Hypoxia-driven Changes in Plasma Membrane Protein Expression Reveal Molecular Radiotherapy Targets

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Background and Aim

- Molecular Radiotherapy (MRT): Targets receptors on cancer cells for effective lymphoma and prostate cancer treatment
- Plasma Membrane Proteins (PMPs): Key in cell function, accessible targets for MRT
- Hypoxia is Common in cervical cancer, alters PMP expression, affects radiotherapy response
- Cervical Cancer Stats: 5-year survival ~58%; 30-50% recurrence, often therapy-resistant metastases

Aim: Utilise biotinylation to selectively isolate PMPs for mass spectrometry characterisation in the SiHa cervical cancer cell line under hypoxic conditions

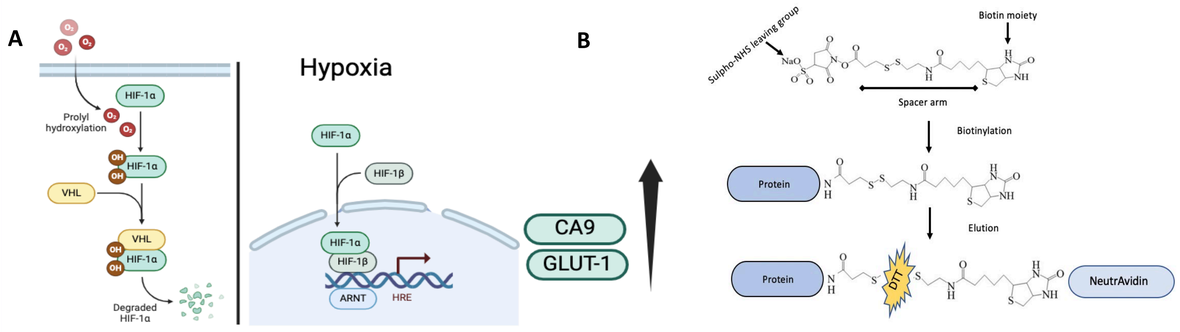


Figure 1: A) HIF-1α degradation and stabilisation pathways in normoxia versus hypoxia. B) Sulpho-NHS-SS-biotin structure

Method

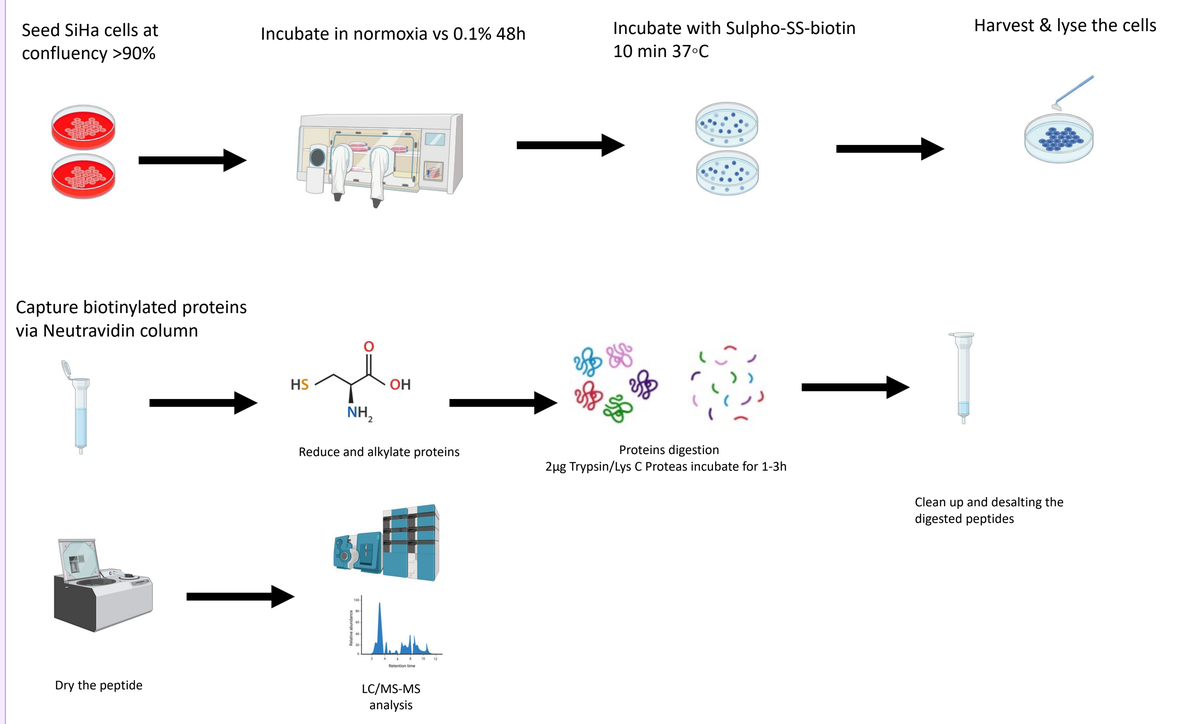
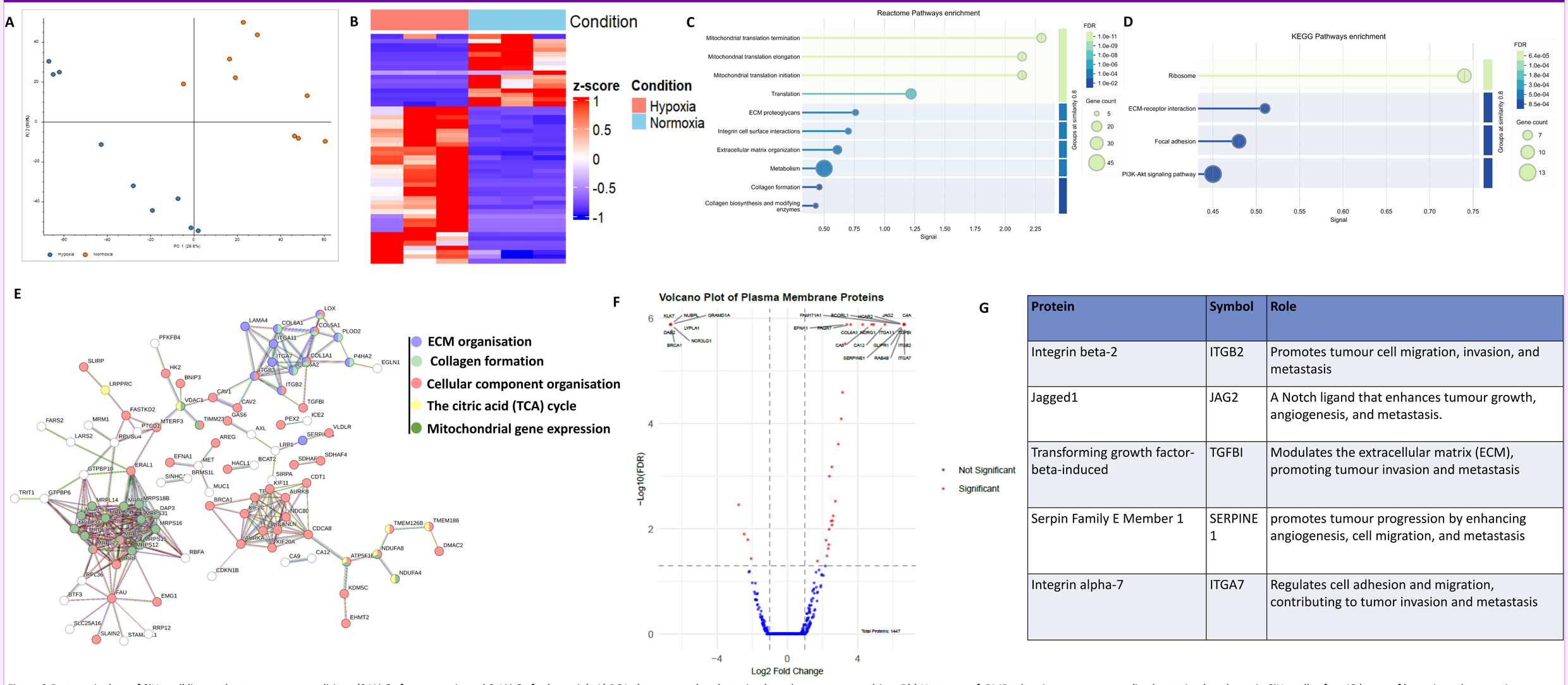


Figure 2: Diagram illustrating the experimental workflow. **Hypoxia was induced using Whitley H35 Hypoxystation (Don Whitley Scientific, UK).**

Results



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Protein	Symbol	Role
Integrin beta-2	ITGB2	Promotes tumour cell migration, invasion, and metastasis
Jagged1	JAG2	A Notch ligand that enhances tumour growth, angiogenesis, and metastasis.
Transforming growth factor-beta-induced	TGFBI	Modulates the extracellular matrix (ECM), promoting tumour invasion and metastasis
Serpin Family E Member 1	SERPINE 1	promotes tumour progression by enhancing angiogenesis, cell migration, and metastasis
Integrin alpha-7	ITGA7	Regulates cell adhesion and migration, contributing to tumor invasion and metastasis

Figure 3:Proteomic data of SiHa cell line under two oxygen conditions (21% O_2 for normoxia and 0.1% O_2 for hypoxia). A) PCA shows samples clustering based on oxygen condtion. B)) Heatmap of PMPs showing z-score-normalized protein abundance in SiHa cells after 48 hours of hypoxia and normoxia. Proteins with $|\log_2 FC| \geq 1$ and FDR < 0.05 were clustered using Euclidean distance, revealing distinct expression patterns. Data represent three biological replicates (n=3. C) Reactome pathway enrichment analysis of differentially expressed proteins in siha cells under hypoxia . D) . Kegg pathway enrichment analysis of differentially expressed proteins in siha cells under hypoxia E) PPI Network of Hypoxia-Upregulated Proteins in SiHa Cervical Cancer Cells . F) Volcano plot displaying differential protein abundance between hypoxia and normoxia in SiHa cells. Significantly expressed proteins are identified based on FDR < 0.05 and $|\log_2 FC| > 1$.G) Table showes the top 5 upregulated proteins and their role in cancer.

Discussion

- SiHa cells under hypoxia showed significant protein expression changes compared to normoxia
- 4,143 overlapping proteins were identified between biotinylation-based enrichment and whole-cell lysates
- Gene Ontology (GO) analysis highlighted enriched plasma membrane functions, including: ABC-type transporter activity transmembrane receptor kinase activity
- Top upregulated PMPs in hypoxia are ITGB2, JAG2, TGFBI, SERPINE1, and ITGA7 – promote migration, angiogenesis, and metastasis.
- Hypoxia drives aggressive tumor behaviour in cervical cancer, warranting further investigation for therapeutic potential

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