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GATA-6 is a novel transcriptional repressor of the human *Tenascin-C* gene expression in fibroblasts

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Abstract

In this study we show that GATA-6 is a novel repressor of *TN-C* gene expression. We demonstrated that overexpression of GATA-6 in fibroblasts inhibited basal levels, as well as markedly decreased IL-4- and TGF-β-induced TN-C mRNA and protein levels. A GATA-6 response element was mapped to position −467 to −460 of the TN-C promoter. In addition, we showed that GATA-6 binds this site both *in vitro* and *in vivo*.

Keywords: *Tenascin-C*; Transcriptional regulation; GATA-6

1. Introduction

TN-C is a modular extracellular matrix glycoprotein composed of a series of epidermal growth factor-like repeats, fibronectin type III-like repeats and a C-terminal fibrinogen-like globular domain [1]. TN-C is highly expressed during development in organogenesis and at sites of epithelial–mesenchymal transition [2]. In the adult, TN-C expression is less abundant, but is induced during wound healing and in pathological conditions such as tumorigenesis, vascular hypertension and myocardial infarction [3–6]. TN-C is an important regulator of cell adhesion, migration and proliferation during tumorigenesis and vascular remodeling. In cell culture, TN-C interferes with integrin-dependent spreading of most cell types by binding to fibronectin and preventing its interaction with syndecan-4 [7]. Syndecan-4, a transmembrane heparan sulfate proteoglycan, works in synergy with integrin α5β1 to activate Rho signaling and subsequently, actin stress fiber assembly and cell spreading on fibronectin [8]. Disruption of syndecan-4 signaling in cancer cells stimulates a migratory behavior and proliferation [9,10]. TN-C also plays a critical role in the vascular remodeling during pulmonary arterial hypertension (PAH) by promoting proliferation and survival of vascular smooth muscle cells (VSMCs) via its ability to cross-modulate the activity of EGF and FGF-2 receptors [11]. Given the importance of TN-C during the development and progression of tumorigenesis and vascular disease, identification of factors that regulate TN-C expression is important in understanding the site-specific and transient nature of its expression during these pathological conditions.

The GATA family of transcription factors is an evolutionary conserved family of DNA-binding proteins that contain two tandem zinc fingers that interact with other transcriptional regulators and bind to the canonical DNA motif (G/A)GATA(A/T) [12]. Six family members have been identified in vertebrates and based on their sequence homology and expression patterns are divided into two subfamilies: GATA-1, -2, and -3, which are involved mainly in the development of hematopoietic cells and GATA-4, -5 and -6, which function in the development of mesoderm- and endoderm-derived organs such as the heart and gastrointestinal tract, respectively [13]. Human GATA-6 is expressed in a wide array of adult tissues including heart, lung, liver, kidney, pancreas, spleen, ovary and small intestine, where it is believed to maintain the differentiated phenotypes of cells within these tissues [12,14]. Loss of GATA-6 in ovarian carcinomas leads to a loss of epithelial-specific markers like laminin and Disabled-2 (Dab2) [15]. It has been suggested that GATA-6 is a

Abbreviations: *TN-C*, *Tenascin-C*; CAT, chloramphenicol acetyltransferase; ChIP, Chromatin Immunoprecipitation; IL-4, Interleukin-4; TGF-β, Transforming growth factor-β; ECM, extracellular matrix; COL1A2, α2(I) collagen

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regulator of the cell cycle in vascular myocytes and embryonic fibroblasts [14,16,17]. In VSMCs, GATA-6 mRNA is downregulated after mitogenic stimulation and forced expression of GATA-6 inhibits cell proliferation [18]. GATA-6 has also been shown to be downregulated in balloon-injured rat carotid arteries and when restoration of GATA-6 levels was performed with transduction of a GATA-6-encoding adenovirus, vessels exhibited a higher degree of VSMC differentiation and a reduced level of intimal hyperplasia [18]. Furthermore, GATA-6 has been shown to regulate genes involved in cell–cell and cell–matrix interactions associated with synthetic VSMC function, such as the response to vascular injury [19]. Therefore, GATA-6 may be one of the key regulators of the VSMC phenotype during proliferative vascular diseases like PAH and atherosclerosis.

Because TN-C and GATA-6 have been shown to be key players in both cancer and vascular remodeling, and the human TN-C promoter contains seven putative GATA binding sites, we decided to investigate whether GATA-6 can regulate *TN-C* gene expression. In this study, we demonstrate that GATA-6 is a functional repressor of TN-C transcription and binds to at least one GATA site within the TN-C promoter *in vivo*.

2. Materials and methods

2.1. Cells

Human foreskin fibroblast cultures were obtained from foreskins of healthy newborns and propagated as previously described [20]. 293T cells were purchased from ATCC (Manassas, VA, USA) and grown in the same conditions.

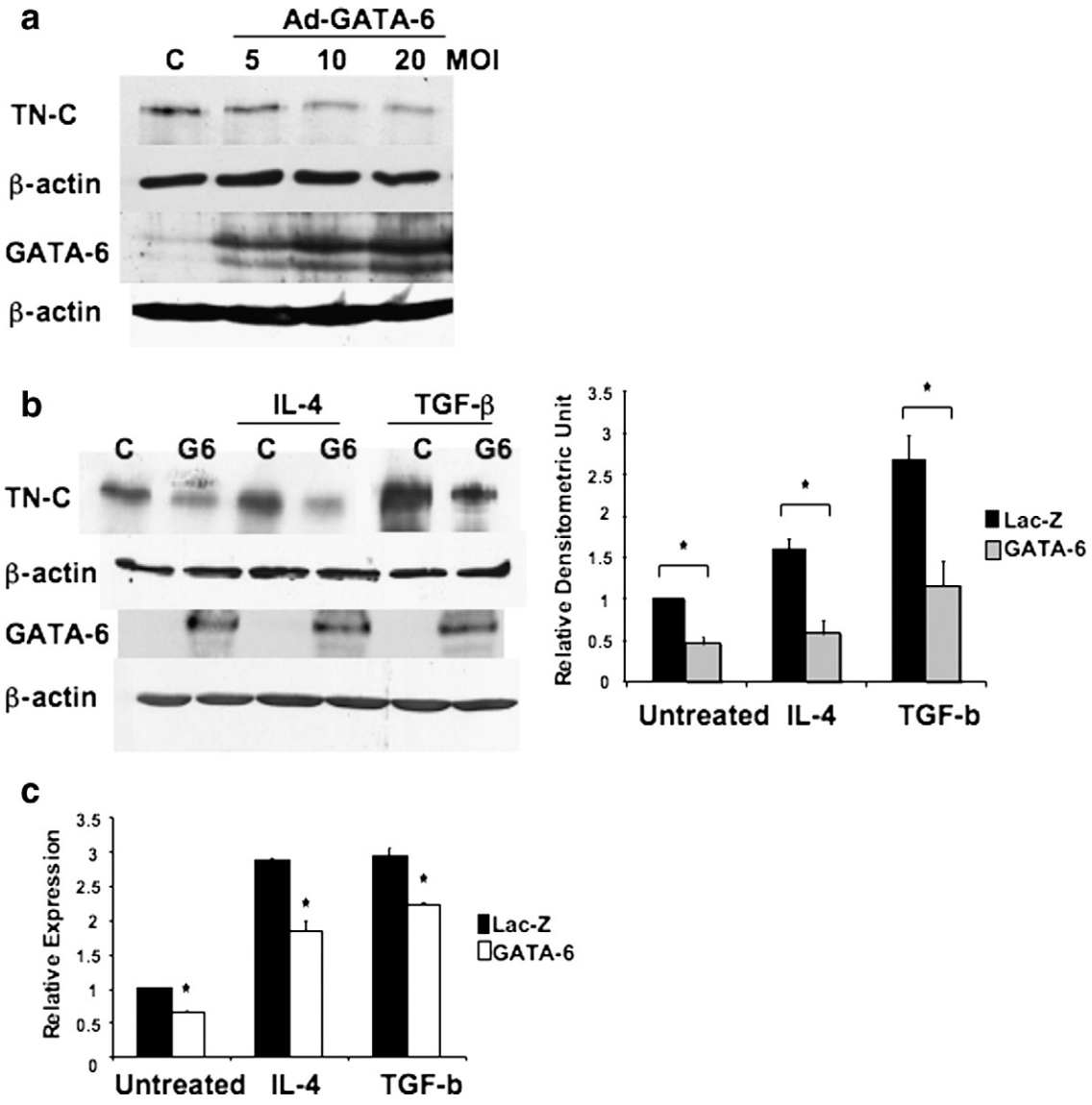

Fig. 1. GATA-6 negatively regulates *TN-C* gene expression. (a) Cell lysates from fibroblasts transduced with 5, 10, and 20 MOI of Ad-GATA-6 or 20 MOI of Ad-LacZ (control) for 48 h were subjected to immunoblot analysis with antibodies against TN-C and GATA-6. (b) Fibroblasts were transduced with 10 MOI of Ad-GATA-6 or control virus for 24 h, then treated with IL-4 (10 ng/ml) or TGF-β (2.5 ng/ml) for 24 h. Samples were assayed by immunoblot analysis with antibodies against TN-C and GATA-6 or (c) quantitative RT-PCR to determine TN-C mRNA levels. One representative Western blot of four independent experiments is shown. Band intensities were quantitated by densitometric analysis and are shown relative to the level of untreated fibroblasts transduced with control virus. *$P<0.01$.