Abnormal Transforming Growth Factor-ß Expression in Mesenchymal Stem Cells from Patients with Osteoarthritis

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ABSTRACT. ***Objective.*** To determine the expression of the genes that code for the isoforms of transforming growth factor-ß (TGF-ß) and TGF-ß receptors (TBR) in mesenchymal stem cells (MSC) from patients with osteoarthritis (OA).

Methods. Total RNA was extracted from primary cultures of MSC and quantitative real-time reverse transcription-polymerase chain reaction was performed to analyze gene expression.

Results. MSC from patients with OA showed significantly increased total TGF-ß, TGF-ß1 isoform, TBR-II, and TBR-III mRNA expression compared to controls.

Conclusion. Our study is the first reporting the gene expression levels of TGF-ß and its isoforms and receptors in patients with OA. These findings might have pathological significance for OA disease. (First Release April 1 2008; J Rheumatol 2008;35:904–6)

Key Indexing Terms:
OSTEOARTHRITIS
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Osteoarthritis (OA) is a common joint disease that mainly affects elderly people and is characterized by degeneration of the cartilage in the joint. Once some of the weight-bearing cartilage is lost, its lack of repair and associated subchondral bone changes are considered crucial for progression of the disease[1].

The primary reservoir for mesenchymal stem cells (MSC) is bone marrow. MSC can differentiate into various tissue types including bone, cartilage, fat, and muscle[2]. Hypothetically, and supported by some preliminary experimental data, MSC in bone marrow are able to migrate to damaged tissues and initiate and/or enhance the wound repair process[2].

Given that transforming growth factor-ß (TGF-ß) is known to play an important role in directing cell fate choices in mesenchymal cells[3], the possibility of altered TGF-ß expression in MSC from the bone marrow of patients with OA warrants evaluation. Our objective was to compare the expression of genes encoding the 3 isoforms of TGF-ß (1, 2, 3) and TGF-ß receptors (TBR-I, TBR-II, TBR-III) in primary cultures of MSC isolated from the bone marrow of patients with endstage OA and healthy control subjects.

MATERIALS AND METHODS

Patients and specimens. Fresh bone marrow was obtained from the femur channel of 21 patients with endstage knee OA (mean age 74.7 yrs, range 61–89), at the time of total joint replacement surgery, and 10 controls with no known history of joint disease (mean age 66.6 yrs, range 44–90), at the time of organ/tissue procurement. The diagnosis of knee OA was based on American College of Rheumatology criteria[4]. All research procedures involving human subjects were conducted according to the Declaration of Helsinki. The study protocol was approved by our institute's Review Board, and informed consent was obtained from all the subjects.

The isolation and culture expansion of MSC were carried out, as described[5].

RNA extraction and reverse transcription (RT). Total RNA was extracted from the third passage MSC cultures using the QIAamp RNA Mini Kit (Qiagen, Hilden, Germany); then all samples were digested using the Deoxyribonuclease I Kit (Sigma-Aldrich, St. Louis, MO, USA), and finally, RT was carried out with the Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics, Mannheim, Germany).

Real-time quantitative RT-polymerase chain reaction (PCR). Quantitative RT-PCR was conducted in a Rotor-Gene 3000 real-time cycler (Corbett Research, Sydney, Australia). Each sample was analyzed in duplicate for each one of the genes, and rRNA18S was used as internal control for relative quantification, as described[6]. Table 1 shows the sequences used in the quantitative RT-PCR: (1) the primers and probes used in the TaqMan assay for the expression of total TGF-ß and the rRNA 18S; and (2) primer pairs used in the SyBr Green assay for the TGF-ß (isoforms and receptors) and rRNA 18S.

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Table 1. Sequences of primers and probes used in quantitative real-time PCR for expression of total TGF-ß and TGF-ß isoforms and their receptors.

	Primer/Probes	Sequence (5'→ 3')
TGF-ß	F-primer	GTG ACA GCA GGG ATA ACA CAC TG
	R-primer	CAT GAA TGG TGG CCA GGT C
	Taqman probe	FAM-ACA TCA ACG GGT TCA CTA CCG GC-TAMRA
TGF-ß1	F-primer	5' - TGC GGC AGC TGT ACA TTG A - 3'
	R-primer	5' - TGG TTG TAC AGG GCC AGG A - 3'
TGF-ß2	F-primer	5' - GGC TCA GTG GGC AGC TTG T - 3'
	R-primer	5' - GCT CAA TCC GTT GTT CAG GC - 3'
TGF-ß3	F-primer	5' - CCC AGC TCT AAG CGG AAT GAG - 3'
	R-primer	5' - GCG CTG TTT GGC AAT GTG - 3'
TBR-I	F-primer	5' - CAA CTC AGT CAA CAG GAA GGC A - 3'
	R-primer	5' - AAA GAT GAT CTC CAG CAC AGC A - 3'
TBR-II	F-primer	5' - ATG AGC AAC TGC AGC ATC ACC - 3'
	R-primer	5' - TCC AGG ATG ATG GCA CAG TG - 3'
TBR-III	F-primer	5' - TGT CAC CTG GCA CAT TCA TT - 3'
(betaglycan)	R-primer	5' - TCT CAG CAC TGT CTT GGT GG - 3'
rRNA18S*	F-primer	5' - GCC CGA AGC GTT TAC TTT GA - 3'
	R-primer	5' - TCC ATT ATT CCT AGC TGC GGT ATC - 3'
rRNA18S**	F-primer	GCC CGA AGC GTT TAC TTT GA
	R-primer	TCC ATT ATT CCT AGC TGC GGT ATC
	Taqman probe	FAM-AAA GCA GGC CCG AGC CGC C-TAMRA

F-primer: forward PCR primer; R-primer: reverse PCR primer; PCR: polymerase chain reaction; TGF: transforming growth factor. * For relative quantification of TGF-ß isoforms and their receptors. ** For relative quantification of total TGF-ß expression.

Statistical analysis. Data are reported either as a normalization ratio (NR) mean ± SD in normal conditions or as the NR median and percentiles (25th–75th percentile) if the variables did not follow a Gaussian distribution. Normally distributed variables were compared using the Student's t-test and non-normally distributed data using the Mann-Whitney U-test. The level of significance was set at $p < 0.05$.

RESULTS

Significantly higher total TGF-ß mRNA expression was observed in MSC from patients with OA compared to controls ($p = 0.042$). Given this upregulation of TGF-ß mRNA expression in MSC from the patients with OA, we then examined the expression of the major isoforms of TGF-ß (TGF-ß1, TGF-ß2, and TGF-ß3) and of the 3 TGF-ß family receptors TBR-I, TBR-II, and TBR-III. Of these isoforms, only TGF-ß1 showed significantly increased expression levels in OA MSC compared to controls ($p = 0.036$). Expression levels of the TBR-I receptor failed to differ significantly between patients and controls, while the expression of both TBR-II and TBR-III was significantly higher in MSC from patients with OA compared to controls ($p = 0.001$; $p = 0.021$, respectively). These data are provided in Table 2.

DISCUSSION

Our results clearly demonstrate significantly increased total TGF-ß mRNA expression in MSC from patients with OA. However, only expression levels of the TGF-ß1 isoform were found to be increased. TGF-ß1 is known to regulate cell growth, differentiation, migration, and extracellular matrix production[7]. It has also been shown[8] that TGF-ß increases smooth muscle α-actin expression in MSC. Further, TGF-ß induces the chondrogenic differentiation of MSC in the presence of dexamethasone or 3-dimensional cell aggregates[9]. Collectively, these data indicate that TGF-ß may act in conjunction with other microenvironmental factors on MSC differentiation.

Our findings also indicate increased TBR-II and TBR-III expression levels in MSC from patients with OA compared to controls. TBR-I and TBRII are responsible for the biological effects of TGF-ß1 in mammalian cells. TBR-III, also known as betaglycan, is a proteoglycan coreceptor that enhances signaling by increasing the affinity of TGF-ß[10]. Betaglycans are widespread in mesenchymal cells, epithelial cells, and neurons and bind to TGF-ß1 via their extracellular portions. It has been suggested that TBR-III controls the availability of TGF-ß1 in the local extracellular microenvironment and regulates its active appearance as functional TBR-I and TBR-II[11]. Silva, *et al*[12] examined the profile of gene expression of human bone marrow MSC and found that TGF-ß-induced mRNA is the third most abundant transcript, thus confirming the important role of the TGF-ß signaling pathway in this cell population.

It seems clear that MSC, when delivered by intravenous infusion, are capable of specific migration to a site of

Table 2. Expression of TGF-ß and its isoforms and receptors in the MSC of patients with OA determined by quantitative real time RT-PCR.

Gene	NR OA Patients	NR Controls	p
TGF-ß	0.61 ± 0.28	0.40 ± 0.24	0.042*
TGF-ß1	2.07 ± 0.99	1.20 ± 1.05	0.036*
TGF-ß2	2.25 (0.78–5.34)	2.94 (0.78–6.44)	0.982
TGF-ß3	18.13 (11.20–27.29)	14.54 (0.35–25.5)	0.734
TBR-I	0.17 (0.05–0.76)	0.11 (0.06–0.31)	0.441
TBR-II	0.17 (0.08–0.26)	0.05 (0.02–0.06)	0.001*
TBR-III	0.18 (0.07–1.38)	0.06 (0.03–0.35)	0.021*

* Values < 0.05 considered significant. NR: normalization ratio; OA: osteoarthritis; MSC: mesenchymal stem cells; RT-PCR: reverse transcription-polymerase chain reaction (25th–75th percentile).

injury[13]. Moreover, tissue-committed stem cells appear to reside in the bone marrow and can be mobilized into the peripheral blood following damage[14]. A possible explanation for the upregulated TGF-ß expression observed in the bone marrow-derived MSC from our patients with OA could be a stimulatory effect on mesenchymal cell proliferation in bone marrow, thus allowing expansion of the MSC population and/or the osteoblast- and chondrocyte-committed cells in response to the bone and cartilage damage characteristic of this disease.

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