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13. ABSTRACT (Maximum 200 Words) Bone morphogenetic proteins (BMPs), a family of growth factors, play diverse roles in cell proliferation and survival. In order to investigate the signal transduction of BMPs in prostate carcinogenesis, we designed a strategy to express a constitutively active BMP receptor Alk3 and a downstream signaling protein SMAD1 as well as a dominant negative BMP receptor Alk3 in prostate epithelial cells driven by a rat probasin promoter. By simply comparing the prostate weights of transgenic males with non-transgenic males and prostate histology, we did not detect any significant differences among these different groups at different ages. We have further crossed these transgenic lines with a strain of mice with prostate cancer (TRAMP). By this approach, we are unable to observe obvious influence of Alk3 and Smad transgenes in the progression of prostate cancer of the TRAMP mice. Therefore, our conclusion is that alteration in BMP signaling in transgenic mice with this strategy fails to reveal a possible role of BMP signaling in prostate carcinogenesis.

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INTRODUCTION

There is increasing amount evidence supporting the roles of Bone Morphogenetic Proteins (BMP) in cell proliferation, differentiation, and survival (Heldin et al., 1997; Hogan, 1996; Kingsley, 1994; ten Dijke et al., 1996). BMP signaling components (BMP4, BMP7, BMP receptors, and their downstream signal transducers) are expressed in the adult prostate epithelium of the mouse. The objective of our research is to investigate the possible roles of BMP signaling transduction in the proliferation of prostate epithelium and the tumorigenesis of prostate epithelial cells. We have tested the potential roles of BMP signaling in prostate development and tumorigenesis in transgenic mice by over-expressing constitutively active and dominant negative BMP receptors and a constitutively active downstream signal transducer SMAD1. However, our effort was not able to reveal a role of BMP signaling in prostate carcinogenesis.

BODY

Task1. Study the effect of constitutively active BMP receptor type IA (CA BMPR-IA) on prostate tumorigenesis in transgenic mice.

a. Make PBI-CA BMPR-IA construct. Our initial approach was to use the rat probasin promoter to drive gene expression in prostate (Greenberg et al., 1995; Kasper et al., 1998). However, new studies on the rat probasin promoter (PB) by Dr. Robert Matusik's group at Vanderbilt University indicated that to use the original PB promoter driving transgene expression in the prostate yields a low percentage of high level expressing lines (Zhang et al., 2000). Therefore, it will take considerable amount of work to generate enough transgenic mouse lines before a useful mouse line can be obtained. In order to reduce the load of future works and more importantly to obtain a more powerful promoter for prostate expression, we set forth to construct an artificial prostate promoter (PBI) as shown in Figure 1. Therefore, our plan for the first year was slightly modified.

In the new vector pPBI, several critical features have been added in an attempt to enhance prostate-specific and androgen-dependent expression. First, the original PB promoter (426 bp) is present with all its components including TATA box (Greenburg et al., 1994). Therefore, PBI should be at least as potent as the original PB promoter. Second, a region of 340-bp 5' to the minimal PB promoter (containing two copies of the androgen response element (ARE)) is linked to additional 3 copies of ARE to enhance the androgen response. Moreover, this whole region with 5 copies of ARE is repeated 5 times. Therefore, PBI contains the PB promoter and 25 copies instead of 2 copies of ARE. Thirdly, an artificial intron, a multiple cloning site (MCS), and an SV40 late poly (A) signal are linked 3' to the PB promoter to increase the transcription efficiency and to facilitate the insertion of different cDNAs. Furthermore, internal ribosome entry site (IRES) and enhanced green fluorescent protein (EGFP) was introduced for monitoring the transgene expression. In PBI CA BMPR-IA construct, CA BMPR-IA was inserted between Xho I and Sma I sites (Figure 2).

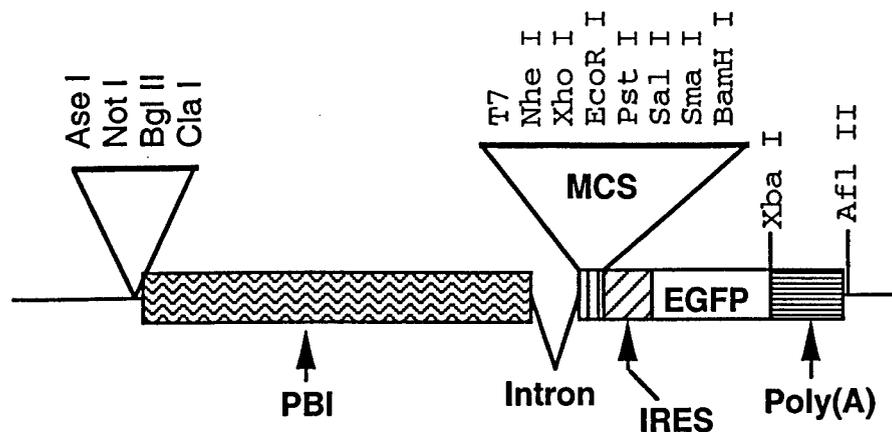


Figure 1. Schematic representation of PBI vector. PBI contains 25 copies of androgen responsive element, 5 copies of rat probasin (PB) promoter sequence without TATA box, and a full 426 bp PB promoter. An intron followed by a multiple cloning sites (MCS), IRES, EGFP, and an SV 40 late poly (A) signal (derived from pSI vector, Promega). Therefore, different cDNAs can be easily cloned into the MCS. Various restriction enzyme digestion sites and T7 promoters are indicated.

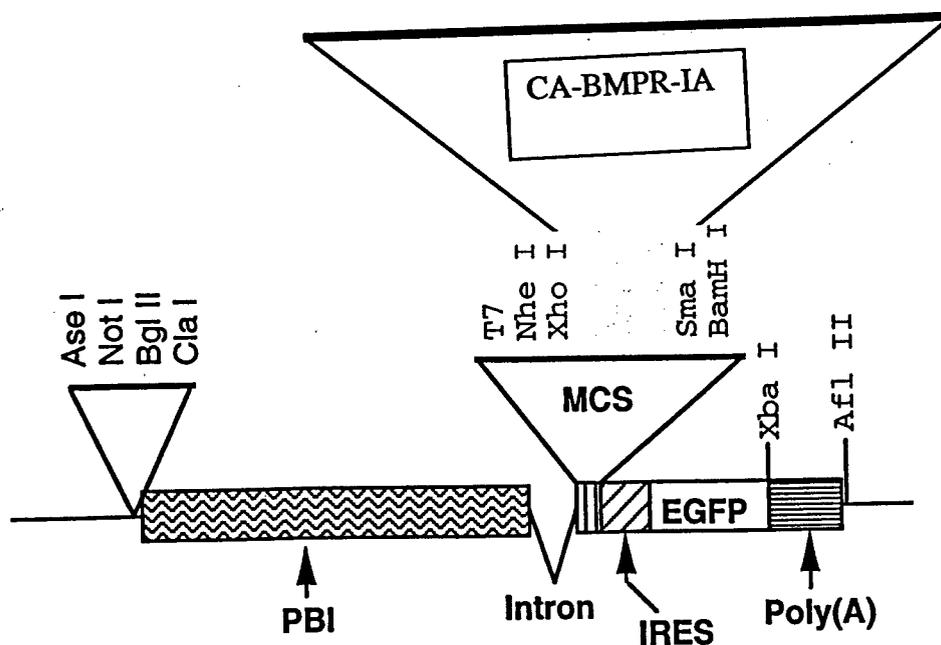


Figure 2. Schematic representation of PBI CA BMPR-IA. Constitutively active human BMP receptor type IA cDNA was inserted between Xho I and Sma I using standard molecular biology techniques.

b. Generation of PBI CA BMPR-IA transgenic mice. Using standard microinjection techniques (Hogan et al., 1994), we have generated six transgenic lines. Instead of performing Northern blotting to check gene expression, we tried to use EGFP to monitor the expression of the transgenes in the prostate. However, we were unable to observe definitive GFP expression in any transgenic line. Bearing in mind that with bi-cistronic expression by IRES, the second open reading frame is usually expressed at 10% of the first open reading frame and GFP is not very sensitive in comparison with other markers such as luciferase, we continued with our research plan to examine prostate histology and weights at different age groups.

c. Comparison of prostate weights and histology. We examined all available transgenic lines obtained at ages 6-12 months. No obvious difference in prostate weights and histology were observed between transgenic and non-transgenic controls.

d. Examine whether the PBI CA BMPR-IA transgene affects the development and progression of PB-Tag induced prostate cancer (or TRAMP). Although PBI CA BMPR-IA transgene alone does not affect prostate, there was a possibility that the transgene could affect cancer development. We, therefore, crossed PBI CA BMPR-IA transgenic lines with PB-Tag to generate double transgenic mice. Prostates of double transgenics and PB-Tag transgenics were compared in terms of their weights and histology. No obvious differences were detected.

Task 2. Study the effect of dominant negative BMPR-IA (DN-BMPR-IA) on prostate carcinogenesis in transgenic mice.

a. Make PBI DN BMPR-IA construct. This construct was made the same way as PBI CA BMPR-IA (Figure 2).

b. Generation of PBI DN BMPR-IA transgenic mice. Seven transgenic lines were generated. Once again, none of them seem to have detectable levels of GFP expression.

c. Examine whether expression of DN BMPR-IA affects prostate weight and histology. We examined all transgenic lines and were unable to detect any obvious change in prostate weights and histology in comparison with wild-type controls. This part was performed essentially in the same way as for CA BMPR-IA transgenic mice.

d. Examine whether DN BMPR-IA affects the development of prostate cancer in PB-Tag transgenic mice. We crossed DN BMPR-IA transgenic lines with PB-Tag mice in the same way as for CA BMPR-IA transgenic mice. No obvious increase or decrease of prostate weights of PB-Tag mice was observed.

Task 3. Study the effect of constitutively active SMAD1 (CA SMAD1) on prostate carcinogenesis in transgenic mice.

a. Make CA-SMAD1 transgene DNA construct. CA SMAD1 was inserted into PBI vector in the same way as described in Figure 2 for CA BMPR-IA.

b. Generation of PBI CA SMAD1 transgenic mice. Six lines of transgenic mice were obtained. Once again, using GFP as a marker, we were unable to detect obvious expression.

c. Examine whether CA SMAD1 transgene affects prostate biology. We examined prostate weights and histology in the same way as for CA BMPR-IA transgenics and were unable to observe obvious differences between the transgenics and the control wild-type mice at different ages (6-12 months).

d. Examine whether CA SMAD1 transgene affects the progression of prostate cancer in PB-Tag mice. We crossed the SMAD1 transgenics with PB-Tag mice and were unable to detect obvious differences in prostate weights and histology between the double transgenics and PB-transgenics at different ages (6-12 months).

KEY RESEARCH ACCOMPLISHMENTS:

Constructed a potential useful vector PBI.

Made PBI CA BMPR-IA DNA construct.

Made PBI DN BMPR-IA DNA construct.

Made PBI CA SMAD1 DNA construct.

Generated transgenic mice using the above constructs.

Examined the potential role of BMP signaling in prostate development and carcinogenesis.

REPORTABLE OUTCOMES:

Except the above accomplishments, the funding of this grant has been used to train the following Researchers in experimental design, molecular biology, genetics, and mouse handling and maintenance:

Liang-Xue Lai, Don Carpenter, Amy Marble, Yongzhong Wei, and Xiaoming Liu as well as five undergraduate students and veterinary students at University of Missouri-Columbia.

CONCLUSIONS:

We attempted to address the possible functions of BMP signaling in prostate development and carcinogenesis using transgenic mouse approach. However, we were unable to observe a positive correlation of BMP signaling with prostate development or carcinogenesis. Because the transgenes do not appear to drive high levels of expression the exact role of BMP signaling in prostate biology could not be ascertained at this time. Nevertheless, we would recommend a possible trial with adenovirus-driven BMPR-IA or SMAD1 to locally infect prostate epithelium. Reportedly adenovirus can drive high levels of genes expression.

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