00533 GNA13 Drives CXCL5 Gene Expression Through the Trans-activation of NF-kB in Prostate Cancer Cells

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Aims: G protein-coupled-receptors and heterotrimeric G proteins have been implicated in many cancers. A heterotrimeric G protein, GNA13, is upregulated in solid tumors and drives tumor initiation, drug resistance and metastasis in solid tumors including prostate cancers. Here, we sought to understand how GNA13 contributes to cancer advancement. Particularly, the potential role of GNA13 in the regulation of a pro-tumorigenic chemokine, CXCL5, is examined.

Methodology: RNA sequencing and Ingenuity Pathway Analysis were used to identify targets impacted by loss of GNA13 in PC3 cells. Experiments were conducted in vitro using sh-RNA knock-down of GNA13 (PC3 & Du145: High GNA13 cell lines) and over-expression of GNA13 (LnCAP cells; low GNA13). RNA expression was determined by qPCR and protein levels by immunoblotting/ELISA. Luciferase reporters driven by a CXCL5 promoter fragment containing base pairs -2324 to +325 relative to the transcriptional start site, and serial truncations of the same were designed for promoter studies. Transient knock-down of NF-kB was done using siRNA targeting p65.

Result: Various CXC-chemokines were identified through total RNA sequencing as impacted by GNA13 levels. Validation studies confirmed impact on CXCL5, where its RNA and protein expression consistently reflected GNA13 levels across 3 different prostate cancer cells. Promoter analysis suggested that the -505/+325 fragment is essential for GNA13 response. Using Transcription Factor-binding prediction, a NF-kB site (amongst others) was found in the -505/+325 region. Loss of GNA13 suppressed FBS-induced I?B and p65 phosphorylation and luciferase expression of a NF-kB-driven promoter-reporter construct in these cells. Furthermore, p65 knock-down significantly reduced GNA13-dependent CXCL5 RNA expression.

Conclusion: Our findings suggest GNA13 drives CXCL5 mRNA expression and protein secretion in three different prostate cancer cells. Mechanistically, GNA13- induced NF-kB signaling pathway is seemingly a key mediator of CXCL5 expression. Since CXCL5 is an established pro-angiogenic chemokine, the effect of GNA13-driven CXCL5 on tumor angiogenesis should also be further examined.