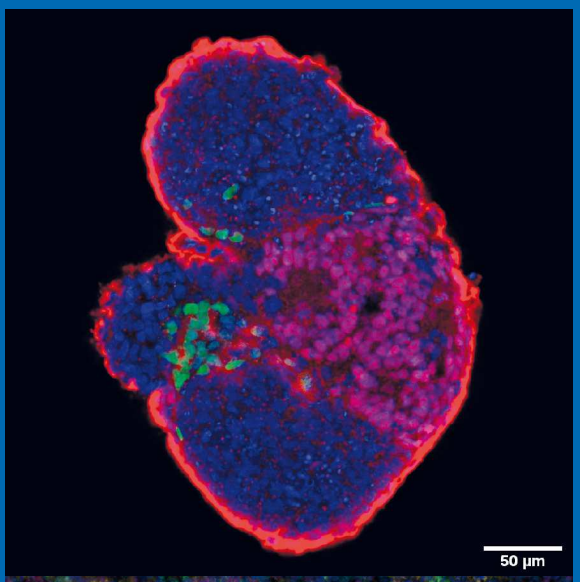
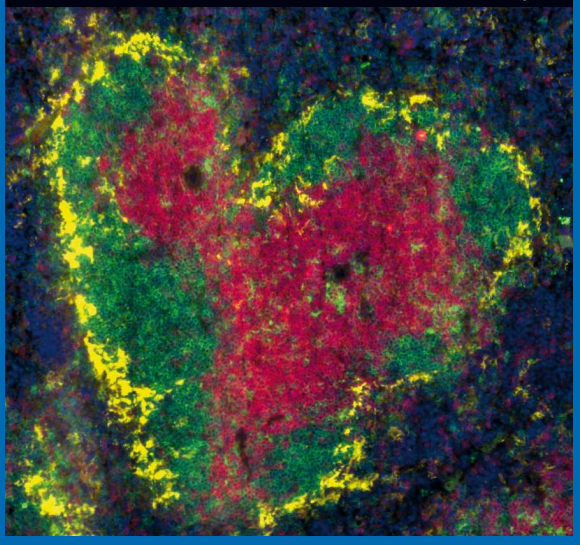
Gene Function in Cell Growth, Differentiation & Development





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Introduction

Elucidating cell functions and cell fate decisions has been the major focus of the institute during the year, which includes work on pluripotent and hematopoietic stem cells, cell adhesion and migration, cell tracking, DNA methylation and mechanobiology (Fig. 1). More specifically, induced pluripotent stem cells (iPS cells) were employed for disease modeling (leukemia, pain) and compound screening. Hematopoietic stem cells and their development in antigen presenting dendritic cells (DC) were studied.

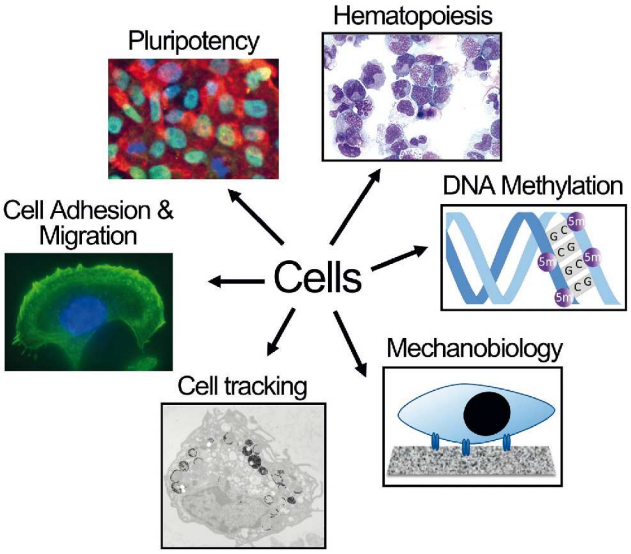

Fig. 1: Major research topics of the year 2019 are depicted.

We use genome editing with CRISPR/Cas as a particular versatile toolbox for precision genome engineering in pluripotent embryonic stem cells and adult somatic stem cells to generate cells with wanted properties. The Epi-BloodCount project aims at quantification of leukocytes in blood samples based on their DNA methylation signature.

KIT D816V iPS Cells for Precision Medicine

Patient specific iPS cells are engineered stem cells obtained from somatic cells of patients by reprogramming. iPS cells can differentiate into all cell types of our body and thus provide unique opportunities for disease modeling, drug development and regenerative medicine. We generated KIT D816V iPS cells from patients with aggressive systemic mastocytosis and mast cell leukemia to develop a disease model for mechanistic and drug discovery studies (Toledo et al., 2019; in collaboration with the Department of Hematology, Oncology, Hemostaseology and Stem Cell Transplantation, RWTH Aachen University Hospital; Fig. 2).

KIT D816V iPS cells differentiated into neoplastic hematopoietic progenitor cells and mast cells with patient-specific phenotypic features, which reflects the heterogeneity of the disease. The KIT D816V mutation was also introduced into human embryonic stem cells (ES cells) by CRISPR/Cas9n genome editing (Fig. 3). Such KIT D816V ES cells, when differentiated into hematopoietic cells, recapitulated the phenotype observed for KIT D816V iPS cells (Toledo et al., 2019).

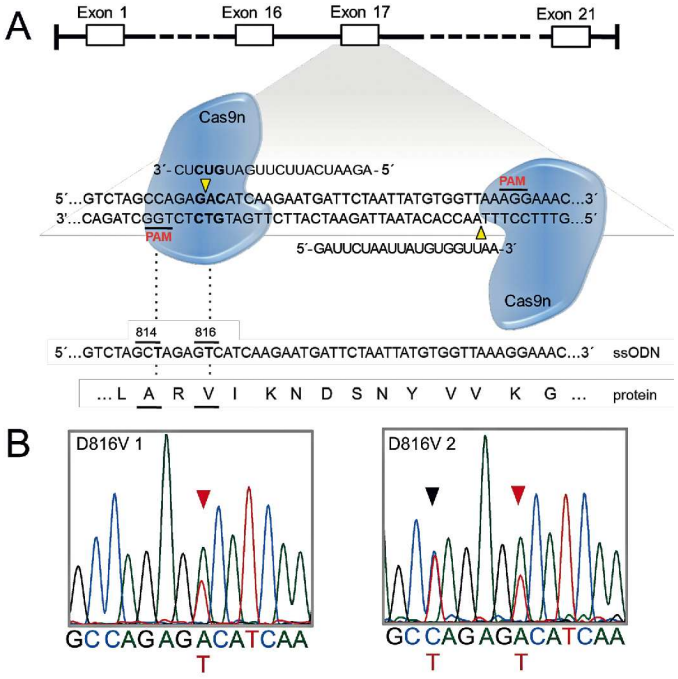

Fig. 3: Precision genome engineering of KIT D816V human ES cells by CRISPR/Cas9 nickase (Cas9n). (A) The A-T mutation and a silent C-T mutation in exon 17 of KIT gene were introduced by Cas9n and a synthetic oligonucleotide (ssODN). (B) DNA sequence of mutated KIT gene of (A) (Toledo et al., 2019).

KIT D816V causes constitutive activation of the KIT tyrosine kinase receptor and we exploited our KIT D816V iPS cells and ES cells to investigate new tyrosine kinase inhibitors targeting KIT D816V. Our study identified a new selective KIT D816V inhibitor and thus a new drug candidate for KIT D816V targeted therapy of advanced systemic mastocytosis and mast cell leukemia.

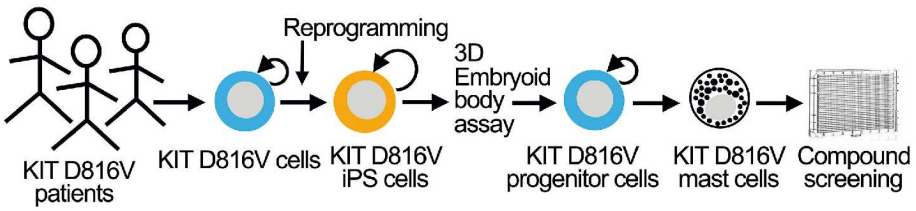

Fig. 2: Schematic representation of KIT D816V iPS cell generation from KIT D816V patients by reprogramming and of KIT D816V iPS cell differentiation into hematopoietic progenitor cells and mast cells for compound screening (Toledo et al., 2019).

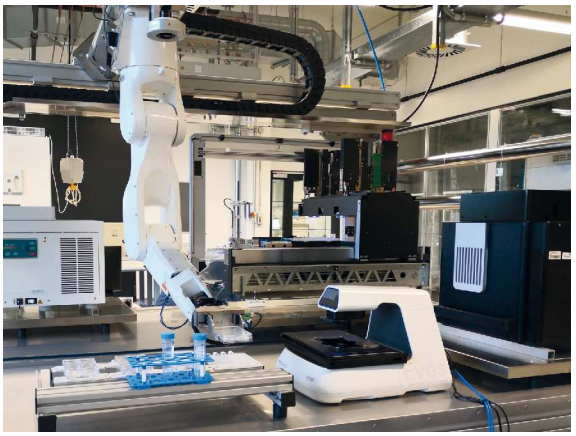

Fig. 4: iPS cell production and differentiation in automatic cell production facility (iCellFactory).

In 2019 we also followed up on our efforts to further develop our automatic cell production facility for iPS cells, and of cells derived thereof, to meet the ever increasing need of these cells for various biomedical applications, such as compound screening (Fig. 4; see also above; in collaboration with Laboratory for Machine Tools and Production Engineering, WZL, RWTH Aachen University and Fraunhofer Institute for Production Technology, IPT, Aachen, Germany).

Patient-specific iPS cells for Studying Chronic Pain

Chronic pain represents a particularly devastating disease that requires intensive research on the underlying molecular mechanisms. To this end we generated iPS cells of inherited erythromelalgia (IEM) patients with chronic pain harboring a sodium channel Nav1.7 mutation (Meents* et al., 2019).

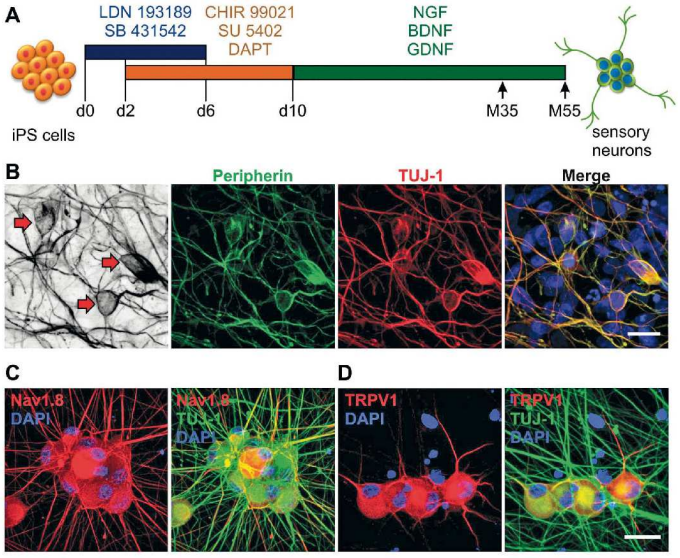

Fig. 5: Sensory neurons are obtained from IEM patient-specific iPS cells. (A) Schematic representation of iPS cell differentiation into sensory neurons. (B-D) IEM neurons express peripherin, TUJ-1, Nav1.8 and TRPV1 (Meents et al., 2019).*

These Nav1.7 mutation iPS cells were differentiated into sensory neurons (Fig. 5) and subjected to electrophysiology analysis. We found that the IEM mutation caused a hyperpolarizing shift of Nav1.7 activation (Meents* et al., 2019). Our model provides a new rational for Nav1.7 action and should be most valuable for developing more efficacious clinical analgesics.

TGF-β/BMP and HGF Receptors are Regulators of EMT and MET Programs in Dendritic Cell Development and Migration

DC are important regulators of adaptive immunity and act as sentinels in almost all peripheral tissues of our body. Accordingly, in the life cycle of DC, changes in the states of sedentariness and migration are closely related to their development. We propose the concept that genetic programs of mesenchymal-to-epithelial transition (MET) and epithelial-to-mesenchymal transition (EMT) regulate homing and migration of DC, respectively (Hieronymus et al., Semin. Cell Dev. Biol., 2015; Sagi and Hieronymus, Front. Immunol., 2018). We particularly focus on investigating signaling via TGF-β receptor and hepatocyte growth factor (HGF) receptor, which have a specific impact on regulating EMT and MET programs and thus act as relays during DC development, function and migration (Fig. 6).

DC develop from hematopoietic stem cells in bone marrow and migrate as precursors into peripheral tissues, such as skin (Fig. 6). Depending on TGF-β receptor signaling DC are functionally embedded there to act as guardians of the immune system. A differential and sequential role of BMP7 and TGF-β1 in differentiation of Langerhans cells, the contingent of DC in stratified squamous epithelia, was recently identified in collaboration with A.-H. Hovav from Hebrew University, Jerusalem, Israel (Capucha et al., J. Exp. Med., 2018).

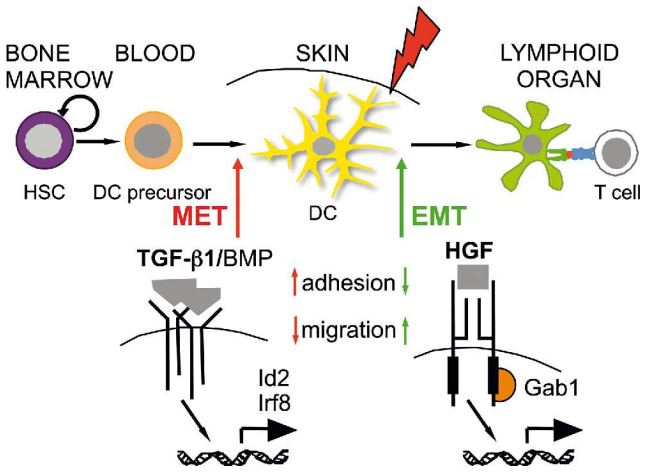

Fig. 6: DC development and migration are differentially regulated by TGF-β/BMP and HGF receptor signaling via MET and EMT programs, respectively.

The role of TGF-β/BMP-target genes Id2 and IRF8 in MET and thus in adhesion and migration regulation are further addressed. Following antigen uptake, DC are activated, leave the peripheral tissue and migrate via lymphatic vessels to lymphoid organs for antigen-specific T cell stimulation. We previously identified HGF receptor signaling in skin DC as essential in this EMT-like process. We now further study the underlining signaling process with a particular focus on the role of the adaptor protein Gab1.

Biotinylated and Near-Infrared Cellulose Nanocrystals for Cellular Labeling and Bioimaging

Cellulose nanocrystals (CNC) are a promising candidate for biomedical applications due to their special surface chemistry, low toxicological risk, negligible inflammatory response and the ability to penetrate cells. Higher tissue penetration, lower biological auto-fluorescence and reduced light scattering have greatly increased the interest of near-infrared fluorescent probes. Known limitations of these probes include dye aggregation, low solubility in water and undesired changes of photophysical properties.

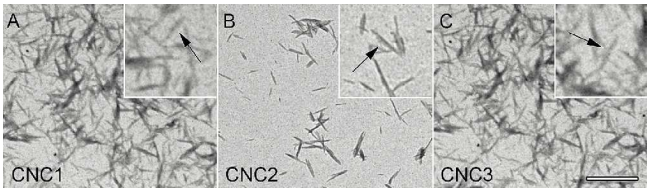

Fig. 7: Representative transmission electron microscopy images of CNC derivatives showing their typical needle-like structure (arrows in insets). CNC1: biotinylated CNC; CNC2: PDI-based NIR-CNC, CNC3: biotinylated and NIR-CNC. Scale bar: 500 nm.

To overcome these limitations, we are working on a joint project with Luiz H.C. Mattoso (LNNA, Embrapa Instrumentation, São Carlos, Brazil) centered on the development of near-infrared cellulose nanocrystals (NIR-CNC). We developed chemically modified CNC derivatives by covalent incorporation of PEGylated biotin, perylenediimide (PDI) based NIR organic dye or a combination of both (Fig. 7) and evaluated their suitability for labeling and imaging of different cell lines.

PDI-labeled CNC showed a superior photostability compared to similar commercially available dyes under long periods of constant and high intensity illumination. All CNC derivatives displayed excellent cytocompatibility towards all cell types. Moreover, CNC were effectively internalized and localized in the cytoplasm around perinuclear areas. Our findings demonstrate the suitability of these new CNC derivatives for labeling, imaging and long-time tracking of a variety of cell types.

Epi-Blood-Count: Quantification of Leukocytes Based on DNA Methylation

White blood cells (leukocytes) are vital components of our immune system and leukocyte numbers are indicative for many diseases. Traditionally, leukocytes are quantified in fresh blood samples based on their size and granularity or on specific proteins on their surface. We developed an innovative Epi-Blood-Count approach to quantify leukocytes in fresh as well as in frozen blood samples based on DNA methylation.

DNA methylation is a chemical modification of cytosine residues in the DNA molecule by which cells regulate their gene expression. Each cell type has a specific gene expression and thus specific methylation patterns in the DNA. For each major leukocyte type, we identified a single cytosine residue in the DNA whose methylation pattern discriminates it from all other leukocyte types. By measuring the DNA methylation level in blood at these cytosines (e.g. via pyrosequencing or digital droplet PCR) we can determine the number of leukocytes in any given sample. We have shown to perform relative (%) and absolute (cells/µl) cell counting for major leukocyte types, such as granulocytes, CD4+ T cells, CD8+ T cells, NK cells, B cells and monocytes (Frobel et al., Clin. Chemistry, 2018).

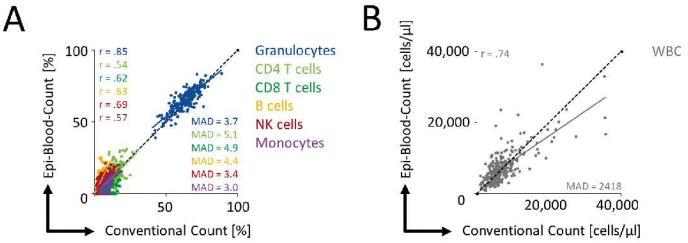

Fig. 8: Epi-Blood-Count for leukocyte quantification. (A) Epigenetic prediction for 6 blood cell types in comparison to conventional cell counts. (B) Epi-Blood-Counts for absolute cell numbers in comparison to conventional white blood cell (WBC) counts. Correlation coefficient r and mean absolute deviation (MAD) are given for each cell type.

By the end of 2019, we have collected and analyzed more than 900 blood samples from healthy donors and patients in collaboration with the Department of Hematology, Oncology, Hemostaseology and Stem Cell Transplantation and the Institute of Immunology, both at RWTH Aachen University Hospital. The correlation of relative Epi-Blood-Counts to conventional leukocyte counts is good as indicated by the coefficient of determination r and mean absolute deviation (MAD) (Fig. 8A). We can determine total leukocyte numbers by addition of a reference DNA molecule to each blood sample. The reference contains a human DNA sequence with an unmethylated cytosine residue whose counterpart in leukocytes is fully methylated. Consequently, the analysis of the DNA methylation level at this reference cytosine in blood (e.g. via pyrosequencing or digital droplet

PCR) provides the total leukocyte number for any given blood sample (Fig. 8B). In the future, we aim for further validation of the Epi-Blood-Count with pediatric and capillary blood samples and its application as *in vitro* diagnostic device.

Spatial Organization of Pluripotency Markers in Colonies and Aggregates of iPS cells

During development of tissues the stem cells progressively differentiate into multiple cell types. This process is tightly controlled by the preserved sequences and patterns of the differentiating tissues. The patterning is influenced by chemical gradients of morphogens and by mechanical stimuli. We are using iPS cells to model and study this phenomenon. We have shown that the shape of iPS cell colonies impact on the gradual expression of pluripotency factors (Abagnale et al., Stem Cell Reports, 2017).

The iPS cells are cultured on defined areas - using microcontact printing of adhesion proteins - and then the distribution of pluripotency factors (read-outs for pluripotency/differentiation pathways) is measured (Fig. 9). Also, 3D aggregates of iPS cells are used to study how they will be

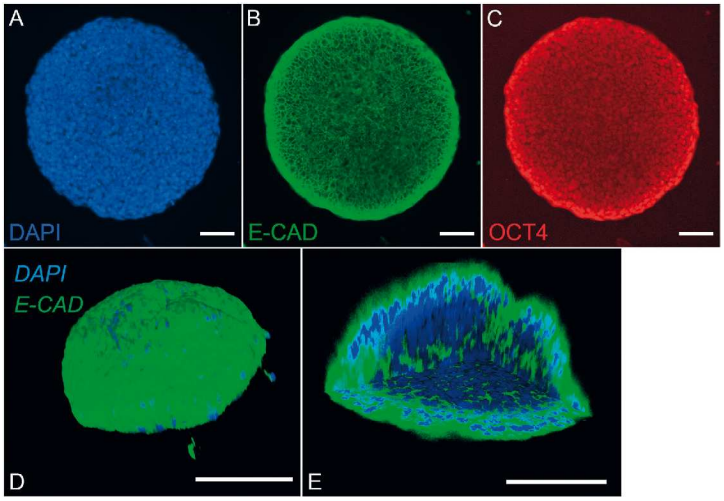

Fig. 9: (A-C) iPSC colony with circular shape showing high expression of pluripotency factors (OCT4/E-CAD) on rim region compared to its center. (D-E) 3D aggregate of iPSCs showing the same overexpression on the aggregate periphery.

patterned in 3D under similar conditions. The expression pattern of the pluripotency factors is closely related to the size of colony. Smaller colonies exhibited no patterning while the larger colonies showed constant width of the over-expression ring. We are currently evaluating the relationship between mechanical-related factor on the patterning process.

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Team

Lab retreat in Ghent, Belgium