Gene Function in Cell Growth, Differentiation & Development

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Introduction

Our research focuses on elucidating genetic and epigenetic regulation of stem cells, including hematopoietic stem cells (HSC), mesenchymal stem cells (MSC) and embryonic stem cells (ES cells). A particular focus is on the molecular mechanisms driving the differentiation of HSC into specific lineages, such as antigen presenting dendritic cells (DC).

A further emphasis is on the engineering of stem cells from somatic cells, including the generation of induced pluripotent stem cells (iPS cells). Given the enormous potential of engineered stem cells and their differentiated progeny, our studies are expected to provide the basis for novel biomedical applications and cell-based therapies. Further projects concentrate on (i) recapitulating conditions of the stem cell niche with biomaterials and specific factors and (ii) tracking of stem cells and immune cells in vivo with magnetic nanoparticles. This involves also studies on the cell-to-cell communication and cell migration.

Pluripotent stem cells are immortal and escape normal processes of aging (Koch et al., 2013). Thus, we study the underlying mechanisms of cellular ageing and how cellular aging can be measured molecularly.

Our studies build on a strong expertise in bioinformatics and computational biology. Therefore methods are developed and applied for genetic and epigenetic analysis of cell differentiation and disease, including predictions and associations.

Induced Pluripotent Stem Cells

iPS cells are engineered stem cells with properties very similar to ES cells. The molecular characteristics of iPS cells and their generation from somatic cells are now being increasingly better understood. We found that knockdown of the polycomb group protein Ezh2 severely impairs iPS cell generation (Fig. 2). Ezh2 is a protein involved in chromatin remodelling and our findings emphasize the impact of chromatin architecture on reprogramming and iPS cell generation (Ding et al., 2013).

Dendritic Cell Development Requires Histone Deacetylase Activity

DC are the most potent antigen-presenting cells of our immune system. They develop from multipotent hematopoietic progenitors (MPP) in two steps of cell fate decision (Fig. 4A). In step 1, MPP progress into common dendritic cell progenitors (CDP). In step 2, CDP further differentiate into classical DC.
(cDC) and plasmacytoid DC (pDC). Cell fate decisions are mediated by epigenetic changes, such as histone acetylation. The level of histone acetylation is regulated by histone acetyltransferases (HAT) and histone deacetylases (HDAC), which add and remove acetyl groups, respectively (Fig. 4B).

Fig. 4: (A) Development of MPP into DC. Step 1: DC commitment. Step 2: DC differentiation. (B) Level of histone acetylation is determined by the balanced activity of HAT and HDAC.

We studied the impact of histone acetylation on DC development by employing the HDAC inhibitor trichostatin A (TSA). We observed that DC commitment of MPP into CDP (step 1) was attenuated by HDAC inhibition (Fig. 5A). Furthermore, we found that differentiation of CDP into DC was restrained (step 2) and that pDC development was specifically blocked (Fig. 5B). Thus, our work demonstrates that chromatin modifiers, such as HDAC, are required for efficient DC development (Chauvistré et al., 2013).

Fig. 5: DC development requires HDAC activity (Chauvistré et al., 2013). (A) MPP/CDP cultures are treated with TSA and analyzed by flow cytometry. CDP are progressively lost with increasing concentrations TSA. (B) HDAC inhibition during DC differentiation results in reduced numbers of CD11c+ DC and lack of pDC.

Magnetic Nanoparticle-labelling of Cells and Tracking by MRI

Cellular therapies using stem cells and immune cells, such as DC, are increasingly applied in clinical trials. Accurate delivery of cells to target organs is crucial for the success of such cell-based therapies. Engineered magnetic nanoparticles (MNP) are currently emerging as promising tools in numerous medical applications, including the development as contrast agents for magnetic resonance imaging (MRI). We demonstrate tailoring of MNP properties using layer-by-layer assembly of polyelectrolytes (PE). Such PE-coated MNPs proved to be effective cell-labelling agents for DC (Schwarz et al., Nanomedicine, 2012; in collaboration with W. Richtering, Institute of Physical Chemistry and J. E. Wong, Chemical Process Engineering (AVT.CVT), Faculty of Mechanical Engineering, RWTH Aachen University, Aachen, Germany; U. Himmelreich and M. Hodenius, Biomedical NMR Unit/MoSAIC, Faculty of Biomedical Sciences and Laboratory of BioNanoColloids, Interdisciplinary Research Centre, Katholic University Leuven, Belgium; M. Hoehn, In vivo NMR Research Group, MPI for Neurological Research, Cologne, Germany). Currently, we investigate PE-coated and lipid-shell MNPs for labelling of HSC derived from mouse bone marrow or human umbilical cord blood (Hodenius et al., Nanotechnology, 2012).

Fig. 6: (A) Cell labelling with synthetic and biogenic MNP for cell tracking by MRI. (B) Electron micrographs of lipid-shell MNP, magnetosomes and polyelectrolyte MNP (PEI-MNP) after uptake into HSC (left). T2*-weighted 3D gradient echo MRI of MNP-labelled HSC in agarose phantoms to determine cell detection limits (right).
Epigenetic Tracks of Aging

Aging affects all tissues of our organism and there is a growing perception that this process is associated with tightly regulated epigenetic modifications. These modifications – such as DNA methylation and histone modifications – influence the DNA conformation without changing the DNA sequence. Age-associated DNA methylation changes are tissue specific and tightly regulated.

We have invented a method to estimate the donor age of blood samples based on DNA methylation at only three specific CpG sites. These genomic regions can be analyzed relatively cost effective by pyrosequencing. Based on the three DNA methylation levels the age is predicted with a mean absolute deviation of about 5 years (Fig. 7). Lifestyle parameters, gender and certain blood diseases affect these predictions – hence, our “Epigenetic-Aging-Signature” seems to reflect the biological age of blood rather than the chronological age (Weidner et al., 2013; patent pending).

Computational Biology of
Cell Differentiation and Gene Regulation

We develop methods for analyzing epigenetic signatures to investigate gene regulation during cell differentiation and disease. We propose a computational framework (Fig. 8) to uncover regulatory gene networks for DC development by combining gene expression data, sequence-based motif matching and data from chromatin immunoprecipitation (ChIP) deep sequencing (ChIP-seq).

Additionally, we develop methodologies to detect cell specific open chromatin regions to reduce the search space for transcription factor binding sites (Gusmao et al., 2013). This strategy greatly enhances the accuracy of cell specific binding site detection (Fig. 9). These methods are implemented in the Regulatory Genomics Toolbox (http://reg-gen.googlecode.com).

Fig. 7: Epigenetic-Aging-Signature. The donor age of blood samples is estimated by epigenetic analysis of three genomic regions (predicted age). This method provides a simple biomarker to estimate the biological age of blood.

Fig. 8: Strategy of integration of gene expression data, sequence-based motif matching and ChIP-seq data for inference of cell specific regulatory networks.

Fig. 9: Example of histone modification and DNase hypersensitive sequencing to detect open chromatin regions (footprints).

Another line of research is the use of statistical methods and machine learning methods to deduce mechanism of disease from patient specific DNA sequences and gene expression profiles. We have investigated the benefit of distinct proximity indices in the task of clustering patient specific expression profiles (Jaskowiak et al., 2013). Gene Set Variance Analysis allows transforming gene expression data into pathway space, thereby improving downstream analysis of cancer gene expression (Hänzelmann et al., 2013).

In Araujo et al., 2013 we propose a methodology of combining gene networks for improving the association of single nucleotide polymorphisms (SNP) from genome-wide associations. We could detect novel associations in distinct stages of Alzheimer’s disease progression. Moreover, we developed an approach to detect sequencing errors from Illumina technology, which avoids the miscalling SNP in DNA sequencing studies (Allhoff et al., 2013).

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Selected References in 2013


**Patent applications**

Method for determining a human individual's predisposition to contract a malignant disease (Epimutation of DNMT3A/DNMT3B); 2013; EP 13167411.1; Wagner W, Just E, Walenda T, Weidner C, Brümmerendorf TH.
Team


Foto 2: Anette Christ (4th from left) receives first Joint PhD of RWTH Aachen University and Maastricht University.

Foto 3: Professor W. Wagner (right) receives Vision4 Life Sciences Price for Regenerative Medicine together with Professor K. De Greef, Antwerp, Belgium (left); Professor A. Ramon, President of ITERA (center).