RNA-Seq to identify putative mechanistic differences and similarities between HUVECs and HDLECs.

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Human umbilical vein endothelial cells (HUVECs) and Human dermal lymphatic endothelial cells (HDLECs) are commonly used *in vitro* models of the blood and lymphatic endothelium. These are routinely applied in the study of endothelial cell (EC) behaviour in conditions such as angiogenesis, lymphangiogenesis and inflammation. While both blood endothelial cells (BECs) and lymphatic endothelial cells (LECs) are key components of the vascular system and derive from a shared origin, each exist in a different microenvironment and present differing phenotypes. Understanding the transcriptomic profiles of HUVECs and HDLECs may offer opportunities to uncover novel biological insight into the processes of the blood and lymphatic endothelium and apply to vascular diseases including tumour metastasis.

To this end, bulk RNA-seq analysis was employed. RNA was extracted from HUVECs and HDLECs (n=3) and samples were sequenced by Novogene with concomitant preliminary bioinformatics to achieve quantified read counts. Subsequent bioinformatics analyses were carried out based on the workflow described in [1] including sample quality control, count normalisation, differential expression and functional enrichment with the DESeq2 R package and Webgestalt webserver. RNA-seq thresholds of fold change > 2 and Benjamini-Hochberg adjusted p-value of < 0.05 were used to identify differentially expressed genes (DEGs). Functional terms and pathways were deemed significant with false discovery rate (FDR) < 0.05.

3307 DEGs were identified, with 1397 and 1910 found to be enriched in HUVECs and HDLECs respectively. To validate the results, known markers for BECs and LECs were identified as enriched. Functional enrichment analyses recognised shared and exclusive functional terms and pathways. Shared terms included cell adhesion, cytokine, leukocyte, MAPK, tube and vascular development related processes. HUVEC enriched terms included Wnt signalling, CD4 and T-cell related. HDLEC enriched terms included immune response, calcium, chemokine production, endothelial and epithelial cell, interferon and interleukin related. Further potential markers and putative drivers of phenotype were identified based on the DE results. The top 5 HUVEC enriched genes ranked by log2FC were XIST, NOVA1, CTC-546K23.1, SLITRK4 and GSTM1. The top 5 HDLEC enriched genes were PDPN, TRCP6, RBM20, ANKRD36BP2 and RAMP3. These results may give further insight into the specific mechanisms of BECs and LECs and shed further light on potential therapeutic targets for blood and lymphatic vascular diseases.