Mechanical stretching induces the apoptosis of parametrial ligament fibroblasts via the actin cytoskeleton and Nr4a1

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Abstract
Background The anatomical positions of pelvic floor organs are maintained mainly by ligaments and muscles. Long-term excessive mechanical tension stimulation of pelvic floor tissue beyond the endurance of ligaments or muscles will lead to the occurrence of pelvic organ prolapse (POP). In addition, cytoskeletal reconstitution is a key process by which cells respond to mechanical stimulation. The aim of the present study was to investigate the protective effect of actin cytoskeleton in resist mechanical stretching (MS)-induced apoptosis of parametrial ligament fibroblasts (PLFs) and the underlying mechanisms.

Methods 8 women who underwent hysterectomy surgery for reasons excluding the presence of malignant tumors and POP served as controls, and 7 patients who underwent hysterectomy surgery for only advanced POP comprised the POP group. MS was provided by a fourpoint bending device. We examined the effects of MS on actin cytoskeleton and apoptosis of PLFs. Then the apoptosis was detected after latrunculin A (Lat-A, a potent inhibitor of actin) exposure and the interference of Nr4a1.

Results MS could significantly induce apoptosis of PLFs from non-POP patients, which exhibited an apoptosis rate close to that of PLFs from POP patients, and the apoptosis rate was higher following latrunculin A treatment. In addition, Nr4a1 and Bax expression was increased while Bcl-2 and caspase-3 expression was clearly decreased after treatment with MS and Lat-A. However, the MS-induced apoptosis of PLFs was reduced when treatment with siRNA targeting Nr4a1 was used to downregulate the level of Nr4a1.

Conclusions These outcomes reveal a novel mechanism that links the actin cytoskeleton and apoptosis in PLFs by Nr4a1; this mechanism will provide insight into the clinical diagnosis and treatment of POP.

Full Text
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Figures
Identification of primary parametrial ligament fibroblasts (PLFs). Immunofluorescence staining for vimentin (A), cytokeratin (B) in cultured PLFs and a merged image (C). (D), Primary cultured PLFs visualized by light microscopy (magnification: 200×).
Detection of apoptosis of the uterine sacral ligament. A, TUNEL staining of the uterine sacral ligament, magnification: 200×. B, The ratio of TUNEL-positive cells to the total number of cells. C and D, Protein levels in the uterine sacral ligament of POP and control groups were determined by Western blotting and normalized to those of GAPDH. * indicates p < 0.05, ** indicates p < 0.01, *** indicates p < 0.001. (CON: the uterine sacral ligament obtained from patients without POP; POP: the uterine sacral ligament obtained from patients with POP).
Mechanical stretching increased the apoptosis rate and actin cytoskeleton disassembly in PLFs. A, Cell apoptosis was assessed by flow cytometry analysis. The apoptosis rate was determined as the percentage of PE-positive cells, with early apoptotic cells negative for 7-AAD and late apoptotic cells positive for 7-AAD. B, Quantified apoptosis rates in each group. C, PLFs were stained with phalloidin and imaged by fluorescence microscopy. Red fluorescence delineates the cell cytoplasm; blue fluorescence delineates nuclei (magnification: 200×). D, The relative cell surface areas were quantified by ImageJ software. E, Protein levels in PLFs were determined by Western blotting and normalized to those of GAPDH. F, Band intensities were quantified by Quantity One. G, mRNA levels in PLFs were quantified by real-time RT-PCR and normalized to those of GAPDH. *** indicates p < 0.001. (CON: PLFs isolated from patients without POP; MS: PLFs isolated from patients without POP that were exposed to mechanical stretching; POP: PLFs isolated from patients with POP).
Figure 4

The effect of mechanical stretching on apoptosis after actin cytoskeleton disassembly. A, Cell apoptosis was detected by flow cytometry analysis; B, Quantified apoptosis rates in each group. C, PLFs were stained with phalloidin and imaged by fluorescence microscopy (magnification: 200×). D, Relative cell surface areas were quantified by ImageJ software. E, Protein levels in PLFs were determined by Western blot analysis and normalized to those of GAPDH. F, Band intensities were quantified by Quantity One. G, mRNA levels in PLFs were quantified by real-time RT-PCR and normalized to those of GAPDH. * indicates p < 0.05, ** indicates p < 0.01 and *** indicates p < 0.001 compared with the CON group; # indicates p < 0.05, ## indicates p < 0.01 compared with the MS group; ^ indicates p < 0.05, ^^
indicates p < 0.01 and ^^^ indicates p < 0.001 compared with the Lat-A group. (CON: PLFs isolated from patients without POP; MS: PLFs isolated from patients without POP and exposed to mechanical stretching; Lat-A: PLFs isolated from patients without POP and exposed to Lat-A; Lat-A+MS: PLFs isolated from patients without POP and exposed to Lat-A and mechanical stretching).
Figure 5

The effect of mechanical stretching on apoptosis after Nr4a1 deficiency. A, Cell apoptosis was detected by flow cytometry analysis. B, Protein levels in PLFs were determined by Western blot analysis and normalized to those of GAPDH. C, The levels of Nr4a1 after Nr4a1 gene interference. D, Quantified apoptosis rates in each group. E, Band intensities were quantified by Quantity One. F, mRNA levels in PLFs were quantified by real-time RT-PCR and normalized to those of GAPDH; * indicates p < 0.05, ** indicates p < 0.01 and *** indicates p < 0.001 compared with the CON group; # indicates p < 0.05, ## indicates p < 0.01 compared with the MS group; ^ indicates p < 0.05 ^^ ^ indicates p < 0.001 compared with the si-Nr4a1 group. (CON: PLFs isolated from patients without POP; MS: PLFs isolated from patients without POP and exposed to mechanical stretching; si-Nr4a1: si-Nr4a1-mediated transfection was used to silence Nr4a1 in PLFs; si-Nr4a1+MS: si-Nr4a1-treated cells treated with mechanical stretching).
