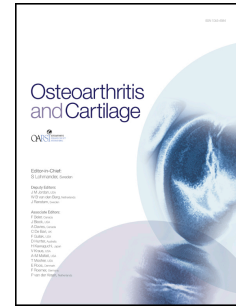


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Osteoarthritis Year in Review 2017: Genetics and Epigenetics

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1 Title: Osteoarthritis Year in Review 2017: Genetics and Epigenetics

2

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9

10 **Summary**

11 Objective: The purpose of this review is to describe highlights from original research
12 publications related to osteoarthritis (OA), epigenetics and genomics with the intention of
13 recognising significant advances.

14 Design: To identify relevant papers a Pubmed literature search was conducted for articles
15 published between April 2016 and April 2017 using the search terms 'osteoarthritis' together
16 with 'genetics', 'genomics', 'epigenetics', 'microrna', 'lncRNA', 'DNA methylation' and
17 'histone modification'.

18 Results: The search term OA generated almost 4000 references. Publications using the
19 combination of descriptors osteoarthritis and genetics provided the most references (82
20 references). However this was reduced compared to the same period in the previous year;
21 8.1% to 2.1% (expressed as a percentage of the total publications combining the terms
22 osteoarthritis and genetics). Publications combining the terms osteoarthritis with genomics

23 (29 references), epigenetics (16 references), lncRNA (11 references; including the
24 identification of novel lncRNAs in OA), DNA methylation (21 references), histone
25 modification (3 references) and microRNA (79 references) were reviewed. Potential OA
26 therapeutics such as histone deacetylase inhibitors have been identified. A number of non-
27 coding RNAs may also provide targets for future treatments.

28 Conclusion: There continues to be a year on year increase in publications researching
29 microRNAs in OA (expressed as a percentage of the total publications), with a doubling over
30 the last 4 years. An overview on the last year's progress within the fields of epigenetics and
31 genomics with respect to OA will be given.

32 **Keywords**

33 Osteoarthritis, genetics, epigenetics, non-coding RNA

34 **Introduction**

35 Epigenetics are a group of genome function mechanisms that do not solely result from the
36 DNA sequence. The term epigenetics encompasses DNA and chromatin modifications and
37 their associated functions as well as non-coding RNAs (ncRNAs). Epigenetic control of gene
38 expression is essential for normal organismal development and cellular function. Abrogation
39 of epigenetic regulation is evident in osteoarthritis (OA). In addition to understanding the
40 pathogenesis of OA through epigenetic research, abnormal epigenetic profiles may act as
41 biomarkers for disease stratification or predictors of disease outcome. Thus epigenetics is a
42 crucial area in the diagnosis, prognosis, and treatment of this disease.

43 Interestingly not all OA tissues or joints are studied to the same extent, and thus it is difficult
44 to gain a complete, integrated understanding of the epigenetics systems which contribute to
45 OA. Whilst this review summarises the main levels of epigenetic control studied over the last

46 12 months (between May 2016- May 2017), we also highlight potential additional directions
47 required by the field.

48 **GENETICS**

49 **Genetics of Osteoarthritis**

50 OA is known as a complex heterogeneous disease, in which one of the contributing factors to
51 disease progression is a genetic component [1, 2]. Genome-wide association studies (GWAS)
52 has enabled the discovery of novel genetic variants that could be used as prognostic
53 biomarkers for early diagnosis, or establish risk groups prone to the disease development.
54 There have been five articles published employing GWAS for discovery of genetic variants
55 associated with OA this year [3-7] . Most of the variants were found in the non-translated
56 regions within the genes or on the areas remote from the gene, suggesting the regulatory
57 changes in genes involved in OA. Whereas changes within the gene itself point to structural
58 changes of the synthesized proteins related to early OA onset. Styrkarsdottir *et al.* (2017) [5]
59 demonstrated a missense variant of the COMP gene (p.Asp369His) and a frameshift mutation
60 in the CHADL gene (p.Val330Glyfs*106), corresponding to hip replacement surgery on
61 average 13.5 years and 4.9 years earlier in these patients, respectively. Results from these
62 studies have discovered novel gene variants, suggesting additional genes involved in OA
63 progression. Although each has a small effect size, combined with other factors these may
64 contribute to OA.

65 **Endophenotype studies**

66 Sample size is an important factor in GWAS studies, increasing the power and
67 consequentially, the number of single nucleotide polymorphisms (SNPs) tested in the
68 experiment [8]. However, it is not the only feature that can be used in order to find more

69 statistically significant genetic variants in heterogeneous diseases such as OA. Stratification
 70 of endophenotypes, in OA particularly can lead to the discovery of novel variants. Recently
 71 published articles (Table 1) clearly demonstrate that using intermediate endophenotypes such
 72 as site of maximal joint space narrowing (maxJSN), bone remodeling, cartilage thickness and
 73 radiographic progression can help to yield more loci than previously reported.
 74 Panoutsopoulou *et al.* (2016) [3] compared variants in hypertrophic with non-hypertrophic
 75 OA. The most significant variant was located between STT3B and GADL1 genes
 76 (rs6766414), and this association was fully attenuated in non-stratified analyses of all hip OA
 77 cases versus population controls.

78 Table 1. The use of endophenotypes in OA-related GWAS studies

Joint	Endophenotype	Sample size	Population; study	Variants	Reference
Hip	Radiographic; max JSN, bone remodelling	OA;2118, Cx;6500	<i>European;</i> arcOGEN	LRCH1, STT3B, GADL1, STT3B	Panoutsopoulou <i>et al.</i> , 2016 [3]
Hip	Radiographic; min JSN, cartilage thickness	OA;13,013, Cx;8227	<i>European,</i> <i>US, Asian;</i> Rotterdam Study I Rotterdam Study II TwinsUK, SOF MrOS	TGFA PIK3R1 FGFR3 TREH	Castaño- Betancourt <i>et</i> <i>al.</i> , 2016 [4]
Hip	No	OA;5657, Cx;207,514	<i>Icelandic;</i> novel	COMP CHADL	Styrkarsdottir <i>et al.</i> , 2017 [5]
Knee	Radiographic progression; KL	CHECK;431, meta:1603	<i>European;</i> <i>CHECK,</i> <i>OAI, Spain</i>	mtDNA variants; superhaplogroup JT	Fernandez- Moreno <i>et al.</i> , 2016 [6]
Knee	Radiographic; definite osteophytes, min JSN, TJR	OA;3,898, Cx;3,168	<i>North</i> <i>American;</i> Osteoarthritis Initiative, Johnston County Osteoarthritis	LSP1P3	Yau <i>et al.</i> , 2017 [7]

			Project, Multicenter Osteoarthritis Study, Genetics of Osteoarthritis study		
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79 OA; osteoarthritis, TJR; total joint replacement, JSN; joint space narrowing, Cx; control,
80 mtDNA; mitochondrial DNA; CHECK; cohort hip and cohort knee, meta; meta-analysis.

81

82 **EPIGENETICS**

83 Epigenetics play a key role in the development of OA and explains the relationship between
84 heritable traits, the environment, other factors (particularly ageing in relation to OA, as it is
85 an age-related disease) and OA itself. DNA plasticity is mediated in part by epigenetic
86 changes, and it is proposed that it can be passed to subsequent generations. This was studied
87 in the epidemiological study of the consequences of the Dutch famine which concluded that
88 early-life environmental conditions can cause epigenetic changes in humans that persist throughout
89 life [9]. Thus epigenetics establishes that joint health can be affected by the interplay of our
90 genes and environment in addition to the proposed inherited effects of our ancestors' genes
91 and environment. Epigenetic traits are both highly dynamic, and tissue specific (indeed even
92 down to the level of different areas of the same tissue). Epigenetics enables tight control at
93 the transcriptional level via gene expression (DNA methylation and histone modification;
94 through methylation and acetylation of histones) resulting in changes to chromatin 3D
95 structure, and the translational level (ncRNAs; microRNAs (miRs), long non-coding RNAs
96 (lncs), small nucleolar RNAs (snoRNAs); mRNA editing and mRNA stability) affecting
97 protein expression (reviewed [10]).

98

99 **DNA Methylation**

100 The methylation of base cytosines (5-methyl cytosine) within CpG containing nucleotides is
101 a stable epigenetic marks that results in gene silencing [11] and is known as DNA
102 methylations. In previous years genome-wide DNA methylation studies have concentrated
103 principally upon cartilage tissue. This is because it is composed of a single cell type; the
104 chondrocyte, making it less problematic to study. Hence genuine alterations in DNA
105 methylation can be assessed as these are not affected by disease-related changes in cell
106 proportions [12]. Table 2 summarises the genome-wide DNA methylation studies published
107 in the last year. OA-related studies prior to this have already been discussed [13]. The
108 methodology of choice published continues to be the Human IlluminaMethylationBead-Chip
109 450K array. Studies published in the last year are distinct from those previously reported as
110 currently experiments involving other tissues such as subchondral bone [14] and fibroblast-
111 synoviocytes [15] are being undertaken. Additionally a number of studies have investigated
112 OA progression [14, 16]. These investigated different regions within the tibial plateau as
113 indicators of OA development. Methylation changes appeared to occur at a later stage of
114 disease indicating that these are a consequence rather than a cause of OA. Similar to other
115 studies [17] it was found that joint specific methylation patterns are independent of disease,
116 indicating location specific epigenetic marks [15]. However Ai *et al.* [15] also identified OA
117 or rheumatoid arthritis (RA) specific methylation patterns.

118

119 Three studies undertook RNA sequencing in parallel with DNA methylation analysis [15, 18,
120 19]. This is becoming an increasingly used methodology enabling methylation variation, and
121 the functional consequences at the transcriptional level to be assessed together. Each of these
122 studies found different correlations between the DNA methylated genes within contrasts and
123 level of gene expression, both identified and their direction. It appears that in these studies
124 most CpG sites with variable methylation are unconnected to gene expression variation. The

125 properties of these associations seem complex, with the location of CpG probes with respect
 126 to the corresponding gene offering little information with regards to the type of correlation.
 127 All studies in Table 2 contain limited sample sizes which reduces the ability to detect weak
 128 associations that may be pertinent to our understanding of this epigenetic mark in OA.

129 The role of the epigenetic clock was investigated in OA for the first time (including work by
 130 Jefferies *et al* [20]). DNA methylation age-measures (DmAM), which combine methylation
 131 levels at CpG sites that experience methylation changes with ageing are potential biomarkers
 132 of epigenetic ageing [21]. The potential role of DNA methylation in cartilage ageing in OA
 133 was investigated. Studying methylation changes at both the local and systemic level Vidal-
 134 Bralo *et al.* identified premature epigenetic ageing due to DNA methylation changes specific
 135 to OA cartilage [22]. Interestingly similar findings were not extended to blood cells and bone.
 136 Other joint tissues and larger sample sizes are required for clarification of these interesting
 137 findings.

138 Table 2. Genome-wide methylation studies in OA

Study; joint and disease	Number of donors	Tissue/cell type	Contrasts	Technique	Results (DE DMS)	Reference
Knee; OA progression	12 donors; early, inter, late OA from each	Subchondral bone	Early vs inter, Early vs severe, inter vs severe	450K array	72, 397, 257	Zhang <i>et al.</i> , 2016 [14]
Knee; OA progression	12 donors; early, inter, late OA from each	Chondrocytes	Early vs inter, Early vs severe	450K array	0, 519	Zhang <i>et al.</i> , 2016 [16]
Hip; fracture and OA	22 fracture, 18 OA	MSCs	Fracture vs OA	450K array* <i>parallel RNASeq</i>	9038	Del Real <i>et al.</i> , 2017 [19]
Hip and knee; RA, OA	30 RA; 12 knee, 10 hip 16 OA; 10 knee, 5 hip	Fibroblast-like synoviocytes	RA vs OA RA hip vs RA knee RA vs OA	450K array* <i>parallel RNASeq</i>	1714 3739 9589 2172	Ai <i>et al.</i> , 2016 [15]

			knee RA vs OA hip			
Knee; normal and OA	11 normal, 12 OA	Cartilage	Normal vs OA	450K array* <i>parallel RNASeq</i>	929	Alvarez -Garcia <i>et al.</i> , 2016 [18]
Knee and hip; cartilage neocartilage derived from knee chondrocyte s or hip MSCs	6 knee, 6 hip cartilage; 4 neocartilage from MSCs, 4 neocartilage from chondrocytes	Chondrocytes and MSCs	Neocartilage MSC vs chondrocytes	450K array	5884	Bomer <i>et al.</i> , 2016 [23]

139 OA; osteoarthritis, RA; rheumatoid arthritis, MSC; mesenchymal stem cells, DE DMS;
140 differentially expressed DNA methylation sites.

141 **Histone modifications**

142 DNA is wrapped around an octamer of histone proteins forming the complex structure of
143 chromatin. Posttranscriptional modifications of the histone tails can alter the accessibility of
144 chromatin, and change gene transcription by allowing promoter site transcription factor
145 binding and initiating transcription [24]. These modifications are dynamic meaning that these
146 changes can be altered in response to stimuli. Posttranslational modifications occur through
147 sets of enzymes such as histone deacetylases (HDACs). Two studies in the last year have
148 investigated the effects of pharmacological intervention points through targeting these
149 enzymes in order to provide insights into the role of epigenetics in OA and identify
150 exploitable targets for treatments. In one study Vorinostat, a HDACI and II inhibitor was
151 demonstrated as a suppressor of catabolic marker expression in OA through inhibition of IL-
152 6 signaling [25]. Further work by the group showed that it functioned through increased
153 recruitment of CEBPalpha to the MCPIP1 promoter, relieving the miR-9-mediated inhibition
154 of MCPIP1 expression in OA chondrocytes [26]. A further study found that H3K27me3
155 demethylases regulated *in vitro* chondrocyte activity in OA through the inhibition of TGF β

156 induced gene expression. Targeting the inhibition of H3K27me3 demethylases could provide
157 potential OA therapeutics [27].

158

159 **NON-CODING RNAs**

160 **MicroRNAs**

161 MiRs are small (19-25 nucleotides (nt)) ncRNAs that function at the post-transcriptional level
162 by binding and suppressing the expression of specific mRNA targets [28]. MiRs are involved
163 in different cellular pathways, highlighting the role of these molecules in maintaining tissue
164 homeostasis as well as being implicated in disease [29]. Over the last few years the roles of
165 miRs in OA has been reviewed extensively with studies identifying numerous miR candidates
166 involved in cartilage homeostasis and/or OA pathogenesis [30-34]. Most of the studies
167 published during the last year focused on miRs which have been previously reported to play a
168 role in chondrocyte function and OA, such as miR-140 [35] miR-29 [32], miR-34a [34, 36],
169 miR-9 [37, 38] and miR-98 [33, 39]. Identification of miR-181a-5p and miR-4454 as
170 mediators of facet cartilage degeneration was presented at OARSI 2017 [40]. However, some
171 studies reported for the first time the implication of specific miRs in OA, such as miR-15-5p
172 [41] and miR-410 [42]. The methodologies remain similar, with human primary OA
173 chondrocytes treated with miR mimics and/or inhibitors and qRT-PCR being commonly
174 utilized in order to measure the expression of both specific miRs and cartilage-associated
175 genes (Table 3, a full summary table of MiR studies reviewed is in Supplementary File 1).
176 Additionally many of these studies identified and validated using luciferase reporter assays
177 putative target genes of the miRs in question, and these findings were usually integrated into,
178 or associated with important signalling pathways, such as the NF-kB and AP1 (c-fos/c-jun)
179 [43, 44].

180 Regarding genome-wide approaches undertaken in the last year, four studies used microarray
 181 analysis to test for differentially expressed (DE) miRs in OA chondrocytes treated with
 182 various stimulating factors or whole mouse joints from a destabilization of the medial
 183 meniscus (DMM) model. Each study identified several DE miRs [31, 32, 45, 46]. One study
 184 undertook a next generation sequencing approach using human cartilage from different OA
 185 stages [47]. Finally, Kung *et al.* (2017) measured DE circulating miRs in the serum of DMM
 186 mice with the results suggesting that two miRs, miR-3102-5p and miR-3081-5p,
 187 demonstrated higher expression in late stage OA compared to controls, although findings
 188 were not validated [48]. A role of circulating miRs in OA was presented by Rousseau *et al* at
 189 OARSI 2017 [49].

190 Although the number of research publications and miRs involved in OA pathogenesis is
 191 rising constantly, no miR biomarkers have been validated that could be utilised in early
 192 diagnosis of the disease. This is due partially to the fact that OA is a multifactorial
 193 heterogeneous disease. As a result, the miR signature responds differently to the type of
 194 stimuli involved in OA initiation and progression. Similarly, therapeutic options based on
 195 miRs are also hindered by the heterogeneity of the disease, the need for targeted delivery
 196 approaches and the lack of evidence on the molecular and cellular processes that orchestrate
 197 OA. This clearly highlights the importance of an in-depth understanding of the signalling
 198 pathways behind OA but at the same time steps should be taken to integrate the multiple miR
 199 findings into the clinical setting, especially for some of the well-studied miRs which provide
 200 promising therapeutic targets.

201 Table 3. Selected miRs studies focusing on cartilage associated genes

MicroRNA	Target genes	Cellular/Biological process	Tissue	Reference
mir-9	SIRT1	Oxidative stress-induced chondrocyte death	Cartilage	D'Adamo <i>et al.</i> (2017) [37]

miR-15a	VEGFA	Matrix degradation	Cartilage	Chen <i>et al.</i> (2017) [41]
miR-23a	SMAD3	OA development	Cartilage	Kang <i>et al.</i> (2016) [50]
miR-26a/b	KPNA3	NF- κ B signaling pathway	Cartilage	Yin <i>et al.</i> (2017) [44]
miR-29 family	FZD3, FZD5, DVL3, FRAT2, CK2A2	OA development and progression	Cartilage	Le <i>et al.</i> (2016) [32]
miR-34a	SIRT11	Chondrocyte apoptosis	Cartilage	Yan <i>et al.</i> (2016) [34]
miR-98	BCL-2	Chondrocyte apoptosis	Cartilage	Wang <i>et al.</i> (2016) [39]
miR-221	SDF1	ECM degradation	Cartilage	Zheng <i>et al.</i> (2017) [47]
miR-381	HDAC4	Chondrocyte Hypertrophy	Mouse forelimbs	Chen <i>et al.</i> (2016) [51]
mir-410	WNT3A	Chondrogenic differentiation	Bone marrow MSCs	Zhang <i>et al.</i> (2017) [42]

202

203 Other non-coding RNAs

204 The relevance of ncRNAs to OA has mainly focussed on the widespread disruption of miR
 205 expression. However we are beginning to understand and study the nature and involvement of
 206 other ncRNAs in OA such as piwi-interacting RNAs (piRNAs), snoRNAs and large-
 207 intergenic non-coding RNAs (lincRNAs). In the last year an insight into their potential roles
 208 in OA has emerged for the first time.

209 SnoRNAs mediate enzymatic modifications of other RNA species, such as ribosomal RNAs,
 210 by forming ribonucleoprotein complexes with enzymes [52]. These modifications include
 211 ribose methylation and pseudouridylation. Little data exists on the role of these RNA species
 212 in OA. A pubmed search gave only one result regarding the implication of snoRNAs in OA
 213 during last year; Steinbusch *et al.* (2017) undertook snoRNAseq analysis in OA joints and
 214 serum from DMM mice [53]. Several DE snoRNAs, such as SNORA64, SNORD46 and
 215 SNORD116, were identified and validated and the authors concluded that snoRNAs could be
 216 used as potential biomarkers for joint degeneration.

217 PiRNAs (24-32 nt) form RNA-protein complexes with piwi proteins and are linked to both
 218 epigenetic and post-transcriptional gene silencing of genetic elements, thus protecting cells
 219 from invasive transposable elements in the germline [54]. For the first time piRNAs and their

220 binding partners were identified in OA and RA synovial fibroblasts and synovial fluid. The
 221 study concluded that PIWI/piRNA pathways are involved in innate immunity and may have a
 222 role in the pathogenesis of RA [55].

223

224 **Long non-coding RNAs**

225 There has been an increase in studies published on lncRNAs in OA (up from four in 2015-
 226 2016 to 11 in the same period 2016-2017). LncRNAs are an RNA molecule greater than 200
 227 nt. Dysregulated expression of lncRNAs performs a significant role in inflammation-related
 228 diseases, and has been demonstrated as being associated with OA progression and cartilage
 229 degradation (reviewed [56]). Two discovery studies were undertaken (Table 4). In one study
 230 a role in mediating inflammation driven cartilage degeneration for the novel lncRNAs
 231 CILinc01 and Clinc02 was identified [57]. Additionally a number of targeted lncRNA studies
 232 on both novel [36, 58] and previously studied lncRNAs [59, 60] were undertaken (Figure 1).

233 Table 4. Long non-coding RNA discovery studies in OA

Study	Species	Technology	Method and N	Findings	Reference
Post-traumatic OA	Mouse	RNA sequencing	Joints; 1 day (n=5), 1(n=5), 6 (n=3), 12 (n=3) week post injury	18 DE lncRNAs (at least one time point)	Chang <i>et al.</i> , 2016 [61]
Normal versus OA cartilage	Human	RNA sequencing	Chondrocytes; hip OA±IL-1β (n=3 each)	983 lncRNAs identified, 125 DE	Pearson <i>et al.</i> , 2016 [57]

234 N; number donors, OA; osteoarthritis, IL-1β; interleukin 1β, DE; differentially expressed

235 **Emerging areas for future study**

236 Understanding the biology of RNA modifications represents one of the next potential
 237 frontiers in arthritis research. [62]. We realise that the control of the transcriptome is
 238 pertinent to the diverse aspects of gene regulation, cellular functionality and development,

239 and that alterations can result in disease. There is an emerging field of research termed
240 ‘epitranscriptomics’; the identification and characterisation of changes in biochemical RNA
241 modifications that do not comprise alterations to the RNA sequence. Epitranscriptomic
242 analysis was the Nature method of the year 2016 [63]. Epitranscriptomics includes
243 modifications to rRNA, tRNA and mRNA. However the role and function of snoRNAs,
244 lncRNAs, anti-sense, and small RNAs derived from tRNAs remains largely unrealized.

245 A further layer of gene expression control is through alterations in genetic information by
246 RNA editing (epitranscriptomics) or via the establishment of RNA covalent modifications.
247 Interestingly disease-related exome sequencing has contributed to the pivotal attributions of
248 mutations in RNA modifying enzymes to many human diseases [64]. In OA the risk gene fat
249 mass and obesity associated protein (FTO) is an m⁶A mRNA eraser [65]. Improved
250 technologies (reviewed [66]) will enable RNA modifications signatures and dynamics to be
251 discovered.

252 Within the context of this emerging discipline (as an additional molecular level on control in
253 physiology and disease), and with expanding omics technological advances the discipline of
254 ‘systems biology’ is becoming increasingly influential in our understanding of OA [67]
255 (Figure 2). Its aim is to systematically and comprehensively obtain quality data from all
256 biological hierarchies’ whilst assimilating the data to develop predictive models of the system.
257 Some of the challenges of systems biology in OA research include that not all tissues are
258 evenly represented in systems studies, not all levels are explored systematically (for instance
259 there are limited studies on histone modifications) and there is difficulty in integrating and
260 correlating the different levels of the system. These challenges thus represent further
261 opportunities to address.

262 **Author Contributions**

263 Mandy Peffers, Panagiotis Balaskas and Aibek Smagul searched the literature, summarised
264 results and wrote the manuscript.

265

266 **Conflict of interest**

267 We have no conflicts of interest.

268

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459 **Figure Legend**

460 Figure 1. LncRNA studies in OA targeting different mechanisms of action of lncRNAs .
461 Three functions of lncRNAs have been investigated; through acting as sponges for miRs,
462 transcriptional activation and repression and the regulation of the chromatin state (miR;
463 microRNA, TMSB4; thymosin- β 4, SMSCs; synovium-derived mesenchymal stem cells, IL-
464 1β ; interleukin 1β , MMP; metalloproteinase). References; 1. Zhang *et al.*, 2016 [36], 2. Liu *et*
465 *al.*, 2016 [58], 3. Zhang *et al.*, 2017 [60], 4. Zhang *et al.*, 2016 [59].

466 Figure 2. Schematic diagram of a systems orientated approach to develop novel diagnostic
467 and treatment solutions to OA. Omics studies enable pictures of the biological hierarchy. Red
468 boxes highlight the areas covered in this review [67].

469 **Supplementary Files**

470 Supplementary File 1 Summary table of miR studies reviewed.

471

1 Figure Legend

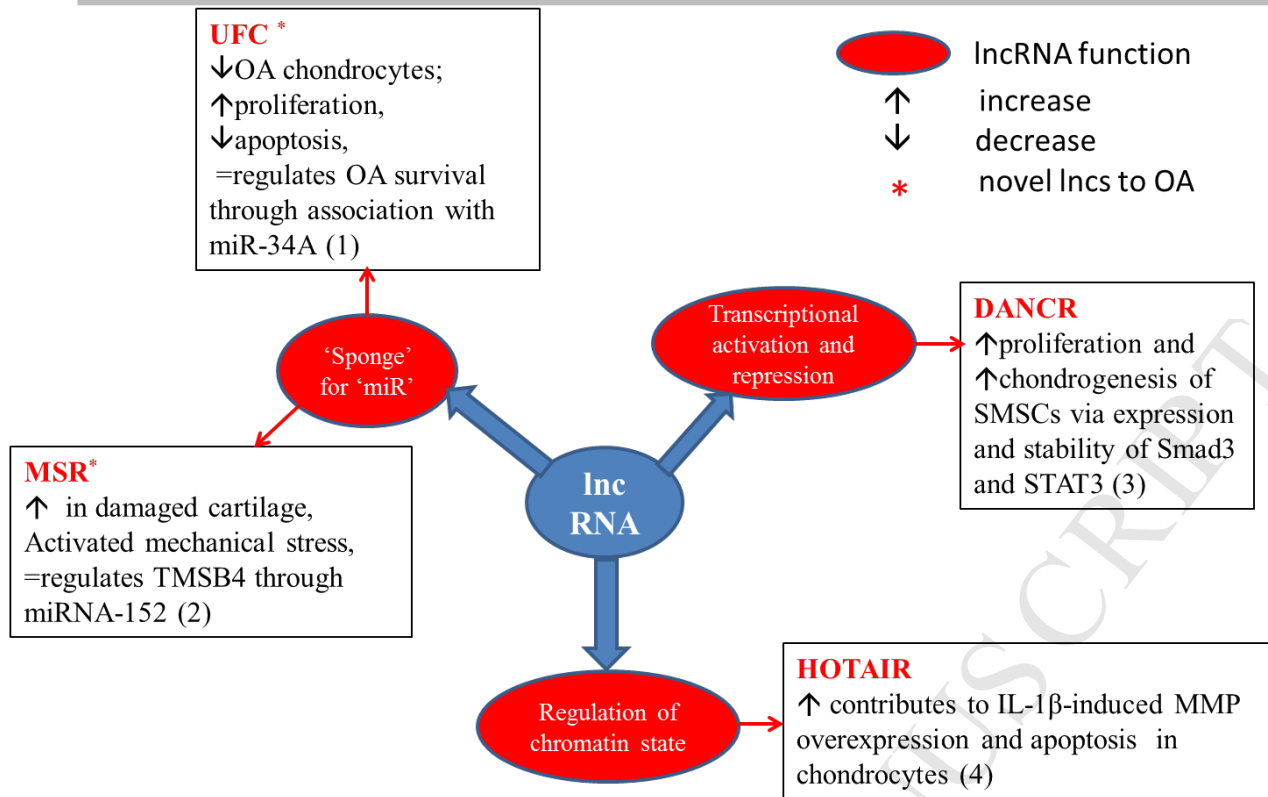
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3. Zhang *et al.*, 2017
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