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14. ABSTRACT A large percentage of prostate cancers show either loss or mutational inactivation of the Rb tumor suppressor gene. Rb mediates its tumor suppressor function through its association with other cellular proteins. Our study focuses on Thoc1 protein, which interacts with the N terminal region of Rb and thereby may mediate some Rb functions. Previous reports show that Thoc1 is upregulated in breast and lung cancers and is required for survival of transformed cells, suggesting Thoc1 may play a role in tumorigenesis. To test our hypothesis, we are using a mouse model of prostate cancer where prostate-specific deletion of Rb and p53 genes leads to development of metastatic adenocarcinoma. We find that deletion of Thoc1, Rb and p53 genes leads to increased survival of mice compared to mice with loss of Rb and p53. Histopathological analyses of prostate tissue revealed that Thoc1 is required at early stages of prostate tumorigenesis. Additional studies are underway, which will enable us to understand mechanisms of Thoc1 action in prostate tumorigenesis. Our preliminary findings and previous reports showing differential requirement for Thoc1 in tumor cells compared to normal cells suggests that Thoc1 may be a potential target for therapy of prostate cancer.					
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Table of Contents

	<u>Page</u>
Introduction.....	5
Body.....	5
Key Research Accomplishments.....	7
Reportable Outcomes.....	7
Conclusion.....	7
References.....	7
Appendices.....	N/A

Introduction

Prostate cancer is the second leading cause of cancer related death in men in the US. Prostate tumors harbor mutations or loss of Rb tumor suppressor gene in nearly half of the cases. The tumor suppressor function of Rb is mediated by its association with other cellular proteins. Thoc1 is an Rb-binding protein that has been found to be upregulated in prostate cancers (unpublished data), in cells derived from the TRAMP mouse model of prostate cancer and other cancer types such as breast (Guo et al., 2005) and non-small cell lung cancer (Yang et al., 2008) compared to matched normal tissue, suggesting that Thoc1 may play a role in tumorigenesis. We also found that E1A/Ras transformed MEFs were dependent on Thoc1 for their survival whereas normal MEFs did not require Thoc1 for survival (Li et al., 2007). Also, conditional deletion of Thoc1 in mouse mammary gland did not affect normal mammary gland development suggesting that tumor cells may be dependent on Thoc1 for survival and growth (unpublished observation). Although Thoc1 physically interacts with Rb, the functional significance of their interaction in the context of tumorigenesis is not known. Rb and Thoc1 seem to have opposite effects on cellular physiology in tumor cells. For example, Rb is either mutated or lost in prostate cancers whereas Thoc1 has been found to be upregulated in prostate tumors suggesting that they may interact in an antagonistic manner. Based on this, we propose to determine if Rb and Thoc1 functionally interact in prostate tumorigenesis and if Thoc1 plays a role in initiation and development of adenocarcinoma and metastasis using a mouse model of prostate cancer.

Body

Research and Training Accomplishments

Task 1. Test if Rb and Thoc1 functionally interact in mouse prostate tumorigenesis.

Task 1b. Histopathological analysis of prostate tissues from mice of test and control genotypes was performed to study the effect of compound loss of Thoc1 and Rb on the development of hyperplasia. Analysis of H&E (Hematoxylin and Eosin) stained prostate tissue at 36 weeks of age in control and test genotypes did not reveal development of hyperplastic lesions. It is likely that in this mouse model (Zhou et al, 2006), development of hyperplasia and prostate intraepithelial neoplasia occurs with increased latency.

Task 1c. Immunohistochemistry (IHC) and Western blot analysis for Thoc1 expression was performed in prostate tissue to show decreased Thoc1 expression in $Rb1^{F/F};Thoc1^{F/F};PB-Cre4$ (test genotype) compared to $Rb1^{F/F};Thoc1^{+/+};PB-Cre4$ (control genotype). However, immunohistochemistry for cyclin A, cyclin E and cyclin D were not performed since these mice did not develop hyperplastic lesions at this time point.

Task 2. Determine whether Thoc1 mediates mouse prostate adenocarcinoma and metastasis.

To determine if Thoc1 plays a role in mouse prostate tumorigenesis, we used a mouse model where deletion of Rb and p53 genes in prostate epithelium leads to metastatic adenocarcinoma of the prostate gland (Zhou et al, 2006). We crossed $Rb1^{F/F};p53^{F/F};PB-Cre4$ mice with $Thoc1^{F/F}$ mice to generate $Rb1^{F/F};p53^{F/F};Thoc1^{F/F};PB-Cre4$ (test genotype) and $Rb1^{F/F};p53^{F/F};Thoc1^{+/+};PB-Cre4$ (control genotype). We found that $Rb1^{F/F};p53^{F/F};Thoc1^{F/F};PB-Cre4$ mice (test genotype) had significantly longer survival compared to mice in control genotype group suggesting that Thoc1 may be required for tumorigenesis process initiated by loss of Rb1 and p53 in the mouse prostate. Although most of the test mice developed prostate tumors with longer latency, the tumors retained expression of Thoc1 protein, which suggested that tumors in mice of test group arose due to inefficient recombination of Thoc1 floxed allele.

To understand the role of Thoc1 in tumor formation process, we performed histopathological analysis of prostate tissue as outlined in Tasks 2c, d and e. Serial sectioning of prostate tissue at 20 weeks of age was performed to identify early and advanced tumor lesions in test and control genotypes. As outlined in Table1, most of the mice in test group were normal at 20 weeks of age but all of the control mice had developed early (mPIN) or advanced

(invasive carcinoma) lesions. All the lesions in prostates of test group expressed Thoc1 protein and retained atleast one unrecombined allele of Thoc1 as determined by PCR genotyping (Fig 1). These findings suggest that Thoc1 loss affects early stages of tumor development. Further studies are underway to understand the mechanism of inhibition of tumorigenesis upon loss of Thoc1 in mouse prostate.

Performance of the above-mentioned tasks gave me an opportunity to learn various techniques such as PCR genotyping, H&E staining, immunohistochemistry, histopathological analysis of lesions in prostate, liver and lungs tissues and mouse husbandry. In addition, I got an opportunity to work on the design of experiments, analysis and interpretation of data, skills that are highly important for my training. My training program also included attending weekly seminar meetings of the Genitourinary Program at Roswell Park Cancer Institute. Investigators from within Roswell Park Cancer Institute and invited speakers working on a wide range of aspects of prostate cancer present their work at these seminars, which has helped broaden my understanding of the disease as well as research in the field of prostate cancer. I got an opportunity to present a poster based on the above findings at AACR sponsored Frontiers in Basic Cancer Research Meeting held in Boston, MA (October 8-11, 2009). Attending this meeting gave me an opportunity to present and discuss my research with students, post-doctoral fellows and scientists from other institutions.

Genotype	Normal	mPIN	Invasive Carcinoma	Metastasis (macroscopic)
Rb^{F/F}: p53^{F/F}: Thoc1^{+/+}: PB-Cre4	0	2	9	0/11
Rb^{F/F}: p53^{F/F}: Thoc1^{F/F}: PB-Cre4	8	1	3	0/12
Rb^{F/F}: p53^{F/F}: Thoc1^{F/F}	13	0	0	0/13

Table 1. Histopathological analysis of mice at 20 weeks of age. mPIN = mouse prostate intraepithelial neoplasia.

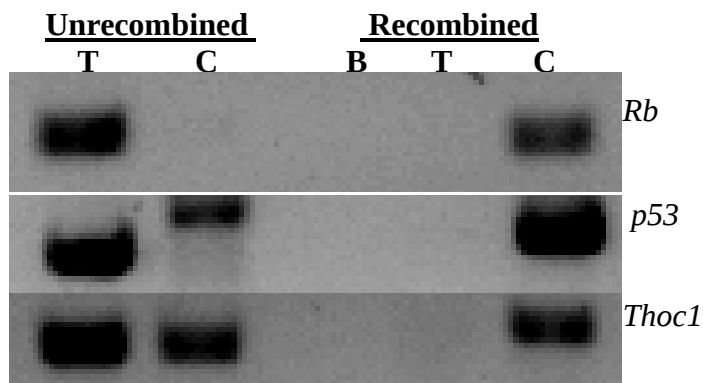


Fig 1. PCR analysis of Rb, p53 and Thoc1 genes in tumor tissue of Rb1^{F/F}:p53^{F/F}:Thoc1^{F/F}:PB-Cre4 (test genotype) mouse. T=tail DNA, C=tumor cell DNA, B=blank.

Key Research Accomplishments

1. Compound loss of Thoc1, Rb and p53 in mouse prostate inhibits early stages of tumorigenesis.

Reportable Outcomes

Meenalakshmi Chinnam, Yanqing Wang, Xiaoling Wang, Xiaojing Zhang, David L. Gold, Alexander Yu Nikitin, and David W. Goodrich. Abstract B24: Thoc1 is required for mouse prostate tumorigenesis initiated by loss of Rb and p53. Cancer Res. 2009 69: B24. Poster presentation at AACR Frontiers in Basic Cancer Research Meeting held in Boston, MA, October 8-11, 2009.

Conclusion

Thoc1 protein is an Rb–associating protein that has been found to be upregulated in breast, lung and prostate tumors. Therefore, Thoc1 may play a role in tumorigenesis process. Reports from our lab has shown the requirement of Thoc1 protein in the survival of E1a/Ras transformed cells and cells of numerous human cancer cell lines. These findings suggest that transformed cells may require Thoc1 for their survival. To study the role of Thoc1 in tumorigenesis process, we used a mouse model of prostate cancer and found that loss of Thoc1 specifically in the prostate epithelium significantly increased the survival of mice. Moreover, the tumors developing in the test mice retained Thoc1 expression possibly due to inefficient recombination of Thoc1 floxed allele. Analysis of prostate tissue at 20 weeks of age revealed that Thoc1 loss affects early stages of tumor development. We are further investigating the mechanisms of Thoc1 requirement for tumor development.

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