INTRODUCTION: Lumbar spinal stenosis (LSS) remains the most common indication for spinal surgery in patients over the age of 65. Though numerous studies have examined causative factors of LFH, studies evaluating protective factors are lacking. Recently, microRNA-29a has been identified as a ‘master fibromRNA’ regulator, as decreased levels of microRNA-29a have been linked to fibrosis in the heart, liver, kidney and skin.6 Additionally, microRNA-29a has been reported to repress TAB1-mediated TIMP-1 production in LSS, and has been associated with LF hypertrophy.3,5 This study’s aim was to test the hypothesis that patients with symptomatic LSS exhibit decreased expression of microRNA-29a in their LF 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. 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 100 nM and high 300 nM concentrations) group, and a microRNA-29a over-expression group. For the over-expression 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, 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 (±2.0) 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 500 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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