Discovery and characterization of small molecules targeting the DNA-binding ETS domain of ERG in prostate cancer

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Introduction

Intrachromosomal rearrangements fusing androgen receptor (AR)-regulated TMPRSS2 promoter to ERG coding sequences (TMPRSS2-ERG) occur in 50% of prostate cancers (PC). TMPRSS2-ERG fusions are early events in PC initiation and are associated with high Gleason score, aggressive disease, and poor prognosis due to activation of aberrant ERG-driven transcriptional programs that promote migration, invasion and epithelial-mesenchymal transition. Despite initial dependence on AR, ERG expression can become self-driven, resist to antiandrogens, and persist in castration-resistant PC through a feed-forward regulation. There are currently no drugs that can directly target the ERG protein.

Results

Discovery of small molecules that target the DNA-binding ETS domain of the ERG protein

In silico pipeline for discovering small molecule inhibitors of ERG

While targeting transcription factors is a challenging task, we applied our established rational drug discovery pipeline, specialized in targeting protein-DNA interaction sites, to develop anti-ERG small molecules.

In silico modeling

- Protein modeling
- Molecular docking
- Chemical similarity
- Medicinal chemistry
- Molecular dynamics
- Structure-activity relationship

In vitro and in vivo experiments

- Transcriptional activity
- Direct binding with ERG
- Primary/DNA interaction
- Cell viability assay
- Cell migration and invasion
- Solubility and stability
- Xenograft models

VPC-18005 is not cytotoxic

- Concentration (µM)
  - 0
  - 0.03
  - 0.1
  - 0.3
  - 1
  - 3
  - 5
  - 10
  - 25

Direct binding of VPC-18005 to the DNA-binding ETS domain of the ERG protein

Figure 4. In silico pipeline for discovering small molecule inhibitors of ERG.

VPC-18005 inhibits ERG-driven transcription

Figure 5. A) Overexpression of ERG in VCAp and PNT1B-ERG cell lines. B) VPC-18005 reduced ERG-driven transcriptional activity in luciferase assays.

VPC-18005 disrupts ERG binding to DNA

Figure 6. A) Dose-dependent response of VPC-18005 in PNT1B-ERG and VCAp cells on ERG-mediated luciferase activity with IC50 values of 3 µM and 6 µM, respectively. B) Dose-dependent response of YK-4-279 (a published experimental ERG inhibitor) in PNT1B-ERG and VCAp cells on ERG-mediated luciferase activity with IC50 values of 5 µM and 16 µM, respectively. The toxicity of YK-4-279 is shown in red filled dots.

VPC-18005 inhibits migration and invasion of ERG-overexpressing cells in vitro

Figure 7. After treatment with up to 25 µM VPC-18005, no effect on cell viability (MTS) was observed with ERG-expressing cells (PNT1B-ERG and VCAp) and non-ERG expressing cells (PC3). In contrast, the inhibitor YK-4-279 demonstrated cytotoxic effects starting at 1 µM in all three cell lines.

Figure 8. A) NMR data shows direct binding of VPC-1805 at the ERG-ETS domain. Insert: chemical shifts were observed with increasing concentrations of VPC-1805 (red to purple), but not DMSO. B) Amino acid residues exhibiting large chemical shift perturbations were mapped to their corresponding locations on the ERG-ETS domain.

Figure 9. EMSA shows that VPC-1805 disrupts ERG binding to CIC/4 promoter DNA elements containing the GGA4 ETS recognition motif. C) The inhibition of ERG-DNA interactions by VPC-1805 is not due to ERG protein degradation as shown by Western blots.

Figure 10. VPC-1805 reduced cell migration of ERG-expressing cells but not MDCK cells. A) PNT1B-Mock cells and B) PNT1B-ERG cells were treated with 5 µM compounds or DMSO (control) at 24 hrs in a real-time cell analysis system (xCelligence). Dotted lines: standard deviations (N=3). The horizontal dotted line: level of migration the MDCK cells reached at 48 h in comparison to ERG cells. C) Rate of migration was inhibited in the presence of VPC-1805 (* p-value = 0.03). YK-4-279 was cytotoxic and resulted in no migration. D) Pretreatment of VPC-1805 (10 µM) inhibits the subsequent invasion of PNT1B-ERG spheroids into the surrounding matrix (* p-value < 0.05). YK-4-279 was cytotoxic and resulted in no invasion from day 0.

Results

VPC-18005 inhibits migration of ERG-overexpressing cells in vivo

Figure 11. A) Five days following injection of PNT1B cells (green arrows), only ERG expressing cells invaded and metastasized into the head and tail region of the fish (white arrows). B) Following 5 days of daily treatment, VPC-1805 reduced occurrence of metastasis in zebrafish grafted with PNT1B-ERG and VCAp cells. DMSO versus 1 µM (\(^*\) p-value = 0.03) and 10 µM (\(^*\) p-value = 0.002) VPC-1805.

Figure 12. A) VPC-1805 is soluble and metabolically stable, with a half-life of 19 hours. B) VPC-1805 serum level reached approximately 1 µM in a 4 week oral dosing study in mice (n=3). These mice maintained normal body weight, with no toxicity detected.

Summary

Using a rational drug discovery pipeline, we have developed a new class of anti-ERG chemicals. A representative compound VPC-1805 has been shown to 1) inhibit ERG-driven transcription at low µM concentrations; 2) directly bind to ERG and disrupt its interaction with DNA; 3) inhibit migration and invasion of ERG-expressing PC cells; and 4) reduce metastasis in zebrafish xenograft models. Further assessment of VPC-1805 demonstrated its metabolic stability and oral bioavailability without overt toxicity from prolonged treatment in mice. Future work will focus on optimizing potency of VPC-1805 derivatives through computer-aided medicinal chemistry and establishing in vivo efficacy through mouse xenograft models.

Developing small molecules targeting ERG represents a major unmet opportunity for new PC therapeutics. With available urine tests for ERG-positive PC, future anti-ERG drugs can offer a precision medicine approach to specifically treat these patients.

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