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Expression of FSHR in chondrocytes and the effect of FSH on chondrocytes

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ABSTRACT

Objective: Chondrocytes express many kinds of hormone receptors. The function of Follicle stimulating hormone (FSH) in the ovary is mediated by FSH receptor (FSHR). FSH receptor (FSHR) is found in many non-ovarian tissues, however it has been unclear if chondrocytes express FSHR. The purpose of this study is to determine it.

Methods: Mouse primary chondrocytes and human articular cartilage tissues were examined. The expression and sequence of FSHR mRNA by reverse transcription polymerase chain reaction (RT-PCR) and sequenced, respectively, and its protein expression was tested using western blotting and location was observed under immunofluorescence microscopy. Ovarian tissue was as a positive control. After FSH stimulated mouse chondrocytes, intracellular cAMP levels were assessed by ELISA, and gene expression relative to Mouse WNT Signaling Pathway was tested by RT^2 Profiler PCR Arrays.

Results: FSHR was detected at the transcriptional level and confirmed to have the same sequence as that of ovary-derived mRNA of FSHR. FSHR proteins presented at the same line as the positive proteins of ovary, in mouse chondrocytes and human cartilage tissue, respectively. FSHR proteins were located at the cell membrane. Intracellular cAMP contents were significantly elevated up to 7-fold in mouse chondrocytes by forskolin (100 mM) ($P < 0.001$); however, different doses of FSH did not change the cAMP contents in mouse primary chondrocytes. RT^2 Profiler PCR Arrays demonstrated that FSH could cause changes in gene expression among the 84 preordained genes, such as Fosl1, Rhou, and Dkk1, in mouse chondrocytes relative to the control.

Conclusion: Mouse chondrocytes and human articular cartilage express functional FSHR. Moreover, FSH can act on chondrocytes and cause genetic changes.

1. Introduction

Follicle stimulating hormone (FSH) is a pituitary glycoprotein that has an important role in the maturation and development of follicles. The effect of FSH is mediated by binding of the hormone with a specific receptor (namely FSHR), which is specifically located on the granulosa cells of the ovary [1]. FSHR belongs to the G protein-coupled receptor family and consists of 10 exons, 9 introns, and the promoter region at chromosome 2p21 [2].

In prior studies, FSHR mRNA was expressed in primary murine and RAW cell precursors during differentiation. FSHR is located on

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the membrane of osteoclast surface [3]. However, in one recent study, an opposite view was presented in which the expression of FSHR was not detected in bone, cultured osteoblast or osteoclast RNA preparations. Some results suggested that FSH can aggravate alveolar bone loss by FSHR, independent of estrogen [4].

Osteoarthritis is a joint disease in humans that occurs primarily after age 50, especially in postmenopausal women. Cartilage erosion and subchondral sclerosis are characteristic cartilage pathological changes. We hypothesized that there may be a certain correlation between FSH and osteoarthritis.

In previous studies, glucocorticoid receptor was found in growth plate chondrocytes [5], and thyroid hormone receptors were also detected in growth plate cartilage in rats [6]. Many signals have been shown to regulate the function of growth plate including growth hormone, insulin-like growth factor I, glucocorticoids, thyroid hormone, estrogen, androgen, vitamin D, and leptin. Chondrocytes are highly likely to express FSHR. Thus far, there has been no study concerning FSHR expression in cartilage tissue or chondrocytes. To our knowledge, chondrocytes are the only cells in cartilage tissue. If chondrocytes do express FSHR, then we need to determine the expression mechanism. In the future, perhaps we will find a new therapy for osteoarthritis.

In the present study, we extensively investigated the mRNA and protein expression and elementary function of FSHR in mouse chondrocytes. Our results have important implications for a novel physiopathological role of FSH.

2. Methods and materials

2.1. Mouse chondrocyte culture

Fresh cartilage tissue from newborn mice was isolated and digested in DMEMF12(HyClone GE Healthcare Life Sciences) (+glutamine) medium containing 10% FBS, P/S, cysteine and collagenase P for 18.5 h at 37 °C. Upon addition of complete medium to the matrix digestion solution, the digestion was terminated. Then, the digest-solution was collected in a fresh tube, and the tube was centrifuged at 300G for 15 min. The supernatant was removed, the pellet was resuspended in 10 ml DMEMF12 + P/S + FBS, and the suspension was filtered through a 100 μm-Falcon filter in a fresh 50 ml tube. Next, the tube was centrifuged at 300G for 5 min, the pellet was resuspended in 10 ml DMEMF12 + P/S + FBS, and the suspension was then filtered through a 40 μm-Falcon filter into a used 50 ml tube. The filter was rinsed with 10 ml of DMEMF12 + P/S + FBS, and the suspension was filtered through the used filter again. This centrifugation and suspension cycle was carried out twice. Finally, chondrocytes were obtained and cultivated in cell culture dishes at a density of 5 × 105 cells/ml. Chondrocytes were maintained in complete medium DMEM/F-12 supplemented with 10% fetal bovine serum (FBS; TRACE, Sydney, Australia), 0.1% P/S gentamycin and 50 mg/ml L-ascorbic acid (Gibco, New York, USA), cysteine-solution (35.1 mg/ml), and ascorbic acid + ascorbate-2-phosphate stock diluted 1:500 at 37 °C in a humid environment with 5% CO2. The culture medium was changed every 2 days. Once a chondrocyte confluence of over 90% was achieved, the cells were lysed for subsequent RNA/protein extraction. Primary chondrocytes were used for FSHR detection by reverse transcription polymerase chain reaction (PCR) and Western blotting, whereas FSH treatment was performed on first passage chondrocytes.

2.2. Sample collection

Human cartilage tissue was obtained from patients who received knee joint replacement surgery because of severe osteoarthritis in the Department of Orthopedics' ward of Shandong Provincial Hospital. Human ovarian tissue was obtained from patients who had undergone oophorectomy in the gynecology ward of Shandong Provincial Hospital due to a malignant ovarian tumor.

All tissue samples were obtained with the informed consent of the patients. The study conformed to the principles of the Declaration of Helsinki and was approved by the Ethics Committee of the Shandong Provincial Hospital. All animals received humane care in compliance with the guidelines from the Animal Care and Use Committee of Shandong University.

2.3. RNA isolation, cDNA synthesis, reverse transcription RT-PCR and sequencing

Total RNA of mouse chondrocytes was isolated using RNAiso Plus (TaKaRa BIO INC, 525–0058, Japan). DNase/RNase-free water (Beijing Solarbio Science & Technology Co Ltd) were used to treat isolated RNA in order to eliminate genomic DNA contamination. cDNA was synthesized from 1 μg of the isolated RNA performed with a PrimeScriptTM RT reagent Kit with gDNA Eraser (TaKaRa Biotechnology, Dalian, China). Equal amounts of cDNA were subjected to reverse transcription PCR (TaKaRa Biotechnology, Dalian, China) with specific primers for human FSHR or for mouse FSHR (extramembrane section, transmembrane section and intramembrane section).

Human cartilage tissue was obtained from patients who received knee joint replacement surgery because of severe osteoarthritis in the Department of Orthopedics' ward of Shandong Provincial Hospital. Human cartilage tissue RNA was isolated using an RNeasy Plus Universal Mini Kit (Qiagen). Cartilage tissues were ground in liquid nitrogen followed by RNA extraction with the RNeasy Plus Universal Mini Kit according to the manufacturer's instructions.

Primer sequences and their corresponding annealing temperatures used in PCR are provided in Table 1. Table 1 and the PCR conditions are described in the appendix 1.

2.4. Western blot analysis of FSHR

Western blot analysis was used to confirm the expression of FSHR in mouse chondrocytes and human cartilage. Mouse primary chondrocytes were got as before said. Primary chondrocytes were then harvested and lysed using modified Ripa lysis buffer (5% Tris/pH¼7.5, 15% NaCl, 1% Nonidet P-40/IGEPAL, 0.1% sodium dodecyl sulphate, and 1% deoxycholate) supplied with Sigma FAST protease inhibitor solution (1:100; Sigma Aldrich, UK). Human cartilage tissue was obtained from patients who received knee joint replacement surgery because of severe osteoarthritis in the Department of Orthopedics' ward of Shandong Provincial Hospital. Human ovarian tissue was obtained from patients who had undergone oophorectomy in the gynecology ward of Shandong Provincial Hospital due to a malignant ovarian tumor. Mouse ovarian tissue and human ovarian tissue were used as positive control in this assay. Thirty micrograms of each protein lysate were electrophoresed onto a nitrocellulose gel and probed with a 1:500 dilution of primary rabbit polyclonal anti-FSHR antibody (Abcam, UK) was utilized as an internal control. The membrane was then exposed to Western blotting detection reagents (Amersham, UK) and visualized by exposure to an LAS-3000 imaging system (Fuji Photo Film Co., Ltd., Japan).

2.5. Immunofluorescence

The cellular location of FSHR was examined in mouse primary chondrocytes by immunofluorescence microscopy. Briefly, cells

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