Inhibition of versican expression by siRNA facilitates tropoelastin synthesis and elastic fiber formation by human SK-LMS-1 leiomyosarcoma smooth muscle cells *in vitro* and *in vivo*

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Abstract

Versican is an extracellular matrix (ECM) molecule that interacts with other ECM components to influence ECM organization, stability, composition, and cell behavior. Versican is known to increase in a number of cancers, but little is known about how versican influences the amount and organization of the ECM components in the tumor microenvironment. In the present study, we modulated versican expression using siRNAs in the human leiomyosarcoma (LMS) smooth muscle cell line SK-LMS-1, and observed the formation of elastin and elastic fibers *in vitro* and also *in vivo* in a nude mouse tumor model. Constitutive siRNA-directed knockdown of versican in LMS cells resulted in increased levels of elastin, as shown by immunohistochemical staining of the cells *in vitro*, and by mRNA and protein analyses. Moreover, versican siRNA LMS cells, when injected into nude mice, generated smaller tumors that had significantly greater immunohistochemical and histochemical staining for elastin when compared to control tumors. Additionally, microarray analyses were used to determine the influence of versican isoform modulation on gene expression profiles, and to identify genes that influence and relate to the process of elastogenesis. cDNA microarray analysis and TaqMan low density array validation identified previously unreported genes associated with downregulation of versican and increased elastogenesis. These results highlight an important role for the proteoglycan versican in regulating the expression and assembly of elastin and the phenotype of LMS cells.

Introduction

Versican is an extracellular matrix (ECM) proteoglycan found in the interstitium of most soft tissues. There are at least four naturally occurring versican isoforms that have been identified and characterized. These isoforms, designated V0, V1, V2, and V3, are generated by alternatively splicing the central α-glycosaminoglycan (α-GAG) and β-glycosaminoglycan (β-GAG) domains [1–3]. Important to the biology and function of versican are its chondroitin sulfate (CS) GAG side chains and the interaction of its protein core with other molecules [4,5].

A number of studies have reported on an inverse relationship between elastin and versican expression [6–11]. Gene array studies of developing mice show coordinated expression patterns of elastin and its associated proteins, which underscore the critical spatial and temporal timing of protein expression required for the generation of elastic fibers [12]. In general, it has been observed that when versican expression and accumulation is high, tropoelastin expression and its polymerization is low [13]. For example, smooth muscle cells (SMCs) isolated from rat pups are typically elastogenic and do not express detectable versican in contrast to SMCs from adult rats, which typically produce little elastin and have measurable levels of versican [13–18]. Correspondingly, downregulation of versican in adult rat SMCs by the use of versican antisense mRNA results in

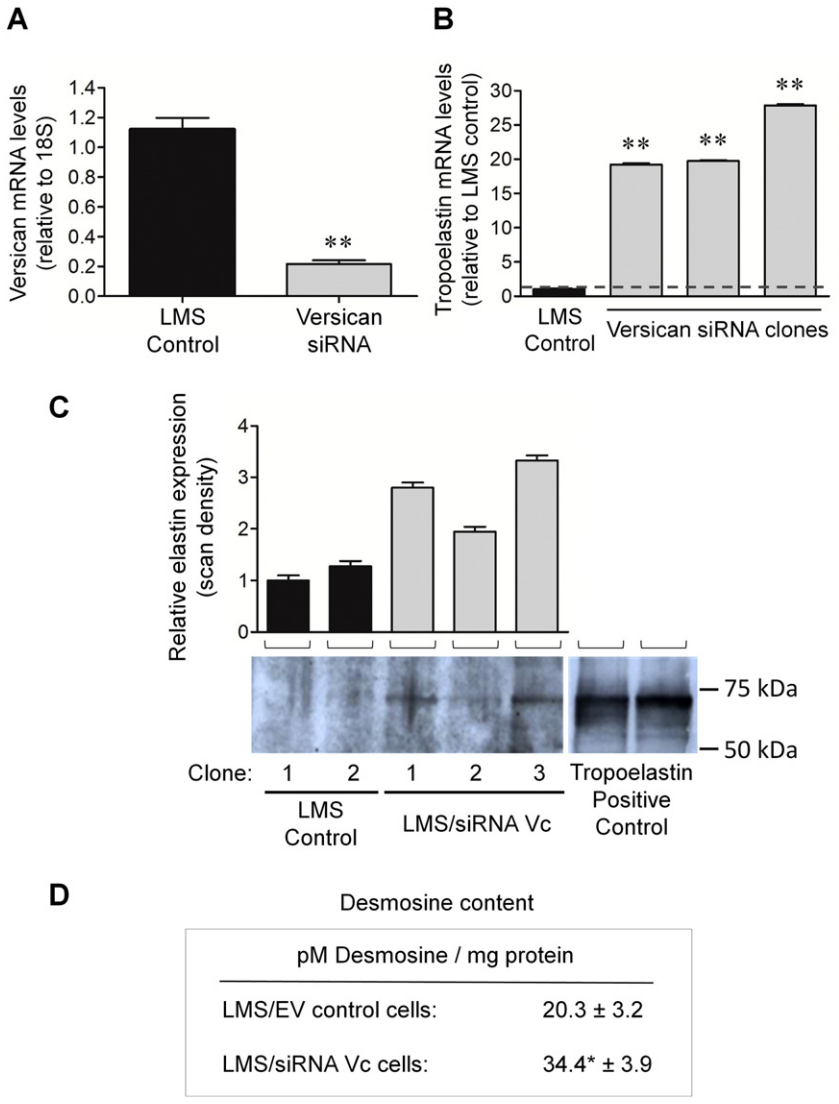

Fig. 1. siRNA-mediated knockdown of versican leads to a corresponding increase in elastin expression, accumulation, and levels of mature elastin (desmosine). (A) qRT-PCR demonstrates on average a 79% knockdown of versican mRNA using versican-directed siRNA (n = 7; gray columns) compared to controls (n = 4; black columns). (B) A corresponding 12- to 29-fold increase in tropoelastin expression (gray columns) is observed in all the LMS clones constitutively expressing versican-directed siRNA compared to LMS/EV controls. The results of three clones are shown. ** $p < 0.01$. (C) Representative Western blot probed with anti-bovine tropoelastin detecting 70 kDa tropoelastin in lysates of empty vector clone and siRNA versican LMS cells in culture. All lanes were loaded with equal protein (20 μg). Lanes 1 and 2 show little or no elastin, while lanes 3, 4, and 5 show detectable elastin levels. Lanes 6 and 7 are positive controls of full-length tropoelastin which migrate at the same molecular weight as the cell culture sample extracts. Molecular weight range of ~50–100 kDa is shown. Densitometry in upper panel represents the relative elastin expression ± SE; LMS control (black) vs. LMS/siRNA Vc cells (gray). The relative elastin expression level of each of the samples was made in comparison to the LMS/EV control in lane 1 (set to one). (D) Desmosine content was measured in LMS/EV control cells (n = 5) and compared to LMS/siRNA Vc cells (n = 5) and was approximately 70% greater in the versican siRNA cells vs. the control LMS cells. Mean ± SE; * $p < 0.05$.

increased deposition of elastin *in vitro* and *in vivo* [8]. Collectively, these studies provide support for the hypothesis that an inverse relationship exists between versican and elastin expression.

Numerous studies link versican to disease states (see recent reviews; [5,19]). An inverse relationship between elastin and versican has been demonstrated in vascular and lung diseases. Versican is prominent in early and advanced stages of lesion formation in atherosclerotic plaques with loss or degradation of elastic lamellae [20]. Increased levels of versican are also found during restenosis, and in vascular grafts, and aneurysms [20,21]. Furthermore, supravalvular aortic hypertrophic stenosis is observed in patients who have mutations and or deletions of the elastin gene [22]. In lung diseases such as lymphangioleiomyomatosis (LAM) and chronic obstructive pulmonary disease (COPD), increased elastin levels with decreased versican levels and vice versa have been observed [7,23–25]. Interestingly, low levels of elastin expression are associated with high proliferation of skin fibroblasts in Costello syndrome [26] and this relationship could be reversed through modulation of versican isoform expression [6]. Furthermore, a relationship exists between elastin deposition and cell proliferation in that the elastin knockout mouse dies at birth due to excessive subendothelial proliferation of SMCs leading to obstruction and closure of the aorta [27]. Thus, there are significant data that suggest an important relationship between versican,