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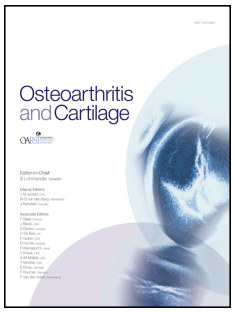

Osteoarthritis Year in Review 2017: Genetics and Epigenetics

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Title: Osteoarthritis Year in Review 2017: Genetics and Epigenetics

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Summary

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

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

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

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

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

Keywords

Osteoarthritis, genetics, epigenetics, non-coding RNA

Introduction

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

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

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

GENETICS

Genetics of Osteoarthritis

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

Endophenotype studies

Sample size is an important factor in GWAS studies, increasing the power and consequentially, the number of single nucleotide polymorphisms (SNPs) tested in the 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	*European;* arcOGEN	LRCH1, STT3B, GADL1, STT3B	Panoutsopoulou et al , 2016 [3]
Hip	Radiographic; min JSN, cartilage thickness	OA;13,013, Cx;8227	*European, US, Asian;* Rotterdam Study I Rotterdam Study II TwinsUK, SOF MrOS	TGFA PIK3R1 FGFR3 TREH	Castaño-Betancourt *et al*., 2016 [4]
Hip	No	OA;5657, Cx;207,514	*Icelandic;* novel	COMP CHADL	Styrkarsdottir *et al*., 2017 [5]
Knee	Radiographic progression; KL	CHECK;431, meta:1603	*European; CHECK, OAI, Spain*	mtDNA variants; superhaplogroup JT	Fernandez-Moreno *et al*., 2016 [6]
Knee	Radiographic; definite osteophytes, min JSN, TJR	OA;3,898, Cx;3,168	*North American;* Osteoarthritis Initiative, Johnston County Osteoarthritis	LSP1P3	Yau *et al*., 2017 [7]

			Project, Multicenter Osteoarthritis Study, Genetics of Osteoarthritis study		

OA; osteoarthritis, TJR; total joint replacement, JSN; joint space narrowing, Cx; control, mtDNA; mitochondrial DNA; CHECK; cohort hip and cohort knee, meta; meta-analysis.

EPIGENETICS

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

DNA Methylation

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

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

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

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

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 *et al.*, 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 *et al.*, 2016 [16]
Hip; fracture and OA	22 fracture, 18 OA	MSCs	Fracture vs OA	450K array* *parallel RNASeq*	9038	Del Real *et al.*, 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* *parallel RNASeq*	1714 3739 9589 2172	Ai *et al.*, 2016 [15]

			knee RA vs OA hip			
Knee; normal and OA	11 normal, 12 OA	Cartilage	Normal vs OA	450K array* *parallel RNASeq*	929	Alvarez-Garcia *et al.*, 2016 [18]
Knee and hip; cartilage neocartilage derived from knee chondrocytes 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 *et al.*, 2016 [23]

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

Histone modifications

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

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

NON-CODING RNAs

MicroRNAs

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

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

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

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 *et al.* (2017) [37]

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

Other non-coding RNAs

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

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

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

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

Long non-coding RNAs

There has been an increase in studies published on lncRNAs in OA (up from four in 2015-2016 to 11 in the same period 2016-2017). LncRNAs are an RNA molecule greater than 200 nt. Dysregulated expression of lncRNAs performs a significant role in inflammation-related diseases, and has been demonstrated as being associated with OA progression and cartilage degradation (reviewed [56]). Two discovery studies were undertaken (Table 4). In one study a role in mediating inflammation driven cartilage degeneration for the novel lncRNAs CILinc01 and CIinc02 was identified [57]. Additionally a number of targeted lncRNA studies on both novel [36, 58] and previously studied lncRNAs [59, 60] were undertaken (Figure 1).

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 *et al.*, 2016 [61]
Normal versus OA cartilage	Human	RNA sequencing	Chondrocytes; hip OA±IL-1β (n=3 each)	983 lncRNAs identified, 125 DE	Pearson *et al.*, 2016 [57]

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

Emerging areas for future study

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

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

A further layer of gene expression control is through alterations in genetic information by RNA editing (epitranscriptomics)or via the establishment of RNA covalent modifications. Interestingly disease-related exome sequencing has contributed to the pivotal attributions of mutations in RNA modifying enzymes to many human diseases [64]. In OA the risk gene fat mass and obesity associated protein (FTO) is an m^6A mRNA eraser [65]. Improved technologies (reviewed [66]) will enable RNA modifications signatures and dynamics to be discovered.

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

Author Contributions

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

Conflict of interest

We have no conflicts of interest.

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References

1. Chapman K, Valdes AM. Genetic factors in OA pathogenesis. Bone 2012; 51: 258-264.
2. Minafra L, Bravata V, Saporito M, Cammarata FP, Forte GI, Caldarella S, et al. Genetic, clinical and radiographic signs in knee osteoarthritis susceptibility. Arthritis Res Ther 2014; 16: R91.
3. Panoutsopoulou K, Thiagarajah S, Zengini E, Day-Williams AG, Ramos YF, Meessen JM, et al. Radiographic endophenotyping in hip osteoarthritis improves the precision of genetic association analysis. Ann Rheum Dis 2017; 76: 1199-1206.
4. Castano-Betancourt MC, Evans DS, Ramos YF, Boer CG, Metrustry S, Liu Y, et al. Novel Genetic Variants for Cartilage Thickness and Hip Osteoarthritis. PLoS Genet 2016; 12: e1006260.
5. Styrkarsdottir U, Helgason H, Sigurdsson A, Norddahl GL, Agustsdottir AB, Reynard LN, et al. Whole-genome sequencing identifies rare genotypes in COMP and CHADL associated with high risk of hip osteoarthritis. Nat Genet 2017; 49: 801-805.

6. Fernandez-Moreno M, Soto-Hermida A, Vazquez-Mosquera ME, Cortes-Pereira E, Pertega S, Relano S, et al. A replication study and meta-analysis of mitochondrial DNA variants in the radiographic progression of knee osteoarthritis. Rheumatology (Oxford) 2017; 56: 263-270.
7. Yau MS, Yerges-Armstrong LM, Liu Y, Lewis CE, Duggan DJ, Renner JB, et al. Genome-Wide Association Study of Radiographic Knee Osteoarthritis in North American Caucasians. Arthritis Rheumatol 2017; 69: 343-351.
8. Hong EP, Park JW. Sample Size and Statistical Power Calculation in Genetic Association Studies. Genomics Inf 2012; 10: 117-122.
9. Heijmans BT, Tobi EW, Stein AD, Putter H, Blauw GJ, Susser ES, et al. Persistent epigenetic differences associated with prenatal exposure to famine in humans. Proc Natl Acad Sci U S A 2008; 105: 17046-17049.
10. Romagnolo DF, Daniels KD, Grunwald JT, Ramos SA, Propper CR, Selmin OI. Epigenetics of breast cancer: Modifying role of environmental and bioactive food compounds. Mol Nutr Food Res 2016; 60: 1310-1329.
11. Jaenisch R, Bird A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet 2003; 33 Suppl: 245-254.
12. Reynard LN. Analysis of genetics and DNA methylation in osteoarthritis: What have we learnt about the disease? Semin Cell Dev Biol 2017; 62: 57-66.
13. van Meurs JB. Osteoarthritis year in review 2016: genetics, genomics and epigenetics. Osteoarthritis Cartilage 2017; 25: 181-189.
14. Zhang Y, Fukui N, Yahata M, Katsuragawa Y, Tashiro T, Ikegawa S, et al. Identification of DNA methylation changes associated with disease progression in subchondral bone with site-matched cartilage in knee osteoarthritis. Sci Rep 2016; 6: 34460.
15. Ai R, Hammaker D, Boyle DL, Morgan R, Walsh AM, Fan S, et al. Joint-specific DNA methylation and transcriptome signatures in rheumatoid arthritis identify distinct pathogenic processes. Nat Commun 2016; 7: 11849.
16. Zhang Y, Fukui N, Yahata M, Katsuragawa Y, Tashiro T, Ikegawa S, et al. Genome-wide DNA methylation profile implicates potential cartilage regeneration at the late stage of knee osteoarthritis. Osteoarthritis Cartilage 2016; 24: 835-843.
17. den Hollander W, Ramos YF, Bos SD, Bomer N, van der Breggen R, Lakenberg N, et al. Knee and hip articular cartilage have distinct epigenomic landscapes: implications for future cartilage regeneration approaches. Ann Rheum Dis 2014; 73: 2208-2212.
18. Alvarez-Garcia O, Fisch KM, Wineinger NE, Akagi R, Saito M, Sasho T, et al. Increased DNA Methylation and Reduced Expression of Transcription Factors in Human Osteoarthritis Cartilage. Arthritis Rheumatol 2016; 68: 1876-1886.
19. Del Real A, Perez-Campo FM, Fernandez AF, Sanudo C, Ibarbia CG, Perez-Nunez MI, et al. Differential analysis of genome-wide methylation and gene expression in mesenchymal stem cells of patients with fractures and osteoarthritis. Epigenetics 2017; 12: 113-122.
20. Jefferies M, Rivas A. Rapid knee OA progression is associated with decelerated peripheral blood DNA methlyation aging: data from the osteoarthritis initiative. Osteoarthritis and Cartilage 2017; 25.
21. Horvath S. DNA methylation age of human tissues and cell types. Genome Biol 2013; 14: R115.
22. Vidal-Bralo L, Lopez-Golan Y, Mera-Varela A, Rego-Perez I, Horvath S, Zhang Y, et al. Specific premature epigenetic aging of cartilage in osteoarthritis. Aging (Albany NY) 2016; 8: 2222-2231.
23. Bomer N, den Hollander W, Suchiman H, Houtman E, Slieker RC, Heijmans BT, et al. Neo-cartilage engineered from primary chondrocytes is epigenetically similar to autologous cartilage, in contrast to using mesenchymal stem cells. Osteoarthritis Cartilage 2016; 24: 1423-1430.

24. Ernst J, Kheradpour P, Mikkelsen TS, Shoresh N, Ward LD, Epstein CB, et al. Mapping and analysis of chromatin state dynamics in nine human cell types. Nature 2011; 473: 43-49.
25. Makki MS, Haqqi TM. Histone Deacetylase Inhibitor Vorinostat (SAHA) Suppresses IL-1beta-Induced Matrix Metallopeptidase-13 Expression by Inhibiting IL-6 in Osteoarthritis Chondrocyte. Am J Pathol 2016; 186: 2701-2708.
26. Makki MS, Haqqi TM. Histone deacetylase inhibitor vorinostat (SAHA, MK0683) perturb miR-9-MCPIP1 axis to block IL-1beta-induced IL-6 expression in human OA chondrocytes. Connect Tissue Res 2017; 58: 64-75.
27. Yapp C, Carr AJ, Price A, Oppermann U, Snelling SJ. H3K27me3 demethylases regulate in vitro chondrogenesis and chondrocyte activity in osteoarthritis. Arthritis Res Ther 2016; 18: 158.
28. Nugent M. MicroRNAs: exploring new horizons in osteoarthritis. Osteoarthritis Cartilage 2016; 24: 573-580.
29. Adams BD, Parsons C, Walker L, Zhang WC, Slack FJ. Targeting noncoding RNAs in disease. J Clin Invest 2017; 127: 761-771.
30. Chen G, Gao X, Wang J, Yang C, Wang Y, Liu Y, et al. Hypoxia-induced microRNA-146a represses Bcl-2 through Traf6/IRAK1 but not Smad4 to promote chondrocyte autophagy. Biol Chem 2017; 398: 499-507.
31. Ji Q, Xu X, Xu Y, Fan Z, Kang L, Li L, et al. miR-105/Runx2 axis mediates FGF2-induced ADAMTS expression in osteoarthritis cartilage. J Mol Med (Berl) 2016; 94: 681-694.
32. Peffers MJ, Collins J, Fang Y, Goljanek-Whysall K, Rushton M, Loughlin J, et al. Age-related changes in mesenchymal stem cells identified using a multi-omics approach. Eur Cell Mater 2016; 31: 136-159.
33. Wang GL, Wu YB, Liu JT, Li CY. Upregulation of miR-98 Inhibits Apoptosis in Cartilage Cells in Osteoarthritis. Genet Test Mol Biomarkers 2016; 20: 645-653.
34. Yan S, Wang M, Zhao J, Zhang H, Zhou C, Jin L, et al. MicroRNA-34a affects chondrocyte apoptosis and proliferation by targeting the SIRT1/p53 signaling pathway during the pathogenesis of osteoarthritis. Int J Mol Med 2016; 38: 201-209.
35. Karlsen TA, de Souza GA, Odegaard B, Engebretsen L, Brinchmann JE. microRNA-140 Inhibits Inflammation and Stimulates Chondrogenesis in a Model of Interleukin 1beta-induced Osteoarthritis. Mol Ther Nucleic Acids 2016; 5: e373.
36. Zhang G, Wu Y, Xu D, Yan X. Long Noncoding RNA UFC1 Promotes Proliferation of Chondrocyte in Osteoarthritis by Acting as a Sponge for miR-34a. DNA Cell Biol 2016; 35: 691-695.
37. D'Adamo S, Cetrullo S, Guidotti S, Borzi RM, Flamigni F. Hydroxytyrosol modulates the levels of microRNA-9 and its target sirtuin-1 thereby counteracting oxidative stress-induced chondrocyte death. Osteoarthritis Cartilage 2017; 25: 600-610.
38. Gu R, Liu N, Luo S, Huang W, Zha Z, Yang J. MicroRNA-9 regulates the development of knee osteoarthritis through the NF-kappaB1 pathway in chondrocytes. Medicine (Baltimore) 2016; 95: e4315.
39. Wang J, Chen L, Jin S, Lin J, Zheng H, Zhang H, et al. MiR-98 promotes chondrocyte apoptosis by decreasing Bcl-2 expression in a rat model of osteoarthritis. Acta Biochim Biophys Sin (Shanghai) 2016; 48: 923-929.
40. Nakamura A, Ramersaud Y, Sharma A, Lewis S.J., Wu B, Datta P, et al. Identification of microrna 181-5p and microrna 4454as mediators of cartilage degenerationthrough the activation ofzinc finger protein 440 mediated NFKB pthway and potential circulating biomarkers for detecting the severity of facet joint osteoarthritis in blood. Osteoarthritis and Cartilage 2017; 25: S33.
41. Chen H, Tian Y. MiR-15a-5p regulates viability and matrix degradation of human osteoarthritis chondrocytes via targeting VEGFA. Biosci Trends 2017; 10: 482-488.

42. Zhang Y, Huang X, Yuan Y. MicroRNA-410 promotes chondrogenic differentiation of human bone marrow mesenchymal stem cells through down-regulating Wnt3a. Am J Transl Res 2017; 9: 136-145.
43. Ji Q, Xu X, Zhang Q, Kang L, Xu Y, Zhang K, et al. The IL-1beta/AP-1/miR-30a/ADAMTS-5 axis regulates cartilage matrix degradation in human osteoarthritis. J Mol Med (Berl) 2016; 94: 771-785.
44. Yin X, Wang JQ, Yan SY. Reduced miR26a and miR26b expression contributes to the pathogenesis of osteoarthritis via the promotion of p65 translocation. Mol Med Rep 2017; 15: 551-558.
45. Rasheed Z, Al-Shobaili HA, Rasheed N, Al Salloom AA, Al-Shaya O, Mahmood A, et al. Integrated Study of Globally Expressed microRNAs in IL-1beta-stimulated Human Osteoarthritis Chondrocytes and Osteoarthritis Relevant Genes: A Microarray and Bioinformatics Analysis. Nucleosides Nucleotides Nucleic Acids 2016; 35: 335-355.
46. Rasheed Z, Rasheed N, Al-Shaya O. Epigallocatechin-3-O-gallate modulates global microRNA expression in interleukin-1beta-stimulated human osteoarthritis chondrocytes: potential role of EGCG on negative co-regulation of microRNA-140-3p and ADAMTS5. Eur J Nutr 2017.
47. Zheng X, Zhao FC, Pang Y, Li DY, Yao SC, Sun SS, et al. Downregulation of miR-221-3p contributes to IL-1beta-induced cartilage degradation by directly targeting the SDF1/CXCR4 signaling pathway. J Mol Med (Berl) 2017; 95: 615-627.
48. Kung LH, Zaki S, Ravi V, Rowley L, Smith MM, Bell KM, et al. Utility of circulating serum miRNAs as biomarkers of early cartilage degeneration in animal models of post-traumatic osteoarthritis and inflammatory arthritis. Osteoarthritis Cartilage 2017; 25: 426-434.
49. Rousseau JC, Sornay-Rendu E, Borel O, Cahpurlat R. Association of circulating miRs with OA. Osteoarthritis and Cartilage 2017; 25: S455.
50. Kang L, Yang C, Song Y, Liu W, Wang K, Li S, et al. MicroRNA-23a-3p promotes the development of osteoarthritis by directly targeting SMAD3 in chondrocytes. Biochem Biophys Res Commun 2016; 478: 467-473.
51. Chen W, Sheng P, Huang Z, Meng F, Kang Y, Huang G, et al. MicroRNA-381 Regulates Chondrocyte Hypertrophy by Inhibiting Histone Deacetylase 4 Expression. Int J Mol Sci 2016; 17.
52. Mleczko AM, Bakowska-Zywicka K. When small RNAs become smaller: emerging functions of snoRNAs and their derivatives. Acta Biochim Pol 2016; 63: 601-607.
53. Steinbusch MM, Fang Y, Milner PI, Clegg PD, Young DA, Welting TJ, et al. Serum snoRNAs as biomarkers for joint ageing and post traumatic osteoarthritis. Sci Rep 2017; 7: 43558.
54. Sai Lakshmi S, Agrawal S. piRNABank: a web resource on classified and clustered Piwi-interacting RNAs. Nucleic Acids Res 2008; 36: D173-177.
55. Plestilova L, Neidhart M, Russo G, Frank-Bertoncelj M, Ospelt C, Ciurea A, et al. Expression and Regulation of PIWIL-Proteins and PIWI-Interacting RNAs in Rheumatoid Arthritis. PLoS One 2016; 11: e0166920.
56. Jiang SD, Lu J, Deng ZH, Li YS, Lei GH. Long noncoding RNAs in osteoarthritis. Joint Bone Spine 2016.
57. Pearson MJ, Philp AM, Heward JA, Roux BT, Walsh DA, Davis ET, et al. Long Intergenic Noncoding RNAs Mediate the Human Chondrocyte Inflammatory Response and Are Differentially Expressed in Osteoarthritis Cartilage. Arthritis Rheumatol 2016; 68: 845-856.
58. Liu Q, Hu X, Zhang X, Dai L, Duan X, Zhou C, et al. The TMSB4 Pseudogene LncRNA Functions as a Competing Endogenous RNA to Promote Cartilage Degradation in Human Osteoarthritis. Mol Ther 2016; 24: 1726-1733.
59. Zhang C, Wang P, Jiang P, Lv Y, Dong C, Dai X, et al. Upregulation of lncRNA HOTAIR contributes to IL-1beta-induced MMP overexpression and chondrocytes apoptosis in temporomandibular joint osteoarthritis. Gene 2016; 586: 248-253.

60. Zhang L, Yang C, Chen S, Wang G, Shi B, Tao X, et al. Long Noncoding RNA DANCR Is a Positive Regulator of Proliferation and Chondrogenic Differentiation in Human Synovium-Derived Stem Cells. DNA Cell Biol 2017; 36: 136-142.
61. Chang JC, Sebastian A, Murugesh DK, Hatsell S, Economides AN, Christiansen BA, et al. Global molecular changes in a tibial compression induced ACL rupture model of post-traumatic osteoarthritis. J Orthop Res 2017; 35: 474-485.
62. Schaefer M, Kapoor U, Jantsch MF. Understanding RNA modifications: the promises and technological bottlenecks of the 'epitranscriptome'. Open Biol 2017; 7.
63. Helm M, Motorin Y. Detecting RNA modifications in the epitranscriptome: predict and validate. Nat Rev Genet 2017; 18: 275-291.
64. Torres AG, Batlle E, Ribas de Pouplana L. Role of tRNA modifications in human diseases. Trends Mol Med 2014; 20: 306-314.
65. arc OC, arc OC, Zeggini E, Panoutsopoulou K, Southam L, Rayner NW, et al. Identification of new susceptibility loci for osteoarthritis (arcOGEN): a genome-wide association study. Lancet 2012; 380: 815-823.
66. Xiong X, Yi C, Peng J. Epitranscriptomics: Toward A Better Understanding of RNA Modifications. Genomics Proteomics Bioinformatics 2017; 15: 147-153.
67. Mueller AJ, Peffers MJ, Proctor CJ, Clegg PD. Systems approaches in osteoarthritis: Identifying routes to novel diagnostic and therapeutic strategies. J Orthop Res 2017.

Figure Legend

Figure 1. LncRNA studies in OA targeting different mechanisms of action of lncRNAs . Three functions of lncRNAs have been investigated; through acting as sponges for miRs, transcriptional activation and repression and the regulation of the chromatin state (miR; microRNA, TMSB4; thymosin-β4, SMSCs; synovium-derived mesenchymal stem cells, IL-1β; interleukin 1β, MMP; metalloproteinase). References; 1. Zhang *et al.*, 2016 [36], 2. *Liu et al.*, 2016 [58], 3. Zhang et al., 2017 [60], 4. Zhang et al., 2016 [59].

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

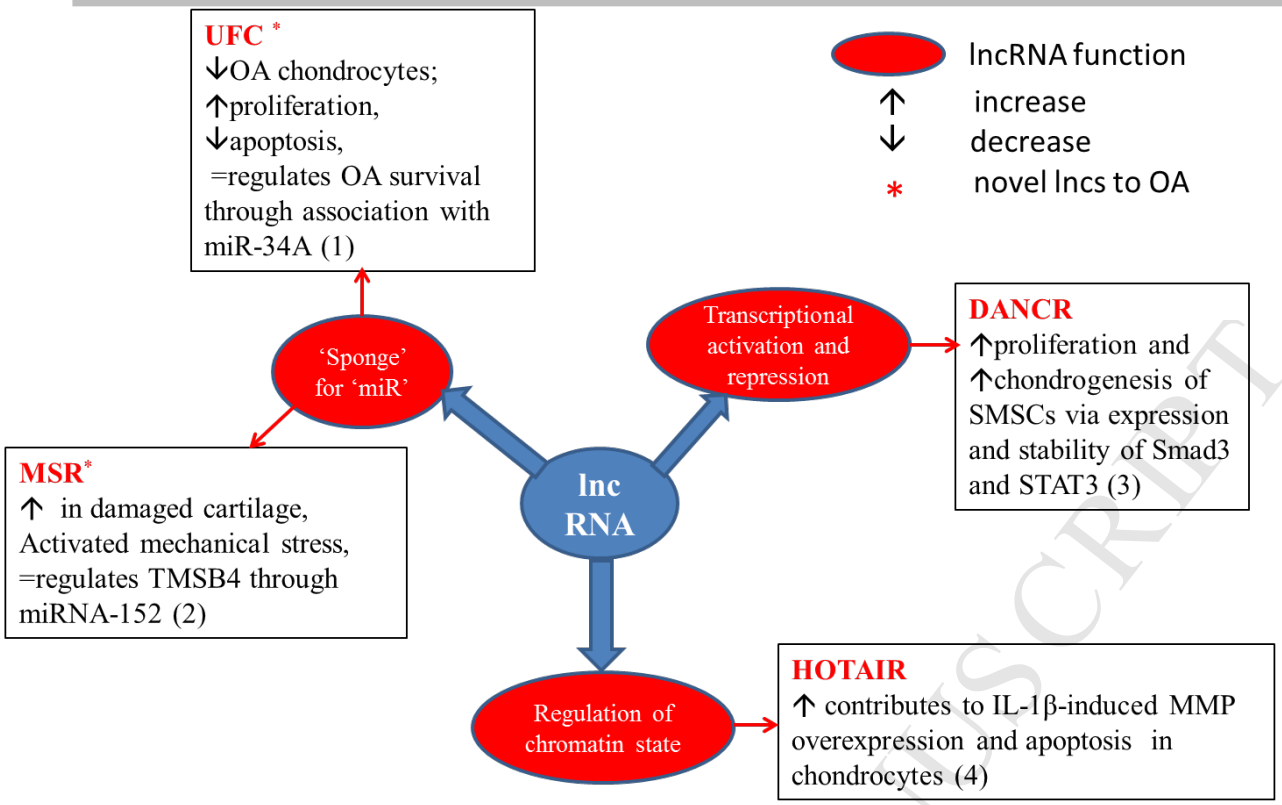
Supplementary Files

Supplementary File 1 Summary table of miR studies reviewed.

Figure Legend

Figure 1. LncRNA studies in OA targeting different mechanisms of action of lncRNAs . Three functions of lncRNAs have been investigated; through acting as sponges for miRs, transcriptional activation and repression and the regulation of the chromatin state (miR; microRNA, TMSB4; thymosin-β4, SMSCs; synovium-derived mesenchymal stem cells, IL-1β; interleukin 1β, MMP; metalloproteinase). References; 1. Zhang *et al.*, 2016 [34], 2. *Liu et al.*, 2016 [53], 3. Zhang et al., 2017 [55], 4. Zhang et al., 2016 [54].

Figure 2. Schematic diagram of a systems orientated approach to develop novel diagnostic and treatment solutions to OA. Omics studies enable pictures of the biological hierarchy. Red boxes highlight the areas covered in this review [62].



1. Zhang *et al.*, 2016
2. Liu *et al.*,2016
3. Zhang *et al.*, 2017
4. Zhang *et al.*, 2016