Peer Review File

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Reviewer A

I have no major concerns with the data and methods within the manuscript.

Minor comments

• It would be beneficial to compare the activity of cGAS/STING signalling pathway described between RA and healthy controls, although access to these samples is not always easy, as higher basal or stimulated activity could indicate a change in destructive RA. It would be nice to see this addressed in the discussion and any relevant findings from the literature mentioned.

Reply 1: Many thanks for the reviewer. We have already compared the cGAS expression in another related article (see reference: https://pubmed.ncbi.nlm.nih.gov/31472320/). The resulted showed that cGAS level increased in RA FLSs compared with healthy controls and correlated with synovitis scores. We already have modified our text as advised (see page 9, line 408-409).

• Synovial fibroblasts are not a single subset and show diversity beyond lining versus sublining (Croft et al, 2019; Zhang et al, 2019). Please discuss how the findings in your manuscript might be specific to particular subsets etc.

Reply 2: Thank you very much for your suggestions. Fibroblast can be classified to different subsets based on single cell transcriptional analysis. In the previous study, FAPα+ THY1+ immune effector fibroblasts located in the synovial sub-lining, and FAPα+ THY1- destructive fibroblasts restricted to the synovial lining layer(Croft et al, 2019). In our manuscript, cGAS regulated the synovial inflammation and invasion. Based on these functions of cGAS, we speculated that cGAS may regulate the function of sub-lining and lining synoviocytes.

• The manuscript would benefit from a final proofread to catch minor grammatical errors etc.

Reply 3: Thank you very much for your suggestions. I am sorry for the grammatical errors. Our revised paper has been polished by medical writing service (American Journal Experts (AJE)).

Croft, A.P., Campos, J., Jansen, K. et al. Distinct fibroblast subsets drive inflammation and damage in arthritis. Nature 570, 246–251 (2019). https://doi.org/10.1038/s41586-019-1263-7

Zhang, F., Wei, K., Slowikowski, K. et al. Defining inflammatory cell states in rheumatoid arthritis joint synovial tissues by integrating single-cell transcriptomics and mass cytometry. Nat Immunol 20, 928–942 (2019). https://doi.org/10.1038/s41590-019-0378-1

Reviewer B

The topics of this manuscript are interesting and important. However, there are several major concerns listed as below.

1.Sample: Synovial cells were prepared from patients with RA by joint replacement surgery or synovectomy. How about the patients' backgrounds for demographics, disease characteristics, and treatment? Negative controls of synoviocytes should be set to conclude that the observation was unique to RA. There are no controls in this study.

Reply 1: Thank you very much for your comments. According to the reviewer's suggestion, we added the backgrounds for RA patients in Supplemental Table S2. We have already compared the cGAS expression in another related article (see reference: https://pubmed.ncbi.nlm.nih.gov/31472320/). The result showed that cGAS level increased in RA FLSs compared with healthy controls and correlated with synovitis scores.

2. Stimulation: How did the authors confirm that IND dsDNA (45bp) is optimal to stimulate STING in synovial fibroblasts and is specifically activating STING pathways, but not others? There are no positive controls. How about the effect of STING-agonists in this assay?

Reply 2: Many thanks for the reviewer's suggestion. So far, cytosolic dsDNA sensors included cGAS, IFI16, DDX41, DAI, AIM2 ,DNA-PK , etc. How the actual source of DNA impacts the activity of various DNA sensors remains fully explored (https://pubmed.ncbi.nlm.nih.gov/33329609/). In our previous study, we used ISD, HSV, E. coli as the cytosolic dsDNA source to stimulate RA FLSs (see reference: https://pubmed.ncbi.nlm.nih.gov/31472320/) and activated cytosolic dsDNA sensor cGAS and STING. So we chose ISD dsDNA as the stimulator of cGAS/STING pathway in this manuscript. STING agonists activate both innate and adaptive antitumor immunity(https://pubmed.ncbi.nlm.nih.gov/34571002/). The STING agonist was more specific for STING activation; however, the effect of STING agonist on cGAS activity is not clear. We focus cGAS/STING pathway in migration and invasion of RA FLSs in this article.

3. Knock down: How did the authors confirm the specificity of knocking down the molecules? Are the protein expressions of cGAS and STING significantly down-regulated? The reviewer did not clearly separate western blot bands among the slots.

How much was the protein expression reduced by siRNA? There are no positive controls such as STING-antagonists. When knocking down STING, was the expression of cGAS up-regulated? If it is the case, what happened in the other pathways, rather than STING in the fibroblasts?

Reply 3: Many thanks for the reviewer's suggestion. We used qPCR and western blotting to confirm the siRNA efficiency and specificity. In the current graph, the protein level of cGAS or STING siRNA can be reduced to 40% or 50% of siRNA control. Previously we only chose the result of 3 independent western blotting experiments. In fact, before performing functional experiments, we repeatedly verify that the knockdown efficiency. We incorporated these data and found that the protein level of cGAS or STING siRNA 50% reduction compared to the siRNA control. We changed presentive pictures and graphs in the revised manuscript. When knocking down STING, the expression of cGAS did not change in our previous study ((see reference: https://pubmed.ncbi.nlm.nih.gov/31472320/)).

4. The experiments in Figure 1 and Figure 2 apereas to be same. The description for Figure 1 and Figure 2 in the results section is completely identical.

Reply 4: Many thanks for the reviewer's suggestion. We are sorry for our mistakes. We modified our text as advised (see page10, line 464-465).