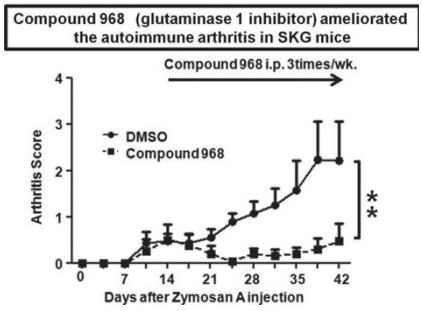
Objectives: We evaluated the role of these metabolic pathways in RA-FLS proliferation and in autoimmune arthritis in SKG mice.
Methods: The expression of glycolysis- or glutaminolysis-related enzymes was evaluated by real-time PCR and Western blotting, and the intracellular metabolites were evaluated by metabolomic analyses. The effects of glucose or glutamine on RA-FLS cell growth were investigated using glucose- or glutamine-free medium. Glutaminase 1 (GLS1) siRNA and the GLS1 inhibitor compound 968 were used to inhibit GLS1 in RA-FLS. Arthritis was induced in SKG mice by zymosan A injection. Compound 968 was used to study the effect of GLS1 inhibition on Zymosan A-injected SKG mice. Ki-67-positive cells were analyzed by immunohistochemistry.
Results: GLS1 expression was increased in RA-FLS, and metabolomic analyses revealed that glutamine and glutamate consumption were increased in RA-FLS. RA-FLS proliferation was reduced under glutamine-deprived, but not glucose-deprived conditions. Cell growth of RA-FLS was inhibited by GLS1 siRNA transfection or GLS1 inhibitor treatment. Silencing of GLS1 in RA-FLS did not affect IL-6 or MMP-3 production in supernatants. GLS1 expression in RA-FLS was not affected by pro-inflammatory cytokine stimulation. Compound 968 ameliorated the autoimmune arthritis and decreased the number of Ki-67-positive synovial cells in SKG mice.



Conclusions: Our findings suggested that glutamine metabolism plays an important role in regulating RA-FLS proliferation, without being affected by pro-inflammatory cytokine stimulation or affecting cytokine production, and may be a novel therapeutic target for RA.
Disclosure of Interest: None declared
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FRI0023 ARTESUNATE CAN INHIBIT MIGRATION AND INVASION OF FIBROBLAST-LIKE SYNOVIOCYTES VIA SUPPRESSION OF MATRIX METALLOPROTEINASE 9 IN RHEUMATOID ARTHRITIS PATIENTS

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Background: Evidences show that antimalarial agents of artemisinin and its derivatives such as artesunate may inhibit proinflammatory cytokines secretion from human rheumatoid arthritis (RA) fibroblast-like synoviocytes (FLS) in vitro. It has also been demonstrated that artesunate may ameliorate the symptoms of arthritis and prevent joint damage in collagen induced arthritis rat, which suggests that artesunate may be used for RA treatment. Recent studies show that RA-FLS is critical for joint destruction in RA because it can migrate and attach to cartilage and bone, and then invade them by secreting proteases such as matrix metalloproteinases (MMP) 9 in RA. However, effects of artesunate on migration and invasion of RA-FLS are poorly understood.
Objectives: To investigated the effects of artesunate on migration and invasion of RA-FLS and its underlying mechanism.
Methods: Synovial tissues were obtained from active RA patients as well as osteoarthritis (OA) and noninflammatory orthopedic arthropathies (Orth.A) patients and immumohistochemical (IHC) staining were performed for MMP9 expression. FLS isolated from these patients were analyzed for MMP9 exprssion by western blot (WB) and incubated with artesunate at different concentrations (0μM, 20μM, 40μM and 60μM), methotrexate (MTX, 10nM) or hydroxychloroquine (HCQ, 20μM) for 24 hours. Effects of artesunate on migration and invasion capacity were detected by transwell and wound healing assays. MMP9 and PI3K/Akt signal transduction protein expression after artesunate treatment was measured by WB.
Results: (1) IHC staining showed that synovial MMP9 expressed in lining and sublining area with intense nuclear and endochylema staining in RA synovium and the percentage of MMP9+ cells was significantly higher in RA (n=32) than that in OA (n=6) or Orth.A (n=6, Figure 1A, B).
(2) Migration and wound healing assays for 12 hours and invasion assay for 24 hours showed that RA-FLS possessed stronger capacity in migration and invasion than OA-FLS or Orth.A-FLS (Figure 1E, F). Artesunate inhabits the migration and invasion capacity of RA-FLS in a dose-dependent manner. MTX also has an inhibition effect on the migration and invasion of RA-FLS, but not HCQ (Figure 2A).
(3) MMP9 expression in RA-FLS was significantly higher than that in OA-FLS or Orth.A-FLS (Figure 1C, D). 40μM or 60μM artesunate markedly inhibited the expression of MMP9 in RA-FLS by WB (Figure 2B).
(4) WB analysis showed artesunate suppressed generation of phophso-Akt in a dose-dependent manner which indicated that Akt activity (phophso-Akt/Akt) in 40μM and 60μM artesunate treatment groups were significantly lower than that in untreated group (Figure 2C).

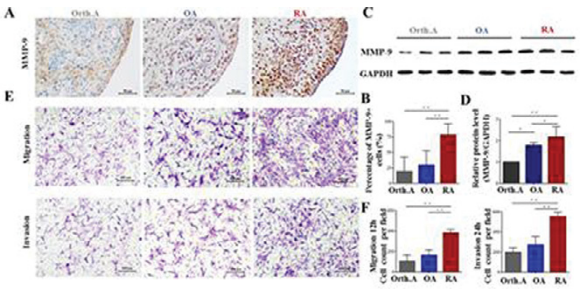

Figure 1 Expression of MMP9 in synovium and FLS and migration and invasion capacity of FLS. **(A and B)** IHC analysis showed that expression of MMP9 from RA patients was significantly higher than OA and Orth.A patients. Data are representative as means ± SD **P<0.01, ***P<0.001. **(C and D)** WB analysis showed that expression of MMP9 from RA-FLS was significantly higher than Orth.A-FLS or OA-FLS. Data were representative as means ± SD, *P<0.05, **P<0.01. **(E and F)** Transwell assays showed that RA-FLS possess stronger capacity of migration and invasion than OA-FLS or Orth.A-FLS. Data were representative as means ± SD, **P<0.01.

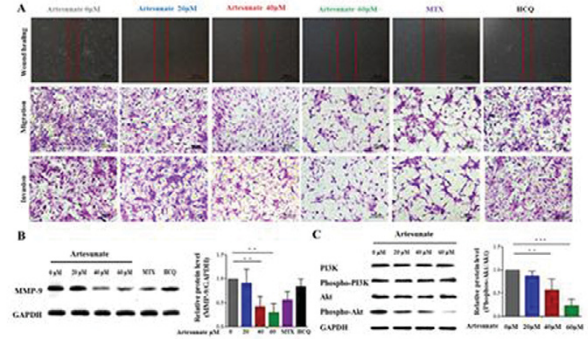

Figure 2 Effects of artesunate on the migration and invasion capacity and MMP9, PI3k/Akt pathway expression. (A) Artesunate inhabits the migration and invasion capacity of RA-FLS in a dose-dependent manner. **(B)** WB analysis of RA-FLS showed that MMP9 expression was siginificantly inhibited with treatment with 40 μM and 60 μM artesunate. **(C)** WB analysis showed that artesunate suppressed the expression of phophso-Akt in a dose-dependent manner. Bar are representative as means ± SD, **P<0.01, ***P<0.001.

Conclusions: Artesunate could inhibit the migration and invasion capacity of RA-FLS and the expression of MMP9 through suppressing Akt activity.
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FRI0024 MICRORNA-146A CONTROLS LOCAL BONE DESTRUCTION BY REGULATING FIBROBLAST INDUCED OSTEOCLASTOGENESIS IN INFLAMMATORY ARTHRITIS

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Background: MicroRNA (MiR-) 146a plays an important role in the regulation of the innate immune response and has also been shown to suppress cancer development in myeloid cells. Although in late stages of arthritis elevated expression of miR-146a in synovial tissue of rheumatoid arthritis patients was detected, the level of this miRNA was found to be down regulated in early disease, but its role in the development of inflammatory arthritis is still elusive.
Objectives: The objective of this study is to analyse the role of miR-146a in arthritis by the use of a chronic arthritis disease model. We aim to investigate the regulatory function of this miRNA in the pathogenic stroma, therefore in fibroblasts but also in immune cells.
Methods: To induce arthritis we used the chronic inflammatory hTNFtg disease model, therefore we crossed miR-146a deficient into hTNFtg mice. Disease severity was assessed clinically and histologically. Blood of arthritis animals was analysed by flow cytometry. Serum cytokine levels were measured by Elisa. Synovial fibroblasts were isolated from metatarsal bones and their proliferation