Journal of Autoimmunity 82 (2017) 74–84

Contents lists available at ScienceDirect

Journal of Autoimmunity

journal homepage: www.elsevier.com/locate/jautimm

MicroRNA-146a governs fibroblast activation and joint pathology in arthritis

Victoria Saferding [a], Antonia Puchner [a], Eliana Goncalves-Alves [a], Melanie Hofmann [a], Michael Bonelli [a], Julia S. Brunner [b], Emine Sahin [b], Birgit Niederreiter [a], Silvia Hayer [a], Hans P. Kiener [a], Elisa Einwallner [c], Ramzi Nehmar [d], Raphael Carapito [d], Philippe Georgel [d], Marije I. Koenders [e], Mark Boldin [f], Gernot Schabbauer [b], Mariola Kurowska-Stolarska [g], Günter Steiner [a, h], Josef S. Smolen [a], Kurt Redlich [a], Stephan Blüml [a, *]

[a] Division of Rheumatology, Internal Medicine III, Medical University of Vienna, Austria
[b] Institute for Physiology, Center for Physiology and Pharmacology, Medical University of Vienna, A-1090, Vienna, Austria
[c] Department of Laboratory Medicine, Medical University of Vienna, Vienna, Austria
[d] INSERM UMR_S 1109, Fédération de Médecine Translationnelle (FMTS), Université de Strasbourg, Centre de Recherche en Immunologie et Hématologie, 1, Place de l'Hôpital, 67085, Strasbourg Cedex, France
[e] Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands
[f] Department of Molecular and Cellular Biology, Beckman Research Institute, City of Hope, Duarte, CA, United States
[g] Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, G12 8TA, United Kingdom
[h] Cluster of Arthritis and Rehabilitation, Ludwig Boltzmann Society, Vienna, Austria

ARTICLE INFO

Article history:
Received 6 March 2017
Received in revised form
16 May 2017
Accepted 17 May 2017
Available online 22 May 2017

ABSTRACT

Synovial fibroblasts are key cells orchestrating the inflammatory response in arthritis. Here we demonstrate that loss of miR-146a, a key epigenetic regulator of the innate immune response, leads to increased joint destruction in a TNF-driven model of arthritis by specifically regulating the behavior of synovial fibroblasts. Absence of miR-146a in synovial fibroblasts display a highly deregulated gene expression pattern and enhanced proliferation *in vitro* and *in vivo*. Deficiency of miR-146a induces deregulation of tumor necrosis factor (TNF) receptor associated factor 6 (TRAF6) in synovial fibroblasts, leading to increased proliferation. In addition, loss of miR-146a shifts the metabolic state of fibroblasts towards glycolysis and augments the ability of synovial fibroblasts to support the generation of osteoclasts by controlling the balance of osteoclastogenic regulatory factors receptor activator of NF-κB ligand (RANKL) and osteoprotegerin (OPG). Bone marrow transplantation experiments confirmed the importance of miR-146a in the radioresistant mesenchymal compartment for the control of arthritis severity, in particular for inflammatory joint destruction. This study therefore identifies microRNA-146a as an important local epigenetic regulator of the inflammatory response in arthritis. It is a central element of an anti-inflammatory feedback loop in resident synovial fibroblasts, who are orchestrating the inflammatory response in chronic arthritis. MiR-146a restricts their activation, thereby preventing excessive tissue damage during arthritis.

1. Introduction

Inflammatory arthritides, such as rheumatoid arthritis (RA) or psoriatic arthritis (PsA), are systemic autoimmune diseases characterized by synovial infiltration of inflammatory cells, leading to clinical symptoms such as tender and swollen joints [1–5]. The local inflammatory milieu of hematopoietic and mesenchymal cells

Abbrevations: OPG, osteoprotegerin; RANKL, receptor activator of NF-κB ligand; miR, micro RNA; FLS, fibroblast like synoviocytes; PsA, psoriatic arthritis; hTNFtg, human tumor necrosis factor transgene; TRAF6, TNF receptor associated factor 6; IRAK-1, interleukin-1 receptor-associated kinase 1.

* Corresponding author. Division of Rheumatology, Internal Medicine III, Medical University of Vienna, Währinger Gürtel 18-20, 1090, Vienna, Austria.
E-mail address: stephan.bluemel@meduniwien.ac.at (S. Blüml).

http://dx.doi.org/10.1016/j.jaut.2017.05.006
0896-8411/

in the joint supports the development of bone damage by specifically equipped cells, namely osteoclasts, which are multinucleated cells derived from hematopoietic monocytic precursors and the only known cell type capable of bone resorption [1,6]. Although intensely studied, many mechanisms controlling joint inflammation and subsequent osteoclastogenesis are yet incompletely understood. The need for a better understanding of the pathogenesis of these diseases is highlighted by the fact that in spite of a steadily growing arsenal of therapies still up to 50% of patients with RA or PsA are not responding well to current treatment regimens [7,8].

Micro RNAs (miRs) are non-coding regulatory RNAs that have been shown to be important in the regulation of many aspects of biology, including cancer development and immunity [9–12]. MicroRNAs regulate gene expression at a post transcriptional level by translational repression or degradation of target gene mRNA [11,13]. Originally thought to be important for fine tuning of cellular responses, the generation of mice lacking specific microRNAs has demonstrated very specific functions of certain microRNAs [14–16]. Several miRs have been implicated in the pathogenesis of systemic autoimmune diseases including RA, although the *in vivo* relevance of individual microRNAs remains largely unknown [17–20].

MiR-146a is mainly expressed in immune cells of the innate and adaptive immune system and it is induced during their activation or maturation through NF-κB. Its main targets are interleukin-1 receptor-associated kinase 1 (IRAK1), TRAF6 and RelB, among others [16,21–23]. Functionally miR-146a regulates inflammatory responses in monocytes and macrophages as well as the resolution of T cell responses and the maintenance of immunological tolerance [16,22,24,25].

MiR-146a expression has been demonstrated to be differentially regulated in patients with RA compared to healthy controls [26–30]. Furthermore, using agonistic miR-146a as a therapeutic agent in a commonly used disease model of arthritis, the collagen induced arthritis, revealed reduction of joint damage, by decreasing the capacity for osteoclastogenic differentiation of myeloid cells [31]. In addition, miR-146a has been shown to be an important regulator of the proinflammatory NF-κB induced cytokines IL-6 and IL-1β in Borrelia burgdorferi-induced arthritis [32]. In this study, we show that miR-146a, by regulating the expression of TRAF6, is a central regulator of the pathogenicity of synovial fibroblasts in inflammatory arthritis. Loss of miR-146a leads to increased proliferation and elevated metabolic activity in synovial fibroblasts and enhances their ability to support the generation of bone destructing osteoclasts during arthritis.

2. Materials and methods

2.1. Mice

Breeding pairs of miR-146a$^{-/-}$ and miR-146a$^{+/+}$ littermate (B6.(FVB)-MIR146 $^{TM1.}$1BAL/J) mice were provided by Mark Boldin/David Baltimore. These mice were crossed into Tg197 human TNF transgenic mice (hTNFtg; genetic background C57BL6; [33]) to obtain miR-146a$^{-/-}$/hTNFtg mice. Mice used in all experiments were age- and sex-matched. All data were generated from littermates.

2.2. Antibodies and reagents

The following antibodies were used: CD11c (Clone: HL3) (Armenian hamster anti-mouse; BD Pharmingen); CD11b (Clone: M1/70) (rat anti-mouse; BD Pharmingen); CD11b (Clone: M1/70) (rat anti-mouse; eBioscience).

2.3. Arthritis disease model

For the human TNF alpha transgenic mouse model of arthritis [33] hTNFtg mice were crossed with miR-146a$^{-/-}$ mice.

2.4. Histological analysis

Mouse hind paws or tibiae were fixed in 4.5% formalin for 6 h, decalcified in 14% EDTA/ammonium hydroxide buffer (pH 7.2; Sigma-Aldrich) at 4 °C until the bones were pliable. Serial paraffin embedded sections from hind paws and tibiae (2 μm) were stained with hematoxylin and eosin (H&E) or for tartrate-resistant acid phosphatase (TRAP) activity. Staining for TRAP was done as previously described [34]. For quantification of inflammation and erosion areas, bone volume per tissue volume, trabecular number, trabecular thickness, trabecular separation, number of osteoclasts per bone perimeter and number of osteoblasts per bone perimeter, Zeiss Axioskop2 Mot microscope (Carl Zeiss Micro-Imaging) and Osteomeasure Analysis System (OsteoMetrics) were used. Osteoclasts were counted in TRAP stained sections of tarsal joints; to distinguish them from other TRAP^{+} cells such as macrophages and dendritic cells, we counted only TRAP^{+} cells displaying a minimum of 3 nuclei located at the eroded sites of bone surfaces in the metatarsal bone as osteoclasts. The sum of inflammation or erosion areas for each mouse was calculated from H&E-stained sections of tarsal joints from each mouse.

Microscopic analysis was performed on Zeiss Axioscop2 Mot microscope, at a magnification of 5× (numerical aperture NA: 0,16), 10× (NA: 0,45) and 20× (NA: 0,75) plan apochromat objective, at an illumination colour temperature of 3200 K, with air as imaging medium. Olympus DP73 was used as imaging camera. Cellsens Dimension was used as acquisition software. Images were processed by contrast optimization.

For immunohistochemistry rat monoclonal F4/80 (Serotec; diluted 1:200) and polyclonal rabbit anti Ki-67 (Abcam; diluted 1:100) were used. To quantify the percentage of F4/80 or Ki-67 positive cells, staining intensity of F4/80 or Ki-67 was analyzed by tissue cytometry using HistoQuest (Tissuegnostics).

Assessment of Ki-67 production in Ki-67 stained sections of tarsal joints, was done using a semi quantitative scoring system, ranging from 0 to 3 (0 = no colouring detectable, 1 = brown colouring on one or two spots, 2 = brown colouring on more than two spots, 3 = brown colouring of the whole section).

2.5. Cell culture

Osteoclast generation was performed as previously described [35].

For *in vitro* fibroblasts and coculture assays, fibroblasts were isolated from hind paws of wt or miR-146a$^{-/-}$ mice as previously described [40]. Fibroblasts were seeded at a density of 1 × 10^5cells/ml and proliferation was assessed by ^{3}H-Thymidine incorporation. For coculture after 24 h of fibroblast adherence, macrophages were added to the culture and stimulated with 10 ng/ml IL-1β [32].

2.6. Transfection

Transfection of fibroblasts with either TRAF6 or control siRNA (Dharmacon) was done as previously described [36].

2.7. Cell metabolism

Seahorse XF-24 cell culture plates (Agilent Technologies) were coated with 15 μg/cm^2 rat collagen (Sigma), cells were seeded at a density of 4 × 10^4 cells/well. The oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) of cultured synovial