European Journal of Obstetrics & Gynecology and Reproductive Biology 174 (2014) 141–145

Contents lists available at ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology

journal homepage: www.elsevier.com/locate/ejogrb

Relationship between the expressions of mitofusin-2 and procollagen in uterosacral ligament fibroblasts of postmenopausal patients with pelvic organ prolapse

Hua-yun Chen [a], Ye Lu [a,*], Yu Qi [b], Wen-pei Bai [a], Qin-ping Liao [a,**]

[a] Department of Obstetrics & Gynecology, Peking University First Hospital, Beijing 100034, China
[b] Experiment Center of Peking University First Hospital, Beijing 100034, China

ARTICLE INFO

Article history:
Received 15 July 2013
Received in revised form 7 November 2013
Accepted 28 November 2013

Keywords:
Pelvic organ prolapse
Laser capture microdissection
Fibroblast
Procollagen
Mfn2

ABSTRACT

Objectives: To compare the mRNA and protein expressions of mitochondrial fusion protein-2 (mitofusin-2, Mfn2), and procollagen 1A1/1A2/3A1 in uterosacral ligament fibroblasts of postmenopausal patients with or without pelvic organ prolapse (POP). The effect of Mfn2 on the expression of procollagen in fibroblasts was also investigated.

Study design: Thirty-seven POP patients and 23 non-POP postmenopausal patients were included in the POP (study) and non-POP (control) groups, respectively. Laser capture microdissection (LCM) was combined with quantitative real-time polymerase chain reaction (qRT-PCR) and western blotting to detect the mRNA and protein expressions of Mfn2, and types I and III procollagen in uterosacral ligament fibroblasts of the two groups, and the differences in expression levels were compared between the groups. The correlation between Mfn2 and procollagens was also investigated.

Results: Fibroblasts were successfully isolated from frozen sections of the uterosacral ligament using LCM. The results of qRT-PCR and western blot showed that the expressions of types I and III procollagen were significantly lower and those of Mfn2 were significantly higher in the POP group than in the non-POP group ($p < 0.05$, all). In POP, opposite trends of protein expression changes of Mfn2 and procollagens were observed along with the duration of postmenopause ($P < 0.05$), while this was not the case in POP accompanied by stress urinary incontinence and frequency of vaginal delivery ($P > 0.05$). The expressions of type I and III procollagen were negatively associated with Mfn2 in POP patients ($-1 < r < 0$, $P < 0.001$, all).

Conclusions: Mfn2 expression changed along with the duration of postmenopause and had a negative association with the expression of procollagens. Our results suggest that the Mfn2 protein may affect the synthesis of procollagen of fibroblasts in postmenopausal patients with POP. Changes in Mfn2 and procollagen expression may play a role in the development of POP.

1. Introduction

Female pelvic organ prolapse (POP) is a condition in which the pelvic organs descend into or prolapse out of the vagina. The symptoms of POP involve the lower genital tract, urinary tract, and gastrointestinal tract, and have an impact on activity and quality of daily life [1]. POP has been shown to be related to functional disorders of the supporting tissues of the pelvic cavity. The uterosacral ligament is one of the main supporting tissues in the female pelvis, and the collagen fibers in the ligament are important structural elements for the maintenance of the position of the uterus and the prevention of uterine prolapse [2]. Collagen and collagen fibers in the uterosacral ligament are mainly generated by crosslinking of different procollagen proteins, which are synthesized and secreted by fibroblasts. Previous research on POP has focused on extracellular matrix (ECM) metabolic changes, such as the number and morphological changes of type I and type III collagens [3–5], but the fibroblasts that synthesize and secrete the collagens have not been studied in detail.

Mitochondrial fusion protein-2 (mitofusin-2, Mfn2) is a protein localizes to the mitochondrial outer membrane and possesses an essential role in mitochondrial fusin, thus regulating mitochondrial morphology and function in mammalian cells [6]. The normal morphology and function of mitochondria ensure the biological functions of cells [7]. Mfn2 is also involved in oxidative stress,

* Corresponding author. Tel.: +86 013651032423.
** Corresponding author. Tel.: +86 013701124527.
E-mail addresses: songqinlu@sina.com, yelu123@yeah.net (Y. Lu), qinping_liao@sohu.com (Q.-p. Liao).

0301-2115/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.ejogrb.2013.11.024

intracellular signal transduction, and regulation of cell proliferation and apoptosis among other biological processes [8,9]. Abnormal expression and loss of function of Mfn2 play important roles in the development and progression of many diseases. Considering its multiple effects on cells, we hypothesized that Mfn2 may affect the function of fibroblasts by modulating mitochondrial fusion or signal transduction pathways leading to changes in collagen synthesis and secretion. Therefore, we speculated that Mfn2 may play a role in the pathogenesis of POP.

2. Materials and methods

2.1. Tissue sample collection and processing

Between August 2011 and July 2013, we collected samples of uterosacral ligament from POP and non-POP patients treated at Peking University First Hospital. Uterosacral ligament biopsy specimens were obtained from uterine samples collected from 37 patients with POP II–IV (according to the POP-Q) (study group) and 23 non-POP patients who underwent hysterectomy because of gynecological benign diseases, and who were matched by age, parity, primiparity age, body mass index (BMI) and duration of postmenopause (control group) (Table 1). The staging of POP was based on the POP-Q evaluation system [10]. None of the patients had a history of hormone replacement therapy, urinary tract infection, or vaginal surgery. None of the women in the two groups had diseases affecting collagen metabolism. All surgeries were performed by the same group of skilled surgeons. Samples were collected from the patients after obtaining informed consent, and ethical approval was obtained from the ethics committee of Peking University First Hospital. All samples were cut into 2 mm × 3 mm sections, separately packaged by optimum cutting temperature compound (OCT), and then stored in liquid nitrogen. Postmenopause is defined according to the 2011 Stages of Reproductive Aging Workshop (STRAW + 10) criteria.

2.2. Frozen sections and laser capture microdissection

Frozen uterosacral ligament specimens were cut into 5 sections 10 μm in thickness using a Leika CM1900 cryostat. The third piece on the siliconized glass slide was loaded for hematoxylin–eosin (HE) staining and used for laser capture microdissection (LCM). The remaining 4 pieces on the glass slide were covered with PEN film and stained with LCM Staining Kit (Ambion AM1935, USA) for 1 min followed by washing in RNase-free water, and then used for LCM. By comparing with the HE stained sections, we selected the areas enriched in fibroblasts and used LCM to isolate the fibroblasts with RNAqueous®-Micro Kit (Ambion AM1931, USA) for extraction of RNA and cytosolic proteins.

Table 1
Demographic data on postmenopausal women recruited into the study divided into two groups: POP and non-POP.

Characteristics	Non-POP (n = 23)	POP (n = 37)	t	p
Age (years)	59.87 ± 7.10	60.19 ± 6.83	0.169	0.886
Body mass index (kg/m^2)	24.62 ± 3.08	25.72 ± 2.46	1.455	0.152
Primiparity age (years)	25.83 ± 3.49	25.32 ± 2.85	0.584	0.562
Parity	2.13 ± 0.82	2.26 ± 0.99	0.501	0.618
Duration of postmenopause	8.61 ± 6.79	9.32 ± 7.01	0.374	0.710
mRNA				
Mfn2	0.61 ± 0.19	0.79 ± 0.26	2.134	0.029
Procollagen 1A1	0.93 ± 0.27	0.63 ± 0.22	2.483	0.012
Procollagen 1A2	1.41 ± 0.43	0.94 ± 0.28	3.077	0.008
Procollagen 3A1	0.81 ± 0.27	0.62 ± 0.14	1.635	0.041
Protein				
Mfn2	0.67 ± 0.26	0.85 ± 0.28	2.474	0.017
Procollagen 1A1	1.21 ± 0.53	0.90 ± 0.31	2.705	0.009
Procollagen 1A2	1.31 ± 0.57	0.93 ± 0.32	3.129	0.003
Procollagen 3A1	0.76 ± 0.31	0.51 ± 0.21	3.516	0.001

Data of two groups are Mean ± S.D.
p Value <0.05 for comparison of the study and control groups using Student's t-test for continuous variables.

2.3. Primer design

According to the GenBank sequences, the detection primers for procollagen 1A1/1A2/3A1, Mfn2, and β-actin mRNAs were designed by Shanghai Sangon Biotech Co., Ltd. Primer sequences for target and internal control genes were as follows:

Forward primers, procollagen 1A1: 5′-CGAGGGCCAAGACGAAGA-3′; procollagen 1A2: 5′-TGGATACGCGGACTTTGTTG-3′; procollagen 3A1: 5′-TCGCCCTCCTAATGGTCAAG-3′; Mitofusin-2: 5′-CATCAGCTACACTGGCTCCAACT-3′; β-actin: 5′-CACGGCTGCTTCCAGCTC-3′.

Reverse primers, procollagen 1A1: 5′-CACGTCTCGGTCATGGTACCT-3′; procollagen 1A2: 5′-GGCTGGGCCCTTTCTTACAG-3′; procollagen 3A1: 5′-GGTCACCATTTCTCCCAGGAA-3′; Mitofusin-2: 5′-GATGAGCAAAGGTCCCAGACA-3′; β-actin: 5′-CACAGGACTCCATGCCCAG-3′.

2.4. RNA isolation and qRT-PCR

The LCM tissue was collected in a plastic lid. Cells were lysed with the cell lysis buffer included in the total RNAqueous®-Micro Kit, and RNA was extracted. The RNA was reverse transcribed into cDNA according to the instructions of the reverse transcription kit (Transgen AE301-02, Beijing). According to the instructions of the qPCR kit (Promega A6001, USA), the target gene and internal control β-actin were generated using qRT-PCR. Two duplicate holes were performed and repeated 3 times. The expression of the target gene relative to that of the internal control was calculated using the $2^{-\Delta Ct}$ method. The target products were electrophoresed using 1.5% agarose gel.

2.5. Protein extraction and western blotting analysis

After centrifugation, the extracted RNA was collected and the precipitate was removed by centrifugation for 10 min. Protein was precipitated out of the supernatant with isopropanol, the mixture was centrifuged for 10 min, and the supernatant was discarded. The protein precipitate was washed with anhydrous ethanol, and the protein was dissolved using 1% SDS after vacuum pumping. Protein concentration was measured using the BCA method, and the protein was denatured at 95 °C for 5 min. Protein samples containing 10 μg were separated using 8% SDS-PAGE electrophoresis, transferred to nitrocellulose filter membranes, blocked with 5% skim milk for 1 h, and incubated in primary antibodies (the dilution of procollagen 1A1/1A2/3A1 monoclonal antibodies (Santa Cruz sc-133179/166572/166333, USA) was 1:200, the dilution of Mfn2 monoclonal antibody (Abcam ab56889, UK) was 1:500, and the dilution of β-actin monoclonal antibody (ZSGB-BIO TA-09, Beijing) was 1:1000). Membranes were incubated at 4 °C overnight. After washing, the membranes were incubated in horseradish peroxidase-labeled goat anti-mouse IgG for 1 h at room temperature. After washing, membranes were reacted with electrochemiluminescence reagent (GE Healthcare Life Sciences RPN 2235, USA) and imaged using a western blot chemiluminescence camera (Kodak Image Station 4000 mm Pro, USA). The grayscale ratio was calculated using the AlphaEaseFC software. Semiquantitative analysis was performed on the expression of the corresponding protein.