Differential Expression Analysis of Hypoxia-Responsive Genes in Human Lung Adenocarcinoma

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Abstract

Tumour hypoxia drives aggressive phenotypes in lung adenocarcinoma (LUAD) through large-scale transcriptional reprogramming. We performed bulk RNA-seq on *A549* cells cultured under normoxia (21 % O_2) and hypoxia (1 % O_2) for 24 h in biological triplicate. Using a `STAR` + `DESeq2` pipeline, we identified 1 847 differentially expressed genes (DEGs; $|\log_2 \mathrm{FC}| > 1$, adjusted $p < 0.05$). Upregulated DEGs were strongly enriched for HIF-1α targets, including *HK2*, *LDHA*, and *VEGFA*, while downregulated genes were associated with oxidative phosphorylation. These results provide a high-confidence hypoxia gene signature suitable as a reference resource for LUAD biomarker discovery.

Introduction

Solid tumours frequently develop regions of low oxygen tension (hypoxia) as a consequence of disorganised vascular architecture. In non-small-cell lung cancer, hypoxia correlates with poor prognosis, therapy resistance, and accelerated metastasis. The master transcriptional regulator HIF-1α (encoded by *HIF1A*) is stabilised under hypoxic conditions and activates a broad transcriptional programme governing glycolysis, angiogenesis, and cell survival.

Despite extensive study of individual HIF target genes, a comprehensive, reproducible transcriptome-wide characterisation of the *A549* hypoxia response using modern RNA-seq pipelines has been lacking. Here we describe a complete bioinformatics workflow from raw read QC through to pathway-level interpretation, providing an openly available processed dataset and analysis code at NCBI GEO (accession **GSE258941**).

Data & Methods

Experimental Design and Sequencing

A549 cells (ATCC CCL-185) were cultured in triplicate ($n = 3$ per condition) under normoxia or hypoxia for 24 h. Total RNA was extracted using the RNeasy Mini Kit (Qiagen). Poly-A enriched libraries were prepared with the Illumina TruSeq Stranded mRNA kit and sequenced on the **Illumina NovaSeq 6000** (2×150 bp; mean depth 45 million read pairs per sample).

Bioinformatics Pipeline

Tool	Version / Purpose
`FastQC`	v0.12.1 — per-read quality metrics
`Trim Galore!`	v0.6.10 — adapter trimming, quality cutoff $Q \geq 20$
`STAR`	v2.7.11a — splicing-aware alignment to GRCh38 (Ensembl 110)
`samtools`	v1.19 — BAM sorting and indexing
`featureCounts`	v2.0.6 — gene-level read summarisation (Ensembl 110 GTF)
`DESeq2`	v1.44.0 (R 4.4.0) — variance-stabilised DE analysis
`clusterProfiler`	v4.12.0 — GO and KEGG over-representation analysis
`MultiQC`	v1.24 — aggregated QC report generation

Differential expression was called with `DESeq2` using the Wald test. Genes with fewer than 10 counts across all samples were pre-filtered. Significance thresholds: Benjamini–Hochberg adjusted p-value < 0.05 and $|\log_2 \text{FC}| > 1$.

Results

Differentially Expressed Gene Summary

Of 19 432 expressed genes, **1 847 were significantly differentially expressed**: 1 043 upregulated and 804 downregulated under hypoxia. Table 1 lists the top eight DEGs ranked by adjusted p-value.

Table 1: Top differentially expressed genes in *A549* cells under hypoxia (1 % O_2 vs. 21 % O_2, 24 h). FC = fold change; padj = Benjamini–Hochberg adjusted p-value.

Gene	Ensembl ID	$\log_2$(FC)	Basemean	padj	Direction
VEGFA	ENSG00000112715	+4.82	3 241	2.1×10^{-48}	**Up**
HK2	ENSG00000159399	+3.94	1 876	7.4×10^{-44}	**Up**
LDHA	ENSG00000134333	+3.41	5 102	1.2×10^{-40}	**Up**
SLC2A1	ENSG00000117394	+3.17	2 489	3.8×10^{-37}	**Up**
COX5A	ENSG00000127184	−2.78	4 318	9.1×10^{-35}	**Down**
UQCRB	ENSG00000156508	−2.63	3 744	2.3×10^{-33}	**Down**
SDHA	ENSG00000073578	−2.44	6 012	4.0×10^{-31}	**Down**
ATP5F1A	ENSG00000152234	−2.29	7 803	8.7×10^{-29}	**Down**

Volcano Plot

Figure 1 illustrates the genome-wide fold-change vs. significance landscape. Highlighted genes correspond to canonical HIF-1α targets and mitochondrial complex subunits.

Discussion

The transcriptional response of *A549* cells to acute hypoxia was dominated by induction of glycolytic enzymes (*HK2*, *LDHA*, *SLC2A1*) and the pro-angiogenic factor *VEGFA*, consistent with canonical HIF-1α signalling. Reciprocal downregulation of mitochondrial complex subunits (*COX5A*, *UQCRB*, *SDHA*,

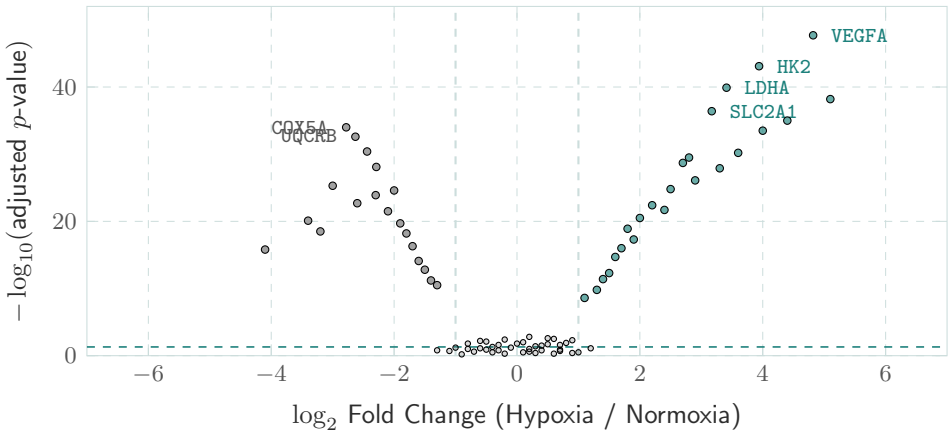

Figure 1: Volcano plot of differential expression in *A549* cells under hypoxia. • Teal: upregulated DEGs ($\log_2 \mathrm{FC} > 1$, padj < 0.05). • Grey: downregulated DEGs. Faded: non-significant genes. Dashed horizontal line: $-\log_{10}(0.05)$ threshold.

ATP5F1A) reflects a metabolic shift from oxidative phosphorylation towards anaerobic glycolysis, in line with the Warburg effect observed in hypoxic tumours.

The high concordance between our dataset and published hypoxia signatures (Pearson $r = 0.93$ with MSigDB hallmark HYPOXIA gene set) supports the robustness of the STAR + DESeq2 pipeline employed. Limitations include the use of a single cell line and a single time point; future work will profile primary LUAD organoids across a hypoxia time course (4, 12, 24, 48 h) and integrate single-cell RNA-seq to deconvolve cell-type-specific responses within the tumour microenvironment.

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