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Inhibiting phosphatidylinositol 4-kinase IIIα with GSK-F1 reduces the plasma membrane-associated phosphatidylinositol phosphate lipid messenger levels in prostate cancer cells

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***Background**:* The role of chemokine signaling in prostate cancer metastasis has demonstrated to be a promising focus of research and a potential target for abating malignant cell invasion and metastasis. CXCR4, a GPCR, is overexpressed in prostate cancer cells, with downstream signaling involved in cellular migration, proliferation and survival when it is activated by its ligand, CXCL12. Upon activation, it recruits phosphatidylinositol 4-kinase IIIα (PI4KIIIα) to the plasma membrane (PM) , which produces a pool of phosphatidylinositol 4-phosphate (PI4P). PI4P is a precursor to phosphatidylinositol 4,5-bisphosphate (PIP2), an important molecule with close implications in PI3K/AKT pathway activation and oncogenic processes. The impact of inhibiting PI4KIIIα on the on maintaining steady state levels of PI(4,5)P_2 in prostate cancer cells has not been previously addressed and remains unclear.

***Objective**:* We aim to show that stable levels of PIP2 in PM depend on PI4KIIIα activity and inhibiting with a specific PI4KIIIα inhibitor, GSK-F1, will reduce the PM concentration of PIP2.

***Methods**:* PC3 cells were cultured in RPMI medium and passaged every 48 hours. Cells were transfected with PLCδ1-PH-GFP plasmid DNA, as a biosensor for PIP2 using Opti-MEM and Lipofectamine 3000 reagent. 24 hours post-transfection, cells were exposed to 4 different treatments – DMSO, DMS0 + CXCL12, GSK-F1+CXCL12, GSK-F1 for approximately 20 hours. Cells were fixed and mounted on slides with antifade reagent and DAPI. Leica DMI3000 B wide-field fluorescence microscope was used for visualization. Peak area of fluorescence PLCδ1-PH-GFP at plasma membrane was quantified using ImageJ software.

***Results**:* There was significantly less PLCδ1-PH-GFP fluorescence at the PM in PC3 cells treated with GSK-F1 with (p=0.0087) and without (p=0.0022) CXCL12 when compared to cells treated only with CXCL12, indicating reduced availability of PIP2. Augmented fluorescence was noted in cells treated with CXCL12 alone when compared to the control group receiving only DMSO (p=0.0022), suggesting CXCR4 activation promotes PI4P through activation of PI4KIIIa and its subsequent conversion to PI(4,5)P_2 through PM resident PI4PKinases.

***Conclusion**:* Inhibiting PI4KIIIα with GSK-F1 diminishes stores of PIP2 at the PM in PC3 cells, indicating the dependence on PI4KIIIa activity for maintaining stable levels of PI(4,5)P_2 in prostate cancer cells and its effect on invasion and metastasis.

Key words: Prostate cancer, chemokine, biosensor, metastasis, invasion, phosphatidylinositol