Peer Review File

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Reply to Reviewer A's comments

In this Editorial Commentary, authors revealed a molecule-based technique for diagnosis of OA. Although this is very important in the field of OA, authors failed to show the importance of this Commentary. For instance, where researchers should focus on? It is important to focus on the molecular signature of OA during different stages of diseases. This might be different in each body fluid. Urine might be an excellent target for discovery of molecular biomarker reflecting metabolic and inflammatory changes in the joint or cartilage.

What about figure 1? How necessary for this Commentary?

Reply: Thank you for your kind comments. The current diagnosis and treatment of OA mainly rely on clinical and imaging manifestations, neglecting its molecular pathophysiology. The mismatch between participants' molecular characteristics and drug therapeutic mechanisms might explain the failure of some disease-modifying drugs in clinical trials and practice. To highlight the importance of molecules in OA diagnosis in the future, we wrote this Editorial Comments. According to your suggestion, we highlighted the areas that future researches need to focus on, such as linking the molecular profiles in body fluids with clinical information, OA stages and the efficacy of some therapeutics, so as to further enhance the feasibility of molecular diagnosis in clinical applications (page 7, line 9). We also suggested that molecular characteristics should be taken in consideration in the development of inclusion and exclusion criteria in future clinical trials (page 7, line 20), which may potentiate the efficacy of some therapeutics.

Besides, in the previous version, we drew the figure 1 to show the post-transcriptional regulation of gene expression. According to your comments, to make the manuscript

better, we changed the structure and focus of this manuscript. Now, the figure 1 has been deleted. We have modified our text as advised (see page 7, line 9; page 7, line 20).

Changes in the text:

In page 7, line 9: Taken together, the molecules in body fluids could provide us with lots of information about OA pathogenesis, which will greatly promote the development of molecular diagnosis of OA. By associating molecular profiles in body fluids with clinical information, OA stages and the efficacy of some therapeutics, a more comprehensive and scientific picture should be created to test the clinical application value of molecular diagnosis of OA.

In page 7, line 20: Since OA is a highly heterogeneous disease, molecule-based diagnosis raises the possibility that different subgroups may be adapted to different modes of intervention. Therefore, molecular characteristics should be considered in the development of inclusion and exclusion criteria in future clinical trials. Stratifying homogenous patients at the molecular level and selecting therapies targeting their pathogenesis may potentiate the efficacy of some therapeutics. We hope the following studies will focus on deepening the knowledge of molecular profile during OA initiation and progression, and validating their relevance to clinical practice, which will contribute to further improvements in therapeutic options for patients with OA.

Reply to Reviewer B's comments

This is a thoughtful and timely editorial relating to this recent publication in Bone research, which uses a transcriptomic approach to identify four molecular endotypes of osteoarthritis in various joint tissues.

a. My main comment is that there is much activity in this area in the search for molecular subtypes in OA, with some other contemporaneous publications of note which are not referenced, and arguably should be alongside some broader discussion of findings of note in this area (given the thrust of the editorial is progress):

Coutinho de Alemeida, Rheumatology 2021.

https://pubmed.ncbi.nlm.nih.gov/32885253/

Soul et al, ARD 2018 https://pubmed.ncbi.nlm.nih.gov/29273645/

Chou et al, Sci Rep 2020 https://doi.org/10.1038/s41598-020-67730-y

Reply: Thank you for your kind suggestion. Based on your advice, we added the discussion about contemporaneous publications on molecular classification of OA (see page 3, line 19; page 5, line 12).

Changes in the text:

In page 3, line 19: In this context, research in the field of molecular diagnosis of OA has been very active in recent years. Since the pattern of gene expression reflects cell responses to pathological condition, transcriptome data has been used in the diagnosis and classification of OA in several studies. For example, Soul et al have investigated the pattern of gene expression in non-OA and OA cartilage by RNA-Sequencing (RNA-Seq) (10). To avoid the gene expression alterations that occur in damaged cartilage, they analyzed the intact cartilage in OA group. They showed 2692 differentially expressed genes between non-OA and OA cartilage, and surprisingly, they found a large increase in the expression of matrix protein genes in the OA group. Further unsupervised clustering analysis stratified OA into two subgroups: Group A showed increased expression of cartilage components like collagen type II, V, IX and XI and less expression of collagen type I; in contrast, Group B showed reduced expression of chondrogenic genes and enhanced expression of osteogenic genes. Similarly, Almeida et al analyzed the whole-transcriptome profiling of OA cartilage and also identified two subgroups (11). Upon integrating radiographic OA data, they found that one subgroup was likely to be characterized by lower osteophyte scores and higher joint space narrowing (JSN) scores. These results clearly show that OA is a highly heterogeneous disease and raises the concept for a more precise molecular diagnosis.

In page 5, line 12: In recent years, RNA-seq (10-12) and single-cell RNA-seq (13, 14) have been extensively studied in order to reveal OA subtypes and pathophysiology at molecular and cellular levels. However, it should be critically noted that the transcriptome-based diagnosis has some limitations. First, although transcriptional networks play a fundamental role in governing cell function and fate, they do not

entirely determine cellular identity due to ubiquitous post-transcriptional regulation, translational regulation, and degradation mechanisms (15). Confirmation of the link among transcriptome atlas, proteome atlas, and clinical information for OA patients is still pending. Second, the aforementioned transcriptome analysis relies heavily on the technically and invasively acquired joint tissue specimens. It is difficult to generalize in clinical practice, not available for early OA diagnosis and cannot dynamically reflect the pathological changes of the disease, especially for post-treatment evaluation.

2. I think the narrative is good but could probably sometimes benefit from some minor language editing. I am not going to be exhaustive here, but a couple of places where meaning is potentially lost:

Lone 25 which is not available in predicting…do you mean not able to predict

Line 29 – manifested. Do you mean measures?

Line 40 contradict

Reply: Thank you for your comments. We have modified our text as advised (see page 2, line 8; page 3, line 19; page 6, line 11; page 5, line 8)

Changes in the text:

In page 2, line 8: Such diagnostic criteria are not able to predict high risk OA individuals and/or provide evidence for early diagnosis of the disease.

In page 3, line 19: In this context, research in the field of molecular diagnosis of OA has been very active in recent years. Since the pattern of gene expression reflects cell responses to pathological condition, transcriptome data has been used in the diagnosis and classification of OA in several studies.

In page 6, line 11: Numerous biomarkers that reflect the pathophysiology of OA can be found in body fluids, including synovial fluid (SF), blood, and urine.

In page 5, line 8: Their findings provide a new approach for the diagnosis of OA, and the transcriptome atlas may allow for precise diagnosis and targeted therapeutics of OA in the future.

3. Therapies for OA are not necessarily even in their infancy. They are mainly in clinical trials, but we have difficulty having 'positive trials' in OA, not through lack of trying (with several decades of effort in this area). Maybe this wording can be re-examined.

Similarly, one could argue that society costs are not just in elderly (at start of intro).

Reply: Thank you very much for your advice on our language. We have modified our text as advised (see page 2, line 9; page 2, line 3).

Changes in the text:

In page 2, line 9: In addition, despite having emerging pharmaceutical therapies in recent decades (3), only a fraction of potentially disease-modifying OA drugs have been applied in clinical practice. Whereas, a portion of these drugs show ambiguous outcomes, and the use of them in clinical guidelines are usually in disagreement (4, 5). This could be due to a mismatch between the molecular mechanisms by which the drug works and the clinical manifestations at the time of the decision to use the drug, since the commonly recognized clinical features cannot elucidate the pathological changes of OA (6). Thus, the lack of pathophysiology-based OA diagnosis impedes the development of targeted therapeutics.

In page 2, line 3: Osteoarthritis (OA) is a common disabling condition worldwide, representing a substantial and growing health burden with extensive socio-economic costs (1).

4. Were there any specific criticisms/limitations of this paper that the editorial wanted to consider (other than not being a protein search, which they obviously expand on at length). Surely suggested treatments (line 45) may not be possible/appropriate from a cross sectional analysis such as this, but the findings should drive further targeted hypothesis testing and experimental medicine in this area?

Reply: Thank you for your comments. There are some limitations of RNA-seq or single cell RNA-seq based molecular diagnosis of OA, for example transcriptional information may cannot reflect cellular function and fate because of ubiquitous post-transcriptional regulation, translational regulation, and degradation mechanisms.

More importantly, the transcriptome analysis relies heavily on the technically and invasively acquired joint tissue specimens. In clinical practice, joint tissue specimens are difficult to obtain unless patients undergo total knee arthroplasty. In this context, it seems impossible to diagnose OA at early stage by transcriptome analysis because the patient has not yet reached surgical indications. And it is impossible to monitor the pathological alteration dynamically. Future studies should pay more attention to the molecules in body fluids, especially link the molecular profiles in body fluids with clinical information, OA stages and the efficacy of some therapeutics to test the clinical application value of molecular diagnosis of OA. In addition, taking molecular profiles in body fluids into consideration may potentiate the efficacy of some therapeutics in clinical trials. We have added this information in the text (see page 5, line 12; page 7, line 9; page 7, line 22).

Changes in the text:

In page 5, line 12: In recent years, RNA-seq (10-12) and single-cell RNA-seq (13, 14) have been extensively studied in order to reveal OA subtypes and pathophysiology at molecular and cellular levels. However, it should be critically noted that the transcriptome-based diagnosis has some limitations. First, although transcriptional networks play a fundamental role in governing cell function and fate, they do not entirely determine cellular identity due to ubiquitous post-transcriptional regulation, translational regulation, and degradation mechanisms (15). Confirmation of the link among transcriptome atlas, proteome atlas, and clinical information for OA patients is still pending. Second, the aforementioned transcriptome analysis relies heavily on the technically and invasively acquired joint tissue specimens. It is difficult to generalize in clinical practice, not available for early OA diagnosis and cannot dynamically reflect the pathological changes of the disease, especially for post-treatment evaluation.

In page 7, line 9: Taken together, the molecules in body fluids could provide us with lots of information about OA pathogenesis, which will greatly promote the development of molecular diagnosis of OA. By associating molecular profiles in body fluids with clinical information, OA stages and the efficacy of some therapeutics, a

more comprehensive and scientific picture should be created to test the clinical application value of molecular diagnosis of OA.

In page 7, line 22: Therefore, molecular characteristics should be considered in the development of inclusion and exclusion criteria in future clinical trials. Stratifying homogenous patients at the molecular level and selecting therapies targeting their pathogenesis may potentiate the efficacy of some therapeutics.

Reply to Reviewer C's comments

The English needs to be revised. In addition, the document lacks clarity in terms of structure and the message to convey.

Reply: Thank you for your suggestion. We have revised the language throughout the article and invited a native English speaker to help us polish the manuscript. According to your comments, to convey the message clearer, we reorganized the structure of this manuscript into five parts: (1) Background; (2) OA is a molecular disorder; (3) Transcriptome atlas-based OA diagnosis; (4) Body fluid: readily accessible molecular pool for OA diagnosis; and (5) Future perspectives. We hope the structure and message in this version is clear.

Changes in the text: We have changed the whole structure and language of this manuscript.