Beyond Gene Sequencing: Profiling DNA Methylation Patterns in Human Aortic and Mitral Valves

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Background: Valve Interstitial cells play a major role in physiological and pathological conditions including rheumatic heart disease. These cells are tightly regulated by a variety of mechanisms including genetic and epigenetic factors, such DNA methylation. Methylation fingerprints of human valves have not been reported to date.

Methods: Pieces of tissue from 7 paired normal aortic and mitral valves (6 males, 1 female, mean age= 59.4 yrs, age range 42-84 yrs) were de-endothelialised using collagenase, washed and frozen immediately. Tissue lysates were separately subjected to DNA isolation, enzyme digestion, bisulphite conversion, bioanalyzer QC, KAPA library quantification, multiplex Next generation sequencing on an Illumina HiSeq2500. Quality control on the Illumina raw reads was done using FASTQC version 0.10.1. After trimming via Trim Galore version 0.3.7, the trimmed reads are mapped to UCSC *Homo Sapiens* (human genome sequence, version hg19) using Bismark, a methylation aware mapper. An enrichment analysis using Panther version 11 was performed on genes with differentially methylated promoters to find GO terms that are overrepresented in categories such as: molecular function, biological process, cellular component, protein class and pathways.

Results:

Different Methylation patterns were encountered in aortic and mitral valves involving several relevant gene clusters that play major roles in processes that affect essential valve functions. These genes include ECM genes such as class L phosphatidylinositol glycan anchor biosynthesis gene (PIGL) and zona pellucida glycoprotein 1 (ZP1). Such genes encode for proteins that are essential for valve movement, protein secretion and heart morphogenesis. Also, matrix metalloproteinases (MMPs), that are implicated in longevity of heart valves, such

as pitrilysin metallopeptidase 1 (PITRM1) and ADAM metallopeptidase domain 6 (ADAM) were found to be differentially methylated. Growth factors such as growth factor independent 1B transcription repressor (GFI1B), latent transforming growth factor beta binding protein 4 (LTBP4) and growth factor receptor-bound protein 7 (GRB7) exhibited also differential methylation patterns. Furthermore, the cardiac specific transcription factor (TCF21), required for the formation of cardiac fibroblasts, was found to be differentially methylated. Finally, Wnt5B, one of the Wnt family members, was found to be hypermethylated. The Wnt signaling pathway plays a crucial role in cardiac development in general and is also involved in cardiac injury response.

Conclusion: The cells of the aortic and mitral valve have different methylation signatures which requires extensive further investigations and might result in further understanding of the pattern in progression of disease in Rheumatic valve affection.