Targeting Kinases Critical for TMPRSS2-ERG Function in Prostate Cancer

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Summer Research Program in Genomics
TMPRSS2-ERG in Prostate Cancer

- TMPRSS2-ERG translocation causes ERG overexpression
- Expressed in 50% of prostate cancers
- Invasion, proliferation, survival
- Targets for prostate cancer therapy
High-throughput screen identifies 40 kinases likely to affect ERG

Potential upstream modulators of ERG

Mechanisms unknown

Several are targets of existing kinase-inhibiting drugs
Methods

Kinase Inhibition

Drug treatment

ERG protein expression

ERG mRNA expression

Downstream target effects

Viability phenotype
PP242 mTOR Inhibition Reduces ERG Protein Expression

- Reduced protein expression with mTOR inhibition by PP242
- No significant change in protein expression with other drugs
Downstream ERG Targets

**ARHGDIB mRNA Expression**

- Control
- pp242 1uM
- pp242 10uM

**PLA1A mRNA Expression**

- Control
- pp242 1uM
- pp242 10uM
PP242 mTOR inhibition downregulates ERG downstream target transcription

**Kinase Inhibition**

- **TMPRSS2**
  - **ERG**
  - **AR**
    - **Testosterone**
      - **ERG mRNA fusion transcript**
      - **ERG Transcription Factor Protein**
        - **Downstream Target Effects**

**Graphs:**

- **ARHGDIB mRNA Expression**
  - Fold Change
  - Treatment: control, pp242 1uM, pp242 10uM

- **PLA1A mRNA Expression**
  - Fold Change
  - Treatment: control, pp242 1uM, pp242 10uM
PP242 mTOR Inhibition Reduces ERG mRNA Expression

Kinase Inhibition

mTOR Inhibition and ERG mRNA Expression

- Control
- pp242 1uM
- pp242 10uM

Fold Change
mTOR Inhibition Reduces Downstream ERG Target Expression

**mTOR Inhibition and ERG mRNA Expression**

**Downstream ERG Targets**

**ARHGDIB mRNA Expression**

**PLA1A mRNA Expression**
Viability Phenotype

Kinase Inhibition

Viability (% of initial) vs. log(drug concentration)

**PP242**

Viability (% of initial) vs. log(drug concentration)

**Torin**

Viability (% of initial) vs. log(drug concentration)
Conclusions

• mTOR inhibition reduces ERG protein levels, increases ERG transcription
  • Post-transcriptional ERG modification

• mTOR is an upstream regulator of ERG
  • Potential therapeutic target for ERG overexpressing tumors
Future Directions

- Determine other phenotypic effects
  - Invasion, serum deprivation

- Confirm result in other mTOR inhibitor drugs

- Mechanism
  - Apply to other transcription factors
## Acknowledgements

<table>
<thead>
<tr>
<th>Hahn Lab</th>
<th>SRPG Program</th>
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<tbody>
<tr>
<td>David Takeda</td>
<td>Bruce Birren</td>
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<td>Francie Latour</td>
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<td>Yaara Zwang</td>
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Questions?
Results

Cell treatment by various kinase inhibitor drugs

Viability by cell titer-glo ATP assay
ERG protein expression by Western Blot
ERG mRNA expression by qRT-PCR
Downstream target mRNA Expression
Mechanism, other mTOR inhibitors, etc.

PP242 mTOR inhibition downregulates ERG protein expression
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VIABILITY PHENOTYPE

ERG transcription factor protein

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HT kinase screen identifies 40 ERG modulators

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Hypothesis:
- If the kinases identified by the shRNA screen are involved in the ERG pathway, then their inhibition by drugs will reduce downstream signaling and phenotype.
Kinase Inhibition and Viability

IC50 Across Cell types

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• Kinase inhibition viability phenotype (toxicity) not specific to ERG
Kinase Inhibition and Viability

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Cell treatment by various kinase inhibitor drugs

Viability by cell titer-glo ATP assay
ERG protein expression by Western Blot
ERG mRNA expression by qRT-PCR
Downstream target mRNA Expression
Mechanism, other mTOR inhibitors, etc.

Toxicity by kinase inhibition is not specific to ERG
Results

- Reduced protein expression at 12, 24 hours
Results

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Results

- Reduced protein expression at 12, 24 hours
- Quantification? Incluir todo con actin?
Results

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PP242 mTOR inhibition downregulates ERG protein expression

Toxicity by kinase inhibition is not specific to ERG
PP242 Increases ERG Transcription

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Results

Cell treatment by various kinase inhibitor drugs

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- ERG mRNA expression by qRT-PCR
- Downstream target mRNA Expression
- Mechanism, other mTOR inhibitors, etc.

Toxicity by kinase inhibition is not specific to ERG

PP242 mTOR inhibition downregulates ERG protein expression

PP242 mTOR inhibition upregulates ERG mRNA expression
Kinase Inhibition and ERG Target Transcription

- PLA1A and ARGHD1B are downstream targets of ERG

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