Metastatic Prostate Cancer Patients Treated with GVAX® Vaccine For Prostate Cancer Develop Immunoreactivity to Filamin: Role of Filamin in Prostate Cancer Metastasis

Allogeneic whole cell immunotherapy for prostate cancer

CG1940 and CG8711
PC3 and LNCaP tumor cells, genetically modified to secrete GM-CSF, irradiated for safety, and injected intradermally into patients

Phase 3 clinical trials are ongoing in patients with metastatic hormone refractory prostate cancer
GVAX®: MECHANISM OF ACTION

CG1940/CG8711 → GM-CSF

SKIN

Dendritic cell

Lymphatics

CD8 T Cell

CD4 T Cell

LYMPH NODE

TUMOR (Systemic)

Antibodies

IFN-γ

CTLs

B CELLS
**IDENTIFICATION OF FILAMIN FROM EARLY STUDIES**

- In a Phase 2 trial, sera from 17/59 patients immunized with GVAX® vaccine for prostate cancer, reacted positively in Western Blots to a ~280 kD band in vaccine cell lysates
  - A favorable survival trend was observed in reactive patients compared with non-reactive patients (p = 0.09)

- Post-immunization serum from one complete responder was reactive to multiple proteins in lysates
  - One major seropositive protein was identified as β-Filamin by mass spectrometry
PATIENT SERA IMMUNOREACT WITH VACCINE CELL LYSATES

CG1940

CG8711

Pt. 211  Pt. 306
Pre  Post  Pre  Post

Anti-Filamin

280 kD
NON-MUSCLE FILAMIN ISOFORMS

α-Filamin (ABP-280)  β-Filamin (ABP-278/276)

- 70% homologous excluding hinge domains
- H1 is unique and H2 is 44% homologous
- Both filamin isoforms are proteolytically processed by Calpain
PATIENT SERUM REACTS TO IMMUNOPRECIPITATED FILAMIN

IP

Purified Filamin

280 kD
190 kD

Pt. 211
Anti-Filamin

Pre Post

280 280 C
190 190
90 90
10 10

CALPAIN
Trypsin

N Y
N Y
N Y

N C

Purified Filamin

280 280 C
190 190
90 90
10 10

CALPAIN
Trypsin

N Y
N Y
N Y

N C
FILAMIN EXPRESSION IN PROSTATE CELL LINES CORRELATES WITH METASTATIC POTENTIAL

LNCaP
Carcinoma derived from lymph node metastasis

PC3
Stage IV prostate adenocarcinoma derived from bone metastasis

DU145
Prostate carcinoma derived from brain metastasis

MW
200
116
97
66
45
31

280 kD
90 kD
GAPDH

CELL GENESYS
FILAMIN IS OVEREXPRESSED IN PROSTATE TUMOR TISSUE

Normal Tissue

Tumor Tissue

Prostate

280 kD
190 kD

280 kD
190 kD

**
Sera from two patients immunized with GVAX® reacts with Filamin
- β-Filamin by mass spectrometry
- α- and/or β-Filamin by antibody reactivity

Filamin is over-expressed in prostate tumor compared to normal prostate tissue

Filamin expression is higher in PC3 and DU145 cells compared to LNCaP correlating with the metastatic potential of these cell lines
WHAT IS THE POTENTIAL ROLE OF FILAMIN IN CANCER METASTASIS?
• α-filamin and tissue factor interact in tumor cell migration
  (Ott et al., The Journal of Cell Biology, 1998)

• Calpain cleavage of adhesion and actin binding proteins are implicated in prostate cancer motility
  (Mamoune et al., Cancer Research, 2003)
  (Rios-Doria et al., JBC 2003 and Cancer Research, 2004)
STEPS IN PROSTATE CANCER METASTASIS TO BONE

WORKING MODEL FOR THE ROLE OF FILAMIN IN PROSTATE METASTASIS

DE-ADHESION (PROSTATE) → NON-MOTILE (PROSTATE) → MOTILE (CIRCULATION) → BONE METASTASIS

Cell Adhesion Molecule
FILAMIN
Actin
TGF-β
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