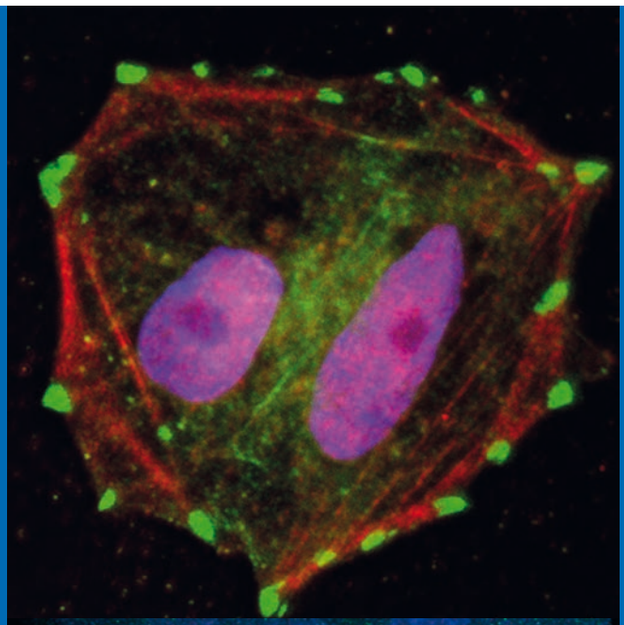
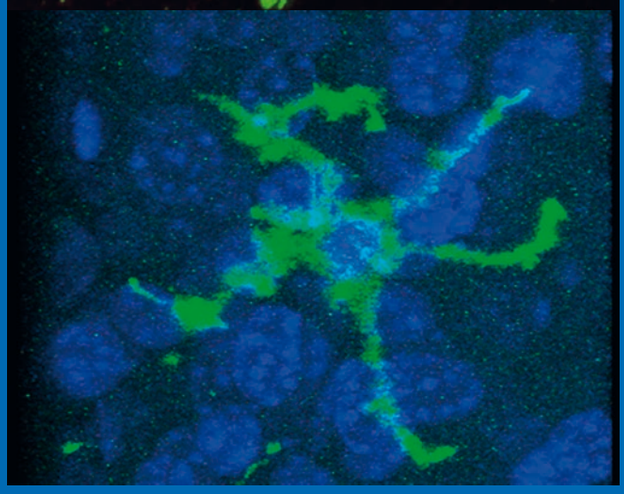
Gene Function in Cell Growth, Differentiation & Development





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Introduction

Precision medicine (also referred to as personalised medicine) aims at tailoring medical therapy to the specific disease and needs of the individual patient. Stem cells are particularly well suited for precision medicine both for diagnosis and cell based therapy.

The institute studies various stem cell types and their differentiated progeny. A particular focus is on pluripotent stem cells, hematopoietic stem cells and mesenchymal stem cells. Additionally, engineered pluripotent stem cells are generated from normal body cells by reprogramming, referred to as induced pluripotent stem cells (iPS cells). Further to this we use precision genome editing by CRISPR/Cas technology for generating iPS cells with wanted properties for e.g. disease modelling.

Hematopoietic stem cells give raise to all cells of blood and we study blood cell composition based on DNA methylation signatures. Dendritic cells (DC) represent a highly specialized blood cell type required for immunity and immune tolerance. We investigate molecular mechanism of DC development from hematopoietic stem cells and DC migration. These studies also extend to general mechanism of cell-biomaterial interaction and their impact on cell motility and adhesion.

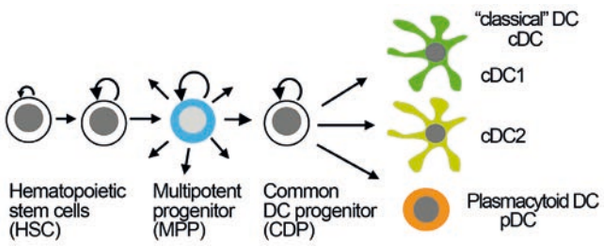

Fig 1: *Hematopoietic stem cells develop into specific DC subsets cDC1, cDC2 and pDC by successive steps of lineage commitment and differentiation.*

Finally, computational approaches are being widely used to study gene expression, chromatin architecture and gene networks in both normal physiological and pathological states. We would like to congratulate our computational expert Ivan Costa for accepting the position as Professor and founding head of the Institute for Computational Genomics, Joint Research Center for Computational Biomedicine, RWTH Aachen University, Aachen, Germany.

iPS Cells for Disease Modelling in vitro

Patient specific cells for studying human diseases are limited or not available. Here iPS cell technology provides a solution: iPS cells represent an inexhaustible cell source and can differentiate into cells of all three germ layers and thus a large array of cell types are readily obtained for disease modelling in vitro (Zenke, 2017; Zenke et al., 2017).

Our focus is on immunodeficiency and hematopoietic malignancies, such as leukemia. We have generated iPS cells deficient in the transcription factor IRF8 by CRIPSR/Cas (Sontag et al., 2017a; 2017b; Sontag and Zenke, 2017; Fig. 2) and patient specific iPS cells harbouring leukemia causing and/or associated mutations, such as those in Jak2, Kit,

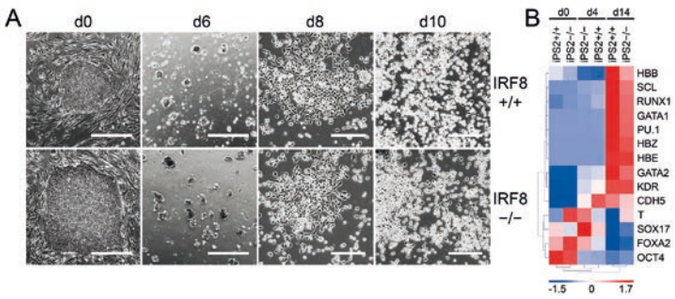

Fig. 2: *iPS cells deficient for IRF8 and controls (IRF8-/- and IRF8+/+, respectively) are differentiated into hematopoietic progenitors in embryoid body assays (A) and analysed for specific changes in gene expression by qRT-PCR (depicted in heat map format in B; red, high gene expression; blue, low gene expression; Sontag et al., 2017a).*

Tet2, NFE2 and Asxl1 (in collaboration with Department of Hematology, Oncology, Hemostaseology and Stem Cell Transplantation, RWTH Aachen University Hospital, Aachen, Germany and Department of Medicine I and Ludwig Boltzman Cluster Oncology, Medical University, Vienna, Austria). Patient specific iPS cells are differentiated into leukemic cells and used for molecular studies and compound screening (in collaboration with Department of Organic Chemistry, RWTH Aachen University, Aachen, Germany).

To study large scale production of patient specific iPS cells a new cell production facility is being set up (iCellFactory, Fig. 3; in collaboration with Laboratory for Machine Tools and Production Engineering, WZL, RWTH Aachen University (mechanical design, automation and control software) and Fraunhofer Institute for Production Technology, IPT (microscopy), Aachen, Germany).

Fig. 3: *New robotic system for automatic iPS cell production under construction.*

EMT Regulators as Switches in DC Development

DC originate from hematopoietic stem cells in bone marrow (Fig. 1) and exit the bone marrow niche as precursors to immigrate into peripheral tissues, such as skin (Fig. 4). Here DC become sessile and functionally embed to act as sentinels of the immune surveillance system. Following antigen uptake DC are activated, emigrate the peripheral tissue and travel via lymphatic vessels to lymphoid organs where they encounter T cells to present processed antigens (Fig. 4). We investigate signaling via TGF-β1 receptor and hepatocyte growth factor (HGF) receptor (also known as MET) in DC development, function and migration. We propose that mechanisms of mesenchymal-to-epithelial transition (MET) and epithelial-to-mesenchymal transition (EMT) are important for generating sessile and migratory DC, respectively (Hieronymus et al., Semin. Cell Dev. Biol., 2015; Sagi and Hieronymus, 2017, in press). In addition, a study in collaboration with Hovav et al., Hebrew University, Jerusalem, Israel revealed a differential and sequential role of BMP7 and TGF-β1 in differentiation of Langerhans cells (LC), the contingent of DC in the stratified squamous epithelia (Capucha et al., 2017). Hence, we propose the concept that EMT and MET programs are regulated in DC/LC development by Met, and TGF-β1 and/or BMP7 signaling, respectively (Fig. 4).

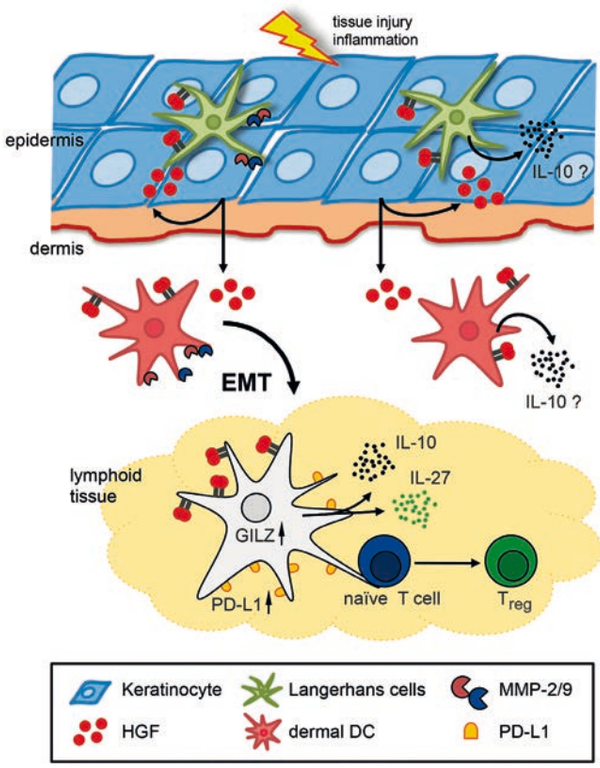

Fig. 4: HGF/Met signaling induces LC and dermal DC emigration from skin in an EMT-like process, including matrix metalloproteinase (MMP) activation to facilitate arrival in lymph nodes and antigen presentation to naive T cells. In addition, HGF induces tolerogenic phenotypes by IL-10 and IL-27 secretion which results in enhanced numbers of regulatory T cells (Tregs).

Epi-Blood-Count: DNAm Based Leukocyte Subset Quantification

Analysis of the cellular composition of blood is a routinely requested laboratory test in hematological diagnostics. So far such measurements done with fresh blood samples and immunophenotypic analysis are labour intensive. Here we established an alternative approach based on DNA methylation (DNAm) measurements at individual CG dinucleotides (CpGs) that reflect the relative composition of leukocytes. The DNAm measurements, referred as "Epi-Blood-Count" show nearly the same precision as conventional hematocytological methods and are applicable also to frozen blood samples (in collaboration with Department of Hematology, Oncology, Hemostaseology and Stem Cell Transplantation and Institute for Immunology, RWTH Aachen University Hospital, Aachen, Germany).

Predicted blood counts for granulocytes, lymphocytes and monocytes – based on our "Epi-Blood-Count" – correlate well with measurements on a hematology analyzer (Fig. 3A). In analogy, the method is also applicable to other leukocyte subsets, such as CD4 T cells, CD8 T cells, B cells and NK cells. Additionally, the calculated absolute cell numbers based on DNAm correlate nicely with absolute cell counts (Fig. 3B). Our Epi-Blood-Count approach allows determining white blood cell composition in frozen blood samples, improves the cost effectiveness and advances the standardization of white blood cell counts (Frobel et al., in press).

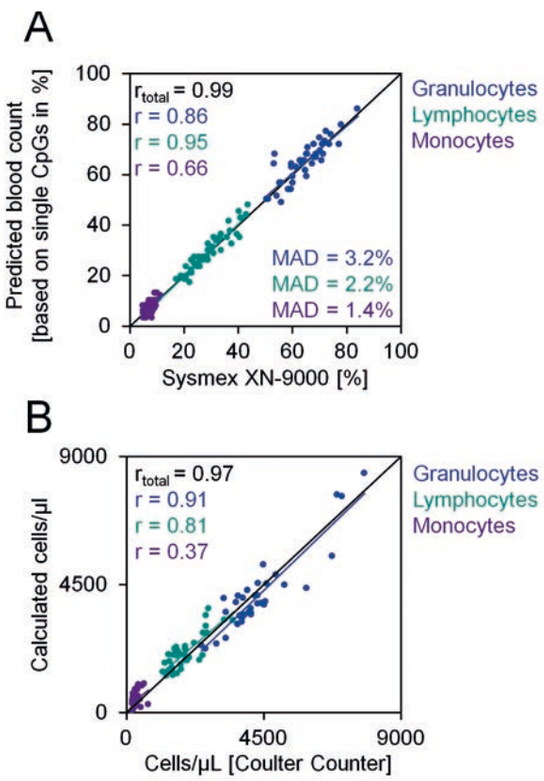

Fig. 5: Leukocyte subset Epi-Blood-Count and absolute quantification of cells based on DNA methylation (Frobel et al., in press).

Solution Blow Spinning Fibres for the Analysis of Cell Motility and Adhesion

New technologies have made possible the development of polymeric biomaterials with controlled geometry and physico-chemical properties. Solution blow spinning technique has the advantage of ease of use allowing the production of nano or microfibres and the direct fibre deposition on any surface in situ. Yet, very little is known about the influence of such fibres on biological functions such as immune response and cell migration. We engineered polymeric fibres composed of either pure poly(lactic acid) (PLA) or blends of PLA and polyethylene glycol (PEG) by solution blow spinning (SBS) and determined their impact on DC and on cell adhesion and motility (Paschoalin et al., 2017).

Cells readily interacted with fibres resulting in an intimate contact characterised by polymerisation of actin and the accumulation of focal adhesion components at sites of cell-fibre interactions (Fig. 6). Remarkably, fibres did not elicit any sizeable increase of activation markers and inflammatory cytokines in DC, which remained in their immature (inactive) state (Paschoalin et al., 2017). These findings will allow the development of new biomaterials for tissue engineering and regenerative medicine.

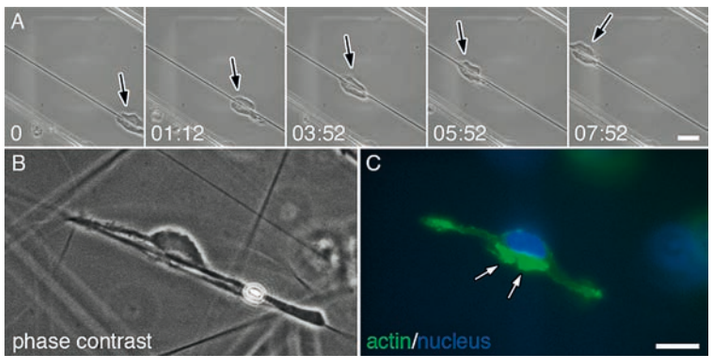

Fig. 6: *Motility of mouse DC along SBS fibres. DC were seeded on fibres for 24 hours before being imaged at 37°C, 5% CO2. The arrow points to a DC moving in a high directional way along one SBS fibre. Numbers indicate elapsed time in minute and seconds. Immunofluorescence microscopy of mouse DC seeded on SBS fibres (B, C). Note the accumulation of actin filaments at cellular regions in contact with SBS fibres (white arrow in C). Cell nucleus stained with DAPI (blue); actin filaments stained with Alexa 488-phalloidin (green). Scale bars: 20 µm (for A), 10 µm (for B, C) (Paschoalin et al., 2017).*

Computational Approaches for Personalized Medicine in Type II Diabetes

Type 2 diabetes (T2D) is a disease with an increasing prevalence in industrialized countries. T2D is currently treated with a combination of lifestyle changes and pharmacological therapies. However, there are no specific guidelines for how to use available anti-diabetic drugs to target the underlying genetic traits. In a collaborative work with Anders Rosengren (University of Gothenburg, Sweden), we have analysed genome-wide data from T2D patients to find novel biomarkers and evaluate therapeutic approaches targeting these genes.

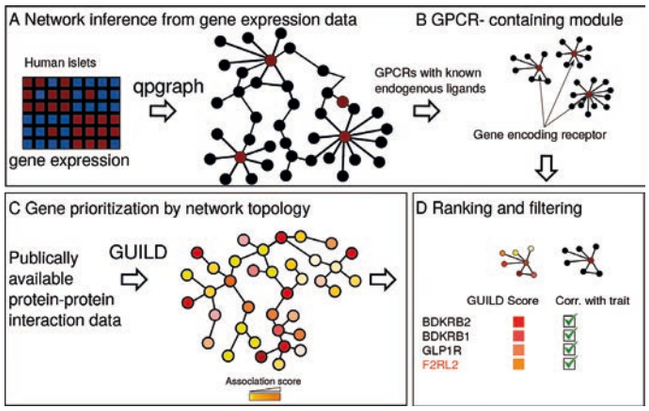

Fig. 7: *Workflow of the computational strategy to find modules of receptor genes dysregulated in T2D patients.*

In a first study, we propose a methodology to identify receptor modules dysregulated in T2D patients using co-expression networks (Fig. 7). We demonstrate that the receptor PAR3 is associated with increase in insulin secretion. Moreover, antibodies blocking PAR3 counteracted the insulin secretion of pancreatic beta cells (Hänzelmann et al., 2016). Next, we performed an integrative analysis of gene expression and open chromatin data on T2D patients. We observed binding of the Sox5 transcription factor in genes, which are down regulated in T2D patients and are associated to open chromatin in precursors of islet cells. This indicates an association of Sox5 with islet cell maturation. Importantly, treatment of T2D diabetic mice with inhibitors of a chromatin remodelling factor rescued Sox5 expression and restore normal insulin secretion (Axelsson et al., 2017).

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Patent applications

[1] Method for determining blood counts based on DNA methylation; 2017; EP17163798.6 (Wagner W, Frobel J)

[2] Verfahren zur Bestimmung der Zellzahl von eukaryotischen Zellen; 2017; Az: 10 2017 004 108.3 (Wagner W, Bozic T)

Team

On the Left: Poster Award to Kristin Seré, PhD at Medical Sciences Day 2017, Medical Faculty, RWTH Aachen University

Figure below: Lab Retreat in Antwerp, Belgium

Visiting the opencast coal mining area Weisweiler in the vinicity of Aachen, Germany