Acid sphingomyelinase regulates the biological functions of fibroblast-like synoviocytes

Sirs,

Acid sphingomyelinase (ASM), a lysosomal protein, hydrolyses sphigomyelin to produce ceramide and plays an important role in regulating apoptosis, immune function, and inflammation, and has been reported as a treatment target in a wide range of diseases, such as tumours, cardiovascular diseases, neurological diseases, and respiratory diseases (1). Rheumatoid arthritis (RA) is a chronic autoimmune disease characterised by synovial inflammation in which fibroblast-like synoviocytes (FLS) play critical roles. Correspondingly, FLS can be the treatment target for RA (2). Previous study reported that serum levels of ASM were increased (3), serum ASM activity was higher in patients with RA than in healthy controls, and the treatment of anti-tumour necrosis factor α (TNF-α) inhibitors could statistically reduce ASM activity, suggesting a possible role of ASM in the RA pathogenesis (4). Beckman *et al*. reported that knockdown ASM or pharmacological inhibition ASM by amitriptyline could alleviate the joint swelling and decrease the production of proinflammatory cytokines (5), and Chen *et al*. reported that ASM could be the target to treat collagen induced arthritis after inhibiting TNF-α-induced NF-κB activation (6), suggesting the treatment role by targeting ASM. Here we show the ASM expression is increased in the synovial tissue of RA patients and ASM affects the biological functions of FLS.

We firstly performed immumohistochemical staining to test the expression level of ASM in synovial tissue originated from RA patients and non-affected controls and results showed expression level of ASM was obviously elevated in RA patients than in controls and ASM was mainly expressed in the infiltrated cells (Fig. 1A). To assess the role of ASM in regulating biological functions of FLS, we used lentivirus to transfect FLS to overexpress or knock down ASM. Results showed ASM level was significantly upregulated, increased by about 5 folds or downregulated, reduced by 84%, detected by qRT-PCR (Fig. 1B), which was also confirmed by western blotting result (Fig. 1C). Then we test the effect of ASM on the biological functions of FLS, results showed ASM had a trend to affect the apoptosis that the apoptosis rates were average 1.8% in the tran-NC group and 3.3% in the over-ASM group while reduced to average 1.42% in the si-ASM group, not reaching statistic meaning owing to large error bar (Fig. 1D), and overexpression of ASM promoted while down-regulated expression inhibited migration and proliferation (Fig. 1E-G). Considering the critical pathological roles of cytokines in the RA (7), we tested the effect of ASM on the mRNA expression levels of proinflammatory cytokines induced by TNF-α, and results suggested overexpression of ASM increased while down-regulated expression suppressed the mRNA expression levels of IL-1β, IL-6, IL-8 (Fig. 1H-J).

The 2021 study by Zhao *et al*. reported ASM inhibitor desopramine or knockdown of ASM could abolish IL-1β-induced expression and secretion of IL-6 in FLS, and desopramine suppressed IL-1β-induced proliferation, migration, and invasion of FLS (3), suggesting the critical role of ASM in regulating the biological functions

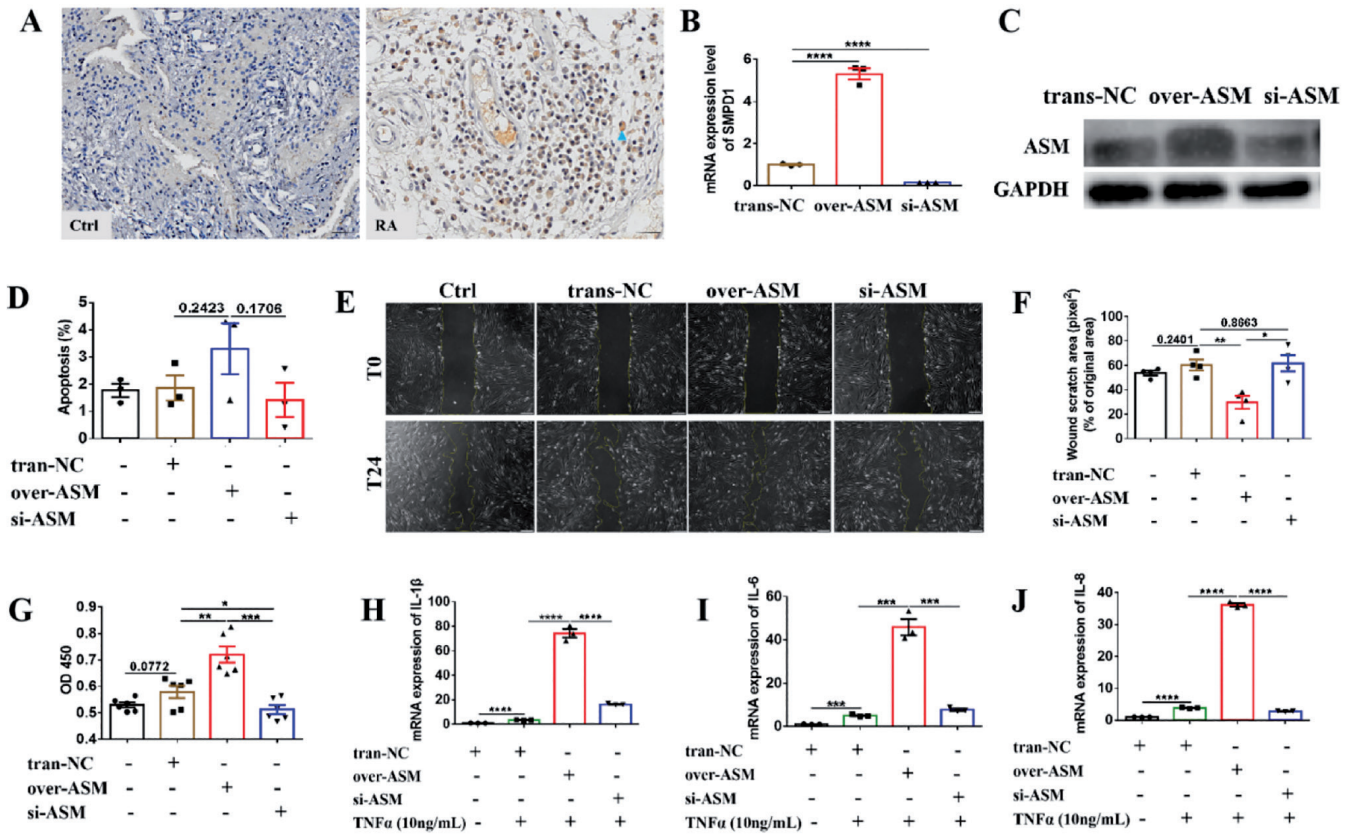

Fig. 1. ASM is involved in regulating the biological functions of FLS.
A: IHC staining of ASM in the synovial tissues originated from RA patients and non-affected controls. Blue arrow head is an example for the positive staining of ASM, scale bar 25μm. **B**-**C**: ASM expression level in FLS after lentivirus transfection (moi=100) to over-expression or knock down ASM detected by qRT-PCR and western blotting. **D**: Affection of ASM on FLS apoptosis, detected by flow cytometry. **E**: Scratch assay to test the migration ability of FLS after over-expression or knock down ASM. **F**: quantification of FLS migration. **G**: CCK8 assay to test the effect of ASM on FLS proliferation. **H**-**J**: mRNA expression levels of cytokines IL-1β, IL-6, IL-8 in FLS after over-expression or knock down ASM detected by qRT-PCR. Data are expressed as mean with standard error and comparisons among experimental groups are analysed by independent one-way ANOVA test. **p*<0.05, ** *p*<0.01, *** *p*<0.001, **** *p*<0.0001. All experiments were performed in triplicate.

of FLS, which were confirmed by our study. To sum up, the elevated expression of ASM and the role in regulating FLS biological functions provide ASM as a potential treatment target in RA. Correspondingly, small molecular drugs that could inhibit the expression or activity of ASM would be the newly kind promising drugs for the treatment of RA.

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