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ORIGINAL ARTICLE

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Expression profiles of two types of human knee-joint cartilage

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Abstract We have performed a comprehensive analysis of gene-expression profiles in human articular cartilage (hyaline cartilage) and meniscus (fibrocartilage) by means of a cDNA microarray consisting of 23,040 human genes. Comparing the profiles of the two types of cartilage with those of 29 other normal human tissues identified 24 genes that were specifically expressed in both cartilaginous tissues; these genes might be involved in maintaining phenotypes common to cartilage. We also compared the cartilage profiles with gene expression in human mesenchymal stem cells (hMSC), and detected 22 genes that were differentially expressed in cells representing the two cartilaginous lineages, 11 specific to each type, which could serve as markers for predicting the direction of chondrocyte differentiation. Our data should also provide useful information about regeneration of cartilage, especially in support of efforts to identify cartilage-specific molecules as potential agents for therapeutic approaches to joint repair.

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Introduction

Several transcriptional factors are thought to be key factors directing differentiation of mesenchymal tissues: molecules of the MYOD family for muscle differentiation (Ferrari et al. 1998), PPARG2 for adipose tissue differentiation (Rosen and Spiegelman 2000), and CBFA1 for osteogenic differentiation (Komori et al. 1997). Although SOX9 and CBFA1 are thought to be key transcriptional factors during cartilage differentiation (Bi et al. 1999), other factors are likely to be involved as well (Takeda et al. 2001). Articular cartilage (hyaline cartilage) of the knee, and the knee meniscus (fibrocartilage), are different (Ross et al. 1995). Identification of common cis-elements in genes expressed specifically in these two types of cartilage should yield insight into cartilage-specific transcriptional regulation.

Investigators interested in cartilage development and regeneration have a few markers available for predicting the direction of differentiation, i.e., type II collagen and aggrecan for hyaline cartilage, and type I collagen for fibrocartilage (Burgeson et al. 1982; Ross et al. 1995; Carlberg et al. 2001). Cartilaginous cells are believed to originate from multipotential mesenchymal cells that resemble human mesenchymal stem cells (hMSCs) (DeLise et al. 2000; Carlberg et al. 2001). Since hMSCs are relatively easy to obtain, hMSC cultures are considered to be good sources of cells for modeling regeneration of mesenchymal tissues such as cartilage and bone (Pittenger et al. 1999; Carlberg et al. 2001; Ochi et al. 2003). Thus, genes that are expressed specifically in either hyaline cartilage or fibrocartilage should be useful markers to indicate the direction of differentiation. To identify such genes, we have applied a cDNA microarray

containing 23,040 genes in a system that provides high-throughput analysis of expression. In the work reported here, we evaluated the power of this system to detect cartilage-specific molecules.

Materials and methods

Clinical samples

All patients were diagnosed and treated by members of the Department of Orthopedic Surgery, Keio University School of Medicine. "Normal" articular cartilage samples were obtained during total knee replacement surgery performed on eight female patients (age range 56–80 years, median 69 years) who were diagnosed with primary osteoarthritis of the knee. In each case a non-affected site of articular cartilage, such as the patellar–femoral joint, was carefully peeled to provide samples for further investigations. "Normal" meniscus tissues were obtained from samples removed during endoscopic meniscectomy of six patients (five male and one female; age range 13–28 years, median 20 years) who were diagnosed with traumatic meniscal injury; again, non-affected tissue corresponding to each sample was obtained for our investigations. All 14 patients (or their families in some cases) gave informed consent according to the guidelines of both Keio University and the University of Tokyo. Resected samples were snap-frozen in liquid nitrogen immediately and sent to the Human Genome Center, Institute of Medical Science at the University of Tokyo, without any personal identifiers.

Preparation, T7-based amplification, and labeling of RNAs

Total RNA was extracted from each frozen clinical sample and from human mesenchymal stem cells (hMSC) purchased from BioWhittaker, Inc. (Walkersville, MD) to serve as a universal control, using TRIzol reagent (Life Technologies, Inc., Rockville, MD). Total RNAs of 29 normal human tissues (mesenteric adipose tissue, bone marrow, brain, heart, kidney, liver, lung, lymph node, mammary gland, pancreas, placenta, prostate, salivary gland, skeletal muscle, small intestine, spinal cord, spleen, stomach, testis, thymus, thyroid, trachea, uterus, colon, ovary, fetal brain, fetal kidney, fetal liver, and fetal lung) were obtained as described previously (Saito-Hisaminato et al. 2002). Equal amounts of RNA from each of the 29 normal tissues were pooled to serve as another universal control (29Mix). All RNA preparations were digested with RNase-free DNase I (Nippon Gene Co., Tokyo, Japan) according to the manufacturer's recommendations. Two rounds of T7-based RNA amplification and the preparation of cDNA probes were carried out as described elsewhere (Kitahara et al. 2001; Ochi et al. 2002). Amplified RNA (2.5 μg) from each cartilage sample (articular cartilage: a mixture of 8 cases; meniscus, 6 patients) was labeled with Cy5-dCTP (Amersham Biosciences, Uppsala, Sweden); an equal amount of amplified RNA from each universal control (29Mix or a pool of amplified RNA from hMSCs) was labeled with Cy3-dCTP (Amersham Biosciences) for hybridization to the cDNA microarray.

Construction and analysis of the cDNA microarray

Fabrication of our cDNA microarray slides has been described previously (Ono et al. 2000; Kitahara et al. 2001). Slides containing a duplicate set of 23,040 cDNA spots were used for each analysis of expression profiles, to reduce experimental fluctuation. Hybridizations were performed to compare hyaline cartilage (Cy5) with 29Mix (Cy3), fibrocartilage (Cy5) with 29Mix (Cy3), hyaline cartilage (Cy5) with hMSC (Cy3), and fibrocartilage (Cy5) with hMSC (Cy3). Hybridization, washing, and scanning were performed as described elsewhere (Ono et al. 2000; Kitahara et al. 2001). The intensity of each duplicated signal was evaluated photometrically using the ArrayVision computer program (Imaging Research, Inc., St Catharines, Ontario, Canada). Intensities of the 29 individual normal tissues (test) and 29Mix (control) were evaluated as described elsewhere (Saito-Hisaminato et al. 2002). The fluorescence intensities of Cy5 (test) and Cy3 (control) for each target spot were adjusted so that the mean Cy5/Cy3 ratio of the 52 housekeeping genes was equal to one. We assigned a cut-off value to each microarray slide, using variance analysis. If both Cy3 and Cy5 signal intensities were less than the cut-off values, the expression level of the corresponding gene in that sample was assessed as low or absent. For other genes we calculated Cy5/Cy3 as a relative expression ratio. A two-dimensional hierarchical clustering method was performed as described before (Saito-Hisaminato et al. 2002).

Semi-quantitative RT-PCR

A 3-μg aliquot of each RNA to be used for microarray analysis was reverse-transcribed to single-stranded cDNA using oligo$(dT)_{12–18}$ primer and Superscript II (Invitrogen, Carlsbad, CA). Semi-quantitative reverse transcription PCR (RT-PCR) was carried out for seven selected genes as described previously (Ono et al. 2000), using the following primer sets: for *UBA52*, 5′-GAT CTT TGT GAA GAC CCT CAC TG-3′ and 5′-CAG ATC ATC TTG TCG CAG TTG TA-3′; for *RPL23*, 5′-CTG TAT ATC ATC TCC GTG AAG GG-3′ and 5′-AAG TCT GCA CAC TCC TTT GCT AC-3′; for *RAB13*, 5′-CAA CAA CAC TTA CAT CTC CAC CA-3′ and 5′-GAG CAC TTG TTG GTG TTC TTC TT-3′; for *IGF2*, 5′-CTT GGA CTT TGA ATC AAA TTG G-3′ and 5′-CCT CCT TTG GTC TTA CTG GG-3′; for *IGL@*, 5′-CAA CTG TAC ACC TAA AGG CTC TC-3′ and 5′-ATC TGA GAG GAG AGG AGA GTG A-3′; for *PDLIM1*, 5′-CCA TAC AAG ATG AAT TTA GCC TCT G-3′ and 5′-GTC AGA CAC GTT ATA TT TGA TTG GG-3′; and for *COL1A1*, 5′-CAT GTT CGG TTG GTC AAA GATA-3′ and 5′-AAT ACA AAA CCA CCA AGA CCT CC-3′. PCR reactions were optimized for the number of cycles to ensure product intensity within the linear phase of amplification.

Results and discussion

Identification of genes that were highly expressed in both hyaline cartilage and fibrocartilage

Comparison of expression profiles in cartilage with those of other normal human tissues should indicate genes that are preferentially expressed in cartilage. We selected 6707 genes for which data were present for hyaline cartilage; 585 of them, including five collagen genes, showed hyaline cartilage/29Mix ratios >10, and 209 also showed hyaline cartilage/hMSC ratios >10 (data not shown). Similarly, we selected 6436 genes for which data were present for fibrocartilage; 605 showed fibrocartilage/29Mix ratios >10 and 145 showed fibrocartilage/hMSC ratios >10 (data not shown).

Identification of "cartilage-specific" genes

Articular cartilage and meniscus are both considered to be cartilaginous tissues (Ross et al. 1995). A hierarchical clustering of the 31 normal human tissues we examined, including the two types of cartilage, indicated that the

latter are very closely related in gene expression profiles (data not shown). Thus we assumed that some phenotypes common to hyaline cartilage and fibrocartilage should be maintained by the same "cartilage-specific transcriptional regulators."

To obtain candidates for these putative "cartilage-specific transcriptional regulators," we searched for genes whose relative expression ratio for either hyaline cartilage/29Mix or fibrocartilage/29Mix were >5 while (i) expression ratios in the 29 non-cartilaginous tissues were <2 or (ii) expression was absent in 90% of the normal tissues, and identified 69 genes. Among them, 24 revealed expression ratios of >5 for both hyaline cartilage/29Mix and fibrocartilage/29Mix; but 22 of the 69 were highly expressed only in hyaline cartilage and 23 were highly expressed only in fibrocartilage (Fig. 1a, b).

Identification of separate marker genes for hyaline cartilage and fibrocartilage

Comparison of expression profiles of articular cartilage (hyaline cartilage) and meniscus (fibrocartilage) with the profile obtained from a mixture of 29 other normal tissues showed a specific character for cartilaginous tissue after final differentiation, but this approach was not sufficient for identifying genes important for understanding development and regeneration of mesenchymal tissues. Thus, we re-examined the expression profiles to investigate genes that showed different levels of expression between each type of cartilage and hMSCs. We first selected 4837 genes for which data were obtained for either hyaline cartilage/hMSC or fibrocartilage/hMSC and then looked for candidate marker genes that could satisfy the criteria of both "hyaline cartilage/hMSC ratio of >2" and "fibrocartilage/hMSC ratio of <0.5," or genes that would satisfy the criteria of both "fibrocartilage/hMSC ratio of >2" and "hyaline cartilage/hMSC ratio <0.5." We detected 22 genes that were, by this definition, differentially expressed in one or the other of the two types of cartilaginous cells (Fig. 2a, b).

In all, we examined 92,160 gene expression patterns in two cartilaginous tissues, in comparison with 668,160 measurements of genes expressed in 29 other normal tissues, by means of a cDNA microarray. To our knowledge this is the first study to apply such a large-scale approach for analyzing expression profiles of cartilaginous tissues. This work has established an expression database for sets of genes that are expressed specifically in cartilage.

We subjected the expression profiles of two types of cartilaginous tissue and 29 normal tissues to a hierarchical clustering analysis. The dendrogram showed that articular cartilage and meniscus fell into the same, closely related terminal branch, indicating that both hyaline cartilage and fibrocartilage were very similar in gene expression patterns. Among several mesodermal tissues included in the 31-tissue panel, only bone marrow and nerve tissues yielded expression patterns that were relatively similar to that of cartilage (data not shown). Our data imply that significant alterations in expression level must occur for a large number of genes during the differentiation process.

Transcriptional regulation of genes in a given tissue is controlled by a combination of mechanisms, for example changes in chromatin structure mediated by the association of histone acetylases and deacetylases (Wade 2001), binding of transcriptional regulators to an enhancer and/or a suppressor (Luscher and Larsson 1999), binding of transcription factors to a promoter region, and methylation of CpG islands in promoter sequences (Ballestar and Wolffe 2001). Although the precise mechanisms of cooperation among these elements are not well understood (Cha et al. 2000), identification of common cis-elements that exist only in cartilage-specific genes should yield insight into the physiology of chondrocytes. Such core sequences might be useful for (a) identifying novel transcription-related factors in cartilage, and (b) establishing cartilage-specific expression vectors for genes that are necessary for regenerating and/or maintaining phenotypes of cartilaginous tissues.

In our experiments, 24 genes were expressed specifically in both hyaline cartilage and fibrocartilage. Among them, *WNT7A* is member of the *WNT* gene family, which consists of structurally related genes encoding secreted signaling molecules. This gene is implicated in the regulation of limb chondrogenesis (Rudnicki and Brown 1997; DeLise et al. 2000; Tufan et al. 2002). *COMP* is normally expressed at high levels in the territorial matrix of chondrocytes and is believed to belong to the family of thrombospondin genes (Newton et al. 1994); mutations of *COMP* have been found in patients with pseudoachondroplasia or multiple epiphyseal dysplasia (Ikegawa et al. 1998), indicating its important role in maintaining cartilaginous structure (Thur et al. 2001). *GLG1* encodes a conserved membrane sialoglycoprotein present in the Golgi apparatus of most cells; this protein binds with basic fibroblast growth factor (FGFB) and is expressed during chondrogenesis (Stieber et al. 1995). *GPS2* encodes a 327-amino-acid polypeptide with no similarity to other proteins or known motifs; however, this molecule potently regulates RAS- and mitogen-activated protein kinase (MAPK)-mediated signals and interferes with JNK activity in yeast and mammalian cells (Spain et al. 1996). Considering that *WNT* genes, *COMP*, *FGFB*, *MAPK*, and *JNK* are known to play important roles in chondrogenesis (Thur et al. 2001), their products might well be critical to the physiology of chondrocytes. SOX9, believed to be a key transcriptional factor in chondrocytes (Bi et al. 1999), is a member of the sex-determining region Y-type HMG (high mobility group) box protein family; it is required for chondrocyte-specific gene expression and chondrogenesis (Bi et al. 1999). In our experiments the SOX9 gene was highly expressed in both hyaline cartilage and fibrocartilage (relative ratios >10), but its expression was relatively ubiquitous (relative ratio >2 in seven of the 29 normal non-cartilaginous tissues examined).

Fig. 1a, b Schema of "cartilage-specific genes." Relative expression ratios were (i) >5 in cartilaginous tissues, while ratios in a mixture of cDNAs from 29 other normal tissues were <2 or (ii) >5 in cartilaginous tissues, while ratios were missing for 90% of individual normal tissues compared with the 29Mix. **a** Twenty-four of the 69 genes were highly expressed in both hyaline cartilage and fibrocartilage (overlapping), while 22 were highly expressed in hyaline cartilage and 23 were highly expressed in fibrocartilage. Each cell in the matrix represents the expression level of a single transcript in a single tissue, with *red* and *green* indicating transcript levels respectively above and below the median for that gene across the 29Mix. *Black* represents unchanged expression; *gray* indicates slight expression or none (intensities of both Cy3 and Cy5 less than the cut-off value). Abbreviations: *H*, relative expression ratio in hyaline cartilage; *F*, relative expression ratio in fibrocartilage. **b** Semi-quantitative RT-PCR of three selected genes using RNAs from two types of cartilage and six other normal tissues. The integrity of each template was controlled through amplification of β2MG

a

Cartilage specific genes (Overlapping)

H	F	Accession	HSID	Symbol	Gene Name
		L32137	1584	*COMP*	cartilage oligomeric matrix protein
		W51788	5798	*PELO*	pelota (Drosophila) homolog
		X05232	83326	*MMP3*	matrix metalloproteinase 3 (stromelysin 1, progelatinase)
		AJ224677	7122	*SCRG1*	scrapie responsive protein 1
		AA780308	98785	*KSP37*	Ksp37 protein
		AI022360	190583	*ESTs*	ESTs
		D79205	300141	*RPL39*	ribosomal protein L39
		AA127483	180139	*SMT3H2*	SMT3 (suppressor of mif two 3, yeast) homolog 2
		U70735	15591	*MOV34*	COP9 subunit 6 (MOV34 homolog, 34 kD)
		U53476	72290	*WNT7A*	wingless-type MMTV integration site family, member 7A
		AA292179	119502	*UBA52*	ubiquitin A-52 residue ribosomal protein fusion product 1
		M74905	79396	*MPG*	N-methylpurine-DNA glycosylase
		AF037261	33787	*SCAM*	vinexin beta (SH3-containing adaptor molecule)
		U28963	296940	*GPS2*	G protein pathway suppressor 2
		X16135	2730	*HNRPL*	heterogeneous nuclear ribonucleoprotein L
		M81758	46038	*SCN4A*	sodium channel, voltage-gated, type IV, alpha polypeptide
		W84565	109494	*SPUF*	secreted protein of unknown function
		AI018632	150101	*LAMP1*	lysosomal-associated membrane protein 1
		AA338449	110695	*ESTs*	ESTs, Weakly similar to B0495.6 [C.elegans]
		U28811	78979	*GLG1*	Golgi apparatus protein 1
		AA703770	4888	*SARS*	seryl-tRNA synthetase
		X76013	79322	*QARS*	glutaminyl-tRNA synthetase
		L07540	171075	*RFC5*	replication factor C (activator 1) 5 (36.5kD)
		AA292834	46901	*KIAA1462*	KIAA1462 protein

Cartilage specific genes (Hyalin cartilage)

H	F	Accession	HSID	Symbol	Gene Name
		U97276	77266	*QSCN6*	quiescin Q6
		X55954	234518	*RPL23*	ribosomal protein L23
		AA609358	5245	*FLJ20643*	hypothetical protein FLJ20643
		AI022075	12210	*FLJ13732*	hypothetical protein FLJ13732 similar to tensin
		AI039548	187899	*ESTs*	ESTs
		AA812932	123515	*ESTs*	Homo sapiens cDNA FLJ13840 fis, clone THYRO1000783
		AA854476	284294	*BCRP2*	Breakpoint cluster region protein, uterine leiomyoma, 2
		AI193935	131250	*LOC63920*	transposon-derived Buster3 transposase-like
		AA836860	287776	*OTRPC4*	vanilloid receptor-related osmotically activated channel
		AF075292	49585	*FGF18*	fibroblast growth factor 18
		AF038603	129758	*PSTPIP1*	proline-serine-threonine phosphatase interacting protein 1
		L02321	75652	*GSTM5*	glutathione S-transferase M5
		BE614190	23495	*FLJ11252*	hypothetical protein FLJ11252
		AA843531	100002	*HSPC162*	HSPC162 protein
		U05877	177559	*IFNGR2*	interferon gamma receptor 2 (interferon gamma transducer 1)
		X91349	3759	*USP5*	ubiquitin specific protease 5 (isopeptidase T)
		AI004706	182364	*ESTs*	ESTs, Weakly similar to 25 kDa trypsin inhibitor [H.sapiens]
		BE502540	171077	*ESTs*	ESTs, Weakly similar to similar to serine/threonine kinase [C.elegans]
		X54412	154850	*COL9A1*	collagen, type IX, alpha 1
		X75450	279651	*MIA*	melanoma inhibitory activity
		J02984	133230	*RPS15*	ribosomal protein S15
		X56932	119122	*RPL13A*	ribosomal protein L13a

Cartilage specific genes (Fibrocartilage)

H	F	Accession	HSID	Symbol	Gene Name
		X05345	77798	*HARS*	histidyl-tRNA synthetase
		N20283	26276	*ESTs*	ESTs
		W02928	9933	*ALTE*	Ac-like transposable element
		AA232823	283728	*PCNP*	PEST-containing nuclear protein
		AA780074	122706	*ESTs*	ESTs
		AI079967	117266	*ESTs*	ESTs
		AA503921	299121	*ESTs*	ESTs
		AA521154	105500	*ESTs*	ESTs
		AA214125	78103	*NAP1L4*	nucleosome assembly protein 1-like 4
		X75593	151536	*RAB13*	RAB13, member RAS oncogene family
		X97544	20716	*TIM17*	translocase of inner mitochondrial membrane 17 (yeast) homolog A
		M94054	102267	*LOX*	lysyl oxidase
		J00306	12409	*SST*	somatostatin
		BF688836	77886	*LMNA*	lamin A/C
		AA583019	31791	*ACYP2*	acylphosphatase 2, muscle type
		AA766527	306127	*ESTs*	ESTs
		N24911	5258	*C11ORF2*	chromosome 11 open reading frame2
		AA284317	6120	*FLJ13222*	hypothetical protein FLJ13222
		L37042	283738	*CSNK1A1*	casein kinase 1, alpha 1
		L20970	172081	*PDE4D*	phosphodiesterase 4D, cAMP-specific
		AA813363	193784	*ESTs*	Homo sapiens mRNA; cDNA DKFZp586K1922
		AF043472	47584	*KCNS3*	potassium voltage-gated channel, delayed-rectifier, subfamily S, member 3
		AA234909	181077	*KIAA1306*	KIAA1306 protein

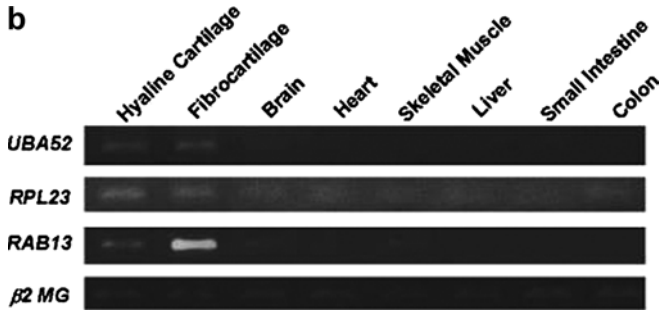


Fig. 1a, b (Continued)

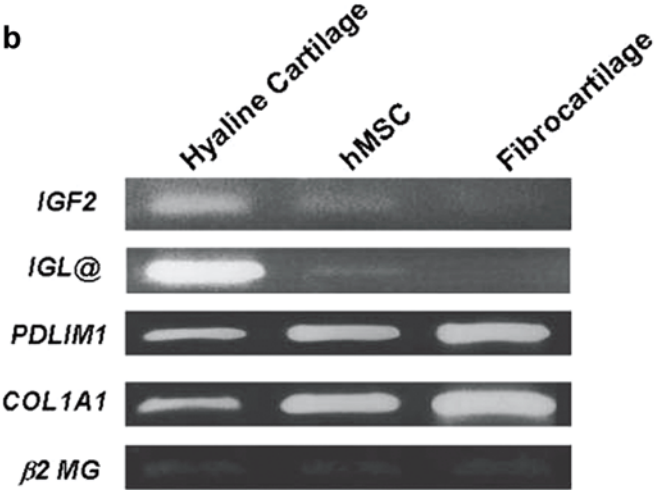
Several known markers are commonly invoked to predict the direction of differentiation in studies of cartilage development and regeneration, specifically collagen type II and aggrecan for hyaline cartilage and type I collagen for fibrocartilage (Burgeson et al. 1982). Our genome-wide cDNA microarray analyses revealed 22 additional genes that were specifically expressed in cartilaginous tissue: 11 in hyaline cartilage and 11 others in fibrocartilage. RT-PCR experiments also supported differences in gene expression patterns among hyaline cartilage, fibrocartilage, and hMSC, indicating that this set of 22 genes can include candidates to serve as markers during chondrogenesis from multipotential mesenchymal cells.

Our data indicated high expression of type I collagen in fibrocartilage, but expression of this gene was very low in hyaline cartilage. On the other hand, type II collagen was extremely abundant in hyaline cartilage (ratio of >100), and relatively so in fibrocartilage (ratio of 15). The consistency of our data with previous reports verifies their quality (Tufan et al. 2002). One of the main goals of regenerative medicine is to replace damaged

Fig. 2a, b Schema of marker genes for hyaline cartilage or fibrocartilage. Relative expression ratios were >2 in either type, and the ratios in the other cartilage were <0.5 compared with hMSC, for all 22 genes listed. **a** Eleven of the 22 genes meeting the strict differential criteria were highly expressed only in hyaline cartilage (H), while 11 others were highly expressed only in fibrocartilage (F). The color scale was constructed from expression ratios of cartilaginous tissue (Cy5):hMSC (Cy3) as described in Fig. 1. **b** Semi-quantitative RT-PCR of four selected genes using RNAs from two types of cartilage and from hMSCs. The integrity of each template was controlled through amplification of β2MG

Marker genes of hyaline cartilage and fibrocartilage **a**

H	F	Accession No.	HSID	Symbol	Gene Name
		X07868	251664	*IGF2*	insulin-like growth factor 2 (somatomedin A)
		AI197946	181125	*IGL@*	immunoglobulin lambda locus
		AA436362	30868	*RTN4R*	reticulon 4 receptor (Nogo receptor)
		AA779724	122113	*ESTs*	ESTs
		L05779	113	*EPHX2*	epoxide hydrolase 2, cytoplasmic
		AA809819	5710	*CREG*	cellular repressor of E1A-stimulated genes
		AA812932	123515	*FLJ13840*	Homo sapiens cDNA FLJ13840 fis, clone THYRO1000783
		AA761311	211563	*BCL7A*	B-cell CLL/lymphoma 7A
		M22430	76422	*PLA2G2A*	phospholipase A2, group IIA (platelets, synovial fluid)
		AW167729	10029	*CTSC*	cathepsin C
		X00129	76461	*RBP4*	retinol-binding protein 4, interstitial
		AF070616	3618	*HPCAL1*	hippocalcin-like 1
		AI268231	130829	*ESTs*	ESTs
		AI183533	300823	*LIMK2*	LIM domain kinase 2
		AA316112	284194	*FLJ20831*	hypothetical protein FLJ20831
		AI265770	75807	*PDLIM1*	PDZ and LIM domain 1 (elfin)
		AI349804	133294	*ESTs*	ESTs
		AA491502	97199	*C1QR*	complement component C1q receptor
		AA977821	172928	*COL1A1*	collagen, type I, alpha 1
		AA780074	122706	*ESTs*	ESTs
		J03464	179573	*COL1A2*	collagen, type I, alpha 2
		AF037335	5338	*CA12*	carbonic anhydrase XII



tissue with regenerated tissue that can function normally. To realize this goal it is important to establish methods to generate the required tissue as well as to maintain the physiological function of the regenerated tissue. The data documented here should provide useful information for both approaches and contribute to research toward regeneration of cartilage tissues in clinical settings.

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