O01 Study on the possible interactions between FGFRL1- and AR-regulated mechanisms in prostate cancer

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Introduction & Objectives: Androgen receptor (AR) is a transcription factor, which plays a key role in development and progression of prostate cancer (PCa) and is also the main therapeutic target of metastatic PCa. In the majority of cases, PCa becomes resistant to androgen deprivation and anti-androgen therapy. In a small subset of patients, tumours progress towards neuroendocrine (NE) differentiation, while others bypass both AR and NE dependence and progress through fibroblast growth factor (FGF) signalling (Bluemn et al., 2017).

Background: We have previously found that expression of the poorly understood FGF receptor FGFRL1 is elevated in a significant portion of primary PCa (n=150). In in vitro cultures of PC3M cells, overexpression of FGFRL1 inhibited FGF-induced phosphorylation of FGFR substrate 2 (FRS2), which is a key transducer of FGFR signaling. This in turn leads to weakening of the downstream pathways like Ras–Raf–MapK and PI3K–Akt. In LNCaP cells, dihydrogen testosterone (DHT) and synthetic androgen (R1881) treatment increased FGFRL1 protein expression. However, adding AR antagonists like Enzalutamide did not block this long-term effect.

Objective: We aimed to study our hypothesis that expression and/or function of FGFRL1 and AR are correlated in PCa cells.

Materials & Methods: Lipofecatmine RNAiMax reagent was used for transient transfection of targeted siRNA (s28781 abcam) leading to knock-down of FGFRL1 expression in AR expressing cell lines like VCaP and LNCaP, followed by analysis of altered protein- and mRNA expression and promoter activity by Western blotting, RT-qPCR and chromatin immunoprecipitation (ChIP) sequencing using Diagenode iDeal CHiP-seq for transcription factors kit (Cat no: C01010170).

Results: We observed that in androgen-deprived cultures, addition of R1881 increases the expression of FGFRL1 and other AR-regulated genes like Calcium/Calmodulin Dependent Protein Kinase Kinase 2 (CAMKK2) and UDP-N-Acetylglucosamine Pyrophosphorylase 1 (UAP1) The results suggest a molecular interaction between FGFRL1- and AR-mediated mechanisms in the regulation of AR target gene expression. To study this association further, we will analyse changes in the number or identity of AR binding sites in FGFRL1 knock down cells versus control cells. This will be performed by chromatin immunoprecipitation, combined with sequencing (ChIP-Seq), in the presence and absence of the synthetic androgen R1881.