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What did we learn from "omics" studies in Osteoarthritis

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Abstract

Purpose of review: "Omics" technologies, developed for the massive analysis of the major biologically relevant molecules (genes, proteins, metabolites) have been applied to the study of osteoarthritis (OA) for more than a decade.

Recent findings: "Omics" studies have undoubtedly contributed to increase the knowledge on pathogenic processes related with OA, and have provided hundreds to thousands of molecules that might have a putative biomarker utility for this disease.

Summary: This review describes the most recent "omics" studies in OA research, their conclusions, and discuss those remaining challenges. Still many validation studies must be performed in large and well characterized cohorts for the translation of the findings from "omics" strategies to clinical applications. The development of tools for the intelligent integration of "omics" data with clinical and imaging information is also mandatory to take full profit of the work that has been already performed.

Keywords: osteoarthritis, genomics, proteomics, metabolomics, biomarkers

Introduction

The “omics” technologies were developed for the large-scale analysis of the major biologically relevant molecules: DNA, RNA, proteins and metabolites (amino acids, lipids, etc.). Over the last decade, these technologies have been extensively applied for the study of osteoarthritis (OA) pathogenesis and for the discovery of novel molecules with marker usefulness for this disease. This review will describe the most recent “omics” studies in OA research and their conclusions, and discuss those remaining challenges.

Recent achievements in genomics studies

In genomics, recent technological advances expanded the amount of “omic” data to higher levels, including genome sequencing, epigenetics, and transcriptomics. The aims of each of these molecular approaches are the understanding of the mechanisms underlying OA, and the identification of molecular markers to predict disease onset and progression (Table 1).

During the last decade, genome-wide association studies (GWAS) have been the preferred tool to study the genetics of late onset knee, hip and hand OA (1-3). Results derived from these studies reported that, as for many complex diseases, there is no single genetic variant responsible for OA. However, up to thousands of loci could be potentially associated, each with a small effect (4). To date, this method of analysis identified 19 independent susceptibility loci for OA (4). Some of them, such as rs1180992 at DOT1L gene, rs2862851 at TGFA, rs10471753 at PIK3R1, rs2236995 at SLBP, rs496547 at TREH and rs10948172 at RUNX2, are also significantly associated with a decreased cartilage thickness in terms of clinical radiographic endophenotyping according to mJSW (minimal joint space width) (5). The study of endophenotypes is a way to increase power

in GWAS, enabling the detection of genes with potential functional importance that were not revealed in previous case-controls studies due to disease heterogeneity. This is the case of a recent meta-analysis of clinically relevant endophenotypes in hip OA, where the authors reported suggestive evidence for association of 6 variants located in novel genes such as LRCH1 or STT3B with increased joint space narrowing and the bone remodeling response (6). A very recent study performed a GWAS of total hip replacements based on variants identified through whole-genome sequencing, concluding that two variants: a missense mutation in the COMP gene and a frameshift variant in the CHADL gene, were significantly associated with an increased risk of hip replacement (7).

Mitochondrial genetics has also been consolidated as a contributor to the risk of knee OA. Two recent meta-analyses showed a significant association of specific mtDNA variants with the rate of incidence and progression of OA in well-defined prospective cohorts such as the Cohort Hip and Cohort Knee (CHECK) and the Osteoarthritis Initiative (OAI). In these studies, haplogroup J and superhaplogroup JT associate with a decreased rate of incident knee OA at 8 years and a decreased rate of radiographic progression, respectively (8, 9).

DNA methylation in OA has also been the focus of many studies during the last years. The first studies relied on the analysis of specific CpG sites in promoter regions of candidate genes involved in the OA process such as the matrix metalloproteinases (10). However, the last works were based on genome-wide DNA methylation analyses in articular cartilage (11, 12). In the last year, a very interesting study analyzed DNA methylation changes in three regions of the subchondral bone of the tibial plateau to represent early, intermediate and late stages of OA, and compare them with those on the site-matched cartilage. Authors concluded that methylation changes in the subchondral bone could precede changes in the cartilage (13). All these studies show a high variable

number of differentially reported sites. However, enrichment analyses of all of them indicate similar pathways, including embryonic morphogenesis, inflammation and skeletal development (14). Finally, according to OA methylation changes, Vidal-Bralo and colleagues concluded that premature epigenetic aging is a characteristic of OA cartilage, being a component of the disease pathogenesis that reflects damage and vulnerability (15).

Several studies have used arrays to investigate gene expression changes in OA, mainly in articular cartilage (16). However, over the last years, these transcriptomic assays were performed primarily to validate methylation analyses (11, 17) and actually are being substituted by the most sensitive RNA-seq assay. Considering the proposed hypothesis that the regenerative potential of human mesenchymal stem cells (hMSCs) is altered in advanced-stage OA, some studies analyzed the methylation and/or expression profile of these cells. One of these works performed a large-scale gene expression profile of bone marrow stromal cells (BMSCs) from osteoarthritic cells compared with healthy, using a microarray from Affymetrix. This study revealed up to 690 intergroup differentially regulated genes between BMSCs from OA donors and healthy controls (18). Another relevant work described a validation transcriptomic analysis by RNA-seq to compare the expression patterns of hMSCs from patients with fractures and OA. In this study, the authors denote two areas of potential interest for discovering new therapeutic targets for bone mass disorders and bone regeneration: those related to the mechanisms stimulating MSCs proliferation and those impairing their terminal differentiation (17). Finally, the availability of data from the Gene Expression Omnibus (GEO) database permitted the bioinformatic analyses and meta-analyses of gene expression profiles. This is the case of one study of disease-related genes of synovial membrane associated with progression of OA, in which the authors identified 401 up-regulated genes involved in the inflammatory

response and 196 down-regulated genes related with cell cycle processes (19). Another work identified a small overlap between the differentially expressed genes of the cartilage compared to those of the synovium. The authors suggest the existence of different pathogenic mechanisms that are specific of the synovium, since a much higher amount of differentially expressed genes were found in this tissue when comparing OA samples with healthy controls (20).

Finally, a relevant number of studies were performed most recently to discover differentially expressed micro-ribonucleic acids (miRNAs) in cartilage or bone between OA and controls. However, there is almost no overlap between those reported to be differentially expressed with statistical significance (14). Analyses of circulating miRNAs in serum or synovial fluid, which may reflect altered tissue expression in OA and have thus a potential use as disease biomarkers, are of special interest. The first study on this area was conducted by Beyer and colleagues in a large prospective cohort consisting of 816 Caucasian individuals, and explored the association between serum levels of miRNAs and the development of severe OA. The authors identified the miRNA let-7e as a potential predictor for severe knee or hip OA (21). A more recent study aimed to identify miRNAs in synovial fluid useful to differentiate between early- and late-stage knee OA, and led to the identification of a panel of seven circulating miRNAs (22).

Characterization of OA related-proteins and identification of putative biomarkers by proteomics

After more than a decade of proteomics studies performed in OA, these approaches have contributed to a better understanding of disease pathogenesis and the identification of novel protein markers. The most recent descriptive studies in this field have been focused in elucidating the molecular composition of cartilage and the disease-dependent changes

that occur in this tissue. This has been achieved by qualitative and quantitative shotgun analyses of the different layers and types (knee or hip) of OA and healthy tissues (23), and also by the evaluation of the response to IL-1α in cartilage using an *in vitro* model of mouse tissue explants (24). The analysis of chondrocyte secretomes has been reported as a valuable strategy to explore the processes related with ECM remodeling and the molecular mechanisms driven by the cell in response to different stimuli (25). Accordingly, a recent proteomic study on secretomes reports the effects of nicotine on both OA and healthy chondrocytes treated with IL-1β, and suggests a negative effect of this drug on the joint (26). Apart from these shotgun studies, the progression of matrix degradation in response to mechanical damage and cytokine treatment of human knee cartilage explants has been also evaluated using targeted proteomics (27). Different protein domains of aggrecan, COMP neoepitopes and collagen pro-peptides were measured throughout a 21-day culture period, being some of them potentially relevant as biomarkers for post-traumatic OA.

Biological fluids such as synovial fluid, plasma and serum, have been also extensively studied for the search of protein markers for OA (Table 2). In a discovery step, suspension beads-based protein arrays were used to screen serum samples from a cohort including OA and RA patients, and healthy controls. After linear regression analysis adjusting for sex, age and body mass index (BMI) three proteins were significantly elevated in serum from OA patients compared to controls: C3, ITIH1 and S100A6. A panel consisting of these three proteins had an area under the curve of 0.82 for the classification of OA and control samples (28). In an analogous study, a panel of 7 proteins was found quantitatively different in sera from OA, RA and healthy controls (29). A targeted proteomic analysis was also performed on sera from OA patients and controls, in this case employing mass spectrometry (30). The authors developed a method

for the multiplexed monitoring of 14 biomarker candidates for OA, and verified the increased amount of Haptoglobin and von Willebrand Factor in OA patients.

Finally, protein modifications have also been recently explored using proteomic approaches. In one of this works, glycated, oxidized and nitrated proteins and amino acids were detected in synovial fluid and plasma of arthritic patients. Their combination with hydroxyproline and anti-CCP antibody status provided a plasma-based biochemical test of 0.92 sensitivity and 0.90 specificity for early-stage OA (31). Using a very different strategy, N-glycosylation of proteins was analyzed by mass spectrometry imaging (MSI) in subchondral bone from knee OA patients (32). The latter study demonstrates the usefulness of this novel technology to complement the proteomic data with valuable spatial information (33).

Metabolomic approaches to profile OA-related processes and identify novel biomarkers

Being the youngest of the "omics" technologies, methods for the study of the metabolome have greatly evolved in the last years, also in the field of OA.

Two metabolomic approaches were recently carried out to gain insight into pathogenic processes characteristic of OA, such as subchondral bone sclerosis or osteophyte formation. In the first study, a metabolic profiling was carried out on subchondral bone from patients with primary OA (34). 68 metabolites were identified to be significantly changed in the sclerotic tissue compared with the non-sclerotic. Metabolites such as taurine, hypotaurine, beta-alanine, L-carnitine, and glycerophospholipids were found to be related with this pathological process. In the work on osteophyte formation (35), authors found metabolic variations between extracts of osteophyte cartilage tissues and uninvolved control cartilages, which are related with processes of collagen dissolution,

destruction of boundary layers, and self-restoration. Phenylalanine metabolism was also highly correlated with osteophyte formation.

Regarding the identification of metabolites with putative biomarker usefulness for the disease, metabolomic analyses have been performed recently in synovial fluid, plasma and urine from OA patients. The conclusions from these studies are summarized in Table 3. In synovial fluid, twenty-eight metabolites (including malate, ethanolamine, squalene, glycerol, myristic acid, oleic acid, lanosterol, heptadecanoic acid, and capric acid) were identified as critical metabolites for discriminating between early and late OA. These were robustly altered along the radiographic stage of knee OA (36). In a similar study, six different metabolites (glutamine, 1,5-anhydroglucitol, gluconic lactone, tyramine, threonine, and 8-aminocaprylic acid) were strongly associated with knee OA. Gluconic lactone concentration was also significantly different between OA and RA (37). Finally, a recent lipidomic analysis has been carried out, describing the identification of thirty-seven lipids in the soluble fraction of SF from OA and RA patients. Among them are polyunsaturated fatty acids and their pro-inflammatory and pro-resolving lipoxygenase (LOX) and cyclooxygenase (COX) pathway markers. This work shows for the first time that resolution pathways are present in SF from OA patients (38).

In plasma, Zhang and colleagues reported the identification of lower arginine concentrations in patients with knee OA compared to controls (39). They hypothesize this is due to an over activity of arginine to ornithine pathway, which leads to an imbalance between cartilage repair and degradation. In another study from the same group, the branched chain amino acids to histidine ratio was confirmed to be associated with advanced knee OA, and also the lysophosphatidylcholines (lysoPCs) to phosphatidylcholines (PCs) ratio. Subjects with this latter ratio ≥0.09 were 2.3 times

more likely to undergo total knee replacement than those with the ratio <0.09 during a 10-year follow-up (40).

Finally, a very interesting study described metabolomic profiles in urine, distinguishing OA progressors (≥0.7 mm decrease in JSW at 18 months) from non-progressors (≤0.35 mm decrease in JSW). Glycolate, hippurate, and trigonelline were among the important metabolites for discriminating these groups at baseline, whereas alanine, N,N-dimethylglycine, glycolate, hippurate, histidine, and trigonelline, were among the metabolites that were important at 18 months. These findings support a role for metabolic factors in the progression of knee OA, and suggest that measurement of metabolites could be useful to predict progression (41). Altogether, metabolomic studies have reported a number of molecules that play a role in the pathogenic process of OA and may be useful markers for disease progression studies.

Remaining challenges

Given the great amount of information that the "omics" approaches has provided to the investigation in OA, still there is a bottleneck in translating these findings to useful tools in clinical routines. Validation studies, capable to monitorize panels of biomarker candidates and qualify them for a clinical application, are still minority. In genomics, several meta-analyses have been performed for the systematic evaluation of the findings obtained in GWAS, and some validation studies have led to the definition of polymorphisms associated with OA susceptibility, severity and rate of progression. Such type of analyses is yet almost absent in the field of proteomics and metabolomics, due to the higher complexity of the multiplexed analysis of metabolites and (even more) proteins. In this field, the ultimate advances in targeted proteomics and metabolomics technologies (such as mass spectrometry instrumentation and protein microarray

platforms) are expected to facilitate their application in larger cohorts and under the frame of clinical trials.

To date, clinical data has been combined with the evaluation of genetic polymorphisms to predict primary knee OA progression (42), and also with imaging markers to generate prediction algorithms of structural progression (43). Furthermore, a recent study evaluated the predictive validity of 18 protein biomarkers in serum and urine samples from the OAI cohort (44). Considering the promising results obtained in these independent works, the next objective would be to develop combined tools (genes+proteins+metabolites+imaging) to identify patients with high risk of progression who will respond to a specific treatment. The integration of "omics" information with clinical and imaging data is a highly promising strategy for the identification of phenotype profiles. The APPROACH project (Applied Public-Private Research enabling OsteoArthritis Clinical Headway), currently ongoing, contributes to this integration by combining biomedical information (clinical, genomic, proteomic, metabolomic, x-ray and MRI) from knee OA patients and controls into a unified bioinformatics platform (45).

Conclusion

"Omics" technologies applied in the last decade for the study of OA have provided thousands of molecules related with this disease. Further validation of these findings will allow moving from single to multiplex biomarkers, defining the so-called molecular signatures related with a specific OA phenotype, or either those that could contribute to an increased diagnostic accuracy, disease progression studies, or to predict the response of each patient to a treatment. Leveraging multiomics technology to combine this information with the clinical data may much better define these biomarker profiles and further the goal of precision medicine strategies in OA.

Key Points

- The high complex nature of osteoarthritis has hindered the development of tools for the precise evaluation and therapeutic management of this disease.

- "Omics" technologies have contributed to increase the knowledge on OA pathogenesis, and have provided lists of molecules related with this disease.

- Further validation studies are still needed to translate the findings from "omics" studies to clinical applications.

- A tool able to combine the molecular information generated by the "omics" studies with imaging and clinical data would be highly valuable to facilitate precision medicine strategies in OA.

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Conflicts of interest

Authors declare no conflicts of interest.

References

1. Evangelou E, Valdes AM, Kerkhof HJ, Styrkarsdottir U, Zhu Y, Meulenbelt I, et al. Meta-analysis of genome-wide association studies confirms a susceptibility locus for knee osteoarthritis on chromosome 7q22. Ann Rheum Dis. 2011;70(2):349-55.

2. Evangelou E, Kerkhof HJ, Styrkarsdottir U, Ntzani EE, Bos SD, Esko T, et al. A meta-analysis of genome-wide association studies identifies novel variants associated with osteoarthritis of the hip. Ann Rheum Dis. 2014;73(12):2130-6.

3. Styrkarsdottir U, Thorleifsson G, Helgadottir HT, Bomer N, Metrustry S, Bierma-Zeinstra S, et al. Severe osteoarthritis of the hand associates with common variants within the ALDH1A2 gene and with rare variants at 1p31. Nat Genet. 2014;46(5):498-502.

4. Warner SC, Valdes AM. Genetic association studies in osteoarthritis: is it fairytale? Curr Opin Rheumatol. 2017;29(1):103-9.

• *An interesting review describing the most robust SNPs associated with osteoarthritis susceptibility derived from GWAS*

5. Castaño-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(10):e1006260.

6. 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(7):1199-206.

•• One of the most important studies to date describing the influence of robust SNPs in radiographic endophenotypes in hip osteoarthritis

7. 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(5):801-5.

8. Fernández-Moreno M, Soto-Hermida A, Vázquez-Mosquera ME, Cortés-Pereira E, Pértega S, Relaño S, et al. A replication study and meta-analysis of mitochondrial DNA variants in the radiographic progression of knee osteoarthritis. Rheumatology (Oxford). 2017;56(2):263-70.

9. Fernández-Moreno M, Soto-Hermida A, Vázquez-Mosquera ME, Cortés-Pereira E, Relaño S, Hermida-Gómez T, et al. Mitochondrial DNA haplogroups influence the risk of incident knee osteoarthritis in OAI and CHECK cohorts. A meta-analysis and functional study. Ann Rheum Dis. 2017;76(6):1114-22.

• *The first meta-analysis of mtDNA haplogroups on incident knee OA and functional explanation of their effects*

10. Roach HI, Yamada N, Cheung KS, Tilley S, Clarke NM, Oreffo RO, et al. Association between the abnormal expression of matrix-degrading enzymes by human osteoarthritic chondrocytes and demethylation of specific CpG sites in the promoter regions. Arthritis Rheum. 2005;52(10):3110-24.

11. 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(8):1876-86.

12. Zhao L, Wang Q, Zhang C, Huang C. Genome-wide DNA methylation analysis of articular chondrocytes identifies TRAF1, CTGF, and CX3CL1 genes as hypomethylated in osteoarthritis. Clin Rheumatol. 2017.

13. 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.

•• The first study to date describing that methylation changes in subchondrial bone could precede methylation changes in cartilage

14. van Meurs JB. Osteoarthritis year in review 2016: genetics, genomics and epigenetics. Osteoarthritis Cartilage. 2017;25(2):181-9.

15. 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(9):2222-31.

16. Steinberg J, Zeggini E. Functional genomics in osteoarthritis: Past, present, and future. J Orthop Res. 2016;34(7):1105-10.

17. Del Real A, Pérez-Campo FM, Fernández AF, Sañudo C, Ibarbia CG, Pérez-Núñez 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(2):113-22.

18. Stiehler M, Rauh J, Bünger C, Jacobi A, Vater C, Schildberg T, et al. Large-scale gene expression profiling data of bone marrow stromal cells from osteoarthritic donors. Data Brief. 2016;8:545-8.

19. Dong S, Xia T, Wang L, Zhao Q, Tian J. Investigation of candidate genes for osteoarthritis based on gene expression profiles. Acta Orthop Traumatol Turc. 2016;50(6):686-90.

20. Park R, Ji JD. Unique gene expression profile in osteoarthritis synovium compared with cartilage: analysis of publicly accessible microarray datasets. Rheumatol Int. 2016;36(6):819-27.

21. Beyer C, Zampetaki A, Lin NY, Kleyer A, Perricone C, Iagnocco A, et al. Signature of circulating microRNAs in osteoarthritis. Ann Rheum Dis. 2015;74(3):e18.

22. Li YH, Tavallaee G, Tokar T, Nakamura A, Sundararajan K, Weston A, et al. Identification of synovial fluid microRNA signature in knee osteoarthritis: differentiating early- and late-stage knee osteoarthritis. Osteoarthritis Cartilage. 2016;24(9):1577-86.

23. Hsueh MF, Khabut A, Kjellstrom S, Onnerfjord P, Kraus VB. Elucidating the Molecular Composition of Cartilage by Proteomics. J Proteome Res. 2016;15(2):374-88.

• *An interesting insight into the proteomic composition of human articular cartilage.*

24. Wilson R, Golub SB, Rowley L, Angelucci C, Karpievitch YV, Bateman JF, et al. Novel Elements of the Chondrocyte Stress Response Identified Using an in Vitro Model of Mouse Cartilage Degradation. J Proteome Res. 2016;15(3):1033-50.

25. Sanchez C, Bay-Jensen AC, Pap T, Dvir-Ginzberg M, Quasnichka H, Barrett-Jolley R, et al. Chondrocyte secretome: a source of novel insights and exploratory biomarkers of osteoarthritis. Osteoarthritis Cartilage. 2017.

26. Lourido L, Calamia V, Fernandez-Puente P, Mateos J, Oreiro N, Blanco FJ, et al. Secretome analysis of human articular chondrocytes unravels catabolic effects of nicotine on the joint. Proteomics Clin Appl. 2016;10(6):671-80.

27. Wang Y, Li Y, Khabut A, Chubinskaya S, Grodzinsky AJ, Onnerfjord P. Quantitative proteomics analysis of cartilage response to mechanical injury and cytokine treatment. Matrix Biol. 2016.

28. Lourido L, Ayoglu B, Fernandez-Tajes J, Oreiro N, Henjes F, Hellstrom C, et al. Discovery of circulating proteins associated to knee radiographic osteoarthritis. Sci Rep. 2017;7(1):137.

• *One of the first proteomic studies in OA using high throughput protein microarrays. .*

29. Sierra-Sanchez A, Garrido-Martin D, Lourido L, Gonzalez-Gonzalez M, Diez P, Ruiz-Romero C, et al. Screening and Validation of Novel Biomarkers in Osteoarticular Pathologies by Comprehensive Combination of Protein Array Technologies. J Proteome Res. 2017;16(5):1890-9.

30. Fernandez-Puente P, Calamia V, Gonzalez-Rodriguez L, Lourido L, Camacho-Encina M, Oreiro N, et al. Multiplexed mass spectrometry monitoring of biomarker candidates for osteoarthritis. J Proteomics. 2017;152:216-25.

31. Ahmed U, Anwar A, Savage RS, Thornalley PJ, Rabbani N. Protein oxidation, nitration and glycation biomarkers for early-stage diagnosis of osteoarthritis of the knee and typing and progression of arthritic disease. Arthritis Res Ther. 2016;18(1):250.

32. Briggs MT, Kuliwaba JS, Muratovic D, Everest-Dass AV, Packer NH, Findlay DM, et al. MALDI mass spectrometry imaging of N-glycans on tibial cartilage and subchondral bone proteins in knee osteoarthritis. Proteomics. 2016;16(11-12):1736-41.

33. Rocha B, Ruiz-Romero C, Blanco FJ. Mass spectrometry imaging: a novel technology in rheumatology. Nat Rev Rheumatol. 2017;13(1):52-63.

•• *Review of one of the most promising proteomic technologies, still in its infancy in osteoarthritis research.*

34. Yang G, Zhang H, Chen T, Zhu W, Ding S, Xu K, et al. Metabolic analysis of osteoarthritis subchondral bone based on UPLC/Q-TOF-MS. Anal Bioanal Chem. 2016;408(16):4275-86.

35. Xu Z, Chen T, Luo J, Ding S, Gao S, Zhang J. Cartilaginous Metabolomic Study Reveals Potential Mechanisms of Osteophyte Formation in Osteoarthritis. J Proteome Res. 2017;16(4):1425-35.

36. Kim S, Hwang J, Kim J, Ahn JK, Cha HS, Kim KH. Metabolite profiles of synovial fluid change with the radiographic severity of knee osteoarthritis. Joint Bone Spine. 2016.

37. Zheng K, Shen N, Chen H, Ni S, Zhang T, Hu M, et al. Global and targeted metabolomics of synovial fluid discovers special osteoarthritis metabolites. J Orthop Res. 2017.

38. Jonasdottir HS, Brouwers H, Kwekkeboom JC, van der Linden HMJ, Huizinga T, Kloppenburg M, et al. Targeted lipidomics reveals activation of resolution pathways in knee osteoarthritis in humans. Osteoarthritis Cartilage. 2017;25(7):1150-60.

• *A novel lipidomic strategy describes interesting pathways activated in OA.*

39. Zhang W, Sun G, Likhodii S, Liu M, Aref-Eshghi E, Harper PE, et al. Metabolomic analysis of human plasma reveals that arginine is depleted in knee osteoarthritis patients. Osteoarthritis Cartilage. 2016;24(5):827-34.

40. Zhang W, Sun G, Aitken D, Likhodii S, Liu M, Martin G, et al. Lysophosphatidylcholines to phosphatidylcholines ratio predicts advanced knee osteoarthritis. Rheumatology (Oxford). 2016;55(9):1566-74.

41. Loeser RF, Pathmasiri W, Sumner SJ, McRitchie S, Beavers D, Saxena P, et al. Association of urinary metabolites with radiographic progression of knee osteoarthritis in overweight and obese adults: an exploratory study. Osteoarthritis Cartilage. 2016;24(8):1479-86.

• *Promising metabolomic analysis in urine reveals metabolites associated with OA progression.*

42. Blanco FJ, Moller I, Romera M, Rozadilla A, Sanchez-Lazaro JA, Rodriguez A, et al. Improved prediction of knee osteoarthritis progression by genetic polymorphisms: the Arthrotest Study. Rheumatology (Oxford). 2015;54(7):1236-43.

43. LaValley MP, Lo GH, Price LL, Driban JB, Eaton CB, McAlindon TE. Development of a clinical prediction algorithm for knee osteoarthritis structural progression in a cohort study: value of adding measurement of subchondral bone density. Arthritis Res Ther. 2017;19(1):95.

44. Kraus VB, Collins JE, Hargrove D, Losina E, Nevitt M, Katz JN, et al. Predictive validity of biochemical biomarkers in knee osteoarthritis: data from the FNIH OA Biomarkers Consortium. Ann Rheum Dis. 2017;76(1):186-95.

•• *A very exhaustive screening of the utility of a panel of protein biomarkers for OA.*

45. https://www.approachproject.eu/

Tables

Table 1. Genomics studies in OA disease from 2016

Study	Type of study	Trait/Phenotype	Findings
Warner, 2017	Review GWAS	OA susceptibility	Description of the most robust SNPs associated with OA with genome-wide significance
Castaño-Betancourt, 2016	GWAS	mJSW	6 variants associated with decreased cartilage thickness
Panotsopoulou, 2017	Meta-analysis	JSN and bone remodeling	6 variants associated with increased JSN and bone remodeling response
Styrkarsdottir, 2017	WGS/GWAS	Hip replacement	2 variants associated with increased risk of hip replacement
Fernández-Moreno, 2017a	Genetic association	Incident knee OA	mtDNA haplogroup J associated with a decreased rate of incident knee OA
Fernández-Moreno, 2017b	Genetic association	Rx knee OA progression	mtDNA cluster JT associated with a decreased risk of Rx knee OA progression
Zhang, 2016	Genome-wide methylation in bone and cartilage	Stages of OA	Changes in subchondral bone precede the methylation changes in cartilage
Vidal-Bralo, 2016	DNA methylation	Epigenetic aging in cartilage	Premature epigenetic aging as a characteristic of OA cartilage
Stiehler, 2016	Gene expression array in BMSCs	Regenerative potential of BMSCs in advanced OA	690 intergroup differentially regulated genes between OA and healthy controls
Del Real, 2017	RNA-seq in hMSCs	Differential expression patterns between OP and OA	Mechanisms stimulating hMSCs proliferation and mechanisms impairing their terminal differentiation as areas of potential interest for new therapeutic targets
Dong, 2016	Bioinformatic analysis of gene expression patterns of SM	OA progression	401 up-regulated genes involved in inflammatory response and 196 down-regulated genes involved in cell cycle
Park, 2016	Gene expression between cartilage and synovium	OA susceptibility	There are different pathogenic mechanisms that are specific for the synovium in OA
Beyer, 2015	Circulant miRNAs in serum	OA severity	miRNA let-7e as a potential predictor for severe knee or hip OA
Li, 2016	Circulant miRNAs in SF	Stages of OA	7 circulating miRNAs differentially expressed between late-stage OA and early-stage OA

OA: osteoarthritis; GWAS: genome-wide association studies; SNP: single nucleotide polymorphism; mJSW: minimum joint space width; JSN: joint space narrow; mtDNA: mitochondrial DNA; WGS: whole-genome sequencing; BMSCs: bone marrow stromal cells; hMSCs: human mesenchimal stem cells; OP: osteoporosis; SM: synovial membrane; SF: synovial fluid; miRNA: micro RNA

Table 2. Circulating proteins with putative biomarker utility for OA found in the most recent proteomic studies.

Protein	Biomarker utility	Reference
C3, ITIH1, S100A6	Knee OA diagnosis	Lourido, 2017
PLTP, NRAM1/SLC11A1	Knee OA diagnosis	Sierra-Sánchez, 2017
HPT, VWF	OA diagnosis	Fernández-Puente, 2017
Glycated, oxidized and nitrated proteins and amino acids	Early OA diagnosis	Ahmed, 2016

C3: Complement C3, HPT: Haptoglobin; ITIH1: Inter-alpha trypsin inhibitor heavy chain H1; OA: osteoarthritis; PLTP: phospholipid transfer protein; NRAM1/SLC11A1: Natural resistance-associated macrophage protein 1; VWF: von Willebrand Factor.

Table 3. Metabolites with putative biomarker usefulness in OA, described in the most recent metabolomic studies.

Metabolite	Type of sample	Biomarker utility	Reference
Malate, ethanolamine, squalene, glycerol, myristic acid, oleic acid, lanosterol, heptadecanoic acid and capric acid.	Synovial fluid	Diagnosis, Progression	Kim, 2016
Glutamine, 1,5-anhidroglucitol, gluconic lactone, tyramine, threonine and 8-aminocaprilyc acid	Synovial fluid	Knee OA diagnosis	Zheng, 2017
Polyunsaturated fatty acids, LOX and COX pathway markers	Synovial fluid	Diagnosis	Jonsadottir, 2017
Arginine (low concentrations)	Plasma	Knee OA diagnosis	Zhang, O&C 2016
Branched chain amino acids/histidine, lysoPCs/PCs	Plasma	Diagnosis, Prognosis	Zhang, Rheumatology 2016
Glycolate, hippurate, trigonelline	Urine	Progression predictors	Loeser, 2016
Alanine, N,N-dimethylglycine, glycolate, hippurate, histidine, trigonelline	Urine	Progression markers	Loeser, 2016

COX: ciclooxygenase; LOX: lipoxygenase; lysoPCs: lysophosphatidylcholines; PCs: Phosphaticylcholines; OA: osteoarthritis.