AD_____

Award Number: W81XWH-04-1-0388

TITLE: The Role of SnoN and Ski in Mammary Epithelial Cell Transformation

PRINCIPAL INVESTIGATOR: **Deng Pan**

CONTRACTING ORGANIZATION: University of California
Berkeley, CA 94720

REPORT DATE: July 2008

TYPE OF REPORT: Annual Summary

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)*
July 2008	Annual Summary	2Dec2007 - 1Jul2008

4. TITLE AND SUBTITLE

The Role of SnoN and Ski in Mammary Epithelial Cell Transformation

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-04-1-0388

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Deng Pan, Kunxin Luo

email: Dengpan@berkeley.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

University California
Berkeley, CA 94720

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, MD 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

The transforming growth factor-β (TGFβ), signaling through the Smad proteins, regulates a wide variety of cellular processes including proliferation, survival, cell-matrix interaction, differentiation and plays a complex role in mammalian tumorigenesis. SnoN and Ski are critical negative regulators of TGFβ signaling by binding to and repressing the activities of the Smad proteins. Our previous work have shown that SnoN and Ski expression is often elevated in breast cancer cells and they play both pro-oncogenic and anti-oncogenic functions in breast cancer development. In this study, we explored the anti-oncogenic activity of SnoN and showed that high levels of SnoN induced premature senescence in mammalian epithelial cells. SnoN interacted with the Promyelocytic leukemia (PML) protein and was recruited to the PML nuclear bodies where it stabilizes p53, leading to premature senescence. Furthermore, overexpression of SnoN inhibits oncogenic transformation induced by Ras and Myc in vitro and significantly blocks papilloma development in vivo in a carcinogen-induced skin tumorigenesis model. The few papillomas that were developed displayed high levels of senescence and spontaneously regressed. Our study has revealed a novel Smad-independent pathway of SnoN function that mediates its anti-oncogenic activity

15. SUBJECT TERMS

None Listed

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

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Table of Contents

Page

Introduction:

The transforming growth factor-β (TGFβ) regulates a wide variety of cellular processes including proliferation, survival, cell-matrix interaction, differentiation and plays a complex role in mammalian tumorigenesis. TGFβ signals through the Smad proteins (Smad2, Smad3) by inducing their phosphorylation and their hetero-oligomerization with Smad4, resulting in accumulation in the nucleus, where they regulate expression of TGFβ-responsive genes. We and others have shown that Ski and SnoN bind to Smad2, Smad3 and Smad4 and repress their ability to activate TGFβ target genes through disruption of the functional R-Smad/Smad4 complex, recruitment of a transcriptional co-repressor complex and blocking the binding of transcriptional co-activators to Smad2/3. In mammals, Ski and SnoN are expressed in all adult cells and tissues at a low level, and this expression is altered in many human cancer cells (1).

The long-term goal of this project is to determine the role of SnoN and Ski, repressors of TGFβ signaling, in mammary epithelial cell transformation. To this end, we had compared expression levels of SnoN and Ski in nontumorigenic versus malignant mammary cells. By perturbing the relative levels of SnoN and Ski in untransformed and malignant mammary cells, we showed that Ski and SnoN promote breast cancer cell proliferation but inhibit tumor cell EMT and metastasis (2). Thus, Ski and SnoN play both pro-oncogenic and anti-oncogenic roles in breast cancer growth and progression. The pro-oncogenic activities of these proteins are likely mediated by their ability to antagonize Smad signaling. However, the molecular basis for the anti-oncogenic role of SnoN and Ski has not been defined.

Body:

In mammary epithelial cells, we found that numerous attempts by us to overexpress the Ski or SnoN proteins resulted in inhibition of cell proliferation and eventual cell death. This is also consistent with SnoN possessing an anti-oncogenic activity. To further understand how SnoN exerts anti-tumorigenic functions and whether this activity is dependent on the Smad pathway, we generated a knockin mice expressing a mutant SnoN that is defective in binding to the Smad proteins and thus cannot antagonize Smad signaling. This mouse strain thus allows us to examine the Smad-independent activities of SnoN. Interestingly, mouse embryonic fibroblasts (MEF) isolated from this mouse strain showed premature senescence. A detailed examination of these cells showed that the expression level of SnoN was significantly elevated, and this elevated SnoN expression was the cause of senescence. Interestingly, high-level expression of WT SnoN also led to senescence, and this could explain our long-standing observation that it is impossible to stably overexpress SnoN in human epithelial cell lines. Subsequently we found that SnoN interacted with the Promyelocytic leukemia (PML) protein and was recruited to the PML nuclear bodies where it stabilizes p53, leading to premature senescence. Furthermore, overexpression of SnoN inhibits oncogenic transformation induced by Ras and Myc in vitro and significantly blocks papilloma development in vivo in a carcinogen-induced skin tumorigenesis model. The few papillomas that were developed displayed high levels of senescence and spontaneously regressed. Our study has revealed a novel Smad-independent pathway of SnoN function that mediates its anti-oncogenic activity.

Key Research Accomplishments:

We have revealed a novel function and signaling pathway by which SnoN exerts anti-tumorigenic activities by inducing premature senescence in human cells.

Reportable Outcomes;

A paper has been submitted to describe this study and later published in the EMBO J.

Pan, D., Zhu, Q. and **Luo, K.** (2009) SnoN functions as a tumor suppressor by inducing premature senescence. *EMBO J.* **28**: 3500-3513.

Conclusion:

Our studies have revealed that SnoN contains both pro-oncogenic function, through antagonizing TGFβ/Smad signaling, and anti-oncogenic activity, through inducing premature senescence by activating p53.

References:

1.Jahchan NS, and Luo K. (2010) SnoN in mammalian development, function and diseases. *Curr. Opin. Pharmacol*. **10**: 670-675.

2. Jahchan NS, You YH, Muller WJ, and **Luo K**. (2010) Transforming growth factor-beta regulator SnoN modulates mammary gland branching morphogenesis, postlactational involution, and mammary tumorigenesis. *Cancer Res.* **70**: 4204-13.