

# 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.

## Materials and 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 was analysed histologically and by  $^3\text{[H]}$ thymidine incorporation. RNA expression levels were measured by qPCR.

### Results:

When we crossed miR-146a<sup>-/-</sup> into hTNFtg mice, histological examination revealed a significant increase in synovial inflammation and even more striking a more than twofold increase in local bone destruction, due to increased generation of osteoclasts in the tarsal joints of miR-146a<sup>-/-</sup>/hTNFtg mice compared to hTNFtg mice. Interestingly, systemic bone loss was comparable in hTNFtg compared to miR-146a<sup>-/-</sup>/hTNFtg mice, suggesting an important local role of miR-146a. Indeed, we detected increased levels of IL-1 $\beta$ , TRAF6, a major target of miR-146a and RANKL, in addition the expression level of OPG was decreased locally in the paws of miR-146a<sup>-/-</sup>/hTNFtg compared to hTNFtg mice. By performing bone marrow transplants we could indeed show a pivotal role for miR-146a in mesenchymal cells in controlling local osteoclast generation and bone destruction. Analysis of important mesenchymal cells in arthritis, the synovial fibroblasts exhibited enhanced proliferation if miR-146a is missing, *in vitro* and *in vivo*. Moreover stimulation of these cells with IL-1 $\beta$ , a prominent cytokine in arthritis which was also shown to be negatively regulated by miR-146a, led to increased expression of RANKL and TRAF6 in miR-146a deficient synovial fibroblasts.

### Conclusion:

These data demonstrate an important mitigating role of the miR-146a in inflammatory arthritis, most importantly in local bone destruction, by controlling mesenchymal expression of osteoclastogenic factors. This shows an important anti-inflammatory role of miR-146a, which might possibly be exploited for therapeutic purposes.