A Novel Proteomic Pipeline for the Identification of Hypoxia-Sensitive Plasma Membrane Proteins.

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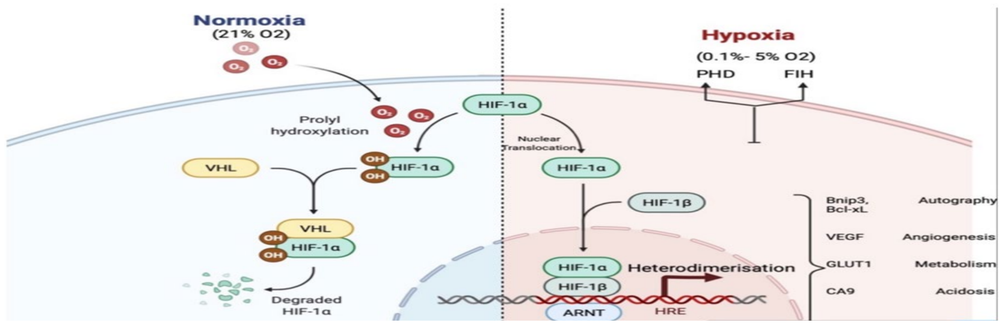
Introduction

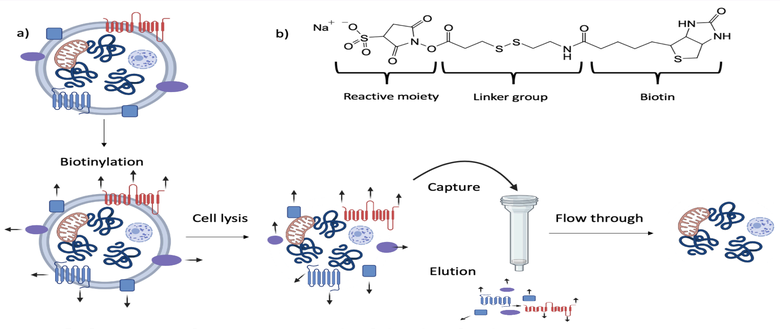
- Hypoxia affects 70% of bladder cancer patients driving treatment resistance and poor prognosis.
- Hypoxia alters cancer cells' plasma membrane protein (PMP) composition, which can be therapeutically exploited.
- Proteomic analysis of PMPs in hypoxic cells can therefore identify novel therapeutic targets
- Protein fractionation techniques are needed to avoid protein masking during proteomic analyses.

Aim: To introduce a novel pipeline to identify hypoxia-induced PMPs.

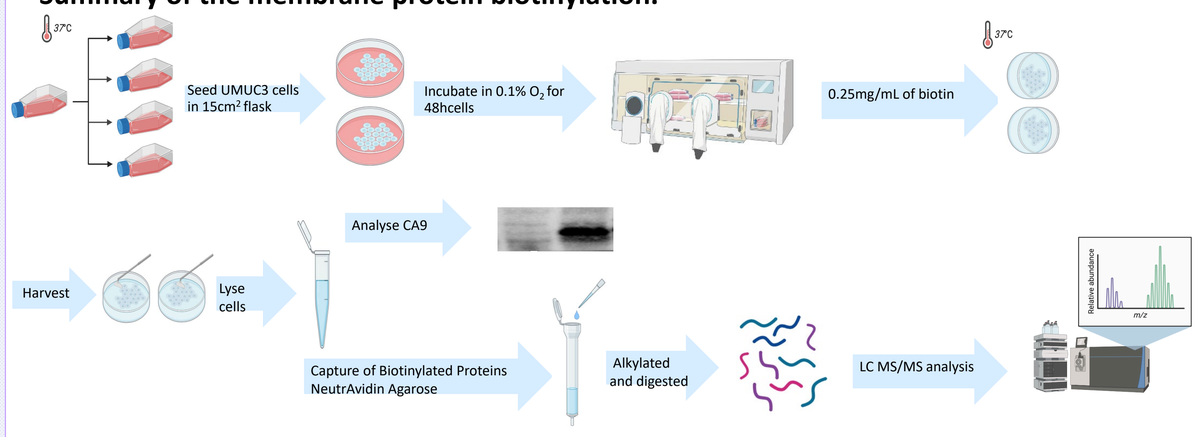


Summary of the transcription factor HIF regulation under different O_2 concentrations.

Method



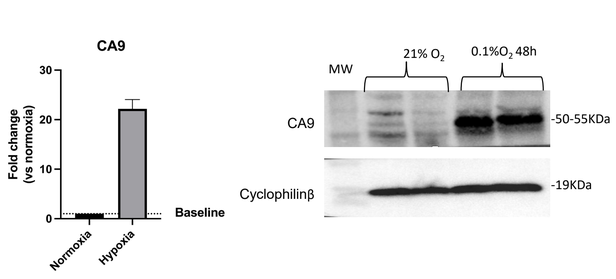
Summary of the membrane protein biotinylation.



Summary schematic of PMPs enrichment using biotin reagent and MS sample preparation.
Hypoxia was induced using a Whitley H35 Hypoxystation (Don Whitley Scientific, UK).

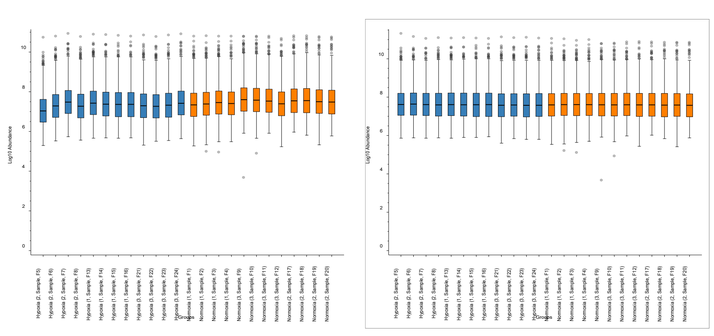
Results

Analysis of CA9 Expression Under Hypoxic Condition



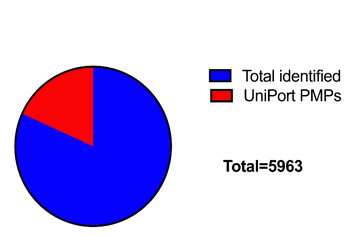
Western blot showing increased CA9 expression under hypoxia (0.1% O_2) compared to normoxia (21% O_2), with an expected band at 50 kDa. n=2 biological repeats

Analysis of CA9 Expression Under Hypoxic Condition



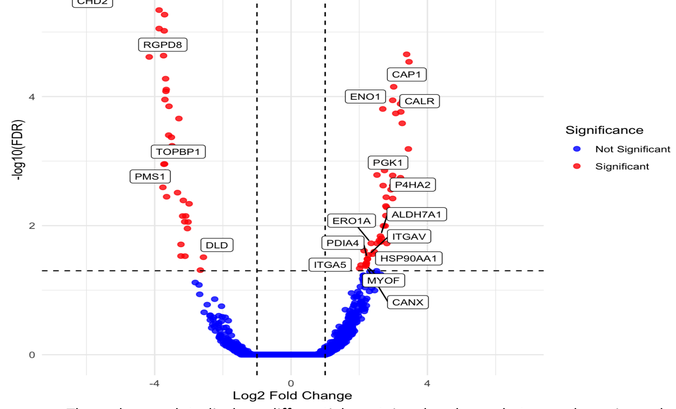
The plots show relative protein abundance in UMUC3 samples across different conditions and time points, comparing biotin-based enrichment before and after normalisation

Enriched Proteomic outcome



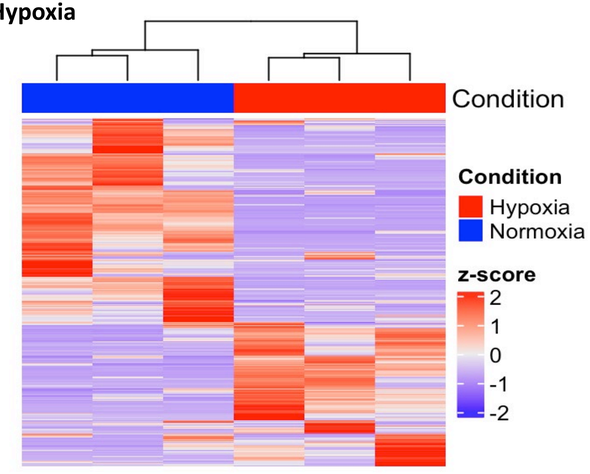
Proteomic analysis in UMUC3 cells identified 5,963 proteins, including 1,332 membrane proteins (UniProt). Each condition was performed in 12 technical repeats.

Volcano plot of Enriched proteomic data



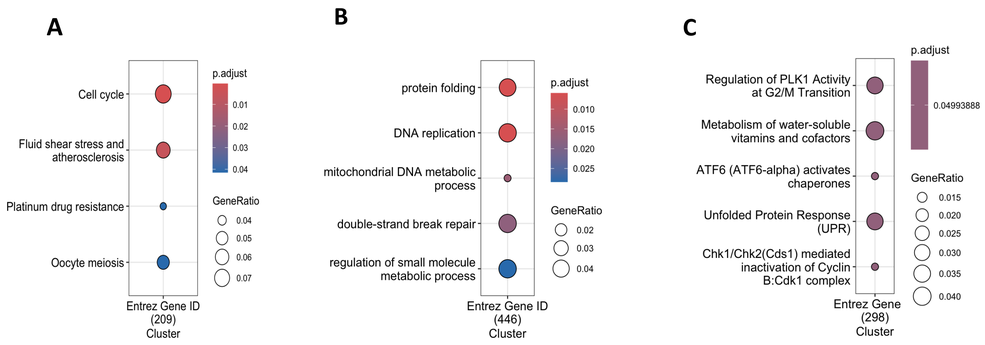
The volcano plot displays differential protein abundance between hypoxia and normoxia in UMUC3 cells. Significantly enriched proteins are identified based on FDR < 0.05 and |log2FC| > 1. The top 30 significant proteins are labelled.

Significant Proteins Expression in UMUC3 Cells Under Hypoxia



This heatmap shows z-score normalised protein abundance in UMUC3 cells after 48 hours of hypoxia and normoxia. Proteins with |log2FC| ≥ 1 and FDR < 0.05 were clustered using Euclidean distance, revealing distinct expression patterns. Data represent three biological replicates (n=3).

Enrichment Analysis of Significant Proteomic Data: KEGG Pathways, Biological Processes, and Reactome Pathways



Enrichment analysis of significant proteomic data (|log₂FC| ≥ 1, FDR < 0.05) identified key pathways across three categories. A. In KEGG, there was an upregulation in the cell cycle category, indicating hypoxia-induced effects. B. The top enriched biological process was protein folding. C. In Reactome, the most significant pathway was the regulation of PLK1 activity at the G2/M transition. These findings highlight the biological relevance of the identified proteins in response to hypoxia.

Top 5 Upregulated and Downregulated Proteins in UMUC3 Enriched Proteomic Data Under Hypoxic Conditions

Gene Symbol	Cellular component	Function
MYOF	Plasma membrane	Promote invasion
ITGAV	Plasma membrane	Promote metastasis
ACTG1	Non-structural extracellular	Promote proliferation
HSP90AA1	Non-structural extracellular	Angiogenesis, invasion and metastasis
PDIA4	Non-structural extracellular	Tumor progression by affecting cell apoptosis and DNA repair
RGPD8	Nucleus	Cell cycle control, and transcriptional regulation
ACTC1	Cytosol	Promotes cell survival, inhibits apoptosis
PARP14	Plasma membrane	Promotes cancer progression and signaling pathway
DLD	Cytoskeleton	Cancer progression and metabolism
CEP192	Cytosol	Cell cycle regulation and cancer progression

The table presents the top 10 differentially expressed proteins in UMUC3 cells under hypoxia versus normoxia. MYOF is the most upregulated, while CEP192 is the most downregulated. Five upregulated (log2FC > 1, FDR < 0.05) and five downregulated (log2FC < -1, FDR < 0.05) proteins were selected, highlighting potential key players in the hypoxic response

Conclusion

1. The biotinylation-based proteomic pipeline identified 1,332 hypoxia-sensitive PMPs in UMUC3 cells, with minimal cytoplasmic contamination.
2. Several hypoxia-associated proteins, including ITGAV, SLC2A1, L1CAM, CA12, GLUT1, MYOF, and CEP192, were significantly enriched. These proteins are all linked to poor cancer outcomes.
3. Consistent protein abundance across replicates validated the reliability of this approach for identifying therapeutic targets.
4. In summary, the optimised method successfully enriched PMPs while reducing cytoplasmic proteins.

Acknowledgment

1. Majmaah University.
2. Don Whitley Scientific UK.

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