The Impact of DNA Methylation on Cellular Differentiation of Immune Cells

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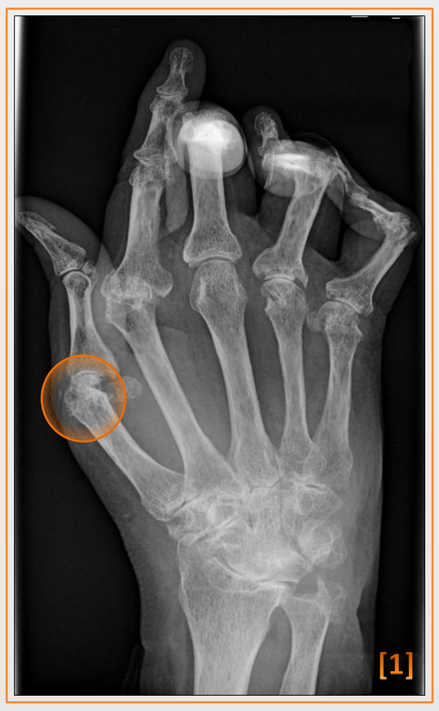
Epigenetic changes in DNA methylation are associated with regulation of gene expression and play an important role in cellular differentiation. Such changes might be of importance in chronic inflammatory diseases where autoreactive immune cells are thought to perpetuate inflammation and organ destruction.

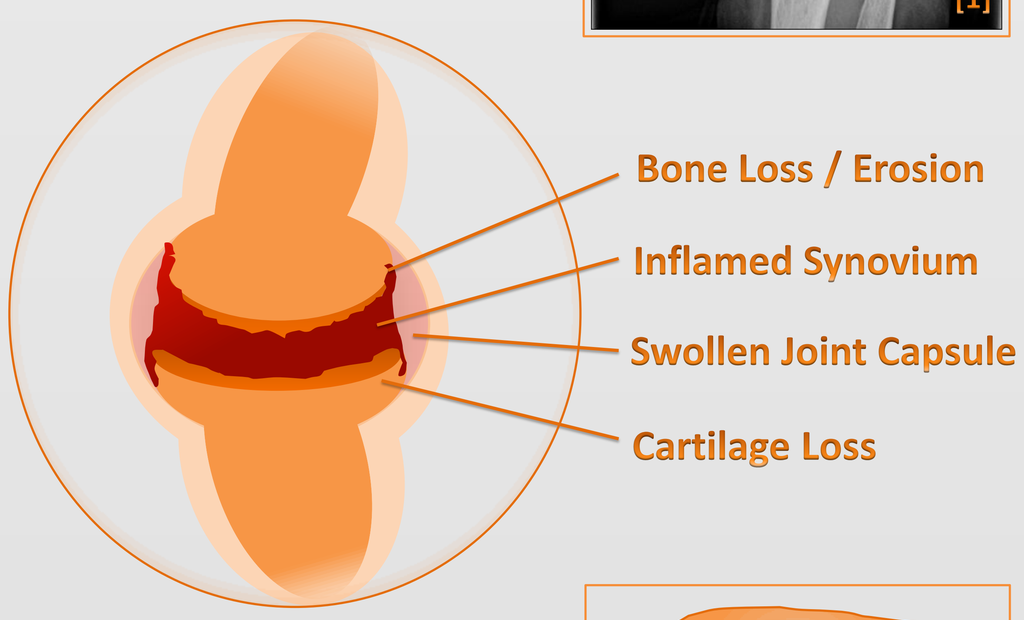


"What are the differences in DNA methylation of immune cells?"

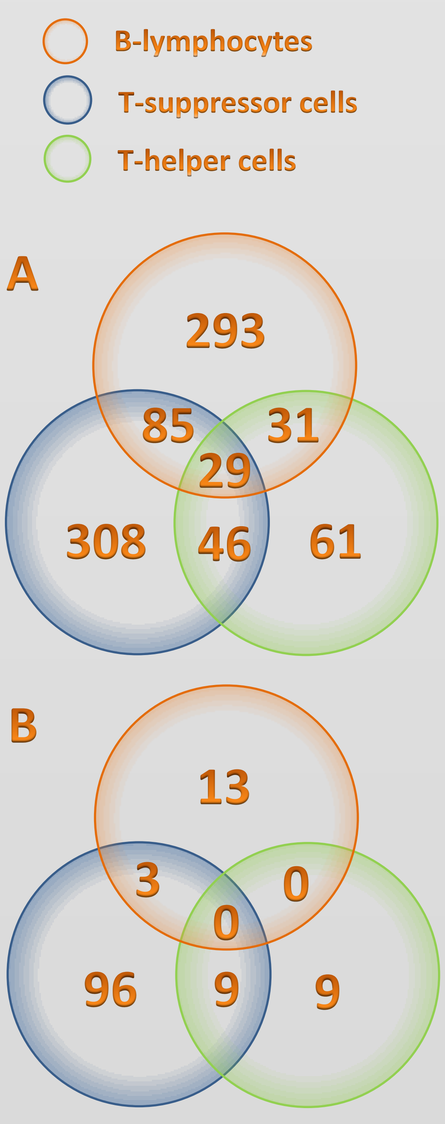
Methylation of cytosins within a CpG dinucleotide is a common epigenetic DNA modification and may arrest cells in a pathogenic state in complex disorders, e.g. cancer[2] or rheumatoid arthritis[3]. CpGs occur mainly in clusters, called **CpG islands** (CPIs), being present in nearly 70 % of the human gene promoter regions[2]. The methylation state of one CpG or a whole CPIs may influence the expression of the corresponding gene due to binding of Methylation-Binding-Domains (MBD) and other methylation dependent proteins. To identify CpGs influencing gene expression and common methylation patterns we used several approaches, e.g. network analysis and machine learning techniques.

"Which immune cells are key players in chronic inflammatory diseases?"

To explore these variations and its regulatory mechanism, we have gathered genome-wide DNA methylation data in human immune cells extracted from peripheral blood, specifically T-helper cells (CD4), T-suppressor cells (CD8), macrophages (CD14), granulocytes (CD15), B-lymphocytes (CD19) and natural killer cells (CD56). Furthermore, we have analysed **methylation patterns** associated to cell differentiation of T- and B-cells and the correlation with gene expression data.



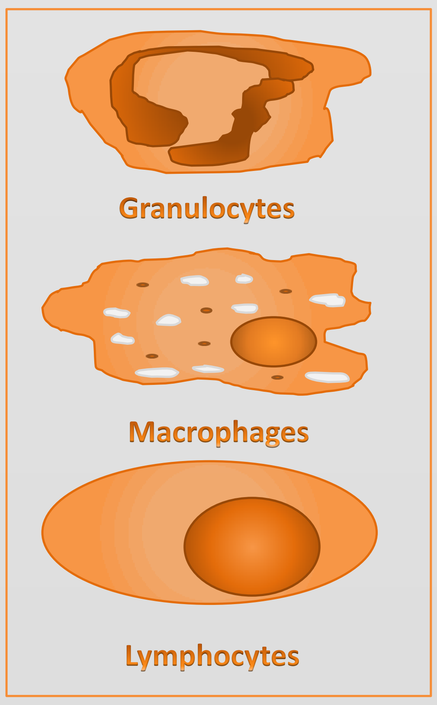
"What is the epigenetic fingerprint of rheumatoid arthritis?"

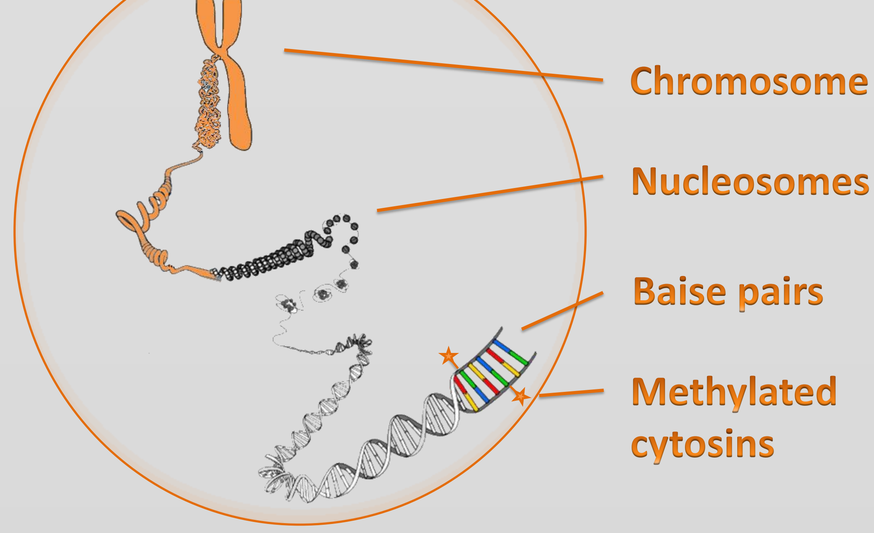


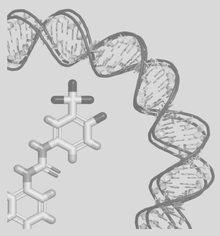
Number of genes which show (**A**) hypo- or (**B**) hyper-methylation in comparison between cells in naïve and memory state (diff. abs. > 0.6, BH-correction).

Illumina HumanMethylation450 BeadChip platform was used to determine the DNA methylation state of cells from four healthy donors after FACS sorting. These arrays provide a genome-wide coverage of more than 485,000 CpG methylation sites per sample at single-nucleotide resolution. Additionally, we were able to identify a substantial number of common CpG methylation sites, which were differentially methylated between naive and memory states of T-(CD4, CD8) and B-cells (CD19). We observed that both, hyper- and hypomethylation of promoter regions of genes involved in cell differentiation. The impact of these alterations on inflammatory diseases will be a subject to further research which will include the analysis of autoreactive immune cells from rheumatoid arthritis patients[4].

Developed tools are integrated into the Workflow management system **Galaxy**[5] to enable reproducible and transparent analyses.







References

[1] Brägelmann B. (http://www.braegelmann.de/bernd) & Steinhoff, M.
[2] Hansen K. D., *et al*. "Increased methylation variation in epigenetic domains across cancer types."; Nat Genet. 2011
[3] Karouzakis E., *et al*. "DNA methylation regulates the expression of CXCL12 in rheumatoid arthritis synovial fibroblasts."; Genes and Immunity 2011
[4] Nakano K., *et al*. "DNA methylome signature in rheumatoid arthritis."; Ann Rheum Dis. 2012
[5] Goecks, J., *et al*. "Galaxy: a comprehensive approach for supporting accessible, reproducible, and transparent computational research in the life sciences."; Genome Biol. 2010

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