THU0059

PLASMA EXOSOMAL MIR-92A ARE INVOLVED IN THE OCCURRENCE AND DEVELOPMENT OF BONE DESTRUCTION IN RA PATIENTS BY INHIBITING APOPTOSIS OF FIBROBLAST-LIKE SYNOVIOCYTES

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Background: Rheumatoid arthritis (RA) is a chronic and progressive autoimmune disease that mainly affects joints. Bone erosion and bone destruction are the characteristic features of RA.¹ The mechanism of bone destruction is not fully understood at present. The decrease of apoptosis of human fibroblast-like synovocytes (FLSs) originating from mesenchymal is involved in the occurrence and development of bone destruction in RA.² Exosomes are important mediators of biological information and play a part in the occurrence and development of various diseases including RA.³

Objectives: The aim of study was to find whether exosomes participate in the pathogenesis of bone destruction in RA.

Methods: plasma was collected from 10 healthy people and 20 RA patients. According to Sharp-van der Heijde score (SHS), patients were divided into two groups named bone destruction group and non bone destruction group. Exosomes were extracted by Total Exosome Isolation reagent and confirmed by transmission electron microscope and western blot. The internalisation of exosomes was detected by immunofluorescence. Normal FLSs were stimulated with exosomes. Flow cytometry was utilised to detect the alteration of cell cycle and apoptosis rate. The cell proliferation was determined by CCK-8 assay. Apoptosis proteins (Bax, BCL-2 and caspase-3) were examined by western blot. The concentrations of TNF- α and IL-1 in the cell supernatants were measured by enzyme-linked immunosorbent assay (ELISA). High-throughput sequencing was used to detect the expression of miRNAs in exosomes.

Results: There is no difference between exosomes of normal people and RA patients in promoting cell proliferation. However, the exosomes of RA patients can prohibit the cell apoptosis and promote the release of TNF- α and IL-1 from FLSs more effectively. Of these two RA groups, the abilities of bone destruction group exosomes are higher. The expressions of Bcl-2 and caspase-3 in bone destruction group are also significantly higher than that in the non bone destruction group and the normal group. Inversely the expression of Bax in bone destruction group is lower. Additionally, exosomal miR-92a are significantly over expressed in bone destruction group.

Abstract THU0059 – Figure 1

Abstract THU0059 – Figure 2

Abstract THU0059 – Figure 3

Abstract THU0059 – Figure 4

Abstract THU0059 – Figure 5

Conclusions: The study showed that exosomes in the serum of RA patients can prohibit the apoptosis of FLSs and enhance the secretion of inflammatory cytokines to promote bone destruction. Exosomes play an important role in the pathogenesis of RA. Exosomes can be used as a potential predictor for early bone destruction.

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THU0060

ALTERATIONS OF SPLICING IN LEUKOCYTES FROM RHEUMATOID ARTHRITIS PATIENTS AND ITS INFLUENCE ON THE AUTOIMMUNE, INFLAMMATORY AND ATHEROTHROMBOTIC PROFILE OF THE DISEASE. POTENTIAL ROLE OF U4ATAC

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Objectives: The aim of this study was the identification, in leukocytes of Rheumatoid Arthritis (RA) patients, of the alterations present in the spliceosome and the machinery responsible for the splicing, as well as their influence on the activity of the disease and its atherothrombotic profile.

Methods: We evaluated, using a microfluidic qPCR array (Fluidigm), a set of 45 elements of the splicing machinery: the complete major and minor spliceosome components and a series of splicing factors with potential pathological role. Monocytes, neutrophils and lymphocytes of purified from 74 RA patients and 29 healthy donors (HD) were assessed. In parallel, extensive clinical/serological evaluation, and correlation and association analyses were carried out.

Results: A significant alteration in several components of the spliceosome was observed in all the three leukocyte subtypes from RA patients compared to HD. Various spliceosome components were specifically altered in different leukocyte subtypes; it should be noted that a general downregulation was observed. Likewise, it was striking that 7 elements, including two small nuclear RNA (snRNA) of the major spliceosome (U1 and U5), the snRNA of the minor spliceosome, U4atac, and the splicing factors RBM3, RBM17, SAM68 and SRSF10 showed the same alteration pattern: all significantly reduced in the 3 leukocyte subtypes of patients with RA, except for U4atac, which was consistently over expressed and virtually absent in HD leukocytes. Although this process needs further analysis, it is likely that the overexpression of U4atac could interfere in the normal functioning of the major spliceosome, by binding to U5, thus altering the splicing of most introns (>99%), favouring non-canonical splicing, and generating aberrant proteins involved in the development of this pathology. Correlation and association studies showed a significant association between the expression levels of the 7 splicing factors cited and several clinical/serological parameters, including the activity of the disease, the positivity for anti-CCP and RF antibodies, and the expression of different inflammatory mediators. Likewise, reduced values of other splicing factors, differentially deregulated in the three leukocyte subtypes, were associated with radiological involvement, as well as with the presence of atheroma plaques, hyperlipidemia and arterial hypertension.

Conclusions: We have identified specific alterations in the splicing machinery of leukocytes from RA patients, associated with the activity of the disease, as well as with its inflammatory and atherothrombotic profile. Altogether, the generalised reduction of multiple elements of the splicing machinery and the consistent elevation of U4atac will deem necessary to examine the possible role of this snRNA in the alteration of the spliceosome in the near future, as well as its specific implication in the regulation of the expression of key proteins in the pathology of RA.

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THU0061

RESTRICTED AXL SYNOVIAL EXPRESSION AND INCREASED CLEAVAGE OF AXL ECTODOMAIN CORRELATE WITH HIGHLY INFLAMED SYNOVITIS AND MORE SEVERE RHEUMATOID ARTHRITIS

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Background: Tyrosine kinase Axl, member of the TAM family, is expressed by antigen presenting cells and behaves as a negative regulator of the inflammatory cascade. Inflammatory stimuli up-regulate Axl expression in bone-marrow-derived macrophages¹. Soluble (s) Axl, generated by ADAM10, is a potent decoy for the TAM-ligand Gas6 and can impair TAM axis activation in lupus². In rheumatoid arthritis (RA) dendritic cells, Axl is epigenetically down-regulated³. Emerging evidence has emphasised the significant role of Axl/sAxl in the pathogenesis and progression of autoimmune diseases, but little is known about TAM expression and regulation within the rheumatoid synovium.

Objectives: We aimed to quantify Axl/ADAM10 in synovial tissue (ST) and sAxl/Gas6 in synovial fluid (SF) and to correlate Axl/sAxl expression with synovial inflammation and disease severity in RA patients.

Methods: ST/SF were sampled from early treatment-naïve RA patients undergoing ultrasound (US)-guided synovial biopsy of the most inflamed accessible joint. RA was diagnosed according to ACR/EULAR2010 criteria. The Krenn's synovitis score of inflammation was determined by H and E. Immunohistochemistry (IHC) staining of CD3/CD20/CD138/CD68 allowed to define the synovial immune infiltrate. Axl/ADAM10/CD68 were assessed in ST by IHC/immunofluorescence (30 patients). sAxl/Gas6 were quantified in 17 SF by ELISA. Synovial gene expression data were obtained by next-generation sequencing (80 patients).

Results: Axl was predominantly expressed only by CD68-positive macrophages of the lining and co-localised with ADAM10. AXL synovial mRNA significantly negatively correlated with the Krenn's score and with the degree of infiltration by B/T lymphocytes, plasma cells, and sub-lining macrophages, but not lining macrophages. In SF, sAxl positively correlated with Gas6 and was significantly more abundant in patients with highly inflamed synovitis and more active disease. AXL synovial gene expression showed strong negative correlation with indexes of disease severity, including ESR, CRP, DAS28, and US synovial thickening/power-doppler, and with the mRNA of pro-inflammatory cytokines, e.g. TNF and IL6.

Conclusions: Our data demonstrate that, despite the highly-inflamed environment characterising RA synovia, Axl expression is restricted to the synovial lining, where it can be cleaved and released into the synovial fluid by ADAM10. There, sAxl can bind Gas6 with high affinity, preventing its interaction with transmembrane functional TAM receptors. Raised levels of sAxl and down-regulated AXL mRNA expression correlate with highly inflamed synovitis and more severe disease. Defects in the TAM system could provide an original mechanistic explanation of the persistent inflammation in the RA joints, representing a novel therapeutic target exploitable to regain tissue homeostasis.

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THU0062

WNT5A INVOLVEMENT IN MIGRATION, INVASION AND THE PRO-INFLAMMATORY PHENOTYPE OF RHEUMATOID SYNOVIOCYTES

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Background: Fibroblast-like synoviocytes (FLS) are pivotal in the inflammation and joint damage of rheumatoid arthritis (RA). These cells acquire an aggressive phenotype; they migrate and invade articular structures perpetuating synovial inflammation. Also, they contribute to cartilage and bone damage by secretion of cytokines, metalloproteinases and cathepsins. The mechanisms modulating migration and invasion of FLS are not yet completely known. Recently, the role of the non-canonical pathway of Wnt5a has been highlighted in these processes, as well as, its contribution to osteoclastogenesis. Moreover, Wnt5a could be involved in other pathogenic aspects of RA, as suggested by its involvement in tissue