Abstract

ODM-207, a novel BET-bromodomain inhibitor as a therapeutic approach for the treatment of patients with castration resistant prostate cancer

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Background

During normal development, the androgen receptor (AR) regulates genes that are essential for the differentiation of the prostate. However, in prostate cancer (PCa), AR-regulated gene transcription is re-directed to drive the malignant phenotype. Also castration resistant prostate cancer (CRPC) is dependent on AR and is characterized by persistent activation of AR signaling by residual tissue androgens. In fact, activation of AR signaling has been shown to be crucial for prostate cancer growth at all stages of the disease and several molecular mechanisms have been proposed to explain the addiction to AR including emergence of AR overexpression, AR ligand binding domain (LBD) mutations and splicing variants lacking the LBD. Unfortunately, despite recent advances in treatment of CRPC, nearly all patients treated with current hormonal therapies eventually will relapse.

Herein, we show that ODM-207, an inhibitor of tandem bromodomain (BD) containing family of transcriptional regulators known as the BET (bromodomains and extraterminal) proteins antagonizes the interaction between the Brd4 and acetylated histone peptides. In cellular studies with AR-positive prostate cancer cell lines, ODM-207 possessed potent growth inhibitory activity. ODM-207 also displayed potent antiproliferative activity in a VCaP model resistant to second generation anti-androgen enzalutamide and inhibited the expression of the AR splice variant AR-V7. In 22Rv1 prostate cancer xenograft, oral administration of ODM-207 was efficacious in suppressing tumor growth at well tolerated doses.

Herein, we performed RNA-sequencing studies and gene set enrichment analysis of ODM-207 treated VCaP cells to study the pathways regulated by ODM-207. Gene set enrichment analysis indicates significant changes in expression of genes involved in e.g. MYC and AR gene signatures. Indeed, in cellular studies with AR-positive prostate cancer cell lines as well as 22Rv1 xenograft model, ODM-207 showed potent down-regulation of Myc, an important oncogenic regulator of cell proliferation and tumorigenesis.

Methods

Biochemical activity: Blocking of ODM-207 to BRM full length recombinant protein was tested by measuring the displacement of fluorescently labeled inhibitor from bromodomain peptides and the TR-FRET assay.

Cells lines: VCaP, 22Rv1 and 293T cells were purchased from ATCC and maintained in the condition recommended by the producer. 22Rv1 cells were derived from a patient suffering from prostate cancer, where the tumor was unresponsive towards endocrine treatment and death occurred 2 months after the treatment.

Cell viability assays: MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; CellTiter-Blue® cell viability assay, a non-radioactive, colorimetric viability assay based on the reduction of an alamarBlue dye by metabolic active cells.

Immunoblotting: Cells were treated with ODM-207 and samples were immunoblotted with antibodies against AR-V7, Myc, LNCaP, VCaP, GAPDH and 53BP1 (Cell Signaling Technology, Danvers, MA).

Results

1. ODM-207 is a novel, potent, and structurally distinct inhibitor of BET proteins

a) Biochemical potency of ODM-207

<table>
<thead>
<tr>
<th>Bromodomain</th>
<th>IC50 (nM)</th>
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<tr>
<td>BRD4 full length</td>
<td>B9</td>
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b) Antiproliferative effects of ODM-207 in prostate cancer cell lines

2. ODM-207 regulated pathways and genes in prostate cancer cell lines

a) Differentially expressed genes in VCaP cells between ODM-207 and DMSO treatment groups

b) Pathways regulated by ODM-207 in VCaP cells

3. ODM-207 inhibits tumor growth in AR-V7 expressing 22Rv1 xenograft

a) Effect of ODM-207 on tumor volumes

b) Effect of ODM-207 on tumor weights

c) ODM-207 inhibits Myc in vivo efficacy study

4. ODM-207 downregulates AR-V7 in VCaP and enzalutamide resistant VCaP cells

5. ODM-207 downregulates Myc in prostate cancer cell lines

Conclusions

ODM-207 is a novel and structurally distinct BET protein inhibitor

ODM-207 inhibits proliferation of androgen receptor dependent prostate cancer cell lines including enzalutamide-resistant models

Causes suppression of AR target genes, Myc signaling and DNA repair pathway and induction of p53 pathway

Induces a dose-dependent reduction in Myc and AR-V7 splice variant protein levels

In vivo, oral administration of ODM-207 potently inhibits tumor growth and Myc expression in 22Rv1 prostate cancer xenograft model