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TITLE: Initiating Events in Prostate Cancer: The Role of Somatic Activation of Beta-Catening

PRINCIPAL INVESTIGATOR: Khashayarsha Khazaie, Ph.D., D.Sc.

CONTRACTING ORGANIZATION: Dana Farber Cancer Institute
Boston, MA 02115-6084

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PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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Murine models of prostate cancer have been developed that rely on the somatic activation of β-catenin. The approach employs Cre-loxP mediated targeted genetic recombination of the Catnb+/-fox(ex3) locus. Expression of Cre was targeted specifically to the prostate secretory epithelium using androgen responsive minimal probasin (PB) or prostate specific antigen (PSA) gene promoters. We were able to demonstrate that the target of transformation by β-catenin in the prostate is the secretory epithelia. We have provided evidence for the benign nature of transformation by β-catenin and the conversion of this benign phenotype to invasive cancer upon heterozygous loss of PTEN. Local inflammatory reactions were shown to be inherently associated with and contribute to the local tumor microenvironment, suggesting a crosstalk between tumor and host immune response that may be contributing to the success of the tumor. Future work will focus on the contribution of the PTEN mutation to tumor progression, the contribution of local inflammatory responses, and studies of downstream targets of β-catenin in the prostate physiology and cancer.
Initiating events in prostate cancer: The role of somatic activation of β-catenin.

**Introduction.**

Histopathological studies of prostate cancers have led to the identification of prostate intra epithelial neoplasia (PIN), a specific type of lesion that represents the primary precursor of human prostate cancer (2). PIN is recognized as a continuum between low-grade and high-grade forms, with high-grade PIN most likely representing the immediate precursor of early invasive carcinoma. Characteristic architectural and cytological features are shared between PIN lesions and early invasive carcinomas including multifocal nature of the lesions, and common chromosomal abnormalities (reviewed in (3)). We have reported characteristic appearance of PINs upon stabilization of β-catenin in the prostate (4). This observation is in line with the deregulation of PI3kinase activity in prostate cancer (5), and recent observations that link PI3kinase activity with the accumulation of β-catenin and stimulation of the androgen receptor signaling (6, 7). In our earlier study, stabilization of β-catenin was achieved through Cre/loxP mediated excision of the third exon of β-catenin. This model was based on the MMTV-LTR driven expression of Cre in a wide range of secretory epithelia, and skin. Preneoplastic lesions were observed only in the prostate, suggesting a specific role for β-catenin in the initiation of prostate cancer. The research underway aims to clarify the role of β-catenin in prostate cancer, its relevance to human prostate cancer, and the cross talk of this signaling pathway with other events associated with human prostate cancer.
Body.

Task 1&2.

To establish the role of β-catenin in the initiation of prostatic intraepithelial neoplasia (PIN) like lesions, & determine targets of oncogenic action of β-catenin.

We have achieved prostate specific expression of Cre under the minimal probasin (PB) promoter (8), or the prostate specific antigen (PSA) promoter. The PSA-Cre mice were described before (9, 10). Both promoters are androgen dependent, while the PSA promoter is also transactivated by β-catenin.

These mice were crossed with Catnb<sup>+</sup>x(ex<sup>3</sup>) mice, and male progeny of 3 months of age were analyzed for prostate lesions. Double transgenic male progenies develop PIN, as revealed by histologic analysis. The PB-Cre mice generate less aggressive and more limited disease, in comparison with the PSA-Cre mice, which appear to have more extensive lesions, with profound inflammatory infiltrates (Fig. 1). The PB-Cre or PSA-Cre mice do not generate lesions in other tissues, and subsequently the mice are otherwise in good health. Loss of the adenomatous polyposis coli (APC) gene is considered to be...
the genetic cause of human colon cancer. In contrast to frequent APC defects, mutations affecting the β-catenin gene are rare in colon cancer. These facts suggest that although the stabilization of β-catenin efficiently promotes pre-neoplasia, by itself it may not be sufficient to cause malignancy. In a collaborative study with Dr. Fotini Gounari we have begun to gain insight into this issue, by comparing the impact of mutations that lead to the loss of APC with those that directly stabilize β-catenin. By introducing the corresponding Cre dependent conditional mutations in lymphocytes, we have shown that loss of APC leads to blockade of mitosis and that escape from this block requires aberrant chromosome segregation. As a result dividing cells that are deficient for APC are inherently genetically unstable. In contrast, direct stabilization of β-catenin leads to aberrant proliferation and differentiation of immature lymphocytes. These observations indicate that genetic instability associated with the loss of APC may be a critical factor in promoting the malignant transition of the lesions, and that the stabilization of β-catenin in the presence of intact APC function may therefore not be sufficient for tumor progression (manuscript submitted).

We are currently comparing prostate specific loss of APC with stabilization of β-catenin to gain further insights into the role of genomic instability in tumor progression in the prostate. So far, we have confirmed occurrence of PINs in mice that have lost APC function in a prostate specific manner. Further work is underway to compare proliferation and genetic stability of prostate epithelial cells in the two types of mice, and to relate these to the ability of the lesions to progress to malignancy.

The lesions caused by PSA-Cre appear to be massively infiltrated with inflammatory cells. We had earlier reported the use of protease activatable fluorescent probes for imaging of adenomatous polyps (1). The presence of proinflammatory leukocytes in the prostatic lesions encouraged us to apply similar imaging techniques to the prostate. To this aim 3-7 months old PSA-Cre x Catnb+/-lox(ex3) mice were injected with Cathepsin-B sensitive probe with emission maximum of 694, 24 hours in advance. The next day the mice were anesthetized, injected with a fluorescent polymer that emits at 750 nm and reveals blood vessels, and prostates were imaged by intra vital fluorescent confocal microscopy using a x4 lens. The imaging analysis revealed in situ Cathepsin activity in diseased prostates, with abundant neo-angiogenesis supplying the lesions (Fig. 2). To reveal the cellular source of the proteolytic activity in the lesions we prepared single cell suspensions of the entire diseased prostates by limited collagenase digestion, and separated the epithelial from the leukocyte cell populations using percoll gradient centrifugation. Cell surface staining and FACS analysis revealed CD11b+Gr1+ cells as the cellular source of the Cathepsin signal. These observations suggest that stabilization of β-catenin and initiation of prostate cancer coincide with local inflammatory reactions that contribute to the elevated proteolytic activity of the tumor microenvironment.

Task 3.
To test the hypothesis that β-catenin induced lesions can progress to carcinomas.

The cooperation of PTEN/Akt-1 pathway with β-Catenin.

Lesions produced by the stabilization of β-catenin in the prostate are preneoplastic and non-invasive, suggesting that additional genetic mutations may be required for tumor progression. PTEN (Phosphatase and tensin deleted on chromosome 10) mutations have been frequently associated with progression in prostate cancer, and are present in 30% of
primary (11) (12) and 63% of metastatic prostate cancers (13). In mice, prostate specific loss of PTEN predisposes the animals to metastatic prostate cancer (14) (15) (16). Interestingly, genetic deficiency in PTEN in humans is associated with at least two hereditary hamartoma disorders, Cowden's syndrome (CS) and Bannayan-Riley-Ruvalcaba syndrome (BRRS). These are typically diseases of inflammatory nature with predisposition to cancer. It was therefore postulated that deficiency in PTEN may promote malignant progression of the β-catenin induced PINs, and that the progressive tumors would have an inflammatory nature. To test this, we introduced a heterozygous PTEN deficient allele in PBCre x Catnb\({\text{+/lox(ex3)}}\) mice. At 3 months of age mice containing all three mutations harbored invasive prostate cancer with a strong inflammatory component (Fig. 3). Imaging of the mice with Cathepsin activated fluorescent probes revealed strong proteolytic activity within the prostate. Imaging of the vasculature feeding the lesions revealed abnormally expanded and thin walled vessels, characteristic of tumor vasculature. Rapid leakage of the imaging probe indicated leakiness of the vessels, which is also characteristic of blood vessels in advanced tumors (Fig. 4).

Figure 3. Progression from benign PIN in PBCre x Catnb\({\text{+/lox(ex3)}}\) mice to invasive carcinoma in PBCre x Catnb\({\text{+/lox(ex3)}}\) x PTEN mice. a) typical PIN lesions in the prostate of double transgenic mice at 3 months of age, b) and c) histologies of prostates from two independent PBCre x Catnb\({\text{+/lox(ex3)}}\) x PTEN mice of 3 months of age. Note absence of inflammation in (a) but profuse inflammatory infiltrates in (b) and (c). Thin arrow point to tumor, thick arrows to inflammatory infiltrates.
These observations are consistent with deregulation of β-catenin in primary prostate cancer, and with the loss of PTEN contributing to tumor progression. Furthermore they implicate inflammation in the process of tumor progression. Recent works from several laboratories have suggested that inflammation may play a determining role in facilitating the growth and progression of tumors. In transgenic models of skin cancer inhibition of proteolytic activity associated with tumor infiltrating pro-inflammatory leukocytes, or depletion of CD4+ T cells leads to significant delay and suppression of tumorigenesis (17-19). These reports implicate host inflammatory responses in the acceleration of tumor progression. In mouse models of colon cancer CD4+CD25+ immune suppressor cells play a significant role in slowing down the growth of adenomas (20, 21). Our unpublished results suggest that this may be due to the suppression of innate inflammatory responses to the intestinal lesions. The major components of these inflammatory reactions are macrophages, neutrophils and mast cells, the same cells seen to infiltrate prostatic lesions in our studies. Altogether, the reports and our unpublished observations suggest that stabilization of β-catenin and the cooperative effect of PTEN lead to the progressive growth of invasive prostate cancer, and that the extent of inflammation in the lesions may be predictive of the advanced/malignant nature of the lesion. We are currently testing these hypotheses by crossing our mice to Cathepsin-B deficient mice, as well as by manipulating the host responses to the tumors.
Key research accomplishments (2004-2005).
- It was demonstrated that stabilization of β-catenin in the prostate secretory epithelia, is responsible for the initiation of PIN.
- It was demonstrated that these lesions in the mouse do not progress to malignancy at least with the first 1/3 life span of the mouse.
- Preliminary observations suggest that the inability of the above lesions to progress may be related to the genetic integrity of aberrant cells; in contrast loss of APC leads to genetic instability.
- Stabilization of β-catenin was linked to local inflammatory reactions.
- Malignant progression of the β-catenin induced lesions was achieved by introducing a heterozygous defect in PTEN.
- Tumor progression was linked with increased inflammation and local proteolytic activity.

Reportable outcomes.
The first year focused our attention on improving and characterizing the animal models, as well as relating our observations to human prostate cancer. In the second year, we established the benign nature of transformation by β-catenin and addressed the possibility that this may be related to the genetic integrity of the lesions. We provided evidence for the cooperation of β-catenin signaling with PTEN deficiency in promoting malignant progression of prostate cancer. Furthermore, we linked tumor progression with local inflammatory reactions.

Conclusions.
Using different transgenic mice we were able to demonstrate that the target of transformation by β-catenin in the prostate is the secretory epithelia. We have provided evidence for the benign nature of transformation by β-catenin and the conversion of this benign phenotype to invasive cancer upon heterozygous loss of PTEN. Local inflammatory reactions are inherently associated with and contribute to the local tumor microenvironment, suggesting a crosstalk between tumor and host immune response that may be contributing to the success of the tumor.
Future work will focus on the contribution of the PTEN mutation to tumor progression, the contribution of local inflammatory responses, and studies of downstream targets of β-catenin in the prostate physiology and cancer.

References


# BIOGRAPHICAL SKETCH

**NAME**

Khashayarsha Khazaie

**POSITION TITLE**

Assistant Professor, Ph.D. D.Sc.

## EDUCATION

<table>
<thead>
<tr>
<th>INSTITUTION AND LOCATION</th>
<th>YEAR</th>
<th>DEGREE CONFERRED</th>
<th>FIELD OF STUDY</th>
</tr>
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<tbody>
<tr>
<td>University of Surrey, Guildford, Surrey, U.K.</td>
<td>B.Sc.</td>
<td>1978</td>
<td>Medical Biochemistry</td>
</tr>
<tr>
<td>National Institute for Medical Research, London, U.K.</td>
<td>PhD</td>
<td>1982</td>
<td>Genetics/Aging</td>
</tr>
<tr>
<td>Rene Descartes University, Paris FR</td>
<td>D.Sc.</td>
<td>1998</td>
<td>Tumor Biology</td>
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## ACADEMIC APPOINTMENTS

- **2003-** Assistant Professor of Immunology, Molecular Imaging/Radiology, MGH, Harvard Medical School. Boston, MA
- **1999-2003** Instructor of Pathology, Harvard Medical School. Boston, MA
- **1998-1999** Visiting Faculty, University of Paris V Medical School, Université René Descartes, Paris, France.
- **1989-1998** Research Group Leader, Division of Tumor Immunology, German Cancer Research Center, Heidelberg, Germany.

## HOSPITAL APPOINTMENTS

- **2002-** Senior NRSA Fellow in Cancer Immunology, Dana Farber Cancer Institute.
- **1999-2003** Instructor, Department of Cancer Immunology & AIDS, Dana-Farber Cancer Institute.
- **1998-1999** Visiting Senior Scientist, National Institute of Science and Medicine Research, Department of Médecine Necker-Enfants Malades, Paris, France.

## POSTDOCTORAL TRAINING

- **1987-1989** Research Fellow, Laboratory of Molecular and Cellular Immunology Department of Medicine Lyon, France.
- **1985-1987** Research Fellow, Differentiation Program, European Molecular Biology Laboratory Heidelberg, Germany.
- **1982-1985** Research Fellow, Department of Gene Structure and Expression, National Institute for Medical Research, London, U.K.

## MEMBERSHIP OF SOCIETIES AND PRIVILEGES:

- **1998-** Mentoring Privilege (HDR), Faculty of Medicine Necker-Enfants Malades, University of Paris, France.
- **2000-** Member of Harvard Cancer Center: Cancer Immunology, Cancer Imaging, GI Malignancies Program.
- **2004-** Member of German Society for Immunology (DGFI)
- **2005-** Member of Committee for Immunology, Harvard Medical School
- **2005-** Active Member of New York Academy of Sciences

## Adhoc Reviewer:


## ORIGINAL REPORTS:


Reviews, Chapters, and Editorials,


Khazaie, K. 1996. The role of EGF receptor in the initiation and progression of malignancy. In EGF Receptor In Tumor Growth And Progression, Edited by R. Lichtner and T. Harkins, pp. 166-180, Springer-Verlag Heidelberg


Research projects completed or ongoing in the last three years (PI: K. Khazaie)

Ongoing Research Support (PI: K. Khazaie)

Idea Award. May 2002 - May 2005
DAMD17-02-1-0361, Department of Defense Breast Cancer Research Program,
Title: Cancer Immunology in an inducible model of breast cancer.
Major goals: To develop an animal model of inducible mammary cancer, and to use this model to study antigen specific immune responses against the mammary gland and mammary tumors.

Idea Award. 2003-2006
DAMD17-03-1-0210, Department of Defense Prostate Cancer Research Program,
Title: Initiating events in prostate cancer: The role of somatic activation of β-catenin.
Major goals: To evaluate the stabilization of β-catenin as an initiating event in prostate cancer.

Dana Farber Cancer Institute
Title: Mast cells and orchestration of local inflammatory reactions in colon cancer.
Major goals: To investigate the cross-talk between tumor epithelium and infiltrating mast cells.

RO1-CA104547-01A1 2004-2009
Title: Imaging Proteolytic Activity in Colon Cancer
Major goals: Image proteolytic activity in animal models of colon cancer, and apply imaging to detection of tumor status and biological response.

Completed Research Support

Senior National Research Council Award Jan 2003 – Aug 2004
National Cancer Institute
(Salary Support only)

Inter-programmatic Research Award 2002-2004
Dana Farber Cancer Institute/Harvard Cancer Center
Title: An animal model for investigating immunosurveillance and immunotherapy in prostate cancer.
Major goals: To develop an animal model of prostate cancer based on the prostate specific activation of the APC/β-catenin pathway, and to use this model to study antigen specific immune responses against the prostate.

National Colorectal Cancer Research Alliance. (Award) 2001
Entertainment Industry
Title: An Animal Model for Designing Targeted Immune Intervention in Colon Cancer.
Major goals: To Investigate antigen specific immune responses in the healthy and neoplastic mouse intestine.

Hershey Prostate Cancer/Survivors Walk. (Award) 2001
Beth Israel Hospital
Title: somatic activation of β-catenin reveals a critical event in the initiation of prostate cancer.
Major goals: To Investigate the role of β-catenin in prostate cancer.

Applied for

RO1-CA112348-01 re-submitted in Nov 2004
Title: Inflammation in Colon Cancer: A Cause or consequence?
Major goals: Define the role of innate immune response in the initiation of polyposis and in progression of invasive carcinomoma.