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Synovial fluid and plasma levels of milk fat globule–epidermal growth factor 8 are inversely correlated with radiographic severity of knee osteoarthritis

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Abstract

Objective: Mounting evidence demonstrates that inflammation plays an important role in the pathogenesis of osteoarthritis (OA). Milk fat globule–epidermal growth factor 8 (MFG-E8) is an important glycoprotein that is involved in anti-inflammatory responses. The present study was performed to assess the MFG-E8 levels in plasma and synovial fluid and explore the association between radiographic severity and MFG-E8 levels in patients with knee OA.

Methods: This study involved 138 healthy controls and 142 patients with knee OA. The MFG-E8 levels in plasma and synovial fluid were evaluated by enzyme-linked immunosorbent assay. The Kellgren and Lawrence classification was used for OA grading.

Results: The plasma MFG-E8 level was significantly lower in patients with knee OA than in healthy controls. The synovial fluid MFG-E8 level was significantly lower than the plasma level in patients with knee OA. More importantly, the MFG-E8 levels in synovial fluid and plasma were

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significantly and inversely associated with radiographic severity among patients with knee OA.
Conclusions: These results demonstrate that the levels of MFG-E8 in synovial fluid and plasma are inversely correlated with the radiographic severity of knee OA.

Keywords
Osteoarthritis, milk fat globule–epidermal growth factor 8, plasma, synovial fluid, severity, inflammation

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Introduction

Osteoarthritis (OA) is a common joint disease characterized by secondary synovial inflammation, sclerosis of subchondral bone, and damage of articular cartilage.[1] As part of the aging process, OA is among the leading causes of poor quality of life and disability in people of advanced age.[2] Little is known about the definite etiology and pathogenesis of OA. However, many growth factors and cytokines have been found to participate in the development of OA.[3,4]

Milk fat globule–epidermal growth factor 8 (MFG-E8), a secreted glycoprotein, was first identified in the mammary glands and subsequently studied in many other tissues.[5–8] Accumulating evidence indicates that MFG-E8 has anti-inflammatory effects.[9] A previous study showed that MFG-E8 plays an important role in the balance of bone metabolism, especially under pathological conditions.[10] Patients with rheumatoid arthritis have a lower serum concentration of MFG-E8 than do healthy controls, and MFG-E8 deficiency augments the severity of arthritis in mice.[11] Moreover, the involvement of proinflammatory cytokines and chronic inflammatory processes in the pathophysiology of OA is generally accepted.[12] However, the levels of MFG-E8 in synovial fluid and plasma in patients with OA remain unknown. In addition, the correlation of the levels of MFG-E8 in synovial fluid and plasma with the radiographic severity of knee OA has not been investigated.

Therefore, the present study was performed to detect the levels of MFG-E8 in plasma and synovial fluid from healthy controls and patients with knee OA. We also explored the correlation of the levels of MFG-E8 in synovial fluid and plasma with the severity of disease in patients with OA.

Materials and methods

Patients

Plasma samples were collected from patients with knee OA and healthy controls from 1 January 2017 to 31 December 2017 in the Department of Orthopaedics, the Second Affiliated Hospital of Nantong University. Synovial fluid samples were also collected from the patients with knee OA. The control group comprised patients with knee injuries with no radiological or clinical evidence of OA. All patients with knee OA were diagnosed with primary knee OA according to the criteria of the American College of Rheumatology.[13] Patients with post-traumatic OA,

autoimmune disorders, knee injuries, joint infections, and systemic inflammatory diseases were excluded. This study was approved by the Research Ethics Committee of the Second Affiliated Hospital of Nantong University. Written informed consent was obtained from all patients. The clinical features of the patients with knee OA and controls are shown in Table 1.

Radiographic assessment

The radiographic severity among patients with knee OA was evaluated using the Kellgren and Lawrence (KL) grading system.[14] KL grading was performed as previously described.[15]

Measurement of MFG-E8 in plasma and synovial fluid

The levels of MFG-E8 in plasma and synovial fluid were determined using an enzyme-linked immunosorbent assay (ELISA) kit (Abcam, Cambridge, UK) according to the manufacturer's instructions. The measurements were performed as previously described.[15] Briefly, synovial fluid, plasma, and recombinant human MFG-E8 standards were added to wells coated with monoclonal antibody to MFG-E8. After incubation for 2 hours at room temperature, each well was washed three times. A horseradish peroxidase-conjugated antibody to MFG-E8 was then added to each well. After incubation for another 2 hours at room temperature, each well was washed three times and substrate solution was added to the wells. After incubation for 30 minutes, the reaction was terminated through the stop solution, and the color intensity was determined by measuring the absorbance at 450 nm. The concentration of MFG-E8 was measured via a standard density–concentration curve. The detection limit for the ELISA was 2.4 pg/mL. Each sample was run in triplicate.

Statistical analysis

All statistical analyses were performed using SPSS 19.0 (IBM Corp., Armonk, NY, USA). Data are expressed as a mean $\pm$ standard error of the mean. The statistical significance of correlations between the levels of MFG-E8 in plasma and synovial fluid and the KL grade was determined via Spearman analysis. The unpaired Student's t-test was used to analyze statistical differences between two groups. Analysis of variance was used to determine the statistical differences among multiple groups. Differences between groups were considered statistically significant at P values of <0.05.

Results

Baseline clinical characteristics

This study included 138 healthy controls (age, 56–85 years) and 142 patients with

Table 1. Baseline clinical characteristics of patients with knee OA and controls.

	Patients with OA	Controls	*P*
Patients, n	142	138	
Age, years	67.8 $\pm$ 0.9 (52.3–83.3)	68.1 $\pm$ 0.8 (54.6–81.6)	0.4
Sex, female/male	118/24	116/22	0.8
BMI, kg/m^2	23.1 $\pm$ 1.2 (20.9–25.3)	22.7 $\pm$ 1.6 (19.4–26.0)	0.5

Data are expressed as number of patients or mean $\pm$ standard error of the mean (95% confidence interval). *P* value, comparison between control and OA groups. OA, osteoarthritis; BMI, body mass index.

knee OA (age, 55–88 years). The analysis showed no statistically significant differences in age, body mass index, or sex between healthy controls and patients with knee OA (Table 1).

MFG-E8 levels in synovial fluid and plasma

ELISA was performed to determine the levels of MFG-E8 in plasma and synovial fluid from patients with knee OA and healthy controls. In total, 240 plasma samples were collected from the patients and controls, and 142 synovial fluid samples were collected from the patients. As shown in Figure 1, the healthy controls had a significantly higher mean plasma MFG-E8 concentration than the patients with OA (538.7 ± 41.1 vs. 300.3 ± 26.8 pg/mL, respectively; $P < 0.001$). Additionally, among patients with knee OA, the level of MFG-E8 was significantly lower in synovial fluid than in plasma (166.4 ± 17.2 vs. 300.3 ± 26.8 pg/mL, respectively; $P < 0.01$). These results suggest that the level of MFG-E8 in plasma is lower in patients with than without knee OA.

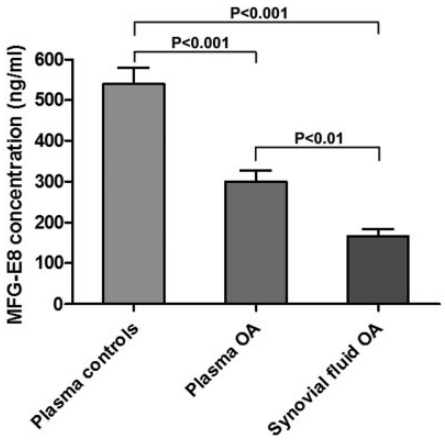

Figure 1. MFG-E8 levels in synovial fluid and plasma of healthy controls and patients with knee OA. MFG-E8, milk fat globule–epidermal growth factor 8; OA, osteoarthritis.

Association of radiographic severity with MFG-E8 concentration in plasma and synovial fluid

The features of the OA subgroups are shown in Table 2. Greater radiographic severity of knee OA was significantly associated with lower MFG-E8 concentrations in both synovial fluid and plasma ($P < 0.05$). We also investigated the relationships between the levels of MFG-E8 in plasma and synovial fluid and the radiographic severity of knee OA. The results indicated that the levels of MFG-E8 in plasma and synovial fluid were inversely correlated with the radiographic severity of knee OA ($P < 0.001$, $r = -0.559$ and $P < 0.001$, $r = -0.586$, respectively) (Table 3; Figures 2 and 3).

Discussion

OA is a common joint disease characterized by secondary synovial inflammation, sclerosis of subchondral bone, and damage of

Table 2. Plasma and synovial fluid MFG-E8 levels in patients with knee osteoarthritis.

	Total	KL grade 2	KL grade 3	KL grade 4	*P*
Patients, n	142	42	53	47	
Plasma MFG-E8, pg/mL	300.3 ± 26.8 (259.1–342.4)	480.5 ± 38.8 (402.0–559.0)	228.7 ± 15.6 (197.4–260.1)	119.1 ± 17.5 (83.9–154.3)	<0.01
Synovial fluid MFG-E8, pg/mL	166.4 ± 17.2 (132.4–200.4)	347.8 ± 43.9 (259.3–436.4)	139.6 ± 21.1 (97.3–182.0)	67.3 ± 15.9 (35.3–99.4)	<0.05

Data are expressed as mean ± standard error of the mean (95% confidence interval). *P* values indicate differences among KL subgroups. MFG-E8, milk fat globule–epidermal growth factor 8; KL, Kellgren and Lawrence.

articular cartilage.[1,16,17] As part of the aging process, OA is among the leading causes of poor quality of life and disability in people of advanced age and affects about 37% of the population over 60 years of age in the United States.[18] The diagnosis of OA is usually delayed because of the low sensitivity of radiological and biological examinations.[19] Therefore, identification of a sensitive biomarker in the early stage of OA is of great importance for improving the prognosis of patients.

Accumulating evidence demonstrates that OA is closely related to inflammation.[20,21] Inflammation has been deemed to play an important role in the development and progression of OA in both the early and late phases of the disease.[22,23] MFG-E8, a secretory glycoprotein, is expressed in various mammalian cell types.[24,25] Many studies have indicated that MFG-E8 takes part in various biological processes and pathophysiological functions, including fertilization,[26] angiogenesis,[27,28] autoimmune diseases,[29,30] and inflammatory responses.[31–33] In addition, MFG-E8 reportedly inhibits the inflammatory response by decreasing the expression of proinflammatory molecules.[34–36] More importantly, a previous study showed that the plasma concentration of MFG-E8 is increased in pregnancy, which is characterized by a chronic, low-grade inflammatory state.[37] These findings prompted us to compare the levels of MFG-E8 in plasma and synovial fluid between healthy controls and patients with knee OA.

Table 3. Correlations between KL grade and MFG-E8 in plasma or synovial fluid of patients with knee osteoarthritis via linear regression analysis.

Variable	MFG-E8 in plasma R^2/*P*	MFG-E8 in synovial fluid R^2/*P*
KL grade	0.312/<0.001	0.343/<0.001

KL, Kellgren and Lawrence; MFG-E8, milk fat globule–epidermal growth factor 8.

Inflammation is the primary pathogenic event that leads to pain and joint damage in

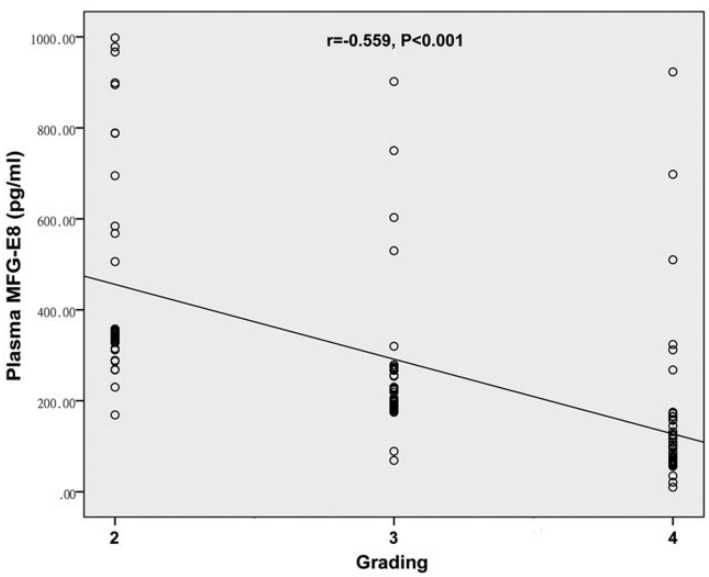

Figure 2. Correlation of plasma MFG-E8 with the radiographic severity of knee osteoarthritis. MFG-E8, milk fat globule–epidermal growth factor 8.

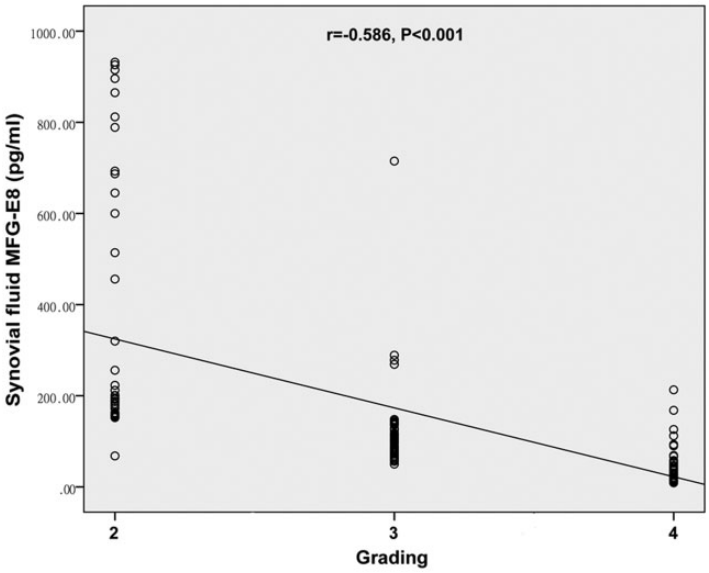

Figure 3. Correlation of synovial fluid MFG-E8 with the radiographic severity of knee osteoarthritis. MFG-E8, milk fat globule–epidermal growth factor 8.

patients with rheumatoid arthritis.[38] This suggests that inflammation is the pathological process common to both rheumatoid arthritis and OA. A previous study indicated that MFG-E8 plays an important role in the balance of bone metabolism, especially under pathological conditions.[10] Moreover, patients with rheumatoid arthritis reportedly have lower serum concentrations of MFG-E8 than do healthy controls, and MFG-E8 deficiency augments the severity of arthritis in mice.[11] Similar to previous findings, the present study demonstrated that the plasma MFG-E8 level was lower in patients with than without knee OA. Furthermore, the levels of MFG-E8 in plasma and synovial fluid were inversely correlated with the radiographic severity of knee OA. Our study also showed that in patients with knee OA, the MFG-E8 level was significantly lower in synovial fluid than in plasma. The reason for the lower level of MFG-E8 in synovial fluid may be the limited transport of MFG-E8 across the synovial membrane barrier because of its complex structure and molecular weight (53 kD).

Our study has several potential limitations. First, definite cause-and-effect relationships could not be established because this was a cross-sectional study with a relatively small sample size. Second, the level of MFG-E8 in synovial fluid was not measured in the healthy controls. Third, the expression of MFG-E8 in local tissues was not measured in either the healthy controls or patients with OA. In view of this, further research should be performed to clarify the potential of MFG-E8 as a diagnostic tool for knee OA.

In summary, we have demonstrated that the MFG-E8 concentration in plasma was obviously lower in patients with knee OA than in healthy controls. Additionally, among patients with knee OA, the levels of MFG-E8 were significantly lower in synovial fluid than in plasma. More importantly, our results indicated that the levels of MFG-E8 in synovial fluid and plasma were inversely correlated with the

radiographic severity of knee OA. These data demonstrate that the levels of MFG-E8 in synovial fluid and plasma are inversely correlated with radiographic severity of knee OA.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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