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Background: Fibroblast-like synoviocytes (FLS) are pivotal in inflammation and joint damage of rheumatoid arthritis (RA). These cells acquire an aggressive and invasive phenotype and secrete inflammatory mediators, metalloproteases and cathepsins that perpetuate inflammation and lead to cartilage and bone damage. We have previously shown that non-canonical Wnt5a pathway is involved in the aggressive phenotype of FLS by increasing their migration and invasion ability, and by stimulating the inflammatory response. The non-canonical Wnt signaling pathway included the planar cell polarity (PCP), with the activation of Rho and Rac GTPases, and the Wnt/Ca2+ pathways. We have also shown that Wnt5a contributes to the aggressive phenotype of RA FLS by binding to RYK receptor, through Rho-ROCK pathway and the activation of MAPKs, ERK and P38, as well as the activation of AKT and GSK3 β .

Objectives: To elucidate the therapeutic potential of the ROCK inhibitor (Y-27632) in the K/BxN serum transfer arthritis model.

Methods: Two groups of C57BL/6J mice were used, in the control group, mice were treated with physiological serum and in the experimental group with a ROCK inhibitor (Y-27632). Arthritis was induced by intraperitoneal injection of 100 μ l of K/BxN serum on days 0 and 2. In the experimental group, mice were treated with intraperitoneal injections of 10 mg/kg from day 0 until sacrifice, on day 10. Control mice were treated with the same volume of physiological serum. Arthritis was assessed by two observers using a semiquantitative clinical score. For histological analysis, it was decided to obtain the right ankle joints and foot. Joints were fixed in formalin for 6h and were decalcified and embedded in paraffin. Sections were stained with hematoxylin and eosin (H&E) and toluidine. Finally, total RNA was obtained from wrist and ankle joints of mice and the expression of inflammatory mediators and metalloproteases was analyzed by real-time PCR.

Results: Arthritis was induced in C57BL/6J mice, which were treated with Y-27632 (ROCK inhibitor) or with physiological serum. The incidence of arthritis was 100% in both groups of mice and there were no differences in the course of the disease. Clinical score was significantly lower in the Y-27632-treated mice, all along the follow-up, compared with controls. Similar results were observed in the histological analysis. We also analyzed the effect of ROCK inhibitor on the inflammatory response of K/BxN serum-transfer induced arthritis. This analysis revealed that expression of IL6, IL1 β , CXCL1, MMP3, MMP9 and MMP13 were significantly decreased in Y-27632-treated mice compared with control mice. In addition, TNF and NOS2 expression was reduced in Y-27632-treated mice to reach the same levels that observed in C57BL/6J mice without arthritis.

Conclusion: These results indicate that the inhibition of the Rho-ROCK pathway decreases the severity of arthritis in the K/BxN serum transfer model, and point to ROCK as potential therapeutic target for RA. Supported: ISCIII / PI17 / 01660 / RETICS Program, RD16 / 0012/0014 / Cofinanced FEDER.

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SAT0016

DEVELOPMENT OF FIBROBLAST-LIKE SYNOVIOCYTE ASSAYS FOR TARGET DISCOVERY IN RHEUMATOID ARTHRITIS

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Background: The rheumatoid arthritis (RA) synovium is characterized by an overabundance of fibroblast-like synoviocytes (FLS), which play a central role in the initiation and perpetuation of disease via multiple mechanisms.¹ FLS promote disease progression by producing high levels of proinflammatory factors, migrating to and invading cartilage and bone, and promoting self-proliferation and resistance to apoptosis. Our current understanding of the molecular mechanisms that govern FLS-mediated pathology in the synovial joint remains incomplete. Importantly, almost 30% of treatment-naïve early RA patients exhibit a strong fibroid phenotype that correlates with relatively poor response to disease-modifying anti-rheumatic drugs.² Yet, current therapies in RA are not directly aimed at FLS pathology, creating an opportunity for novel therapeutic target discovery.

Objectives: Our aim is to develop a broad suite of screening-amenable assays in RA patient-derived FLS for the discovery of target pathways that control multiple pathological properties, including cytokine secretion, migration, and invasion.

Methods: A sensitive high-throughput RA-FLS secretion assay was developed to examine the ability of small-molecule inhibitors to block the production of interleukin (IL)-6 and matrix metalloproteinase (MMP)-3 in response to stimuli. To create a physiologically relevant stimulus, a surrogate synovial fluid cocktail (composed of 12 factors) was defined and titrated for optimal concentration selection. Small-molecule inhibitors (N=170) of diverse biological pathways were screened using the full cocktail or individual stimulation (TNF α , IL-1 α , or IL-17) to characterize assay performance. In addition, an FLS platelet-derived growth

factor (PDGF)-mediated migration screening assay was developed using a live cell imaging system (Incucyte) to quantify real-time FLS migration.

Results: Due to the variability and limited volume of synovial fluid, we developed a surrogate synovial fluid cocktail to mimic the relevant stimulation of RA-FLS in the inflamed joint. The surrogate cocktail was composed of 12 factors: TNF α , IL-1 α , IL-17, IFN γ , OSM, LIF, GM-CSF, IP-10, VEGF, PDGF, AREG, and FGF2. Individual titration of these factors demonstrated that only 3 stimulatory factors (TNF α , IL-1 α , and IL-17) resulted in a robust increase of IL-6 production. Importantly, when all 12 factors were combined, a synergistic increase in IL-6 and MMP-3 production by FLS was observed. Screening results identified several reference compounds, including an inhibitor of transforming growth factor- β -activated kinase 1 (TAK1), that was previously reported to block cytokine secretion in FLS.³ Treatment with this compound showed complete inhibition of IL-6 and MMP-3 secretion. In addition to the cytokine secretion assay, treatment of FLS with this TAK1 inhibitor resulted in almost complete inhibition of migration (Fig. 1).

Conclusion: Novel FLS assays were developed to discover new targets and interrogate pathways involved in multiple disease-driving mechanisms of FLS in RA. In order to mimic the inflammatory environment present in the RA synovium, we developed a 12-factor surrogate synovial fluid cocktail. A synergistic release of both IL-6 and MMP-3 was demonstrated following cocktail stimulation compared to individual cytokines. This points to the important contribution that multiple factors play in the FLS pathogenic processes and will allow us to uncover pathway interactions that may not be captured with single stimuli. In addition, the development of a real-time, 96-well, imaging-based assay to interrogate FLS migration will allow us to identify targets that control this critical pathological function of FLS.

References:

- [1] Bartok B, et al. *J Immunol*. 2014;192(5):2063-2070.
- [2] Humby F, et al. *Ann Rheum Dis*. 2019;78(6):761-772.
- [3] Jones DS, et al. *Nat Chem Bio*. 2017;13(1):38-45.

Figure 1. Treatment of RA-FLS with TAK1 inhibitor over 24h demonstrates complete inhibition of PDGF-mediated migration.

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SAT0017

METABOLIC CHANGES INDUCED BY ANTI-MALONDIALDEHYDE/MALINDIALDEHYDE-ACETALDEHYDE ANTIBODIES PROMOTE OSTEOCLAST DEVELOPMENT

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Background: Malondialdehyde (MDA) is a highly reactive compound produced by lipid-peroxidation in situations associated with oxidative stress. MDA can irreversibly modify proteins residues such as lysine, arginine and histidine. In addition, MDA adducts can further react with acetaldehyde to generate malondialdehyde-acetaldehyde (MAA) modifications. Such modifications can give rise to immunogenic neo-epitopes that are recognized by autoantibodies. In fact, anti-MDA/MAA IgG antibodies are significantly increased in the serum of patients with autoimmune diseases, such as rheumatoid arthritis (RA) (1) and systemic lupus erythematosus (2). Recently, we have shown that anti-MDA/MAA IgG antibodies are able to promote osteoclast (OC) differentiation *in vitro* (1).