Over-expression of miR-223 in T-lymphocytes of early rheumatoid arthritis patients

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Causes of rheumatoid arthritis (RA) are still largely unknown, but it is now well established that genetic, epigenetic and environmental factors are important in the pathogenesis of the disease. Among the epigenetic regulatory mechanisms, micro-RNA (miRNA) activity may be crucial. Increased levels of miR-146 and -155 have been found in RA synovial fibroblasts (1, 2) and we have recently demonstrated that miR-223 is dramatically up-regulated in peripheral blood and synovial CD4+ T-lymphocytes from RA patients compared to healthy controls (3), suggesting that this aberrant over-expression of miR-223 could contribute to the RA pathogenesis.

In the present study we wanted to address the question whether miR-223 is also over expressed in the early stage of the disease. We analysed miR-223 expression in peripheral blood T-lymphocytes from 12 early RA patients (7 females, 5 males) aged between 33 and 81 years (median 59 years) with disease duration between 6 and 12 months (median 7 months). In addition, to verify whether a specific sub-population of T-lymphocytes is responsible for miR-223 over-expression, we sorted CD4+ and CD8+ T-lymphocytes from three different early RA patients. All patients fulfilled the 1987 ACR criteria for RA classification (4), all had active disease according to DAS28 score. Nine (75%) were IgM rheumatoid factor (RF) positive and 8 (66.7%) had anti-cyclic citrullinated peptide (CCP) antibodies in serum. Eight of them were taking no drug at all, whereas 4 patients were under treatment with less than 10 mg of prednisone and paracetamol at the time of blood collection. No patient was on DMARDs or biologic therapy. Control samples (n=10) were taken from local blood donor bank (6 females and 4 males, aged between 30 and 70 years, median 59 years).

IgM RF and anti-CCP antibodies were tested by commercial kits. Cells purification, pan T Cell isolation, RNA extraction and qRT-PCR were performed as previously described (3).

The results can be summarised as follows. Median level of miR-223 was 144,73 in early RA patients, compared with 6,7 in the controls (p-value <0.001, Wilkoxon Rank Sum test). By analysing the expression level of miR-223 in the single individuals, we found that 9/12 (75%) of the early RA patients had high levels (defined as >4 fold compared to healthy controls median), while all controls displayed very low levels (Fig. 1). Furthermore, we did not find any correlation between miR-223 level and erythrocyte sedimentation rate, C-reactive protein, RF and anti-CCP antibody level and DAS28 score. We also found that miR-223 is predominantly expressed in CD4+ T-cells in early RA as well.

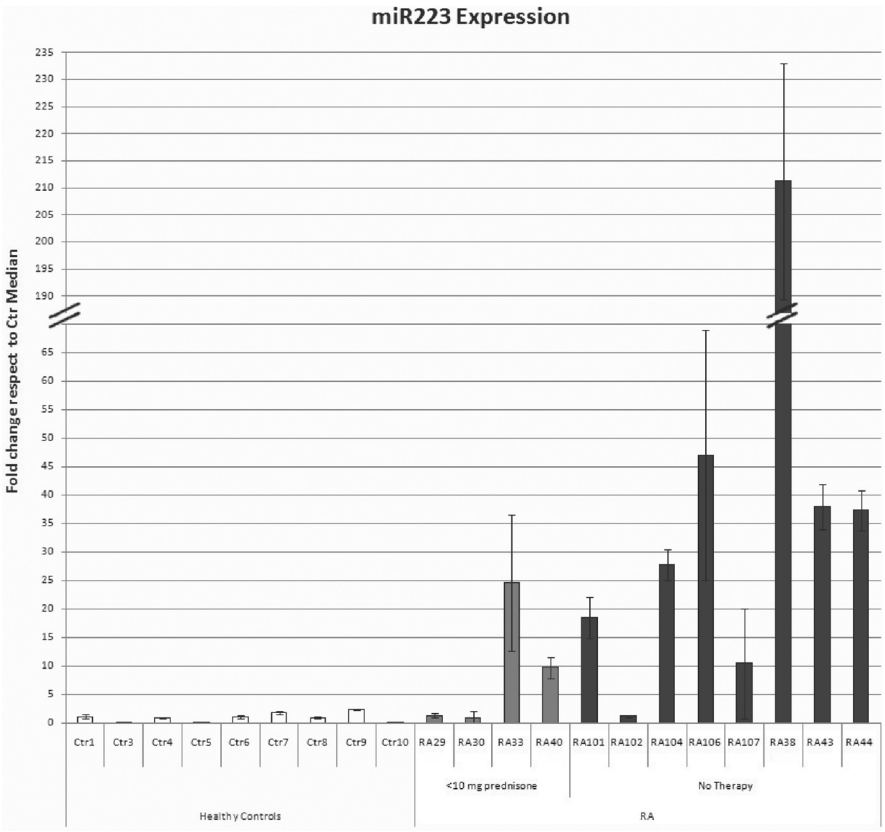

Fig. 1. miR-223 expression in early RA patients and healthy controls. White box: healthy controls (n=10, Ctr2 is missing because out of range); grey box: treated RA patients (n=4); dark grey box: untreated RA patients (n=8). Bars represent standard deviations. qRT-PCR analysis confirms over-expression of miR-223 in peripheral blood T-lymphocytes from 12 early RA patients compared to the median of healthy controls.

Thus, we demonstrated that miR-223 is over-expressed in T-lymphocytes also in early RA patients. In particular CD4+ T cells express miR-223 much more than the CD8+ subpopulation. As CD4+ cells are key players in the RA pathology, our results suggest that miR-223 may play a role even in the early stage of the disease. Indeed, Murata *et al*. recently showed that miR-16, miR-146a, miR-155 and miR-223 were higher in RA synovial fluids than in osteoarthritis (OA) synovial fluids, but not in plasma (5), suggesting a role for these miRNAs in the pathogenesis of the disease.

While the role of miR-223 in the differentiation of the myeloid lineage has been well characterised (6,7), little is known about its function in T-lymphocytes. In this regard, it has been recently demonstrated that a potential target of miR-223 could be E2F1 protein, as shown in acute myeloid leukemia (8). E2F1 protein belongs to the E2F transcription factor family which controls the initiation of DNA synthesis and subsequent transition of cells from G0-G1 to S phase of the cell cycle. Several studies demonstrated that a mutation of the E2F1 gene in mice causes enhanced T-lymphocyte proliferation, leading to systemic and organ specific autoimmunity. Recently Salam *et al*. showed that E2F1 may have a functional effect to induce the development of type 1 diabetes mellitus and Sjögren's syndrome in NOD mice (9). In RA patients over-expression of miR-223 could decrease the expression level of E2F1 protein, thus leading to dysregulation of T lymphocytes and to autoimmunity. In conclusion, our study suggests that miR-223 may have a role in the early stage of RA and that a better characterization of the function of this miRNA in T-lymphocytes may provide further information on the RA pathogenesis.

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