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TITLE:  The Role of beta-TrCP Ubiquitin Ligase Receptor in the Development of Breast Cancer

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Beta-TrCP ubiquitin ligase receptor is required for activation of anti-apoptotic transcription factor NF-kappaB. Beta-TrCP activities are essential for v-Ras-mediated transformation of cells. As beta-TrCP proteins are pivotal to activation of the NF-kappaB pathway, up-regulation of NF-kappaB transactivation via an increase in beta-TrCP levels and activities may contribute to malignant transformation of cells. Under these conditions, an elevated expression of beta-TrCP is expected to promote cell transformation. Anti-apoptotic effect of NF-kappaB is suggested among the mechanisms implicated in NF-kappaB-driven transformation. NF-kappaB has been shown capable of blocking apoptosis induced by TNFalpha, ionizing radiation, or the chemotherapeutic agents. Inhibition of NF-kappaB activities dramatically potentiates apoptosis of cancer cells induced by various pro-apoptotic stimuli. These and other data indicate that NF-kappaB inhibiting agents could become useful adjuvants in anti-tumor therapies. We hypothesize that beta-TrCP activities are essential for development of breast cancer. To this end we will employ new transgenic mice with inducible dominant negative beta-TrCP2 (dn-bTrCP2) in mammary tissues in breast carcinogenesis model and determine whether inhibition of beta-TrCP function will abrogate development of breast tumors. Since beta-TrCP mediates ubiquitination and degradation of IkappaB in response to IKK-inducing stimuli, identifying the mechanisms of beta-TrCP function in mouse mammary tumors may potentially lead to design of the agents capable of inhibiting beta-TrCP function and effective for cancer prevention and therapy. The result of this study may significantly contribute to our understanding of the development of human breast tumors.
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INTRODUCTION:

Beta-transducin repeats-containing proteins (β-TrCP) serve as the substrate recognition subunits for the SCF$^{\beta\text{-TrCP}}$ E3 ubiquitin ligases. These ligases ubiquitinate specifically phosphorylated substrates and play a pivotal role in the regulation of cell division and various signal transduction pathways, which, in turn, are essential for many aspects of tumorigenesis. β-TrCP is known to mediate degradation of proteins, which exhibit anti-growth or pro-apoptotic properties (such as Emi1, Cdc25a, IκB, IFNAR1), whereas β-TrCP-mediated degradation of proteins which promote cell growth and survival (e.g., β-catenin, PRLR) is often impaired in tumors via inhibition of specific phosphorylation (reviewed in (1)). β-TrCP is required for activation of anti-apoptotic transcription factor NF-κB by activated v-Ras and v-RAF. β-TrCP activities are essential for v-Ras-mediated transformation of NIH/3T3 cells (2). As β-TrCP proteins are pivotal to activation of the NF-κB pathway, up-regulation of NF-κB transactivation via an increase in β-TrCP levels and SCF$^{\beta\text{-TrCP}}$ activities may contribute to malignant transformation of mammary epithelial cells (in addition to activation of IKK). Under these conditions, an elevated expression of β-TrCP is expected to promote cell transformation via activation of NF-κB-dependent survival pathways. Over-expression of β-TrCP2 was observed in most of the cell lines derived from human breast tumors as compared to the non-transformed cell lines, and tumor tissue samples from the patients with primary breast cancers (2). β-TrCP1/2 expression and activities are induced by oncogenes which activate MAPK pathway (including v-Ras and v-RAF) (2) and by Wnt/β-catenin signaling pathway (3, 4) and these pathways are often activated in breast cancers. Recent data also demonstrate that inhibition of β-TrCP activities promotes the sensitivity of breast cancer cells to anti-growth and pro-apoptotic effects of anti-cancer drugs (5). In addition, mammary glands of βTrcp1(-/-) female mice display a hypoplastic phenotype; conversely, the mammary epithelia of MMTV-βTrcp1 mice proliferate more and show increased NF-κB DNA binding activity and higher levels of nuclear NF-κB p65/RelA (6). β-TrCP plays an important role in protecting human breast cancer cells from apoptosis. Thus, we are interested to determine whether β-TrCP function is essential in mouse mammary carcinogenesis. We hypothesized that β-TrCP activities are essential for development of breast cancer. To this end we will employ new transgenic mice with inducible dominant negative β-TrCP2 (dnβ-TrCP2) in mammary tissues in a standard DMBA breast carcinogenesis model and determine whether inhibition of β-TrCP function will abrogate development of breast tumors.

BODY:

Task 1. To generate K5.rTA; TRE-dn-beta-TrCP2 double transgenic animals, we have crossed K5.rTA+/− and TRE-dn-beta-TrCP2+/+ mice. The progeny was genotyped for the presence of K5.rTA transgene, and TRE-dn-beta-TrCP2 transgenes using PCR analysis and Southern blot hybridization. Non-transgenic littermates from the same breeding were selected to use as controls in DMBA experiments. The inducibility of transgene expression was analyzed in the mammary gland of transgenic animals after treatment with Doxycycline (Figure 1).

As a result of this task, we have had sufficient number of K5.rTA; TRE-dn-beta-TrCP2 double transgenic mice for carcinogenesis experiments.
Task 2. To test whether over-expression of dominant negative beta-TrCP2 suppresses mammary carcinogenesis, we treated 56-day old virgin female K5-rTA, TRE-dnβ-TrCP2 double transgenic mice, along with non-transgenic (FVB) virgin female mice with DMBA by gavage (70 mg g\(^{-1}\) body weight) once a week for 6 weeks. To induce the expression of dominant negative beta-TrCP2 in mammary epithelium half of the animals of each genotype started receiving Doxycycline in drinking water at the time of first DMBA treatment, and continued receiving Doxycycline for the entire duration of experiment. The experimental groups will be as follows: i) double transgenic mice treated with DMBA, no Doxocycline; ii) double transgenic mice treated with DMBA, and Doxocycline; iii) non-transgenic littermates treated with DMBA, no Doxocycline; iv) non-transgenic littermates treated with DMBA, and Doxocycline. The animals were palpated twice a week for tumor appearance. Although higher than expected DMBA toxicity in mice with FVB genetic background did not allow enough time for mammary tumors to grow, we observed significant effect of transgene expression on DMBA-induced mammary hyperplasia. Histopathology revealed that Doxycycline-induced expression of dnβ-TrCP2 inhibited DMBA-induced hyperplasia in the mammary glands of K5-rTA, TRE-dnβ-TrCP2 double transgenic mice (Figure 2). The analysis of mammary glands revealed reduced nuclear accumulation of p65 in the animals with induced expression of dnβ-TrCP2 confirming that the function of βTrCP is abrogated in mammary epithelia of these mice. These data suggest that the function of βTrCP is important for early stages of mammary carcinogenesis.

The results of this task determined that inhibition of beta-TrCP function inhibits NF-κB activity and abrogates early stages of breast tumors development. K5-rTA, TRE-dnβ-TrCP2 double transgenic mice may be used as a model system to investigate the effect of β-TrCP inhibition on tumorigenesis driven by variety of oncogenes in mammary gland. The future studies will determine whether inducible expression of dnβ-TrCP2 is capable of inhibiting mammary tumorigenesis at the later stages of tumor progression.

**KEY RESEARCH ACCOMPLISHMENTS:**

- We have developed double transgenic mice with inducible expression of dominant negative β-TrCP2 in mammary epithelia.
- Induction of dominant negative β-TrCP2 expression in mammary epithelia inhibited activity of endogenous β-TrCP and resulted in reduced nuclear accumulation of p65/RelA member of NF-κB family of transcription factors.
- Induction of dominant negative β-TrCP2 expression in mammary epithelia attenuated DMBA-induced hyperplasia.
- K5-rTA, TRE-dnβ-TrCP2 double transgenic mice may be used as a model system to investigate the effect of β-TrCP inhibition on tumorigenesis driven by variety of oncogenes in mammary gland.
REPORTABLE OUTCOMES:

We have developed an animal model that can be used to study the function of β-TrCP ubiquitin ligase in mammary glands under variety of physiological and pathophysiological conditions (i.e. tumorigenesis, development, etc.).

The manuscript describing the result of this study is currently in preparation.

CONCLUSION:

The result of this study significantly contributed to our understanding of the development of human breast tumors. Anti-apoptotic effect of NF-κB is suggested among the mechanisms implicated in NF-κB-driven transformation. NF-κB has been shown capable of blocking apoptosis induced by TNFα, ionizing radiation, or the chemotherapeutic agents. Inhibition of NF-κB activities dramatically potentiates apoptosis of cancer cells induced by various pro-apoptotic stimuli. These and other data indicate that NF-κB inhibiting agents could become useful adjuvants in anti-tumor therapies. The results of this study determined that inhibition of beta-TrCP function abrogate early stages of breast tumor development. Our studies provide a rational for the development agents capable of inhibiting β-TrCP function and effective for cancer prevention and therapy.

K5-rTA, TRE-dnβ-TrCP2 double transgenic mice may be used as a model system to investigate the effect of β-TrCP inhibition on tumorigenesis driven by variety of oncogenes in mammary gland. The future studies will determine whether inducible expression of dnβ-TrCP2 is capable of inhibiting mammary tumorigenesis at the later stages of tumor progression. We are planning to cross these mice with TRE-neu mice to determine whether βTrCP activities are essential for development of breast cancer using a mouse model with inducible co-expression of Neu and dominant negative βTrCP in mammary tissues.

REFERENCES:


APPENDICES:
Curriculum vitae is attached.

SUPPORTING DATA:

Figure 1. Induction of transgene expression in mammary gland.

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<th>dox</th>
<th>H</th>
<th>β-</th>
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<td>+</td>
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K5.rTA; TRE-HA-dn-beta-TrCP2 double transgenic animals were treated with 2 mg/ml of Doxycycline in 5% sucrose in drinking water for 7 days. dn-beta-TrCP2 expression was analyzed by immunoblot with HA-specific antibody (Santa Cruz Biotechnology).

Figure 2. Over-expression of dn-beta-TrCP2 inhibits DMBA induced mammary hyperplasia.

<table>
<thead>
<tr>
<th>WT + dox</th>
<th>Tg + dox</th>
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200x magnification

H&E staining of representative mammary glands.
Figure 3. Over-expression of dn-beta-TrCP2 inhibits NF-κB activity in mammary epithelia.

p65 immunostaining was performed as described previously (7). Arrows indicate cytoplasmic localization of p65 in the mammary epithelia of double transgenic animals expressing dn-beta-TrCP2.
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Education:

M.D.; 1993; Russian State Medical University, Moscow, Russia

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Postdoctoral Training

1996-1997 MD Anderson Cancer Center, Science Park Research Division, Department of Carcinogenesis, Smithville TX

1997-1998 AMC Cancer Research Center, Center for Cancer Causation and Prevention, Lakewood, CO
Awards and Honors:


1991 Harvard University, Cambridge MA, Travel Award for the Soviet-American Medical Student Exchange Program

1992 Travel Award of the International Medical Student Association, Aarhus, Denmark


1999 Aspen Cancer Conference Young Investigator Award and Fellowship

2000 "International Skin Carcinogenesis Conference”, Tuscon, AZ. Invited speaker


2002 Aspen Cancer Conference Young Investigator Award and Fellowship


2006 Aspen Cancer Conference Young Investigator Award and Fellowship

2007 National Cancer Center Research Institute, Tokyo, Japan. Invited speaker

2007 International Symposium on Tumor Biology, Kanazawa, Japan. Invited speaker

Lectures:


2001 Regulation of βTrCP ubiquitin ligase receptor. Division of Medical Oncology, Department of Medicine, University of Colorado Health Science Center, Denver, CO. October 30, 2001 (Invited speaker).

2002 Induction of HOS ubiquitin ligase receptor by mitogen signaling and its role in cell transformation. Division of Endocrinology and Program in Hormonal Cancers Research Conference, University of Colorado Health Science Center, Denver, CO. September 25, 2002 (Invited speaker).


2003 The Role of SCF<sup>HOS</sup> Ubiquitin Ligase in Skin Carcinogenesis. Department of Dermatology, University of Wisconsin, Madison, WI. April 2, 2003 (Invited speaker).

2003 The Role of SCF<sup>HOS</sup> Ubiquitin Ligase in Skin Carcinogenesis. Department of Dermatology, University of Michigan, Ann Arbor Mi. (Invited speaker).


2005 The Regulation of β-TrCP Ubiquitin Ligase Receptor. Molecular and Cellular Pharmacology Graduate Program. University of Wisconsin, Madison, WI (Invited speaker).

2006 Mechanisms of stabilization of β-TrCP1 and c-myc mRNA in response to Wnt/β-catenin signaling. University of Texas Health Science Center at San Antonio, San Antonio Cancer Institute, SACI Seminar Series (Invited speaker).

2006 The Regulation of β-TrCP Ubiquitin Ligase Receptor. Molecular and Cellular Pharmacology Graduate Program. University of Wisconsin, Madison, WI (Invited speaker).

2007 Mechanisms of stabilization of β-TrCP1 and c-myc mRNA in response to Wnt/β-catenin signaling. International Symposium on Tumor Biology, Kanazawa, Japan. (Invited speaker)

2007 Mechanisms of stabilization of β-TrCP1 and c-myc mRNA in response to Wnt/β-catenin signaling. National Cancer Center Research Institute, Tokyo, Japan (Invited speaker).


2007 The Role of GLI2 Transcription Factor in Prostate Tumorigenesis. UW O’Brien Urology Research Center annual symposium, May 4, 2007 (Invited speaker).
Positions and Appointments:

1989-1993  Senior Lab Assistant, N.N.Blokhin Cancer Research Center, Institute of Carcinogenesis, Moscow, Russia

1990-1991  Senior Lab Assistant, Engelgardt Institute of Molecular Biology Russian Academy of Sciences, Moscow, Russia

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1997-1998  Senior Program Associate, AMC Cancer Research Center, Center for Cancer Causation and Prevention, Lakewood, CO.

1998-2004  Associate Scientist, AMC Cancer Research Center, Center for Cancer Causation and Prevention, Lakewood, CO.

2004-present  Assistant Professor, University of Wisconsin, Department of Dermatology, Madison, WI.

Grant Review Committees:


Department of Defense, PCTA-1, Prostate Cancer Panel, 2006.

Dutch Cancer Society

Academy of Sciences of the Czech Republic

Ad hoc Reviewer for:

Molecular and Cellular Biology
The Journal of Biological Chemistry
Cancer Research
EMBO reports
International Journal of Cancer
Genomics
Cancer Letters
Molecular Carcinogenesis
**Editorial Board Member:**

International Journal of Biological Chemistry  
International Journal of Cancer Research

**Teaching Experience:**

Supervised several M.S., M.D. and Ph.D. students.  
Thesis advisor for Ph.D. candidate (University of Wisconsin METC program).  
Serve on Thesis Committee of three Ph.D. candidates (University of Wisconsin METC program)  
Supervised undergraduate students in Undergraduate Research Scholar program at the University of Wisconsin  
Have supervised 5 post doctoral fellows.  
Taught the genetics section of Introductory Biology - Bio 151 to undergraduate students.

**Support:**

**Current**

RO1 (Spiegelman)  
NIH NCI  
The Role of CRD-BP-regulated mRNA stability in colorectal cancers.  
2007-2012

RO1 (Spiegelman – Co-PI)  
NIH NCI  
GR impairment in carcinogenesis: tumor suppressor role.  
2005-2010

RO1 (Spiegelman – Co-PI)  
NIH NCI  
Stability of prolactin receptor and prolactin signaling in breast cells.  
2006-2011

RSG-02-140-01-CNE- (Spiegelman)  
American Cancer Society  
Analysis of SCF^{HOG} ubiquitin ligase in skin carcinogenesis.  
2002-2007

Breast Cancer Research Program 2004, (Spiegelman)  
Concept Award  
Agency: Department of Defense  
The role of beta-TrCP ubiquitin ligase receptor in the development of breast cancer.  
2005-2007

University of Wisconsin Medical School Medical Education and Research Committee; The Wisconsin Partnership Fund for a Healthy Future (Spiegelman) 2006-2007  
New Investigator Program Grant  
Gli2 protein stabilization in the activation of Hedgehog signaling pathway in prostate cancer.  
2004-2007

University of Wisconsin Startup Package  
2004-2007
Pending:

Prostate Cancer Research Program 2007, (Spiegelman) 2007-2010
Idea Development Award
Agency: Department of Defense
The Role of GLI2 Transcription Factor in Prostate Tumor Development

Past
ACS IRG/UCCC (Spiegelman) 2002-2003
CANCER RESEARCH SEED
Analysis of novel murine ubiquitin ligase receptor, mHOS, its role in NF-κB activation and skin carcinogenesis.

Oncology Sciences Corporation, Inc. (Spiegelman) 2002-2004
Assay for identifying specific SCF^{HOS} ubiquitin ligase inhibitors

Cancer League of Colorado, Inc (Bhatia – fellowship) 2002-2003
Analysis of β-catenin/Tcf signaling in skin carcinogenesis.

Publications:

Peer Reviewed


Invited reviews

Invited book chapter

Non Peer Reviewed


