MicroRNA-29a: A Novel Therapeutic Target to Treat Lumbar Spinal Stenosis

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INTRODUCTION: Lumbar spinal stenosis (LSS) remains the most common indication for spinal surgery in patients over the age of 65.[1] Despite being a common cause of lumbar spinal stenosis (LSS), the mechanism of ligamentum flavum hypertrophy (LFH) is poorly understood.[2] It has been demonstrated that LF hypertrophy is secondary to fibrosis, but the molecular mechanism by which this occurs has not been fully elucidated.[3] Though numerous studies have examined causative factors of LFH, studies evaluating protective factors are lacking. Recently, microRNA-29a has been identified as a 'master fibromiRNA' regulator, as decreased levels of microRNA-29a have been linked to fibrosis in the heart, liver, kidney and skin.[4] Additionally, microRNA-29a has been reported to repress TAB1-mediated TIMP-1 production, which has been associated with LF hypertrophy.[5,6,7] This study's aim was to test the hypothesis that patients with symptomatic LSS exhibit decreased expression of microRNA-29a in their LFH tissue, and that transfection of LFH cell culture with microRNA-29a inhibitor would increase expression of fibrotic proteins implicated in LFH.

METHODS: Six patients (3 M, 3 F; age 68 ± 10.8 years) underwent L3-L5 laminectomy to address symptomatic spinal stenosis. LF thickness was measured on preoperative axial T1 MRIs to identify hypertrophic and non-hypertrophic levels.[8] In each patient, LFH tissue was collected from L4/L5 and non-hypertrophic LF was removed from L2/L3, which served as normal LF control. LF cell cultures derived from surgical LF tissue were divided into three experimental groups: a control, a microRNA-29a inhibitor (low (100nM) and high (300nM) concentrations) group, and a microRNA-29a over-expressor group. For the over-expressor group, microRNA-29a lentiviral plasmid with precursor-containing sequences from GenScript Biotech were transfected into LF cells from cell culture to induce over-expression of microRNA-29a while the inhibitor group was transfected with MISSION synthetic microRNA inhibitor to decrease cellular expression of microRNA-29a. RT-PCR and the comparative ΔΔCt method were performed to establish relative microRNA-29a levels and gene expression profiles of the genes involved in fibrosis (collagen I, collagen III). . The measurements of LF thickness, collagen I, collagen III, and microRNA-29a levels were compared using one-tailed paired *t*-tests. The correlations among collagen I, collagen III, and microRNA-29a levels were analyzed using Pearson's correlation coefficients. $p < 0.05$ was considered statistically significant.

RESULTS: The thickness of LF in the stenotic levels was significantly higher than in the non-stenotic levels (6.8 ± 0.9 mm vs 4.0 ± 0.9 mm, $p < 0.01$). Additionally, mRNA levels of collagen I were significantly higher ($p = 0.04$) and microRNA-29a levels were significantly lower ($p = 0.03$) in hypertrophic LF compared to control (FIGURE 1A). The mRNA levels of collagen III were higher in the hypertrophic LF, although this was not significant ($p = 0.10$). MicroRNA-29a level was negatively correlated with type I collagen level ($r = -0.82$, $p = 0.02$) and type III collagen level ($r = -0.53$, $p = 0.14$). Over-expression of microRNA-29a resulted in a 0.58 (±0.001) fold decrease in relative gene expression of collagen I. Over-expression of microRNA-29a did not appreciably alter collagen III gene expression in these experiments with a 0.96 (±0.04) fold decrease in relative gene expression of collagen III (Figure 1C). Inhibition of microRNA-29a at 100 nM dosing resulted in a 9.4 (±0.2) fold increase in collagen I expression relative to control. The 300 nM dose resulted in a 14.1 (±1.4) fold increase in the relative gene expression of collagen I (Figure 1B). Similarly, for collagen III the same competitive inhibitor at 100 nM dosing resulted in a 2.0 (±0.1) fold increase in collagen III expression relative to control. The 300 nM dose resulted in a 2.5 (±0.3) fold increase in the relative gene expression of collagen III (Figure 1D).

DISCUSSION: These data suggest that microRNA-29a may potentially play a protective role against LFH and may serve as a therapeutic target in the management of LSS. Our finding demonstrated stimulation of microRNA-29a production attenuates expression of key genes that promote LFH. This pilot study lays the groundwork for further investigations using LF cell cultures to test therapeutic agents that upregulate microRNA-29a to blunt LFH and elucidate the molecular mechanisms through which microRNA-29a controls fibroblast collagen production.

SIGNIFICANCE: This study demonstrates that microRNA-29a may potentially be used to treat LFH and provides the groundwork to initiate the development of a therapeutic product for LSS.

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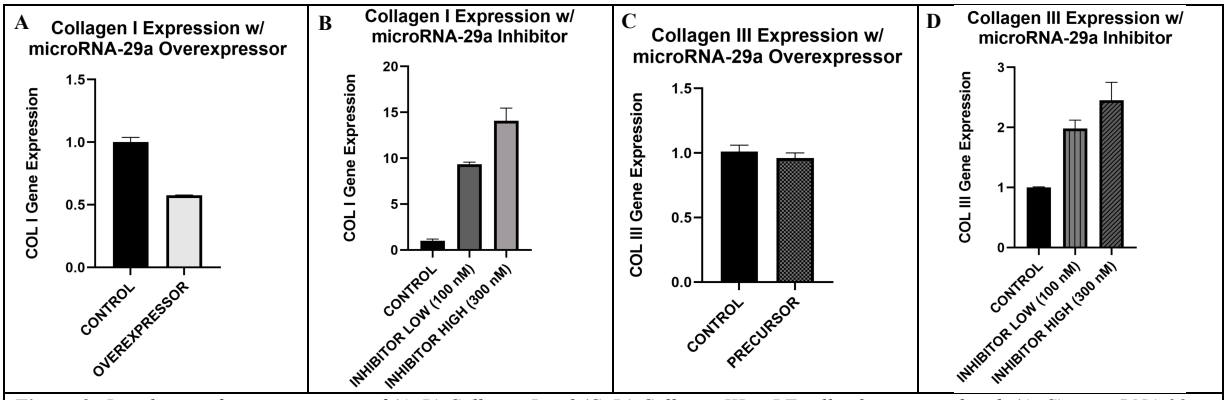

Figure 2: *Regulation of gene expression of (A, B) Collagen I and (C, D) Collagen III in LF cell culture treated with (A, C) microRNA-29a over-expressor and (B, D) microRNA-29a inhibitor*