Applications of transcriptomics in support of drug development for osteoarthritis

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ABSTRACT

Objective: Understanding the heterogeneity and pathophysiology of osteoarthritis (OA) is critical to support the development of tailored disease-modifying treatments. To this aim, transcriptomics tools are highly relevant to delineate dysregulated molecular pathways and identify new therapeutic targets.

Methods: We review the methodology and outcomes of transcriptomics studies conducted in OA, based on a comprehensive literature search of the PubMed and Google Scholar databases using the terms “osteoarthritis”, “OA”, “knee OA”, “hip OA”, “genes”, “RNA-seq”, “microarray”, “transcriptomic” and “PCR” as key words. Beyond target-focused RT-qPCR, more comprehensive techniques include microarrays, RNA sequencing (RNA-seq) and single cell RNA-seq analyses.

Results: The standardization of those methods to ensure the quality of both RNA extraction and sequencing is critical to get meaningful insights. Transcriptomics studies have been conducted in various tissues involved in the pathogenesis of OA, including articular cartilage, subchondral bone and synovium, as well as in the blood of patients. Molecular pathways dysregulated in OA relate to cartilage degradation, matrix and bone remodeling, neurogenic pain, inflammation, apoptosis and angiogenesis. This knowledge has direct application to patient stratification and further, to the identification of candidate therapeutic targets and biomarkers intended to monitor OA progression.

Conclusion: In light of its high-throughput capabilities and ability to provide comprehensive information on major biological processes, transcriptomics represents a powerful method to support the development of new disease-modifying drugs in OA.

1. Introduction

Osteoarthritis (OA) is a highly prevalent chronic musculoskeletal condition, affecting up to 16% of the population worldwide and 23% of individuals aged 40 and over [1]. As one of the most disabling and painful disease, OA has been ranked as the 11th highest priority by the World Health Organization. Furthermore, it causes a huge economic burden estimated to up to 2.5% of Growth National Product in Western countries, as a consequence of significant healthcare expenses and indirect costs associated with comorbidities [2]. As of today, there is no curative treatment for OA, with current interventions solely based on symptomatic non-pharmacological and pharmacological treatments having a limited effect on function recovery and pain. Thus, a high unmet medical need for disease-modifying osteoarthritis drugs (DMOADs) persists to slow down progression of the disease. Ideally, future treatments should provide benefits on both function and pain, while delaying or avoiding the need for surgical joint replacement.

The development of various DMOAD candidates has been unsuccessful to this date, mainly because OA is a heterogeneous disease with various pathogenic factors eventually leading to common clinical and radiographic manifestations. The complexity of OA stems from the possible involvement of several periarticular tissues beyond cartilage, including the subchondral bone, fat pad, tendons, ligaments, synovial tissue and skeletal muscle. Consequently, the cross-talk between cartilage and its surrounding tissues should be recognized as an important contributor to OA, broadly regulating cartilage remodeling and overall...
Several disease processes. Previous attempts to establish patient classifications were based on key underlying pathophysiological mechanisms, clinically relevant patient characteristics, disease stage, nature of joints involved or degree of associated inflammation [3–5]. However, no consensus could be established as these features often overlap in patients. Whereas a stratified representation of OA as endotypes does not exist as of today, it could be facilitated by the recent availability of profiling approaches relying upon omics technologies, well suited to better understand molecular changes associated with the disease [6]. Next generation sequencing (NGS) technologies have made remarkable progress to allow high throughput DNA and RNA sequencing analyses in large cohorts of patients. In particular, RNA-seq allows to measure quantitatively the level of gene expression in the blood or in target tissues at the whole transcriptome level, thus providing for each patient comprehensive information regarding his/her inflammatory, immune and metabolic status, all being relevant to design better tailored treatments.

In this context, we review herein the methodology and quality controls used in transcriptomics studies. We further report on the outcomes of such studies conducted in OA patients, both in affected tissues (cartilage, subchondral bone and synovium) and in the blood. We discuss how those transcriptomics studies are shedding light on dysregulated molecular pathways associated with OA as well as on potential new therapeutic targets or biomarkers useful to inform patient stratification and treatment strategies.

2. Methodology and quality control in transcriptomics

The technologies currently available to assess gene expression are presented in Table 1. Among those, real-time reverse transcription polymerase chain reaction (RT-PCR) has been broadly used as a highly sensitive and reproducible method to measure the expression of specific sets of genes [7]. Microarrays rely upon synthetic oligonucleotides spotted on a microchip to assess the expression of thousands of different genes simultaneously [8]. Whereas those methods can analyze multiple genes at once, RT-PCR does not differentiate between differentially spliced RNAs (high sensitivity, low specificity) and microarray lacks sensitivity and specificity. They are being complemented by new RNA-Seq technologies which do not require species nor transcript-specific probes and can help identifying novel transcripts [9]. RNA-Seq thus encompasses a large range of gene expression quantification methods, allowing to detect differentially expressed genes (DEG) with a higher specificity and sensitivity than microarrays [10].

Table 1

| Characteristics | Research purposes | Advantages | Limitations | Quality Controls |
|-----------------|------------------|------------|-------------|-----------------|
| RT-PCR          | Qualitative and quantitative assessment of the expression of known genes | Wide dynamic range of quantification, High sensitivity | Focused on selected genes (hypothesis driven), Costly, Potential bias increased by cycles of PCR amplification | RINb |
| Microarrays     | Application to the expression of thousands of genes | Species and transcript-specific detection of gene expression, Comprehensive analysis of the whole transcriptome (non hypothesis-driven), High throughput analysis applicable at patient cohort level | Limited dynamic range of detection, Costly and time consuming, Complex data integration and interpretation | RIN |
| RNA-seq         | Qualitative and quantitative analysis of the whole transcriptome, including novel genes | Comprehensive analysis of the whole transcriptome (non hypothesis-driven), High throughput analysis applicable at patient cohort level | Need to analyze sufficient numbers of cells to overcome sequencing coverage bias, Low capture efficiency, Complex data integration and interpretation | Detected genes per cell, UMIc counts per cell, Percentage of detected mitochondrial genes per cell |
| Single cell RNA Seq | Analysis of gene expression at the transcriptome level in individual cells | Comprehensive analyses of biological processes at individual cell level, Possibility to combine with single cell proteomics and spatial representation within tissues | Need to analyze sufficient numbers of cells to overcome sequencing coverage bias, Low capture efficiency, Complex data integration and interpretation | Detected genes per cell, UMIc counts per cell, Percentage of detected mitochondrial genes per cell |

a RT-PCR: real-time reverse transcription polymerase chain reaction.

b RIN: RNA Integrity Number.

c UMI: unique bar code.

d DV200: percentage of RNA fragments with a length over 200 nucleotides.

Recently, single cell RNA-Seq (scRNA-Seq) has been broadly adopted to relate gene expression to a specific cell within a tissue [11]. Single cell RNA-Seq can also be combined with focused proteomics, and further, to spatial representation within a tissue (Table 1). One limitation is that sufficient numbers of cells need to be analyzed to overcome the inter-cell heterogeneity in gene expression profiles.

Irrespective of the technology, both the process of RNA extraction [12] and the quality control of RNA are critical to ensure the reliability of outcomes [13] (Fig. 1 A, B, C). Although RNA is a thermodynamically stable molecule, it is rapidly digested by ubiquitous RNase enzymes present in biological fluids and tissues. A gradual degradation of RNAs leads to a continuous shift towards shorter fragment sizes affecting the results of quantitative analyses. In order to evaluate RNA integrity, bioanalyzer electrophoresis is commonly used to confirm that 28S and 18S RNAs are properly detected. RNA Integrity Number (RIN) is a software algorithm routinely used to assess RNA quality [14], with a RIN value of at least 6, commonly set as a cutoff for an acceptable RNA integrity [15]. When using formalin-fixed, paraffin-embedded (FFPE) tissue samples, RNA signals are often more difficult to detect, resulting in low RIN values. RNA signals are considered as detectable when RIN is above 2 in FFPE tissue [16,17]. Quality control of such FFPE-derived RNA is then better performed by determining percentages of RNA fragments with a length over 200 nucleotides (DV200) [13] (Fig. 1 A, B). A commonly accepted threshold for DV200 to ensure a sufficient RNA quality is over 70 when using an Illumina platform.

QC data combining RIN and DV200 values can be represented by principal component analysis (PCA). A PCA analysis of RNA samples obtained from 286 OA patients included in the APPROACH cohort illustrates that RIN and DV200 values are independent (with a Pearson correlation of 0.56) and thus provide complementary information on RNA quality (Fig. 1D). Samples exhibiting either low RIN or low DV200 values appear as outliers (Fig. 1A and B) and can be excluded from subsequent data analyses. In addition, the measurement of FPKM (fragments per kilobase of exon model per million reads) is a sample normalization method frequently used to overcome the gene-length and library-size effects [14]. Collectively, biases in gene expression may reflect RNA sample or sequencing quality issues, distinguishable in profiles obtained with a Kernel plot representation (Fig. 1C). Specific quality controls for scRNA-Seq are commonly based on three main criteria, including (i) number of genes per cell [15]; (ii) unique bar code (UMI) counts per cell; (iii) percentage of mitochondrial genes per cell. The number of genes detected varies depending on the quality of the library
and cell types used, while further reflecting the heterogeneity of single cell datasets [18]. The two first metrics are often evaluated together to document RNA quantity. Single cell samples exhibiting few (ie below 2000) expressed genes or low UMI counts usually contain small RNA quantities. The third metric reflects samples with damaged/dying cells whose cytoplasmic mRNA leaked out through a broken membrane, thus releasing mitochondrial mRNA [18,19].

3. Overview of transcriptomics studies in OA

Table 2 provides a comprehensive overview of transcriptomic studies conducted in joint tissues and in the blood of patients with OA since the availability of reliable quantitative transcriptomics methods (ie circa 2010). We reviewed the methodology and outcomes of transcriptomics studies conducted in human OA based on a comprehensive literature search of the PubMed and Google scholar databases using the terms “osteoarthritis”, “OA”, “knee OA”, “hip OA”, “genes”, “RNA-seq”, “microarray”, “transcriptomic” and “PCR” as key words. The inclusion criteria were as follows: (1) original articles published in peer-reviewed journals, (2) publications focused on primary knee or hip OA, (3) studies reporting transcriptomic changes at mRNA levels. Publications describing effects on circulating, mitochondrial or long coding RNA only were excluded from our review. When two publications used the same dataset, only the first one was mentioned.

Collectively, gene expression profiling studies in OA patients were performed using a broad range of transcriptomics methods, ranging from target-focused qRT-PCR, and microarrays to next-generation RNA-Seq and scRNA-Seq, as described above, thus providing a genome-wide coverage and broad dynamic range at both research and cohort-patient levels. Eighteen studies focused on gene expression profiles in target tissues highlighting molecular changes related to pathophysiological processes occurring within the joint during the course of OA (Table 2). Such analyses at a tissue level, however, require invasive biopsies difficult to obtain in clinical practice. Two additional studies reported gene expression profiles in peripheral blood leukocytes obtained from OA patients, providing insights on molecular dysregulations at a systemic level (Table 2). Such studies on blood samples are also useful to identify circulating biomarkers of interest to monitor disease status and/or
### Table 2
Transcriptomics studies Focusing on patients with OA.

| Studies | Study objectives | Sample types and patient numbers | Methods | Main Results |
|---------|-----------------|-----------------------------------|---------|--------------|
| Xu J et al., 2012 [22] | Transcriptomic analyses of normal and end-stage OA hip cartilage. | Hip cartilage N = 9 OA/N = 10 CTLR | Microarray (validation by RT-qPCR) | 998 DEG between healthy femoral neck and OA cartilage associated with 71 canonical pathways; potential role of IL-17 signaling in OA. Low overlap between knee and hip OA gene profiles. MMP1, ADAMTS-1 and ADAMTS-5 increased in knee OA but decreased in hip OA cartilage. |
| Chou C.-H et al., 2013 [27] | Identification of OA related genes in site-matched tissues. | Cartilage and subchondral bone from knee N = 50 OA | qPCR | Up to 27 among 61 genes dysregulated in the same direction in both tissues. 19 genes related to OA severity. GDF10 downregulated in both articular cartilage and subchondral bone and associated with severe OA. |
| Soul J et al., 2018 [23] | Study of OA heterogeneity and patient subgroups for stratification. | Knee cartilage N = 60 OA/N = 10 CTLR | RNA-seq | Unsupervised clustering identifies 2 subgroups of patients. A 10 gene-subgroup classifier was validated by RT-qPCR in 16 additional OA patients. Intact OA cartilage sampled in both groups exhibited very low expression and no differential expression of pro-inflammatory cytokines. |
| Steinberg J et al., 2018 [34] | Identification of molecular differences between chondrocytes from osteophytic, low- and high-grade articular cartilages from OA patients. | Primary chondrocytes from hip N = 9 OA | RNA-seq in combination with DNA methylation and proteomics | Higher differences in gene expression and protein abundance in osteophytic versus low-grade articular cartilage compared to high-grade. Fewer significant differences between chondrocytes from high- and low-grade articular cartilage. Identification of 56 genes differentially expressed between osteophytic and low-grade articular cartilages including IL6, ALDH1A2, MMP13, CLIP. Gene ontology enrichment analysis shows that differences are at the level of extracellular matrix organization, skeletal system development, platelet aggregation and regulation of ERK1 and ERK2 cascade. |
| Ji Q et al., 2018 [18] | Understanding molecular mechanisms underlying human cartilage degeneration by analyzing the profile of human chondrocytes at different stages of OA. | Isolated chondrocytes from knee N = 10 OA | sc-RNA seq and histological assays | Identification of 7 different chondrocyte phenotypes including 3 novels populations/gene signatures associated with human OA progression. |
| He A et al., 2018 [20] | Study the genes and functional pathways deregulated in OA patients. | Knee cartilage N = 10 OAN = 5 CTLR | Microarray | Identified 145 common aberrant genes in both DNA methylation and mRNA expression levels, and 26 associated biological pathways in osteoarthritic cartilage. The pathway enrichment analysis showed that the MAPK signaling is associated with OA development. |
| Rai M – F et al., 2018 [25] | Assessment of changes in the medial and lateral tibial plateau cartilage in tandem with obesity in OA patients. | Knee cartilage medial and lateral compartments N = 23 | RT-PCR | BMI has a distinct and differential impact on medial and lateral compartments. Transcripts elevated with obesity in the medial compartment were enriched for metabolic processes including blood pressure. |
| Mimpfen J.Y et al., 2021 [26] | Comparison of the transcriptome of chondrocytes and synovial fibroblasts derived from cartilage and synovium of patients with end-stage knee OA, after treatment with IL-17 A, IL17F or IL-17AF. | Knee cartilage and synovium N = 6 OA | RNA-seq and RT-qPCR | Both genes (IL17RA and IL17RC) were expressed by chondrocytes and synovial fibroblasts. Treatment with these cytokines induced activation of pathways related to inflammatory responses, complement, hypoxia, angiogenesis, and glycolysis and modulated genes associated with experimental arthritis, knee osteoarthritis, and musculoskeletal disease, which further highlights the potential importance of this cytokine in OA. Treatment of these samples with these cytokines and increasing dose of secukinumab (anti-IL17A) significantly inhibited IL17-A induced genes such as MMP1 in chondrocytes but also IL-6, PDNP, NKB17 in synovial fibroblasts. Analysis of unaffected cartilage samples led to the identification of two OA clusters. Cluster A is enriched in cartilage-related pathways while Cluster B is associated with an upregulation of chemokine genes as well as higher joint space narrowing (JSN) and low osteophyte (OP) scores. |
| De Almeida R Coutinho et al., 2021 [21] | Identification of OA subtypes based on pathophysiological processes and/or clinically relevant characteristics. | Knee and hip cartilage N = 66 OA | RNA-seq | Analysis of unaffected cartilage samples led to the identification of two OA clusters. Cluster A is enriched in cartilage-related pathways while Cluster B is associated with an upregulation of chemokine genes as well as higher joint space narrowing (JSN) and low osteophyte (OP) scores. |
| Wang X et al., 2021 [37] | Understanding the changes occurring in the chondrocytes from OA patients, Kashin-Beck and controls. | Knee cartilage N = 1 OA N = 1 CTLR | Singe-cell RNA-seq and RT-qPCR | Identification of a new population of chondrocytes expanded in OA (RegC) while CPCs populations were markedly expanded in healthy controls. |
| Subchondral bone and bone marrow lesions | Assessment of cellular and molecular expression of bone marrow lesions (BML) in OA. | Bone marrow lesions from knee N = 84 OA/N = 12 CTLR | Microarray | Comparison between OA BML and normal bone revealed 218 DEGs. In BML, high expression of genes involved in neuronal development (STMN2, THBS4, PSIP1, NAP2), pain, cartilage/bone formation, ECM turnover (MMP-13, COL16A1) and angiogenesis was documented. |

(continued on next page)
Table 2 (continued)

| Studies          | Study objectives                                                                 | Sample types and patient numbers                  | Methods                         | Main Results                                                                                                                                 |
|------------------|----------------------------------------------------------------------------------|---------------------------------------------------|--------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------|
| Yuen C et al.,   | Classification and determination of the OA subtypes.                            | Synovial and subchondral bone from knee OA        | RNA-seq                        | Unsupervised clustering of OA cartilage samples identifying four distinct OA subtypes:    |
| 2020 [39]        |                                                                                  | N = 227 OA                                        |                                | - the C1-glycosaminoglycan metabolic may present a relatively earlier stage with no specific symptoms        |
|                  |                                                                                  | N = 5 CTLR                                       |                                | - The C2 collagen metabolic disorder represented osteophytes          |
|                  |                                                                                  |                                                   |                                | - in the clinic                                                                                                                                   |
|                  |                                                                                  |                                                   |                                | - The C3-activated sensory neuron OA subtype with high expression of neural markers                                                            |
|                  |                                                                                  |                                                   |                                | - The C4-inflammation OA subtype presented high inflammation and a narrow joint space in the clinic                                          |
| Synovial tissues |                                                                                  |                                                   |                                |                                                                                                                                               |
| Wang Q et al.,   | Determination of the role for the inflammatory complement system in the causation of osteoarthritis. | GSE12317                                          | Microarray qPCR                | Gene expression of complement components (C1s, C4A, factor B, C9, C3, C5) are markedly higher expressed while the complement inhibitors (clusterin, factor H, C4b, C1 inhibitor) are lower expressed in synovial fluids from OA patients compared to healthy synovial membranes. The excessive complement activation is part of the OA synovitis. |
| 2011 [30]        |                                                                                  | GSE12317                                          |                                |                                                                                                                                               |
|                  |                                                                                  | GSE12021                                          |                                |                                                                                                                                               |
|                  |                                                                                  | N = 10 OA                                         |                                |                                                                                                                                               |
|                  |                                                                                  | N = 13 CTLR                                       |                                |                                                                                                                                               |
| Ritter S Y et al., | Gene expression analysis from cartilage and synovium in parallel with proteomic profiling of knee SF from OA patients or controls. | Cartilage & synovial tissue and fluid from knee   | Microarray and comparison with 2-DEG and mass spectrometry | The study identified 66 proteins differentially expressed in OA SF compared to healthy SF. Among those, 3 dominant pathways were identified, related to acute-phase response signaling complement activation, and coagulation. Many complement proteins have a concordant expression in both synovium and SF suggesting that the synovium may be the primary source of these proteins in the SF. Comparison with cartilage microarray data were less clear. |
| 2013 [31]        |                                                                                  | Synovial tissue N = 19                           |                                |                                                                                                                                               |
|                  |                                                                                  | Cartilage                                        |                                |                                                                                                                                               |
|                  |                                                                                  | N = 12 OA/N = 13 CTLR                            |                                |                                                                                                                                               |
| Lambert C et al., | Comparison of the gene expression in synovial cells from inflamed or normal synovial membrane in the same OA patient. | Synovial membrane from knee                       | Microarray                      | Among the 896 DEG identified between normal and inflamed areas, a significant number of genes were categorized as belonging to inflammation (upregulation of IL6, IL8, CCL5, CXCL6, CXCL16, CXCL2, CXCL1, TREM-1 and SI00A9), cartilage extracellular matrix metabolism (upregulation of MMP5, MMP9) showing an imbalance between anabolic and catabolic genes in favor of catabolic genes. DEG also impact the Wnt signaling with an upregulation of Wnt-5a involved in chondrocyte differentiation, and angiogenesis pathway with the upregulation of STC1. Identification of USP46, CPVL, PKRPS, FOSL2, GADD45B, PTGS1, NFKB1, ADAMTS1, and TFAM genes as being dysregulated, confirming their important role in the pathophysiology of OA. |
| 2013 [32]        |                                                                                  | N = 12 OA                                        |                                |                                                                                                                                               |
| Zhang X et al.,  | Identification of DEGs in joint synovial tissues from OA vs normal individuals. | Synovial tissue                                  | Microarray (Validation by RT-qPCR) |                                                                                                                                               |
| 2018 [9]         |                                                                                  | N = 54 OA/N = 43 CTLR                           |                                |                                                                                                                                               |
| Blood studies    | Comparative analysis of gene expression in PBMCs from OA patients compared to non-OA controls. | PBMC from knee OA patients                       | Microarray Validation by qPCR  | Two clusters were identified from symptomatic OA patients based on inflammatory gene signature. Patients with inflammatory gene signature had higher pain scores and were at higher risk of radiographic progression at 2 years. at 2 years. Elevated expression of PGE synthase mRNA in PBMC was observed from patients with symptomatic knee OA vs non-OA controls and is associated with an increased PGE2 production. A dichotomization of patients based on the presence of a "PBL inflammatory phenotype" (above median expression levels of IL1b, TNFÎ± and COX-2 determined by qPCR) revealed that patients expressing these inflammatory genes had more rapid radiographic progression than patients with expressions levels at or below the median. Finally, patients with high levels of IL-1b (continued on next page) |
treatment efficacy. Analyses of genes differentially expressed between damaged and intact cartilage as well as synovial areas or bone marrow lesions within the same joint of OA patients provided deeper insights on the relative molecular changes occurring in periarticular tissues. Such approaches can help relating apparently independent events, such as structural changes, pain, or inflammation, into an integrated view to identify interlinked molecular pathways at the whole joint level.

From these analyses, chondrocytes were found to play a critical role in cartilage degradation, mainly resulting from an activation of MAPK pathway in OA [20] leading to an increased production of cytokines and enzymes involved in the cleavage of extracellular matrix components, such as collagens and proteoglycans [21–25]. Phenotypical alterations of chondrocytes in OA appear as a consequence of major changes in gene expression profiles when compared to normal cartilage. Of note, one hurdle in such analyses is that chondrocytes within articular cartilage are scarce (representing 1–5% of the tissue volume), even more so for OA cartilage. Thus, adapted methods have been set up in order to collect sufficient amounts of high-quality RNA from articular cartilage tissue and perform transcriptomics analyses [24]. Other approaches consisted in prior isolation and expansion of chondrocytes in primary cultures before prior isolation and expansion of chondrocytes in primary cultures before RNA extraction. These transcriptomic analyses provided converging evidence for an upregulation of genes associated with cartilage degradation as well as matrix and bone remodeling during OA [21,22,26]. Differences in the expression of genes primarily associated to cartilage function were linked to biological processes leading to both the formation of osteophytes and OA progression. In addition, chondrocytes were found to express multiple proinflammatory proteins likely contributing to the pathogenesis and evolution of OA. However, it is still unclear whether the association between OA and inflammatory markers is causative or subsequent to cartilage degradation or mechanical dysfunction [27].

Bone marrow lesions, known to be an early event in joint damage, are associated with angiogenesis and fibrosis features, in addition to new cartilage formation and increased bone turnover. Finer-grained transcriptomics analyses of associated dysregulated pathways showed an important contribution of genes related to cartilage remodeling, neurogenesis, pain sensitization, as well as chemokine and cytokine signaling [28]. Besides these findings, the differential gene expression analysis of joint synovial tissues from OA patients and healthy individuals supported the hypothesis of a low-grade inflammation in OA [9,29]. The molecular changes in acute-phase, complement and coagulation pathways suggested that chronic and progressive articular damage observed during OA occurs at least in part through a sustained inflammatory response associated with unresolved tissue injury [30]. Dysregulation of angiogenesis, apoptosis and Wnt signaling pathways were also identified as prominent contributors to cartilage degradation and OA progression [9,20,28,31]. The co-expression of osteogenic and angiogenic genes and their association with tissue remodeling highlight that vascular proliferation and bone formation are coupled events in bone marrow lesions.

Blood vessels being formed within vascularized tubules, the upregulation of genes consecutive to NGF signaling and neurotrophin pathway activation in subchondral bone tissue likely accounts for new nerve formation, neangiogenesis and pain [32,33]. It was also hypothesized that damages to innervated joint structures and OA synovial inflammation may cause peripheral pain as inflammation decreases the activation threshold of local afferent nerve fibers [34]. As mentioned above, the activation of genes encoding for inflammatory mediators such as chemokines or cytokines was also frequently reported in target tissues, either as a consequence of cartilage degradation but also as a possible local cause of OA, raising the hypothesis of inflammation as one of the phenotypic manifestations of this heterogeneous disease [35].

Transcriptomics analyses also revealed differences between hip and knee OA, with evidence for an enrichment in epigenetic modifications such as DNA methylation in the knee. Although there was a poor overlap in differentially expressed genes between both sites, IL11 and CHADL were found as common dysregulated genes. Both were previously linked to genetic susceptibilities [33]. Interestingly, new chondrocyte subtypes, markers of chondrocyte subpopulations and signaling pathways contributing to OA have been identified through the use of scRNA-seq [36,37]. Differential transcriptomics analyses were also applied to better understand the various phenotypes of OA, allowing to define subgroups of patients likely to benefit from personalized treatment strategies. Network analysis of RNA-Seq data allowed to distinguish two subgroups of patients with knee OA. Group A was characterized by changes in chemokine signaling, inflammasome activation, glycosaminoglycan synthesis, Toll receptor activation and innate immune responses. Group B rather exhibited dysregulations in complement regulation, Wnt signaling, eicosanoid receptor signaling and syndecan interactome. Further analysis revealed little evidence for inflammatory pathway activation within intact OA cartilage nor for activation by factors from synovial fluid [23]. Nonetheless, common changes in circadian clock regulators were observed in both OA groups highlighting a possible general mechanism affecting tissue integrity. Furthermore, similar biological processes (including inflammation) exist despite the molecular heterogeneity in OA cartilage and synovium. However, molecularly defined patient clusters differ between tissues, potentially reflecting differences in tissue-specific disease processes [24]. In this regard, two patient subgroups were identified with low-grade OA cartilage associated with gender and proton pump inhibitor prescription, while in synovium two additional patient subgroups were associated with inflammation, extracellular matrix and cell adhesion. Using an independent dataset, similar clusters were defined using unsupervised hierarchical clustering [21] with considerable overlap (45%) of differentially expressed genes with the ones identified by Soul et al. [23]. In the latter study, the intact cartilage in cluster A patients was enriched in genes encoding for cartilage and extracellular matrix-related pathways, whereas genes involved in chemokine signaling pathways were rather upregulated in cluster B. Cluster B was also characterized by high joint space narrowing (JSN) and low osteophyte scores.

Recently, a new classification model was established using an unsupervised clustering of transcriptomic data from OA cartilage samples, allowing the identification of four OA subtypes associated with distinct functional signatures [38]. The C1-glycosaminoglycan metabolic disorder OA subtype presented classical clinical symptoms, while the C2-collagen metabolic disorder OA subtype was associated with clinical symptoms linked to osteophytes. The C3-activated sensory neuron OA subtype, enriched in the youngest patients, displayed a high expression of neural molecules such as LRRRC4C, CTNNNAZ, and GRID2, suggesting a strong susceptibility to pain in this subtype. Lastly, the C4-inflammation
OA subtype presented high inflammation and clinical symptomatology of a narrow joint space.

Early diagnosis and treatment are crucially needed to decrease the burden of OA. For this purpose, biomarkers detected in the blood possibly in the form of specific and easily monitorable gene signatures could facilitate the monitoring of OA patients with different clinical phenotypes. The search for such a signature has been the focus of some transcriptomics studies (Table 2). Blood cell analyses suggested that symptomatic knee OA patients could be classified into two distinct clusters based on levels of inflammatory gene expression. OA patients with an inflammatory gene signature (including IL-1β, IL-8, COX-2) exhibited higher pain scores and were at a higher risk of disease progression in a 2-year follow-up [39]. Elevated baseline expression of IL-1H. Kaplon et al. Osteoarthritis and Cartilage Open 3 (2021) 100221

potential diagnostic and prognostic biomarkers for disease-modifying OA burden of OA. For this purpose, biomarkers detected in the blood of OA patients also appear very interesting, most particularly if they can be in the future combined with gene expression profiling in PBMCs from OA patients can help identifying prognostic and follow-up biomarkers for the disease.

4. Discussion

OA remains a devastating, slowly progressive disease affecting millions of patients worldwide, still in a need for efficient disease-modifying therapies. Relating clinical phenotypes to well-defined patient subgroups in terms of underlying pathophysiological mechanisms remains a key challenge to design better targeted therapies and envision a personalized medicine approach [30]. Also, a better understanding of OA pathophysiology could lead to the identification of new therapeutic targets to address the unmet medical needs of millions of patients [41].

As a first step, the transcriptomics studies described in the present review have contributed to better understand at a molecular level some of the biological processes occurring during OA in articular cartilage, synovial tissue and subchondral bone in association with alternations in joint structure and pain features (Table 2). Gene expression studies conducted on target organs also resulted in a better understanding of patient heterogeneity, with a first proposed classification in the form of two distinct molecularly defined clusters. Given the importance of a patient stratification to envision a differentiated precision-medicine strategy, further replication of these findings in independent datasets with clinical data integration are required. Transcriptomics analyses in the blood of OA patients also appear very interesting, most particularly if they can be in the future combined with gene expression profiling performed in target organs from the same patients. Identification of blood-borne biomarkers reflecting pathophysiological mechanisms occurring in the joints would represent an outstanding achievement with multiple applications to early diagnosis, patient stratification and therapeutic monitoring in OA.

In light of the complexity of OA, transcriptomics should in the future be combined with proteomics, lipidomics, metabolomics, epigenetics, imaging technologies as well as artificial intelligence and robust computational methods to integrate massive and multimodal data coming from those analyses. Such a strategy is currently applied in the context of the APPROACH IMI consortium on a cohort of 300 patients with OA, with a clinical evaluation conducted at baseline and after 1 and 2 years of follow-up [51]. This APPROACH study aims to relate the outcome of multi-omics and imaging analyses with disease progression. Such an integrated analysis, in which blood transcriptomics will undoubtedly play an important role, is well suited to pave the way for precision medicine-based therapies better designed to treat OA patients on the basis of a comprehensive understanding of their biological and clinical specificities.

Author contributions

HK, FDC, LL and PM have conducted the literature search to mine and analyze published transcriptomics studies in OA. YL, AL and SC have generated the data and provided the overview on quality controls for RNA samples. All authors have contributed to writing and critically reviewing the manuscript.

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Declaration of competing interests

All authors are employees at Servier. Servier is a member of the APPROACH IMI consortium. The authors have no conflict of interest to declare in relationship with this study.

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