Identification of TGFβ-related genes regulated in murine osteoarthritis and chondrocyte hypertrophy by comparison of multiple microarray datasets

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ABSTRACT

Objective: Osteoarthritis (OA) is a joint disease characterized by progressive degeneration of articular cartilage. Some features of OA, including chondrocyte hypertrophy and focal calcification of articular cartilage, resemble the endochondral ossification processes. Alterations in transforming growth factor β (TGFβ) signaling have been associated with OA as well as with chondrocyte hypertrophy. Our aim was to identify novel candidate genes implicated in chondrocyte hypertrophy during OA pathogenesis by determining which TGFβ-related genes are regulated during murine OA and endochondral ossification.

Methods: A list of 580 TGFβ-related genes, including TGFβ signaling pathway components and TGFβ-target genes, was generated. Regulation of these TGFβ-related genes was assessed in a microarray of murine OA cartilage: 1, 2 and 6 weeks after destabilization of the medial meniscus (DMM). Subsequently, genes regulated in the DMM model were studied in two independent murine microarray datasets on endochondral ossification: the growth plate and transient embryonic cartilage (joint development).

Results: A total of 106 TGFβ-related genes were differentially expressed in articular cartilage of DMM-operated mice compared to sham-control. From these genes, 43 were similarly regulated during chondrocyte hypertrophy in the growth plate or embryonic joint development. Among these 43 genes, 18 genes have already been associated with OA. The remaining 25 genes were considered as novel candidate genes involved in OA pathogenesis and endochondral ossification. In supplementary data of published human OA microarrays we found indications that 15 of the 25 novel genes are indeed regulated in articular cartilage of human OA patients.

Conclusion: By focusing on TGFβ-related genes during OA and chondrocyte hypertrophy in mice, we identified 18 known and 25 new candidate genes potentially implicated in phenotypical changes in chondrocytes leading to OA. We propose that 15 of these candidates warrant further investigation as therapeutic target for OA as they are also regulated in articular cartilage of OA patients.

Keywords: osteoarthritis, TGFβ, chondrocyte hypertrophy, microarray

1. INTRODUCTION

Osteoarthritis (OA) is characterized by degeneration of articular cartilage and the clinical symptoms are joint pain and functional impairment [1, 2]. The chondrocytes in articular cartilage of OA patients exhibit phenotypic changes that resemble hypertrophic differentiation of chondrocytes during endochondral ossification in the postnatal growth plate [3-8] and in embryonic joint development [9-11]. Since articular cartilage has limited repair capacity, it is essential to prevent cartilage degeneration at an early stage. To accomplish this, the pathogenic mechanisms initiating OA require further elucidation.

The transforming growth factor-β (TGFβ) signaling pathway has been implicated in OA pathogenesis and in hypertrophic differentiation of chondrocytes [12-15]. Polymorphisms in *TGFB1* and *SMAD3,* a signaling molecule that is activated by binding of TGFβ to its receptor, have been associated with multiple joint pathologies in OA [16-20] and mutations in *SMAD3* lead to early-onset of OA in multiple articular joints in humans [21, 22]. We have shown previously that protein expression of Tgfb3 and phosphorylated Smad2 is reduced in two murine models for OA [23]. In mice, deficiency of *Smad3*, *Tgfbr2* or overexpression of a truncated kinase-defective Tgfbr2, result in a degenerative joint disease resembling human OA [24, 25]. Importantly, a decrease in Tgfbr1 in murine and human articular chondrocytes correlates with OA development and elevated expression of markers for chondrocyte hypertrophy [26]. Aside from its involvement in OA, TGFβ signaling plays a crucial role in maintenance of articular cartilage under normal physiological conditions and skeletal development [27, 28]. Chondrocyte-specific deletion of *Tgfbr2*, *Tgfbr1*, *Smad3* or *Smad4* accelerates hypertrophic differentiation of chondrocytes in the growth plate and articular cartilage [29-35]. Moreover, *Tgfb2* knockout mice display severe abnormalities in bone formed by endochondral ossification [36]. Together these data indicate that TGFβ is crucial for maintenance of the articular (pre-hypertrophic) chondrocyte phenotype and that alterations in TGFβ signaling lead to chondrocyte hypertrophy and predispose to OA.

It is currently not precisely known which aspects of TGFβ signaling are associated with chondrocyte homeostasis, hypertrophy or OA development and whether there is overlap. A second question is whether phenotypic changes of articular chondrocytes in OA show similarity to chondrocyte hypertrophy in normal developmental processes (transient growth plate cartilage, joint development). For the identification of molecular targets that may be involved in early onset and progression of OA, microarray analyses have been performed on murine models for OA (early onset, trauma-induced) rather than on human OA cartilage (end-stage disease) [37-40]. One of the most recent studies in the murine OA model compared multiple independent micro-array experiments and identified the TGFβ pathway

as common denominator [39]. Due to the divergent effects of TGFβ signalling in articular cartilage homeostasis and disease, we hypothesized that defining the regulation of genes involved in, or regulated by, the TGFβ signaling pathway in early OA and endochondral ossification would result in the identification of novel targets implicated in phenotypic changes of chondrocytes in OA pathogenesis. In this study, we first generated a list of TGFβ-related genes, which included genes encoding components of the TGFβ signaling pathway (from the Kyoto Encyclopedia of Genes and Genomes; KEGG) [41] and genes shown to be regulated by TGFβ (*i.e.* TGFβ-target genes) in cartilaginous cells by microarray analyses [42-44]. Secondly, we determined which of these TGFβ-related genes are regulated in a murine model for early OA as well as during endochondral ossification in murine growth plates and/or embryonic joint development using available microarray datasets [11, 37, 45]. Finally, genes identified by this approach were further explored in the literature to evaluate whether we identified genes known to be involved in murine and human OA pathology and to determine which genes might be novel candidates implicated in hypertrophic differentiation of articular chondrocytes during OA pathogenesis.

2. METHODS

2.1 List of TGFβ-related genes

A list of genes related to TGFβ signaling was compiled by including murine genes of the TGFβ signaling pathway derived from the KEGG database [41] and genes shown to be regulated by TGFβ in four published microarray experiments: 1x *in vivo* and 3x *in vitro* [42-44]. Takahashi *et al.* analyzed gene expression (Murine Genome Array U74Av2; Affymetrix) in unstimulated and TGFβ1-stimulated (10 ng/mL for 9 hours) chondrocytes from the H4 murine cell line [42, 46]. Sohn *et al.* performed microarray analysis (GeneChip Mouse Genome 430 2.0 Array; Affymetrix) on murine embryonic (E11.5) sclerotome cells cultured in micromass in absence or presence of TGFβ1 (5 ng/mL for 8 hours), and on vertebra isolated from wild-type and *Tgbr2* knock-out mice [43]. Ramaswamy *et al.* evaluated gene expression (GeneChip Bovine Genome Array; Affymetrix) in micromass cultures of chondrocytes (isolated from articular cartilage of 3 cows) that were cultured in absence or presence of TGFβ1 (5 ng/mL for 8 hours) [44]. For the latter study, bovine gene probes (Affymetrix) were translated to mouse orthologs. After merging the results of the three published microarray studies [42-44], one list of 501 unique genes regulated by TGFβ (up and down) was obtained.

To obtain a complete list of TGFβ-related genes, we merged the KEGG TGFβ pathway murine gene list, containing 85 genes, with the list of 501 genes previously

shown to be regulated by TGFβ in murine or bovine chondrogenic cells [42-44]. As 6 genes overlapped between these two lists, the TGFβ-related gene list contained 580 unique genes (Supplementary Table 1).

2.2 Microarray datasets of murine OA cartilage, growth plate zones and developing embryonic joints

2.2.1 Microarray dataset of murine OA cartilage

The murine early OA microarray experiment has been described in detail elsewhere [37]. Briefly, OA was induced in 10-week-old male C57BL/6 mice by surgical destabilization of the medial meniscus (DMM) of the right knee. As control, a sham operation (where the medial menisco-tibial ligament was exposed, but not transsected) was performed on the left knee. At 1, 2 and 6 weeks after surgery, tibial epiphyses were isolated (n=4 mice per time point), decalcified, embedded and snap-frozen. Cryosections were stained with toluidine blue to locate developing OA lesions (loss of toluidine blue staining and cartilage fibrillations) in non-calcified medial tibial plateau articular cartilage for laser-microdissection (Arcturus Bioscience). Anatomic and histologic landmarks were used to laser-microdissect noncalcified articular cartilage from sham-operated mice. After pooling laser-microdissected sections from each individual mouse, total RNA was isolated using TRIzol reagent (Invitrogen) following manufacturer's protocol and RNA was amplified in two rounds using the MessageAmp II aRNA Amplification Kit (Ambion) to obtain over 30 μg amplified RNA per mouse joint. Microarray expression profiling was performed on amplified RNA from cartilage of individual DMM- or sham-operated mouse joints, using microarrays (Cy3/Cy5 dye swap with replicate RNA samples). Labeled RNA was hybridized to 44k whole genome oligo microarray (G4122A; Agilent technologies). The arrays were scanned on a G2565BA DNA microarray Scanner (Agilent technologies) and Agilent Feature Extraction software version 9.5.3 was used to extract the features. The microarray data have been validated by real-time quantitative PCR (qPCR) on amplified RNA [37].

2.2.2 Microarray dataset of murine growth plate zones

Details regarding the performed microarray experiment are described elsewhere [11]. In brief, femoral growth plates were isolated from long bones of a 14-day old female Swiss white mouse, immersed in Tissue-Tek OCT embedding compound (Sakura Finetechnical, Tokyo, Japan) and snap-frozen in isopentane. Using microdissection, approximately 2,000 chondrocytes (per layer) were isolated from the proliferative (PR), pre-hypertrophic (PH) and hypertrophic (H) layer of the growth plate using an ophthalmic scalpel (Feather, Osaka, Japan). Total RNA was extracted using PicoPure RNA isolation kit (Arcturus Bioscience, Mountain View, CA), treated

with DNase to remove contaminating genomic DNA (Qiagen, Hilden, Germany) and linearly amplified using MessageAmp aRNA kit (Ambion) according to manufacturer's protocol. Amplified RNA was labeled with Cy3/Cy5 fluorophores, then hybridized to 44k whole genome oligo microarrays (G4122A; Agilent Technologies) and scanned on an Axon 4000B scanner. Features were extracted using GenePix Pro software (version 4.1; Axon Instruments, Union City, CA, USA). The microarray data have been validated by qPCR on amplified RNA [11].

2.2.3 Microarray dataset of murine embryonic joint

During embryonic limb formation, transient embryonic cartilage undergoes hypertrophy and endochondral ossification to form long bones [9-11]. The interzone is critical for joint formation and consists of two outer zone layers adjacent to the epiphyseal end of the future bones and an intermediate zone. The outer interzone undergoes endochondral ossification, forming the subchondral bone, whereas the intermediate interzone will form articular cartilage [45]. Details regarding the performed microarray experiment are described elsewhere [45]. Hind limbs from murine embryos of CD-1 IGS mice (n=3) recovered on gestational day 15.5 (E15.5) were isolated. Hind limbs were snap-frozen in liquid nitrogen, embedded in frozen section medium (Neg-50, ThermoFisher, Walldorf, Germany) and sectioned along the sagittal axis. Laser capture microdissection (PALM Microbeam system; Carl Zeiss Microscopy GmbH) was used to isolate femorotibial intermediate interzone (II), femorotibial outer interzone (OI), and femoral and tibial transient embryonic cartilage (EC). Three independent biological replicates were collected of II and OI, and two replicates of EC. Each replicate originated from 1 out of 3 individual embryos from different litters. Cells were lysed and total RNA was isolated using RNeasy Micro Kit following manufacturer's instructions (Qiagen). The integrity, purity and quantity of RNA were determined using Agilent Bioanalyzer 2100 (RNA 6000 Pico LabChip® kit; Agilent Technologies). Subsequently, RNA was amplified and labeled with fluorescent Cyanide 3-CTP (Cy3) using the Agilent Low Input Quick Amp Labelling kit (Agilent Technologies). Labeled cRNA was hybridized to Agilent Whole Mouse Genome Oligo Microarrays (Agilent Sureprint G3 mouse 8x60L Microarray; Agilent Technologies), according to the Agilent 60-mer microarray processing protocol. Subsequently, fluorescent signal intensities were detected using Agilent's Microarray Scanner System and processed using Agilent Feature Extraction Software. The microarray data have been validated by qPCR on amplified RNA [45].

2.2.4 Analysis of microarray datasets

Published microarray datasets of the murine DMM (destabilization of the medial meniscus) model for OA [37], 14 days-old mouse femoral growth plates [11] and

developing murine embryonic joints [45] were imported and processed using Genespring 13.1 Multi-Omic software (Agilent Technologies). After uploading experiments as single colour experiments, normalization using the 75th percentile shift was performed. Data separation was confirmed by principle component analyses plot analysis. Samples of the murine OA dataset were grouped into sham and DMM cohorts at 1, 2 and 6 weeks after surgery. Samples of the growth plate dataset were grouped into microdissected material originating from the PR, PH and H zone. The dataset originating from the developing joints of murine embryos at gestational day 15.5 (E15.5), just prior to cavitation, was clustered into laser dissected femorotibial material from the II, OI and EC. Moderated T-test and volcano plots were used to determine cut-off values for fold change and statistical significance (fold change ≥ 3 and $P \leq 0.01$).

2.2.5 Quantitative PCR

Amplified RNA (100 ng) was reverse transcribed with the Transcriptor high fidelity cDNA synthesis kit (Roche, 05081963001). Freeze dried cDNA (10 ng) from the murine OA cartilage experiment was reconstituted in 40 µl ddH_2O and 1 µl sample was used per reaction. Quantitative real-time PCR was performed on a C1000 Touch™ Thermal Cycler (Biorad, 184-1100) with Sybr Green master mix (Eurogentec, RT-SN2X-03+WOUN). Primer sequences (Aplied Biosystems) were: Gapdh (fw: 5'-AAGGGCATCTTGGGCTACAC-3'; RV: 5'-GGCATCGAAGGTGGAAGAGT-3'), Scara3 (fw: 5'-GCCTCCTCCTCTTGGTTGAC-3'; RV: 5'-TGGTCCAGCTTGCTGTTCAT-3'), Pmepa1 (fw: 5'-AGCTCCAGGCTGTGTAAAGG-3'; RV: 5'-ACGTAGGGTACAGGGTCACA-3') , Cdkn2b (fw: 5'-GTGGGTGCAGTCAGTACCTT-3'; RV: 5'-AACCACTTCAGTGCCTCTCA-3'), Nr2f2 (fw: 5'-GACCCTCAGCTTCCCTCTGT-3'; RV: 5'-CAGGTCAGATGCTGTGCTGTA-3'). Relative gene expression was calculated with Gapdh as reference gene using the $2^{-\Delta Ct}$ formula [47]. Based on the micro-array the direction of regulation of genes selected for qPCR validation was known. Therefore an unpaired one-tailed t-test was used to asses statistical significance (Graphpad Prism v5.01). A $P < 0.05$ was considered statistically significant.

3. RESULTS

3.1 TGFβ-related genes regulated in a murine OA model

We first determined which TGFβ-related genes were regulated in murine articular cartilage after DMM-induced OA compared to sham-operation at the same time point. From the 580 TGFβ-related genes, 106 genes were significantly regulated in DMM compared to sham at week 1, 2 and/or 6 (Fig. 1; Supplementary Table 2). The largest number of genes that were differentially expressed between DMM and

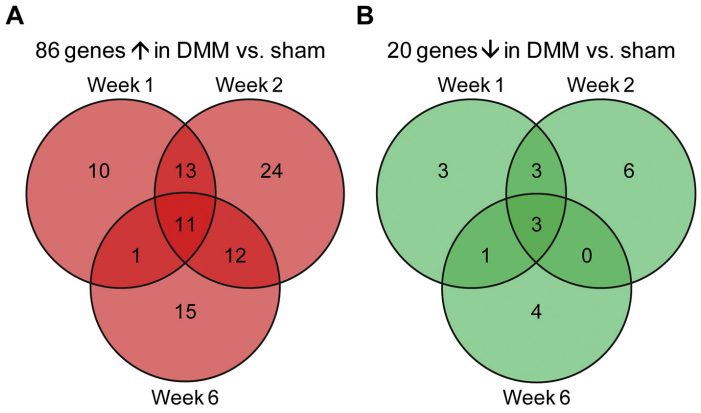

Figure 1. TGFβ-related genes regulated in a murine model for OA.

Microarray analysis was performed on cartilage of mice in which OA was induced by surgical destabilization of the medial meniscus (DMM) of one knee and sham operation of the other knee at 1, 2 and 6 weeks post-surgery. Expression of 580 TGFβ-related genes (Supplementary Table 1) was compared between DMM and sham by a moderated T-test (Supplementary Table 2). Venn diagrams illustrate overlap between the 106 unique genes that were upregulated **(A)** or downregulated **(B)** in DMM versus sham (fold change ≥ 3 and $P \leq 0.01$).

sham was at 2 weeks post-surgery (72 genes in total), where early focal degeneration of cartilage at the medial tibial plateau was observed on histology [37]. Of the 106 DMM-regulated TGFβ-related genes, 86 were upregulated (Fig. 1A) and 20 were downregulated (Fig. 1B) in murine OA cartilage at week 1, 2 and/or 6 post-surgery. Overlap between up or down-regulated genes revealed that 11 genes were up and 3 down regulated at all evaluated time points (Fig. 1).

3.2 TGFβ-related genes regulated in OA and chondrocyte hypertrophy during endochondral ossification

To identify genes implicated in hypertrophic differentiation of chondrocytes during early OA, we determined which of the TGFβ-related genes regulated in DMM-induced OA were regulated in the same direction (up/down) during endochondral ossification. Two independent microarray datasets on murine chondrocyte hypertrophy, mimicking early and late steps of endochondral ossification, were used.

Microarray data from the proliferative (PR), pre-hypertrophic (PH) and hypertrophic (H) zone of the growth plate [11] were filtered for the TGFβ-related genes identified in DMM-induced OA. We found that 25 of the 86 genes upregulated in damaged cartilage following DMM were more highly expressed in the hypertrophic zone compared to pre-hypertrophic or proliferative zones (Table 1). Furthermore, the expression of 5 out of 20 genes that were downregulated in the DMM model was also lower in the hypertrophic zone compared to prehypertrophic or proliferative zones (Table 1).

Table 1. Overlap of TGFβ-related gene regulation in murine OA and the growth plate.
Of the 106 TGFβ-related genes that were differentially expressed in murine OA, expression was evaluated in the hypertrophic (H) vs. pre-hypertrophic (PH) zone and hypertrophic (H) vs. proliferative (PR) zone of the growth plate. The table is separated in two parts for the direction of gene regulation (first genes higher in the hypertrophic zone, then lower). Multiple probe sets shown when applicable.

Gene symbol	Higher in H than in PH zone		Higher in H than in PR zone	
	Fold change	*P*-value	Fold change	*P*-value
Agpat9	–	–	7.56	1.93E-08
Agpat9	–	–	6.45	1.87E-06
Ank	9.77	9.20E-09	5.11	2.62E-07
Arhgap24	3.21	7.62E-04	–	–
Bmp6	–	–	7.28	1.98E-07
Cd44	6.77	2.12E-06	5.12	3.73E-06
Cd44	5.07	3.85E-08	4.61	1.13E-07
Cdkn2b	–	–	4.16	6.59E-07
Dcn Ddit4l	3.08	3.29E-03	–	–
	–	–	27.37	3.32E-08
Ddit4l	–	–	13.19	1.77E-07
Ddit4l	–	–	8.63	3.94E-07
Dnajb9	–	–	3.31	4.55E-07
Dnajb9	–	–	5.02	2.08E-07
Fn1	3.64	1.29E-06	17.61	1.49E-05
Inhba	8.09	7.48E-07	15.83	8.09E-07
Jag1	11.73	1.83E-04	–	–
Kitl	4.48	1.35E-04	10.74	2.53E-04
Map1b	–	–	4.29	5.04E-07
Nr2f2	3.19	3.78E-04	4.10	1.68E-07
Nr2f2	4.16	7.94E-06	–	–
Pcdh17	4.01	2.30E-06	5.43	2.37E-06
Pdgfra	7.64	4.74E-07	3.31	5.09E-06
Pmepa1	4.38	3.02E-07	–	–
Ptgs2	13.26	2.62E-09	49.11	1.58E-09
Ptgs2	22.82	4.94E-10	187.12	9.16E-08
Rgcc	–	–	7.53	2.97E-08
Serpine1	–	–	10.13	1.67E-04
Slit2	3.74	4.87E-04	9.27	8.91E-04
Slit2	3.89	5.04E-07	18.03	2.96E-08
Smad7	–	–	6.94	1.52E-07
Timp3	–	–	7.09	1.36E-06
Tnnt2	–	–	3.82	1.65E-03
Gene symbol	Lower in H than in PH zone		Lower in H than in PR zone	
	Fold change	*P*-value	Fold change	*P*-value
Hhip	–	–	5.95	2.50E-08
Myrip	–	–	10.95	4.88E-07
Ncapg	–	–	4.37	3.82E-06
Ogn	3.43	5.50E-06	18.38	1.06E-08
Thbs4	5.23	1.12E-08	3.95	8.78E-08

Analyses based on moderated *t*-test: Fold change ≥ 3 and $P \leq 0.01$.
– = not significantly regulated.

In addition to chondrocytes in the growth plate, chondrocytes in transient embryonic cartilage also undergo hypertrophic maturation [9-11]. In parallel with the growth plate dataset, we used a dataset on embryonic joint formation to determine which TGFβ-related genes identified in DMM-induced OA were also regulated in endochondral ossification during joint development. Jenner *et al.* have shown that genes relevant to chondrocyte hypertrophy are predominantly expressed in transient embryonic cartilage (EC), to a lesser extent in the outer interzone (OI) and lowest in the intermediate interzone (II) [45]. Therefore, we compared gene expression between EC and both interzone layers. Nine out of the 86 TGFβ-related genes that were upregulated in cartilage of DMM-operated mice were also upregulated in EC when compared to the two interzone layers (OI and II; Table 2). Of the 20 genes that were downregulated in DMM, 7 genes were downregulated in EC compared to OI and II (Table 2).

Table 2. Differential expression of TGFβ-related genes regulated in murine OA and joint development.

Of the 106 TGFβ-related genes that were differentially expressed in murine OA, expression was evaluated in transient embryonic cartilage (EC) vs. the outer interzone (OI) and transient embryonic cartilage (EC) vs. the in- termediate interzone (II). The table is separated in two parts for the direction of gene regulation (first genes higher in EC, then lower). Multiple probe sets shown when applicable.

Gene symbol	Higher in EC than in OI		Higher in EC than in II	
	Fold change	*P*-value	Fold change	*P*-value
6330415B21Rik	–	–	6.86	2.76E-04
Asb4	8.32	6.56E-04	9.01	7.42E-05
Bmp7	–	–	10.30	5.97E-03
Cdkn2b	–	–	3.32	4.66E-03
Dtna	–	–	3.83	3.23E-03
Gna14	–	–	7.52	7.55E-03
Ltbp1	–	–	3.22	7.46E-03
Papss2	3.96	3.84E-03	8.19	2.87E-05
Prkg2	6.58	2.49E-03	6.80	1.49E-04
Gene symbol	Lower in EC than in OI		Lower in EC than in II	
	Fold change	*P*-value	Fold change	*P*-value
Adamtsl2	5.06	6.00E-03	–	–
Ccnjl	–	–	7.43	1.30E-04
Gas6	–	–	4.87	7.24E-03
Hhip	3.92	9.55E-03	–	–
Hhip	11.52	1.94E-03	–	–
Scara3	3.97	5.17E-03	–	–
Thbs4	7.98	3.07E-04	23.66	6.33E-06
Wipf3	4.87	1.35E-03	–	–

Analyses based on moderated *t*-test: Fold change ≥ 3 and $P \leq 0.01$.
– = not significantly regulated.

Finally, the results from TGFβ-related genes identified in the DMM model and either the hypertrophic zone of the growth plate (Table 1) or in transient embryonic cartilage (Table 2) were compared. Sixty-three of the TGFβ-related genes were regulated in murine OA but not in any of the endochondral ossification datasets (Supplementary Figure 1 and Supplementary Table 3). Overall, 43 of 106 TGFβ-related genes were regulated in the same direction in DMM-induced OA as well as in endochondral ossification datasets. *Cdkn2b* overlapped between the 25 genes upregulated in the hypertrophic zone and the 9 genes upregulated in EC, resulting in a total of 33 genes upregulated in murine articular cartilage during OA and in endochondral ossification (Fig. 2). Between the 5 genes downregulated in the hypertrophic growth plate and 7 genes downregulated in EC, *Hhip* and *Thbs4* overlapped. Hence, a total of 10 different genes were downregulated in both the OA and the endochondral ossification datasets (Fig. 2).

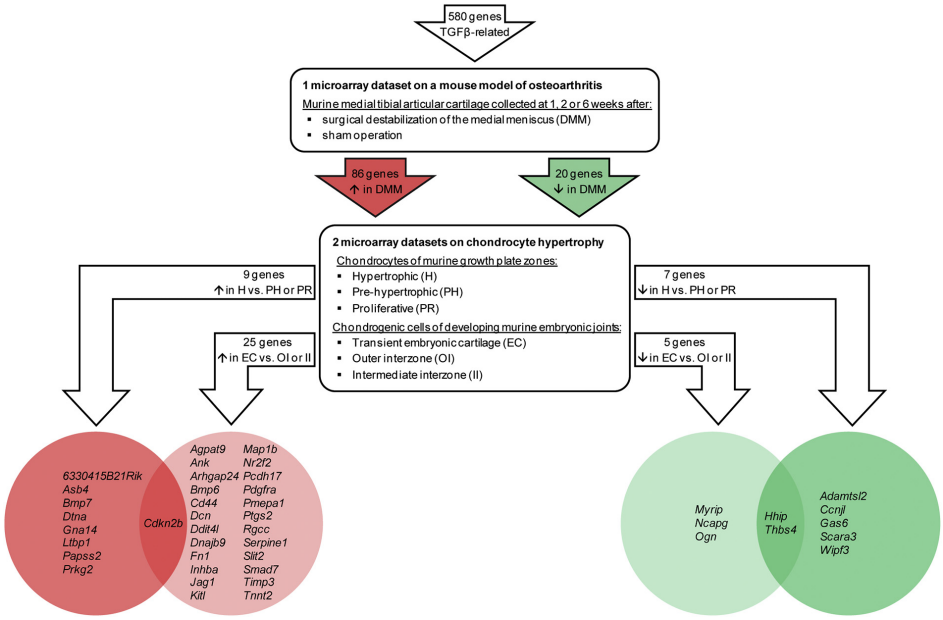

Figure 2. Overlap of TGFβ-related gene regulation in murine OA and endochondral ossification.

TGFβ-related genes regulated in the same direction during murine OA (DMM surgery) and chondrocyte hypertrophy in the growth plate (hypertrophic (H), pre-hypertrophic (PH) and proliferative (PR) zones) or in embryonic joint development (transient embryonic cartilage (EC), outer interzone (IO) and intermediate interzone (II) zones) were determined using published microarray data [11, 45]. Of the 580 TGFβ-related genes, a total of 106 genes were regulated in murine OA. When compared to endochondral ossification datasets, 33 genes were upregulated (left) and 10 genes were downregulated (right) in both murine OA and endochondral ossification datasets (fold change ≥ 3 and $P \leq 0.01$).

3.3 Identification of novel genes implicated in OA

Since 43 TGFβ-related genes were regulated in both DMM-induced OA and either of the endochondral ossification datasets, these genes might be novel candidates implicated in hypertrophy of chondrocytes in OA. To determine whether these genes have previously been associated with OA, a literature search was performed. We found that 18 of the 43 genes (Table 3) are known to be regulated in cartilage of animal models for OA (early-stage OA) or human OA patients (late-stage OA). This observation indicates that our TGFβ-focussed approach identified relevant genes. Because the remaining 25 genes (Table 4) have not been associated with OA, we considered these as novel candidates involved in early-onset of OA. An overview of the regulation of these 25 genes in the DMM model is presented in Supplementary Figure 2.

To obtain further evidence for the expression and regulation of these genes we performed a qPCR based validation on the original samples from the DMM experiment. Four genes (*Scara3*, *Pmepa1*, *Cdkn2b*, *Nr2f2*) were selected based on varying levels of expression in the micro-array (Log2 expression: *Scara3* ≥ 4, Pmepa1 ≥ 0, *Ckdn2b* ≤ 0, *Nr2f2* ≤ -2 a.u.) and up (*Pmepa1*, *Cdkn2b*, *Nf2f2*) or down regulation (*Scara3*) in the DMM samples compared to Sham. Validation measurements revealed a statistically significant 4 fold down regulation at week 1 and 2 for *Scara3*, and a significant up regulation of *Pmepa1* at week 2 (Figure 3). *Cdkn2b* and *Nr2f2* were lower expressed in the micro-array and this resulated in larger variation of replicates within groups in both the micro-arrays and qPCR measurements. Nevertheless a clear statistically significant induction of *Cdkn2b* and *Nr2f2* at week 6 in the DMM group was reproducible by qPCR. Overall, these data indicate that expression differences of genes expressed as little as -2 to -4 in arbitrary units of the micro-array are reliable and reproducible by qPCR.

Deficiency of genes may cause skeletal abnormalities or OA-like features as, for instance, observed in *Smad3* knockout mice [24, 25]. Therefore, we next investigated whether the 25 novel candidates have a potential role in development and/or maintenance of skeletal tissue. To investigate this, we used the *Mouse Genome Informatics* database to evaluate whether mice deficient for any of the 25 genes are known to have a skeletal phenotype [48, 49]. No data was available for 8 out of 25 genes, because no knockout mice have been generated and no skeletal phenotype was reported for knockout mice of 13 of the 25 genes (Table 4). In contrast, mice deficient for *Ltbp1*, *Nr2f2*, *Pdgfra* or *Prkg2* do show a skeletal phenotype (Table 4) [50-53]. This indicates that from the 25 novel candidates *Ltbp1*, *Nr2f2*, *Pdgfra* or *Prkg2* are involved in the development of skeletal tissue. In agreement, we found that these genes are upregulated in early OA and are associated with a hypertrophic chondrocyte phenotype. More specifically, *Nr2f2* and *Pdgfra* were up regulated in the hypertrophic zone of the growth plate (in comparison to both PZ and PR zone),

Table 3. TGFβ-related genes that have been implicated in OA. Overview of genes previously found to be regulated in articulate cartilage of human OA patients or animal models for OA.

Gene symbol	Gene name	Previously shown to be regulated in cartilage of animal model(s) for OA (early OA)	Previously shown to be regulated in human cartilage of patients with OA (late OA)
Adamtsl2	ADAMTS-like 2	–	Snelling et al. (2014)
Ankh	Progressive ankylosis	Du et al. (2016)	Hirose et al. (2002); Johnson, 2004; Sun et al. (2010a; 2010b); Wang et al. (2005)
Bmp6	Bone morphogenetic protein 6	–	Chou et al. (2013); Sanchez-Sabate et al. (2009)
Bmp7	Bone morphogenetic protein 7	–	Bhutia et al. (2014); Bobinac et al. (2008); Chubinskaya et al. (2000); Merrihew et al. (2003); Schmal et al. (2015)
Cd44	CD44 antigen	Rao et al. (2014); Tibesku et al. (2005)	Dunn et al. (2009); Fuchs et al. (2003); Ostergaard et al. (1997); Zhang et al. (2013)
Dcn	Decorin	Adams et al. (1995); Young et al. (2002; 2005)	Bock et al. (2001); Cs-Szabo et al. (1995); Dourado et al. (1996); Little et al. (1996); Liu et al. (2003); Masse et al. (1997); Melrose et al. (2008); Poole et al. (1996)
Fn1	Fibronectin 1	Burton-Wurster et al. (1985; 1986; 1988); Chang et al. (2017); Gardiner et al. (2015); Sandya et al. (2007); Wurster and Lust (1984); Zang et al. (1995)	Aigner et al. (2001); Carnemolla et al. (1984); Chevalier et al. (1992; 1996); Dunn et al. (2016); Gardiner et al. (2015); Homandberg et al. (1998); Jones et al. (1987); Lorenzo et al. (2004); Miller et al. (1984); Parker et al. (2002); Wright et al. (1996); Zack et al. (2006)
Hhip	Hedgehog interacting protein	Shuang et al. (2015)	–
Inhba	Inhibin beta A subunit	Wei et al. (2010)	Hopwood et al. (2007); Wei et al. (2010)
Jag1	Jagged 1	Gardiner et al. (2015); Hosaka et al. (2013)	Gardiner et al. (2015); Karlsson et al. (2008); Sassi et al. (2014)
Kitlg	KIT ligand	Appleton et al. (2007)	Ceponis et al. (1998)
Ogn	Osteoglycin	–	Chou et al. (2013); Juchtmans et al. (2015); Wang et al. (2016)
Papss2	3′-Phosphoadenosine 5′-Phosphosulfate synthase 2	Ford-Hutchinson et al. (2005)	Ikeda et al. (2001); Luo et al. (2014)
Ptgs2	Prostaglandin-endoperoxide synthase 2	Appleton et al. (2007); Dumond et al. (2004); Fukai et al. (2012); Le Graverand et al. (2001)	Amin et al. (1997); Casagrande et al. (2015); Fan et al. (2015); Fukai et al. (2012); Koki et al. (2002); Valdes et al. (2004; 2006; 2008)
Rgcc	Regulator of cell cycle	–	Tew et al. (2007)
Serpine1	Serpin family E member 1	Bao et al. (2009); Le Graverand et al. (2001)	Belcher et al. (1996); Cevidanes et al. (2014); Franses et al. (2010); Martel-Pelletier et al. (1991)
Smad7	SMAD family member 7	–	Kaiser et al. (2004);
Timp3	Tissue inhibitor of metalloproteinase 3	–	Casagrande et al. (2015); Franses et al. (2010); Gardiner et al. (2015); Kevorkian et al. (2004); Li et al. (2014); Morris et al. (2010); Sahebjam et al. (2007); Su et al. (2015)

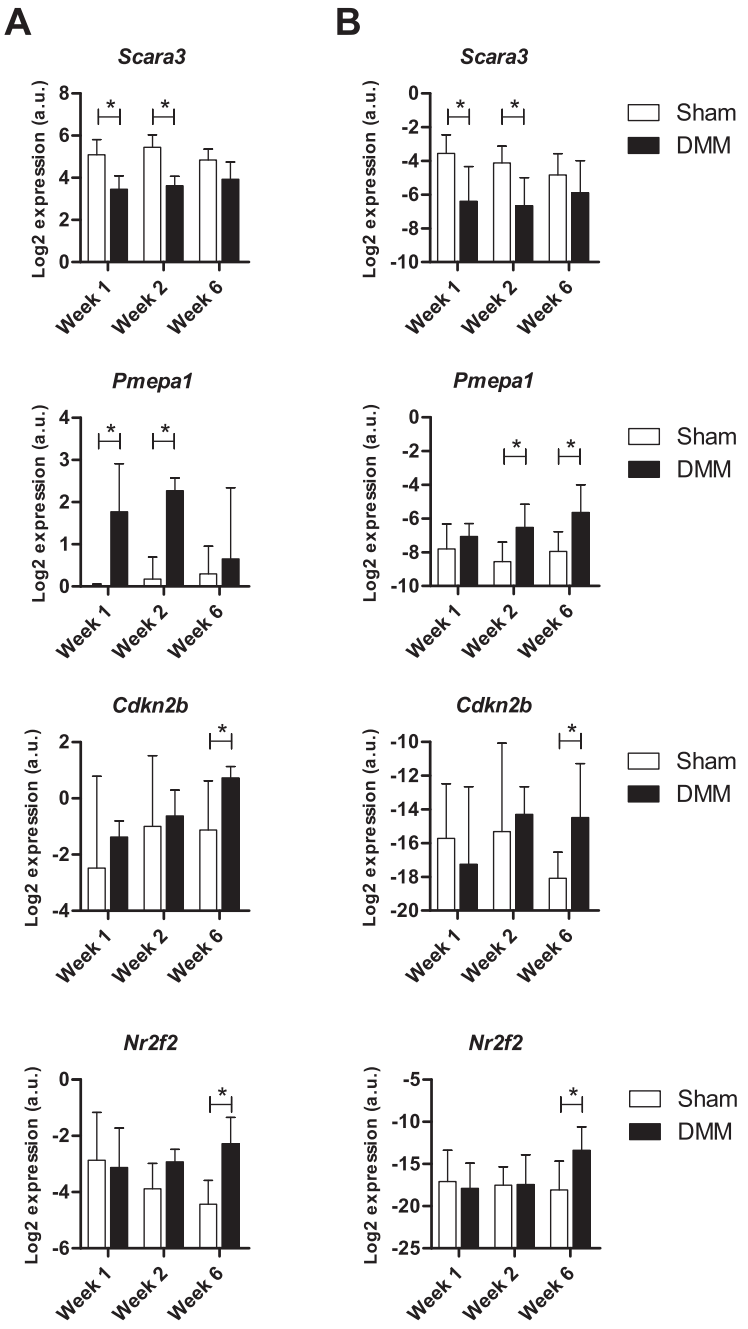

Figure 3. qPCR based validation of four novel candidate genes in murine OA.

Relative gene expression levels of *Scara3*, *Pmepa1*, *Cdkn2b* and *Nr2f2* in A) the micro-array and B) qPCR measurements (n =4 per time point, mean + SD). * = *P* value < 0.05.

Prkg2 was up regulated in embryonic cartilage (compared to either OI or II) and *Ltbp1* was up regulated in EC when compared to II.

To link the 25 genes to human OA, we analyzed their expression in the supplementary data of published human OA microarray studies. For this purpose studies

Table 4. Novel candidate genes associated with phenotypic changes of chondrocytes during osteoarthritis. Overview of novel candidate genes for phenotypical changes in chondrocytes leading to OA. Genes were evaluated for a skeletal phenotype in knock-out animals, based on information from the MGI database, and supplementary data of available human OA micro-array studies

Gene symbol	Gene name	Knockout mice have skeletal phenotype?	Regulated in human OA cartilage?
Ltbp1	Latent transforming growth factor beta binding protein 1	Yes [49]	Yes [53,58]
Nr2f2	Nuclear receptor subfamily 2 group F member 2	Yes [50]	Yes [56,57]
Pdgfra	Platelet derived growth factor receptor alpha	Yes [51]	Yes [59]
Prkg2	Protein kinase, cGMP-dependent, type II	Yes [52]	Yes [57]
Pmepa1	Prostate transmembrane protein, androgen induced 1	No data available	Yes [54–56]
Ddit4l	DNA damage inducible transcript 4 like	No data available	Yes [53,55]
Scara3	Scavenger receptor class A member 3	No data available	Yes [53,55]
Ncapg	Non-SMC condensin I complex subunit G	No data available	Yes [57]
Thbs4	Thrombospondin 4	No	Yes [54,57]
Map1b	Microtubule associated protein 1B	No	Yes [53,57]
Agpat9	Glycerol-3-phosphate acyltransferase 3	No	Yes [57]
Arhgap24	Rho GTPase activating protein 24	No	Yes [57]
Cdkn2b	Cyclin dependent kinase inhibitor 2B	No	Yes [57]
Dnajb9	DnaJ heat shock protein family (Hsp40) member B9	No	Yes [56]
Gas6	Growth arrest specific 6	No	Yes [56]
Ccnjl	Cyclin J like	No data available	No
Myrip	Myosin VIIA and Rab interacting protein	No data available	No
6330415B21Rik	–	No data available	No
Gna14	G protein subunit alpha 14	No data available	No
Asb4	Ankyrin repeat and SOCS box containing 4	No	No
Dtna	Dystrobrevin alpha	No	No
Pcdh17	Protocadherin 17	No	No
Slit2	Slit guidance ligand 2	No	No
Tnnt2	Troponin T2, cardiac type	No	No
Wipf3	WAS/WASL interacting protein family member 3	No	No

TGFβ-related genes that have not been previously implicated in OA. The 25 TGFβ-related genes that have not been previously associated with OA were further studied in literature to obtain indications for a potential role in skeletal tissue development and homeostasis. Moreover, it was evaluated whether these genes were cartilage using published microarray studies.

in which human cartilage from OA patients was compared to that of healthy individuals [54-57] and damaged to intact cartilage of OA joints were used [57-60]. We found that 15 of the 25 novel candidate genes were indeed significantly regulated in human OA cartilage (Table 4). Thus, besides regulation in early OA (DMM model, Supplementary Figure 2), these genes were regulated in late/end-stage OA (human OA). Although these 15 genes have been previously found to be regulated in human OA cartilage, they have not been highlighted for further investigation in OA development.

4. DISCUSSION

In this study we followed a new approach to identify novel candidate genes that are related to TGFβ and implicated in phenotypic changes of chondrocytes, in particular hypertrophic differentiation, during OA. It is currently not precisely known which aspects of TGFβ signaling are associated with chondrocyte homeostasis, hypertrophy or OA development and whether there is overlap. A second question was whether phenotypic changes of articular chondrocytes in OA show similarity to chondrocyte hypertrophy in normal developmental processes (transient growth plate cartilage, joint development). This study revealed 43 genes, of which 25 are novel candidates, that link alterations in TGFβ signaling with enhanced chondrocyte hypertrophy in osteoartritic cartilage.

The expression of genes encoding thrombospondin-4 (Thbs4), hedgehog-interacting protein (Hhip) and cyclin-dependent kinase inhibitor 2B (Cdkn2b) were regulated in all three microarray datasets, providing a strong indication that these proteins may be involved in both chondrocyte maturation and OA. In analogy to *Smad3* knockout mice, mice lacking *Thbs4*, *Hhip* or *Cdkn2b* have a normal skeleton and body size at birth [61-63]. This suggests that these proteins have a redundant role in embryonic joint development, but may still be involved in postnatal maintenance of chondrocyte phenotype. Thbs4 has been identified as a marker of articular cartilage [64]. Moreover, articular cartilage from knee joints of 26 week old *Thbs4* knockout mice is thinner than that of wild-type mice [63]. This indicates that Thbs4 has a protective function in articular cartilage, which is supported by our finding that *Thbs4* was downregulated in DMM-induced OA. Furthermore, Thbs4 has been demonstrated to interact with various cartilage matrix molecules to exert its protective function [65]. It would be interesting to determine the function of Thbs4 in OA models. Similarly, *Hhip* was downregulated in murine cartilage after DMM-induced OA and hypertrophic chondrocytes during endochondral ossification. Previously, Hhip has been shown to antagonize Indian Hedgehog (Ihh) signaling [61, 66]. Ihh signaling is known to induce chondrocyte maturation during endochondral ossification [67]. Therefore, suppression of Ihh signaling by Hhip might

be crucial to prevent chondrocyte hypertrophy. Contrary to our observation, *HHIP* expression is enhanced in human OA cartilage and positively correlates with the OARSI cartilage damage score [68, 69]. *Cdkn2b* was the only gene upregulated in cartilage of DMM-operated mice and in both chondrocyte hypertrophy datasets. It is known to be induced by TGFβ and can induce cell cycle arrest and senescence [70, 71]. A single nucleotide polymorphism in CDKN2A-CDKN2B was associated with type 2 diabetes [72]. Type 2 diabetes is thought to aggravate osteoarthritis [73]. Whether this gene is involved in OA development and/or chondrocyte maturation in human tissue requires further investigation.

Aside from *Cdkn2b* and *Thbs4*, we identified 23 other potential novel candidate genes involved in OA. As the interplay between musculoskeletal tissues has an important role in cartilage homeostasis [74], we evaluated whether deficiency of these genes results in a skeletal phenotype. To the best of our knowledge, no knockout mice have been generated for: *6330415B21Rik*, *Ccnjl*, *Ddit4l*, *Gna14*, *Myrip*, *Ncapg*, *Pmepa1* and *Scara3*. Although mice deficient for *Agpat9*, *Arhgap24*, *Asb4*, *Cdkn2b*, *Dnajb9*, *Dtna*, *Gas6*, *Map1b*, *Pcdh17*, *Slit2*, *Thbs4*, *Tnnt2* or *Wipf3* have been generated, there are no indications that these mice have a skeletal phenotype. Our results indicate that these genes may be important for early OA, which could be evaluated by application of experimentally-induced OA models in these knockout mice. According to literature, deletion of *Ltbp1*, *Nr2f2*, *Prkg2* or *Pdgfra* results in skeletal abnormalities [50-53]. In line with this, these four genes are involved in chondrogenesis and homeostasis of cartilage [75-81]. Conditional or tissue-specific knockout mice may be used to elucidate their role in OA. Although *Gas6*-knockout mice do not have a skeletal phenotype, *Gas6* is involved in survival of articular chondrocytes and regulation of the growth plate [82, 83]. Overall, these findings suggest that *Gas6*, *Ltbp1*, *Nr2f2*, *Prkg2* and *Pdgfra* play a direct role in skeletal development and cartilage homeostasis.

To validate the microarray we performed a qPCR based validation of 4 of the 25 novel identified candidate genes (*Scara3*, *Pmepa1*, *Cdkn2b*, *Nr2f2*), selected to be representative of high and low expressed genes that were either up or down regulated in the DMM model. The PCR could replicate the expression patterns found with microarray in cartilage samples from murine SHAM and DMM operated mice, increasing the validity of our approach to find new candidates. Only for *Pmepa1*, Sham vs DMM at week 1 resulted in a non-statistically significant difference in the qPCR. We think this could be related to the high standard variation combined with the very low expression level detected at week 1 in the micro-array, which is close to the detection limit. However, these conditions combined are not present in the other 25 candidate genes of interest. Overall, this confirmed the validity of our micro-array analysis.

A possible limitation of this study is that we have used the original samples from the micro-array rather then an independent experiment. However, from the 25 novel identified candidate genes, we found evidence for significant regulation of *LTBP1*, *NR2F2*, *PDGFRA*, *PRKG2*, *PMEPA1*, *DDIT4L*, *SCARA3*, *NCAPG*, *THBS4*, *MAP1B*, *AGPAT9*, *ARHGAP24*, *CDKN2B*, *DNAJB9* and *GAS6* in human OA cartilage in the supplementary data provided in previous publications [54-60]. This indicates that these genes are of particular interest for further study in OA pathophysiology. It would be of interest to determine whether manipulation of these genes in articular chondrocytes (*in vitro* and/or *in vivo*) leads to phenotypic changes (*i.e.* hypertrophy) as observed during development of OA. For *Pmepa1* and *Scara3* for example, it would be relevant to generate knock-out mice to establish their role in skeletal development and their function in OA models. Importantly, *Pmepa1* promotor activity is known to be regulated by TGFβ and WNT signalling [84]. TGFβ induced Pmepa1 expression was reported to inhibit R-SMAD signalling and promote non-canonical TGFβ signalling through PI3K/AKT [85]. In our study, *Pmepa1* was also induced in DMM vs Sham conditions. Of relevance, inflammatory signalling pathways can alter R-SMAD function through modulation of the SMAD2/3 linker region that requires non-canonical TGFβ signalling components [86]. Possibly, Pmepa1 also mediates such subtle alterations in TGFβ signalling through non-canonical signaling. *Nr2f2* was increased in DMM vs SHAM in our study and it is known to inhibit SMAD4 dependent transcription [87]. It has been shown that R-SMADs can differentially regulate genes in the absence of SMAD4 [88, 89]. *Scara3* regulation has a function in oxidative stress response and it is a putative tumour suppressor gene. It was shown to be hypermethylated at the DNA level in type II diabetes, which is associated with reduced expression [90]. Type II diabetes is a risk factor for osteoarthritis and in analogy we also found reduced expression in DMM vs SHAM conditions [91].

Of the 106 TGFβ-related genes that were significantly regulated in the DMM model, a higher number was regulated in a similar direction during chondrocyte hypertrophy in growth plate cartilage than in transient embryonic cartilage. This could indicate that the gene expression profile of OA chondrocytes is more similar to hypertrophy in the postnatal growth plate than in transient embryonic cartilage. There are indications that such a difference exists, for example: mice deficient for *Tgfbr2*, *Tgfbr1, Smad3* or *Smad4* exhibit normal joint development and the TGFβ signaling pathway is not enriched in pathway analyses of embryonic joint development [24, 25, 33-35, 45, 92-95]. These observations suggest that the TGFβ signaling pathway is not required for chondrocyte differentiation during embryonic articular cartilage development.

It occurred that most of the identified TGFβ-related genes (33 out of 43) were upregulated, rather than downreglulated, in OA. This might be explained by the

release of TGFβ from the cartilage matrix upon damage [14]. However, some of the TGFβ signaling pathway members, have been reported to be reduced in OA cartilage [23, 26, 96]. A possible explanation for discrepancies in the regulation of TGFβ-related genes could be that they are differentially regulated in late versus early stages of OA [37]. It is conceivable that genes regulated by TGFβ have a distinct role during different stages of OA, but this requires further investigation.

As the transcriptional program initiated by TGFβ is highly cell type and context dependent [97], we generated a comprehensive list of 580 TGFβ-related genes based on data of multiple types of cartilaginous cells stimulated with TGFβ *in vitro* and from cartilaginous tissue of Tgfbr2 knock-out mice (*in vivo*). It is possible that certain target genes were missed by this approach due to timing and concentration dependant differences between these studies. Visa versa it can not be excluded that the observed gene regulation in the DMM model is caused by other cytokines. Our selection criterium (fold change ≥3) for differentially expressed genes was rather strict. Therefore, we might have missed relevant genes that are less strongly regulated. Nevertheless, we found that 106 of these genes were regulated in murine OA (early OA) and we identified 43 candidate genes that are related to hypertrophy of which 18 genes have already been implicated in OA, indicating the validity of our approach. The 63 identified genes that were regulated in cartilage during OA but not during chondrocyte hypertrophy, might be worth further investigation as well since they might represent TGFβ-related genes involved in other processes important in OA development. In summary this new approach of combining different sets of big data, in this case microarray data, is a useful tool to find new potential targets in OA pathogenesis. This study supports the enduring policy to encourage re-use of existing data as well as combining big data. Moreover, this type of meta-analyses of animal experiments fits within the framework of reduction, refinement and replacement [98].

5. CONCLUSIONS

The TGFβ signalling pathway has been implicated in both articular cartilage maintenance and development of osteoarthritis. Although differences in receptor signalling and utilization of different SMAD transcription factors has been reported, it is unclear which downstream target genes mediate positive and negative aspects of TGFβ signalling. One characteristic of early OA that is closely linked to TGFβ signalling is chondrocyte hypertrophy. Chondrocyte hypertrophy is otherwise only observed during the physiological process of endochondral ossification. We hypothesized that defining the regulation of genes involved in, or regulated by, the TGFβ signaling pathway in early OA and endochondral ossification would result in the identification of novel targets implicated in phenotypic changes of chondrocytes in

OA pathogenesis. Using an approach of combining different microarray data sets, we identified 43 unique TGFβ-related genes that were significantly regulated in the same direction in a model for early OA and in one of two independent datasets representing endochondral ossification processes; three genes (*Cdkn2b* upregulated, *Hhip* and *Thbs4* downregulated) were even significantly regulated in the same direction in all three datasets. Both *Cdkn2b* and *Thbs4* have not been previously studied in OA and we found that both were significantly regulated in supplementary data of human OA transcriptome studies. Based on our hypothesis we expected to identify both known and unknown genes involved in OA pathogenesis. Review of the literature revealed that 18 of these 43 TGFβ-related genes (42%) were indeed reported to be involved in OA pathogenesis. The remaining 25 genes were considered new candidate genes potentially implicated in phenotypical changes in chondrocytes leading to OA. Of these 25 genes, knock-out mice were generated for *Ltbp1*, *Nr2f2*, *Pdgfra* or *Prkg2* that exhibit a skeletal phenotype, for 8 of these genes (*6330415B21Rik, Ccnjl, Ddit4l, Gna14, Myrip, Ncapg, Pmepa1, Scara3*) it is not clear whether knock-out leads to a skeletal phenotype, while the remaining 13 available gene knock-outs (*Agpat9, Arhgap24, Asb4, Cdkn2b, Dnajb9, Dtna, Gas6, Map1b, Pcdh17, Slit2, Thbs4, Tnnt2 and Wipf3*) do not show an overt skeletal phenotype. We obtained additional evidence for the relevance of 15 of these genes (*Agpat9, Arhgap24, Cdkn2b, Ddit4l, Gas6, Ltbp1, Map1b, Ncapg, Nr2f2, Pdgfra, Pmepa1, rkg2, Scara3, Thbs4*) since they were reported to be regulated in supplementary data of published human OA micro-array studies. We propose that these 15 candidates warrant further investigation in gain and loss of function models for OA, as they may represent important downstream effector proteins of altered TGFβ signalling in (early) phenotypic changes of articular chondrocytes in OA.

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COMPETING INTEREST

The authors have declared no conflicts of interest.

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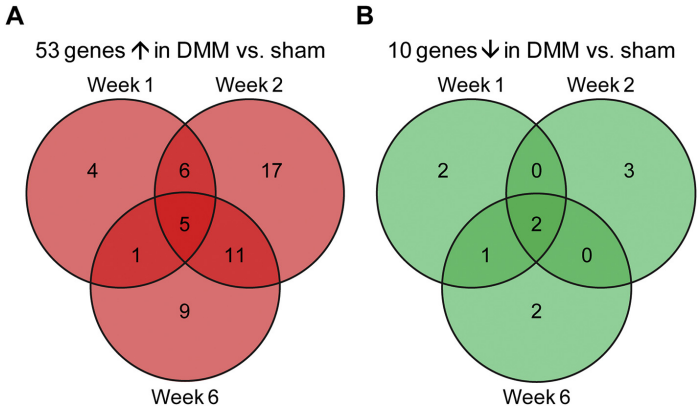
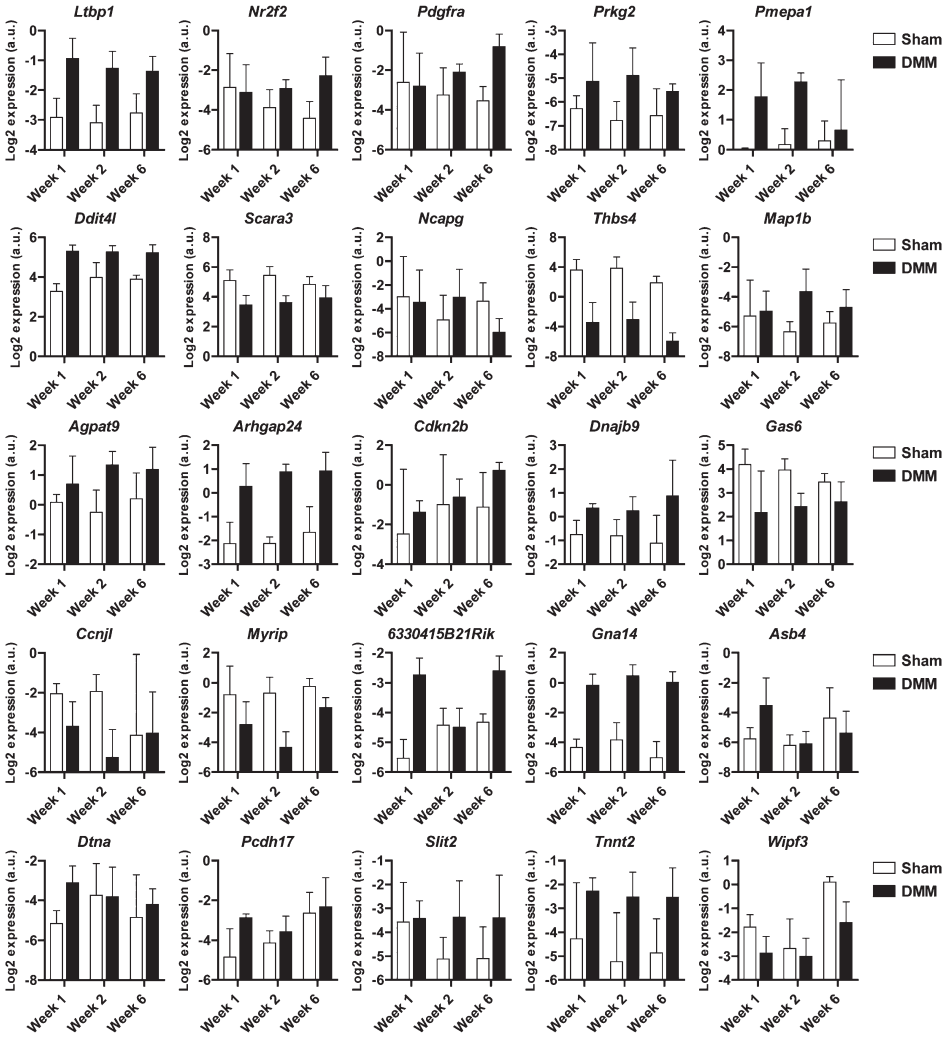
SUPPLEMENTAL MATERIAL



Supplementary Figure 1. Overview of TGFβ-related genes in murine OA that were not regulated in endochondral ossification.

TGFβ-related genes regulated during murine OA (DMM), but not in endochondral ossification in the growth plate or in embryonic joint development, were determined using published microarray data [11, 45]. Of the 106 TGFβ-related genes regulated in murine OA, a total of 63 genes (53 upregulated **(left)**, 10 downregulated **(left)**) were only regulated in murine OA and not in the endochondral ossification datasets (fold change ≥ 3 and $P \leq 0.01$).



Supplementary Figure 2. Regulation of the identified candidate genes in murine OA.

Relative gene expression levels of the 25 new candidate genes for OA were derived from the normalized microarray dataset [36]. Gene expression in murine articular cartilage following sham and DMM operation is shown at 1, 2 and 6 weeks post-surgery (n=4 mice per time point).

Supplementary Table 1. List of 580 TGFβ-related genes.

List of unique genes regulated by TGFβ and/or part of the KEGG pathway, generated as described in the Methods section.

Gene symbol	Gene name	Gene ID
1500015O10Rik	RIKEN cDNA 1500015O10 gene	78896
4930544G11Rik	RIKEN cDNA 4930544G11 gene	67653
5530601H04Rik	RIKEN cDNA 5530601H04 gene	71445
5730478J17Rik	RIKEN cDNA 5730478J17 gene	70580
5830408B19Rik	RIKEN cDNA 5830408B19 gene	74756
5930405F01Rik	RIKEN cDNA 5930405F01 gene	320550
6330415B21Rik	RIKEN cDNA 6330415B21 gene	70753
9330177L23Rik	RIKEN cDNA 9330177L23 gene	77246
9430085L16Rik	RIKEN cDNA 9430085L16 gene	77375
9530059O14Rik	RIKEN cDNA 9530059O14 gene	319626
9630021D06Rik	RIKEN cDNA 9630021D06 gene	319926
9630023C09Rik	RIKEN cDNA 9630023C09 gene	320378
Aard	alanine and arginine rich domain containing protein	239435
Abca9	ATP-binding cassette, sub-family A (ABC1), member 9	217262
Abi3bp	ABI gene family, member 3 (NESH) binding protein	320712
Acox2	acyl-Coenzyme A oxidase 2, branched chain	93732
Actb	actin, beta	11461
Actr2	ARP2 actin-related protein 2	66713
Acvr1	activin A receptor, type 1	11477
Acvr1b	activin A receptor, type 1B	11479
Acvr1c	activin A receptor, type IC	269275
Acvr2a	activin receptor IIA	11480
Acvr2b	activin receptor IIB	11481
Adam12	a disintegrin and metallopeptidase domain 12 (meltrin alpha)	11489
Adamts1	a disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 1	11504
Adamts20	a disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 20	223838
Adamts6	a disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 6	108154
Adamtsl2	ADAMTS-like 2	77794
Adarb1	adenosine deaminase, RNA-specific, B1	110532
Adgrb3	adhesion G protein-coupled receptor B3	210933
Adh5	alcohol dehydrogenase 5 (class III), chi polypeptide	11532
Adm	adrenomedullin	11535
Adra2a	adrenergic receptor, alpha 2a	11551
Adss	adenylosuccinate synthetase, non muscle	11566
Agpat9	1-acylglycerol-3-phosphate O-acyltransferase 9	231510
Agtr1a	angiotensin II receptor, type 1a	11607
Agtr2	angiotensin II receptor, type 2	11609
AI448005	expressed sequence AI448005	98965
AI504432	expressed sequence AI504432	229694

Gene symbol	Gene name	Gene ID
Ak1	adenylate kinase 1	11636
Ak5	adenylate kinase 5	229949
Ak6	adenylate kinase 6	102216272
Alcam	activated leukocyte cell adhesion molecule	11658
Aldh1a1	aldehyde dehydrogenase family 1, subfamily A1	11668
Alg5	asparagine-linked glycosylation 5 (dolichyl-phosphate beta-glucosyltransferase)	66248
Amh	anti-Mullerian hormone	11705
Amhr2	anti-Mullerian hormone type 2 receptor	110542
Ampd3	adenosine monophosphate deaminase 3	11717
Angptl4	angiopoietin-like 4	57875
Ank	progressive ankylosis	11732
Anp32a	acidic (leucine-rich) nuclear phosphoprotein 32 family, member A	11737
Anxa11	annexin A11	11744
Anxa8	annexin A8	11752
Apc	adenomatosis polyposis coli	11789
Aplnr	apelin receptor	23796
Apod	apolipoprotein D	11815
Arap2	ArfGAP with RhoGAP domain, ankyrin repeat and PH domain 2	212285
Arhgap20	Rho GTPase activating protein 20	244867
Arhgap24	Rho GTPase activating protein 24	231532
Arhgef5	Rho guanine nucleotide exchange factor (GEF) 5	54324
Arsi	arylsulfatase i	545260
Asb4	ankyrin repeat and SOCS box-containing 4	65255
Aspn	asporin	66695
Ass1	argininosuccinate synthetase 1	11898
Asxl3	additional sex combs like 3 (Drosophila)	211961
AU015680	expressed sequence AU015680	552875
Avpr1a	arginine vasopressin receptor 1A	54140
Axin2	axin 2	12006
B3galnt2	UDP-GalNAc:betaGlcNAc beta 1,3-galactosaminyltransferase, polypeptide 2	97884
B3galt2	UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase, polypeptide 2	26878
Bach1	BTB and CNC homology 1, basic leucine zipper transcription factor 1	12013
Bag6	BCL2-associated athanogene 6	224727
Bambi	BMP and activin membrane-bound inhibitor	68010
Barx1	BarH-like homeobox 1	12022
Bhlhe40	basic helix-loop-helix family, member e40	20893
Bmp2	bone morphogenetic protein 2	12156
Bmp4	bone morphogenetic protein 4	12159
Bmp5	bone morphogenetic protein 5	12160
Bmp6	bone morphogenetic protein 6	12161
Bmp7	bone morphogenetic protein 7	12162
Bmp8a	bone morphogenetic protein 8a	12163
Bmp8b	bone morphogenetic protein 8b	12164
Bmper	BMP-binding endothelial regulator	73230
Bmpr1a	bone morphogenetic protein receptor, type 1A	12166
Bmpr1b	bone morphogenetic protein receptor, type 1B	12167

Gene symbol	Gene name	Gene ID
Bmpr2	bone morphogenetic protein receptor, type II (serine/threonine kinase)	12168
C030026M15Rik	RIKEN cDNA C030026M15 gene	77378
C1qtnf3	C1q and tumor necrosis factor related protein 3	81799
C4a	complement component 4A (Rodgers blood group)	625018
C4b	complement component 4B (Chido blood group)	12268
Cadps2	Ca2+-dependent activator protein for secretion 2	320405
Calml3	calmodulin-like 3	70405
Camk2n1	calcium/calmodulin-dependent protein kinase II inhibitor 1	66259
Car5b	carbonic anhydrase 5b, mitochondrial	56078
Cav1	caveolin 1, caveolae protein	12389
Cav2	caveolin 2	12390
Cbx6	chromobox 6	494448
Ccdc142	coiled-coil domain containing 142	243510
Ccdc28b	coiled coil domain containing 28B	66264
Ccdc58	coiled-coil domain containing 58	381045
Ccl21a	chemokine (C-C motif) ligand 21A (serine)	18829
Ccl21b	chemokine (C-C motif) ligand 21B (leucine)	100042493
Ccl21c	chemokine (C-C motif) ligand 21C (leucine)	65956
Ccnjl	cyclin J-like	380694
Cd40	CD40 antigen	21939
Cd44	CD44 antigen	12505
Cd59b	CD59b antigen	333883
Cda	cytidine deaminase	72269
Cdc42ep3	CDC42 effector protein (Rho GTPase binding) 3	260409
Cdca5	cell division cycle associated 5	67849
Cdh10	cadherin 10	320873
Cdh2	cadherin 2	12558
Cdkn2b	cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4)	12579
Cgref1	cell growth regulator with EF hand domain 1	68567
Chac1	ChaC, cation transport regulator 1	69065
Chad	chondroadherin	12643
Chdh	choline dehydrogenase	218865
Chek1	checkpoint kinase 1	12649
Chrd	chordin	12667
Cilp	cartilage intermediate layer protein, nucleotide pyrophosphohydrolase	214425
Cldn1	claudin 1	12737
Clu	clusterin	12759
Cmklr1	chemokine-like receptor 1	14747
Cntn3	contactin 3	18488
Col14a1	collagen, type XIV, alpha 1	12818
Col6a1	collagen, type VI, alpha 1	12833
Col8a2	collagen, type VIII, alpha 2	329941
Cotl1	coactosin-like 1 (Dictyostelium)	72042
Cplx2	complexin 2	12890
Crebbp	CREB binding protein	12914
Csrp2	cysteine and glycine-rich protein 2	13008

Gene symbol	Gene name	Gene ID
Ctdspl	CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) small phosphatase-like	69274
Ctgf	connective tissue growth factor	14219
Ctsw	cathepsin W	13041
Cul1	cullin 1	26965
Cux2	cut-like homeobox 2	13048
Cxcl5	chemokine (C-X-C motif) ligand 5	20311
D18Ertd232e	DNA segment, Chr 18, ERATO Doi 232, expressed	52492
D430019H16Rik	RIKEN cDNA D430019H16 gene	268595
D5Ertd505e	DNA segment, Chr 5, ERATO Doi 505, expressed	52485
Dab1	disabled 1	13131
Dancr	differentiation antagonizing non-protein coding RNA	70036
Dcn	decorin	13179
Dctpp1	dCTP pyrophosphatase 1	66422
Ddah1	dimethylarginine dimethylaminohydrolase 1	69219
Ddit4	DNA-damage-inducible transcript 4	74747
Ddit4l	DNA-damage-inducible transcript 4-like	73284
Ddx18	DEAD (Asp-Glu-Ala-Asp) box polypeptide 18	66942
Ddx3y	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, Y-linked	26900
Ddx6	DEAD (Asp-Glu-Ala-Asp) box polypeptide 6	13209
Dio2	deiodinase, iodothyronine, type II	13371
Dkk2	dickkopf WNT signaling pathway inhibitor 2	56811
Dnajb9	DnaJ heat shock protein family (Hsp40) member B9	27362
Dock4	dedicator of cytokinesis 4	238130
Dok1	docking protein 1	13448
Dpysl2	dihydropyrimidinase-like 2	12934
Dtna	dystrobrevin alpha	13527
Dubr	Dppa2 upstream binding RNA	68190
Dusp26	dual specificity phosphatase 26 (putative)	66959
Dyrk1b	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1b	13549
E2f4	E2F transcription factor 4	104394
E2f5	E2F transcription factor 5	13559
Ebf1	early B cell factor 1	13591
Ebf3	early B cell factor 3	13593
Edil3	EGF-like repeats and discoidin I-like domains 3	13612
Efemp1	epidermal growth factor-containing fibulin-like extracellular matrix protein 1	216616
Eif2s3y	eukaryotic translation initiation factor 2, subunit 3, structural gene Y-linked	26908
Emp3	epithelial membrane protein 3	13732
Enc1	ectodermal-neural cortex 1	13803
Endod1	endonuclease domain containing 1	71946
Enpep	glutamyl aminopeptidase	13809
Enpp1	ectonucleotide pyrophosphatase/phosphodiesterase 1	18605
Enpp2	ectonucleotide pyrophosphatase/phosphodiesterase 2	18606
Ep300	E1A binding protein p300	328572
Epas1	endothelial PAS domain protein 1	13819
Erg	avian erythroblastosis virus E-26 (v-ets) oncogene related	13876

Gene symbol	Gene name	Gene ID
Esrrg	estrogen-related receptor gamma	26381
F3	coagulation factor III	14066
Fam134b	family with sequence similarity 134, member B	66270
Fam13c	family with sequence similarity 13, member C	71721
Fam199x	family with sequence similarity 199, X-linked	245622
Fam32a	family with sequence similarity 32, member A	67922
Fat3	FAT atypical cadherin 3	270120
Fat4	FAT atypical cadherin 4	329628
Fbln5	fibulin 5	23876
Fbp1	fructose bisphosphatase 1	14121
Fcf1	FCF1 rRNA processing protein	73736
Fgf18	fibroblast growth factor 18	14172
Fgf21	fibroblast growth factor 21	56636
Fgfr1op2	FGFR1 oncogene partner 2	67529
Fgfr2	fibroblast growth factor receptor 2	14183
Fgfr3	fibroblast growth factor receptor 3	14184
Fjx1	four jointed box 1 (Drosophila)	14221
Flnb	filamin, beta	286940
Flt4	FMS-like tyrosine kinase 4	14257
Fmod	fibromodulin	14264
Fn1	fibronectin 1	14268
Fndc3a	fibronectin type III domain containing 3A	319448
Foxp1	forkhead box P1	108655
Foxq1	forkhead box Q1	15220
Frmd5	FERM domain containing 5	228564
Frmd6	FERM domain containing 6	319710
Fst	follistatin	14313
Fstl3	follistatin-like 3	83554
G0s2	G0/G1 switch gene 2	14373
Gabrg3	gamma-aminobutyric acid (GABA) A receptor, subunit gamma 3	14407
Gadd45b	growth arrest and DNA-damage-inducible 45 beta	17873
Gadd45g	growth arrest and DNA-damage-inducible 45 gamma	23882
Gal	galanin	14419
Galc	galactosylceramidase	14420
Gas6	growth arrest specific 6	14456
Gdf10	growth differentiation factor 10	14560
Gdf5	growth differentiation factor 5	14563
Gdf6	growth differentiation factor 6	242316
Gdf7	growth differentiation factor 7	238057
Gfpt2	glutamine fructose-6-phosphate transaminase 2	14584
Ggps1	geranylgeranyl diphosphate synthase 1	14593
Gjb3	gap junction protein, beta 3	14620
Gm10591	predicted gene 10591	100504239
Gm13304	predicted gene 13304	100504346
Gm13552	predicted gene 13552	100038999
Gm15421	predicted gene 15421	100042049

Gene symbol	Gene name	Gene ID
Gm1987	predicted gene 1987	100504362
Gm21541	predicted gene, 21541	100862177
Gm21949	predicted gene, 21949	100505386
Gm2260	predicted gene 2260	100039484
Gm2274	predicted gene 2274	100039503
Gm5424	predicted gene 5424	432466
Gm9840	predicted gene 9840	100043674
Gna14	guanine nucleotide binding protein, alpha 14	14675
Gng2	guanine nucleotide binding protein (G protein), gamma 2	14702
Gpc2	glypican 2 (cerebroglycan)	71951
Gprc5b	G protein-coupled receptor, family C, group 5, member B	64297
Gprin2	G protein regulated inducer of neurite outgrowth 2	432839
Gpsm2	G-protein signalling modulator 2 (AGS3-like, C. elegans)	76123
Grb10	growth factor receptor bound protein 10	14783
Grid2	glutamate receptor, ionotropic, delta 2	14804
Grin2c	glutamate receptor, ionotropic, NMDA2C (epsilon 3)	14813
Gsg1l	GSG1-like	269994
Gt(ROSA)26Sor	gene trap ROSA 26, Philippe Soriano	14910
Hacd3	3-hydroxyacyl-CoA dehydratase 3	57874
Hand2	heart and neural crest derivatives expressed transcript 2	15111
Has2	hyaluronan synthase 2	15117
Hes1	hairy and enhancer of split 1 (Drosophila)	15205
Hhip	Hedgehog-interacting protein	15245
Higd1c	HIG1 domain family, member 1C	380975
Hmgcr	3-hydroxy-3-methylglutaryl-Coenzyme A reductase	15357
Hook1	hook microtubule tethering protein 1	77963
Hoxa10	homeobox A10	15395
Hoxa9	homeobox A9	15405
Hoxc10	homeobox C10	209448
Hoxd10	homeobox D10	15430
Hoxd3os1	homeobox D3, opposite strand 1	73429
Hoxd9	homeobox D9	15438
Hpgd	hydroxyprostaglandin dehydrogenase 15 (NAD)	15446
Hsd11b2	hydroxysteroid 11-beta dehydrogenase 2	15484
Hsd17b11	hydroxysteroid (17-beta) dehydrogenase 11	114664
Hspb2	heat shock protein 2	69253
Hspb8	heat shock protein 8	80888
Htra1	HtrA serine peptidase 1	56213
Htra3	HtrA serine peptidase 3	78558
Ibsp	integrin binding sialoprotein	15891
Id1	inhibitor of DNA binding 1	15901
Id2	inhibitor of DNA binding 2	15902
Id3	inhibitor of DNA binding 3	15903
Id4	inhibitor of DNA binding 4	15904
Ifng	interferon gamma	15978
Il1rap	interleukin 1 receptor accessory protein	16180

Gene symbol	Gene name	Gene ID
Inhba	inhibin beta-A	16323
Inhbb	inhibin beta-B	16324
Inhbc	inhibin beta-C	16325
Inhbe	inhibin beta-E	16326
Itgbl1	integrin, beta-like 1	223272
Itih5	inter-alpha (globulin) inhibitor H5	209378
Jag1	jagged 1	16449
Kank4	KN motif and ankyrin repeat domains 4	242553
Kcnd3	potassium voltage-gated channel, Shal-related family, member 3	56543
Kcnk1	potassium channel, subfamily K, member 1	16525
Kcnk6	potassium inwardly-rectifying channel, subfamily K, member 6	52150
Kcnmb4	potassium large conductance calcium-activated channel, subfamily M, beta member 4	58802
Kdm5d	lysine (K)-specific demethylase 5D	20592
Kif11	kinesin family member 11	16551
Kif5c	kinesin family member 5C	16574
Kitl	kit ligand	17311
Krt19	keratin 19	16669
Lamp2	lysosomal-associated membrane protein 2	16784
Lcat	lecithin cholesterol acyltransferase	16816
Ldb2	LIM domain binding 2	16826
Ldlrad4	low density lipoprotein receptor class A domain containing 4	52662
Lefty1	left right determination factor 1	13590
Lefty2	left-right determination factor 2	320202
Limk2	LIM motif-containing protein kinase 2	16886
LOC100041504		100041504
LOC100041593		100041593
LOC552873		552873
LOC552911		552911
Lox	lysyl oxidase	16948
Lpl	lipoprotein lipase	16956
Lrp1b	low density lipoprotein-related protein 1B (deleted in tumors)	94217
Lrp8	low density lipoprotein receptor-related protein 8, apolipoprotein e receptor	16975
Lrr1	leucine rich repeat protein 1	69706
Lrrtm1	leucine rich repeat transmembrane neuronal 1	74342
Lsamp	limbic system-associated membrane protein	268890
Ltbp1	latent transforming growth factor beta binding protein 1	268977
Ltbp2	latent transforming growth factor beta binding protein 2	16997
Lypd6	LY6/PLAUR domain containing 6	320343
Maf	avian musculoaponeurotic fibrosarcoma oncogene homolog	17132
Mafb	v-maf musculoaponeurotic fibrosarcoma oncogene family, protein B (avian)	16658
Map1b	microtubule-associated protein 1B	17755
Map2k6	mitogen-activated protein kinase kinase 6	26399
Map3k5	mitogen-activated protein kinase kinase kinase 5	26408
Mapk1	mitogen-activated protein kinase 1	26413
Mapk3	mitogen-activated protein kinase 3	26417

Gene symbol	Gene name	Gene ID
Matn2	matrilin 2	17181
Mbnl3	muscleblind-like 3 (Drosophila)	171170
Mbp	myelin basic protein	17196
Mcm5	minichromosome maintenance complex component 5	17218
Methig1	methyltransferase hypoxia inducible domain containing 1	554292
Mettl7a1	methyltransferase like 7A1	70152
Mettl7a2	methyltransferase like 7A2	393082
Mettl7a3	methyltransferase like 7A3	668178
Mfsd2a	major facilitator superfamily domain containing 2A	76574
Mgll	monoglyceride lipase	23945
Mgp	matrix Gla protein	17313
Mgst1	microsomal glutathione S-transferase 1	56615
Mir22hg	Mir22 host gene (non-protein coding)	100042498
Mkx	mohawk homeobox	210719
Mlph	melanophilin	171531
Mrpl50	mitochondrial ribosomal protein L50	28028
Mrpl53	mitochondrial ribosomal protein L53	68499
Mrps18b	mitochondrial ribosomal protein S18B	66973
Mtus1	mitochondrial tumor suppressor 1	102103
Myc	myelocytomatosis oncogene	17869
Myo18a	myosin XVIIIA	360013
Myo1d	myosin ID	338367
Myocd	myocardin	214384
Myrip	myosin VIIA and Rab interacting protein	245049
Nbl1	neuroblastoma, suppression of tumorigenicity 1	17965
Ncam1	neural cell adhesion molecule 1	17967
Ncapg	non-SMC condensin I complex, subunit G	54392
Ndnf	neuron-derived neurotrophic factor	68169
Ndp	Norrie disease (pseudoglioma) (human)	17986
Nedd4l	neural precursor cell expressed, developmentally down-regulated gene 4-like	83814
Nedd9	neural precursor cell expressed, developmentally down-regulated gene 9	18003
Nefl	neurofilament, light polypeptide	18039
Nfatc1	nuclear factor of activated T cells, cytoplasmic, calcineurin dependent 1	18018
Nfatc2	nuclear factor of activated T cells, cytoplasmic, calcineurin dependent 2	18019
Ngf	nerve growth factor	18049
Nkd2	naked cuticle 2 homolog (Drosophila)	72293
Nme1	NME/NM23 nucleoside diphosphate kinase 1	18102
Nodal	nodal	18119
Nog	noggin	18121
Nov	nephroblastoma overexpressed gene	18133
Nptx1	neuronal pentraxin 1	18164
Nr2f2	nuclear receptor subfamily 2, group F, member 2	11819
Nrip3	nuclear receptor interacting protein 3	78593
Nrp1	neuropilin 1	18186
Nrp2	neuropilin 2	18187
Nrxn1	neurexin I	18189

Gene symbol	Gene name	Gene ID
Nsg2	neuron specific gene family member 2	18197
Nt5e	5' nucleotidase, ecto	23959
Ntf5	neurotrophin 5	78405
Ntm	neurotrimin	235106
Nuak1	NUAK family, SNF1-like kinase, 1	77976
Nucks1	nuclear casein kinase and cyclin-dependent kinase substrate 1	98415
Nupr1	nuclear protein transcription regulator 1	56312
Ogn	osteoglycin	18295
Olfm2	olfactomedin 2	244723
Orc5	origin recognition complex, subunit 5	26429
Osr1	odd-skipped related 1 (Drosophila)	23967
Otor	otoraplin	57329
Palld	palladin, cytoskeletal associated protein	72333
Palm2	paralemmin 2	242481
Pamr1	peptidase domain containing associated with muscle regeneration 1	210622
Papss2	3'-phosphoadenosine 5'-phosphosulfate synthase 2	23972
Pcdh17	protocadherin 17	219228
Pcdh9	protocadherin 9	211712
Pcgf5	polycomb group ring finger 5	76073
Pclo	piccolo (presynaptic cytomatrix protein)	26875
Pcnp	PEST proteolytic signal containing nuclear protein	76302
Pcp4	Purkinje cell protein 4	18546
Pcsk5	proprotein convertase subtilisin/kexin type 5	18552
Pde7a	phosphodiesterase 7A	18583
Pde7b	phosphodiesterase 7B	29863
Pdgfra	platelet derived growth factor receptor, alpha polypeptide	18595
Pdgfrl	platelet-derived growth factor receptor-like	68797
Pdia6	protein disulfide isomerase associated 6	71853
Pdk3	pyruvate dehydrogenase kinase, isoenzyme 3	236900
Pdxdc1	pyridoxal-dependent decarboxylase domain containing 1	94184
Penk	preproenkephalin	18619
Phex	phosphate regulating endopeptidase homolog, X-linked	18675
Pik3r1	phosphatidylinositol 3-kinase, regulatory subunit, polypeptide 1 (p85 alpha)	18708
Pim1	proviral integration site 1	18712
Pitpnb	phosphatidylinositol transfer protein, beta	56305
Pitx2	paired-like homeodomain transcription factor 2	18741
Pkp1	plakophilin 1	18772
Plek2	pleckstrin 2	27260
Plekha4	pleckstrin homology domain containing, family A (phosphoinositide binding specific) member 4	69217
Plod2	procollagen lysine, 2-oxoglutarate 5-dioxygenase 2	26432
Plscr2	phospholipid scramblase 2	18828
Plxnc1	plexin C1	54712
Plxnd1	plexin D1	67784
Pmaip1	phorbol-12-myristate-13-acetate-induced protein 1	58801
Pmepa1	prostate transmembrane protein, androgen induced 1	65112

Gene symbol	Gene name	Gene ID
Polr3k	polymerase (RNA) III (DNA directed) polypeptide K	67005
Postn	periostin, osteoblast specific factor	50706
Ppa1	pyrophosphatase (inorganic) 1	67895
Ppp1r3b	protein phosphatase 1, regulatory (inhibitor) subunit 3B	244416
Ppp2ca	protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoform	19052
Ppp2cb	protein phosphatase 2 (formerly 2A), catalytic subunit, beta isoform	19053
Ppp2r1a	protein phosphatase 2, regulatory subunit A, alpha	51792
Ppp2r1b	protein phosphatase 2, regulatory subunit A, beta	73699
Prg4	proteoglycan 4 (megakaryocyte stimulating factor, articular superficial zone protein)	96875
Prkcdbp	protein kinase C, delta binding protein	109042
Prkg2	protein kinase, cGMP-dependent, type II	19092
Prm1	protamine 1	19118
Prom1	prominin 1	19126
Prpf39	pre-mRNA processing factor 39	328110
Prps1	phosphoribosyl pyrophosphate synthetase 1	19139
Prps1l3	phosphoribosyl pyrophosphate synthetase 1-like 3	328099
Prss12	protease, serine 12 neurotrypsin (motopsin)	19142
Prss35	protease, serine 35	244954
Psap	prosaposin	19156
Psmd10	proteasome (prosome, macropain) 26S subunit, non-ATPase, 10	53380
Pstpip2	proline-serine-threonine phosphatase-interacting protein 2	19201
Ptgs2	prostaglandin-endoperoxide synthase 2	19225
Pth1r	parathyroid hormone 1 receptor	19228
Pthlh	parathyroid hormone-like peptide	19227
Ptprd	protein tyrosine phosphatase, receptor type, D	19266
Ptprr	protein tyrosine phosphatase, receptor type, R	19279
Pxylp1	2-phosphoxylose phosphatase 1	235534
Pygl	liver glycogen phosphorylase	110095
Rab10	RAB10, member RAS oncogene family	19325
Rasal2	RAS protein activator like 2	226525
Rasl11b	RAS-like, family 11, member B	68939
Rbl1	retinoblastoma-like 1 (p107)	19650
Rbx1	ring-box 1	56438
Reps2	RALBP1 associated Eps domain containing protein 2	194590
Rgcc	regulator of cell cycle	66214
Rgs10	regulator of G-protein signalling 10	67865
Rgs2	regulator of G-protein signaling 2	19735
Rhoa	ras homolog family member A	11848
Rhob	ras homolog family member B	11852
Rnf144b	ring finger protein 144B	218215
Rnf19b	ring finger protein 19B	75234
Rock1	Rho-associated coiled-coil containing protein kinase 1	19877
Rpl22l1	ribosomal protein L22 like 1	68028
Rps6ka3	ribosomal protein S6 kinase polypeptide 3	110651
Rps6kb1	ribosomal protein S6 kinase, polypeptide 1	72508

Gene symbol	Gene name	Gene ID
Rps6kb2	ribosomal protein S6 kinase, polypeptide 2	58988
Rspo2	R-spondin 2	239405
Runx1	runt related transcription factor 1	12394
Runx1t1	runt-related transcription factor 1; translocated to, 1 (cyclin D-related)	12395
Runx2	runt related transcription factor 2	12393
Ryr3	ryanodine receptor 3	20192
S100a6	S100 calcium binding protein A6 (calcyclin)	20200
Saa1	serum amyloid A 1	20208
Sar1b	secretion associated Ras related GTPase 1B	66397
Scara3	scavenger receptor class A, member 3	219151
Scara5	scavenger receptor class A, member 5	71145
Scd1	stearoyl-Coenzyme A desaturase 1	20249
Schip1	schwannomin interacting protein 1	30953
Scx	scleraxis	20289
Sdpr	serum deprivation response	20324
Sdr39u1	short chain dehydrogenase/reductase family 39U, member 1	654795
Sec22b	SEC22 homolog B, vesicle trafficking protein	20333
Selenbp1	selenium binding protein 1	20341
Serpine1	serine (or cysteine) peptidase inhibitor, clade E, member 1	18787
Sesn3	sestrin 3	75747
Sfrp2	secreted frizzled-related protein 2	20319
Sfxn3	sideroflexin 3	94280
Shisa3	shisa family member 3	330096
Skap2	src family associated phosphoprotein 2	54353
Skp1a	S-phase kinase-associated protein 1A	21402
Slc17a1	solute carrier family 17 (sodium phosphate), member 1	20504
Slc20a1	solute carrier family 20, member 1	20515
Slc29a1	solute carrier family 29 (nucleoside transporters), member 1	63959
Slc4a7	solute carrier family 4, sodium bicarbonate cotransporter, member 7	218756
Slco3a1	solute carrier organic anion transporter family, member 3a1	108116
Slit2	slit homolog 2 (Drosophila)	20563
Slitrk1	SLIT and NTRK-like family, member 1	76965
Smad1	SMAD family member 1	17125
Smad2	SMAD family member 2	17126
Smad3	SMAD family member 3	17127
Smad4	SMAD family member 4	17128
Smad5	SMAD family member 5	17129
Smad6	SMAD family member 6	17130
Smad7	SMAD family member 7	17131
Smad9	SMAD family member 9	55994
Smarca4	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4	20586
Smoc2	SPARC related modular calcium binding 2	64074
Smurf1	SMAD specific E3 ubiquitin protein ligase 1	75788
Smurf2	SMAD specific E3 ubiquitin protein ligase 2	66313
Snai2	snail family zinc finger 2	20583

Gene symbol	Gene name	Gene ID
Sned1	sushi, nidogen and EGF-like domains 1	208777
Sox11	SRY (sex determining region Y)-box 11	20666
Sox4	SRY (sex determining region Y)-box 4	20677
Sp1	trans-acting transcription factor 1	20683
Spp1	secreted phosphoprotein 1	20750
Spsb1	splA/ryanodine receptor domain and SOCS box containing 1	74646
Srl	sarcalumenin	106393
Ssr3	signal sequence receptor, gamma	67437
St8sia1	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 1	20449
St8sia4	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 4	20452
Stc2	stanniocalcin 2	20856
Steap3	STEAP family member 3	68428
Syt1	synaptotagmin I	20979
Taf9	TATA-box binding protein associated factor 9	108143
Tasp1	taspase, threonine aspartase 1	75812
Tbx1	T-box 1	21380
Tenm2	teneurin transmembrane protein 2	23964
Tesc	tescalcin	57816
Tet1	tet methylcytosine dioxygenase 1	52463
Tfdp1	transcription factor Dp 1	21781
Tfrc	transferrin receptor	22042
Tgfb1	transforming growth factor, beta 1	21803
Tgfb2	transforming growth factor, beta 2	21808
Tgfb3	transforming growth factor, beta 3	21809
Tgfbi	transforming growth factor, beta induced	21810
Tgfbr1	transforming growth factor, beta receptor I	21812
Tgfbr2	transforming growth factor, beta receptor II	21813
Tgfbr3	transforming growth factor, beta receptor III	21814
Tgif1	TGFB-induced factor homeobox 1	21815
Tgif2	TGFB-induced factor homeobox 2	228839
Thbs1	thrombospondin 1	21825
Thbs4	thrombospondin 4	21828
Thsd7a	thrombospondin, type I, domain containing 7A	330267
Timp3	tissue inhibitor of metalloproteinase 3	21859
Tle2	transducin-like enhancer of split 2	21886
Tle3	transducin-like enhancer of split 3	21887
Tmeff1	transmembrane protein with EGF-like and two follistatin-like domains 1	230157
Tmem2	transmembrane protein 2	83921
Tmem204	transmembrane protein 204	407831
Tmem26	transmembrane protein 26	327766
Tmem27	transmembrane protein 27	57394
Tmem30a	transmembrane protein 30A	69981
Tnf	tumor necrosis factor	21926
Tnfaip6	tumor necrosis factor alpha induced protein 6	21930
Tnfrsf11b	tumor necrosis factor receptor superfamily, member 11b (osteoprotegerin)	18383
Tnk2	tyrosine kinase, non-receptor, 2	51789

Gene symbol	Gene name	Gene ID
Tnnt2	troponin T2, cardiac	21956
Tns2	tensin 2	209039
Tomm70a	translocase of outer mitochondrial membrane 70 homolog A (yeast)	28185
Tpr	translocated promoter region, nuclear basket protein	108989
Trim47	tripartite motif-containing 47	217333
Trpc3	transient receptor potential cation channel, subfamily C, member 3	22065
Trps1	trichorhinophalangeal syndrome I (human)	83925
Tspan12	tetraspanin 12	269831
Tspan2	tetraspanin 2	70747
Ttc30b	tetratricopeptide repeat domain 30B	72421
Ttyh1	tweety family member 1	57776
Tulp1	tubby like protein 1	22157
Ucma	upper zone of growth plate and cartilage matrix associated	68527
Ugcg	UDP-glucose ceramide glucosyltransferase	22234
Ugdh	UDP-glucose dehydrogenase	22235
Unc5d	unc-5 netrin receptor D	210801
Upf1	UPF1 regulator of nonsense transcripts homolog (yeast)	19704
Utp18	UTP18 small subunit processome component	217109
Uty	ubiquitously transcribed tetratricopeptide repeat gene, Y chromosome	22290
Vamp7	vesicle-associated membrane protein 7	20955
Vegfa	vascular endothelial growth factor A	22339
Vegfc	vascular endothelial growth factor C	22341
Vstm2a	V-set and transmembrane domain containing 2A	211739
Wif1	Wnt inhibitory factor 1	24117
Wipf3	WAS/WASL interacting protein family, member 3	330319
Wisp1	WNT1 inducible signaling pathway protein 1	22402
Wls	wntless homolog (Drosophila)	68151
Wnt5a	wingless-type MMTV integration site family, member 5A	22418
Xiap	X-linked inhibitor of apoptosis	11798
Xist	inactive X specific transcripts	213742
Yipf5	Yip1 domain family, member 5	67180
Zfa-ps	zinc finger protein, autosomal, pseudogene	22639
Zfhx3	zinc finger homeobox 3	11906
Zfp185	zinc finger protein 185	22673
Zfp800	zinc finger protein 800	627049
Zfp811	zinc finger protein 811	240063
Zfx	zinc finger protein X-linked	22764
Zfyve16	zinc finger, FYVE domain containing 16	218441
Zfyve9	zinc finger, FYVE domain containing 9	230597
Zic1	zinc finger protein of the cerebellum 1	22771

Supplementary Table 2. One hundred and six TGFβ-related genes regulated in murine OA.

List of differentially regulated TGFβ-related genes in DMM operated compared to SHAM operated mice.

Week 1 Gene Symbol	Fold change (abs)	Regulation (DMM vs. sham)	*P*-value
6330415B21Rik	6.740	up	6.65E-03
Ank	5.835	up	7.46E-06
Arhgap24	5.275	up	2.45E-04
Asb4	4.776	up	6.28E-03
Bmp2	7.795	up	3.10E-03
Bmp6	3.871	up	9.38E-03
Bmp7	5.545	up	2.06E-04
Cd44	3.557	up	7.15E-03
Cda	3.358	up	1.72E-03
Col6a1	3.052	up	7.21E-04
Dcn	15.311	up	8.30E-03
Ddit4l	4.082	up	1.36E-05
Dtna	4.092	up	2.81E-04
Enpep	11.362	up	5.56E-03
F3	13.534	up	8.58E-08
Fn1	9.771	up	3.49E-05
Gna14	18.098	up	8.86E-08
Gt(ROSA)26Sor	3.472	up	1.69E-03
Hspb8	3.468	up	4.70E-03
Inhba	11.650	up	1.93E-05
Ltbp1	3.952	up	1.35E-04
Ltbp2	4.995	up	2.22E-04
Nbl1	3.621	up	8.07E-04
Nefl	4.402	up	4.24E-04
Ngf	5.696	up	5.09E-04
Nov	5.620	up	6.23E-06
Nt5e	15.131	up	9.37E-05
Papss2	4.089	up	5.21E-03
Pcdh17	3.965	up	2.03E-03
Penk	7.451	up	2.10E-04
Pmepa1	3.388	up	1.34E-03
Ptgs2	53.168	up	3.13E-06
Rgcc	3.071	up	8.70E-04
Sar1b	3.572	up	6.31E-04
Timp3	4.315	up	2.64E-05
Tnfrsf11b	6.628	up	4.10E-04
Adamtsl2	8.499	down	5.28E-04
Agtr1a	3.617	down	1.19E-03
Ccnjl	3.120	down	4.82E-03
Col8a2	5.208	down	2.01E-04
Gas6	4.033	down	7.81E-03

Week 1 Gene Symbol	Fold change (abs)	Regulation (DMM vs. sham)	*P*-value
Hhip	9.704	down	9.32E-04
Mgll	3.554	down	1.20E-04
Ndnf	4.543	down	5.88E-03
Scara3	3.108	down	9.35E-04
Selenbp1	5.704	down	1.29E-04
Week 2 **Gene Symbol**	**Fold change (abs)**	**Regulation** **(DMM vs. sham)**	***P*-value**
Acvr1	3.015	up	5.87E-05
Adamts6	3.028	up	8.74E-04
Agpat9	3.029	up	7.04E-05
Agtr2	3.688	up	6.44E-03
Ank	8.336	up	1.06E-07
Arhgap24	8.050	up	3.31E-11
Bmp2	9.044	up	3.20E-04
Bmp6	5.891	up	9.94E-05
Bmp7	6.863	up	2.79E-06
Cd44	5.009	up	1.44E-06
Cilp	4.251	up	2.57E-03
Col14a1	4.239	up	3.89E-03
Dcn	58.593	up	4.40E-05
Dpysl2	3.401	up	3.84E-08
Efemp1	8.485	up	4.38E-06
Enpep	12.170	up	4.06E-06
F3	8.186	up	2.20E-03
Fcf1	3.167	up	1.07E-04
Fn1	6.073	up	2.90E-08
Gdf6	6.175	up	6.44E-05
Gjb3	26.314	up	3.65E-08
Gna14	19.907	up	4.59E-08
Hoxd10	3.011	up	2.54E-03
Hspb8	3.180	up	3.63E-05
Htra1	4.459	up	1.72E-05
Id2	4.061	up	6.73E-08
Inhba	15.564	up	5.08E-06
Jag1	4.501	up	4.31E-05
Kitl	3.367	up	3.19E-03
Ltbp1	3.566	up	1.25E-05
Ltbp2	8.714	up	2.09E-07
Map1b	6.478	up	1.25E-04
Nbl1	4.872	up	4.37E-06
Nedd4l	4.955	up	1.06E-03
Nedd9	3.226	up	6.44E-04
Nfatc1	3.651	up	1.18E-04

Week 2 **Gene Symbol**	**Fold change (abs)**	**Regulation (DMM vs. sham)**	***P*-value**
Ngf	4.944	up	8.36E-05
Nov	13.164	up	2.75E-06
Nt5e	17.635	up	1.63E-08
Pcsk5	5.079	up	1.76E-05
Penk	19.513	up	8.16E-08
Pmepa1	4.285	up	1.16E-07
Ppp2cb	4.537	up	1.05E-04
Prkg2	3.700	up	1.06E-03
Ptgs2	50.611	up	7.73E-11
Rgcc	5.544	up	1.36E-08
Rps6ka3	3.950	up	8.33E-04
Scd1	5.096	up	1.09E-07
Serpine1	4.069	up	5.22E-03
Slit2	3.379	up	5.53E-03
Smad7	6.654	up	3.03E-06
Smurf2	4.703	up	3.26E-06
Tgfbi	8.747	up	8.39E-09
Tgif1	3.744	up	1.40E-04
Timp3	3.239	up	7.91E-05
Tmem204	3.748	up	1.49E-06
Tnfaip6	12.220	up	1.26E-05
Tnfrsf11b	6.333	up	2.18E-06
Tnnt2	6.566	up	1.52E-03
Tspan2	3.865	up	1.62E-04
Adamtsl2	5.274	down	3.46E-04
Ccnjl	10.018	down	8.21E-06
Col8a2	7.032	down	7.78E-09
Hhip	10.383	down	7.53E-04
Ibsp	9.333	down	8.29E-08
Map2k6	3.012	down	4.66E-05
Myrip	12.545	down	1.68E-06
Ogn	3.762	down	4.61E-04
Scara3	3.515	down	6.48E-06
Selenbp1	10.249	down	2.76E-08
Thbs4	3.174	down	2.94E-03
Wif1	3.586	down	4.74E-06
Week 6 **Gene Symbol**	**Fold change (abs)**	**Regulation (DMM vs. sham)**	***P*-value**
Acvr2a	4.477	up	1.03E-04
Adss	3.422	up	3.38E-03
Agtr2	5.179	up	6.59E-03
Ank	5.615	up	1.99E-04
Arhgap24	5.961	up	1.57E-05

Week 6 Gene Symbol	Fold change (abs)	Regulation (DMM vs. sham)	*P*-value
Bhlhe40	3.389	up	7.28E-05
Bmpr1b	4.309	up	2.62E-03
Cd44	3.641	up	2.11E-04
Cdkn2b	3.599	up	4.16E-03
Ddah1	3.103	up	3.95E-05
Dnajb9	3.942	up	3.80E-03
Dpysl2	3.250	up	7.82E-04
Enpep	6.167	up	1.97E-03
Gdf6	21.734	up	4.19E-07
Gjb3	15.345	up	7.54E-04
Gna14	33.466	up	7.56E-09
Gprc5b	3.371	up	6.99E-05
Hoxd10	3.035	up	7.69E-03
Inhba	5.547	up	1.40E-04
Jag1	5.027	up	3.91E-04
Kif5c	7.399	up	1.04E-06
Nedd9	3.286	up	1.41E-03
Ngf	3.082	up	5.20E-04
Nov	10.575	up	9.41E-05
Nr2f2	4.450	up	7.97E-05
Nrip3	3.189	up	2.42E-04
Nt5e	6.519	up	3.46E-05
Pdgfra	6.624	up	4.48E-07
Pkp1	5.452	up	2.79E-05
Ppa1	3.022	up	2.19E-03
Ptgs2	124.771	up	2.84E-12
Rgcc	3.055	up	9.92E-05
Sar1b	3.134	up	3.54E-04
Scd1	4.356	up	9.22E-06
Tgfbi	4.401	up	4.14E-05
Tmem27	3.482	up	3.52E-03
Tnfaip6	3.349	up	1.51E-03
Tnfrsf11b	3.137	up	5.92E-03
Tnnt2	5.009	up	1.63E-03
Adamtsl2	5.282	down	2.27E-05
Amhr2	3.663	down	9.45E-05
Chac1	5.335	down	4.17E-04
Col8a2	3.779	down	4.48E-04
Mgll	3.447	down	5.15E-05
Ncapg	6.095	down	5.21E-04
Selenbp1	5.224	down	1.26E-05
Wipf3	3.229	down	6.67E-05

Supplementary Table 3. Sixty-three TGFβ-related genes regulated in murine OA but not in any of the endochondral ossification datasets.

List of differentially regulated TGFβ-related genes in DMM operated compared to SHAM operated mice, which were not differentially expressed in the growth plate or embryonic joint development dataset.

Week 1 **Gene Symbol**	**Fold change (abs)**	**Regulation (DMM vs. sham)**	***P*-value**
Nt5e	15.131	up	9.37E-05
F3	13.534	up	8.58E-08
Enpep	11.362	up	5.56E-03
Bmp2	7.795	up	3.10E-03
Penk	7.451	up	2.10E-04
Tnfrsf11b	6.628	up	4.10E-04
Ngf	5.696	up	5.09E-04
Nov	5.620	up	6.23E-06
Ltbp2	4.995	up	2.22E-04
Nefl	4.402	up	4.24E-04
Nbl1	3.621	up	8.07E-04
Sar1b	3.572	up	6.31E-04
Gt(ROSA)26Sor	3.472	up	1.69E-03
Hspb8	3.468	up	4.70E-03
Cda	3.358	up	1.72E-03
Col6a1	3.052	up	7.21E-04
Mgll	-3.554	down	1.20E-04
Agtr1a	-3.617	down	1.19E-03
Ndnf	-4.543	down	5.88E-03
Col8a2	-5.208	down	2.01E-04
Selenbp1	-5.704	down	1.29E-04
Week 2 **Gene Symbol**	**Fold change (abs)**	**Regulation (DMM vs. sham)**	***P*-value**
Gjb3	26.314	up	3.65E-08
Penk	19.513	up	8.16E-08
Nt5e	17.635	up	1.63E-08
Nov	13.164	up	2.75E-06
Tnfaip6	12.220	up	1.26E-05
Enpep	12.170	up	4.06E-06
Bmp2	9.044	up	3.20E-04
Tgfbi	8.747	up	8.39E-09
Ltbp2	8.714	up	2.09E-07
Efemp1	8.485	up	4.38E-06
F3	8.186	up	2.20E-03
Tnfrsf11b	6.333	up	2.18E-06
Gdf6	6.175	up	6.44E-05
Scd1	5.096	up	1.09E-07
Pcsk5	5.079	up	1.76E-05
Nedd4l	4.955	up	1.06E-03

Week 2 Gene Symbol	Fold change (abs)	Regulation (DMM vs. sham)	*P*-value
Ngf	4.944	up	8.36E-05
Nbl1	4.872	up	4.37E-06
Smurf2	4.703	up	3.26E-06
Ppp2cb	4.537	up	1.05E-04
Htra1	4.459	up	1.72E-05
Cilp	4.251	up	2.57E-03
Col14a1	4.239	up	3.89E-03
Id2	4.061	up	6.73E-08
Rps6ka3	3.950	up	8.33E-04
Tspan2	3.865	up	1.62E-04
Tmem204	3.748	up	1.49E-06
Tgif1	3.744	up	1.40E-04
Agtr2	3.688	up	6.44E-03
Nfatc1	3.651	up	1.18E-04
Dpysl2	3.401	up	3.84E-08
Nedd9	3.226	up	6.44E-04
Hspb8	3.180	up	3.63E-05
Fcf1	3.167	up	1.07E-04
Adamts6	3.028	up	8.74E-04
Acvr1	3.015	up	5.87E-05
Hoxd10	3.011	up	2.54E-03
Map2k6	-3.012	down	4.66E-05
Wif1	-3.586	down	4.74E-06
Col8a2	-7.032	down	7.78E-09
Ibsp	-9.333	down	8.29E-08
Selenbp1	-10.249	down	2.76E-08
Week 6 **Gene Symbol**	**Fold change (abs)**	**Regulation (DMM vs. sham)**	***P*-value**
Gdf6	21.734	up	4.19E-07
Gjb3	15.345	up	7.54E-04
Nov	10.575	up	9.41E-05
Kif5c	7.399	up	1.04E-06
Nt5e	6.519	up	3.46E-05
Enpep	6.167	up	1.97E-03
Pkp1	5.452	up	2.79E-05
Agtr2	5.179	up	6.59E-03
Acvr2a	4.477	up	1.03E-04
Tgfbi	4.401	up	4.14E-05
Scd1	4.356	up	9.22E-06
Bmpr1b	4.309	up	2.62E-03
Tmem27	3.482	up	3.52E-03
Adss	3.422	up	3.38E-03
Bhlhe40	3.389	up	7.28E-05

Week 6 Gene Symbol	Fold change (abs)	Regulation (DMM vs. sham)	*P*-value
Gprc5b	3.371	up	6.99E-05
Tnfaip6	3.349	up	1.51E-03
Nedd9	3.286	up	1.41E-03
Dpysl2	3.250	up	7.82E-04
Nrip3	3.189	up	2.42E-04
Tnfrsf11b	3.137	up	5.93E-03
Sar1b	3.134	up	3.54E-04
Ddah1	3.103	up	3.95E-05
Ngf	3.082	up	5.20E-04
Hoxd10	3.035	up	7.69E-03
Ppa1	3.022	up	2.19E-03
Mgll	-3.447	down	5.15E-05
Amhr2	-3.663	down	9.45E-05
Col8a2	-3.779	down	4.48E-04
Selenbp1	-5.224	down	1.26E-05
Chac1	-5.335	down	4.17E-04

Supplementary Table 4. Details of the references listed in Table 3.

Gene name and corresponding references used for generation of Table 3.

Gene symbol	References
Adamtsl2	Snelling S, Rout R, Davidson R, Clark I, Carr A, Hulley PA, et al. A gene expression study of normal and damaged cartilage in anteromedial gonarthrosis, a phenotype of osteoarthritis. Osteoarthritis Cartilage 2014; 22: 334-343.
Ankh	Du G, Zhan H, Ding D, Wang S, Wei X, Wei F, et al. Abnormal Mechanical Loading Induces Cartilage Degeneration by Accelerating Meniscus Hypertrophy and Mineralization After ACL Injuries In Vivo. Am J Sports Med 2016; 44: 652-663. Hirose J, Ryan LM, Masuda I. Up-regulated expression of cartilage intermediate-layer protein and ANK in articular hyaline cartilage from patients with calcium pyrophosphate dihydrate crystal deposition disease. Arthritis Rheum 2002; 46: 3218-3229. Johnson K, Terkeltaub R. Upregulated ank expression in osteoarthritis can promote both chondrocyte MMP-13 expression and calcification via chondrocyte extracellular PPi excess. Osteoarthritis and Cartilage 2004; 12: 321-335. Sun Y, Mauerhan DR, Honeycutt PR, Kneisl JS, Norton HJ, Zinchenko N, et al. Calcium deposition in osteoarthritic meniscus and meniscal cell culture. Arthritis Res Ther 2010; 12: R56. Sun Y, Mauerhan DR, Honeycutt PR, Kneisl JS, Norton JH, Hanley EN, Jr., et al. Analysis of meniscal degeneration and meniscal gene expression. BMC Musculoskelet Disord 2010; 11: 19. Wang W, Xu J, Du B, Kirsch T. Role of the progressive ankylosis gene (ank) in cartilage mineralization. Mol Cell Biol 2005; 25: 312-323.
Bmp6	Chou CH, Lee CH, Lu LS, Song IW, Chuang HP, Kuo SY, et al. Direct assessment of articular cartilage and underlying subchondral bone reveals a progressive gene expression change in human osteoarthritic knees. Osteoarthritis Cartilage 2013; 21: 450-461. Sanchez-Sabate E, Alvarez L, Gil-Garay E, Munuera L, Vilaboa N. Identification of differentially expressed genes in trabecular bone from the iliac crest of osteoarthritic patients. Osteoarthritis Cartilage 2009; 17: 1106-1114.
Bmp7	Bhutia SC, Singh TA, Sherpa ML. Production of a polyclonal antibody against osteogenic protein-1, and its role in the diagnosis of osteoarthritis. Singapore Med J 2014; 55: 388-391.

Gene symbol	References
	Bobinac D, Spanjol J, Marinovic M, Zoricic Cvek S, Maric I, Cicvaric T, et al. Expression of bone morphogenetic proteins, cartilage-derived morphogenetic proteins and related receptors in normal and osteoarthritic human articular cartilage. Coll Antropol 2008; 32 Suppl 2: 83-87. Chubinskaya S, Merrihew C, Cs-Szabo G, Mollenhauer J, McCartney J, Rueger DC, et al. Human articular chondrocytes express osteogenic protein-1. J Histochem Cytochem 2000; 48: 239-250. Merrihew C, Kumar B, Heretis K, Rueger DC, Kuettner KE, Chubinskaya S. Alterations in endogenous osteogenic protein-1 with degeneration of human articular cartilage. J Orthop Res 2003; 21: 899-907. Schmal H, Henkelmann R, Mehlhorn AT, Reising K, Bode G, Sudkamp N, et al. Synovial cytokine expression in ankle osteoarthritis depends on age and stage. Knee Surgery Sports Traumatology Arthroscopy 2015; 23: 1359-1367.
Cd44	Dunn S, Kolomytkin OV, Waddell DD, Marino AA. Hyaluronan-binding receptors: possible involvement in osteoarthritis. Modern Rheumatology 2009; 19: 151-155. Fuchs S, Dankbar B, Wildenau G, Goetz W, Lohmann CH, Tibesku CO. Expression of the CD44 variant isoform 5 in the human osteoarthritic knee joint: correlation with radiological, histomorphological, and biochemical parameters. Journal of Orthopaedic Research 2004; 22: 774-780. Ostergaard K, Salter DM, Andersen CB, Petersen J, Bendtzen K. CD44 expression is up-regulated in the deep zone of osteoarthritic cartilage from human femoral heads. Histopathology 1997; 31: 451-459. Rao ZT, Wang SQ, Wang JQ. Exploring the osteoarthritis-related genes by gene expression analysis. European Review for Medical and Pharmacological Sciences 2014; 18: 3056-3062. Tibesku CO, Szuwart T, Ocken SA, Skwara A, Fuchs S. Increase in the Expression of the Transmembrane Surface Receptor CD44v6 on Chondrocytes in Animals With Osteoarthritis. Arthritis and Rheumatism 2005; 52: 810-817. Zhang FJ, Luo W, Gao SG, Su DZ, Li YS, Zeng C, et al. Expression of CD44 in articular cartilage is associated with disease severity in knee osteoarthritis. Modern Rheumatology 2013; 23: 1186-1191.
Dcn	Adams ME, Matyas JR, Huang D, Dourado GS. Expression of proteoglycans and collagen in the hypertrophic phase of experimental osteoarthritis. J Rheumatol Suppl 1995; 43: 94-97. Bock HC, Michaeli P, Bode C, Schultz W, Kresse H, Herken R, *et al.* The small proteoglycans decorin and biglycan in human articular cartilage of late-stage osteoarthritis. Osteoarthritis Cartilage 2001; 9: 654-663. Cs-Szabo G, Roughley PJ, Plaas AH, Glant TT. Large and small proteoglycans of osteoarthritic and rheumatoid articular cartilage. Arthritis Rheum 1995; 38: 660-668. Dourado GS, Adams ME, Matyas JR, Huang D. Expression of biglycan, decorin and fibromodulin in the hypertrophic phase of experimental osteoarthritis. Osteoarthritis Cartilage 1996; 4: 187-196. Little CB, Ghosh P, Bellenger CR. Topographic variation in biglycan and decorin synthesis by articular cartilage in the early stages of osteoarthritis: an experimental study in sheep. J Orthop Res 1996; 14: 433-444. Liu W, Burton-Wurster N, Glant TT, Tashman S, Sumner DR, Kamath RV, *et al.* Spontaneous and experimental osteoarthritis in dog: similarities and differences in proteoglycan levels. J Orthop Res 2003; 21: 730-737. Masse PG, Carrino DA, Morris N, Wenger L, Mahuren JD, Howell DS. Loss of decorin from the surface zone of articular cartilage in a chick model of osteoarthritis. Acta Histochem 1997; 99: 431-444. Melrose J, Fuller ES, Roughley PJ, Smith MM, Kerr B, Hughes CE, *et al.* Fragmentation of decorin, biglycan, lumican and keratocan is elevated in degenerate human meniscus, knee and hip articular cartilages compared with age-matched macroscopically normal and control tissues. Arthritis Research & Therapy 2008; 10. Poole AR, Rosenberg LC, Reiner A, Ionescu M, Bogoch E, Roughley PJ. Contents and distributions of the proteoglycans decorin and biglycan in normal and osteoarthritic human articular cartilage. J Orthop Res 1996; 14: 681-689.

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