

Poster # 1665: Hypoxia Induces EMT Transition and Promotes Invasive Transcriptional Programs in Lung Adenocarcinoma

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Introduction

Lung adenocarcinoma (LUAD) is the most common subtype of lung cancer and remains a leading cause of cancer-related mortality worldwide due to its strong invasive and metastatic potential.

Tumor hypoxia is a hallmark of the LUAD microenvironment and plays a critical role in promoting tumor progression, therapeutic resistance, and metastatic dissemination. Increasing evidence suggests that hypoxia promotes epithelial–mesenchymal transition (EMT), a cellular reprogramming process that enables epithelial tumor cells to acquire migratory and invasive properties.

However, the transcriptional mechanisms by which hypoxia drives EMT-mediated invasion in LUAD remain incompletely characterized. Here, an in vitro LUAD hypoxia model was established to investigate the transcriptional mechanisms by which hypoxic stress activates EMT-associated programs that facilitate the transition of LUAD cells from epithelial states toward invasive phenotypes, thereby initiating metastatic progression.

Objective

This study aimed to characterize how hypoxic stress activates epithelial-to-mesenchymal transition (EMT) programs in LUAD cells, driving their transition from an epithelial to an invasive, mesenchymal phenotype. To do this, an in vitro LUAD hypoxia model was established, comparing normoxic and hypoxic culture conditions to capture hypoxia-induced transcriptional changes via bulk RNA sequencing.

Materials and Methods

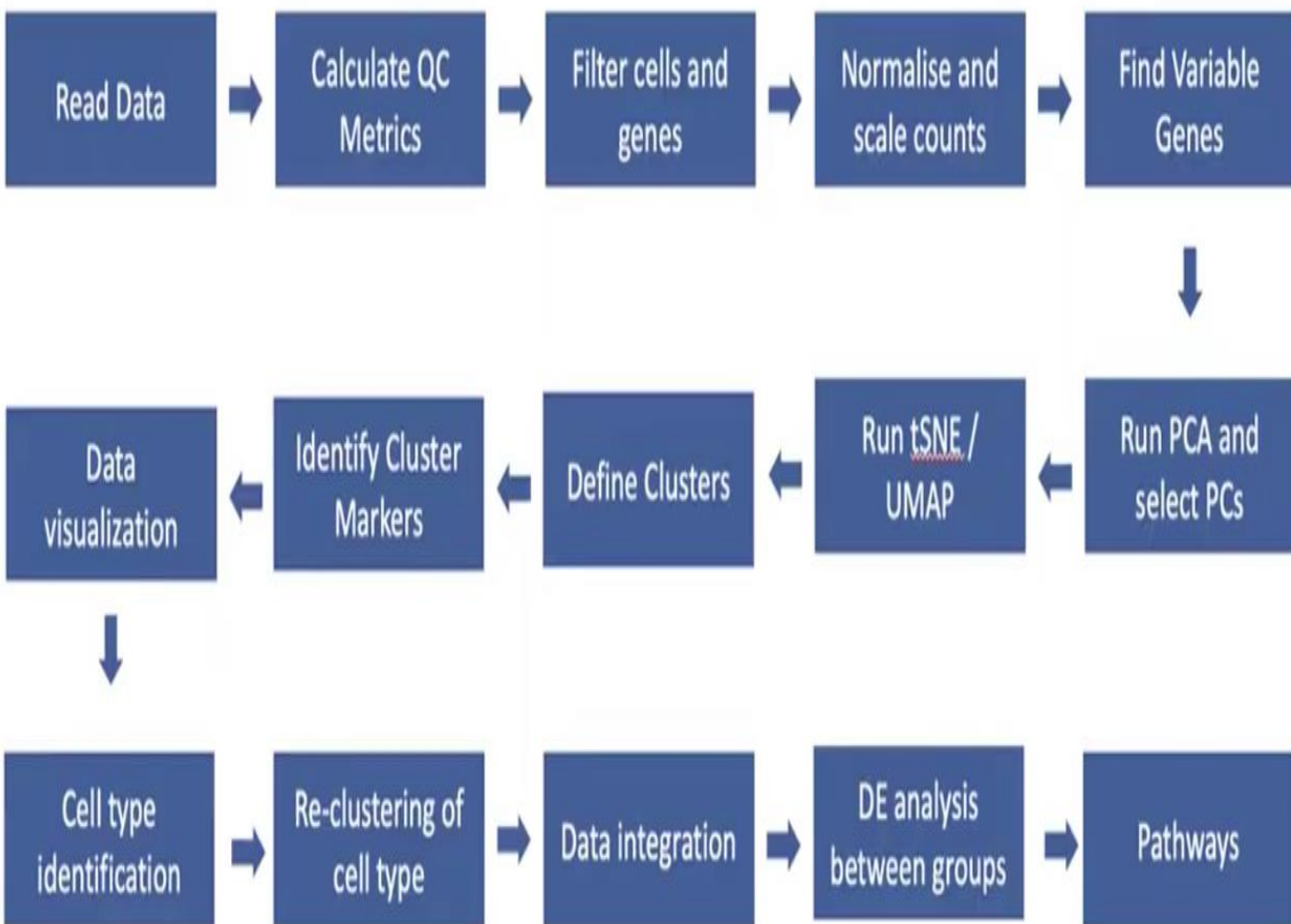
1. Raw reads were quality-assessed with FastQC/MultiQC, then trimmed of adapters and low-quality bases using fastp before alignment to the human reference genome GRCh38/hg19 with STAR; gene-level counts were quantified using featureCounts.

2. Counts were normalized with calcNormFactors (edgeR).

3. DEGs between hypoxic and normoxic conditions were called at $FDR \leq 0.01$ and $\log_2FC > 0.5$, then classified as upregulated or downregulated. DEG heatmaps and curated hypoxia-, angiogenesis-, and glycolysis-related gene panels were visualized using pheatmap, with violin plots generated in ggplot2; all analyses were performed in RStudio.

4. Pathway characterization combined formal GO enrichment analysis (biological process, cellular component, molecular function) with targeted heatmap visualization of curated hypoxia-responsive, angiogenic, and glycolytic gene sets.

Data Analysis Pipeline



Results

1. Principal Component Analysis (PCA) of RNA-seq Data

The PCA plot (Fig. 1) showed clear separation between H1437 LUAD cells cultured under normoxic conditions (21% O₂; blue) and hypoxic conditions (1% O₂; red), demonstrating that hypoxia induced substantial changes in the global transcriptomic profile.

2. Hypoxic exposure for 24 hours induced extensive transcriptomic remodeling in H1437 LUAD cells, with 3,504 genes significantly upregulated and 3,634 genes significantly downregulated, demonstrating a broad transcriptional response to hypoxia

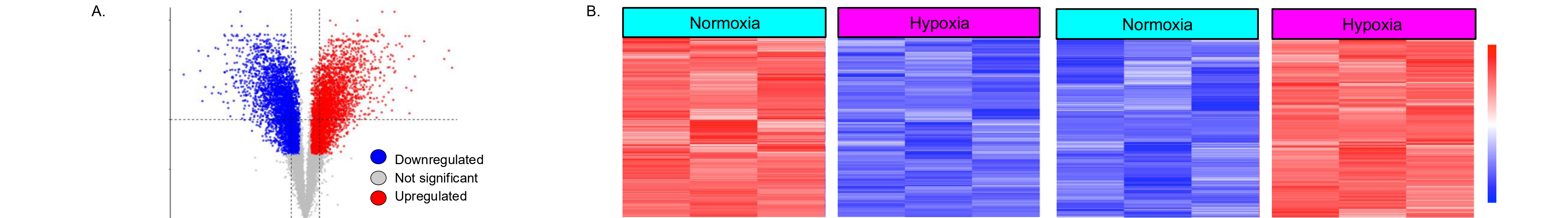


Figure 2. Differential gene expressions (DEGs) in H1437 LUAD cells following hypoxic exposure. (A) Volcano plot showing 3,504 upregulated genes, 3,634 downregulated genes, and 4,097 non-significantly changed genes in H1437 LUAD cells after 24 hours of hypoxia compared with normoxic controls. (B) Heatmaps showing the expression patterns of significantly upregulated and downregulated DEGs induced by hypoxia.

3. Hypoxia activated extracellular matrix remodeling, angiogenic, migration, and EMT-related pathways, accompanied by changes in hypoxia-inducible transcription factors associated with invasive tumor progression

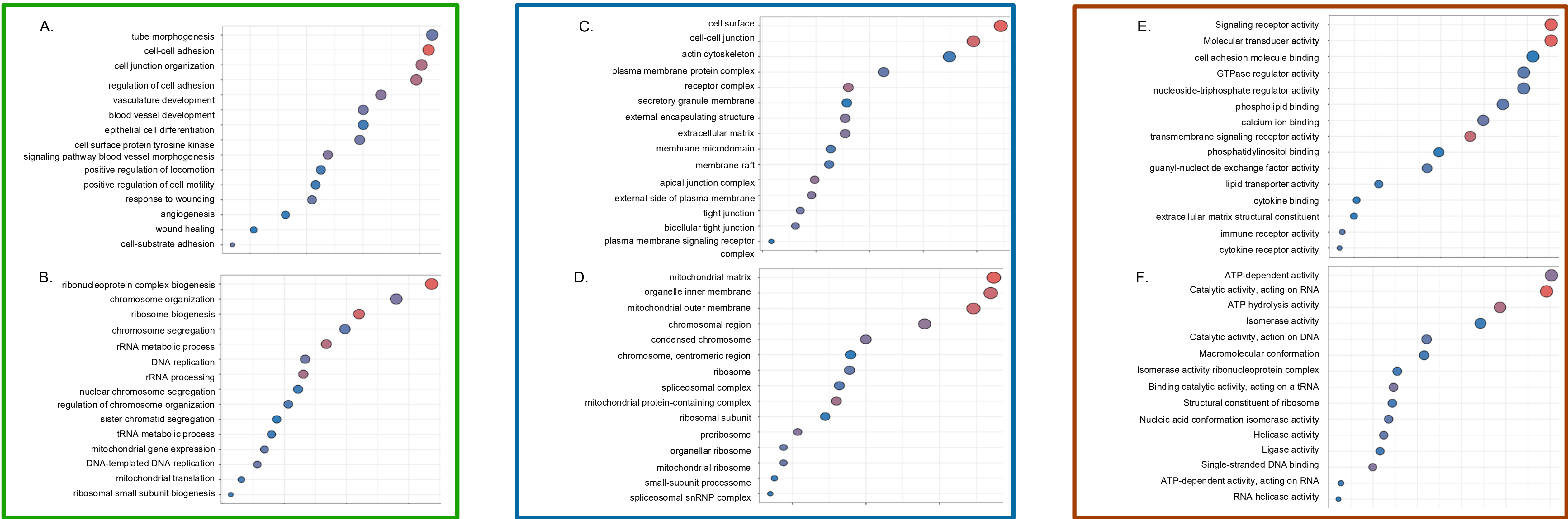
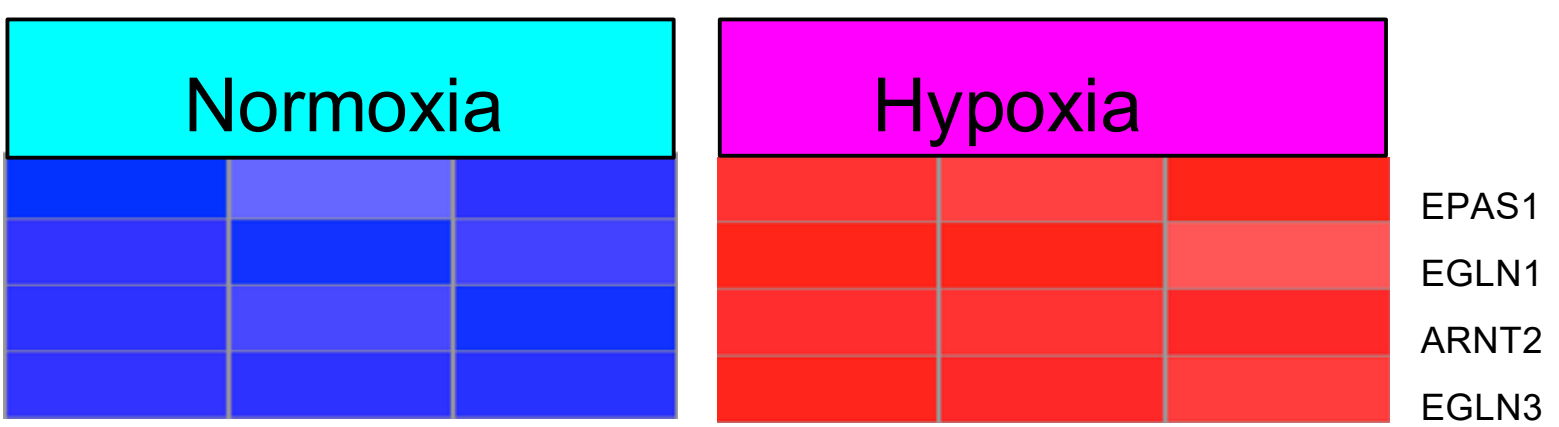


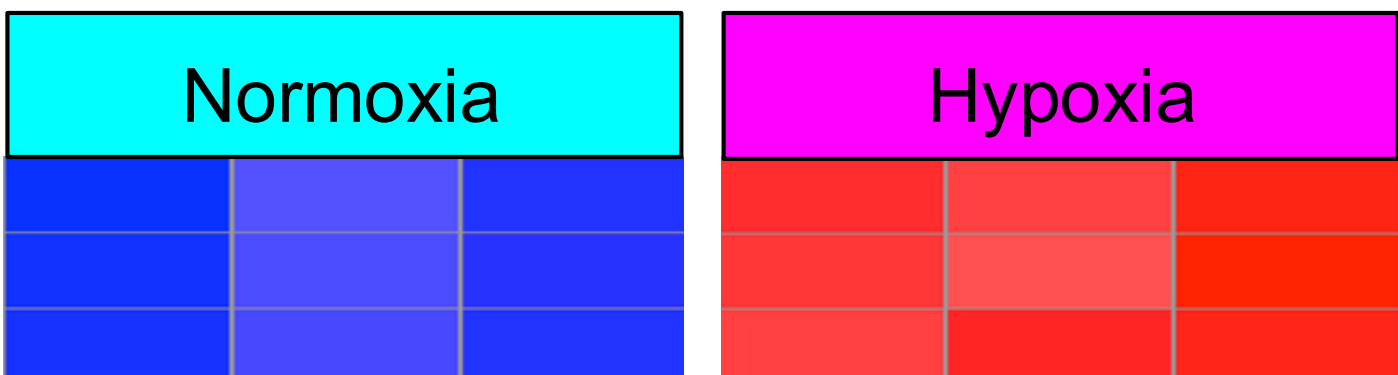
Figure 3. Gene Ontology (GO) pathway analysis of the DEGs in H1437 LUAD cells following hypoxic exposure.

(A.-B.) The biological process (BP) pathways of the up-regulated (A.) and down-regulated (B.) DEGs; (C.-D.) The cellular components (CC) pathways of the up-regulated (C.) and the down-regulated (D.) DEGs; (E.-F.) The molecular component (MF) pathways of the up-regulated (E.) and the down-regulated (F.) DEGs

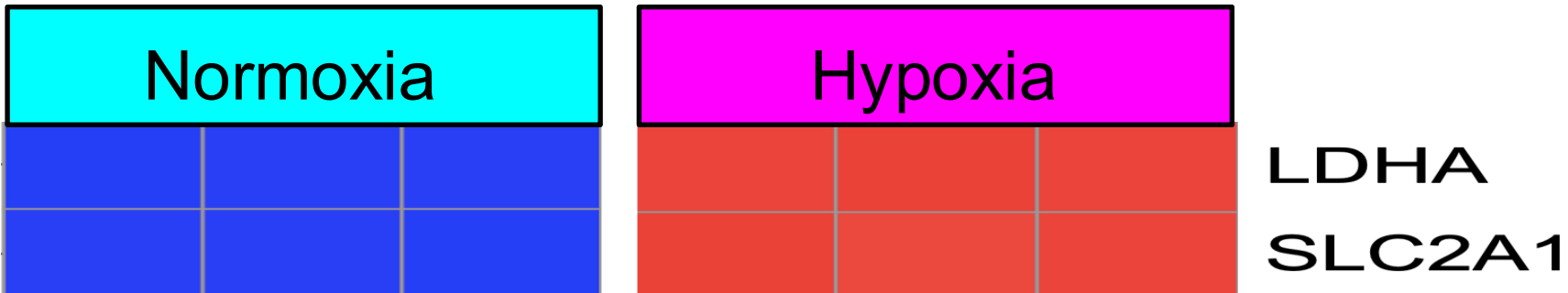
4. Hypoxia significantly upregulated canonical hypoxia-responsive regulators



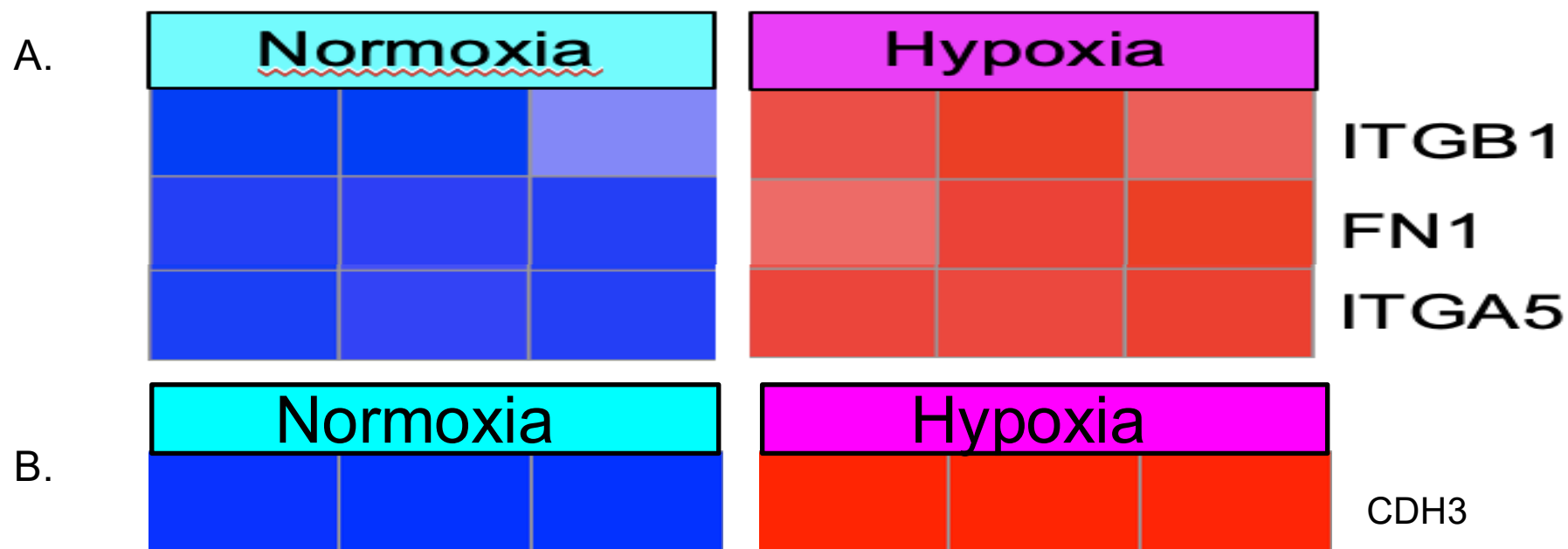
6. Hypoxic conditions induced robust activation of EMT-associated transcription factors



5. Hypoxia significantly upregulated glycolytic pathway genes



7. Hypoxic conditions increased the expression of mesenchymal markers (A.) and epithelial marker (B.)



Discussion

- Hypoxia induced extensive transcriptional reprogramming in LUAD cells, with 3,504 genes upregulated and 3,634 genes downregulated.
- Increased expression of hypoxia-responsive genes (*EPAS1*, *ARNT2*, *EGLN1*, *EGLN3*, *LDHA*, and *SLC2A1*) confirmed activation of hypoxic signaling, while induction of EMT-associated regulators (*STAT3*, *NFKB2*, and *FOSL2*) and mesenchymal genes (*ITGA5*, *ITGB1*, and *FN1*) supported a shift toward an invasive phenotype.
- Enrichment of extracellular matrix remodeling, angiogenesis, and migration pathways further suggests that hypoxia promotes LUAD progression through coordinated metabolic and EMT-related transcriptional changes.

Conclusion

- ❖ These findings demonstrate that hypoxia induces coordinated transcriptional activation of EMT-associated pathways in LUAD cells, supporting a mechanistic role for hypoxia in initiating tumor invasion and metastatic progression.
- ❖ This study also provides a robust in vitro hypoxia model of LUAD that may be useful for anti-tumor drug screening and therapeutic development.

Future Research

- ✓ A key limitation of this study is that invasive behavior was inferred from transcriptional changes alone, without direct functional evidence that hypoxia-exposed LUAD cells acquire migratory or invasive capacity.
- ✓ Future work should incorporate transwell invasion assays, wound-healing assays, or 3D spheroid models to test whether hypoxic cells show increased migration and invasion relative to normoxic controls. Pairing this with targeted knockdown of key EMT transcription factors identified here, such as TWIST1 or SNAI1, would clarify whether these genes are required for the invasive phenotype, not just correlated with it.
- ✓ This would move the study from a descriptive signature toward a mechanistically validated model of hypoxia-driven invasion in LUAD.

References

- Dong YJ, Cai YR, Zhou LJ, et al. Association between the histological subtype of lung adenocarcinoma, EGFR/KRAS mutation status and the ALK rearrangement according to the novel IASLC/ATS/ERS classification. *Oncol Lett.* 2016;11(4):2552–2558. doi:10.3892/ol.2016.4233.
- Enatsu S, Iwasaki A, Shirakusa T, et al. Expression of hypoxia-inducible factor-1 alpha and its prognostic significance in small-sized adenocarcinomas of the lung. *Eur J Cardiothorac Surg.* 2006;29(6):891–895. doi:10.1016/j.ejcts.2006.03.027.
- Xiao Z, Long Q, Liao J, Huang F, Liao L, Dong M. The role of SLC2A1 in lung adenocarcinoma: from tumorigenesis to patient survival. *PLoS One.* 2025;20(8):e0324043. doi:10.1371/journal.pone.0324043.
- Zhan P, Wang J, Lv X, et al. Prognostic value of vascular endothelial growth factor expression in patients with lung cancer: a systematic review with meta-analysis. *J Thorac Oncol.* 2009;4(9):1094–1103. doi:10.1097/JTO.0b013e31819a7e31.
- Liu KH, Tsai YT, Chin SY, Lee WR, Chen YC, Shen SC. Hypoxia stimulates the epithelial-to-mesenchymal transition in lung cancer cells through accumulation of nuclear β -catenin. *Anticancer Res.* 2018;38(11):6299–6308. doi:10.21873/anticancer.12998.
- Goudar RK, Vlahovic G. Hypoxia, angiogenesis, and lung cancer. *Curr Oncol Rep.* 2008;10(4):277–282. doi:10.1007/s11912-008-0043-6.
- Jackson AL, Zhou B, Kim WY. HIF, hypoxia and the role of angiogenesis in non-small cell lung cancer. *Expert Opin Ther Targets.* 2010;14(10):1047–1057. doi:10.1517/14728222.2010.511617.
- Yang SL, Ren QG, Wen L, Hu JL. Clinicopathological and prognostic significance of hypoxia-inducible factor-1 α in lung cancer: a systematic review and meta-analysis. *J Huazhong Univ Sci Technol Med Sci.* 2016;36(3):321–327. doi:10.1007/s11596-016-1586-7.
- Muz B, de la Puente P, Azab F, Azab AK. The role of hypoxia in cancer progression, angiogenesis, metastasis, and resistance to therapy. *Hypoxia (Auckl).* 2015;3:83–92. doi:10.2147/HP.S93413.
- Gilkes DM, Semenza GL. Hypoxia and the extracellular matrix: drivers of tumour metastasis. *Nat Rev Cancer.* 2014;14(6):430–439. doi:10.1038/nrc3728.
- Rankin EB, Giaccia AJ. Hypoxic control of metastasis. *Science.* 2016;352(6282):175–180. doi:10.1126/science.aaf4405.
- Semenza GL. Hypoxia-inducible factors in physiology and medicine. *Cell.* 2012;148(3):399–408. doi:10.1016/j.cell.2012.01.021.
- Keith B, Johnson RS, Simon MC. HIF1 α and HIF2 α : sibling rivalry in hypoxic tumour growth and progression. *Nat Rev Cancer.* 2012;12(1):9–22. doi:10.1038/nrc3183.
- Tam SY, Wu VWC, Law HKW. Hypoxia-induced epithelial-mesenchymal transition in cancers: HIF-1 α and beyond. *Front Oncol.* 2020;10:486. doi:10.3389/fonc.2020.00486.
- Erieff JT, Bennewith KL, Nicolau M, et al. Lysyl oxidase is essential for hypoxia-induced metastasis. *Nature.* 2006;440(7088):1222–1226. doi:10.1038/nature04695.
- Yang MH, Wu MZ, Chiou SH, et al. Direct regulation of TWIST by HIF-1 α promotes metastasis. *Nat Cell Biol.* 2008;10(3):295–305. doi:10.1038/ncb1691.
- Cannito S, Novo E, Compagnone A, et al. Redox mechanisms switch on hypoxia-dependent epithelial-mesenchymal transition in cancer cells. *Carcinogenesis.* 2008;29(12):2267–2278. doi:10.1093/carcin/bgn216.
- Lu X, Kang Y. Hypoxia and hypoxia-inducible factors: master regulators of metastasis. *Clin Cancer Res.* 2010;16(24):5928–5935. doi:10.1158/1078-0432.CCR-10-1360.
- Finger EC, Giaccia AJ. Hypoxia, inflammation, and the tumor microenvironment in metastatic disease. *Cancer Metastasis Rev.* 2010;29(2):285–293. doi:10.1007/s10555-010-9224-5.
- Harris AL. Hypoxia—a key regulatory factor in tumour growth. *Nat Rev Cancer.* 2002;2(1):38–47. doi:10.1038/nrc704.