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Original article

In vivo expression of a conditional TGF-β1 transgene: no evidence for TGF-β1 transgene expression in SM22α-tTA transgenic mice

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Abstract

Transforming growth beta-1 (TGF-β1) appears to play a critical role in the regulation of arterial intimal growth and the development of atherosclerosis. TGF-β1 is expressed at increased levels in diseased arteries; however, its role in disease development remains controversial. Experiments in which TGF-β1 is overexpressed in the artery wall of transgenic mice could clarify the role of TGF-β1 in the development or prevention of vascular disease. However, constitutive overexpression of a TGF-β1 transgene in the mouse artery wall is embryonically lethal. Therefore, to overexpress TGF-β1 in the artery wall of adult mice, we generated mice that were transgenic for a conditional, tetracycline operator (tetO)-driven TGF-β1 allele. These mice were viable, and when crossed with mice expressing a tetracycline-regulated transactivator (tTA) in the heart, expressed the TGF-β1 transgene in a cardiac-restricted and doxycycline-dependent manner. Nevertheless, breeding of the tetO-TGF-β1 transgene into three lines of mice transgenic for a smooth muscle-targeted tTA (SM22α-tTA mice; reported elsewhere to transactivate tetO-driven alleles in smooth muscle cells of large arteries) did not yield expression of the TGF-β1 transgene. Moreover, tTA expression was not detected in aortae of the SM22α-tTA mice. Transgenic mice that express tTA at high levels in vascular smooth muscle and reliably transactivate tetO-driven transgenes would be useful for deciphering the role of TGF-β1 (or other proteins) in normal arterial physiology and in the development of arterial disease. Currently available SM22α-tTA mice were not useful for this purpose. Generation of higher-expressing lines of SM22α-tTA mice appears warranted.

Keywords: Smooth muscle cells; Transforming growth factor beta-1; SM22α; αMHC; Conditional transgenesis; Doxycycline

1. Introduction

Transforming growth factor-β1 (TGF-β1) plays important roles in cardiovascular development and disease. TGF-β1 appears to affect cardiac development, cardiac hypertrophy and fibrosis, arterial intimal growth, and atherosclerosis in both native and transplanted vessels [1]. A more precise elucidation of the roles of TGF-β1 in these processes will likely deepen our understanding of normal development and physiology and may also provide clues for developing novel therapies for cardiovascular diseases.

Gene knockout and overexpression studies, performed in mice, are powerful approaches for elucidating biological roles of genes. Gene knockout studies aimed at manipulating TGF-β1 signaling have been both productive and problematic. Disruption of TGF-β1 signaling in mice by germ line deletion of the genes encoding TGF-β1 (*tgfb1*) or either of the two TGF-β1 receptors (*tgfbr1* and *tgfbr2*) established critical roles for TGF-β1 in immune regulation and early vascular development but also caused embryonic and perinatal lethality [2–4]. Thus, it has not been possible to determine the consequences of loss of TGF-β1 expression or signaling in cardiovascular tissues of adult mice.

Abbreviations: αMHC, alpha myosin heavy chain; β-gal, β-galactosidase; SMC, smooth muscle cell; tetO, tetracycline operator; TGF-β1, transforming growth factor-beta 1; tTA, tetracycline transactivator.

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Germ line overexpression of TGF-β1 in cardiovascular tissues has also been both productive and problematic. Constitutive cardiac overexpression of TGF-β1 in mice, using the α-myosin heavy chain (αMHC) promoter, was compatible with survival to adulthood and revealed a potential role for TGF-β1 in cardiac fibrosis [5]. However, constitutive overexpression of TGF-β1 in murine smooth muscle cells (SMC), using the SM22α promoter, caused early embryonic lethality [6]. Thus, it has not been possible to determine the consequences of chronic overexpression of TGF-β1 in the vasculature of adult mice. This is unfortunate, because mice with increased vascular expression of TGF-β1 would be an attractive experimental setting for testing hypotheses regarding the role of vascular TGF-β1 expression in normal arterial physiology and in the development or prevention of arterial disease.

Conditional transgenic approaches offer powerful means for bypassing embryonic lethality and achieving overexpression in otherwise normal adult mice. The most widely used conditional transgenic approach involves use of a tetracycline-regulatable activator of transcription (tTA) to activate expression of a transgene that includes the tetracycline operator (tetO) [7]. Administration of tetracycline or an analogue during development prevents the tTA from activating tetO-regulated transgene expression; withdrawal of tetracycline postnatally allows the tTA to activate tetO-regulated transgene expression [8]. We used this conditional transgenic approach to attempt to bypass the embryonic lethality of SM22α-TGF-β1 transgenic mice and generate adult mice that overexpress TGF-β1 in the artery wall. We intended to use these mice to unravel the role of vascular TGF-β1 expression in arterial injury, experimental atherosclerosis, and organ transplantation. We began by generating mice with a conditional, tetO-driven TGF-β1 allele. We verified that expression of this allele could be activated in tTA-expressing adult mouse hearts in a tissue-specific, pharmacologically controlled manner. We then obtained lines of mice transgenic for a SM22α-tetracycline transactivator allele (SM22α-tTA). These mice were reported, elsewhere, to drive expression of tetO-driven alleles specifically in SMC of large arteries [9–11]. However, in our hands, the SM22α-tTA allele did not drive expression of the tetO-TGF-β1 allele in adult murine vascular SMC in vivo.

2. Materials and methods

2.1. Transgenic mice

Three lines of mice expressing the tTA from a fragment of the SM22α promoter (SM22α-tTA; C57BL/6 background) [9] were generously provided to us by Dr. Mansoor Husain (University of Toronto, Ontario, Canada). These three lines, each descended from an independent founder, were designated as lines “19”, “21”, and “36”. SM22α-tTA mice expressed the tTA in a tissue-specific pattern at increasing levels (21 < 19 < 36; personal communication, M. Husain). Because of a concern regarding potentially leaky transgene expression and embryonic lethality [6], we initially requested and received lines 19 and 21. In spring 2000, when we reported difficulties in detecting transactivation of a tetO-regulated allele in mice from these two lines, two mice from line 36 were promptly and kindly provided to us. It is our understanding that line 36 is the line used in the published studies that report use of these SM22α-tTA mice [9–11] and that data for these published studies were generated between 1998 and 2003 (personal communication, Dr. M. Husain). The majority of our expression studies on line 36 were carried out on first-generation offspring of the two mice we received in spring, 2000. Mice expressing the tTA from the cardiac αMHC gene promoter (αMHC-tTA; 93% FVB/N background) were obtained from Dr. Glenn Fishman (NYU, New York, NY) [12]. Mice transgenic for a tetO-driven β-galactosidase (β-gal) allele (“Ro1” mice; FVB/N background) were obtained from Dr. Bruce Conklin (Gladstone Institutes, San Francisco, CA) [13]. Mice transgenic for a SM22α–β-gal transgene (C57BL/6 background) were obtained from Dr. Li Li (Wayne State University, Detroit, MI) [14]. The SM22α promoter sequence in the SM22α–β-gal transgene in Dr. Li’s mice is the same sequence used by Dr. Husain’s group to generate the SM22α-tTA transgenic mice [9]. All mice were genotyped by Southern analysis of tail-tip DNA. The αMHC-tTA and SM22α-tTA transgenic mice were genotyped by hybridization of digested tail DNA to an 833 bp *Xba*I-*Sph*I fragment of plasmid pUHD17-1 [15], which contains tTA sequences. This probe detected a 1.8 kb band in *Eco*RI-digested DNA of αMHC-tTA mice and a 4.5 kb band in *Hin*dIII-digested DNA of SM22α-tTA transgenic mice. No other tTA-transgenic mice were present in our transgenic mouse colony.

We generated mice that were transgenic for a conditional, tetracycline-regulated TGF-β1 allele. The transgene included a rat TGF-β1 cDNA driven by a tetO-containing promoter. To begin to construct this transgene, we obtained two plasmids, one containing a 5′ rat TGF-β1 cDNA fragment and the other containing an overlapping 3′ rat TGF-β1 cDNA fragment (Dr. Anita Roberts, National Cancer Institute, Bethesda, MD). We obtained the plasmid pUHG10-3 [13], containing the tetO upstream of a minimal CMV promoter from Dr. Bruce Conklin. The 5′ and 3′ TGF-β1 fragments were ligated in pBluescript to generate a complete 1173 bp rat TGF-β1 cDNA. This plasmid was used as a substrate for site-directed mutagenesis (QuikChange, Stratagene, La Jolla, CA) to convert G to C at position 668 and T to A at position 673. These changes convert two cysteine residues to serine residues and, by analogy with simian and porcine TGF-β1 [16,17], generate a constitutively active TGF-β1 molecule. The mutated rat TGF-β1 cDNA was excised and inserted at the *Xba*I site of pUHG10-3, downstream of the tetO and CMV promoter, to yield the plasmid pUHG10-3-TGF-β1. This plasmid was digested with *Alw*44I and *Bgl*II to release the 2.7 kb tetO-TGF-β1 construct (Fig. 1). This construct was injected into C57BL/6 × SJL F1 hybrid zygotes, which were implanted into pseudopregnant mothers. Offspring were