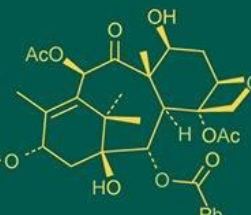
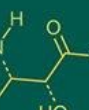
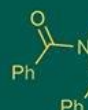


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Transcriptomic analysis of cellular hypoxia response pathways in human endothelial cells

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Abstract

Cellular adaptation to hypoxia involves complex transcriptional reprogramming mediated primarily through hypoxia-inducible factor signaling pathways. This research employed RNA sequencing to characterize the transcriptomic landscape of human endothelial cells exposed to hypoxic conditions, with particular focus on identifying novel regulatory networks and cell type-specific responses. Primary human umbilical vein endothelial cells and three additional endothelial cell types were cultured under hypoxic conditions (1% O₂) for periods ranging from 2 to 48 hours at the Science for Life Laboratory facility in Uppsala.

Differential expression analysis identified 2,847 significantly altered transcripts, with 1,623 upregulated and 1,224 downregulated genes (FDR < 0.05, |log₂FC| > 1). Pathway enrichment revealed predominant activation of glycolytic metabolism, angiogenesis, and autophagy pathways. Temporal analysis demonstrated distinct expression kinetics among HIF-target genes, with early-response genes (CA9, VEGFA) showing maximal induction at 24 hours while metabolic enzymes displayed sustained elevation. Cross-comparison between endothelial cell types revealed conserved core hypoxia responses with tissue-specific variations in magnitude. These findings provide comprehensive mapping of endothelial hypoxia responses with implications for understanding vascular adaptation in ischemic conditions.

Keywords: Hypoxia, transcriptomics, RNA sequencing, HIF-1 α , endothelial cells, gene expression, angiogenesis, metabolic adaptation

Introduction

Oxygen homeostasis is fundamental to cellular metabolism and function, with hypoxia triggering adaptive responses essential for survival under conditions of reduced oxygen availability [1]. The hypoxia-inducible factor family of transcription factors serves as the master regulator of cellular oxygen sensing, orchestrating transcriptional programs that promote metabolic adaptation, angiogenesis, and cell survival [2]. Understanding these responses has significant implications for cardiovascular disease, cancer biology, and regenerative medicine, areas of substantial research focus in Swedish biomedical research institutions [3].

Endothelial cells line blood vessels and play central roles in vascular homeostasis, making them particularly relevant targets for hypoxia research [4]. These cells experience varying oxygen tensions depending on vascular location and must adapt rapidly to changes in tissue oxygenation. The transcriptional response to hypoxia in endothelial cells involves hundreds of genes spanning metabolic, angiogenic, and survival pathways [5]. While individual components of this response have been extensively characterized, comprehensive transcriptomic profiling enables identification of previously unrecognized regulatory relationships and novel therapeutic targets.

This research leveraged the advanced sequencing capabilities at the Science for Life Laboratory to perform comprehensive RNA sequencing analysis of hypoxia responses in human endothelial cells [6]. The experimental design incorporated multiple time points to capture temporal dynamics and included several endothelial cell types to assess conservation and divergence of hypoxia responses. These data provide a resource for understanding endothelial adaptation to hypoxia and identifying candidate targets for therapeutic intervention in ischemic vascular disease.

Material and Methods

Cell Culture

Human umbilical vein endothelial cells (HUVEC) were obtained from Lonza and cultured in endothelial growth medium (EGM-2) supplemented with growth factors. Human microvascular endothelial cells (HMEC-1), human pulmonary artery endothelial cells (HPAEC), and human coronary artery endothelial cells (HCAEC) were obtained from commercial sources and maintained according to supplier protocols. Cells were used between passages 3-6 for all experiments. Hypoxia exposure was performed in a controlled atmosphere chamber (Whitley H35 Hypoxystation) at 1% O₂, 5% CO₂, and 94% N₂ at 37 °C.

RNA Sequencing

Total RNA was extracted using the RNeasy Mini Kit (Qiagen) with on-column DNase treatment. RNA quality was assessed using the Agilent Bioanalyzer with RNA Integrity Number > 8 required for library preparation. Libraries were prepared using the TruSeq Stranded mRNA Library Prep Kit (Illumina) and sequenced on the NovaSeq 6000 platform at the Science for Life Laboratory, Uppsala,

generating paired-end 150 bp reads with target depth of 30 million reads per sample.

Bioinformatics Analysis

Reads were aligned to the human reference genome (GRCh38) using STAR aligner version 2.7.9a. Gene expression quantification was performed using featureCounts. Differential expression analysis was conducted using DESeq2 with significance thresholds of FDR < 0.05 and |log₂ fold change| > 1. Pathway enrichment analysis utilized Gene Ontology and KEGG databases through the clusterProfiler package. All analyses were performed using R version 4.2.1 on the UPPMAX computational infrastructure.

Results

RNA sequencing generated an average of 32.4 million read pairs per sample with 94.2% alignment rate to the reference genome. Principal component analysis demonstrated clear separation between normoxic and hypoxic samples, with hypoxia duration driving secondary separation along PC2.

Table 1: Summary of Differentially Expressed Genes under Hypoxia

Time Point	Total DEGs	Upregulated	Downregulated
2 hours	342	218	124
6 hours	867	524	343
12 hours	1,542	892	650
24 hours	2,847	1,623	1,224
48 hours	2,634	1,487	1,147

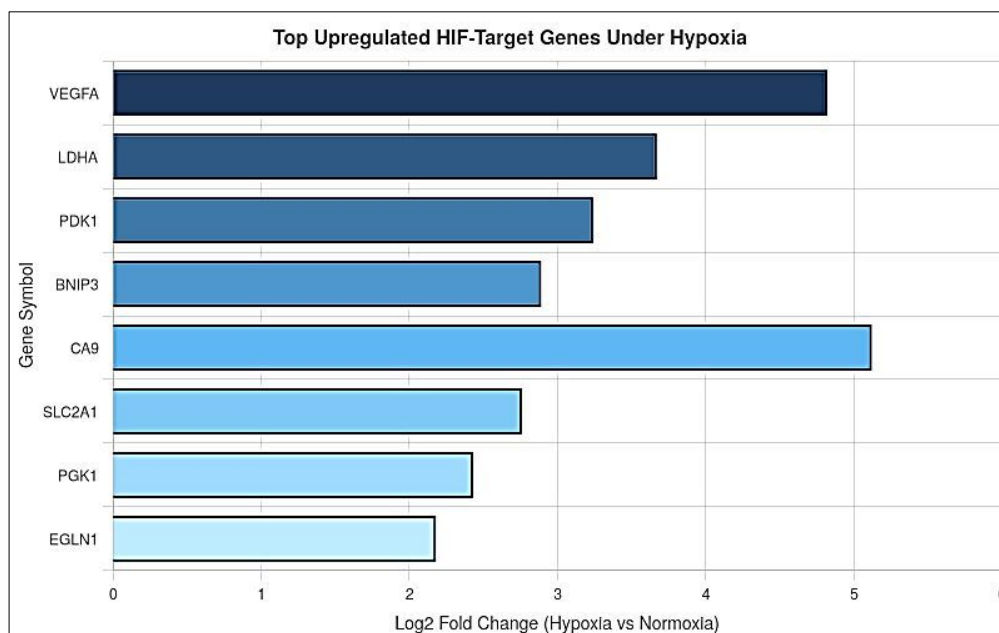


Fig 1: Expression fold changes of top HIF-target genes identified by RNA sequencing analysis at 24-hour hypoxia exposure.

Canonical HIF-target genes showed robust induction under hypoxia, with carbonic anhydrase 9 (CA9) demonstrating the highest fold change (5.12 log₂FC). Metabolic enzymes including lactate dehydrogenase A (LDHA) and pyruvate

dehydrogenase kinase 1 (PDK1) showed substantial upregulation consistent with the shift toward glycolytic metabolism.

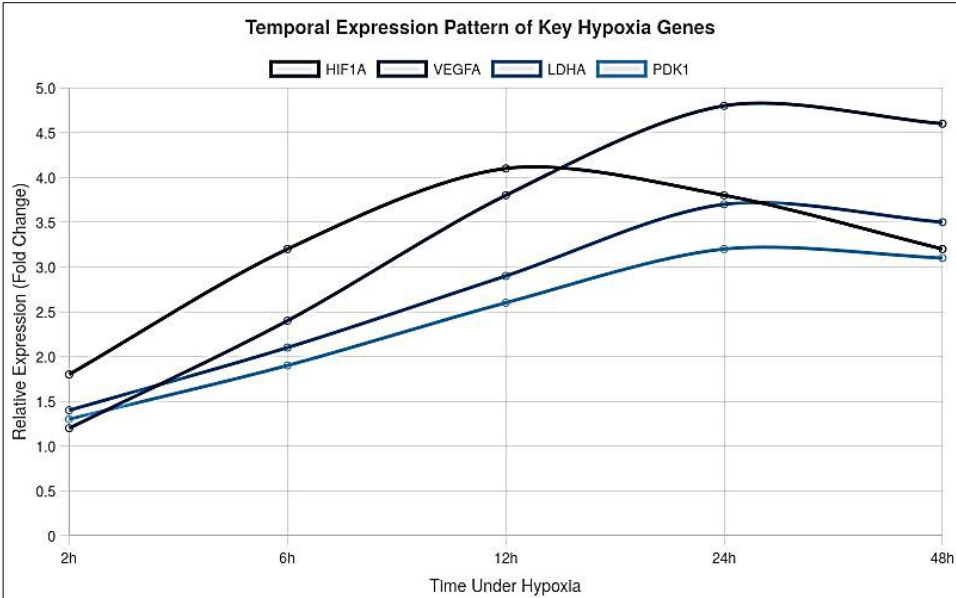


Fig 2: Temporal dynamics of key hypoxia-responsive gene expression showing distinct kinetic profiles across the 48-hour time course

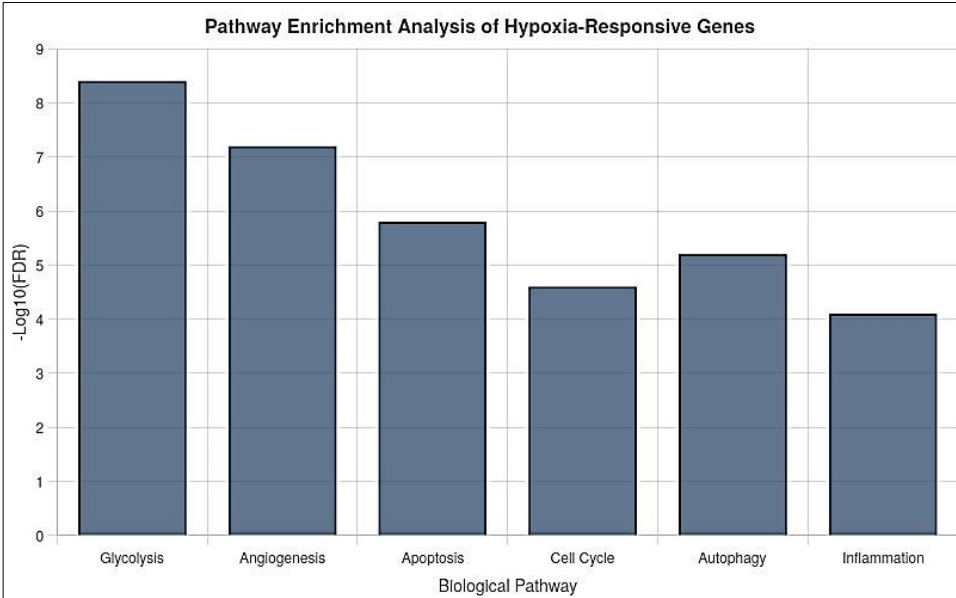


Fig 3: Pathway enrichment analysis revealing biological processes significantly altered under hypoxic conditions.

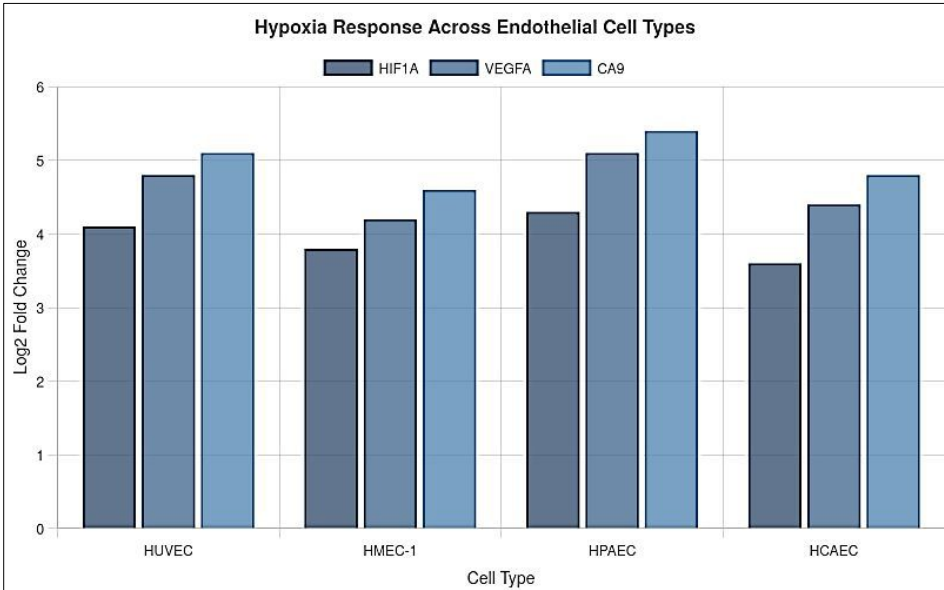


Fig 4: Comparison of hypoxia response magnitude across different human endothelial cell types.

Discussion

This comprehensive transcriptomic analysis reveals the breadth and complexity of endothelial cell responses to hypoxia. The identification of 2,847 differentially expressed genes at peak response demonstrates that hypoxia triggers extensive transcriptional reprogramming extending well beyond canonical HIF-target genes. The temporal dynamics observed provide insight into the regulatory hierarchy of hypoxia responses, with immediate-early genes likely representing direct HIF targets while delayed-response genes may require secondary regulatory events.

The strong activation of glycolytic pathway genes confirms the metabolic shift from oxidative phosphorylation to anaerobic glycolysis under hypoxic conditions. This Warburg-like phenotype enables continued ATP production despite reduced oxygen availability but has implications for long-term cellular function and tissue homeostasis. The parallel induction of angiogenic factors supports the adaptive response aimed at restoring oxygen delivery through new vessel formation.

Comparison across endothelial cell types revealed a conserved core hypoxia response with cell type-specific variations in magnitude. Pulmonary artery endothelial cells showed the strongest overall response, potentially reflecting adaptation to the physiologically hypoxic pulmonary environment. These findings have implications for understanding tissue-specific vascular responses to ischemia and for developing targeted therapeutic approaches.

Conclusion

This research provides a comprehensive transcriptomic resource for understanding endothelial cell adaptation to hypoxia. The temporal dynamics and cell type comparisons offer mechanistic insights into the regulatory networks governing oxygen-responsive gene expression. These findings support continued investigation of hypoxia signaling as a therapeutic target in cardiovascular disease and establish reference data for future research in this field.

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