

### **HYPOXIA INDUCES BLADDER CANCER CELL INVASION BY ANTAGONIZING THE EXTENSION OF PROTEIN O-GLYCOSYLATION**

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Invasive bladder tumours express the cell-surface Sialyl-Tn (STn) antigen, which stems from a premature stop in protein O-glycosylation. The STn antigen favours invasion, immune escape, and possibly chemotherapy resistance, making it an attractive biomarker for target therapeutics. However, the events leading to the deregulation of protein glycosylation are mostly unknown. Since hypoxia is a salient feature of advanced stage tumours, we search into how it influences bladder cancer cells glycophenotype, with emphasis on STn expression. Three bladder cancer cell lines with distinct genetic and molecular backgrounds (T24, 5637 and HT1376) were submitted to hypoxia. To disclose HIF-1 $\alpha$ -mediated events, experiments were also conducted in the presence of Deferoxamine Mesilate (Dfx). In both conditions all cell lines overexpressed HIF-1 $\alpha$  and its transcriptionally-regulated protein CA-IX. This was accompanied by increased lactate biosynthesis, denoting a shift towards anaerobic metabolism. Concomitantly, all cell lines overexpressed the STn antigen and its main biosynthesis enzyme ST6GalNAc.I, in an HIF-1 $\alpha$ -dependent manner. These effects were reversed by reoxygenation, demonstrating that oxygen levels act as an on-off switch for O-glycan extension. The cells behaved similarly when grown under Dfx exposure, suggesting that HIF-1 $\alpha$  plays a key role in O-glycosylation modulation. Moreover, hypoxia enhanced cell invasion, which was inhibited by exposure to anti-STn monoclonal antibody TKH2. Associations between HIF-1 $\alpha$  and STn overexpressions and muscle invasion were further validated in tumour samples. In conclusion, STn overexpression may, in part, result from a HIF-1 $\alpha$  mediated cell-survival strategy to adapt to the hypoxic challenge by enabling invasion. Moreover, the STn antigen offers potential to address tumour hypoxic areas that harbour more aggressive and highly resistant cancer cells and to control disease dissemination.