Disruption of Fibroblast Growth Factor Receptor (FGFR) Signaling as an Approach to Prostate Cancer Gene Therapy

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Clinical Staging of Prostate Cancer

Primary tumor

- No palpable tumor
- Confined to gland
- Capsular invasion
- Extension beyond confines of gland
- Invasion of neighboring structures

Evaluation of local involvement and spread beyond confines of gland follows diagnosis of prostate cancer
• Fibroblast growth factor (FGF) family:
  – includes at least 22 different genes (FGF1-22) encoding related polypeptide mitogens.
  – can stimulate the proliferation of a wide variety of cells.
  – are expressed abundantly in prostate tissue.
  – are required for prostate cells to grow in vitro.
• **FGF Receptors:**
  – located in cell membrane
  – bind FGFs and mediate the action of FGFs.
  – include four high affinity tyrosine kinase receptors (FGFR1-4).
  – involved in the regulation of cell growth, development, and differentiation in a variety of tissues.
  – have increased tyrosine kinase activity in response to binding FGF
  – work in pairs at cell surface
HYPOTHESIS

• Prostate cancer cells are dependent upon FGFR signaling for survival and proliferation.

• Disruption of this signaling pathway by expression of a DN FGFR protein might contribute to death of cancer cells and can be used as gene therapy adjuvant to current treatment options.

• Analysis of gene expression profile in DN FGFR transfected cells might help our understanding of how DN FGFR works and the differentially expressed genes determined by microarray analysis can be used as targets for prostate cancer therapy.
FGFs and their receptors are increased in prostate cancer
Dominant negative FGF receptor

**What is a Dominant Negative Mutation?**

**Answer:** A mutated gene produces a mutated protein that blocks the function of a normal protein.
COLONY FORMATION

DU 145  LNCaP  PC-3

FGFR-1 DN

FGFR-2 DN

pCEP-4 control
FGFR DN markedly decreases phosphorylated FGFR-1

IP with α-PT, probed with α-FGFR1
FGFR DN inhibits proliferation of prostate cancer cells

A. LNCaP

B. DU145
FGFR DN blocks cells at G2/M

A: LNCaP

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<th>48 hrs</th>
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<tr>
<td>LacZ</td>
<td>LacZ</td>
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<tr>
<td>FGFR DN</td>
<td>FGFR DN</td>
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% G2 = 15.3
% G2 = 39.3
% G2 = 15.3
% G2 = 41.5

B: DU145

<table>
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</tbody>
</table>

% G2 = 20.5
% G2 = 31.3
% G2 = 21.4
% G2 = 43.1
FGFR DN treated cells showed more cells at G2
FGFR DN decrease cdc2 kinase activity and increase p21 expression
Treatment of prostate cancer xenografts with AdDN FGFR

![Graphs showing the treatment of prostate cancer xenografts with AdDN FGFR. The graphs compare the number of tumors and mean tumor size at the start and end of treatment for AdGFP and AdDN FGFR.]
Effect of AdFGFR DN on LNCap cells in vivo

- AdDN FGFR
- AdGFP Control

Days: D27, D38, D54, D62, D76, D90

Mean tumor size (mm)

Numbers of mice

Graphs showing the effect of AdFGFR DN on LNCap cells in vivo.
Micro-array Experiment

Prepare cDNA Probe

- Control Cells
- DN FGFR Infected Cells
- Reverse Transcription
- Label with Fluorescent Dyes Cy3 Cy5
- Combine Equal Amounts
- Hybridize probe to microarray

Prepare Microarray

SCAN
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<tr>
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CDC25c ➔ Microarray spot

b-actin ➔ 2.1 fold reduction
FGFR DN down-regulates AKT3
CONCLUSIONS

- FGFs are important survival factors for prostate cancer cells.

- AdDN FGFR inhibits growth of human prostate cancer cell lines LNCaP and DU-145 in vitro and LNCaP in vivo as tested.

- This inhibition is due to the cell cycle arrest at G2 check point. Degradation of cdc25C and up-regulation of p21 molecules in DN FGFR infected cells might contribute to this inhibition.
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