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JNMU
Journal of Nanjing Medical University, 2007, 21(5):321–323
www.elsevier.com/locate/jnmu
Research Paper

Expression of OPGmRNA and ODFmRNA in the patients of hip fracture due to osteoporosis

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Received 4 June 2007

Abstract

Objective: To investigate the gene expression of osteoprotegerin(OPG) and osteoclast differentiation factor(ODF) in the bone tissue of patients with hip fracture due to osteoporosis. **Methods:**OPGmRNA and ODFmRNA in the bone tissue in 50 cases of osteoporosis sufferers(over 50 years old) with hip fracture(Observer Group) and 30 cases of hip facture sufferers with no osteoporosis(Control group) were analyzed with the Semi-Quantitative RT-PCR method. **Results:**The mRNA expressed of ODF, OPG were both high in the patients with hip fracture. In the control group, the expression of OPG mRNA was observed, while the expression of ODF mRNA was very slight. **Conclusion:**Aged patients contained all signals including OPG, ODF that are essential for inducing osteoclastogenesis and promoting bone resorption.

Keywords: osteoporosis; fracture; osteoprotegerin; osteoclast differentiation factor

INTRODUCTION

Osteoporosis(OPG) and osteoclast differentiation factor(ODF) can adjust the differentiation and the bone absorption of the osteoclast. In the bone micro environment, the ratio of ODF mRNA/OPG mRNA decides the bone metabolic direction(anabolism or catabolism). From July 2004 to October 2006, we examined OPGmRNA and ODFmRNA in the bone tissue in 50 cases of hip fracture patients with osteoporosis sickness and 30 cases of non-osteoporosis sickness hip fracture patients (control group).

MATERIAL AND METHODS

Clinical materials

50 aged patients with osteoporosis fracture(age from 51~76 years old) were taken as observation group (4 males, 46 females); 30 examples of aged patients with non-osteoporosis hip fracture were as the control group. All the patients had cancellous bone removed from the end of the marrow cavity, when they were taken hip joint replacement operation or column femoris fixation. All the patients in those two groups had no heptic dysfunction, renal dysfunction, alcoholist, malnutrition, rheumatoid arthritis, ankylosing spondylitis, bone tumor, or endocrinopathy. All the patients in the two groups had not used any medicine that could affect bone metabolism half a year prior to operation.

The measurement of bone density.

DPXL style Dual Energy X-ray Bone Densitometer was used to measure all the patients' lumbar vertebrae bone density.

The measurement of OPGmRNA and ODFmRNA

Fractional collagenase digestion method was used to digest the cancellous bones taken from the operations. DMEM that fetal bovine serum was not contained was used to end the digestion. Osteoblasts were put into different culture flasks to culture in 5%CO_2, at 37℃, after

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being counted. Total RNA was prepared with TRIzol regent from hip fracture sample and normal bone tissue by a first strand complementary DNA(cDNA) synthesis kit. cDNA was synthesized from 20 μg RNA according to the manufacturer's instructions, then cDNA was amplified by electrophoresis on a 1.5% agarose gel and stained with EB. The relative quantity of the PCR products were determined and the mRNA levels were compared with that of the normal bone.

Statistical analysis

The SPSS 10.0 statistic program was adopted to analyze OPG, ODF and OPG/ODF ratio by the analysis of variance.

RESULTS

The patients with hip fracture highly expressed mRNA of ODF and OPG. The expression of OPG mRNA was observed, while the expression of ODF mRNA was very slight in normal bone.(shown in ***Tab 1***)

Tab 1 **OPG and ODF mRNA expression in bone tissue(comparative optical density)** ($\bar{x} \pm s$)

groups	Cases	OPGmRNA	ODFmRNA	OPG/ODF
Control group	30	0.764 ± 0.135	0.486 ± 0.138	1.89 ± 0.41
Observation group	50	0.865 ± 0.089*	0.833 ± 0.230*	1.12 ± 0.42*

Compared with the Control Group,*$P < 0.05$

DISCUSSION

Low Bone Mass is the most important risk factor in senile osteoporosis. There are many factors that influence the bone mass, and among these factors, the lack of estrogens is a very important reason that causes the loss of the bone mass. This is proved by epidemiology and animal experiments[5, 6]. When postmenopausal or post ovariotomy women have a low level of estrogens, bone turnover is increased, and bone loss is faster. This stimulates the creation and differentiation of osteoclast progenitor cells and the increased osteoclast activity advancing bone absorption. The risk of osteoporosis and fracture will be increased[7].

The intactness of the skeleton depends on the dynamic balance of the bone formation and absorption. The balance is controlled by a complex net that is formed by many hormones and cell factors. Osteoblast and osteoclast are two main functional cells that participate in osteogenesis and bone reconstruction. Osteoblast can secrete some kinds of cell factors, which can induce the formation of osteoclast and regulate its function. The balance of osteoprotegerin ligand and its soluble receptors regulates the absorption of the bone. This reasonably explained this phenomenon, and can help to research bone diseases.

Osteoprotegerin(OPG) is a glycoprotein that contains 401 amino acid residues. It has 21 amino acid signal peptides and 380 mature peptides. Its coding area cDNA length is 1 203 bp. In the bone micro environment, osteoblast may express OPG and ODF. ODF stimulates the differentiation of osteoclast in the bone tissue, enhances the activity of the mature osteoclast, and inhibits osteoclast to perish. The summation of all these effects is to add the number of the viable osteoclasts. OPG is the bait receptor of ODF. It prevents the connection between ODF and RANK which is the true functional acceptor on the surface of the osteoclast, inhibits the differentiation of osteoclast, controls the function of the mature osteoclast, and induces the osteoclast to perish [8]. OPG knockout mice exhibit a sharp decrease in bone density and strength.

Transgene mice that excessively expressed OPG display severe osteopetrosis. These all proved that OPG plays a very important role in bone absorption. OPG can be effectively and safely used to inhibit bone absorption and prevent osteoporosis has been proved in animal experiments and clinical experiments[9]. ODF is the central factor during the formation of osteoclast. When macrophage colony stimulating factor exists, ODF may induce the mouse spleen cell and the human peripheral blood mononuclear cell to produce osteoclast[2,10]. By RT-PCR, we have found that there is rich ODFmRNA expression in the bone tissue of the hip fracture patients with postmenopausal osteoporosis sickness. The ratio between ODFmRNA and GAPDH is bigger than that in the normal bone tissue. This proved that the micro environment in the bone tissue has essential condition that can make osteoclast form. Atkins[11] has separated stem cells from postmenopausal women's bone tissue, and has found ODFmRNA expression by RT-PCR.

OPG, the non-functional receptor of ODF, has expression both in the bone tissue of osteoporosis sickness patients and in the normal bone tissue, but is relatively more in the former tissue. Pehaps that is because osteoclasts form actively under the stimulation of ODF.

As the negative feedback adjustment factor, the expression quantity of OPG is relatively elevated. In the bone microenvironment, the ratio of ODF and OPG decides whether it is absorption or formation of the bone. If the expression of ODF is higher than OPG, osteoclast forms actively; otherwise the formation of osteo-

clast will be inhibited. The ratio of OPG and ODF in the bone tissue of osteoporosis sickness patient is far lower than that in the normal bone tissue. This prompts the formation of osteoclasts far more than in the normal bone tissue. These results tally with their histological characteristics. This might be one of the pathology mechanisms of the bone fracture of the osteoporosis sickness patient.

The result of this research demonstrates that the expression of OPG in postmenopausal women's bone tissue is relatively low. This showed no difference with previous clinical findings, that bone loss takes place in postmenopausal women. Further study needs to be done in finding the importance of estrogen levels in OPG and OPGL coupling phenomenon.

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