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AWARD NUMBER: W81XWH-05-1-0415

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

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REPORT DATE: June 2006

TYPE OF REPORT: Annual

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
01-06-2006	Annual	20 Jun 2005 – 19 Jun 2006

4. TITLE AND SUBTITLE

The Role of Beta-TrCP Ubiquitin Ligase Receptor in the Development of Breast Cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-05-1-0415

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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E-Mail:

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Wisconsin
Madison, Wisconsin 53706-1490

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
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-κB-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.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	UU	5	**19b. TELEPHONE NUMBER** *(include area code)*

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INTRODUCTION:

Beta-transducin repeats-containing proteins (β-TrCP) serve as the substrate recognition subunits for the $SCF^{\beta TrCP}$ 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-TrCP2 expression and activities are induced by oncogenes which activate MAPK pathway (including v-Ras and v-RAF), and this pathway is often activated in breast cancers (2) .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 (*3*). 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 (4). 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 **hypothesize** 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:

We have encountered unanticipated problems with the breeding of our transgenic mice. Although we had re-established our breading colony, and are now working to generate K5.rTA; TRE-dn-beta-TrCP2 double transgenic animals (Task 1), the tumor experiments proposed in the Task 2 had to be delayed. The no-cost extension approved by US Army Medical Research and Material Command will allow us to complete the Task 2 in which we will establish the role of $SCF^{\beta TrCP}$ ubiquitin ligase in mammary tumorigenesis.

KEY RESEARCH ACCOMPLISHMENTS: N/A

REPORTABLE OUTCOMES: N/A

CONCLUSION: N/A

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APPENDICES: N/A

SUPPORTING DATA: N/A