

Role of G0/G1 Switch Gene 2 in TGF- β 1-Induced Hypertrophy of Ligamentum Flavum

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INTRODUCTION: Lumbar spinal stenosis (LSS) is a major spinal disorder in elderly patients contributing to back pain. Thickening of ligamentum flavum (LF) due to tissue hypertrophy is the main etiology of LSS. Compression of Dural sac and nerve roots by the hypertrophied LF causes various symptoms such as low back pain, leg pain, paresthesia, numbness, and intermittent claudication, leading to disability and socioeconomic burden. Available evidence suggests that non-surgical measures to treat LSS have mixed efficacies, so there is an urgent need for the development of therapies to treat or prevent hypertrophy of LF (HLF) thereby to decrease cost and morbidity associated with LSS. Histological changes in HLF include a loss of elastic fibers and increased collagen fibers and proteoglycans, led by upregulation of collagens, cell differentiation to myofibroblasts in addition to several growth factors such as transforming growth factor-beta (TGF- β), inflammatory cytokines, angiogenesis factors etc. contributing to pathological changes in HLF. G0/G1 switch gene 2 (G0S2), first identified in activated lymphocytes to regulate lipid metabolism to control cell proliferation, is implicated in the regulation of diverse biological and pathological processes such as cell proliferation, apoptosis, inflammation, metabolism, and carcinogenesis. Recent studies have shown that G0S2 mediates the renal inflammation and fibrosis leading to chronic kidney disease, however its role in HLF is not known. Here, we identified that G0S2 expression is increased upon TGF- β 1 stimulation of LF cells and regulates the expression of genes involved in fibrogenesis.

METHODS: Surgical LF tissue specimen collected during surgery were used to isolate the LF cells from hypertrophied LF (HLF) and adjacent non-hypertrophied LF (control) tissue. Informed consent was obtained from each patient and the study was approved by of an institutional review board. Isolated cells were first stimulated with TGF- β 1 (5 or 10 ng/ml) for 24h before being transfected with control siRNA (siCN) or G0S2-specific siRNA (siG0S2) for and additional 48h (**Figure 1A**). 100 pmol of siRNA was used for all experiments except for the initial dosing experiment (**Figure 1C**). Samples were harvested for RNA isolation and real-time RT-PCR was performed. Data are analyzed in GraphPad Prism 10.0 and Unpaired *t* test was performed to calculate the significance.

RESULTS: Stimulation of control or hypertrophic LF cells with TGF- β 1 induced the expression of *G0S2* (**Figure 1B**). *G0S2* gene expression was successfully knockdown over 70% using siRNA (**Figure 1C**). TGF- β 1 increased the expression of *TGF β 1*, collagen-1 (*COL1*), versican (*VCAN*), alpha smooth muscle actin (α -SMA/*ACTA2*) in both normal and HLF cells, confirming the induction of extracellular matrix fibrosis and myofibroblast differentiation by TGF- β 1 (**Figure 1D**). Knockdown of G0S2 by using siRNA significantly inhibited the TGF- β 1 mediated expression of *TGF β 1*, *VCAN* and *ACTA2* but not *COL1* (**Figure 1D**), suggesting the potential role of G0S2 in LF fibrosis and hypertrophy.

DISCUSSION: This study identified G0S2 as a potential regulator of pathologic development of hypertrophy of LF. Our results show that G0S2 is implicated in fibrotic signaling in LF cells, as it can be induced by TGF- β 1. G0S2 knockdown decreased the TGF- β -induced expression of *TGF β 1*, *VCAN* and *ACTA2* in LF cell culture. Specifically, *VCAN* and *ACTA2*, which refers to proteoglycans and myofibroblast differentiation respectively, and are the pathological markers for thickened ligamentum flavum, are inhibited by G0S2 knockdown. Future studies are necessary to establish the molecular mechanisms behind G0S2 regulation of fibrogenesis and its regulation of HLF.

SIGNIFICANCE/CLINICAL RELEVANCE: This study highlights the possibility of using G0S2 as a suitable target for gene therapy to regulate the TGF- β 1 signaling pathway to reduce hypertrophy of LF relief the LSS.

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FIGURE:

