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Intervertebral Disc/Bone Marrow Cross-Talk with Modic Changes

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Abstract

Study design—Cross-sectional cohort analysis of patients with Modic Changes (MC).

Objective—Our goal was to characterize the molecular and cellular features of MC bone marrow and adjacent discs. We hypothesized that MC associate with biologic cross-talk between discs and bone marrow, the presence of which may have both diagnostic and therapeutic implications.

Background Data—MC are vertebral bone marrow lesions that can be a diagnostic indicator for discogenic low back pain. Yet, the pathobiology of MC is largely unknown.

Methods—Patients with Modic type 1 or 2 changes (MC1, MC2) undergoing at least 2-level lumbar interbody fusion with one surgical level having MC and one without MC (control level). Two discs (MC, control) and two bone marrow aspirates (MC, control) were collected per patient. Marrow cellularity was analyzed using flow cytometry. Myelopoietic differentiation potential of bone marrow cells was quantified to gauge marrow function, as was the relative gene expression profiles of the marrow and disc cells. Disc/bone marrow cross-talk was assessed by comparing MC disc/bone marrow features relative to unaffected levels.

Results—Thirteen MC1 and eleven MC2 patients were included. We observed pro-osteoclastic changes in MC2 discs, an inflammatory dysmyelopoiesis with fibrogenic changes in MC1 and MC2 marrow, and upregulation of neurotrophic receptors in MC1 and MC2 bone marrow and discs.

Conclusion—Our data reveal a fibrogenic and pro-inflammatory cross-talk between MC bone marrow and adjacent discs. This provides insight into the pain generator at MC levels and informs novel therapeutic targets for treatment of MC-associated LBP.

Keywords

Modic change; cross-talk; pathobiology; bone marrow; neurotrophic; inflammation; myelopoiesis; osteoclastogenesis; fibrosis; pain

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INTRODUCTION

Low back pain (LBP) is the world's most disabling condition with detrimental consequences due to increased disability and use of health care services[1]. Although LBP associates with different spinal pathologies, vertebral bone marrow lesions visualized as Modic changes (MC) on magnetic resonance imaging (MRI) have a high specificity for discogenic LBP[2]. Three interconvertible types of MC have been described based on their appearance in T1-weighted (T1w) and T2-weighted images (T2w)[3].

The clinical importance of MC has been previously summarized: 1) MC prevalence is high in LBP patients[4]; 2) LBP patients with MC report a greater frequency and duration of LBP episodes[4–7]; and 3) LBP correlates with MC lesion size[8]. While the pathobiology behind MC-associated pain is unknown[4], it is speculated to result from increased numbers of PGP-9.5 nerve fibers and TNF-α positive cells observed in damaged vertebral endplates adjacent to MC regions [9,10]. Since endplate damage is associated with MC, it is assumed to be a critical prerequisite for MC development[3,11–13]. Endplate damage increases communication between the bone marrow and the disc[14,15] because it allows hydraulic disc/vertebra coupling and increased convective flow induced by cyclic spinal loading[14–16]. As a result, pro-inflammatory and pro-osteoclastic factors from MC discs can drain into the adjacent bone marrow and perturb normal bone marrow hematopoiesis[17–20]. For example, the maturation of granulocytes is amplified with pro-inflammatory cytokines[21]. Additionally, bone marrow cells can aggravate disc degeneration, suggesting bi-directional disc/vertebra communication in the presence of endplate damage[22].

A limited histologic sample of MC lesions has demonstrated fibrosis, inflammation, and high bone turnover in MC marrow [3]. Yet, little else has been published about the nature of MC bone marrow. Thus, the purpose of this study was to characterize the molecular and cellular features of MC bone marrow and adjacent MC discs compared to autologous control. We hypothesized that MC are a consequence of biologic communication between disc cells and the bone marrow compartment, a notion that may have important diagnostic and therapeutic consequences.

MATERIALS AND METHODS

The study was approved by the Institutional Review Board at UCSF and Stanford University (31955, 13-10863, 14-13246). The study was a cross-sectional cohort analysis of patients with Modic type 1 (MC1) or type 2 (MC2) changes undergoing lumbar interbody fusion with pedicle screw fixation of at least two levels for degenerative conditions. Patients were considered for inclusion if one surgical level had MC1 or MC2, and a second surgical level was absent MC. Additionally, patients with surgical indications including tumors, infectious disease, or prior instrumented back surgery were excluded. Four samples were collected per patient: two intervertebral discs and two bone marrow aspirates. One disc and one aspirate was from the MC level and the other disc and aspirate was from the non-MC (control) level (Figure 1).

Cellular composition of the bone marrow was analyzed using flow cytometry. As a measure of bone marrow function, myelopoietic differentiation potential of the bone marrow cells was quantified with a colony-forming unit (CFU) assay, and relative gene expression of the bone marrow and the disc were quantified with quantitative polymerase chain reaction (qPCR). Abbreviations are explained in Table 1 and Supplement Digital Content 1.

Tissue collection and RNA isolation

The correct surgical levels for disc resection and bone marrow aspirate was discussed and confirmed during pre-operative conference as well as immediately prior to the start of the procedure. Intraoperatively, disc tissue was aseptically removed and transferred to phosphate buffered saline (PBS) on ice. Bone marrow aspirates were taken with a 13G Jamshidi needle through pedicle screw insertion trajectories into the vertebral bodies prior to final screw placement. Aspirates were transferred into K2-EDTA blood tubes and 100 µl were transferred to 600 µl RNAlater.

Disc specimens (mainly nucleus pulposus) were washed with PBS, agitated overnight in RNAlater, and pulverized using a freezer-mill (Retsch MM301, Newtown, PA, USA). Approximately 200 mg were dissolved in 2 ml Trizol and RNA was isolated with two steps of chloroform extraction and purified with RNeasy (Qiagen, Valencia, CA, USA) mini columns including DNase digestion.

The bone marrow aliquots in RNAlater were centrifuged at 2000 g for 10 min at room temperature and the supernatant was removed. The pellet was resuspended in 1 ml Trizol, and pressed three times through a 33G needle. RNA was isolated as for disc tissue.

Gene expression analysis

After reverse transcription, expression of 46 genes related to inflammation, innate and adaptive immune response, cartilage/bone deposition/resorption, and neurotrophism were quantified with TaqMan probes (see Table, Supplemental Digital Content 1). Gene expression was normalized to ribosomal protein L30 (RPL30) and fold-change to autologous control sample was calculated. Normal distribution was confirmed with Shapiro-Wilk test and significant differences were detected by comparing ΔCq values of MC and control level with t-tests. Expression levels for each gene were compared between disc and bone marrow using Pearson's correlations. Significance level was $\alpha=0.05$. A trend was defined as $p<0.1$.

Flow cytometry

Whole bone marrow was diluted 1:1 in PBS and stained with a cocktail of fluorescent-coupled antibodies (see Table, Supplemental Digital Content 2) for 30. Cells were fixed and red blood cells lysed with Cal-Lyse™ (LifeTechnologies, Grand Island, NY, USA). 500,000 events were recorded on a BD LSR Fortessa X-20 (Becton Dickinson & Co, San Jose, CA, USA). Sixteen different cell populations were quantified with the gating strategy provided in Supplemental Digital Content 2. Relative cell populations were calculated as percentages of total cell count. Significant differences between MC and control and between MC1 and MC2 were detected with Wilcoxon tests.

Colony-forming unit assay

Isolation and culture of myelopoietic progenitor colonies was done according to manufacturer protocol (MethoCult^{TM}, StemCell Technologies, Vancouver, BC, Canada). Progenitor colonies of granulocyte/macrophage (CFU-GM), granulocyte (CFU-G), macrophage (CFU-M), multi-potential erythroid-macrophage-megakaryocyte (CFU-GEMM), and burst-forming unit erythroids (BFU-E) were counted after 12 days. MC colony counts were normalized to counts from autologous control bone marrow and significant differences ($p<0.05$) between MC and control and between MC1 and MC2 were detected with t-tests.

Cross-Talk assessment

Disc/bone marrow cross-talk was assessed by pairwise correlating the expression of each gene in the disc with the same gene in the bone marrow. If not stated otherwise, a correlation was considered significant if $p<0.05$, if all data points were within Cook's distance ($d=0.5$), and if more than three data pairs existed. All statistical analysis was performed using R (version 2.15.1).

RESULTS

Twenty-two patients were included in the study (MC1: n=13, MC2: n=11). Two patients were included in both groups, because they had both MC1 and MC2 at different levels (three surgical levels collected total). There was no significant difference in age, pain score, weight, height, BMI, and sex between MC1 and MC2 groups (Table 2). Based on Pfirrmann classification, degenerative disease was more severe in MC2 discs compared to control discs ($p<0.05$) but not in MC1 discs compared to control discs.

Gene expression

Neurotrophic receptors of the tropomyosin receptor kinase (Trk) family were up-regulated in MC disc and bone marrow (Table 3 and 4, Figure 2). TrkA was up-regulated in MC1 (6.01-fold, $p<0.05$) and MC2 (2.69-fold, $p<0.01$) discs but its high affinity ligand 'nerve growth factor' (NGF) was down-regulated in MC2 discs (0.30-fold). TrkB was up-regulated in MC1 (2.01-fold, $p< 0.05$) and MC2 (2.98-fold, $p<0.05$) bone marrow and TrkC was up-regulated in MC2 bone marrow (4.33-fold, $p<0.05$).

Pro-fibrotic bone marrow changes were observed in MC1 as increased collagen type 1 alpha (COL1A) expression (1.94-fold, $p=0.06$) and reduced interferon gamma (IFNG) (0.76-fold, $p=0.05$), and in MC2 bone marrow as reduced matrix metalloproteinase 9 (MMP9) (0.20-fold, $p<0.05$) and increased interleukin-4 (IL4) (1.99-fold, $p=0.09$). COL1A was also upregulated in MC2 discs (3.52-fold, $p<0.05$).

Four pro-osteoclastic genes were up-regulated in MC2 discs: colony stimulating factor 1 (CSF1) (1.82-fold, $p<0.05$), peroxisome proliferator-activated receptor gamma (PPARG) (1.83-fold, $p=0.07$), chemokine C-C motif ligand 2 (CCL2) (13.41-fold, $p<0.05$) and interleukin-6 (21,48-fold, $p=0.07$, $p<0.05$). Two anti-osteoclastic genes were down-regulated in MC2 discs: IL4 (0.46-fold, $p=0.08$) and osteoprotegrin (OPG) (0.36-fold, $p<0.05$).

The pro-inflammatory nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) was down-regulated in MC2 bone marrow (0.16-fold, $p<0.05$) and MC1 (0.41-fold, $p=0.08$) and MC2 (0.23-fold, $p=0.07$) discs.

Furthermore, the growth factor granulin (GRN) was down-regulated in MC2 bone marrow (0.70-fold, $p<0.05$) and the transcription factor RAR-related orphan receptor gamma (RORC) was down-regulated in MC1 (0.29-fold, $p=0.07$) and MC2 discs (0.24-fold, $p<0.05$).

Bone marrow cell population

In both MC1 and MC2, the granulocyte cell population was significantly different from control. In MC2, there were less granulocyte progenitors but more mature granulocytes (Figure 3A). In contrast, in MC1, there was a trend towards less mature granulocytes, and the ratio of progenitors to mature granulocytes was significantly increased and higher than in MC2.

Furthermore, in MC2 more innate lymphoid cells (ILC) were found. The increase was due to more ILC type 1 to 3 and not due to more natural killer cells (NKC) (Figure 3B). CD45-negative cells were significantly reduced in MC2 but tended to be increased in MC1 (Figure 3C). Erythroblasts, the largest CD45-negative cell population, was also decreased in MC2.

Myelopoietic differentiation potential

In both MC1 and MC2, we observed more CFU-G (Figure 3D). Furthermore, there were substantially more CFU-GEMM in MC1. In MC2, BFU-E was reduced.

Correlation analysis

A fibrogenic (COL1A, STAT3, IL4) and pro-inflammatory (MyD88, TRAF6, NFκB1, IL1R, TLR4) disc/bone marrow cross-talk was noted through significant correlations of gene expression in MC discs and bone marrow (Table 5). The number of significant correlations was higher than expected by chance ($p<0.05$ for MC1 and MC2).

DISCUSSION

We characterized the cellular and molecular changes in MC, and questioned whether these associated with features of adjacent discs. We observed pro-osteoclastic behaviors in MC2 discs, an inflammatory dysmyelopoiesis along with fibrogenic changes in both MC1 and MC2 marrow, and upregulation of neurotrophic receptors in MC1 and MC2 bone marrow and discs. Further, there was evidence of cross-talk between MC bone marrow and adjacent discs, where fibrogenic and pro-inflammatory gene expression correlated. Overall, these data suggest that disc-secreted factors are important in MC pathobiology, a feature that should motivate new treatment approaches that target MC spinal levels.

Disc/Bone Marrow Cross-Talk

MC are associated with endplate damage, which causes a hydraulic coupling of the disc and the bone marrow[12,13,15,16]. Here, we also show that there is a pro-inflammatory and

fibrogenic coupling of the disc and the marrow in MC, which is likely the consequence of biologic communication between the two compartments. Although the causality of cross-talk remains unknown, the typical synchronous occurrence of MC in both the cranial and caudal vertebra adjacent to the disc, and the correlation of the lesion size with the severity of disc degeneration, suggest that the disc is the trigger in MC pathobiology[23].

Despite the correlation of pro-inflammatory and fibrogenic genes between MC disc and marrow and individually large changes, cross-talk related genes (IL1R, TLR4, MyD88, TRAF6, and STAT3) were not significantly different on an individual basis due to large donor-to-donor variations. This suggests that there may be pathobiological sub-phenotypes within each MC type, that cannot be appreciated using standard T1w and T2w MRI. This observation motivates future studies to establish whether Modic sub-phenotypes exist and whether they have clinical significance.

Fibrotic Granulation Tissue, Inflammation, and Osteoclastogenesis

Granulation tissue and fibrotic tissue have been described in MC1 and MC2 surgical tissues [3]. Granulation tissue is a sign of inflammation during active healing processes that is typically characterized by the deposition of loose extracellular matrix and infiltration of myeloid cells. Fibrotic tissue has abundant collagen, and is formed from repeated healing attempts and chronic inflammation[24]. Our study corroborates at the molecular and cellular level the existence of both, granulation and fibrotic tissue, indicating that MC are a consequence of cycles of inflammation and healing attempts. More granulocyte progenitors in MC1 and more mature granulocytes in MC2 are indicative of granulation tissue and inflammation. Upregulated COL1A in MC1 and downregulated MMP9 in MC2 are indicative of fibrosis.

Discs adjacent to MC also show fibrotic changes (IL6, CCL2, COL1A). IL-6 induces CCL2 expression in monocytes and fibroblasts and increases collagen production in fibroblasts[25]. CCL2 expression typically follows the initial granulocytic phase of inflammation[26] and persistent CCL2 up-regulation indicates chronic inflammation[25]. Therefore, up-regulation of CCL2, IL6, and COL1A in MC2 discs suggests that fibroblasts invaded the adjacent disc or that disc cells underwent fibroblastic conversion[25]. It is conceivable, that disc/bone marrow coupling induces fibrogenic changes of the bone marrow as well. This is supported by the disc/marrow correlation of several genes associated with tissue fibrosis (COL1A, IL4, and STAT3)[24,27]. Chronic TLR-signaling generally accompanies chronic injuries and fibrosis[28]. Therefore, the correlation of gene expression from the TLR/IL1R pathway between the MC disc and bone marrow further substantiates the coupling of fibrogenic changes in both compartments.

Surprisingly, we did not observe signs of active inflammation in MC bone marrow, although MC discs have increased CCL2 and IL6 expression. Inflammation (IL-8 and PGE2) in MC1 and MC2 discs has been reported[18] but recently challenged with reports of no inflammationin MC disc[17,20]. The contradictory findings may reflect the dynamic nature of MC, where short phases of inflammation are followed by longer phases of reconciliation and healing attempts with osteoclast activation and high bone turn-over[17,29]. We also report pro-osteoclastic changes in MC2: pro-osteoclastic CSF1 and PPARG were up-regulated and anti-

osteoclastic IL4 and OPG were down-regulated. Due to the biological coupling of the disc with the bone marrow, these pro-inflammatory and pro-osteoclastic genes can also directly exert their myelopoietic effects in the bone marrow. For instance, IL-6 activates myelopoiesis while suppressing erythopoiesis[19,30], IL-4 governs T helper cell 2 differentiation and is a potent neutrophilic maturation factor[31], CSF1 instructs hematopoietic stem cells to differentiate along the myeloid lineage and supports neutrophil differentiation[32,33], and CCL2 recruits monocytes/macrophages and myeloid progenitors[34]. It is therefore not surprising that myelopoiesis is dysregulated in MC. This is supported by recent studies reporting increased CSF1 and CSF2 in MC discs[17,20]. More granulocyte progenitors in MC closely fit a mouse model of acute and chronic inflammation[21], where a single injection of IL-1β (which simulates acute inflammation after infection or injury) instructed the normally quiescent hematopoietic stem cell population to undergo rapid division to meet the increased myeloid demand, resulting in more myeloid progenitors similar to MC1[21]. In our study IL-1β was not significantly up-regulated in MC1 but trend to be upregulated in MC2 disc and bone marrow. However, CCL2 and IL-6 draining from the adjacent disc into MC also can drive myelopoiesis. Since IL-6 suppresses erythropoiesis, this can explain fewer BFU-E in MC2 [30]. Interestingly, NFκB1 was down-regulated in MC2. As mentioned above this could be because the inflammatory phase is rather short and is followed by a regulatory feedback during the reconciliation phase[3]. Taken together, our data suggest that in MC, localized granulocyte production by hematopoietic progenitors is increased as a result of chronic inflammation with different stages of granulocyte maturity represented in MC1 and MC2.

Neurotrophism

The neurotrophic receptors TrkB and TrkC were upregulated in MC bone marrow and TrkA was up-regulated in MC discs. In the bone marrow, many different cells express TrkB and TrkC[35] which can be due to both a change in cellularity and in transcription activity. Given the association of MC with endplate damage[3,12,13], the strong expression of TrkA, TrkB, and TrkC by osteoblasts and stromal cells in the callus during fracture healing is noteworthy[36–39]. They generally have an osteogenic and angiogenic effect, but also pro-osteoclastic effects for TrkB have been reported[36,37,39,40]. In combination with the pro-osteoclastic factors released by MC2 discs, this may cause a high bone turn-over as described in microarchitectural analysis of MC biopsies[29]. Importantly, skeletal pain after fracture and osteoarthritic pain and bone destruction can be reduced with Trk blockage without inhibiting fracture healing[38,41–44]. Similar to bone callus, MC endplates show high numbers of PGP-9.5 nerve fibers[9,10]. Therefore, MC pain may relate to a healing response. In contrast to stabilized long bone fractures, the persistent load on the disc/vertebra interface may impede proper healing and promotes fibrogenic changes with chronic expression of Trk's.

Granulin

The growth factor granulin was significantly down-regulated in MC2 bone marrow. Granulin is expressed during the inflammatory phase after injury by fibroblasts, endothelial and inflammatory cells. Granulin recruits neutrophils, macrophages, and fibroblasts, and induces

angiogenesis[45]. Therefore, lower granulin levels in MC2 bone marrow further corroborates the absence of an active inflammation.

In cartilage, granulin is chondroprotective and knock-out mice show accelerated disc degeneration[46]. Due to the disc/bone marrow coupling, MC2 discs may suffer from the reduced chondroprotective activity of lower bone marrow granulin levels. This may have added to the higher degree of degeneration of MC2 discs in this study.

Limitations

Several study limitations may affect the generalizability of our results. First, the varying primary diagnoses of the patient population precludes us to draw clinical conclusions. Second, MC2 discs were more degenerated than their control discs. Therefore, changes seen in MC2 discs may also relate to disc collapse and changes in endplate load distribution. Third, despite pre-operative discussions with the surgeons and some intra-procedural radiographic guidance, it is possible that bone marrow aspirates from MC were partially or entirely taken from healthy bone marrow. Fourth, gene expression analysis was performed on unsorted bone marrow cells. Therefore, a change in mRNA concentration may be due to a change of the cellular composition or a change in gene transcription. Fifth, the definition of the ILC population was done by exclusion rather than by positive selection, possibly inflating ILC count. And sixth, disc samples were not cultured or sequenced to exclude bacterial colonization, although all patients were afebrile with unremarkable white blood cell counts preoperatively.

CONCLUSIONS

We provide evidence that disc/bone marrow cross-talk is central to MC pathobiology, thereby offering an etiology of pain associated with MC. These findings establish a broad framework from which novel ideas for MC treatment may be identified, such as inhibiting the synthesis of pro-osteoclastic and myelopoietic-active factors in the disc. Furthermore, the effective pain reduction with Trk antagonists in osteoarthritis and in fracture healing suggests that Trk inhibitors may be a potential option for treating painful MC levels.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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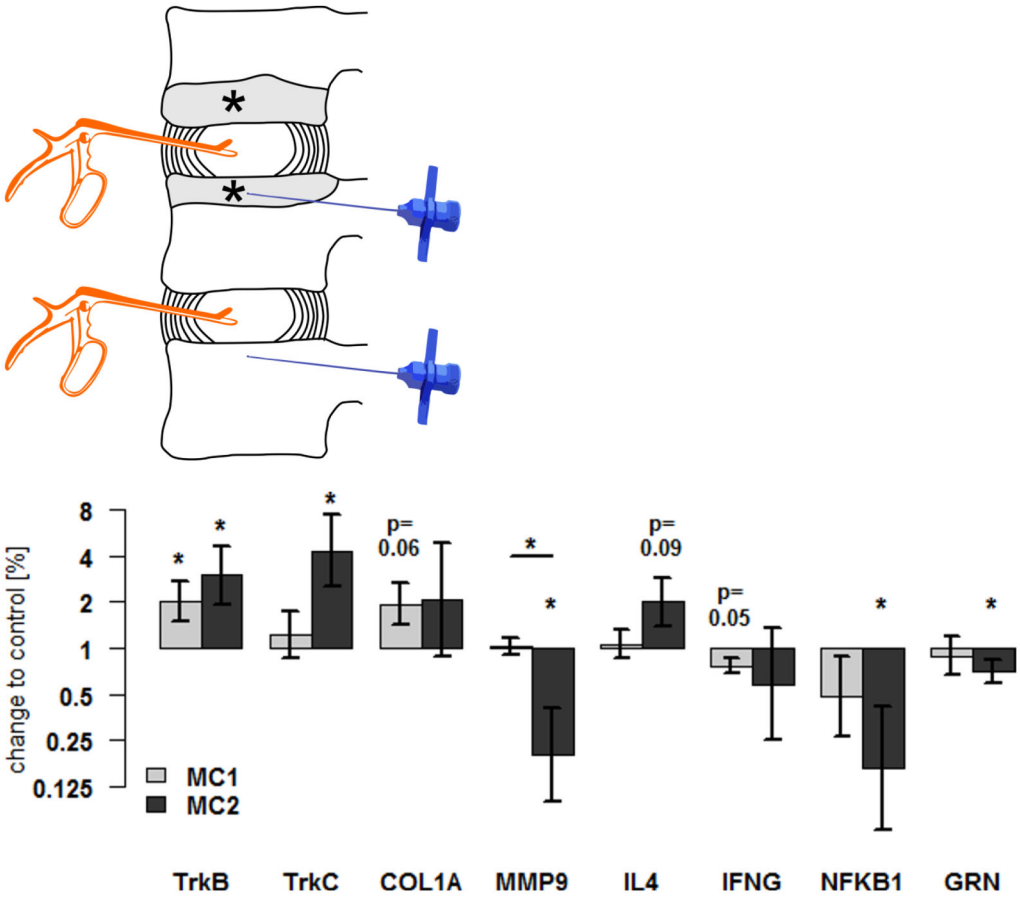

Figure 1.

Schematic drawing of tissue collection. Here, the upper level presents with Modic changes (asterisks). Four samples were collected per patient, two discs and two BM aspirates, one MC and one control tissue each. Disc tissue is collected with a bone rangeur (orange), bone marrow aspirates are taken with a Jamshidi needle (blue).

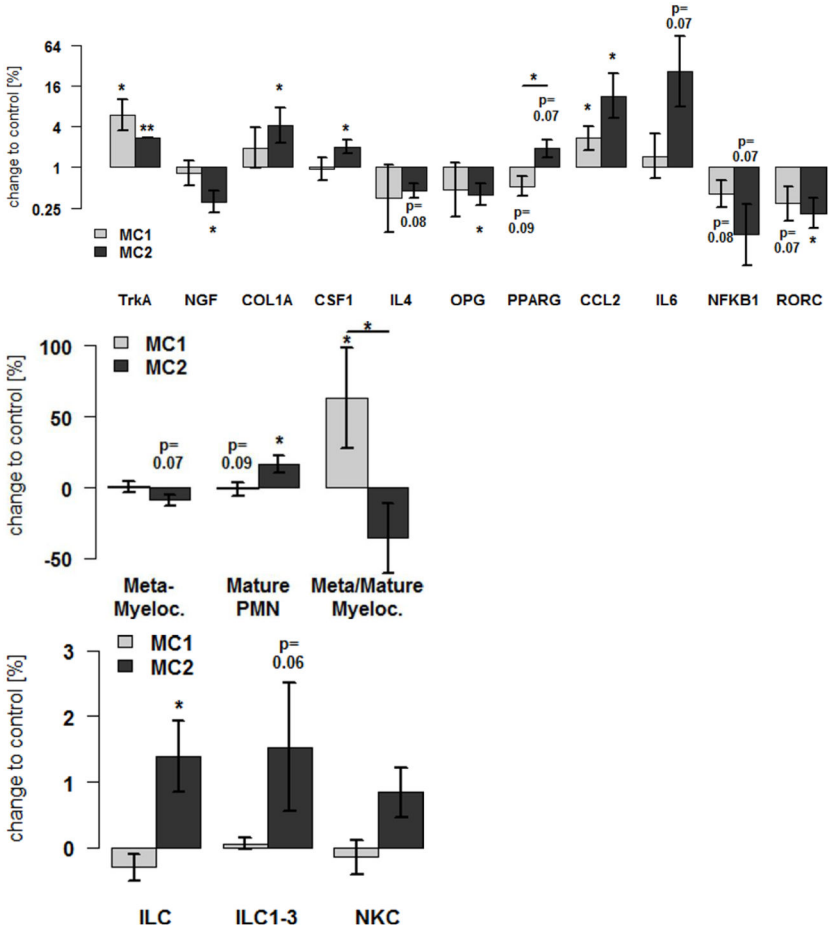

Figure 2.

Genes with significant expression changes in Modic type 1 (MC1) and type 2 changes (MC2) in the **(A)** bone marrow and in the **(B)** adjacent intervertebral disc.

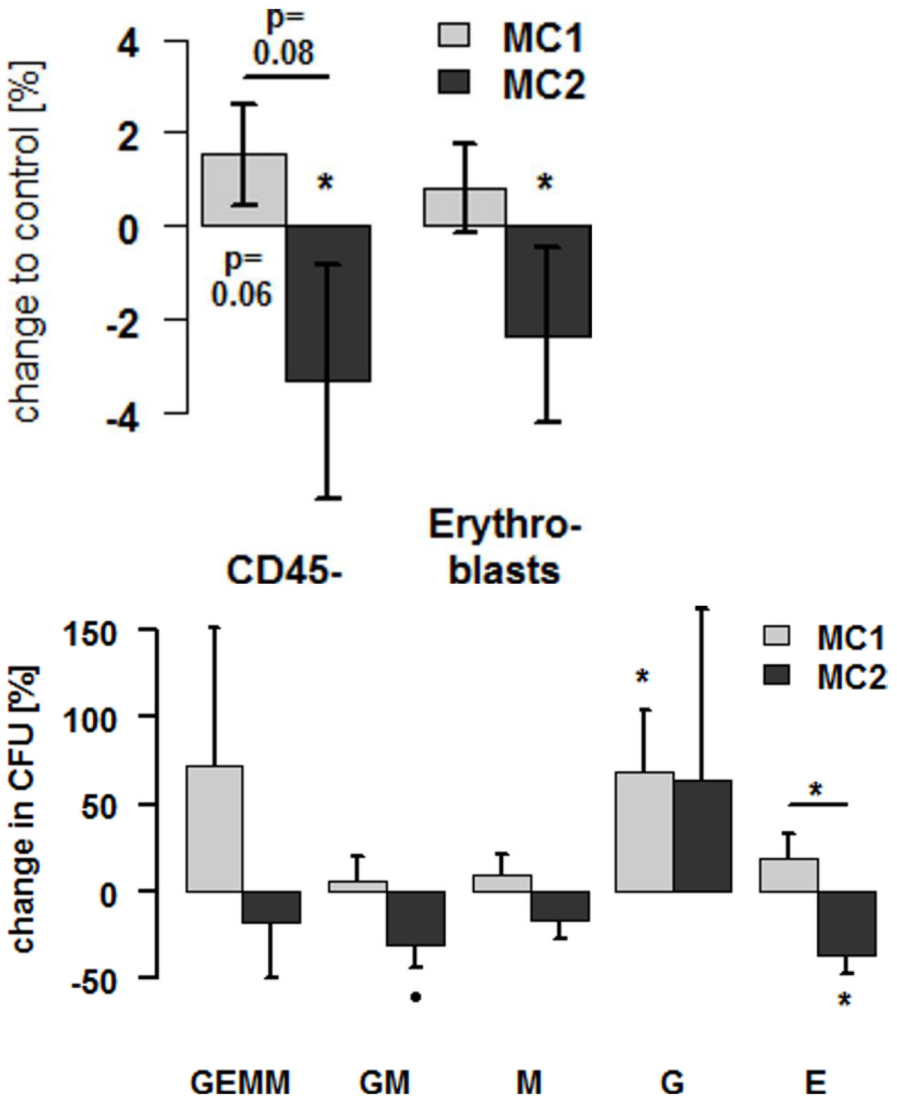

Figure 3.

Changes in bone marrow cell populations in Modic type 1 (MC1) and type 2 (MC2) changes. **(A)** Metamyelocytes, mature polymorphonuclear cells (PMNs), and the ratio of metamyelocytes to mature PMNs. **(B)** Innate lymphoid cells (ILC), ILC type 1–3 (ILC1-3), and natural killer cells (NKC), **(C)** CD45-negative cells, and erythroblasts, **(D)** Differentiation potential of myeloid progenitor cells quantified as colony-forming units (CFUs) of nucleated cells. Changes relative to autologous control bone marrow are indicated. CFUs of multi-potential erythroid-macrophage-megakaryocyte progenitors (GEMM), granulocyte/macrophage progenitors (GM), granulocyte progenitors (G), macrophage progenitors (M), and of burst-forming unit erythroids (E) were analyzed.

Table 1

List of abbreviations. A list of all genes analyzed can be found in Supplement Digital Content 1.

BFU-E	Burst-forming unit erythroids
CCL2	Chemokine C-C motif ligand 2
CFU-G	Colony forming unit granulocytes
CFU-GM	Colony forming unit granulocytes/macrophages
CFU-M	Colony forming unit macrophages
CFU-GEMM	Colony forming unit granulocyte, erythrocyte, monocyte, megakaryocyte
COL1A	Collagen type 1 helix α
CSF1	Colony stimulating factor 1
DD	Disc degeneration
DDD	Degenerative disc disease
GRN	Granulin
IFNG	Interferon gamma
IL1R	Interleukin-1 receptor
IL4	Interleukin-4
IL6	Interleukin-6
IL8	Interleukin-8
ILC	Innate lymphoid cells
LBP	Low back pain
MC	Modic change
MC1, MC2	Modic type 1 change, Modic type 2 change
MMP9	Matrix metalloproteinase 9
MRI	Magnet resonance imaging
MyD88	Myeloid differentiation primary response gene 88
NFκB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NGF	Nerve growth factor
NKC	Natural killer cells
OPG	Osteoprotegrin
PBS	Phosphate buffered saline
PGE2	Prostaglandine E2
PMN	Polymorphonuclear granulocytes
PPARg	Peroxisome proliferator-activated receptor gamma
qPCR	Quantitative polymerase chain reaction
RORC	RAR-related orphan receptor gamma
RPL30	Ribosomal protein L30 (reference gene)
STAT3	Signal transducer and activator of transcription 3
T1w, T2w	T1-weighted, T2-weighted
TLR4	Toll-like receptor 4
TNF	Tumor necrosis factor

TRAF6	TNF receptor associated factor 6
Trk	Tropomyosin receptor kinase

Table 2

MC patient population of the prospectively collected disc and bone marrow samples. Last row indicates p-value for t-test or ANOVA when comparing Modic change type 1 (MC1) population to Modic change type 2 (MC2) population. Control (ctrl), DD (disc degeneration), DDD (degenerative disc disease)

	MC1		MC2		p-value
Gender [n]	f=5, m=8		f=7, m=4		0.20
Age [yr]	62.7±10.8		64.4±11.0		0.71
Pain [1–10]	5.8±2.2		6.5±1.9		0.46
Weight [kg]	77.9±15.0		76.8±18.9		0.88
Height [cm]	172±6.6		167.3±8.6		0.17
BMI	26.3±4.8		27.2±4.8		0.69
Surgical indication					0.71
Spondylolisthesis	1		3		
Deformity	7		6		
Stenosis	2		0		
DDD	2		2		
Operated level	MC1	Ctrl	MC2	Ctrl	0.22 (MC), 0.27 (Ctrl)
T12/L1 to L2/L3	0	1	0	2	
L3/4	4	6	4	1	
L4/5	5	3	3	5	
L5/S1	4	3	4	3	
MC vs Ctrl (p-value)[1]	0.15		0.22		
Degree of DD	MC1	Ctrl	MC2	Ctrl	0.21 (MC), 0.63 (Ctrl)
Moderate: Pfirmann 3–4	6	11	2	8	
Severe: Pfirmann 5	7	2	9	3	
MC vs Ctrl (p-value)[1]	0.10		**0.03***		

[1] p-value for ANOVA comparing MC to control disc from the same patient.

Table 3

Gene expression in intervertebral discs adjacent to Modic type 1 (MC1) and type 2 changes (MC2) relative to autologous control discs. Fold-change, confidence interval (CI) and p-values (p) are indicated for all genes analyzed. Significant p-values are in bold. Last column (1vs2) provides p-values for the correlation of MC1 and MC2 for genes with $p < 0.1$.

gene	MC1			MC2			1vs2 p
	fold change	CI	p	fold change	CI	p	
BMP2	0.57	0.09–3.49	1.00	0.58	0.00–4.36	0.16	
CCL2	2.69	1.09–6.66	**0.03***	13.41	1.58–81.0	**0.02***	
COL1A	1.93	0.42–8.85	0.36	3.52	0.99–17.1	0.05	
CSF1	0.95	0.41–2.21	0.89	1.82	1.17–3.42	**0.02***	
CSF2	NA	NA	NA	NA	NA	NA	
CSF3	NA	NA	NA	NA	NA	NA	
FGF	0.56	0.05–6.58	0.60	1.29	0.04–3.41	1.00	
GRN	0.78	0.26–2.32	0.61	1.16	0.37–3.91	0.72	
IFNG	NA	NA	NA	NA	NA	NA	
IL12B	0.03	0-Inf	0.38	0.08	0.00–4.87	0.08	
IL17A	0.91	0.01–144	0.94	0.01	NA	NA	
IL1A	0.04	NA	NA	1.99	0-Inf	0.80	
IL1B	0.46	0.12–1.77	0.21	2.25	0.27–5.99	0.63	
IL1R2	4.61	0.01–1751	0.38	0.59	0.10–7.55	0.88	
IL23A	0.63	0.31–1.29	0.17	0.25	0.05–1.53	0.11	
IL23R	0.15	NA	NA	NA	NA	NA	
IL4	0.35	0-Inf	0.52	0.46	0.16–1.25	0.08	
IL6	1.46	0.22–9.56	0.64	21.48	0.56–1218	0.07	0.07
IL8	0.55	0.12–2.48	0.39	1.46	0.21–4.90	0.99	
MMP13	1.56	0.40–6.06	0.48	0.53	0.02–9.98	0.51	
MMP3	0.91	0.23–3.59	0.88	1.07	0.25–2.78	0.72	
MMP9	0.97	0.15–6.41	0.97	1.71	0.09–9.80	0.94	
MYD88	0.82	0.28–2.42	0.69	1.32	0.57–2.64	0.55	
NFATC1	0.96	0.58–1.58	0.85	0.89	0.60–1.60	0.94	
NFKB1	0.41	0.15–1.12	0.08	0.23	0.01–1.30	0.07	

gene	MC1			MC2			1vs2 p
	fold change	CI	p	fold change	CI	p	
NGF	0.82	0.32–2.08	0.65	0.30	0.12–0.76	**0.02***	
OPG	0.47	0.06–3.45	0.42	0.36	0.17–0.93	**0.04***	
OSCAR	2.38	0.20–28.7	0.41	0.51	0.13–9.10	0.55	
PPARG	0.53	0.24–1.15	0.09	1.83	0.92–3.98	0.07	**0.01***
RANK	2.47	0.02–267	0.49	1.79	NA	NA	
RANKL	0.75	0.22–2.59	0.62	0.94	0.37–2.29	0.83	
RORC	0.29	0.08–1.11	0.07	0.24	0.06–0.72	**0.02***	
RUNX1	1.11	0.59–2.10	0.72	1.43	0.53–2.64	0.64	
STAT3	0.76	0.40–1.47	0.38	0.86	0.57–1.66	0.92	
TACR1	1.55	0.08–29.9	0.67	3.92	0.46–16.9	0.13	
TGFB1	0.64	0.26–1.57	0.29	0.92	0.68–1.41	0.88	
TLR2	1.00	0.37–2.67	1.00	1.26	0.59–4.92	0.27	
TLR4	0.97	0.41–2.34	0.95	1.26	0.78–2.79	0.18	
TLR6	0.86	0.54–1.39	0.51	0.91	0.49–1.44	0.47	
TNF	0.42	0.03–6.79	0.40	1.18	0.00–670	0.80	
TRAF2	0.89	0.44–1.78	0.70	0.70	0.25–1.88	0.39	
TRAF6	0.73	0.34–1.54	0.37	1.10	0.75–1.90	0.38	
TrkA	6.01	1.11–32.5	**0.04***	2.69	2.47–2.94	**0.004****	
TrkB	0.44	0.16–1.22	0.10	0.40	0.25–1.87	0.47	
TrkC	0.91	0.21–3.86	0.87	2.03	0.66–4.95	0.19	
VCAM1	1.97	0.74–5.22	0.23	4.11	0.80–9.15	0.09	

Table 4

Gene expression in bone marrow with Modic type 1 (MC1) and type 2 changes (MC2) relative to autologous control bone marrow. Fold-change, confidence interval (CI) and p-values (p) are indicated for all genes analyzed. Significant p-values are in bold. Last column (1vs2) provides p-values for the correlation of MC1 and MC2 for genes with $p < 0.1$.

gene	MC1			MC2			
	fold change	CI	p	fold change	CI	p	1vs2 p
BMP2	1.05	0.74–1.50	0.76	0.38	0.10–1.37	0.11	
CCL2	1.49	0.86–2.61	0.14	2.30	0.65–8.11	0.17	
COL1A	1.94	0.97–3.85	0.06	2.08	0.29–15.0	0.16	
CSF1	0.97	0.68–1.37	0.84	0.90	0.41–1.97	0.84	
CSF2	0.53	0.10–2.78	0.37	1.17	0.53–2.57	0.62	
CSF3	1.12	0.30–4.13	0.85	0.89	0.34–2.34	0.76	
FGF	2.36	0.02–256	0.51	3.18	0.58–17.5	0.12	
GRN	0.74	0.47–1.68	0.69	0.70	0.47–1.05	**0.02***	
IFNG	0.76	0.59–0.97	0.05	0.58	0.08–3.99	0.53	
IL12B	0.92	0-Inf	0.96	0.59	0.14–2.53	0.39	
IL17A	NA	0-Inf	0.83	0.36	0.00–27.0	0.20	
IL1A	1.27	0.51–3.14	0.55	1.53	0.47–5.01	0.42	
IL1B	1.00	0.72–1.39	1.00	1.79	0.81–3.96	0.13	
IL1R2	1.10	0.30–4.02	0.83	1.30	0.74–2.30	0.46	
IL23A	0.96	0.60–1.54	0.84	0.97	0.29–3.20	0.95	
IL23R	1.24	0-Inf	0.88	1.25	0.19–8.17	0.76	
IL4	1.05	0.66–1.69	0.81	1.99	0.88–4.51	0.09	0.10
IL6	1.27	0.93–1.72	0.11	1.87	0.44–7.93	0.35	
IL8	0.89	0.56–1.43	0.61	1.07	0.26–4.38	0.91	
MMP13	1.63	0.06–41.5	0.72	NA	NA	NA	
MMP3	0.80	0.03–19.0	0.53	NA	NA	NA	
MMP9	1.03	0.77–1.37	0.85	0.20	0.04–1.01	**0.04***	**0.03***
MYD88	1.12	0.69–1.81	0.63	1.52	0.81–2.87	0.16	
NFATC1	0.99	0.69–1.41	0.94	1.03	0.58–1.81	0.91	
NFKB1	0.48	0.13–1.80	0.27	0.16	0.02–1.41	**0.04***	

gene	MC1			MC2			1vs2 p
	fold change	CI	p	fold change	CI	p	
NGF	NA	NA	NA	NA	NA	NA	
OPG	1.34	0.50–3.55	0.52	1.25	0.39–3.98	0.67	
OSCAR	0.73	0.35–1.52	0.37	0.88	0.33–2.34	0.77	
PPARG	1.12	0.86–1.46	0.36	1.09	0.62–1.94	0.73	
RANK	1.14	0.37–3.56	0.79	1.51	0.09–24.0	0.70	
RANKL	1.30	0.77–2.20	0.30	1.21	0.36–4.02	0.71	
RORC	1.26	0.54–2.96	0.55	1.56	0.62–3.89	0.28	
RUNX1	1.18	0.76–1.84	0.42	1.17	0.68–2.00	0.52	
STAT3	0.90	0.55–1.48	0.66	0.96	0.58–1.58	0.85	
TACR1	2.41	0.44–13.1	0.20	1.73	0.46–6.49	0.35	
TGFB1	1.01	0.64–1.59	0.97	0.99	0.54–1.81	0.36	
TLR2	1.06	0.73–1.54	0.73	1.69	0.81–3.53	0.14	0.08
TLR4	0.84	0.51–1.40	0.48	1.46	0.76–2.80	0.21	
TLR6	0.89	0.58–1.36	0.56	1.36	0.68–2.69	0.33	
TNF	0.94	0.58–1.50	0.76	1.57	0.65–3.77	0.27	
TRAF2	0.94	0.64–1.38	0.74	0.81	0.55–1.19	0.24	
TRAF6	1.13	0.73–1.76	0.54	1.04	0.57–1.90	0.89	
TrkA	1.13	0.60–2.14	0.67	0.58	0.26–1.29	0.15	
TrkB	2.01	1.04–3.91	**0.04***	2.98	1.05–8.50	**0.04***	
TrkC	1.22	0.55–2.70	0.59	4.33	1.07–17.5	**0.03***	0.09
VCAM1	1.41	0.77–2.58	0.24	0.74	0.12–4.39	0.71	

Table 5

Correlation of gene expression between discs and bone marrow in Modic type 1 (MC1) and type 2 (MC2) changes. P-value and R^2 of Pearson's correlation are provided.

	MC1		MC2	
	p	**R^2**	**p**	**R^2**
COL1A	0.05	0.16		
STAT3	0.05	0.3		
IL4			0.05	0.96
MyD88	<0.05	0.2		
TRAF6	<0.01	0.41		
NFκB1	0.06	0.14	<0.05	0.38
IL1R			<0.05	0.58
TLR4			0.06	0.28
TrkB	0.02	0.51		