Effects of KDM5 Inhibitor on the Growth of Early Stage and Advanced Human Prostate Cancer Cells

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ABSTRACT
Prostate cancer (PCa) remains a leading cause of cancer deaths because many patients ultimately develop an incurable castration-resistant form of metastatic PCa. While chromatin alterations are linked to PCa, the role of histone demethylases in the PCa progression has not been fully elucidated. Members of the Jumonji AT-rich interactive domain 1 (JARID1) or lysine demethylase 5 (KDM5) family of lysine demethylases are known for their ability to demethylate H3K4me3. However, the genes encoding these proteins are amplified in metastatic PCa. Literature suggests members of the KDM5 family enhance expression and function of androgen receptor (AR). Here, we have tested this hypothesis by characterizing the effects of PBIT alone and in combination with KDM5B or other KDM5 isoforms modulate the activity of PPARγ. A second nuclear receptor that has been reported to regulate prostate cancer progression is the peroxisome proliferator activated receptor gamma (PPARγ). These data suggest PBIT suppresses PC3 cell proliferation at concentrations greater than or equal to 5 µM. LNCaP cells were more sensitive to 15d-PGJ2. In this cell line, a significant reduction in proliferation was detected only in cells exposed to 20 µM 15d-PGJ2. These data suggest that members of the KDM5 family play a role in the growth of these cell lines.

BACKGROUND
With more than 3 million new cases every year, prostate cancer is the most common cancer and the second leading cause of cancer death in men of the United States. Patients with early stage prostate cancer are treated with surgery or radiation, while metastatic prostate cancer is addressed using androgen deprivation therapy (ADT). Unfortunately, there is no cure for metastatic prostate cancer once it becomes castration-resistant and no longer responds to ADT. Recent studies have shown that alterations in chromatin regulation promote tumorigenesis and progression of PCa. Androgen receptor (AR) signaling is critical for PCa development. A second nuclear receptor that regulates chromatin may serve as effective treatments for prostate cancer. Currently, the only cancer drugs in clinical use that target chromatin regulators are inhibitors of histone deacetylases and DNA methyltransferases, neither of which are used to treat prostate cancer patients. The isoforms of histone lysine demethylase 5 (KDM5) are members of the family of Jumonji C (JmJC) domain-containing histone demethylases that specifically remove dimethyl (me2) and trimethyl (me3) marks from histone 3 lysine 4 (H3K4). KDM5B was initially discovered as a gene upregulated in breast cancer. New studies have demonstrated KDM5B is also overexpressed in metastatic prostate cancer. KDM5B was found to enhance androgen receptor (AR) transcription activity in human prostate cancer cells. AR belongs to the steroid nuclear receptor family and plays a pivotal role in prostate cancer development. A second nuclear receptor that has been reported to regulate prostate cancer progression is the peroxisome proliferator activated receptor gamma (PPARγ). However, it is not known if KDM5B or other KDM5 isoforms modulate the activity of PPARγ or other nuclear receptors that regulate prostate cancer development. 2-(4-(methylphenyl)-1,2-benzisothiazol-3(2H)-one (PBIT) has been suggested as a potential agonist to activate PPARγ. The isoforms of histone lysine demethylase 5 (KDM5) are members of the family of Jumonji C (JmjC) domain-containing histone demethylases that specifically remove dimethyl (me2) and trimethyl (me3) marks from histone 3 lysine 4 (H3K4). KDM5B was initially discovered as a gene upregulated in breast cancer. New studies have demonstrated KDM5B is also overexpressed in metastatic prostate cancer. KDM5B was found to enhance androgen receptor (AR) transcription activity in human prostate cancer cells. AR belongs to the steroid nuclear receptor family and plays a pivotal role in prostate cancer development. A second nuclear receptor that has been reported to regulate prostate cancer progression is the peroxisome proliferator activated receptor gamma (PPARγ). However, it is not known if KDM5B or other KDM5 isoforms modulate the activity of PPARγ or other nuclear receptors that regulate prostate cancer development.

METHODS
Each cell line was plated in 24 well tissue culture plates at a density of 10,000 cells/well and allowed to attach overnight. The cells were then treated with vehicle control (DMSO) or the designated concentration of PBIT for 72 hours (PC3 cells) or 96 hours (LNCaP cells). Cells were then incubated with the Presto Blue Cell Viability Reagent. The level of proliferation was assessed according to the Presto Blue protocol. Each bar represents the mean ± SD for three wells. **P<0.05 compared to the DMSO vehicle. (n=3)

Table: Index Average

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<th>Group</th>
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<th>LNCaP</th>
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<td>50 µM PBIT</td>
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Figure 1. PBIT reduces prostate cancer cell proliferation.

Figure 2. 15d-PGJ2 reduces prostate cancer cell proliferation.

Figure 3. 15d-PGJ2/PBIT combination treatment synergistically reduces prostate cancer cell proliferation.

Figure 4. PBIT reduces PC3 cell migration.

Figure 5. 15d-PGJ2/PBIT combination treatment reduces PC3 cell invasion.

SUMMARY & CONCLUSIONS
- The KDM5 Inhibitor PBIT reduces proliferation of LNCaP and PC3 prostate cancer cells. It also suppresses PC3 cell migration.
- PBIT in combination with 15d-PGJ2 synergistically reduces PC3 cell proliferation and invasion.
- Combining PPARγ agonists and KDM5 inhibitors may provide an effective alternative therapy for advanced metastatic human prostate cancers.
- Next steps will be to elucidate the mechanisms by which PBIT reduces proliferation and migration of human prostate cancer cells.

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CONTACT INFORMATION
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