Gene Silencing in Androgen-Responsive Prostate Cancer Cells from the Tissue-Specific Prostate-Specific Antigen Promoter

Jun Song,1 Shen Pang,1 Yingchun Lu,1 Kazunari K. Yokoyama,4 Jun-Ying Zheng,1 and Robert Chiu1,2,3

1Dental Research Institute, University of California Los Angeles (UCLA) School of Dentistry, 2Department of Surgery/Oncology, UCLA School of Medicine, and 3Jonsson Comprehensive Cancer Center, UCLA, Los Angeles, California; and 4BioResource Center, RIKEN, Ibaraki, Japan

Abstract

The success of gene therapy using a RNA interference approach relies on small interfering RNA (siRNA) expression from a highly tissue-specific RNA polymerase II promoter rather than from ubiquitous RNA polymerase III. Accordingly, we have developed a prostate-specific vector that expresses siRNAs from the human prostate-specific antigen promoter, a RNA polymerase II promoter. Our data demonstrate androgen-dependent and tissue-specific siRNA-mediated gene silencing in the androgen-responsive prostate cancer cell line, LNCaP. The biological significance was evidenced by altered apoptotic activity through the inhibition of the apoptosis-related regulatory gene. These results demonstrate that siRNA-mediated gene silencing from a tissue-specific RNA polymerase II promoter could be a potential tool for tissue-specific gene therapy.

Introduction

Expression of small interfering RNA (siRNA) transcripts from a cytoplasmic virus is capable of generating siRNA-mediated gene silencing in vitro and in vivo (1). Therefore, it is conceivable that a functional siRNA could be expressed directly from a tissue-specific promoter when suitable modifications are made both close juxtaposition of the hairpin to the transcriptional start site and a proper polyadenylation signal. However, until now, there has been no study on siRNA-mediated gene silencing directly from the tissue-specific RNA polymerase II promoter.

The prostate-specific antigen (PSA) is a well-characterized protein (2–5). Its promoter is androgen responsive and tissue specific (6–9). This tissue specificity makes the PSA promoter an ideal regulatory element for prostate-specific transgene expression (10). Here, we demonstrate for the first time that expression of a siRNA driven directly from the PSA promoter is capable of specific gene silencing in an androgen-dependent and tissue-specific fashion.

Materials and Methods

Plasmids. Sequences of fragments of the human PSA enhancer, the PSA promoter, the target sequence for green fluorescent protein (GFP), and the polyadenylation signal (AATAAA) were obtained by polymerase chain reaction amplification using pPSAR2.4K-PCPSA-P-Lux as a template (9). For cloning purposes, forward primer 5′-ATGAATTAAAAGCAATGCAATACACCAATCGAAGG-3′ was flanked with Xhol at the 5′ end (underlined), and reverse primers 5′-ATGAATTATATAAGCAATGCAATACACCAATCGAAGG-3′ (PSA-GFP), 5′-ATGAATTATATAAGCAATGCAATACACCAATCGAAGG-3′ (PSA-PSARNAi), and 5′-ATGAATTATATAAGCAATGCAATACACCAATCGAAGG-3′ (PSA-PSARNAi) were used as a probe. Hybridization was performed as described previously (14).

Western Blot Analyses. Whole-cell lysates were electrophoresed and immunoblotted according to the protocol provided by Santa Cruz Biotechnology (Santa Cruz, CA). Anti-JNK1, anti-JNK2, anti-PI3K, and the anti–phospho-c-Jun polyclonal antibody were purchased from Santa Cruz Biotechnology. The anti–c-Jun polyclonal antibody was purchased from Calbiochem (San Diego, CA), anti-GFP polyclonal antibody was from BD Biosciences Clontech (Palo Alto, CA), anti-FKBP12 polyclonal antibody was from Affinity Bioreagents, Inc. (Golden, CO), and anti–poly(ADP-ribose) polymerase (PARP) polyclonal antibody was obtained from Oncogene Research (Boston, MA).

Terminal Deoxynucleotidyl Transferase-Mediated Nick End Labeling Staining. Programmed cell death was detected with the In Situ Cell Death Detection Kit, TM red (Roche Applied Science, Indianapolis, IN). Terminal deoxynucleotidyl transferase-mediated nick end labeling (TUNEL) staining was performed according to the manufacturer’s protocol.

Results and Discussion

To determine whether the PSA promoter is suitable for expressing siRNA, we developed a vector, pPSARNAi-GFP (Fig. 1A), using the human PSA promoter and its enhancer to express siRNAs to target the GFP gene, a commonly used indicator (6, 9). In the presence of androgen treatment, the GFP expression plasmid was cotransfected with either pPSARNAi-GFP or empty vector pBluescript II KS+ into the prostate-derived, androgen-responsive LNCaP cell line. Cervix-derived HeLa cells and kidney-derived 293T cells were used as control cell lines. Forty-eight hours after transfection, cells were subjected to fluorescence microscopic analysis. The expression of...
Western blot analysis. Expression of FKBP12 was used as an internal control. Either left untreated or treated with 10 nmol/L androgen. GFP expression was detected by cotransfected with the GFP expression plasmid into LNCaP or HeLa cells. The cells were from the PSA infected LNCaP cells, not in HeLa or 293T cells (Fig. 1). Inhibition of GFP expression only occurred in pPSARNAi-GFP–transfected LNCaP cells (Fig. 1A, compare Lanes 5 and 6). These results suggest that siRNA expression from the PSA promoter is androgen dependent and tissue specific.

We subsequently investigated siRNA-mediated endogenous gene silencing from the PSA promoter and enhancer. PSARNAi-JNK was constructed in a lentiviral-based vector to silence the human JNK1 and JNK2 genes by virtue of a shared stretch of identical sequence. Forty-eight hours after infecting LNCaP cells with lentiviral PSARNAi-JNK in the presence or absence of androgen, cell extracts were prepared for Western blot analysis. Significant inhibition of JNK1 and JNK2 was observed only in the androgen-treated lentiviral PSARNAi-JNK–infected LNCaP cells (Fig. 2, A and B), suggesting that siRNA expression from the PSA promoter effectively targets endogenous genes in a tissue-specific manner and is also androgen dependent. Similarly, silencing of the endogenous gene PI3K (15) by lentiviral PSARNAi-PI3K is also androgen dependent and tissue specific (Fig. 2, D and E).

To determine whether the inhibition of JNK resulted from the expression of siRNAs, we examined siRNA expression using dot hybridization. We detected hybridization signal only in LNCaP cells that were infected with lentiviral PSARNAi-JNK in the presence of androgen (Fig. 2C), whereas no hybridization signals were present in HeLa and 293T cells infected with lentiviral empty vector or lentiviral PSARNAi-JNK (Fig. 2C). These results demonstrate that the inhibi-
tion of JNK is dependent on expression of siRNAs. β-Actin was used as a control probe.

To determine whether JNK knockdown affects the phosphorylation status of the downstream target c-Jun, 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced phosphorylated c-Jun was used to assess JNK activity. Phosphorylated c-Jun was enhanced in cells infected with a control viral vector (Fig. 3A, Lane 2, top panel) but not in cells infected with lentiviral PSARNAi-JNK, even in the presence of TPA treatment (Fig. 3A, Lane 4, top panel). Western blot analysis of unphosphorylated c-Jun was used as a control (Fig. 3A, bottom panel). These results clearly demonstrate that the cells with knockdown JNKs lose their responsiveness to TPA-induced JNK activity for phosphorylation of c-Jun and suggest that siRNA-mediated gene silencing by a tissue-specific promoter has a great impact on the regulation of signaling pathways.

Additionally, we examined the effect of the JNK gene silencing in TPA-induced apoptosis of LNCaP cells, using the cleaved M₉ 90,000 PARP fragment as an apoptosis indicator. As shown in Fig. 3B, Lane 4, the detection of the M₉ 90,000 PARP indicated that TPA induced apoptosis in empty lentiviral vector-infected LNCaP cells. In contrast, TPA did not enhance the M₉ 90,000 PARP fragment in LNCaP cells infected with lentiviral PSARNAi-JNK, suggesting that knockdown JNK prevents cells from undergoing apoptosis in response to TPA treatment (Fig. 3B, compare Lane 2 with Lane 1). To detect apoptosis at the single cell level, LNCaP cells infected with either lentiviral PSARNAi-JNK or control lentivirus in the presence or absence of TPA treatment were subjected to TUNEL staining. DNA strand breaks in the cells were then detected by fluorescence microscopy. As shown in Fig. 3C, TPA-treated control cells displayed enhanced TUNEL staining signals, whereas a minor stained signal was seen in LNCaP cells with knockdown of JNK (Fig. 3C), suggesting that knockdown of JNK protects cells from TPA-induced apoptosis in LNCaP cells.

To our knowledge, this is the first evidence that siRNA can be expressed from a tissue-specific promoter. This finding may lead to new directions for application of RNAi technology and demonstrate the possibility that many superior RNA polymerase II-mediated mammalian expression vectors can be used to drive the corresponding small hairpin RNA to silence targeted gene expression in a tissue-specific manner. Furthermore, an inducible polymerase II-mediated expression vector, as described by us, may be used to control the expression of small hairpin RNA for functional analysis of genes essential to cell viability.

In summary, these data demonstrated that a siRNA expressed from either a vector- or lentiviral-based system using the PSA promoter not only specifically reduced the expression of ectopic and endogenous genes in cells but also acted in a tissue-specific and hormone-dependent manner. Further study of the effectiveness of siRNA-mediated gene silencing by the PSA promoter in an animal system will lay the groundwork for creating a potential gene therapy approach for the treatment of prostate cancer.

Acknowledgments

We thank Dr. Luk Van Parijs for providing a lentivirus-based system, John Rossi for valuable advice, Kristen Lum for critical review of the manuscript, and Susan Chou for assistance with experiments.

References

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*Cancer Res* 2004;64:7661-7663.

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