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14. ABSTRACT

Androgen receptor (AR) mediates the action of male sex hormones and plays a crucial role in the therapy of prostate cancer. Post-translational modification has significant impacts on gene expression, but how it affects AR activity is largely unknown. In this study, we demonstrate that AR is covalently modified by NEDD8. We show that PIASy is the E3 ligase catalyzing AR neddylation. We also find that Jab1 interacts with AR and enhances its transcriptional activity through de-neddylation. In the past year, we further established Jab1 shRNA expressing cell lines and characterized the effects of Jab1 silencing on prostate cancer cell growth and proliferation. We show that silencing of Jab1 inhibits prostate cancer cell proliferation.

15. SUBJECT TERMS
NEDD8, Androgen Receptor, Post-translational modification

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Table of Contents

Introduction

We have previously demonstrated that Jab1 physically interacted with AR *in vivo* and *in vitro*. Overexpression of Jab1 enhanced androgen-dependent transcriptional activation of AR. The JAMM motif of Jab1 is required for this enhancement. Endogenous neddylated AR was accumulated in Jab1 knockdown cells and NEDD8 was rapidly removed upon androgen administration. We found a potential E3 ligase that catalyzed this modification and inhibited AR's transcriptional activity. Our results linked neddylation and de-neddylation pathways with regulation of AR and other nuclear receptor's function. We hypothesize that AR is subject to modification by NEDD8, and this process may be a therapeutic target to control prostate cancer growth.

Body

In year 4 (no-cost extension year), we proposed to continue to finish up Specific Aim 4 in the original proposal. In Aim 4, we proposed to determine the effects of silencing endogenous Jab1 in the growth of prostatic cancer cells.

In Aim 4-1, we proposed to generate prostatic cancer cell lines with expression of shRNA against Jab1 or scramble control shRNA. DNA encoding the shRNA sequences against endogenous Jab1 was subcloned into the LentiLox 3.7 vector under the control of the U6 promoter. The Jab1 shRNA LentiLox 3.7 vector was mixed with the ViralPower™ Packaging Mix (containing pLP1, PLP2 and pLP/VSVG DNAs) and used for transfection into 293T cells. After viral particles were packaged and released, culture media were harvested to infect LNCaP and PC3 cell lines and silencing effect by shRNA were determined by detecting the diminishing protein levels of endogenous Jab1. Furthermore, the mRNA and protein levels of PSA or Probasin, which are known AR target genes, were characterized after Jab1 silencing.

This aim was described in Task 4-1, which was to be completed in months 24-27. We planned to generate prostatic cancer cell lines with expressing of shRNA against Jab1 and scramble control. Ultimately, stable cell lines with Jab1 expression inhibited will be established.

Several Jab1 shRNA expressing lines have been successfully established. These cell lines were used to characterize their effects on Jab1 expression and cell growth.

In Aim 4-2, we proposed to characterize the effects of endogenous Jab1 silencing on protein stability of p53 and p27 and cell growth. The effects of Jab1 silencing on the cell proliferation were determined by MTT assay. We plated both LNCaP and PC3 with Jab1 silencing or control at $1X10^4$ cells per well in a 96 well microtiter plate, and the effects

on cell proliferation and growth curve were analyzed. We found that silencing Jab1 impede on cell proliferation of both LNCaP and PC3 cells. To understand growth inhibition mediated by Jab1 silencing, cell cycle analyses were conducted to determine which phase of cell cycle was arrested. Sample were collected and subjected to FACS flow cytometer analysis. So far, the evidence is inconclusive, and we found little evidence of Jab1 silencing on affecting protein stabilities of p53 and p27.

Key Research Accomplishments:

Year 4:

- We have established several Jab1 shRNA expressing cell lines in LNCaP and PC3 cells.
- We have characterized the effect of Jab1 silencing on cell growth and proliferation of prostate cancer cells and found that silencing of Jab1 inhibits prostate cancer cell growth.

Reportable Outcomes:

- Jab1 silencing affects prostate cancer cell growth.

Conclusions:

We have completed the proposed tasks in the third year. We expect to wrap up the study in the following few months during the additional extension of no-cost extension period.

References:
None

Appendices:
None

Supporting Data:
None