Tuberculosis xxx (2016) 1–7

Contents lists available at ScienceDirect

Tuberculosis

journal homepage: http://intl.elsevierhealth.com/journals/tube

REVIEW

Immunomodulating microRNAs of mycobacterial infections

Paulo Bettencourt [a, 1], David Pires [a, b], Elsa Anes [a, b, *]

[a] Research Institute for Medicines, iMed-ULisboa, Faculdade de Farmácia da Universidade de Lisboa, Portugal
[b] Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal

ARTICLE INFO

Article history:
Received 22 June 2015
Received in revised form
17 November 2015
Accepted 21 December 2015

Keywords:
Tuberculosis
MicroRNAs
Mycobacteria
Host–pathogen interactions

SUMMARY

MicroRNAs are a class of small non-coding RNAs that have emerged as key regulators of gene expression at the post-transcriptional level by sequence-specific binding to target mRNAs. Some microRNAs block translation, while others promote mRNA degradation, leading to a reduction in protein availability. A single miRNA can potentially regulate the expression of multiple genes and their encoded proteins. Therefore, miRNAs can influence molecular signalling pathways and regulate many biological processes in health and disease. Upon infection, host cells rapidly change their transcriptional programs, including miRNA expression, as a response against the invading microorganism. Not surprisingly, pathogens can also alter the host miRNA profile to their own benefit, which is of major importance to scientists addressing high morbidity and mortality infectious diseases such as tuberculosis. In this review, we present recent findings on the miRNAs regulation of the host response against mycobacterial infections, providing new insights into host–pathogen interactions. Understanding these findings and its implications could reveal new opportunities for designing better diagnostic tools, therapies and more effective vaccines.

Contents

1. Introduction

The regulation of gene expression by microRNAs (miRNAs) has been investigated extensively for a number of infectious diseases. MicroRNAs are 19–22 nucleotides complementary region to the target mRNA leading to protein translation silencing. Although early studies were focused on the role of miRNAs during viral and parasitic infections [1,2], the crucial role of miRNAs in the interplay between host and bacteria has been assessed as well [3]. Not

* Corresponding author. Faculdade de Farmácia da Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal. Tel.: +351 217946443; fax: +351 217934312.
E-mail addresses: paulo.bettencourt@ndm.ox.ac.uk (P. Bettencourt), dpires@ff.ulisboa.pt (D. Pires), eanes@ff.ulisboa.pt (E. Anes).
[1] Present address: The Jenner Institute, University of Oxford, Old Road Campus Research Building, Roosevelt Drive, Headington, Oxford OX3 7DQ, UK.

http://dx.doi.org/10.1016/j.tube.2015.12.004
1472-9792/

surprisingly, an increased understanding of the roles of miRNAs in major infectious human diseases such as tuberculosis (TB) is now attracting researchers' attention. The genus *Mycobacterium* includes highly pathogenic species such as the agents of tuberculosis and leprosy, caused by *Mycobacterium tuberculosis* (Mtb) and *Mycobacterium leprae*, respectively, but also opportunist pathogens such as *Mycobacterium avium*, which can also cause disseminated infections in immune-compromised people including AIDS patients [4].

Genomic studies have revealed that while extensive portions of the metazoan genome do not encode proteins, they do for bioactive RNA species including the 19–22 nucleotides long miRNAs. MiRNAs are involved in post-transcriptional gene expression control [5], influencing many biological systems, including mammalian immune systems [6]. The silencing of a target protein occurs when the miRNA complex binds the complementary region on the 3′-Untranslated Region (3′-UTR) of the target mRNA in order to terminate translation or promote its degradation [7,8]. Only the 7-base sequence between 2nd and 8th nucleotide is called the "seed region", and a complete match of this sequence is required [9].

MicroRNAs are expressed in a tissue-specific fashion, and in a spatially and temporarily dependent manner, controlling a multidimensional response simultaneously. Considering the (1) sequence complementarities between the seed region and the 3′-UTR of the target mRNAs, (2) the secondary structure of the RNAs, (3) its spatial and (4) timely localization, there is a high probability that a single miRNA may target hundreds of different mRNAs. Conversely, several different miRNAs can also target the same mRNA, resulting in enhanced translational inhibition [10].

The involvement of miRNAs in mycobacterial infections has only been recently addressed, yet the conclusions drawn from those studies have provided promising targets that need to be explored for better understanding the host–pathogen interaction and to design efficient strategies to control the disease, as it has been highlighted in recent reviews [11,12]. In this review, we summarize the recent findings on the role of host miRNAs in response to mycobacterial infection.

2. MicroRNAs in immunity and infection

A plethora of miRNAs has been found to regulate key cells of the immune system. There is evidence of miRNA-mediated regulation in T-cell differentiation and function [13–16] and in the innate function of macrophages, dendritic cells (DCs) and natural killer cells [17,18]. Moreover, miRNAs have not only been found in tissues but also in serum and other body fluids protected from RNAse activity through a stable association with the RNA-induced silencing complex (RISC), and circulating freely [19] or bound to exosomes [20]. The level of regulation promoted by miRNAs is very specific and fast, and may not require *de novo* synthesis of genes products to answer to situations of stress. Therefore, this defence mechanism that is highly conserved from plants to mammals is crucial in the context of the host control against pathogens.

The first miRNAs described as being directly involved in the immune response were the miR-132, miR-146 and miR-155, in the human monocytic cell line THP-1, by David Baltimore's group [18]. In the context of inflammation, miR-146a is expressed as a response to a variety of microbial components and pro-inflammatory cytokines. MiR-146a was found to target the mRNA of the TNF receptor-associated factor 6 (TRAF6) and Interleukin-1 (IL-1) receptor-associated kinase 1 (IRAK1), which are both downstream of Toll-Like Receptors (TLRs) signalling upon activation by pathogen-associated molecular patterns (PAMPs). This led to the proposal that miR-146, which is an NF-κB-dependent gene, controls TLRs and cytokine signalling through a negative-feedback regulation loop. The authors suggested that miR-146 acts as a regulator of the extent of the inflammatory response, by targeting genes transcribed after NF-κB activation [18]. These initial studies showed that miRNAs establish a new paradigm of activation of inflammatory factors when host cells are targeted by bacterial pathogens. Many studies have emerged since then, to characterize the miRNome of host cells upon contact with invading pathogens or PAMPs, with some examples shown in Table 1. Interestingly, the well-known oncogene miR-155 that is induced upon TLR4 activation identifies a potential link between cancer and inflammation in an unprecedented way [21,22]. Indeed, that is the case of the expression of host miRNAs in human gastric tissues infected with *Helicobacter pylori*, which targets the mRNA of genes involved in cancer development [23,24].

Diverse microorganisms have been described to code for small RNAs with regulatory properties. To our knowledge, there are no reports on bacterial RNA described to interfere with host mRNAs, as described for viral RNAs. The Kaposi Sarcoma-Associated Herpes Virus (KSHV) miRNAs directly target cellular mRNAs, as is the case of miR-155 and miR-142-3p orthologues, to manipulate the host mRNA and, consequently, control viral replication and pathogenesis [25,26].

3. Immunomodulating microRNAs of mycobacterial infections

The host miRNAs in response to mycobacterial infection varies significantly in function of the mycobacterial species (Table 2), the strains, and the host itself. Indeed, in infected macrophages, miRNAs are differentially expressed between virulent and avirulent Mtb [27], between the highly virulent Beijing/W strains non-Beijing/W clinical strains [28], and deep sequencing analysis of multidrug-resistant (MDR) Mtb strains revealed that miRNAs were differentially expressed compared to antibiotic sensitive Mtb strains [29]. Upon contact with the host, pathogenic bacteria induce a sequence of mechanisms to promote its invasion and the establishment of a successful infection within the host. This is followed by an immediate and relatively unspecific innate response, eventually leading to a very specific adaptive immune response. Here we will review what is known about the roles of miRNAs on these two different but complementary aspects of the host response to mycobacterial infections: innate and adaptive immunity.

3.1. Innate immunity

After being inhaled into the lung, mycobacteria that bypass the muco-ciliary system, have contact with airway epithelial cells [30], and are then quickly surrounded and engulfed by alveolar macrophages [31,32] and dendritic cells [33]. Once inside macrophages, pathogenic mycobacteria are enclosed in a phagosome whose maturation into phago-lysosome can be blocked by as a microbial evasion strategy. Indeed, we have shown that three distinct processes are targeted during this blockage: actin assembly, fusion with lysosomes, and acidification [34,35]. Actin is a key player in the phagocytic process and its dynamics are involved in the motility of the cell, in the formation of pseudopodia for the internalization of extracellular particles and in vesicle trafficking [36]. For example, our results have indicated a role for miR-142-3p in controlling actin dynamics during infection with implications on bacteria internalization by macrophages [37]. miR-142-3p regulates N-WASP [37], a signal transducer for cell surface receptors leading to actin assembly via the Arp2/3 complex [36]. Furthermore, mycobacteria promote the over-expression of this miRNA at early times of phagocytosis in macrophages. Finally, by manipulating miR-142-3p, we were able to reduce the burden of

Please cite this article in press as: Bettencourt P, et al., Immunomodulating microRNAs of mycobacterial infections, Tuberculosis (2016), http://dx.doi.org/10.1016/j.tube.2015.12.004