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miR-146a controls CXCR4 expression in a pathway that involves PLZF and can be used to inhibit HIV-1 infection of CD4$^+$ T lymphocytes

Maria Teresa Quaranta [a,1], Eleonora Olivetta [b,1], Massimo Sanchez [c], Isabella Spinello [a], Rosa Paolillo [a], Claudia Arenaccio [b,d], Maurizio Federico [b], Catherine Labbaye [a,*]

[a] Department of Hematology, Oncology and Molecular Medicine, Istituto Superiore di Sanità, Viale Regina Elena, 299, 00161 Rome, Italy
[b] National AIDS Center, Istituto Superiore di Sanità, Rome, Italy
[c] Department of Cell Biology and Neurosciences, Istituto Superiore di Sanità, Rome, Italy
[d] Department of Science, University Roma Tre, Rome, Italy

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ABSTRACT

MicroRNA miR-146a and PLZF are reported as major players in the control of hematopoiesis, immune function and cancer. PLZF is described as a miR-146a repressor, whereas CXCR4 and TRAF6 were identified as miR-146a direct targets in different cell types. CXCR4 is a co-receptor of CD4 molecule that facilitates HIV-1 entry into T lymphocytes and myeloid cells, whereas TRAF6 is involved in immune response. Thus, the role of miR-146a in HIV-1 infection is currently being thoroughly investigated.

In this study, we found that PLZF mediates suppression of miR-146a to control increases of CXCR4 and TRAF6 protein levels in human primary CD4$^+$ T lymphocytes. We show that miR-146a upregulation by AMD3100 treatment or PLZF silencing, decreases CXCR4 protein expression and prevents HIV-1 infection of leukemic monocytic cell line and CD4$^+$ T lymphocytes.

Our findings improve the prospects of developing new therapeutic strategies to prevent HIV-1 entry via CXCR4 by using the PLZF/miR-146a axis.

Introduction

The ability of human immunodeficiency virus type 1 (HIV-1) to bind to and enter into CD4$^+$ T cells depends on optimal expression of CD4$^+$ protein on the surface of these cells (Wilen et al., 2012). However, chemokine receptors, in particular CCR5 and CXCR4, are essential coreceptors of CD4$^+$ molecules on cell membrane of T lymphocytes and other cell types, such as monocytes/macrophages and dendritic cells, to allow HIV-1 entry into these cells (Wilen et al., 2012). Thus, receptor-coreceptors which determine viral entry into various cell types, together with the cellular tropism which defines viral phenotype, are among the major factors influencing HIV pathogenesis (Wu and Yoder, 2009; Naif, 2013).

At the present, the current classification of cellular tropism of HIV-1 relies on the differential expression of CCR5 and CXCR4 in monocytes/macrophages and T-cell lines (Wilen et al., 2012; Naif, 2013). Monocytes/macrophages serve as vehicles for viral dissemination between different tissues of the body (Ryan et al., 2002; Ciborowski and Gendelman, 2006; Carter et al., 2010, 2011), thus acting as reservoirs for HIV-1 in tissues (Van Lint et al., 2013).

Then, CCR5 and CXCR4 are relevant targets for pharmaceutical intervention that aims to block HIV-1 entry into macrophages and primary T lymphocytes and to prevent viral dissemination (Proudfoot, 2002; Henrich and Kuritzkes, 2013).

Coreceptor binding of their natural ligands, such as RANTES/CCL5, MIP-1alpha/CCL3, and MIP-1beta/CCL4 for CCR5 and stromal-derived factor, SDF-1/CXCL12, for CXCR4, inhibits HIV-1 entry into CD4$^+$ T cells, monocytic and CD4$^+$ T cell lines (Naif, 2013). However, any change in co-receptors conformation and surface density on cell membrane impacts the overall susceptibility of a given cell type for HIV infection (Lapham et al., 2002; Sloane et al., 2005; Richardson et al., 2012) and may alter the effectiveness of small drugs targeting HIV co-receptors (Proudfoot, 2002; Choi et al., 2012a, 2012b). Then, the discovery of HIV-1 cellular entry inhibitors remains an important objective in molecular pharmacology.

The first clinical trials with the highly specific CXCR4 antagonist, AMD3100 (Mozobil, plerixafor), were designed for treatment of HIV (Hendrix et al., 2000). Approved by Food and Drug Administration (FDA) as a mobilizer of hematopoietic CD34$^+$ cells from the bone marrow to the circulation (Hendrix et al., 2000; DiPersio et al., 2009), AMD3100 is also used to inhibit growth and metastasis

* Corresponding author. Tel.: +39 649902418; fax: +39 649387087.
E-mail address: catherine.labbaye@iss.it (C. Labbaye).
[1] These authors contributed equally to this work.

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of experimental tumors in animal models, as CXCR4 is also involved in metastatic dissemination, growth and survival of cancer cells, including leukemic cells (Domanska et al., 2013).

However AMD3100 is not being further developed for antiretroviral therapy (Hendrix et al., 2004). Highly active antiretroviral therapy (HAART) is currently the only option to control the progression of HIV-1 infection, improve the quality of life of HIV patients and reduce morbidity. Unfortunately, HAART that restores immunological function and helps prevent further diseases transmission, has also developed the phenomenon of drug resistance, a critical factor responsible for failure of this combined therapy (Deeks et al., 2012). In this context, the search for new anti-HIV drugs has been extended to compounds that act against cellular rather than viral target to minimize viral mutagenesis that give rise for drug resistance.

More recently, manipulation of microRNAs (miRNAs) has been proposed as a novel approach to use against HIV-1 infection and for purging the HIV-1 reservoir (Van Lint et al., 2013; Tyagi and Bukrinsky, 2012). miRNAs are small non-coding RNA involved in posttranscriptional gene regulation that participates in RNA interference (RNAi)-mediated gene silencing (Bartel, 2009). RNAi activity is involved in many eukaryotic cellular processes and the deregulated expression of miRNAs and other RNAi components has been described in cancers, metabolic disorders, and infectious diseases (Triboulet et al., 2007; Bivalkar-Mehla et al., 2011; Yeung and Jeang, 2011; Klase et al., 2012). These miRNAs represent a new generation biomarkers for diagnostics and prognostics and, given their stability in vivo (Yeung and Jeang, 2011), new tools to control specific target in disease, including AIDS (Klase et al., 2012).

The role of viral-encoded miRNAs derived from viral genomes (Pfeffer et al., 2004) in virus replication and virus-host interaction is currently under investigation (Ouellet et al., 2013; Zhang et al., 2014), as well as the role of cellular miRNAs in the regulation of HIV-1 viral replication (Lecellier et al., 2005; Huang et al., 2007) and in the complex interactions with HIV-1 (Klase et al., 2012).

miRNA 146a (miR-146a) is considered as a crucial modulator of differentiation and function of cells of the innate and adaptive immunity, acting as a negative feedback regulator of the innate immune response by targeting two adapter proteins, TRAF6 (Tumor necrosis factor receptor -associated factor 6) and IRAK1(IL-1 receptor-associated kinase 1), acting in pro-inflammatory signaling (Taganov et al., 2006; Hou et al., 2009). The latent membrane protein 1 (LMP1), a major oncoprotein of the Epstein-Barr virus (EBV), induces cellular miR-146a expression that may contribute to cellular immortalization and tumorigenesis (Cameron et al., 2008). miR-146a also promotes vesicular stomatitis virus (VSV) replication in macrophages (Hou et al., 2009). The chemokine CCL8/MCP-2, ligand for CCR5 but also potent inhibitor of CD4/CCR5-mediated HIV-1 entry and replication, is also a target of miR-146a in HIV-1 infected microglia (Rom et al., 2010).

Beside its role in immune responses and in disease (Taganov et al., 2006; Boldin et al., 2011; Burger et al., 2014), miR-146a has been also involved in the control of hematopoiesis (Labbaye et al., 2008; Labbaye and Testa, 2012).

We have demonstrated that CXCR4 is a direct target of miR-146a in hematopoietic normal and leukemic cells and identified the transcription factor PLZF (promyelocytic leukemia zinc finger) as a repressor of miR-146a expression in megakaryocytic (Mk) cells (Labbaye et al., 2008).

PLZF was initially identified as a protein whose functions are subverted through chromosomal rearrangements resulting in acute promyelocytic leukemia (Suliman et al., 2012). It is known to regulate progenitor maintenance in multiple tissues (Kelly and Daniel, 2006). Subsequently, PLZF has been found to be a key stem cell maintenance factor in both the hematopoietic system and male germline (Suliman et al., 2012). PLZF was also shown to be a critical regulator of immune system development and function, also implicated in carcinogenesis as a tumor suppressor gene (Suliman et al., 2012). More recently, we have described the regulation of CXCR4 by miR-146a targeting in monocytic (Mo) cells and showed that the enforced expression of miR-146a by AMD3100 treatment, decreases total CXCR4 protein expression and impairs Mo leukemic cell proliferation (Spinello et al., 2011).

In the present study, we showed that PLZF, miR-146a and CXCR4 are expressed and regulated during activation of human primary $CD4^+$ T lymphocytes. TRAF6 expression and regulation was also analyzed in the same cells, as another target gene of miR-146a. In addition, we studied the effects of AMD3100 treatment on miR-146a and CXCR4 expression levels in $CD4^+$ T lymphocytes and during HIV-1 infection of these cells.

We found that PLZF mediates suppression of miR-146a to control increases of CXCR4 and TRAF6 expression levels in $CD4^+$ T lymphocytes, whereas miR-146a upregulation by AMD3100 treatment or PLZF silencing, decreases CXCR4 protein expression level and inhibits HIV-1 infection of $CD4^+$ T lymphocytes.

Altogether, we showed that the PLZF/miR-146a axis that controls CXCR4 expression in $CD4^+$ T lymphocytes, can be used to prevent HIV-1 entry into CD4/CXCR4 expressing target cells in new therapeutic strategies against HIV-1 infection.

Results

PLZF, miR-146a, CXCR4 and TRAF6 expression and regulation in human primary $CD4^+$ T lymphocytes

We previously described in human Mk cells that PLZF suppresses miR-146a transcription and thereby activates CXCR4 translation (Labbaye et al., 2008). To investigate whether this cascade pathway may be present and functional in human primary $CD4^+$ T lymphocytes, we first analyzed PLZF and miR-146a expression and regulation in $CD4^+$ T lymphocytes purified from human peripheral blood (PB), activated by PHA treatment and maintained in culture for few days, as compared to resting $CD4^+$ T lymphocytes (day 0). Then, we examined CXCR4 and TRAF6 expression in the same cells.

Western blot analysis showed that PLZF protein expression, undetectable in resting cells, increases during PHA-activation of $CD4^+$ T lymphocytes (Fig. 1A), whereas miR-146a expression, analyzed by real time PCR, decreases in the same cells (Fig. 1B).

By using real time PCR analysis, we found that CXCR4 mRNA, expressed at very high level in non activated $CD4^+$ T lymphocytes (day 0, Fig. 1C), rapidly decreases during the first hours of activation (0 to 6 hours of culture, Fig. 1C), then remains at a lower but constant level in activated $CD4^+$ T cells (16 to 48 hours of culture, Fig. 1C), in a range of expression quite similar to CXCR4 mRNA levels found in Jurkat and U937 cells (Fig. 1C).

Western blot analysis performed as compared to Jurkat cells, a positive control expressing the 45 kDa isoform of CXCR4 protein, showed that this isoform, almost undetectable in quiescent $CD4^+$ T lymphocytes, increases with the time of culture and activation of $CD4^+$ T lymphocytes (Fig. 1D). Therefore CXCR4 protein expression pathway, inversely correlated to its mRNA expression (Fig. 1C), indicates a posttranscriptional control of CXCR4 mRNA by miR-146a in these cells.

We also examined the expression and regulation of another target of miR-146a, TRAF6, in activated $CD4^+$ T lymphocytes. As observed for CXCR4, TRAF6 mRNA level decreases during the first hours of activation of $CD4^+$ T cells, then remains at a lower level later in culture (from 16 to 24 hours), decreasing slightly at 48 hours (Fig. 1E), whereas the decrease of miR-146a (Fig. 1B) may unblock TRAF6 mRNA translation and then increase TRAF6 protein expression level in these cells (Fig. 1F).

Altogether, our data suggest that the regulation of CXCR4, but also of TRAF6, protein expression level in resting and activated