Combined analysis of epigenetic and transcriptional profiles in different immune cells identifies hot spots of gene regulation by DNA methylation

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Background:

Methylation of DNA may contribute to the regulation of gene expression. Chip technology enables to analyse for methylation of CpG sites but requires a pre-selection of potential hot spots. Such a selection of potential sites is represented on the HumanMethylation450 array (Illumina). However, it is still necessary to validate, experimentally, which CpG selection is functionally relevant.

Objective:

In order to test these CpG sites for possible functional effects, we assumed that cell type specific gene expression in different immune cells like T-cells and monocytes are influenced and maintained by DNA methylation.

Methods:

Cells from 4 healthy donors were sorted by FACS technology for naive and memory T-cells (CD4m, CD4n, CD8m, CD8n), B-cells (CD19m, CD19n), NK-cells (CD56), monocytes (CD14), and granulocytes (CD15). Genome-wide DNA methylation was assessed using the Illumina HumanMethylation450 BeadChip platform. Analysis of data was performed using Genome-Studio (Illumina). Gene expression data were collected from Affymetrix HG-U133Plus 2.0 transcriptomes analysed in the BioRetis database. Mapping of CpG sites with genes was performed using the ensemble genome assembly GRCh37 genomic location map.

Results:

The number of differentially expressed genes or methylated CpG sites were highest between very different cell types like CD14 monocytes and CD4 T-cells (4624 genes; 19261 sites) and lower between naive and memory cells of the same lymphocyte subtype (CD4: 638 genes; 9412 sites). There was a tendency towards more methylation in memory (CD4m: 5433 sites = 2694 genes) compared to naive cells (CD4n: 3979 sites = 2258 genes) for more than 2-fold change while the overall change was dominated by a decrease from naive to memory status. Overlap of differential expression with corresponding changes in methylation was found in only 629 (279) of 1951 increased (2673 decreased) expressed genes for CD14 versus CD4 comparison and 57 (53) of 332 (306) genes for CD4m versus CD4n cells. Of all CpG sites annotated to these identified genes, only about 10% were concordant with expression. These CpG site were within or immediately upstream of the annotated start of the gene with a maximum distance of ≈1500 nucleotides, indicating that overlap with the promoter site is most likely. A common sequence motif around these CpG sites was not immediately detectable but requires more detailed analysis.

Conclusion:

Microarray based comparative analysis of transcriptional and epigenetic differences suggests a detailed picture of methylation associated gene regulation and enables to generate an epigenetic map of relevant CpG site for genes expressed and regulated in immune cell types. As many of the microarray based suspected CpG sites of a defined gene did not match with differential gene expression, epigenetic profiling with microarrays has to be interpreted carefully.