academicJournals

Vol. 7(19), pp. 1179-1182, 22 May, 2013
DOI 10.5897/AJPP12.302
ISSN 1996-0816 © 2013 Academic Journals
http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Expression of CD44 in osteoarthritis cartilage tissue and its clinical significance

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Accepted 25 May, 2012

The expression of CD44 in osteoarthritis cartilage tissue and its effect in the generation and development of osteoarthritis (OA) were studied. Forty patients with primary OA who received joint arthroplasty were randomly included in this study as OA group. The normal articular cartilages as control group were obtained following Outerbridge standard from twenty patients who received amputation. The cartilages were fixed, decalcified, paraffin embedded and stained with hematoxylin and eosin (HE). Immunohistochemical staining method was used to determine CD44 expression. The fragments were divided into normal group, mild group, moderate group and severe group according to the severity of degeneration. CD44 expressed both in the control and OA group, and mainly localized in the cell membrane. The level of CD44 in OA group was significantly higher than that in the control group ($P<0.05$); the level of CD44 gradually decreased with the severity of OA lesions, that is, mild group > moderate group > severe group, and the three groups had significant differences ($P<0.05$). Pearson correlation analysis showed a significant negative correlation between the CD44 expression levels and the Mankin pathology score in the OA cartilage tissue ($r = -6.013$, $P<0.01$). Adhesion molecule CD44 highly expressed in osteoarthritis tissue, and gradually decreased with the severity of osteoarthritis lesions.

Key words: Adhesion molecule CD44, osteoarthritis, cartilage tissue, immunohistochemical staining.

INTRODUCTION

Osteoarthritis (OA), also known as osteoarthrosis, degenerative joint disease and senile arthritis, is a common chronic disabling disease. It is also a serious threat to the health of the elderly and can affect their daily activities. It is reported that in the population of 50 years and above, OA is only second to cardiovascular disease among the diseases that lead to long-term disability (Burnett et al., 2006). Commonly, adult articular cartilage regenerative ability is limited, and articular cartilage degeneration seems to be irreversible pathological change. It is generally agreed that causation is multi-factorial, involving age, genetic predetermination, acute and chronic joint trauma, as well as endocrine disorders and, perhaps, dietary factors and local myodynamia (Stove et al., 2006; Li et al., 2009). Due to the lack of effective treatment of OA, the exact pathogenesis is not yet fully elucidated. Therefore, it is particularly important to explore its etiology and pathogenesis to prevent and cure it. CD44 is a transmembrane adhesion molecule, and as a member of the cell surface adhesion molecules, it mainly participates in heterogeneous adhesion. It has been demonstrated that CD44 plays an important role in local clearance of hyaluronan and mediates cell-matrix interactions involved in tumor cells metastasis, tumor formation and T cell extravasation (Liang et al., 2007). A study showed that CD44 is correlated with animal OA

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model (Smith et al., 2008). Nevertheless, we did not find any report which showed its relationship with human articular cartilage cataplasia of OA. Therefore, in our present work, the expression of CD44 in OA and different degree retrogression of articular cartilage was measured to reveal the correlation of CD44 with OA, and to further elucidate the mechanism of OA, providing theoretical basis for early diagnosis and treatment for OA.

MATERIALS AND METHODS

According to OA diagnosis standard in "the osteoarthritis treatment guidelines" (2007) revised by Association of Osteology Branch, a total of 40 OA patients who received joint arthroplasty at our hospital between August 2009 and August 2011 were randomly included in the study (Osteoarthritis treatment guidelines, 2007). These patients were all in hospital for primary knee OA, and exclude secondary OA like traumatic OA, and other types of arthritis, such as rheumatoid and suppurative arthritis. They never receive any regular treatment before admission to the hospital. These patients comprised 26 males and 14 females and were average (63.17 ± 11.35) years old (range from 52 to 79 years). Out of these, 27 suffered from hip arthritis and 13 from knee arthritis. Samples were cartilages collected from different lesion location. Correspondingly, 20 normal articular cartilages were collected from knee (patients received amputation because of trauma) or femoral head (patients suffered from femoral neck fracture because of violence) as control group. The onset age of the patients (13 males and 7 females) ranged from 50 to 75 years with average of (61.56 ± 15.89) years. Preoperative X-ray and postoperative clinical diagnosis were conducted to exclude the structural damage specimens, such as degeneration, tuberculosis, infection, tumor, rheumatoid inflammation and marked osteoporosis. And in this study, sex and age were not significantly different in OA and control groups (P>0.05).

Specimen processing

The specimens were obtained following Outerbridge standard from OA group who did joint replacement and control group who received amputation. After fixation with 4% paraformaldehyde and decalcification with buffered EDTA (15% ethylene diamine tetraacetic acid), the tissue samples were dehydrated and embedded in paraffin. Sections (5 μm thick) were cut and stained with HE. The expression of CD44 was investigated with immunohistochemical staining and high-pressure-temperature treatment for antigen retrieval. Of the 50 cartilages, normal group, mild group, moderate group and severe group were divided according to the severity of degeneration guided by the improved Mankin standard (Mankin et al., 1971).

Immunohistochemical staining of CD44

PBS other than primary antibody was used as negative control. The product of immune response was observed. It showed that CD44 protein accumulated on the cell membrane with brown color and fine grain, and nuclei was not stained. 10 regions were selected randomly in the articular cartilage images collected from CD44 immunohistochemical staining slices. Static gray-scale in the MIAS medical image analysis software was used to measure their gray value and the average gray value was used to assess the CD44 expression intensity.

Statistic analysis

Average gray value was applied to analysze the expression differences of CD44 in the four groups and the correlation of CD44 expression with Mankin score. All values were expressed as mean ± SE of mean. Student's t-test and χ^2 test were used to assess an overall difference among the groups for each of the variables.

Pearson correlation was used for analysis. Probability values less than 0.05 were considered statistically significant (analysis was performed using SPSS for Windows, Version 16.0).

RESULTS

Morphology of cartilage tissue in the control and OA group

Histomorphology observation showed that in the control group, the surface of the cartilage tissue was slightly unsmooth with no crevice and a little bit of proliferation, and the cells were relatively structured. While in the OA group, there were visible erosion and fractures with local fibrosis or all fibrosis. There was disorder in cells proliferation, and there was decrease in the number of cluster or cells, damage in tidal line and there was capillary elapse or disappearance (Figure 1).

Histological grading of OA

The 50 cartilages were classified according to the severity of degeneration guided by the improved Mankin score. The score and group are shown in Table 1.

The expression of CD44 in the control and OA group

The results revealed that adhesion molecule CD44 expressed both in the control and OA groups and mainly concentrated at the cytomembrane (Figure 2). Nonetheless, the level of CD44 in the OA group was significantly higher than that in the control group (P<0.05); the concentration of CD44 gradually declined with the severity of OA, that is, mild group > moderate group > severe group, and the three groups have significant difference (P<0.05) (Table 2).

The correlation of the CD44 levels in OA cartilage tissues with Mankin score

Pearson correlation analysis indicated that CD44 level in the OA group was negatively correlated with Mankin pathologic score (r =-6.013, P<0.01).

DISCUSSION

Articular cartilage degenerative change, bone reactive hyperplasia of the joint edge and subchondral bone are

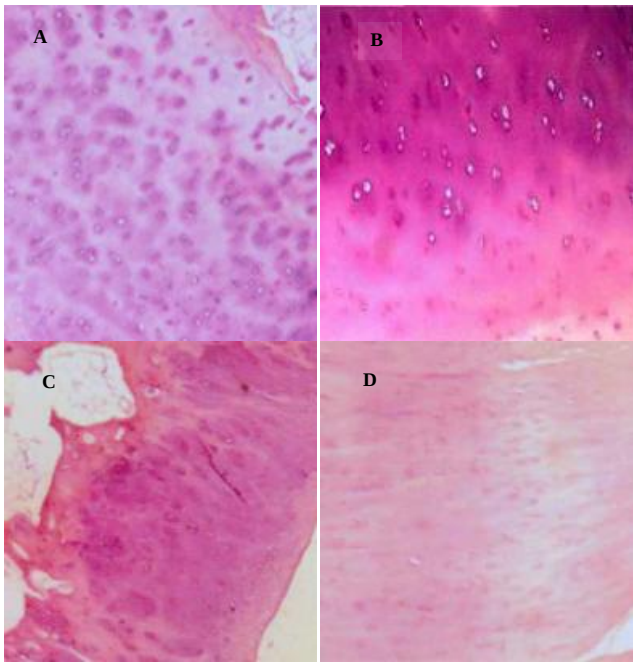

Figure 1. Morphological observations of cartilaginous tissue in the control and OA group (stained with HE, 100×). (A: In the control group, the surface was smooth, and cells were orderly arranged and evenly distributed; B: In the mild group, the surface was slightly unsmooth, and cells were irregularly arranged and unevenly distributed; C: In the moderate group, the surface was fibrosis, and cells were disarray in clusters with incomplete tidal line; D: In the severe group, the surface was fibrosis, and the numbers of cells were decreased obviously).

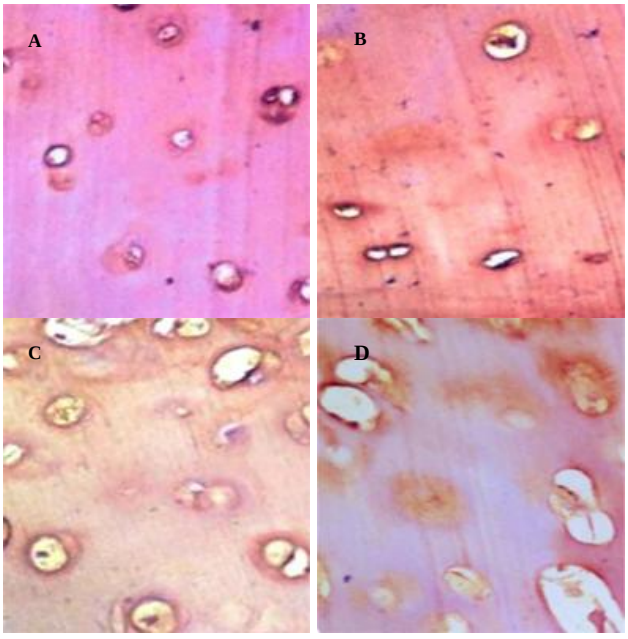

Figure 2. CD44 expressions in the control and OA group (stained with HE, 400×). Groups A, B, C and D show the expression of CD44 in the control, mild, moderate and severe OA groups, respectively.

Table 1. Mankin score.

Group	No.	Mankin score
Control	20	0.57±0.19
Mild	11	3.38±1.13
Moderate	13	7.18±2.39
Severe	16	12.61±4.20

Table 2. Gray value of CD44 in the control and OA group ($\bar{x} \pm s$).

Group	No.	CD44
Control	20	181.17
OA	50	140.60*
Mild	11	165.33*
Moderate	13	146.52*#
Severe	16	118.80*#▲

*Comparison of the value in the OA group with control group: *P<0.05. Comparison of the value in the OA group with mild group: #P<0.05 and with moderate group: ▲P<0.05.

the main pathological changes of OA. Here, series of changes have taken place in the articular cartilage, synovium and synovial cartilage, collagen, proteoglycan as well as histocyte. However, the mechanism of its pathogenesis is not yet fully elucidated presently, especially the understanding of the joint cartilage degeneration in cell and molecular field. Therefore, to explore the pathogenesis of OA at cell and molecular level would help to make clearer, the clinical significance of its prevention and treatment.

CD44 is a kind of stranded cell surface-glycoprotein (GP) encoded by a single gene, which is widely present on the membrane of blood cells, fibroblasts, epithelial cells and endothelial cells (Lou et al., 1999). CD44 gene is also a member of the cell surface adhesion molecules that is encoded by 20 highly conservative exons and can be classified as constitutive (c) and variant (v) isoform by different transcription of the 20 exons. That is, the constitutive type called CD44s, has 10 exons and the transcription segment exists in all CD44 transcripts; another one is alternative spliced by 10 variant exons, called CD44v. Extracellular matrix components have been described as ligands for the CD44, such as hyaluronic acid (HA), fibronectin and collagen. And functional diversity of CD44 seems to be influenced by the multiple natures of ligands (Naor and Nedvetzki., 2003). To sum up, the functions of CD44 including (Chel et al., 2006; Mckallip et al., 2002): homing lymphocytes rolling on the vessels through mediating adhesion of lymphocytes to high endothelial venules; involving in lymphocyte mediation; combining with HA and laminin (LN); binding to cytoskeleton protein and participating in formation of locomotion cells and CD44 which has been

shown to have a crucial role in the cell migration.

Research by Weber et al. (2002) showed that in an induced OA rabbit model, CD44 was highly expressed in different stages of the cartilage by immunohistochemical staining, and the level was associated with histological score criteria. Nedvetzke et al. (1999) injecting anti-CD44 mAbs into type II collagen-induced OA mice showed that anti-CD44 mAb markedly reduced the synovial inflammatory cellular response and the consequent damage to the joint. In addition, eighteen sheep had bilateral lateral meniscectomy to induce OA and sections of synovium were immunostained. Smith et al. (2008) found that CD44 was increased in the synovial lining of OA joints, and HA treatment reduced features of the pathology and improved joint mobility and function in OA. Furthermore, clinical research (Tibesku et al., 2006) revealed that in the serum and synovium, CD44 expression in OA patient was markedly up-regulated than that in healthy subjects. CD44, known to be the principle cell surface receptor for HA, plays a vital role in the extracellular matrix components metabolism, especially HA metabolism.

This study used immunohistochemical staining to determine CD44 concentration in the normal and OA cartilage tissue. Results showed that the level of CD44 in OA group was significantly higher than that in the control group, and there were significant differences between them ($P<0.05$); the level of CD44 gradually decreased with the severity of OA lesions, that is, mild group > moderate group > severe group, and the three groups were significantly different ($P<0.05$). The findings suggested that CD44 could promote the development of articular cartilage lesion of OA, and the possible mechanism may be that highly CD44 expression enhances endocytosis and the capacity of its binding to HA in the extracellular cartilage matrix. Thus, the HA's decomposition finally results to imbalance of extracellular cartilage matrix metabolism, which accelerates cartilage degradation. We also found a significant negative correlation between the CD44 expression levels and the Mankin pathology score in the OA cartilage tissue ($r = -6.013$, $P<0.01$), which showed that CD44 gradually decreased with the severity of OA.

In conclusion, adhesion molecule CD44 is highly expressed in cartilage tissue of OA, and shows a gradual decline with the severity of OA, which provide some clue to further study of the pathogenesis of OA. However, the complexity of OA pathogenesis should be noticed. It is not enough to represent OA progression only by the articular cartilage degeneration degree which cannot reflect the comprehensive pathological process of OA. Moreover, the mechanism of CD44 is not certain at present. Consequently, further studies are needed.

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