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Isolation of Target Genes for Nkx3.1 in Prostate Carcinogenesis

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Nkx3.1 is a prostate-specific tumor suppressor whose loss-of-function represents a critical step in prostate cancer initiation. However, the molecular basis is still largely unknown. We have been utilizing microarray analysis to pursue the gene expression profiling of prostatic lesions in the Nkx3.1 mutant mouse model relative to normal prostate epithelium. Our findings suggest that Nkx3.1 loss-of-function leads to a deregulated secretory function of prostate which representing a defect differentiation of prostate epithelium, thus may contribute to the increased susceptible to carcinogenesis. Moreover, Nkx3.1 mutant prostates are deficient for anti-oxidative protection as a consequence of aging, which underlies its role in cancer predisposition. In an effort to explore the role of Nkx3.1 in advanced stages of prostate cancer progression, gene expression profiling has been performed using Nkx3.1; Pten double mutant and Nkx3.1;Pten;p27kip1 triple mutant prostatic lesions. Our preliminary data have shown that Nkx3.1 loss-of-function cooperates with heterozygosity of p27kip1 in promotion of prostate carcinogenesis through up-regulation of cyclin D1. Our findings provide insight into the roles of Nkx3.1, by itself or in cooperation with other broad-spectrum tumor suppressors, in prostate carcinogenesis.
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I. Training accomplishments

During the funding period of March 2003 through February 2004, I have received extensive training under the guidance of the supervisor, Dr. Cory Abate-Shen, as I have made considerable progress toward identifying Nkx3.1-responsive genes involved in prostate carcinogenesis. With this guidance and training, I have established and optimized the methodologies needed for this study, including laser capture microdissection, RNA isolation and amplification, Affymetrix microarray and data analysis, real-time RT-PCR, in situ hybridization and immunohistochemistry. With the help of Whitney Banach-Petrosky, a senior technician of our laboratory, I have also set up a tissue/cell recombination assay for functional study of candidate target genes of Nkx3.1 during prostate differentiation and/or carcinogenesis.

In addition to the technical support, intellectually, my study has also benefited from the guidance and assistance of Dr. Abate-Shen and other laboratory members. On a weekly basis, I discuss the research project in detail with Dr. Abate-Shen; and on a bi-weekly basis, I present the data at the laboratory meetings. These discussions of the progress and pitfalls of my research project have promoted critical evaluation of the data on a consistent basis. Moreover, many research investigators at CABM and Cancer Institute of New Jersey have provided valuable advices on my research project in many aspects. These include our collaborators, Dr. Michael Shen, who has extensive expertise in prostate biology; and Drs. Yong Lin and Weichung J. Shih, who have provided considerable assistance in microarray data analysis.

Finally, I had the opportunities to attend several symposiums/retreats at CABM, Cancer Institute, and the UMDNJ-Rutger’s life science community during last funding period. I will also be presenting two abstracts (one on myself and one as a coauthor) at the 95th annual meeting of American Association for Cancer Research in March 2004.

II. Research accomplishments

A. Introduction

Our hypothesis is that Nkx3.1 homeobox gene functions as a transcription factor to regulate gene expression during normal prostate growth and differentiation, and that loss of Nkx3.1 leads to the aberrant expression of target genes that ultimately contribute to prostate carcinogenesis. Therefore, identification of Nkx3.1 target genes will provide insight into the molecular pathways associated with prostate carcinoma.

We have demonstrated that the Nkx3.1 mutant mice display histopathological features of prostatic intraepithelial neoplasia (PIN), the presumed precursor of human prostate cancer, which increase in severity with age (1, 2). Furthermore, prostatic-specific loss-of-function of Nkx3.1 cooperates with loss-of-function of broad-spectrum tumor suppressors such as Pten and/or p27kip1 in cancer progression (3, 4).

Using Affymetrix GeneChip expression profiling, as validated by real-time RT-PCR and immunohistochemistry, we have now found that loss-of-function of Nkx3.1 in mice leads to a deregulated secretory function in prostate, which represents a less differentiated secretory tissue and may contribute to increased susceptibility to prostate carcinogenesis. Furthermore, our findings suggest Nkx3.1 loss-of-function leads to accumulation of oxidative damage in prostate as a consequence of aging, which underlies its role in cancer predisposition.

To explore Nkx3.1-responsive genes during more advanced stages of prostate cancer progression, and to verify the synergistic activity of Nkx3.1, Pten and/or p27kip1, Nkx3.1;Pten and Nkx3.1;Pten;p27 compound mutant prostates were analyzed microarray analysis accompanied with the laser capture microdissection (LCM) technique. Our findings suggest that the molecular mechanisms that mediate this cooperativity include the activation of cyclin D1, a key modulator of cell proliferation.
Taken together, our findings underscore the significance of Nkx3.1 loss-of-function, by itself, or cooperating with other putative tumor suppressors such as Pten and p27kip1, in prostate cancer initiation and progression.

B. Body Below we list our goals for this year (from the statement of work) and a description of the status.

Task 1. Isolation of Nkx3.1-responsive genes by microarray analysis.

This goal has been successfully implemented. We compared mRNA from prostates (anterior and dorsolateral lobes) of Nkx3.1 homozygous mutant and wild-type mice at 15-month-old of age. Microarray analysis was done in triplicate using mRNA samples comprised of 3 mutant or 3 wild-type prostates; this ‘3x3 protocol’ minimizes artifacts that arise from individual mRNA samples. Data analyses were done using ANOVA method followed by multiplicity adjustment using Holm or B&H procedure. As visualized by two-way hierarchical clustering, a total of 638 genes were differentially expressed following Nkx3.1 loss-of-function. Among them are deregulated expression of 4 genes encoding seminal vesicle secretory proteins and prostatic specific probasin (Figure 1A). Moreover, of particular interest was aberrant levels of several antioxidant enzyme in Nkx3.1 mutant prostates, suggesting loss-of-function of Nkx3.1 predisposes to prostate cancer through increased oxidative damage (Figure 2A). Validations of microarray findings have been done as described in progress of Task 4.

We have also performed gene expression profiling of LCM samples using 4 prostatic lesions (hyperplasia & low-grade PIN) of Nkx3.1 mutant mice and 4 normal prostatic epitheliums of age-matched wild-type mice. Data analysis is now carrying out.

However, the comparison of prostatic lesions vs. adjacent normal prostatic epithelium of Nkx3.1−/− mice is technically difficult since the heterogeneity and multifocality of the lesions in Nkx3.1 mutant prostate made them hard to be captured from cryosections. Instead, comparison of lesions and normal prostatic epitheliums was performed using different samples.

Task 2. Isolation of target genes for synergistic activity of Nkx3.1 and Pten loss-of-function by microarray analysis.

Microarray experiment has been performed using LCM samples to compare the high-grade or low-grade PIN lesions of Nkx3.1;Pten compound mutants (n=9) vs. normal prostate epitheliums of age-matched Nkx3.1+/− mice (n=4). However, I have not further pursued the comparison of PIN lesions vs. adjacent normal epitheliums Nkx3.1;Pten compound mutants because of the similar aforementioned technical impracticability.
Task 3. Exploration of Nkx3.1 regulated genes in the development of PIN to prostate cancer.

We proposed to explore Nkx3.1-responsive genes in advanced stages of prostate carcinogenesis using a tissue recombination approach. However, our recent studies have shown that Nkx3.1;Pten and Nkx3.1;Pten; p27kip1 compound mutants recapitulate various stages of prostate carcinogenesis from low-grade PIN to high-grade PIN, to invasive adenocarcinoma, and metastases to the distant tissues (3-5). Therefore, we have not pursued the tissue recombination approach. Instead, by using the double (Nkx3.1;Pten) and triple (Nkx3.1;Pten;p27kip1) compound mutants, we extended our proposed task 3 not only to explore Nkx3.1-responsive genes, but also to explore the cooperativity of prostate-specific tumor suppressor Nkx3.1 and broad-spectrum tumor suppressors such as Pten and p27kip1 during multi-stages of prostate cancer progression.

For this purpose, we have performed gene expression profiling using LCM samples of 9 double (Nkx3.1;Pten) mutants and 7 triple (Nkx3.1;Pten;p27kip1) mutants, which representing the progression of prostate cancer from PIN lesions to invasive adenocarcinoma. Our preliminary microarray data analyses of triple mutants have revealed that Nkx3.1 loss-of-function cooperates with heterozygosity of p27kip1 in promotion of prostate carcinogenesis through up-regulation of cyclin D1 (Figure 3).

Task 4. Validation of Nkx3.1-responsive genes.

As mentioned in summary of Task 1, microarray analyses of Nkx3.1 mutant and wild-type prostates have revealed a robust increase in expression of 4 genes encoding secretory proteins that are normally expressed by the seminal vesicle: (i) seminal vesicle secretary protein 2 (SVP2; ~500 fold); (ii) seminal vesicle secretion 5 (SVS5; ~210 fold); (iii) seminal vesicle secretion 6 (SVS6; ~38 fold); and (iv) seminal vesicle autoantigen (SV4; ~20 fold). Meanwhile, genes encoding prostatic secretory proteins, such as probasin, were reduced (by ~3 fold) in Nkx3.1 mutant prostate (Figure 1A). These findings have been further confirmed by Real-time RT-PCR (Figure 1B&C) and immunohistochemistry study (Figure 1D). We thus propose that Nkx3.1 is required for prostatic epithelial specification; in its absence the prostatic epithelial of Nkx3.1 mutants represents defects in differentiation which may contribute to the increased susceptible to carcinogenesis. To test this hypothesis, we have developed two immortalized cell lines from embryonic urogenital epithelium (UGE) or seminal vesicle epithelium (SVE) for functional study of Nkx3.1 using a tissue/cell recombination approach. We are using the Nkx3.1 mutants and UGE/SVE cells as models to study the consequences of aberrant differentiation for cancer susceptibility in prostate.
Furthermore, gene expression profiling, as validated by real-time RT-PCR, have also revealed aberrant expression of several anti-oxidation enzymes following Nkx3.1 loss-of-function, including glutathione peroxidase 2&3 (GPX2, GPX3), and peroxiredoxin 6 (Prdx6) (Figure 2A&B). Further examine of oxidative DNA damage by analyzing the levels of 8-oxo-2'-deoxyguanosine (8-oxo-dG) in genomic DNA has demonstrated increased 8-oxo-dG levels in the Nkx3.1 mutant prostates compared with age-matched controls (Figure 2C). We have also observed a marked enhancement in induced nitric oxide synthase (iNOS) immuno-reactivity in Nkx3.1 mutant prostate, compared to the age-matched wild-type prostate (Figure 2D), suggesting that the mutant may have elevated levels of nitric oxide, a radical, nitrosating agent, and indirect mutagenic oxidant. Taken together, loss-of-function of Nkx3.1 may lead to accumulation of oxidative damage in prostate as a consequence of aging, which underlies its role in cancer predisposition.

C. Key research accomplishments

- Gene expression profiling of Nkx3.1 wild-type and mutant prostates reveal a total of 638 genes are differentially expressed following Nkx3.1 loss-of-function;
- Deregulated secretory function in Nkx3.1 mutant prostate reflects a defective differentiation of prostate which may contribute to the increased susceptible to carcinogenesis;
- Loss-of-function of Nkx3.1 leads to accumulation of oxidative damage in prostate
- Nkx3.1 cooperates with heterozygosity of p27kip1 in promotion of prostate carcinogenesis through up-regulation of cyclin D1.

- A large-scale microarray analysis has been done, including 4 wild-type normal prostatic epitheliums, 4 hyperplastic/low-grade PIN lesions from Nkx3.1+/− prostates, 9 PIN lesions or adenocarcinomas from Nkx3.1;Pten double mutant prostates, and 7 PIN lesions or adenocarcinomas from Nkx3.1;Pten;p27kip1 triple mutant prostate. Data analysis is carrying out in collaboration with Drs. Yong Lin and Weichung J Shih at Cancer Institute of New Jersey.

D. Reportably outcomes

- Abstracts


E. Conclusions

Several lines of evidence have implicated that Nkx3.1 is a prostatic-specific tumor suppressor whose loss-of-function represents a critical step in prostate cancer initiation. However, the molecular basis is still largely unknown. Using the Nkx3.1 mutant mouse model, we have performed a broad exploration using Affymetrix gene expression profiling approach, followed by a validation of several specific genes using real-time RT-PCR and immunohistochemistry. Our findings suggest that Nkx3.1 is required for prostatic epithelial specificity and its loss-of-function leads to deficient secretory function in prostate, which may contribute to the increased susceptible to carcinogenesis.

Moreover, our findings have revealed that loss-of-function of Nkx3.1 lead to a loss of anti-oxidative protection in prostate as a consequence of aging, which underlies its role in cancer predisposition.

To explore the role of Nkx3.1 in advanced stages of prostate cancer progression, gene expression profiling has been performed using Nkx3.1;Pten double mutant and Nkx3.1;Pten;p27kip1 triple mutant prostatic lesions. Our preliminary data have shown that Nkx3.1 loss-of-function cooperates with heterozygosity of p27kip1 in promotion of prostate carcinogenesis through up-regulation of cyclin D1. Upon the completion of microarray data analysis, more interesting genes will be followed up for functional studies to define the succession of molecular events that culminate in prostate carcinoma.

F. References


