Developing novel PTGES3 inhibitors as next generation agents for mCRPC

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Background:

The androgen receptor (AR) is a key driver of prostate cancer. While AR-targeted therapies have shown survival benefits in multiple phase III trials, aggressive prostate cancers often evade these treatments by reactivating AR signaling. Thus, discovering new ways to target AR is crucial for improving outcomes in metastatic castration-resistant prostate cancer (mCRPC). To address this from the perspective of AR regulators, we developed live cell quantitative AR fluorescent reporters. Using these reporters, we conducted genome-wide CRISPRi flow cytometry sorting screens to identify genes modulating AR protein levels. This approach revealed known AR regulators as well as unexpected hits, including prostaglandin E

synthase 3 (PTGES3), a poorly characterized gene in prostate cancer (PCa). PTGES3 repression led to loss of AR protein in AR-driven models, and its expression is linked to resistance to AR-directed therapies. Mechanistically, PTGES3 binds directly to AR, forms a nuclear complex, regulates AR stability, and modulates AR function. These findings highlight PTGES3 as a promising therapeutic target, though specific inhibitors are lacking. Therefore, we set out to develop the first small-molecule inhibitors of PTGES3.

Methods and Results:

We employed a disulfide tethering screen using a library of 1800+ disulfide fragments for covalent modification of CysLite PTGES3, screened via intact protein mass spectrometry, and selected hits that modified the target >65% for further testing. Hits were prioritized based on their EC50 values and ability to covalently modify CysLite PTGES3. The top fragments increased PTGES3's thermal melt temperature, modulated PTGES3-AR interaction (far-western blot), and disrupted the in vitro PTGES3-AR interaction. We optimized these fragments into a covalent compound, rac-18. Treating LNCaP cells with rac-18 significantly reduced AR protein levels. We further showed, using proximity ligation assays, that rac-18 disrupts the AR-PTGES3 interaction in cells. RNA-seq analysis confirmed that rac-18 repressed AR target gene expression, while ATAC-seq analysis revealed a significant loss of accessible DNA regions in the promoters of hallmark androgen response genes following treatment. Notably, rac-18 inhibited the growth of AR-dependent PCa cells but had no effect on AR-independent PCa or non-PCa cells. Additionally, rac-18 significantly reduced 22Rv1 tumor xenograft growth, decreased AR expression, and repressed downstream AR gene expression in vivo.

Conclusions:

We have developed a first-in-class covalent PTGES3 inhibitor, rac-18, which disrupts the PTGES3-AR interaction and suppresses AR signaling in prostate cancer cells. This work presents a novel therapeutic strategy with the potential to overcome resistance to AR-targeted therapies in mCRPC.

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