provide evidence that regenerative properties of stem and progenitor cells are exerted for the most part on their secretion of soluble factors. In line with this notion, we have demonstrated that endothelial progenitor cells derived conditioned medium (EPC-CM) promoted survival of cultured neuronal cells. The present study aimed at investigating whether paracrine factors released from cultured EPC support brain endothelial cell angiogenesis after injury. In addition, we focused on the signaling pathways involved in EPC-CM mediated effects.

**Methods:** Cultures from rat brain endothelial cells were incubated with EPC-derived conditioned medium EPC-CM. Angiogenic response of the microvascular endothelial cells was assessed by measuring tubulogenesis and cell number after incubation with EPC-CM. Cell numbers in culture were measured by the MTT assay after 24 hours incubation. The morphogenic response of endothelial cells to EPC-CM was investigated by means of tube formation assay on growth factor reduced Matrigel™ for 8 hours. In vitro cell migration was measured by wound closure in the scratch assay. The specific PI3K/AKT inhibitor LY294002 and the MAPK/ERK inhibitor PD98059 were used to analyze the involvement of these two signaling pathways in the transduction of the effects of EPC-CM.

**Results:** Incubation of brain microvascular endothelial cells with EPC-CM resulted in a significant increase of tubule and network complexity as compared to controls. The cell number was likewise augmented after incubation with EPC-CM. The wound closure was significantly augmented after incubation with EPC-CM. Importantly to note, inhibition of the PI3K/AKT signaling pathway resulted in a significant reduction of the length, the complexity of the tubule network formation, the overall cell number as well as cell migration. On the other hand inhibition of the MAPK/ERK pathway did not affect the angiogenic response of the brain microvascular cells to EPC-CM.

**Conclusion:** Taken together, our findings demonstrate that EPC derived paracrine factors substantially promote angiogenesis and repair of brain vessels. Thereby, the PI3K/AKT pathway appears to play a major role in the EPC-CM mediated effects. These observations may offer new therapeutical approaches for the repair of injured brain microvessels.

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

Nowell Craig, Wagner Erwin, Radtke Freddy

*EPFL SV ISREC, 1015 Lausanne*

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/ $\beta$ -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.

## **Forkhead transcription factor Foxk2 – a guardian of long-term hematopoietic stem cells**

Fasnacht Nicolas, Lorini Doris, Dubey Christelle and Radtke Freddy

*Ecole Polytechnique Fédérale de Lausanne, Swiss Institute for Experimental Cancer Research (IS-REC), Lausanne, Switzerland*

Forkhead transcription factors belong to an evolutionary conserved gene family and regulate a broad spectrum of biological processes, ranging from cell cycle regulation, survival to differentiation. Mutations in genes of several Fox subfamilies are related to tumorigenesis and cancer progression. However, very little is known about the function of Foxk2. This gene was recently suggested to be a G/T mismatch DNA binding protein or a regulator of the AP-1 transcriptional complex. In order to investigate the *in vivo* function of Foxk2 in the hematopoietic system, we generated a Foxk2 conditional knockout mouse (Foxk2lox/lox). Although null mice had no gross abnormalities and did not show any obvious phenotype, we discovered a defect in hematopoietic stem cell (HSC) function and repopulation capacity. Foxk2ko mice were more susceptible to repeated 5FU injections, a drug that depletes cycling cells. In this situation, HSCs were unable to repopulate the mice, eventually leading to stem cell exhaustion. Moreover, Foxk2-deficient HSCs revealed increased cell cycling kinetics, enhanced apoptosis and generated more reactive oxygen species (ROS) after stress induced by poly:IC or 5FU, resulting in progressive loss of ckit+Sca1+Lin- (KLS) cells and more particular the KLS CD150+CD48-long-term HSC (LT-HSC) fraction. When we assessed the long-term reconstitution capacity of Foxk2-deficient HSCs, it was shown that donor cells are progressively lost during serial transplantation experiments in BM chimera settings. Moreover, in competitive BM chimeras, Foxk2-deficient HSCs were outcompeted already during the primary transplant when the cells were given in a 1:1 or 1:10 ratio with competitor BM cells. These findings suggest that Foxk2 is a regulator of LT-HSCs and protects these cells from apoptosis and excessive cycling due to competitive pressure, injury or stress responses.

## Identification of Hes1 target genes in murine and human T-ALL

Silvia Wirth, Agnieszka Wendorff, Ute Koch and Freddy Radtke

*Ecole Polytechnique Fédérale de Lausanne (EPFL), Swiss Institute for Experimental Cancer Research (ISREC), Station 19, 1015 Lausanne, Switzerland*

T cell acute lymphoblastic leukemia (T-ALL) is the most common pediatric hematopoietic malignancy. Improved chemotherapy cures 80% of pediatric T-ALL patients, whereas relapse patients have poor prognosis. In addition, more than 50% of all T-ALL patients carry mutations in the Notch1 receptor. Although deregulation of the Notch signaling pathway and certain downstream target genes are important players in T-ALL, the precise molecular pathways that control disease development are incompletely understood. T-ALL can be induced in mice either by expressing the Notch intracellular domain (NICD) genetically from the Rosa-26 locus or via retroviral transduction of hematopoietic progenitors. Our studies show that deletion of the direct transcriptional Notch1 target Hes1 affects the development of Notch1 induced T-ALL in the genetic and retroviral model (Wendorff et al., *Immunity*, 2010). We performed RNA-seq on early stage tumour cells in the presence and absence of Hes1 to find differences in gene expression. We also investigated Hes1 in human T-ALL and could show that the knock down of Hes1 in two patient derived cell lines (T-ALL1, CUTL-1) with shRNA results in severe growth retardation accompanied by increased cell death compared to cells expressing scrambled shRNA. In order to identify genome wide targets of Hes1 we applied ChIP-seq, a technique that combines Chromatin Immunoprecipitation (ChIP) with high through put (Illumina) DNA sequencing) in T-ALL1 and CUTL-1. We aim to integrate the ChIP-seq and RNA-seq data sets to find targets regulated by Hes1.