P06 SLFN5 regulates amino acid metabolism by altering LAT1 expression in CRPC

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Introduction: Despite early diagnosis and initial treatment of prostate cancer, many patients relapse and develop aggressive castration resistant prostate cancer (CRPC). The molecular mechanisms behind CRPC are diverse, which makes the development of a single therapeutic strategy difficult. From a proteomic comparison of *in vivo* CRPC and hormone naïve models, Schlafen family member 5 (SLFN5) emerged as a potential biomarker for castration resistance. SLFN5 has not yet been reported in prostate cancer, and has displayed both pro and anti tumorigenic properties in other cancer types. Therefore we sought to elucidate the role of SLFN5 in CRPC.

Materials & Methods: Untargeted proteomic analysis of three different isogenic hormone naïve and CRPC pairs was used to identify proteins that had altered expression under *in vivo* castration conditions. KO and control cells were developed using CRISPR/Cas9 technology and were orthotopically injected into castrated nude mice. Tumour volume was measured weekly by ultrasound scanning. Proteomic, transcriptomic and metabolomic analysis was performed on the tumour samples, as well as on the *in vitro* cell models. Methionine intake and protein synthesis were measured by incorporation of radiolabelled [^{35}S]-Methionine.

Results: From the initial *in vivo* screening, SLFN5 emerged as a protein commonly upregulated in CRPC in all three model pairs. *In vitro* SLFN5 expression levels matched the *in vivo* proteomics, being higher in the CRPC cells and could also be modulated by addition or removal of androgens from the media. SLFN5 KO tumours showed reduced growth compared to control ones. Transcriptomic and proteomic analysis of these tumours highlighted the two components of the LAT1 transporter, SLC7A5 and SLC3A2, as severely down-regulated. This downregulation was further confirmed in *in vitro* cultured SLFN5 KO cells. Since LAT1 exports glutamine out of the cell to intake other amino acids in exchange, we performed a metabolomic analysis of our SLFN5 KO models. Results showed accumulation of glutamine and reduced levels of several LAT1-transported amino acids upon loss of SLFN5. As a consequence, SLFN5 KO cells showed impaired activation of the mTOR pathway as well as reduced rate of protein synthesis, counterbalanced by an increased level of basal autophagy.

Conclusions: The effects observed in the SLFN5 KO models point to an altered metabolism due to reduced intracellular availability of several essential amino acids. Limited ability to obtain the nutrients necessary to synthesise new proteins lead the cells to reduce protein synthesis rate, as well as increase autophagy to potentially reuse essential amino acids. LAT1 has previously been shown to increase under androgen deprivation conditions, driving amino acid intake. Our results point to SLFN5 as a regulator of that metabolic change, allowing cancer cells to obtain the necessary essential amino acids they need to thrive under castration.