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REVIEW

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The potential roles of high mobility group box 1 (HMGB1) in musculoskeletal disease: A systematic review

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Abstract

There is increasing evidence for the involvement of high mobility group box 1 (HMGB1) in inflammation, angiogenesis, and tumorigenesis. However, no studies have reviewed the role of HMGB1 in musculoskeletal disease. This systematic review aimed to evaluate the literature regarding the potential roles of HMGB1 in musculoskeletal disease (joint, tendon, ligament, intervertebral disk, and bone). After searching PubMed, MEDLINE, and EMBASE databases up to 01-01-2020, 66 articles that measured HMGB1 expression in musculoskeletal disease were included. Immune and tissue-resident stromal cells expressed HMGB1, and both diseased human tissues and animal disease models showed increased HMGB1 expression relative to controls. Administration of recombinant HMGB1 to diseased musculoskeletal tissues induced inflammation, whereas blocking HMGB1 ameliorated histopathologic and clinical severity of disease. HMGB1 redox status was investigated in only 3% of the articles: Fully reduced HMGB1 promoted chemotaxis of leukocytes and tissue repair, whereas disulfide HMGB1 acted as a pro-inflammatory mediator. Our review highlights that while HMGB1 is an important mediator in musculoskeletal disease, its redox status remains understudied. Identification of HMGB1 redox status in musculoskeletal tissues is critical to advance understanding of the diverse biological functions of HMGB1 in musculoskeletal disease. Importantly, this will inform future therapeutic strategies to target HMGB1.

KEYWORDS

high mobility group box 1, HMGB1, inflammation, musculoskeletal disease, redox status

1 | INTRODUCTION

Musculoskeletal disorders are a global disease burden in our aging population.[1,2] Adult musculoskeletal tissues do not regenerate due to minimal turnover rates of tendon and cartilage.[3,4] Therefore, the majority of musculoskeletal diseases in adults, including tendinopathy and arthritides (eg, rheumatoid arthritis (RA) and osteoarthritis (OA), rotator cuff tears), have the tendency to develop into chronic disease.[5-7] Chronic pain and immobility can lead to reduced quality of life and physical inactivity, predisposing to metabolic and cardiovascular disease.[8,9] Over the past few decades, there has been increasing evidence implicating the involvement of underlying inflammatory mechanisms in

musculoskeletal diseases such as tendinopathy and arthritis.[7,10] More recently, it has been shown that damaged cells release intracellular proteins called alarmins or damage-associated molecular patterns (DAMPs), which can activate and mobilize the immune system via pattern recognition receptors.[10-12] The most extensively investigated DAMP, high mobility group box 1 (HMGB1), has been shown to exhibit diverse cellular functions regulating tissue health and disease.[13-15]

1.1 | HMGB1 as a DAMP

HMGB1 is a ubiquitous protein that is highly conserved within mammals.[13-17] It usually resides within the nucleus, where it supports the chromatin structure of DNA and regulates gene expression.[16,17] In cases of severe cellular stress, HMGB1 is released from the cell, regulating the balance between the maintenance and resolution of inflammation as a DAMP by switching between different redox states.[13-15,18,19] The redox status of HMGB1 depends on the oxidation of three cysteines (cysteines 23, 45, and 106).[13,14,20-22] From work in other diseases such as cancer, we know that the function of extracellular HMGB1 depends on its redox status.[13,14,20-22] Fully reduced HMGB1, with all cysteine residues having intact thiol groups, forms a heterocomplex with CXCL12 and activates the CXCR4 receptor, initiating chemotaxis of leukocytes and other immune cells. In its intermediate state, a disulfide bridge is formed between cysteine 23 and 45, which activates immune cells via the MD2-TLR4 receptor to produce more HMGB1, chemokines, cytokines, and reactive oxygen species. Finally, fully oxidized HMGB1, in which all three thiol groups have been oxidized to sulfonyl groups, has no inflammatory activity (nor any other known functions).[13,14,20-22]

1.2 | HMGB1 in musculoskeletal disease

Recently, there has been increasing evidence documenting the involvement of HMGB1 in musculoskeletal disease.[23-27] These studies suggest diverse biological functions of HMGB1, including the involvement in both promotion of inflammation and tissue repair.[15,23,24,27] To our knowledge, no reviews have compared the role of HMGB1 in different musculoskeletal diseases. The overarching aim of this review was to establish the potential roles of HMGB1 in musculoskeletal diseases. Our objectives were to investigate the biological role of HMGB1 in human musculoskeletal disease, investigate the role of HMGB1 in animal models of musculoskeletal disease, and determine whether redox status influenced the biological activity of HMGB1 in musculoskeletal disease.

2 | METHODOLOGY

This review was conducted following the published guidelines by the International Committee of Medical Journal Editors (ICMJE) and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).[28,29] The review protocol was registered at PROSPERO (registration number: CRD42018091796).[30]

2.1 | Information sources and search strategy

PubMed, MEDLINE, and EMBASE databases were searched up to 01-01-2020. There were no date restrictions. The search strategy was composed by two researchers (HG and MB) and can be found in Appendix S1.

2.2 | Study selection and eligibility criteria

Abstract screening and study selection were managed through an online tool (Covidence, Cochrane).[31] The abstracts of the articles were individually screened for eligibility by two researchers (HG and JM). All articles reporting on HMGB1 in musculoskeletal disease were assessed for eligibility. Musculoskeletal disease was defined as diseases of tendon, ligament, synovium, cartilage, joint capsule, intervertebral disk (IVD), and bone. Systemic diseases were included if there was a significant musculoskeletal focus (eg, systemic lupus erythematosus [SLE]). Articles were excluded if there was no measurement of HMGB1 expression, or if the effect of adding/blocking HMGB1 on diseased musculoskeletal tissue was not investigated. Other exclusion criteria were diseases without a primary musculoskeletal focus, inappropriate study designs for this review (conference abstracts, meta-analyses, systematic reviews, and letters to the editor), studies without an appropriate comparator group, and the effects of exogenous substances on HMGB1. We also excluded publications in other languages than Dutch, German, or English, and articles that have not been reported in a peer-reviewed journal. Full-text articles were screened to confirm that HMGB1 was studied in musculoskeletal diseased tissue and that an appropriate control group was used. Disagreements were solved by means of consensus or a third reviewer (MB).

2.3 | Data extraction and synthesis of results

One researcher (HG) extracted the data from the included articles using a standardized data-extraction sheet in Microsoft Excel. The study results were tabulated and

summarized qualitatively in the Results section. The second researcher checked the tables for accuracy (JM). Results are divided into paragraphs based on the disease type: musculoskeletal soft tissue disease (tendinopathy, muscle injury, frozen shoulder), bone disease (fractures, IVD disease), and arthritis (OA, RA, juvenile idiopathic arthritis (JIA), SLE, (pseudo)gout).

3 | RESULTS

A total of 4376 abstracts were screened, and 168 articles were selected for full-text article screening (Figure 1). After full-text article screening, 66 articles were included in the systematic review as they fulfilled all selection criteria.[13,15,23-27,32-91] Although most studies focused on either human musculoskeletal disease (n = 31) or animal models of musculoskeletal disease (n = 31), four studies investigated HMGB1 in both human and animal models of musculoskeletal disease (Tables 1 and 2).[45,56,60,71] In the past two decades, there has been an increase in publication frequency of articles on HMGB1 in musculoskeletal disease (Figure 2).[15,27] However, only 2 of 66 of the included studies looked into the redox status of HMGB1 in musculoskeletal disease.[15,27] All included studies used established laboratory methods.[13,15,23-27,32-91] The results of HMGB1 expression in musculoskeletal diseased tissues of both human and animal studies are summarized in Table 3. The effects of local administration and/or inhibition of HMGB1 *in vitro* and *in vivo* are summarized in Table 4.

3.1 | HMGB1 in human musculoskeletal disease

There were 35 studies that investigated HMGB1 in human musculoskeletal diseased tissue, including arthritis (27), fractures (1), IVD degeneration (4), tendinopathy (2), and frozen shoulder (1) (Table 1).[23-26,32,33,36,38,39,41-43,45,46,56-58,60,62,64,66,68,69,71,75-77,80-82,85,88-91] Most studies that investigated HMGB1 expression (26/28) showed that HMGB1 expression is increased in human musculoskeletal diseased tissues compared to control tissues (Table 3).[24-26,32,33,36,38,39,41-43,45,46,56-58,60,62,64,66,68,69,71,75-77,80-82,85,88,89,91]

The redox status of HMGB1 was not investigated in these studies of human musculoskeletal disease.

3.1.1 | HMGB1 expression in human soft tissue joint disease

Three studies investigated the role of HMGB1 in tendinopathy and frozen shoulder.[23-25] In tendinopathy, Akbar et al[24] showed increased gene and protein expression of HMGB1

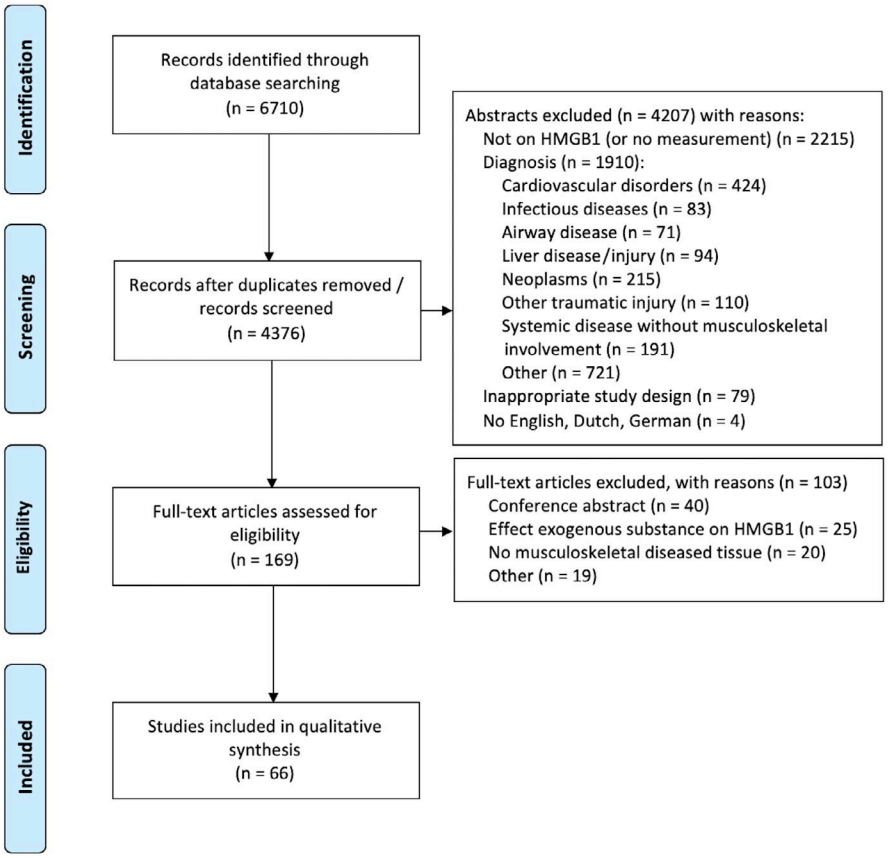

FIGURE 1 Flow diagram

TABLE 1 HMGB1 in human musculoskeletal disease

Author (y)	Country	Disease	Tissue type	HMGB1 measured in tissue	Administration of HMGB1	Blocking of HMGB1	No. of patients	Mean age in years (range)	Female
Soft MSK disease									
Akbar (2017)	UK	Tendinopathy	RC tendon (whole tissue)	✓			16 early/advanced disease[a] 6 control (hamstring)	50 (35-64) 21 (16-25)	NR
Mosca (2017)	UK	Tendinopathy	Supraspinatus tendon (whole tissue)	✓			13 painful disease 6 control (hamstring) 5 post-treatment pain-free[b]	Median: 45 (36-62) Median: 24 (19-26) Median: 57 (43-72)	NR
Cher (2018)	UK	Frozen shoulder	Glenohumeral joint capsule (whole tissue)	✓			10 frozen shoulder 10 unstable shoulder	54 (42-65) 33 (18-46)	50% 20%
Bone disease									
Gruber (2015)	USA	IVD degeneration	Degenerated disk (whole tissue)	✓			14 disease 3 control (donor disk)	45 (21-79) 28 wk	43% 0%
Klawitter (2014)	Switzerland	IVD degeneration	Cultured IVD cells[c]		✓		IVD disease (n = NR)	NR	NR
Liu (2019)	China	IVD degeneration	Degenerated nucleus pulposi (whole tissue)	✓			15 IVD disease[d]: - mild disease - severe disease	47 (37-63)	47%
Shah (2019)	USA	IVD degeneration	Degenerated/herniated/ spinal stenosis disks (whole tissue)	✓			17 IVD disease[e]: • early disease • intermediate disease • advanced disease	NR	NR
Li (2015)	China	Fracture	Fracture hematoma	✓			31 fracture hematomas 31 control (surgical bleeding bone)	NR	NR
Arthritis									
Amin (2014)	USA	OA	1. Cartilage (whole tissue)	✓			6 OA 6 control (fracture)	60-85 24-75	72% 63%
			2. Cultured chondrocytes		✓		OA (n = NR)	NR	NR
Benedetti (2016)	Japan	RA	Cultured synovial cells		✓		RA (n = NR) Control (donor) (n = NR)	NR	NR
Bobek (2014)	Croatia	JIA and SLE	Synovial fluid	✓			97 JIA (+ control serum) 19 SLE (+ control serum)	7 (0.4-12.3) 14 (10.6-15.9)	36% 26%
Cai (2019)	China	RA	Cultured synovial fibroblasts	✓			14 RA 14 control (OA)	59 (SD 8.2) 89 (SD 4.9)	71% 57%
Ding (2019)	China	Post-traumatic OA	Synovial fluid	✓			47 acute ACL injury 35 chronic ACL injury	24 (SD 10)	36% 49%

(Continues)

TABLE 1 (Continued)

Author (y)	Country	Disease	Tissue type	HMGB1 measured in tissue	Administration of HMGB1	Blocking of HMGB1	No. of patients	Mean age in years (range)	Female
Garcia-Arnandis (2010)	Spain	OA	1. Synovium (whole tissue)	✓			15 OA 3 control (cadaveric)	69 (SEM 1) 72 (SEM 1.5)	80% 67%
			2. Cultured chondrocytes		✓		4-8 OA	NR	NR
Hamada (2008)[i]	Japan	RA and pseudogout.	Synovial fluid	✓			41 RA 22 pseudogout 41 control (OA)	66 (39-88) 74 (33-90) 66 (39-88)	93% 73% 71%
He (2013)	China	RA	Cultured synovial fibroblasts		✓		10 RA	56 (36-70)	NR
Huang (2019)	China	OA	Cartilage (whole tissue)	✓			6 OA 6 control (trauma)	NR	NR
Jiang (2017)[i]	China	OA	Cartilage (whole tissue)	✓			6 OA 6 control (fracture)	NR	NR
Kim (2014)	Korea	RA	Cultured synovial fibroblasts		✓		RA (n = NR)	NR	NR
Kokkola (2002)[i]	Sweden	RA	Synovium (whole tissue)	✓			3 RA 1 control (OA)	40, 55 and 80 60	33% 0%
Leclerc (2013)	Sweden	RA and JIA	Cultured synovial fibroblasts		✓		6 RA 4 JIA	NR	NR
Arthritis									
Li (2016)	China	RA	Synovium (whole tissue)	✓			7 RA 7 control (OA)	median: 65 (55-69) median: 61 (52-70)	86% 71%
Li (2011) (#1)	China	OA	Synovial fluid	✓			78 OA 30 control (traumatic)[f]	68.8 (SD 8.4) 39 (SD 10.6)	68% 57%
Mitroulis (2011)	Greece	Gout	Synovial fluid	✓			6 active gout attack 6 acute gout (serum PMNs) 6 control (serum PMNs)	NR	NR
Park (2015)[i]	Korea	RA	1. Synovium (whole tissue)	✓			5 RA 6 control (OA)	58 (SD 2.61) 67 (SD 3.66)	80% 83%
			2. Cultured synovial fibroblasts		✓		5 RA 6 control (OA)	58 (SD 2.61) 67 (SD 3.66)	80% 83%
Qin (2014)	China	RA	Cultured synovial fibroblasts		✓		3 RA 8 OA 5 control (non-OA/RA)[g]	NR	NR

(Continues)

TABLE 1 (Continued)

Author (y)	Country	Disease	Tissue type	HMGB1 measured in tissue	Administration of HMGB1	Blocking of HMGB1	No. of patients	Mean age in years (range)	Female
Rosenberg (2017)	USA	OA	1. Cartilage (whole tissue)	✓			5 hip OA 5 knee OA	71 (60-94) 65 (53-73)	80% 80%
			2. Cultured chondrocytes	✓			OA (n = NR) control (healthy) (n = NR)	NR	NR
Schierbeck (2013)	Sweden	JIA	Synovial fluid	✓			23 JIA 10 control (healthy serum)	median: 12 (2-18) median: 8 (2-14)	65% 30%
Sun (2016)	China	OA	Synovium (whole tissue)	✓			108 OA 75 control (non-OA)[h]	56 (43-75) 55 (40-72)	42% 48%
Taniguchi (2003)	Japan	RA	1. Synovial fluid	✓			30 RA 30 control (OA)	59 (SD 10.7) 72 (SD 7.6)	97% 63%
			2. Cultured synovial macrophages		✓		RA (n = NR)	NR	NR
Terada (2011)	Japan	OA	Cartilage (whole tissue)	✓	✓		31 OA (n = 27 patients) 4 control (healthy cartilage from osteosarcoma patients)	71 (48-82) 16 (9-29)	NR
Arthritis									
Thankam (2016)	USA	OA	Biceps tendon (whole tissue)	✓			4 OA + SLAP tears 11 control (SLAP tears)	40-65	NR
Wahamaa (2011)	Sweden	RA	Cultured synovial fibroblasts		✓		4 RA 5 control (OA)	NR	NR
Wang (2019)	Switzerland	OA	Cartilage (whole tissue)	✓			12 OA 12 control (trauma)	62 (SD 5.6) 27 (SD 4.3)	58% 50%
Xu (2015)	China	RA	Cultured synovial fibroblasts	✓			7 RA 7 control (OA)	median: 61 (55-75) median: 68 (58-70)	71% 29%

Abbreviations: ACL, anterior cruciate ligament; IVD, intervertebral disk; JIA, juvenile idiopathic arthritis; MSK, musculoskeletal; NR, not reported; OA, osteoarthritis; PMN, polymorphonuclear cells; RA, rheumatoid arthritis; RC, rotator cuff; SD, standard deviation; SEM, standard error of the mean; SLAP, lesion of the superior labrum anterior and posterior; SLE, systemic lupus erythematosus; wk, weeks; y, year.

[a]Early tendinopathy is described in the original study as matched intact subscapularis tendon biopsies from the same shoulder of which the torn supraspinatus tendon biopsies were collected.

[b]Post-treatment pain-free samples were collected 1-3 y after treatment using a biopsy needle.

[c]IVD cells: consist of both annulus fibrosus and nucleus pulposus cells.

[d]The Pfirrmann score was used to distinguish between a mild disease group (grade II-III) and a severe disease group (grade IV-V).

[e]The Pfirrmann score was used to distinguish between an early disease group (grade I-II), an intermediate disease group (grade III), and an advanced disease group (grade IV-V)

[f]Described in original study as children admitted to the hospital for reasons not related to autoimmune, inflammatory, or infectious diseases.

[g]Described in original study as non-arthritic knee joints during arthroscopic surgery.

[h]Described in original study as patients without history of joint diseases or gross lesions from traumatic injuries, cruciate ligament tears, discoid cartilage or meniscus injuries, and also clinically and pathologically confirmed without joint lesion.

[i]Both human and animal studies.

TABLE 2 HMGB1 in animal models of musculoskeletal disease

Author (y)	Country	Disease model	Tissue type	HMGB1 measured in tissue	Administration of HMGB1	Blocking of HMGB1	No. of animals used for HMGB1 experiments	Age	Female
Soft MSK disease									
Lee (2018)	UK	Muscle injury	Muscle (whole tissue + in vivo)		✓		4 mice (fr-HMGB1) 4 mice (3S-HMGB1) 4 vehicle mice (PBS)	12-14 wk	100%
Tirone (2018)	Italy	Muscle injury	Muscle (whole tissue)		✓		9 injured mice (fr-HMGB1) 9 injured mice (ds-HMGB1) 9 injured mice (3S-HMGB1) 9 injured vehicle mice 9 control mice (no injury)	8 wk	NR
Thankam (2018)	USA	Tendinopathy	Rotator cuff tendon (whole tissue)	✓			7 rats (3-5 d post-injury) 7 rats (10-12 d post-injury) 7 rats (22-24 d post-injury)	8-10 wk	0%
Venereau (2012)	Italy	Muscle injury	Muscle (whole tissue)	✓			injured mice (CTX injection) (n = NR) vehicle mice (PBS injection) (n = NR)	8 wk	NR
Zhao (2019)	USA	Tendinopathy	Patellar and Achilles tendon (whole tissue)	✓			12 MTR mice 12 ITR mice 12 OTR mice 12 control (no treadmill running) mice	3 mo	100%
Bone disease									
Chen (2019)	China	Fracture	Bone (whole tissue + in vivo)		✓		30 injured rats (HMGB1) 30 injured control rats (no HMGB1)	NR	0%
Horst (2015)	Germany	Fracture	Fracture hematoma	✓			15 trauma pigs 5 sham pigs	3 mo	0%
Horst (2016)	Germany	Fracture	Fracture hematoma	✓			15 trauma pigs 5 sham pigs	3 mo	0%
Lee (2018)	UK	Fracture	Bone (in vivo)		✓	✓	10 mice (fr-HMGB1) 10 mice (3S-HMGB1) 10 mice (glycyrrhizin) 10 vehicle mice (PBS)	12-14 wk	100%

(Continues)

TABLE 2 (Continued)

Author (y)	Country	Disease model	Tissue type	HMGB1 measured in tissue	Administration of HMGB1	Blocking of HMGB1	No. of animals used for HMGB1 experiments	Age	Female
Xue (2017)	China	Fracture	Bone (whole tissue + in vivo)		✓		32 rats (64 tibial osteotomies): • 16 gelatin/HMGB1-CS • 16 gelatin/HMGB1 • 16 gelatin • 16 control (untreated)	12-13 wk	0%
Arthritis									
Ashour (2018)	Egypt	Arthritis (RA model)	Synovium (whole tissue)	✓			8 IA rats 8 control rats (no IA)	NR	NR
Biscetti (2016)	Italy	Arthritis (RA model)	Synovium (whole tissue + in vivo) and synovial fluid		✓	✓	30 IA mice: • 5 HMGB1 • 5 BoxA • 5 IA control (untreated)	8-12 wk	0%
Bitto (2011)	Italy	Arthritis (RA model)	Cartilage (whole tissue)	✓			7 IA mice 7 control mice (healthy)	6-7 wk	0%
Arthritis									
Guo (2011)	China	Arthritis (RA model)	Synovium (whole tissue)	✓			18 IA rats: • 9 early arthritis (28 d) • 9 advanced arthritis (49 d) 12 control (acetic acid) rats: • 6 early control (28 d) • 6 advanced control (49 d)	8-12 wk	NR
Hamada (2008)[c]	Japan	Arthritis (RA model)	Knee and paw joints (whole tissue + in vivo)	✓			IA mice (n = NR) control mice (n = NR)	5 wk	100%
						✓	5 IA rats (IgY anti-HMGB1 treatment) 5 IA control rats (PBS)	8-10 wk	NR
Heinola (2010)	Finland	OA	Cartilage (whole tissue)	✓			7 OA from 6 dairy bulls 6 control dairy bulls (healthy)	30-61 mo <20 mo	NR
Heinola (2013)	Finland	OA	Cartilage (whole tissue) and synovial fluid	✓			54 OA from 27 dairy bulls 6 control dairy bulls (healthy)	30-61 mo <20 mo	NR
Heinola (2014)	Finland	OA	Cartilage (whole tissue)	✓			54 OA from 27 OA culled bulls 6 control bulls (healthy + young)	mean: 56 mo mean: 19 mo	NR

(Continues)

TABLE 2 (Continued)

Author (y)	Country	Disease model	Tissue type	HMGB1 measured in tissue	Administration of HMGB1	Blocking of HMGB1	No. of animals used for HMGB1 experiments	Age	Female
Jiang (2017)[c]	China	OA	Cartilage (whole tissue)	✓			5 ACLT mice 5 sham mice	NR	NR
Kokkola (2002)[c]	Sweden	Arthritis (RA model)	Synovium (whole tissue)	✓			IA rats (n = NR) Control rats (n = NR)	NR	100%
Kokkola (2003)	Sweden	Arthritis (RA model)	Synovium (whole tissue + in vivo)			✓	42 IA mice: • 6 A-box • 6 untreated • 12 anti-HMGB1 antibody • 11 control antibody 11 IA rats (A-box)	6-8 wk 8-12 wk	0% 100%
Kyostioo-Moore (2011)	USA	OA	Synovium, ligament, meniscus (whole tissue)	✓			8 OA mice 5 control mice (healthy)	20 wk	0%
Arthritis									
Ley (2009)	Sweden	OA	Synovium (whole tissue)	✓			11 OA horses 10 OCF horses 4 control horses (healthy)	Mean: 4 (2-6) y Mean: 6 (2-12) y Mean: 4 (1-10) y	NR
Li (2011) (#2)	USA	Arthritis (RA model)	Cultured synovial fibroblasts		✓		RA and control mice (n = NR): • pre- and post-HMGB1 treatment (3-4 independent experiments)	7-8 wk	NR
Maicas (2010)	Spain	Arthritis	Ankle joint (whole tissue)	✓			8 arthritic mice[a] 8 naïve mice	10-12 wk	0%
Ostberg (2010)	Sweden	OA	Synovium (whole tissue + in vivo)	✓		✓	DNase II -/- x IFNRI -/- mice[b]: • 11 untreated • 7 BoxA • 8 vehicle (PBS) 4 control mice (WT)	6-13 wk	NR

(Continues)

TABLE 2 (Continued)

Author (y)	Country	Disease model	Tissue type	HMGB1 measured in tissue	Administration of HMGB1	Blocking of HMGB1	No. of animals used for HMGB1 experiments	Age	Female
Park (2015)[c]	Korea	Arthritis (RA model)	Knee joint (whole tissue)			✓	IA mice: • 5 vehicle • 5 treated with anti-HMGB1 antibody 5 control mice	8-12 wk	0%
Palmblad (2007)	Sweden	OA	Ankle joint (whole tissue)	✓			32 rats, 4 rats per time point: • 4 control rats/ non-OA (day 0) • 12 early OA (day 3,6 and 10) • 4 expected onset OA rats (day 15) • 4 peak severity OA rats (day 21) • 4 chronic OA rats (day 28) • 4 late OA rats (day 38)	NR	0%
Arthritis									
Pullerits (2003)	Sweden	OA	Knee joint (whole tissue)		✓		4 d: • 6 mice (1 μg HMGB1) • 13 mice (5 μg HMGB1) • 3 control mice (0.3 ng LPS) • 5 control mice (1 ng LPS) • 3 control mice (2 ng LPS) 7 d: • 19 mice (5 μg HMGB1) • 3 control mice (0.4 ng LPS) • 5 control mice (1 ng LPS) • 3 control mice (2 ng LPS) • 6 control mice (unmanipulated) 28 d: • 6 mice (5μg HMGB1) • control mice (LPS, n = NR)	6-8 wk	100%
Pullerits (2006)	Sweden	OA	Knee joint (whole tissue + in vivo)		✓		15 mice (PBS) 10 mice (2μg HMGB1)	6-8 wk	100%

(Continues)

TABLE 2 (Continued)

Author (y)	Country	Disease model	Tissue type	HMGB1 measured in tissue	Administration of HMGB1	Blocking of HMGB1	No. of animals used for HMGB1 experiments	Age	Female
Pullerits (2008)	Sweden	OA	Knee joint (whole tissue + in vivo)		✓		18 TNF-$\alpha^{-/-}$ mice: • 6 HMGB1 + LPS • 12 HMGB1 19 TNF-$\alpha^{+/+}$ mice: • 5 HMGB1 + LPS • 14 HMGB1	NR	NR
Seol (2012)	USA	(Traumatic) OA	Cultured chondrocytes from osteochondral explants		✓	✓	traumatic OA cattle explants (n = NR) and 3 human non-OA explants (bone tumor): • 4 impacted + HMGB1 • 4 impacted + glycyrrhizin • 4 impacted control (no HMGB1)	15-24 mo 29, 42, 46 y	N.R 0%.
Shafik (2019)	Egypt	Arthritis (RA model)	Paw joint (whole tissue)	✓			15 IA rats 15 control rats (healthy)	NR	100%
Arthritis									
Wang (2015)	China	Arthritis (RA model)	Synovium (whole tissue)	✓			10 RA rats 10 control rats	10-12 wk	0%
Yamada (2015)	Japan	Arthritis (RA model)	Muscle (whole tissue)	✓			IA rats (n = 3-6 muscles) vehicle control rats (PBS, n = 3-6 muscles)	8 wk	0%
Zhang (2019)	China	OA	Synovium (whole tissue)	✓			8 OA rats (ACLT) 8 control rats (healthy)	3 mo	0%

Abbreviations: 3S-HMGB1, all serine mutant HMGB1; ACLT, anterior cruciate ligament transection; BoxA (or A-box), HMGB1 antagonist; CTX, cardiotoxin; CXCL12, CXC motif chemokine12; DNase II, deoxyribonuclease type II; ds-HMGB1, disulfide HMGB1; fr-HMGB1, fully reduced HMGB1; HMGB1-CS, HMGB1 controlled-release composite combined with cell sheets; IA, induced arthritis; IFNRI, (gene) interferon receptor type I; ITR, intensive treadmill running; LPS, lipopolysaccharide; mo, months; MSK, musculoskeletal; MTR, moderate treadmill running; NR, not reported; OA, osteoarthritis; OCF, osteochondral fragments; OTR, one-time treadmill running; PBS, phosphate-buffered saline; RA, rheumatoid arthritis; TNF-α, tumor necrosis factor α; wk, weeks; WT, wild type; y, year(s).

[a]Arthritis model: arthritis was induced in these mice by injecting serum from arthritic mice intraperitoneally at 10mg/kg/day from days 0 to 10, after which the animals were sacrificed on day 11.

[b]Arthritis model: DNase $II^{-/-}$ X $IFNRI^{-/-}$ mice developed symmetric polyarthritis with strong extracellular HMGB1 expression in synovial tissue.

[c]Both human and animal studies.

in tendinopathic tissues compared to asymptomatic controls. Conversely, Mosca et al[23] showed an increase in HMGB1 protein expression in post-treatment pain-free tendons compared to tendinopathic and healthy tendons, with no differences between tendinopathic and healthy tendons. In frozen shoulder, HMGB1 protein expression was increased compared to control tissue.[25] In both diseases, HMGB1 was expressed by tissue-resident stromal cells and was mostly localized in the nuclear and perinuclear regions.[23-25]

3.1.2 | HMGB1 expression in human bone disease

Five studies investigated the role of HMGB1 in human bone disease, including IVD degeneration and fractures.[36,38,43,58,66] In both diseases, HMGB1 expression was increased in diseased tissues compared to control tissues, and was mainly localized intracellularly.[36,38,43,66] In IVD degeneration, HMGB1 was expressed by cells in the outer annulus (not specified), whereas in fracture hematoma, it was expressed by macrophages.[43,66] Lastly, Klawitter et al[58] investigated the administration of exogenous HMGB1 to cultured cells derived from degenerated IVDs and showed inhibition of IL-6 expression and no effect on IL-8 expression.

3.1.3 | HMGB1 expression in human arthritis

Twenty-seven studies investigated the role of HMGB1 in human arthritis, including (post-traumatic) OA, RA, JIA, SLE, and (pseudo)gout.[26,32,33,39,41,42,45,46,56-58,60,62,64,68,69,71,75-77,80-82,85,88-91] In 20 of 21 studies, HMGB1 expression was increased in diseased tissues compared to control tissues (Table 3).[26,32,39,41,42,45,56,60,64,68,69,71,76,77,80-82,88,89,91] The remaining study[90] found no difference between HMGB1 protein expression in fibroblast-like synovial cells derived from OA compared to RA patients. HMGB1 was mainly localized in nuclear and perinuclear regions, whereas three studies also showed extracellular HMGB1 expression in tissues from OA and RA patients.[32,42,71] Several cells expressed HMGB1, including chondrocytes, synovial fibroblasts, tenocytes, (vascular) endothelial cells, macrophages, and infiltrating inflammatory cells. Local administration of exogenous HMGB1 in arthritic tissues resulted in a pro-inflammatory environment in 11 of 11 studies (Table 4).[32,33,42,46,57,62,71,75,80,81,85]

3.2 | HMGB1 in animal models of musculoskeletal disease

Thirty-five studies investigated HMGB1 in animal models of musculoskeletal disease, including arthritis (26), fractures (5), muscle injury (3), and tendinopathy (2) (Table 2).[13,15,27,34,35,37,40,44,45,47-56,59-61,63,65,67,70-74,78,83,84,86,87] Lee *et al* (2018) investigated the role of HMGB1 in both animal models of fracture and muscle injury.[27] HMGB1 expression was increased in animal models of musculoskeletal disease in 22 of 23 studies (Table 3).[13,37,40,44,45,47-52,54-56,60,61,63,67,70,83,84,87]

3.2.1 | HMGB1 expression in animal models of soft tissue joint disease

Five studies investigated HMGB1 expression in animal models of muscle injury and tendinopathy.[13,15,27,48,83] Thankam et al[83] showed that HMGB1 protein expression was higher in early tendinopathic tissues compared to late tendinopathic and control tendons. Zhao et al[48] found that HMGB1 protein expression was increased in tendinopathic tissues compared to control tissue. Interestingly, tendinopathic tissues showed extracellular HMGB1 expression compared to control tissues where it was mainly localized in the nuclei of tenocytes.[48] In an animal model of muscle injury,[13] HMGB1 protein expression was increased in

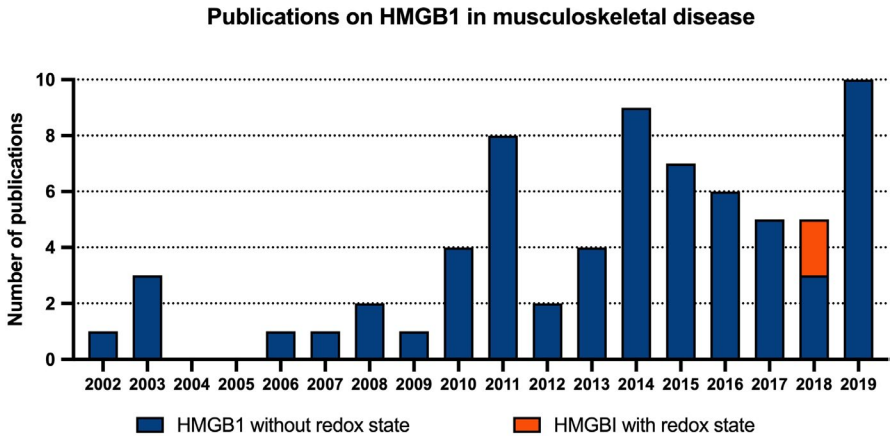

FIGURE 2 Number of publications on HMGB1 in musculoskeletal disease. Number of publications on HMGB1 in musculoskeletal disease throughout the years. There is an increase in publication frequency of articles on HMGB1 in musculoskeletal disease over the past two decades. Yet, only two articles investigated the redox status of HMGB1 in musculoskeletal disease

TABLE 3 Results—HMGB1 expression in musculoskeletal tissue

Author (y)	Disease	Methods	Control type	Localization HMGB1	Cell types expressing HMGB1	HMGB1 compared to control?	HMGB1: pro-inflammatory	HMGB1: non-inflammatory
Human								
Soft MSK disease								
Akbar (2017)	Tendinopathy Early tendinopathy[a]	ELISA Immunostaining PCR	Hamstring tendon (whole tissue)	Nuclear and perinuclear regions	Stromal cells, immune cells	↑ ↑↑	✓	
Mosca (2017)	Tendinopathy Post-treatment pain-free tendinopathy[b]	Immunostaining	Hamstring tendon (whole tissue)	Nuclear and perinuclear regions	Stromal cells, macrophages	= ↑		✓
Cher (2018)	Frozen shoulder	Immunostaining	Unstable shoulder (whole tissue)	Nuclear and perinuclear regions	Stromal cells	↑	✓	
Bone disease								
Gruber (2015)	IVD degeneration	Immunostaining Micro-array analysis	Donor disk (whole tissue)	Intracellular	Cells in the outer annulus	↑	✓	
Liu (2019)	IVD degeneration	PCR Western blotting	Mild disease nucleus pulposus (whole tissue)[c]	NA	NA	↑	✓	
Shah (2019)	IVD degeneration	PCR	Early and intermediate disease disk (whole tissue)[d]	NA	NA	↑	✓	
Bone disease								
Li (2015)	Fracture	ELISA PCR Western blotting	Hematoma from surgical bleeding bone	Intracellular (mainly cytosol)	Macrophages	↑	✓	
Arthritis								
Amin (2014)	OA	Gene expression arrays Immunostaining PCR	Fracture cartilage (whole tissue)	Nuclei, cytosol and extracellular	Chondrocytes	↑	✓	
Bobek (2014)	JIA and SLE	ELISA	JIA and SLE serum	NA	NA	↑	✓	
Cai (2019)	RA	PCR	Cultured synovial fibroblasts (OA)	NA	NA	↑	✓	

(Continues)

TABLE 3 (Continued)

Author (y)	Disease	Methods	Control type	Localization HMGB1	Cell types expressing HMGB1	HMGB1 compared to control?	HMGB1: pro-inflammatory	HMGB1: non-inflammatory
Ding (2019)	Post-traumatic OA	ELISA	Synovial fluid acute ACL injury	NA	NA	↑	✓	
Garcia-Arnandis (2010)	OA	ELISA Immunostaining PCR Western blotting	Cadaveric synovium (whole tissue)	Control: nuclei OA: cytoplasm (and extracellular)	Cells of the synovial (sub) lining, vascular endothelial cells, infiltrated cells	↑	✓	
Hamada (2008)[g]	RA and pseudogout.	ELISA	OA synovial fluid	NA (see animal model)	NA (see animal model)	↑	✓	
Huang (2019)	OA	Immunostaining	Control synovium (whole tissue)	NR	Chondrocytes	↑	✓	
Jiang (2017)[g]	OA	PCR Western blotting	Fracture cartilage (whole tissue)	NA	NA	↑	✓	
Kokkola (2002)[g]	RA	Immunostaining	OA synovium (whole tissue)	OA: mainly nuclei RA: mainly cytoplasm	Synovial cells, chondrocytes, macrophage-like cells	↑	✓	
Li (2016)	RA	Immunostaining PCR Western blotting	OA synovium (whole tissue)	NR	Synovial fibroblasts	↑	✓	
Human								
Arthritis								
Li (2011)	OA	ELISA	Traumatic synovial fluid[e]	NA	NA	↑	✓	
Mitroulis (2011)	Gout	Immunostaining Western blotting	Serum (PMNs)	NR	Synovial cells and PMNs	↑	✓	
Park (2015)[g]	RA	Immunostaining	OA synovium (whole tissue)	Cytoplasm and extracellular	Synovial cells	↑	✓	
Rosenberg (2017)	OA	Immunostaining PCR	OA cartilage hip (whole tissue)	Nuclei	Chondrocytes	↑	✓	
Schierbeck (2013)	JIA	ELISA	Healthy serum	NA	NA	↑	✓	

(Continues)

TABLE 3 (Continued)

Author (y)	Disease	Methods	Control type	Localization HMGB1	Cell types expressing HMGB1	HMGB1 compared to control?	HMGB1: pro-inflammatory	HMGB1: non-inflammatory
Sun (2016)	OA	Immunostaining PCR Western blotting	Non-OA synovium[f] (whole tissue)	Mainly cytoplasm, less in nuclei	Synovial lining cells, inflammatory cells, vascular endothelial cells	↑	✓	
Taniguchi (2003)	RA	ELISA Immunostaining PCR Western blotting	OA synovial fluid	OA: NR RA: mainly cytoplasm	Sublining cells, synovial macrophages	↑	✓	
Terada (2011)	OA	Immunostaining PCR	Control cartilage (whole tissue)	Control: mainly nuclei OA: mainly cytoplasm	Chondrocytes	↑	✓	
Thankam (2016)	OA	Immunostaining PCR	Biceps tendon (SLAP tear) (whole tissue)	intracellular	Inflammatory cells, tenocytes	↑	✓	
Wang (2019)	OA	PCR	Control cartilage (whole tissue)	NA	NA	↑	✓	
Xu (2015)	RA	Western blotting	Cultured synovial fibroblasts (OA)	NA	Synovial fibroblasts	=	✓	
Animal								
Soft MSK disease								
Thankam (2018)	Early tendinopathy (3-5 d post-injury)	Immunostaining	10-12 d post-injury rat tendon (whole tissue)	NR	NR	↑	✓	
			20-22 d post-injury rat tendon (whole tissue)			↑↑		
Zhao (2019)	Tendinopathy	ELISA Immunostaining Western blotting	Healthy control mice tendon (whole tissue)	Control/MTR: nuclei ITR/OTR: extracellular	tenocytes	↑	✓	
Venereau (2012)	Muscle injury	Western blotting	Vehicle mice (PBS) muscle (whole tissue)	NA	NA	↑	✓	✓

(Continues)

TABLE 3 (Continued)

Author (y)	Disease	Methods	Control type	Localization HMGB1	Cell types expressing HMGB1	HMGB1 compared to control?	HMGB1: pro-inflammatory	HMGB1: non-inflammatory
Bone disease								
Horst (2015)	Fracture (t = 0h)	ELISA	Fracture hematoma at t = 48h in injured pigs	NA	NA	↓	✓	
Horst (2016)	Fracture (t = 0h)	ELISA	Fracture hematoma at t = 14h, 24h, and 48h in injured and sham pigs	NA	NA	↓	✓	
Arthritis								
Ashour (2018)	Arthritis (RA model)	ELISA Western blotting	Healthy rat synovium (whole tissue)	NA	NA	↑	✓	
Bitto (2011)	Arthritis (RA model)	PCR	Healthy mice cartilage (whole tissue)	NA	NA	↑	✓	
Guo (2011)	Arthritis (RA model)	FCM Immunostaining	Synovium control (acetic acid) rats (whole tissue)	Control: mainly nuclei arthritis: cytoplasm and extracellular	Synoviocytes	↑	✓	
Hamada (2008)[g]	Arthritis (RA model)	Immunostaining	Healthy mice paw joint (whole tissue)	Arthritis: cytoplasm and extracellular colocalized with tissue hypoxia	Synovial cells, inflammatory cells	↑	✓	
Animal								
Arthritis								
Heinola (2010)	OA	Immunostaining	Healthy bull cartilage (whole tissue)	Healthy: nuclei mild OA: mainly nuclei moderate/severe OA: mainly extracellular and cytoplasm	Chondrocytes	↑	✓	
Heinola (2013)	OA	ELISA Immunostaining	Healthy bull cartilage (whole tissue)	HEALTHY: nuclei OA: mainly nuclei, cytoplasm in severe OA	Chondrocytes	↑	✓	
Heinola (2014)	OA	Immunostaining	Healthy bull cartilage (whole tissue)	Healthy: nuclei OA: cytoplasm severe OA: extracellular	Chondrocytes	↑	✓	
Jiang (2017)[g]	OA	Immunostaining	Healthy (sham) mice cartilage (whole tissue)	NR	Chondrocytes	↑	✓	

(Continues)

TABLE 3 (Continued)

Author (y)	Disease	Methods	Control type	Localization HMGB1	Cell types expressing HMGB1	HMGB1 compared to control?	HMGB1: pro-inflammatory	HMGB1: non-inflammatory
Kokkola (2002)[g]	Arthritis	Immunostaining	Healthy rat synovium (whole tissue)	Healthy: mainly nuclei arthritis: mainly cytoplasm	Fibroblast-like cells, macrophage-like cells, synovial cells, chondrocytes	↑	✓	
Kyostioo-Moore (2011)	OA	Immunostaining	Healthy mice synovium, cartilage, ligament, meniscus, periosteum (whole tissue)	Mainly intracellular and occasionally extracellular	Multiple cell types (eg, chondrocytes, synovial cells, osteoclasts)	↑	✓	
Ley (2009)	OA	Immunostaining	Healthy horse synovium (whole tissue)	Healthy: mainly nuclei OA: cytoplasm, extracellular	Cells of the lining layer, subintimal stroma and endothelium (chondrocytes)	↑	✓	
Animal								
Arthritis								
Maicas (2010)	Arthritis	Immunostaining PCR	Healthy mice ankle joint (whole tissue)	NR	Synovial cells, chondrocytes, cellular infiltrates	↑	✓	
Ostberg (2010)	OA	Immunostaining ELISA Western blotting	Synovium (whole tissue) of 1) non-arthritic DNase $II^{-/-}$ X $IFNRI^{-/-}$ mice and 2) healthy control mice.	Both controls: mainly nuclei OA: cytoplasm (in lysosomal vacuoles), extracellular	Synovial cells, macrophages	↑	✓	
Palmblad (2007)	OA	Immunostaining	Healthy mice ankle joint (whole tissue)	Healthy: mainly nuclei OA: cytoplasm and as severity increases extracellular.	Endothelium cells, chondrocytes, synovial cells, macrophage-like cells	↑	✓	

(Continues)

TABLE 3 (Continued)

Author (y)	Disease	Methods	Control type	Localization HMGB1	Cell types expressing HMGB1	HMGB1 compared to control?	HMGB1: pro-inflammatory	HMGB1: non-inflammatory
Shafik (2019)	Arthritis (RA model)	ELISA	Healthy rat paw joint (whole tissue)	NA	NA	↑	✓	
Wang (2015)	Arthritis (RA model)	PCR Western blotting	Healthy rat synovium (whole tissue)	NA	NA	↑	✓	
Yamada (2015)	Arthritis (RA model)	Western blotting	Healthy rat muscle (whole tissue)	NA	NA	↑	✓	
Zhang (2019)	OA	PCR Western blotting	Healthy rat synovium (whole tissue)	NA	NA	↑	✓	

Abbreviations: ACL, anterior cruciate ligament; DNase II, deoxyribonuclease type II; ELISA, enzyme-linked immunosorbent assay; FCM, flow cytometry; h, hours; IFNRI, (gene) interferon receptor type I; ITR, intensive treadmill running; IVD, intervertebral disk; JIA, juvenile idiopathic arthritis; MSK, musculoskeletal; MTR, moderate treadmill running; NA, not applicable; NR, not reported; OA, osteoarthritis; OTR, one-time treadmill running; PBS, phosphate-buffered saline; PCR, quantitative polymerase chain reaction; PMN, polymorphonuclear cells; RA, rheumatoid arthritis; SLAP, lesion of the superior labrum anterior and posterior; SLE, systemic lupus erythematosus; y, year.

[a]Early tendinopathy is described in the original study as matched intact subscapularis tendon biopsies from the same shoulder of which the torn supraspinatus tendon biopsies were collected.

[b]Post-treatment pain-free samples were collected 1-3 y after treatment using a biopsy needle.

[c]The Pfirrmann score was used to distinguish between a mild disease group (grade II-III) and a severe disease group (grade IV-V).

[d]The Pfirrmann score was used to distinguish between an early disease group (grade I-II), an intermediate disease group (grade III), and an advanced disease group (grade IV-V)

[e]Described in original study as patients with traumatic intra-articular knee joint injury (eg, meniscal or cruciate ligament tears).

[f]Described in original study as patients without history of joint diseases or gross lesions from traumatic injuries, cruciate ligament tears, discoid cartilage or meniscus injuries, and also clinically and pathologically confirmed without joint lesion.

[g]Both human and animal studies.

TABLE 4 Results—Local administration or inhibition of HMGB1 to musculoskeletal tissue

Author (y)	Disease	Tissue	Addition of HMGB1	Blockage of HMGB1	Exogenous HMGB1	Outcomes
Human						
Bone disease						
Klawitter (2014)	IVD degeneration	Cultured IVD cells[a]	✓		Recombinant human HMGB1 (Abnova)	• inhibits IL-6 expression. • no effect on IL-8 expression.
Arthritis						
Amin (2014)	OA	Cultured chondrocytes	✓		Recombinant *E coli* generated HMGB1[b]	• stimulates mRNA of 2 NF-κB gene enhancers, 16 different CCL and CXC chemokines. • induces inducible NO synthase, NO and IL-8.
Benedetti (2016)	RA and OA	Cultured synovial cells	✓		Recombinant human HMGB1 (R&D systems)	• increases Amigo2 expression. • decreases Cd-mediated cellular toxicity.
Garcia-Arnandis (2010)	OA	Cultured chondrocytes	✓		Recombinant human HMGB1 (HMGBiotech)	in combination with IL-1β: • increases NF-κB activation and expression of IL-6, IL-8, CCL2, CCL20, MMP-1, and MMP-3. • increases phosphorylated ERK1/2 and p38 levels
He (2013)	RA	Cultured synovial fibroblasts	✓		Recombinant human HMGB1 (Sigma-Aldrich)	• increases MMP-13 expression. • in combination with LPS: increases IL-6, MMP-3, and MMP-13, and upregulates RAGE and TLR4 expression. • in combination with LPS: increases phosphorylated p38 and phosphorylated IκB.
Kim (2014)	RA	Cultured synovial fibroblasts	✓		Recombinant human HMGB1 (R&D systems)	• increases tube formation in microvascular endothelial cells. • increases expression of HIF-1α, VEGF, phosphorylated IκB, phosphorylated NF-κB p65, and SIRT1.
Leclerc (2013)	RA and JIA	Cultured synovial fibroblasts	✓		Recombinant rat HMGB1 expressed in *E coli* [b]	in combination with IL-1β: increases mPGES-1, COX-2, PGE_2 production.
Park (2015)[f]	RA	Cultured synovial fibroblasts	✓		Recombinant human HMGB1 (R&D systems)	• increases tube formation in microvascular endothelial cells. • increases expression of HIF-1α and VEGF.

(Continues)

TABLE 4 (Continued)

Author (y)	Disease	Tissue	Addition of HMGB1	Blockage of HMGB1	Exogenous HMGB1	Outcomes
Qin (2014)	RA and OA	Cultured synovial fibroblasts	✓		Not clear.	In combination with LPS: • induces transformation of human synovial fibroblasts to RA-like synovial fibroblasts. • reduces apoptosis and enhances autophagy. • upregulates chemokines, adhesion molecules, inflammatory cytokines, and MMPs.
Taniguchi (2003)	RA	Cultured synovial macrophages	✓		Purified calf thymus HMGB1 (Shino-Test Corporation)	• increases expression of TNF-α, IL-1β, and IL-6. • upregulation of RAGE.
Terada (2011)	OA	Cultured chondrocytes	✓		Recombinant human HMGB1[c]	• stimulates production of IL-1β and TNF-α.
Wahamaa (2011)	RA	Cultured synovial fibroblasts	✓		Recombinant rat HMGB1 expressed in *E coli*[b]	• in combination with LPS, IL-1α or IL-1β: enhances production of TNF, IL-6, and IL-8. • in combination with IL-1β: increases MMP production.
Animal						
Soft MSK disease						
Lee (2018)	Muscle injury	Muscle (whole tissue + in vivo)	✓		Recombinant fr-HMGB1 and 3S-HMGB1 (HMGBiotech)[d]	• in vivo: both fr-HMGB1 and 3S-HMGB1 accelerate muscle repair (even if administered 2 wk before the injury). • ex vivo: increased muscle fiber cross-sectional area
Tirone (2018)	Muscle injury	Muscle (whole tissue)	✓		Recombinant WT fr-HMGB1 or 3S-HMGB1 (HMGBiotech)[d]	• fr-HMGB1 coordinates muscle and liver regeneration via CXCR4 by acting on resident muscle stem cells, hepatocytes, and infiltrating cells. • 3S-HMGB1 does not promote cytokine production but promotes muscle and liver regeneration more efficiently.
Bone disease						
Chen (2019)	Fracture	Bone (whole tissue + in vivo)	✓		Recombinant human HMGB1 (Sigma-Aldrich)	• in vivo: increases clinical scores (radiographs, peak torque, torsional stiffness, bone volume). • ex vivo: accelerates healing by increasing expression of osteogenesis-related genes.

(Continues)

TABLE 4 (Continued)

Author (y)	Disease	Tissue	Addition of HMGB1	Blockage of HMGB1	Exogenous HMGB1	Outcomes
Lee (2018)	Fracture	Bone (in vivo)	✓		Recombinant human fr-HMGB1 and 3S-HMGB1 (HMGBiotech)[d]	both fr-HMGB1 and 3S-HMGB1 promote/accelerate osteogenic differentiation and fracture healing (even if administered 2 wk before the injury).
Xue (2017)	Fracture	Bone (whole tissue + in vivo)	✓		Recombinant human HMGB1 (Prospec) on gelatin sponge scaffolds.	• in vivo: HMGB1 gelatin sponge scaffolds increase bone formation • ex vivo: promotes osteogenic differentiations of mesenchymal stem cells through the STAT3 pathway.
Arthritis						
Biscetti (2016)	Arthritis (RA model)	Synovium (whole tissue + in vivo) and synovial fluid	✓		Recombinant human HMGB1 (HMGBiotech)	• in vivo: worsening of arthritis • ex vivo: upregulates VEGF, and increases synovial angiogenesis and overall worsening of arthritis
				✓	BoxA (HMGBiotech)	• in vivo: clinical reduction of arthritis • ex vivo: reduced inflammatory infiltrate, reduced angiogenesis, preservation of joint morphology (histology), and reduced levels of TNF-α, IL-1β, IL-6, VEGF, and IL-17A in synovial fluid
Hamada (2008)[f]	Arthritis (RA model)	Knee and paw joints (whole tissue + in vivo)		✓	IgY anti-HMGB1[e]	• in vivo: ameliorates clinical joint inflammation • ex vivo: reduces histopathologic abnormalities
Kokolla (2003)	Arthritis (RA model)	Synovium (whole tissue + in vivo)		✓	A-box and anti-HMGB1 antibodies[f]	• in vivo: reduces mean arthritis score and disease-induced weight loss • ex vivo: reduces the histologic severity of arthritis (preservation of joint morphology and less IL-1β–producing cells)
Li (2011)	Arthritis (RA model)	Cultured synovial fibroblasts	✓		Recombinant human HMGB1 (Prospec)	increases β-arrestin 1 expression (and not β-arrestin 2).
Ostberg (2010)	Arthritis (RA model)	Synovium (whole tissue + in vivo)		✓	BoxA (HMGBiotech)	• in vivo: improves clinical arthritis scores • ex vivo: ameliorates histologic severity of arthritis (reduces synovial hyperplasia, inflammatory cell influx, depletion of cartilage matrix, and bone erosions)

(Continues)

TABLE 4 (Continued)

Author (y)	Disease	Tissue	Addition of HMGB1	Blockage of HMGB1	Exogenous HMGB1	Outcomes
Park (2015)[f]	Arthritis (RA model)	Knee joint (whole tissue)		✓	Anti-HMGB1–neutralizing antibody (Shino-test Co.)	• ameliorates inflammation features and histologic arthritis scores • attenuates HIF-1α and VEGF • reduces number of synovial vessels
Pullerits (2003)	OA	Knee joint (whole tissue + in vivo)	✓		Recombinant *E coli* generated mouse HMGB1[b]	• in vivo: induces clinical arthritis. • ex vivo: activates NF-κB pathway leading to IL-1 production
Pullerits (2006)	OA	Knee joint (whole tissue + in vivo)	✓		Recombinant *E coli* generated mouse HMGB1[b]	• in vivo: induces clinical arthritis • ex vivo: influx of inflammatory, mostly mononuclear, cells throughout synovial tissue
Arthritis						
Pullerits (2008)	OA	Knee joint (whole tissue + in vivo)	✓		Recombinant *E coli* generated mouse HMGB1[b]	• in vivo: induces clinical arthritis • no differences were found between normal mice and TNF-α knockout mice (thus HMGB1-induced arthritis is not mediated via the TNF pathway)
Seol (2012)	(Traumatic) OA	Cultured chondrocytes from osteochondral explants	✓		Purified HMGB1 (not further specified)	Enhances chemotaxis on injured explants
				✓	Glycyrrhizin	Inhibits cell migration/ proliferation on injured explants

Note: Production and purification of A-box protein of HMGB1, and production of anti-HMGB1 antibodies as described in original study by Kokkola et al, 2003.

Abbreviations: 3S-HMGB1, all serine mutant HMGB1; CCL, chemokine group CC; Cd, cadmium; COX, cyclooxygenase; CXC, chemokine group CXC; CXCR, CXC chemokine receptors; *E coli*, *Escherichia coli*; ERK, extracellular signal–regulated kinase; fr-HMGB1, fully reduced HMGB1; HIF-1α, hypoxia-inducible factor 1-alpha; IL, interleukin; IVD, intervertebral disk; IκB, nuclear factor of kappa light polypeptide gene enhancer in B-cell inhibitor; JIA, juvenile idiopathic arthritis; LPS, lipopolysaccharides; MMP, matrix metalloproteinases; mPGES-1, microsomal prostaglandin E synthase-1; mRNA, messenger ribonucleic acid; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NO, nitric oxide; OA, osteoarthritis; p38, group of mitogen-activated protein kinases; p65, nuclear factor NF-κB p65 subunit; PGE_2, prostaglandin E2; RA, rheumatoid arthritis; RAGE, receptor for advanced glycation end products; siRNA, small interfering ribonucleic acid (RNA); SIRT1, Sirtuin 1, also known as NAD-dependent deacetylase sirtuin-1; STAT, signal transducer and activator of transcription; TLR, Toll-like receptor; TNF-α, tumor necrosis factor α; VEGF, vascular endothelial growth factor; WT, wild type; y, year.

[a]IVD cells: consist of both annulus fibrosus and nucleus pulposus cells.

[b]Complement DNA was cloned and expressed in *Escherichia coli*, and recombinant HMGB1 was generated as described in original studies Wang et al, 1999, Paonessa et al, 1987, or Knapp et al, 2004.

[c]LPS-free, recombinant human HMGB1 was produced in Sf9 cells. Full-length human HMGB1 DNA was amplified from human microvascular endothelial cells (Nippon Gene, Tokyo, Japan). See full article by Terada et al for a detailed description of how recombinant HMGB1 was obtained.

[d]A mutant (3S) isoform of HMGB1 in which the cysteines are replaced with serines, which are resistant to oxidation. This isoform behaves as the fully reduced HMGB1; that is, it has a chemoattractant function.

[e]A neutralizing antibody against HMGB1 was prepared by isolating and purifying IgY-class antibody from egg yolks of hens immunized with whole HMGB1 protein (as previously done in original study Hassl & Aspock, 1988).

[f]Both human and animal studies.

injured tissue compared to control tissue. The effects of addition of exogenous HMGB1[15,27] depended on the redox status of HMGB1: fully reduced HMGB1 resulted in accelerated muscle repair, whereas disulfide HMGB1 did not (Table 4). Interestingly, a dose-response has also been demonstrated; a non-oxidizable mutant form of fully reduced HMGB1 (3S-HMGB1) does not promote inflammation, but does promote muscle repair more efficiently than fully reduced HMGB1 (as it does not need CXCL12 to bind to the CXCR4 receptor).[15,27]

3.2.2 | HMGB1 expression in animal models of bone disease

Five studies investigated the role of HMGB1 in animal models of fractures.[27,53-55,86] Two animal fracture model studies showed that HMGB1 expression was higher in early fracture hematoma compared to late fracture hematoma.[54,55] In addition, Horst et al[54] showed that HMGB1 protein expression was higher in fracture hematoma compared to sham animals. The localization of HMGB1 within the cell was not investigated.[54,55] The addition of exogenous HMGB1 on fracture sites increased the expression of osteogenesis-related genes and promoted osteogenic differentiation (Table 4).[27,53,86] In accordance, Lee et al[27] showed that treatment with glycyrrhizin (a known HMGB1 inhibitor) resulted in delayed fracture healing. Lastly, just as in muscle injury, fully reduced HMGB1 (and the non-oxidizable mutant 3S-HMGB1 even more so) promoted fracture repair.[27] Other redox states of HMGB1 were not investigated.

3.2.3 | HMGB1 expression in animal arthritis models

Twenty-six studies investigated the role of HMGB1 in animal models of arthritis, including OA and RA.[13,15,27,34,35,37,40,44,45,47-56,59-61,63,65,67,70-74,78,83,84,86,87] In all 18 studies, HMGB1 expression was increased in diseased tissues compared to control tissues.[35,37,40,44,45,47,49-52,56,60,61,63,67,70,84,87] In control tissues, HMGB1 was mainly localized in the nuclei, whereas arthritic tissues showed more cytoplasmic and extracellular HMGB1 expression as the severity increased. Both stromal cells and infiltrating inflammatory cells expressed HMGB1, including chondrocytes, synovial cells, endothelial cells, osteoclasts, and fibroblast- and macrophage-like cells (Figure 3). Local administration of exogenous HMGB1 in arthritic tissues resulted in a pro-inflammatory environment in 6 of 6 studies, including worsening of clinical and histopathologic arthritis, increase in synovial angiogenesis, and enhancement of chemotaxis (Table 4).[34,65,72-74,78] Finally, 5 of 6 studies found that directly blocking HMGB1 in arthritic tissues (with anti-HMGB1 antibodies or BoxA) ameliorates arthritis by improving clinical arthritis scores, reducing

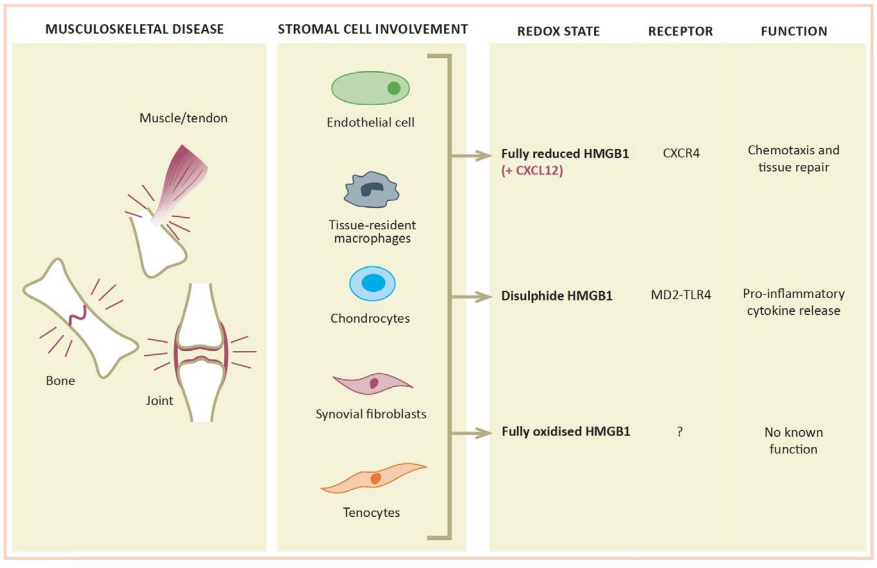

FIGURE 3 HMGB1 and its redox status in musculoskeletal disease. Schematic highlighting the diverse roles of HMGB1 as a driver of inflammation and mediator of tissue repair in musculoskeletal disease. Upon tissue injury, tissue-resident stromal cells (and later infiltrative immune cells, not shown) release HMGB1 into the extracellular environment. We hypothesize that depending on the redox state, HMGB1 will act on several receptors promoting either chemotaxis, tissue repair, or inflammation. Fully reduced HMGB1 will form a heterocomplex with CXCL12 after which it acts on the CXCR4 receptor to promote chemotaxis and tissue repair. Disulfide HMGB1 will act on the MD2-TLR4 receptor (and other receptors which are not shown here), which will promote pro-inflammatory cytokine release. Receptors for fully oxidized HMGB1 have not yet been identified, and therefore, its potential immunomodulatory function is unknown

inflammatory infiltrate, reducing number of synovial vessels, and preserving joint morphology.[34,45,59,70,71] The remaining study[78] found that treatment with glycyrrhizin reduced the number of migrating cells (ie, chemotaxis) on injured explants. The redox states of HMGB1 were not investigated.

4 | DISCUSSION

Since the beginning of this century, studies on HMGB1 in musculoskeletal disease have emerged. The growing number of publications in this field demonstrates a rising interest of HMGB1 in musculoskeletal disease. However, to date no studies have curated these data and compared the role of HMGB1 in a variety of musculoskeletal diseases (Figure 2). Therefore, the overall aim of this study was to perform a systematic review to compare the roles of HMGB1 across multiple common musculoskeletal diseases.

In general, HMGB1 expression is increased in tissues of both human and animal models of musculoskeletal disease compared to control tissues, as well as in early (acute) disease compared to late (chronic) disease.[13,24-26,32,33,35-52,54-58,60-64,66-71,75-77,80-85,87-89,91] HMGB1 is expressed by both tissue-resident stromal cells and infiltrating immune cells.[24-26,32,33,42-48,50-52,56-58,60-64,66,67,69-71,75,76,80-82,85,88] Local administration of HMGB1 to (cultured cells derived from) arthritic tissues worsened both clinical and histopathologic arthritis, increased synovial angiogenesis, and enhanced chemotaxis.[32-34,42,46,57,62,65,71-75,78,81,85,92] In contrast, addition of HMGB1 to cultured cells derived from human degenerate IVDs reduced IL-6 expression and did not affect IL-8 expression.[58] Therefore, one could conclude that HMGB1 might have a protective effect in IVDs, whereas it has a pro-inflammatory effect in arthritic tissues. Another explanation is that these divergent biological functions may be attributed to differential HMGB1 redox states as a consequence of an extracellular environment.[14,93-97] However, the HMGB1 redox status remains understudied in human musculoskeletal disease.

4.1 | Cellular localization of HMGB1

In healthy control tissues, HMGB1 was mainly localized in the nucleus, while in diseased tissues, HMGB1 was localized mainly in the cytoplasm. Although a few articles showed extracellular expression of HMGB1 in arthritic human and animal tendinopathic tissues, the majority of histologic studies did not reveal extracellular expression of HMGB1. Since extracellular HMGB1 has a relatively short half-life, it might have degraded before the tissue is used for experiments.[98] Interestingly, several histologic studies showed there was an increase in cytoplasmic HMGB1 expression (number of immunopositive cells or area of positive immunostaining). Several studies have shown that cytoplasmic translocation of HMGB1 promotes autophagy, limits the apoptotic pathway, and does not only enable the release but also actively promotes its own secretion.[99,100] So far, literature has shown that HMGB1 contains nuclear localization sequences, which contain lysine and serine residues that are susceptible to modification (eg, acetylation, methylation, phosphorylation).[18,19,101] Once modified, HMGB1 is actively secreted out of the nucleus and consecutively out of the cell via secretory lysosomes.[19] Interestingly, necrotic cells passively leak non-modified HMGB1, still able to initiate/sustain inflammation, whereas cells undergoing apoptosis do not leak any HMGB1.[102] It is hypothesized that in apoptotic cells, HMGB1 inextricably binds to chromatin, making it unable to promote inflammation. However, these processes are not yet fully understood. Therefore, future studies should not only investigate the extracellular functions of HMGB1, but also, the intracellular functions of HMGB1 during inflammation should be studied.

4.2 | Extracellular HMGB1: redox states

Extracellular HMGB1 has the ability to initiate and/or sustain an inflammatory microenvironment by switching between different redox states. Although the redox status is crucial feature of HMGB1 as this determines its biological role, few articles discussed these redox states in musculoskeletal disease and only 2 of 66 of the included articles investigated the redox status of HMGB1 in musculoskeletal diseased tissues.[15,27] These studies identified that fully reduced HMGB1 promotes not only chemotaxis, but can also promote tissue repair by acting on the CXCR4 receptor after forming a heterocomplex with CXCL12, whereas disulfide HMGB1 cannot.[15] In addition, pre-treatment with (fully reduced) HMGB1 2 weeks before the fracture accelerated fracture repair.[27] Lee et al[27] found that this is due to resident stem cells being primed (ie, G_{Alert} phase) by fully reduced HMGB1, so that they can re-enter the cell cycle quicker upon new injuries. Moreover, a non-oxidizable mutant form of fully reduced HMGB1 (3S-HMGB1) promoted muscle and fracture repair even more effectively by binding directly to the CXCR4 receptor on resident stem cells without needing the chemokine CXCL12.[15,27] Fully oxidized HMGB1 was not investigated, and whether it has a function remains unknown. Although not in musculoskeletal diseased tissues, Venereau et al (2012), Lee et al (2018), and Tirone et al (2018) investigated the effects of different HMGB1 redox states in cell lines, which were in line with research on HMGB1 in other areas.[13-15,20-22,27,93,95,96] Therefore, it is likely that these redox states have the same function in musculoskeletal disease

(Figure 3). Thus, these findings are in line with literature in other inflammatory diseases and support the idea that the localization and the diverse biological functions of HMGB1 depend on its post-translational modifications and its redox status.[13,14,20-22,93,95,96]

4.3 | Challenges in identifying the redox status of HMGB1 in musculoskeletal disease

Studying the redox states of HMGB1 is challenging as few techniques can distinguish between different redox states of HMGB1 in human tissue. Since there are no specific antibodies available for each HMGB1 redox state, researchers are urged to be creative with other existing methods. One of the most successful techniques has been liquid chromatography-tandem mass spectrometry, which has been successfully used to identify the redox states of HMGB1 in liquid samples such as serum or cerebrospinal fluid, although it remains technically challenging in tissue samples.[103-106] Furthermore, mass spectrometry requires tissue preparation and protein extraction steps before analysis with the mass spectrometer, which raises the question whether alterations in redox state occur before the samples can be analyzed due to the short half-life of HMGB1. Although variations in half-life have been found in different extracellular environments, fully reduced HMGB1 has been shown to have a half-life of approximately 17 minutes in serum and saliva.[98] However, the half-life of HMGB1 in musculoskeletal tissues is unknown.[98] The difficulties of identifying the different redox states of HMGB1 in musculoskeletal disease are thus partially inherent to the complexity of HMGB1, and partially because current methodologies have yet to be optimized or developed.

4.4 | Limitations

The heterogeneity of the included study populations and measurement methods of HMGB1 can both be considered a limitation and a strength of this study. Although all included studies are covered by the term musculoskeletal disease, these diseases differ in pathogenesis. However, this heterogeneity is also a strength since this is the first study to compare the different roles of HMGB1 across a variety of musculoskeletal diseases, thereby exploring whether the observed effects of HMGB1 can be applied to all musculoskeletal tissues and diseases. The small number of studies published on HMGB1 in musculoskeletal disease reflects that this is still a small but growing field. Although recently more studies have looked at HMGB1 in musculoskeletal disease, they often lack a (healthy) control or do not make use of human tissues or cells. A risk with the small number of studies on relatively large number of different conditions in this review is that results might be generalized, especially as the only results from studies that could be included is the concentration of HMGB1 in diseased tissue compared to a healthy control. However, most studies that have been included in this review have come to similar findings. Finally, a quality assessment was not included in this review due to the lack of validated quality assessments available for the type of *in vitro* and *in vivo* studies included. New quality assessment tools should be validated for these types of studies.

4.5 | Therapies targeting HMGB1 in musculoskeletal diseases

The results from the studies included in this article suggest that the addition or direct blocking of HMGB1 could be further investigated as a potential treatment for a variety of musculoskeletal diseases. In human and animal arthritic tissue, the addition of exogenous HMGB1 exacerbated disease, while directly blocking HMGB1 reduced disease severity by ameliorating inflammation. As there are currently no disease-modifying treatments available for osteoarthritis and clinical remission of RA is still a large issue, HMGB1 inhibitors could provide a new treatment option. In animal models of fractures and muscle injuries, addition of the fully reduced redox state of HMGB1 in tissue injury accelerated tissue repair, especially the non-oxidizable mutant form of fully reduced HMGB1 (3S-HMGB1), which was even more efficient than the native fully reduced HMGB1.[15,27] Therefore, administering 3S-HMGB1 should be investigated as a potential therapeutic agent that can accelerate tissue repair after trauma. Interestingly, pre-treatment with (fully reduced) HMGB1 2 weeks before the fracture also accelerated fracture repair in an animal model.[27] Therefore, this could be further investigated as a treatment for patients with an increased fracture risk (eg, elderly patients with osteoporosis and patients undergoing elective surgery). Future therapeutic strategies targeting HMGB1 could also be aimed at preventing HMGB1 from leaving the cell and therefore making it unable to initiate and/or sustain an inflammatory environment. However, as a few articles in our review highlight, extracellular (fully reduced) HMGB1 also plays an important role in tissue repair.[15,27] Therefore, preventing the release of HMGB1 might prevent ongoing inflammation, but it also might prevent tissue repair. Improved understanding of the complexities of the intra- and extracellular roles of HMGB1 is required before (human) clinical trials can address the effectiveness of targeting HMGB1 in musculoskeletal disease.

4.6 | Conclusion

This systematic review highlights that HMGB1 is an important mediator in musculoskeletal disease and its biological

function is dependent on the redox status and target tissue. The redox status of HMGB1 is understudied in musculoskeletal disease. Musculoskeletal tissues from diseased patients and animal models show increased HMGB1 expression relative to control tissues. Specifically, HMGB1 was expressed by tissue-resident stromal cells and infiltrating immune cells. Our systematic review suggests that the effects of local administration of recombinant HMGB1 to diseased musculoskeletal tissues were diverse. These effects ranged from inducing inflammation by adding recombinant HMGB1 (unspecified redox status or disulfide HMGB1), to promoting chemotaxis and accelerating tissue repair (fully reduced HMGB1). Future studies should aim to identify the redox status of HMGB1, including its half-life, in musculoskeletal tissues to advance understanding of the diverse biological functions of this mediator. Second, we must further identify the distinct redox status of recombinant HMGB1, which is given to either induce injury or promote tissue repair. Collectively, these efforts will inform therapeutic strategies targeting HMGB1 in musculoskeletal disease.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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