TRANSCRIPT PROFILING OF THE ENDOTHELIAL CELL'S TEMPORAL RESPONSE TO HYPOXIA

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ABSTRACT

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The human endothelium is a highly involved organ system that responds in a systematic manner to fluctuations of oxygen levels in the body. The physiological responses of coagulation, vascular tone, inflammation and angiogenesis are all regulated in part by the endothelium. Reduced oxygen tension, hypoxia, can initiate these physiological responses and potentially induce a response that is ultimately pathological. Myocardial ischemia, pulmonary hypertension and tumor metastasis are all exacerbated by hypoxia. Therefore, an understanding of the molecular events governing the pathophysiological responses of the endothelium to hypoxia will aid in the identification of novel therapeutic targets. The goals of this study were to develop a high-resolution transcriptome map of the endothelium's temporal response to hypoxia and expand current knowledge of the mechanism of action of hypoxia. Human umbilical vein endothelial cells (HUVECs) were exposed to a reduced oxygen environment of <40mmHg for 2, 3.5, 8, 14, and 26 hours in triplicate cultures and transcript profiling performed using Affymetrix HGU95av2 oligonucleotide microarrays. Statistical analysis revealed the transcriptional regulation of an increasing number of genes in a time dependent manner. Quantitative RT-PCR was used to validate several genes whose regulation by hypoxia in endothelial cells was deemed novel: cyclooxygenase-1 (COX-1), human protein tyrosine phosphatase receptor β (HPTPR-β) and angiotensin converting enzyme (ACE). Furthermore, downregulation of ACE protein activity was found to trend in a functional assay. The molecular map

developed from this investigation will provide a tool to enable target identification of hypoxia responsive genes, biomarker development and further the understanding of disease pathologies involving hypoxia.

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