DNA methylation signature of Factor VIII secreting as well as other endothelial cells in comparison to blood

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Introduction

Endothelial cells are present in all organs with organ-specific characteristics as well as common characteristics between each other.[1] For example liver sinusoidal endothelial cells (HHSEC) are distinguished as a major site of F8 secretion as compared to other endothelial cells.[2,3]

Materials and Methods

To unravel this property of HHSECs to secrete F8 we compared whole human genome methylation (Illumina 450k Infinium Methylation) of blood samples with each of 5 endothelial cells “Human Pulmonary Artery Endothelial Cells (HPAEC)”, “Human Pulmonary Microvascular Endothelial Cells (HPMEC)”, “Human Cardiac Microvascular Endothelial Cells (HCMEC)”, “Human Umbilical Vein Endothelial Cells (HUVECs)”, HHSEC, and Hepatocytes (HH). We compared methylation status in promoter region for every endothelial cells and hepatocytes to blood to identify specific differentially methylated CpG sites and the potential transcription factors binding to these specific CpG sites.

Results and Discussion

Methylation in promoter region of blood showed significant differences as compared to endothelial cells or hepatocytes. High numbers of significant CpG sites (HHSEC=5495, HPAEC=4653, HPMEC=6277, HCMEC=5571, HUVEC=3704 and HH=7070) were found at FDR of 5% with methylation differences greater than 10% [Figure 1]. Pathway analysis showed that the hypomethylated region in Blood are representing function of different subtype of blood cells [Figure 2].

Most of the CpG sites were hypermethylated in endothelial cells when compared to blood [Figure 2]. We found 5 transcription factors predicted to be specific for HHSEC and Hepatocytes in comparison to blood. Moreover, these transcription factors found to be targeting 6 CpG sites which were commonly differentially methylated for both HHSEC and Hepatocytes in comparison to blood [Figure 3].

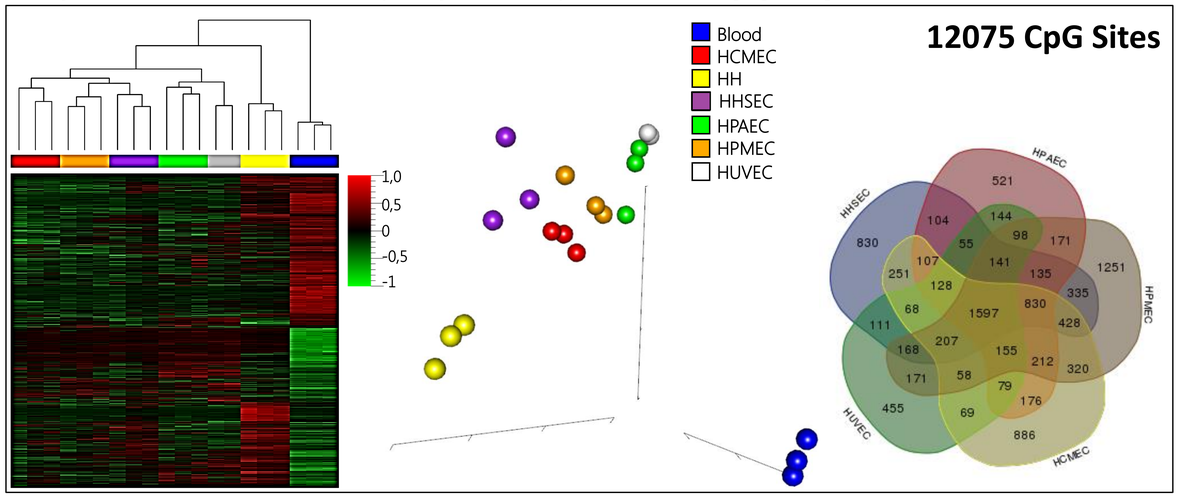

Figure 1: Heatmap, PCA and Venn Diagram of combined significant CpG sites from different endothelial cells and hepatocytes in comparison to Blood at FDR of 5%

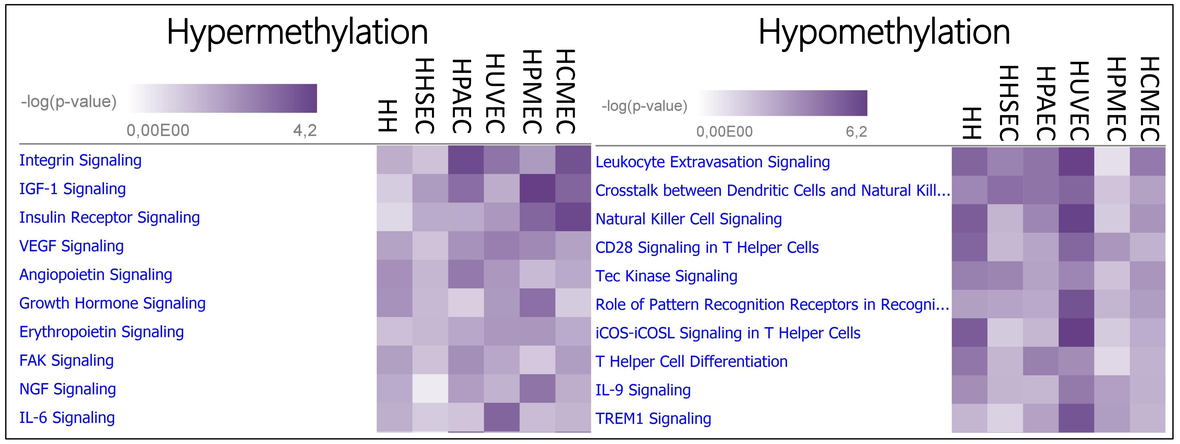

Figure 2: Significant pathways of hypermethylated genes and hypomethylated genes in blood against hepatocytes and endothelial cells

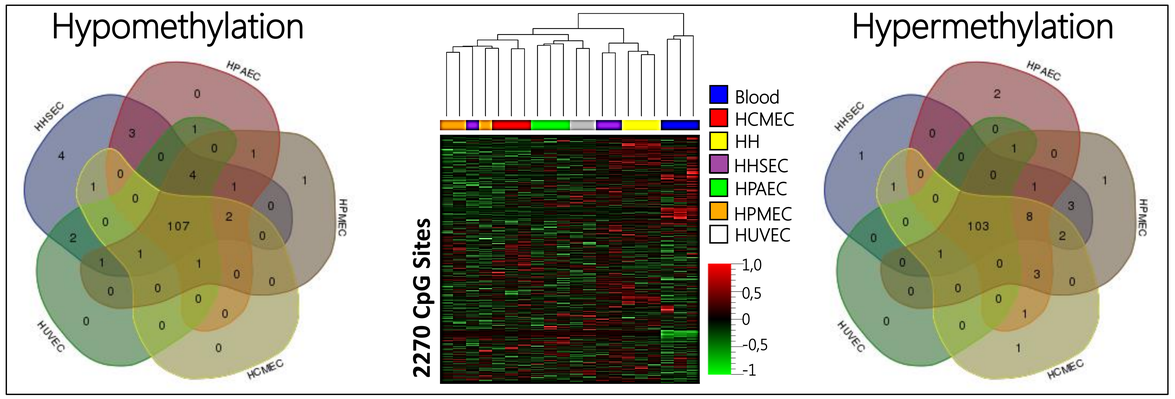

Figure 3: Venn diagrams of predicted transcription factors binding to hypomethyled and hypermethylated CpG sites in blood against hepatocytes and endothelial cells; and Heatmap of CpG sites in promoter region of the predicted transcription factors

Conclusion

The epigenetic/methylation data of HHSEC and hepatocytes suggest that endothelial cells share similar methylation marks like the hosting organ.

References

[1] *Nolan, Daniel J., et al. "Molecular signatures of tissue-specific microvascular endothelial cell heterogeneity in organ maintenance and regeneration."Developmental cell 26.2 (2013): 204-219.*
[2] Shahani, T., et al. *"Human liver sinusoidal endothelial cells but not hepatocytes contain factor VIII."* Journal of Thrombosis and Haemostasis 12.1 (2014): 36-42.
[3] Shahani, Tina, et al. "*Activation of human endothelial cells from specific vascular beds induces the release of a FVIII storage pool.*" *Blood* 115.23 (2010): 4902-4909.