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ORIGINAL RESEARCH

Increase in mast cell marker expression in the synovium of obese patients with osteoarthritis of the knee

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Kentaro Uchida[1]
Shotaro Takano[1]
Gen Inoue[1]
Dai Iwase[1]
Jun Aikawa[1]
Ken Takata[1]
Ryo Tazawa[1]
Ayumu Kawakubo[1]
Hiroyuki Sekiguchi[2]
Masashi Takaso[1]

[1]Department of Orthopedic Surgery, Kitasato University School of Medicine, Sagamihara City, Kanagawa 252–0374, Japan; [2]Shonan University of Medical Sciences Research Institute, Chigasaki City, Kanagawa 253–0083, Japan

Purpose: While research suggests that obesity is a risk factor for knee osteoarthritis (KOA), the mechanisms are not fully understood. Mast cell (MC) numbers are increased in the osteoarthritic synovium and in the adipose tissue of obese individuals. We hypothesized that MC numbers are increased in the synovium of obese KOA patients. This study investigated MC marker and MC-generated cytokine/growth factor expression in the synovium of obese KOA patients.

Patients and methods: Patients radiographically diagnosed with KOA (male: 38, female: 132) were allocated to three groups based on their body mass index (BMI): normal (<25 kg/m^2), overweight (25–29.99 kg/m^2) and obese (≥30 kg/m^2), according to the World Health Organization BMI classification. We used real-time polymerase chain reaction to compare the expression of MC markers (*CD117, CD203c*) and growth factors/cytokines (*FGF2, VEGFA, TNFA*, and *IL8*) in patients' synovium among the groups.

Results: *CD117* expression was significantly higher in the obese group than in the normal and overweight groups. *CD203c* and *FGF2* expression were higher in the obese group than in the normal group. *FGF2* expression levels were significantly correlated with those of *CD117* (ρ=0.487) and *CD203c* (ρ=0.751).

Conclusion: MC markers *CD117* and *CD203c*, and *FGF2* were highly expressed in the synovium of obese KOA patients. Further investigations are needed to reveal the role of MCs in the relationship between obesity and osteoarthritis pathology.

Keywords: mast cells, obese, synovium, osteoarthritis

Introduction

Several reports have suggested that obesity is a risk factor for osteoarthritis (OA), especially knee osteoarthritis (KOA).[1–5] Previous studies have suggested that an obese level of body mass results in excess joint loading and increased risk of OA.[6,7] Interestingly, reports have also suggested that altered metabolic factors as a result of obesity affect the production of cytokines and growth factors that are associated with OA pathology.[8,9] Moreover, the fact that OA is observed in non-weight-bearing joints of obese patients[10,11] implies that mechanical loading may not be the sole contributor, and that other factors may additionally play a role in OA progression. However, these mechanisms are not well established.

Mast cells (MCs) can be found in the synovial tissue, and increased MC numbers have been observed in KOA and rheumatoid arthritis patients, where they are thought to contribute to both acute and chronic inflammatory

Correspondence: Kentaro Uchida
Department of Orthopedic Surgery
Kitasato University School of Medicine,
Kentaro Uchida 1–15–1 Minami-ku,
Kitasato, Sagamihara City, Kanagawa
252–0374, Japan
Tel +81 042 778 9217
Fax +81 042 778 9217
Email kuchida@med.kitasato-u.ac.jp

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processes.[12] Recent studies suggest that MCs are associated with the severity of radiographic KOA.[13] Interestingly, increased MC numbers have also been observed in the adipose tissue of obese individuals, where they are thought to contribute to inflammation.[14] However, whether MC numbers are increased in the synovium of obese KOA patients remains to be determined.

Previous studies have implicated several inflammatory cytokines and growth factors in KOA pathology, including tumor necrosis factor (TNF)-α,[15] interleukin-8 (IL8),[16] fibroblast growth factor-2 (FGF2),[17] and vascular endothelial growth factor (VEGF).[18] Activated MCs synthesize TNF-α,[19–21] IL8,[22,23] FGF2[24,25] and VEGF.[26–28] However, the relationship between obesity and the expression of inflammatory cytokines and growth factors is not well understood.

Here, we investigated MC marker and MC-generated cytokine/growth factor expression in the synovium of obese KOA patients.

Patients and methods

Patients

All subjects underwent total knee arthroplasty (TKA) at our hospital between January 2015 and October 2018. Synovial tissue was extracted during TKA from the subjects (male: 38, female: 132), who were diagnosed with radiographic KOA (unilateral Kellgren/Lawrence grade 3: n=59/170, 34.8%; and grade 4: n=111/170, 65.2%). A portion of each synovial sample was snap frozen in liquid nitrogen and placed in a −80 °C freezer prior to RNA extraction.

This protocol was approved by the Ethics Review Board of Kitasato University (approval number: KMEO B13–113). Participants provided written informed consent to participate in this study and for the removal and use of their synovial tissue one day prior to surgery. This study was conducted in accordance with the Declaration of Helsinki.

Real-time (RT)-polymerase chain reaction (PCR) analysis

All subjects were allocated to three groups based on their body mass index (BMI): normal (<25 kg/m^2), overweight (25–29.99 kg/m^2) and obese (≥30 kg/m^2), according to the World Health Organization (WHO) BMI classification. Patients' clinical characteristics by group are summarized in Table 1.

Table 1 Patients' clinical characteristics by body mass index group

	Normal (n=65)	Overweight (n=71)	Obese (n=34)	*P*-value
Age (years)	75.5±7.9	72.6±6.7	69.6 ±8.4[a,b]	0.004
Male/Female, n	10/55	27/44	6/28	0.077
KL (3/4), n	20/45	27/44	12/22	0.679
BMI (kg/m^2)	22.2±1.7	27.5±1.4[a]	32.8 ±2.2[a,b]	<0.001

Notes: Data are mean ± SD unless otherwise indicated. [a]Statistical difference between normal and obese groups. [b]Statisitcal difference between overweight and obese groups.
Abbreviations: KL, Kellgren/Lawrence grade; BMI, body mass index.

We evaluated MC markers (*CD117, CD203c*) inflammatory cytokines (*TNFA, IL8)* and growth factors (*VEGFA, FGF2)* on the basis of previous studies which reported that these markers were elevated in OA patients[15–18] and were produced by MC.[19–28] RNA extraction, cDNA synthesis and RT-PCR were performed using methods reported previously.[29] Primers used for RT-PCR are listed in Table 2. We used RT-PCR to compare the expression of *CD117, CD203c, FGF2, VEGFA, TNFA*, and *IL8* in the synovium among the groups.

Statistical analysis

SPSS 25.0 was used for statistical analysis. Continuous variables were compared using one-way analysis of variance followed by Fisher's least significant difference test as a post-hoc test.[30] Meanwhile, categorical variables were

Table 2 Sequences of primers used in this study

Primer	Sequence (5′–3′)	Product size (bp)
CD117-F	TGACTTACGACAGGCTCGTG	126
CD117-R	CCACTGGCAGTACAGAAGCA	
CD203c-F	CGACTGCACTATGCCAAGAA	164
CD203c-R	GGTCCATGTGCCAGAAAGAT	
FGF2-F	AGAGCGACCCTCACATCAAG	80
FGF2-R	ACGGTTAGCACACACTCCTT	
VEGFA-F	TTGCCTTGC TGCTCTACCTC	104
VEGFA-R	AGCTGCGCTGATAGACATCC	
TNFA-F	CTTCTGCCTGCTGCACTTTG	118
TNFA-R	GTCACTCGGGGTTCGAGAAG	
IL8-F	ACACTGCGCCAACACAGAAA	89
IL8-R	ACCTCGTGGAGACGCTTTAC	
GAPDH-F	TGCCACTCAGAAGACTGTGG	129
GAPDH-R	TTCAGCTCTGGGATGACCTT	

compared using the Fisher exact test. Spearman's correlation coefficient was used to evaluate the relationship between the expression of *FGF2* and MC markers (*CD117* and *CD203c*). Statistical significance was defined by $P<0.05$.

Results

Clinical characteristics of patients in normal, overweight, and obese groups

Patients were allocated to three WHO BMI classification groups: normal, overweight and obese. Patients in the obese group were significantly younger than those in the normal and overweight groups (Table 1). Male/female ratio and Kellgren/Lawrence grade 3/4 ratio were similar among the groups (Table 1).

Expression of MC markers in normal, overweight, and obese groups

To determine whether MC numbers are increased in obese OA patients, we examined the expression level of MC markers in the synovium of KOA patients. *CD117* expression was significantly elevated in the obese group compared to the normal ($P=0.038$) and overweight groups ($P=0.031$), but was comparable between the normal and overweight groups ($P=0.944$) (Figure 1A). *CD203c* expression was significantly elevated in the obese group compared to the normal group ($P=0.046$) but was comparable with the overweight group ($P=0.136$) (Figure 1B).

Expression of MC-generated cytokines and growth factors in normal, overweight, and obese groups

FGF2 expression was significantly elevated in the obese group compared to the normal group ($P=0.029$) but was comparable to the overweight group ($P=0.207$) (Figure 2A). *VEGFA, TNFA*, and *IL8* expression were similar among the three groups (*VEGFA,* $P=0.389$; *TNFA,* $P=0.552$; *IL8,* $P=0.232$; Figure 2B–D).

Correlation between MC markers and *FGF2* expression

Given that MC markers *CD117* and *CD203c* and the MC-generated growth factor *FGF2* were elevated in the obese group, we examined the correlation between the expression of *CD117* and *FGF2*, and *CD203c* and *FGF2. FGF2* expression levels were significantly correlated with those of *CD117* ($\rho=0.487$, $P<0.001$; Figure 3A) and *CD203c* ($\rho=0.751$, $P<0.001$; Figure 3B).

Discussion

A number of studies have reported a strong link between the development of KOA and obesity.[1,31,32] One study showed that obese KOA patients are 6.8 times more likely to experience KOA progression that those of normal weight.[1] Another study reported that the odds ratio for obese individuals developing KOA was 2.6 relative to normal-weight subjects.[32] In our study, we found that obese KOA patients underwent TKA at a younger age than normal-weight and overweight patients. Together with previous studies,[1,30,31] our findings suggest that obesity is associated with KOA progression.

Studies have reported increased MC numbers in patients with obesity-related glomerulopathy[33] and skin tags,[34] and obese patients.[14] The number of MCs in patients with obesity-related glomerulopathy is significantly and positively correlated with BMI.[33] Obese patients with skin tags have significantly more MCs than overweight patients.[34] MC numbers are also increased in

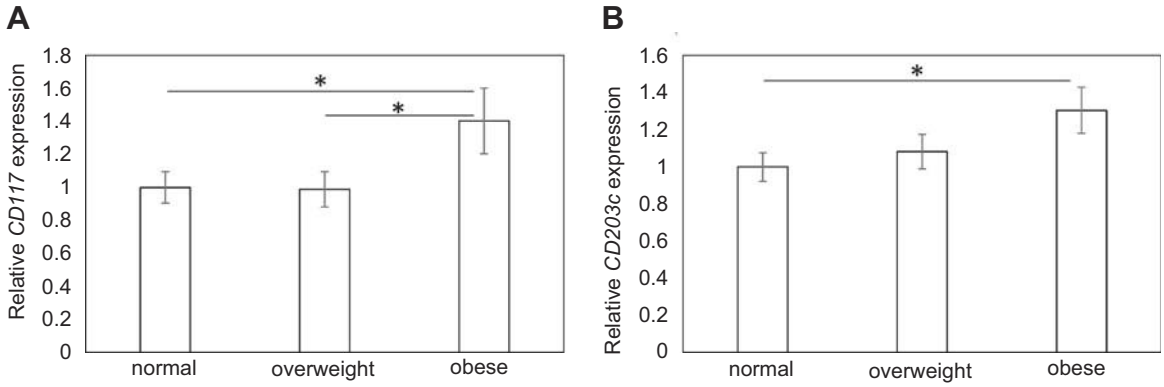

Figure 1 Effect of obesity on mast cell marker expression in synovial tissue. *CD117* (**A**) and *CD203c* expression (**B**) in normal, overweight, and obese groups. *$P<0.05$.

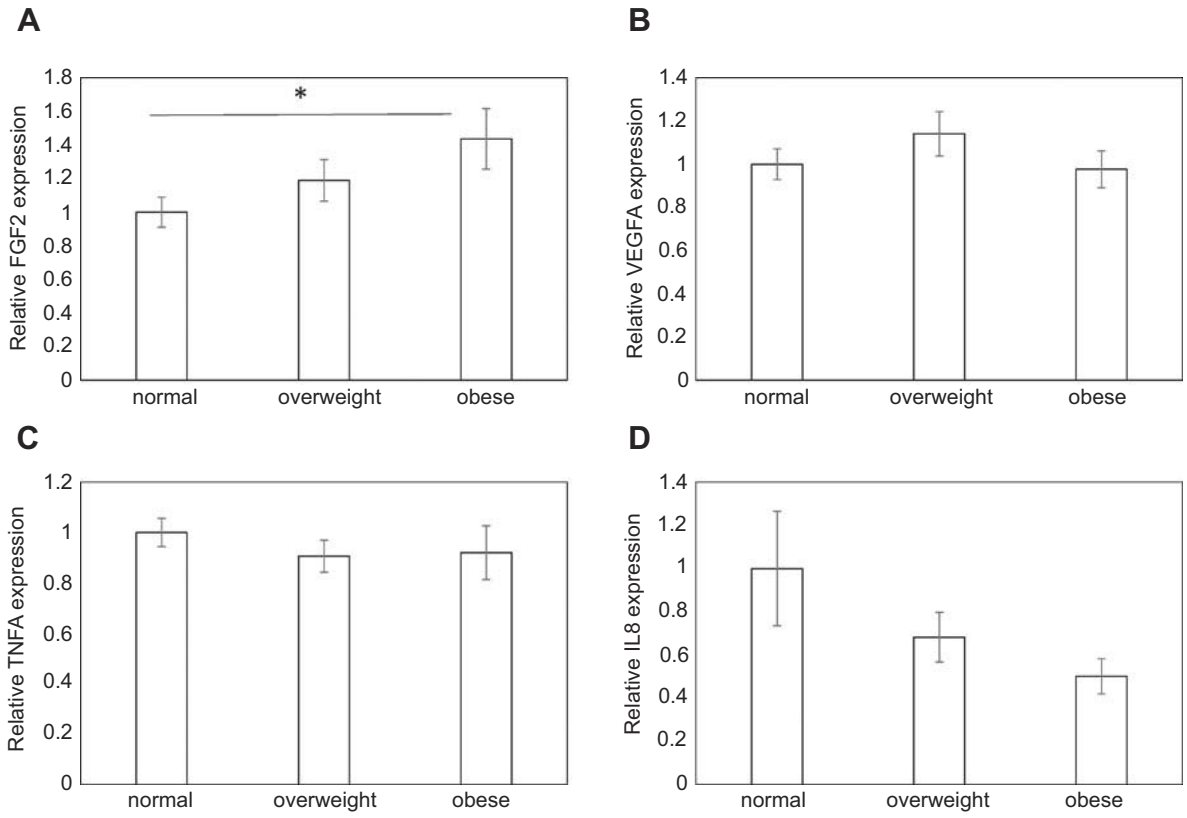

Figure 2 Effect of obesity on the expression of inflammatory cytokines and growth factors in synovial tissue. *FGF2* (**A**), *VEGFA* (**B**), *TNFA* (**C**), and *IL8* (**D**) expression in normal, overweight, and obese groups.*$P<0.05$.

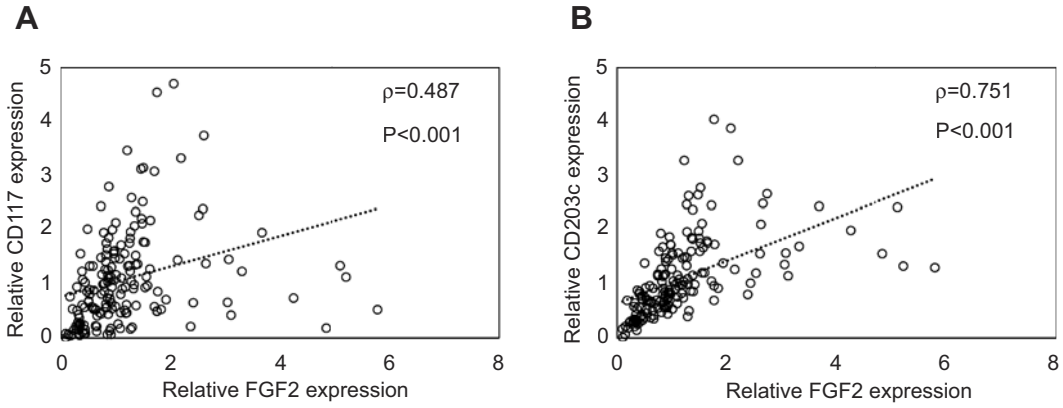

Figure 3 Correlation between the mRNA levels of *CD203c*, *CD117* and *FGF2* in synovial tissue. Relationship between *FGF2* and *CD117* (**A**) and *CD203c* (**B**) in synovial tissue.

the adipose tissue of obese patients, where they are thought to contribute to inflammation in white adipose tissue.[14] In this study, the synovium of obese KOA patients showed higher expression levels of the MC marker *CD117* than that of normal-weight and overweight KOA patients. A recent study showed that there is a relationship between KOA severity and the number of MCs.[13] Further investigations are needed to reveal the role of MCs in the relationship between obesity and osteoarthritis pathology.

MCs produce several mediators that contribute to the inflammation process. FGF2 is one growth factor produced by MCs in the inflammatory state.[24,25] In our study, *FGF2* expression was higher in the synovium of obese KOA patients than that of normal-weight KOA patients, and was correlated with the expression of MC markers

CD117 and *CD203c*. *FGF2* promotes matrix metalloprotease-13 production in articular chondrocytes.[35,36] FGF2 concentrations in synovial fluid and plasma are correlated with the Kellgren/Lawrence grade.[17] Together, our findings and those of previous studies suggest that FGF2 elevation may be associated with increased MC numbers in the synovium of obese KOA patients. However, it is unclear whether FGF2 elevation in obese KOA patients contributes to OA pathology.

Several limitations of the present study warrant mention. First, the inclusion of a non-KOA population is needed to confirm whether MC numbers are increased in obese individuals and if this directly contributes to OA progression. Second, the mechanism by which MCs contribute to OA pathology remains to be determined. Finally, it remains unclear whether the evaluated biomarkers are of sufficient significance to warrant assessment in KOA patients. Other biomarkers should be evaluated in the future.

In conclusion, MC markers *CD117* and *CD203c*, and *FGF2* are highly expressed in the synovium of obese KOA patients. Further investigations are needed to reveal the role of MCs in the relationship between obesity and osteoarthritis pathology.

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Disclosure

The authors report no conflicts of interest in this work.

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