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Decline of miR-124 in myeloid cells promotes regulatory T-cell development in hepatitis C virus infection

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Summary

Myeloid-derived suppressor cells (MDSCs) and microRNAs (miRNAs) contribute to attenuating immune responses during chronic viral infection; however, the precise mechanisms underlying their suppressive activities remain incompletely understood. We have recently shown marked expansion of MDSCs that promote regulatory T (Treg) cell development in patients with chronic hepatitis C virus (HCV) infection. Here we further investigated whether the HCV-induced expansion of MDSCs and Treg cells is regulated by an miRNA-mediated mechanism. The RNA array analysis revealed that six miRNAs were up-regulated and six miRNAs were down-regulated significantly in myeloid cells during HCV infection. Real-time RT-PCR confirmed the down-regulation of miR-124 in MDSCs from HCV patients. Bioinformatic analysis suggested that miR-124 may be involved in the regulation of signal transducer and activator of transcription 3 (STAT-3), which was overexpressed in MDSCs from HCV patients. Notably, silencing of STAT-3 significantly increased the miR-124 expression, whereas reconstituting miR-124 decreased the levels of STAT-3, as well as interleukin-10 and transforming growth factor-β, which were overexpressed in MDCSs, and reduced the frequencies of Foxp3$^+$ Treg cells that were developed during chronic HCV infection. These results suggest that reciprocal regulation of miR-124 and STAT-3 in MDSCs promotes Treg cell development, thus uncovering a novel mechanism for the expansion of MDSC and Treg cells during HCV infection.

Keywords: hepatitis C virus; microRNA-124; myeloid-derived suppressor cells; regulatory T cells; signal transducer and activator of transcription 3

Introduction

Hepatitis C virus (HCV) infection is characterized by a high rate (over 80%) of chronic infection due to evasion of host immunity, and so is considered as an excellent model to study the mechanisms of persistent viral infections in humans.[1,2] Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of immature myeloid cells that are generated as a result of aberrant myelopoiesis, which occurs under certain pathological conditions, such as cancer and inflammatory and infectious diseases.[3–5] MDSCs have gained special attention because of their potent suppressive functions, as they attenuate both innate and adaptive immune responses.[6–10] However, the mechanisms that regulate MDSC generation and suppressive activities are still incompletely understood, especially in the context of HCV infection.

MicroRNAs (miRNAs) are short (22–23 nucleotides) non-coding RNAs that regulate gene expression and function through translation inhibition or degradation of target mRNA.[11] Compelling evidence suggests that some miRNAs regulate HCV replication, immune functions and liver diseases directly by interacting with the HCV genome or indirectly via controlling virus-associated host immune pathways.[12–18] How HCV induces the expression of specific miRNAs in HCV-infected individuals, particularly in MDSCs, to suppress immune responses and promotes viral persistence *in vivo*, is largely unknown.

We have recently shown MDSC expansion in patients with chronic HCV infection that can inhibit T-cell functions by promoting regulatory T (Treg) cell development.[19] In this study, we further investigated the mechanisms that regulate MDSC expansion and suppressive activities, focusing on miRNA-mediated mechanisms. We provide evidence that HCV infection can activate MDSCs to express interleukin-10 (IL-10), transforming growth factor-β (TGF-β), signal transducer and activator of transcription 3 (STAT-3), and to promote Treg cell development through deregulation of miR-124, which is reciprocally regulated by STAT-3. These findings represent a novel mechanism for MDSC expansion and Treg cell development, providing a novel approach for immunotherapy of chronic viral diseases.

Materials and methods

Subjects

The study protocol was approved by the joint institutional review board at East Tennessee State University and James H. Quillen VA Medical Center (ETSU/VA IRB, Johnson City, TN). Written informed consent was obtained from all participants. The study participants composed two populations: 91 chronically HCV-infected individuals and 28 healthy participants. HCV genotype (70% type 1, 30% type 2 or 3) and viral load (ranging from 12 300 to 50 000 000 IU/ml) were performed by Lexington Veteran Affairs Medical Center (Lexington, KY), and all participants were virologically and serologically positive for HCV, before antiviral treatment. Healthy participants were negative for hepatitis B virus, HCV, and human immunodeficiency virus infection, and blood buffy coats were obtained from Key Biologics (Memphis, TN) or Physician's Plasma Alliance LLC (Gray, TN).

Peripheral blood mononuclear cell preparation and flow cytometry

Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood by Ficoll gradient (GE Heathcare, Piscataway, NJ) centrifugation and analysed immediately. For phenotypic analysis of MDSCs, PBMCs were stained with anti-CD33-allophycocyanin, anti-HLA-DR-FITC (BD Bioscience, San Jose, CA), or isotype control antibodies. The cells were acquired on an AccuriTM C6 flow cytometer (BD, Franklin Lakes, NJ) and analysed using FlowJo software (Tree Star Inc., Ashland, OR). For intracellular cytokine staining, PBMCs were stimulated with 1 μg/ml lipopolysaccharide plus 2·5 μg/ml R848 (LPS/R848; both Santa Cruz Biotechnology, Santa Cruz, CA) for 6 hr, and Brefeldin A (BioLegend, San Diego, CA) was added 5 hr before harvest. The cells were fixed and permeabilized using Inside Stain Kit (Miltenyi Biotec, Auburn, CA), and then stained with IL-10-phycoerythrin (PE), and TGF-β-PE or isotype controls. Phosphorylation of STAT-3 (pY705)-PE staining was performed according to the BD Phosflow™ (BD Biosciences, San Jose, CA) protocol. The fluorescence minus one strategy was used to determine background levels and to adjust multicolour compensation for cell gating.

miScript miRNA PCR array and validation

$CD33^+$ myeloid cells were purified from PBMCs using anti-human CD33-conjugated magnetic beads according to the manufacturer's instructions (Miltenyi Biotec., cell purity > 95%). Total cellular RNA from $CD33^+$ cells, pooled from six patients with chronic HCV infection and six healthy participants, was isolated using the miRNeasy Mini kit (Qiagen, Valencia, CA). RNA quality and quantity were analysed using a BioPhotometer spectrophotometer UV/VIS, and RNA integrity was determined using gel electrophoresis. The miScript miRNA array, which includes 384 annotated miRNAs, was performed by Qiagen Inc.. Data were analysed using RT^2 PROFILER PCR array analysis software and expression values of more than twofold change or less than twofold change (HCV versus healthy participants) were considered as up-regulated or down-regulated miRNAs, respectively. To validate the up- or down-regulated miRNAs by real-time PCR, cDNA was generated from total RNA by miScript II RT kit (Qiagen). Expression levels of miR-29a and miR-124 were assessed by real-time PCR using miScript SYBR Green PCR kit (Qiagen) on CFX96TM Real-time PCR Detection System (Bio-Rad Laboratories Inc., Hercules, CA). Levels of miR-29a and miR-124 were quantified with the $2^{-\Delta\Delta ct}$ relative quantification method, normalized to U61 small nuclear RNA (SNORD61).

Western blot

$CD33^+$ myeloid cells, isolated from HCV-infected patients or following small interfering RNA (siRNA) and miRNA transfections, were subjected to Western blot analysis to measure the expression of ERI-1 and STAT3 using anti-ERI-1 (Santa Cruz Biotechnology, Santa Cruz, CA), anti-STAT3 (Cell Signaling Technology, Danvers, MA) primary antibodies, followed by a horseradish peroxidase-conjugated secondary antibody (Cell Signaling). Membranes were stripped and re-probed with anti-β-actin antibody (Santa Cruz Biotechnology) as an internal control. The proteins were visualized using the Amersham ECL Prime Western Blotting Detection Reagents (GE Healthcare Biosciences, Pittsburgh, PA) and Bio-Rad chemiDoc^{MP} imaging system (Bio-Rad, Philadelphia, PA).

MicroRNA transfection

$CD33^+$ myeloid cells purified from PBMCs of HCV-infected patients were transfected with 30 pmol of

mirVana microRNA mimic for miR-29a or miR-124 or negative control #1, or 30 pmol of STAT-3 siRNA or ERI-1 siRNA or control siRNA (Life Technologies, Grand Island, NY) using the Human Monocyte Nucleofector Kit and Nucleofector I Device (Lonza, Allendale, NJ). The transfected cells were cultured in 10% fetal bovine serum Iscove's modified Eagle's medium (Lonza, Allendale, NJ), stimulated with LPS/R848 for 6 hr and Brefeldin A for 5 hr before harvesting the cells at 24 or 48 hr post-transfection, quantified miR-124 by quantitative PCR, or immune stained with anti-HLA-DR-FITC (BD Bioscience), anti-IL-10, anti-TGF-β, or anti-pSTAT-3 as described above. Additionally, the transfected $CD33^+$ cells were co-cultured with healthy $CD4^+$ T cells in a ratio of 1 : 2 in the presence of 1 μg/ml anti-CD3 and 2 μg/ml anti-CD28 (BD Bioscience) for 5 days, followed by analysis of Treg cell frequencies by immunostaining with anti-CD4-PE, anti-CD25-Alexa488 and anti-Foxp3-Pe-Cy5 (eBioscience, San Diego, CA) using a Foxp3 Staining Set (MACS) and analysed by flow cytometry as described above.

Statistical analysis

The parametric data were presented as mean ± SEM. Comparisons between groups were made by one-way analysis of variance (ANOVA) using PRISM version 5. One-tail paired *t*-test was used to compare two groups with transfection by miR-124 mimic and negative control. The non-parametric data (Fig. 3f) were presented as medium and analysed by one-tail Mann–Whitney *U*-test. Values of $P < 0{\cdot}05$ or $P < 0{\cdot}01$ were considered significant or very significant, respectively.

Results

Differential regulation of miRNAs in myeloid cells from HCV-infected patients

We and others have recently shown marked expansion of MDSCs, which suppress T-cell responses, in patients with chronic viral infections;[19–29] however, the underlying mechanisms for their suppressive activities remain incompletely understood. The differentiation and maturation of the myeloid lineage (myelopoiesis) are orchestrated by interdependent interactions between cytokine receptors, transcription factors, and as recently described, miRNAs.[30,31] Emerging evidence suggests that miRNAs underlie myelopoiesis, and the outcome of distorted myelopoiesis is the generation of MDSCs.[32–34] To identify specific miRNAs that regulate myelopoiesis during HCV infection, we performed miRNA expression profiling in $CD33^+$ myeloid cells isolated from six HCV-infected patients and six healthy participants. This miRNA array included 384 annotated miRNAs. Differentially expressed miRNAs in $CD33^+$ myeloid cells from HCV-infected patients versus healthy participants were shown in a heat map (Fig. 1a) and a scatter plot (Fig. 1b). Expression of 362 miRNAs was unchanged between HCV-infected patients and healthy participants (within a twofold change). A total of 12 miRNAs were differentially expressed in $CD33^+$ myeloid cells from HCV-infected patients compared with the healthy participants; six miRNAs were up-regulated and six miRNAs were down-regulated significantly (Fig. 1c).

Of the miRNAs that were down-regulated in myeloid cells during HCV infection, we were particularly interested in miR-124 and miR-29a, because bioinformatic analysis and recent data revealed that these two miRNAs were involved in regulating the expression of multiple target mRNAs; this includes STAT-3 by a mechanism that involves direct binding to its 3′-untranslated region and hence inhibition of STAT-3 mRNA translation in inflammatory or malignant cells.[32–34] These two miRNAs were therefore carried forward for further analysis. We validated their expression levels by real-time RT-PCR in $CD33^+$ myeloid cells isolated from HCV-infected patients and healthy participants. As shown in Fig. 1(d), miR-124 and miR-29a were significantly decreased in $CD33^+$ myeloid cells from HCV-infected patients compared with healthy participants, revealing a decline in miR-124 and miR-29a expression in myeloid cells in the setting of chronic HCV infection.

STAT-3 contributes to the decline of miR-124 expression in myeloid cells of HCV-infected patients

Functional analysis (see below) revealed that miR-124, but not miR-29a, was involved in the regulation of STAT-3, IL-10 and TGF-β expression in MDSCs, so we focused our investigation on the possible mechanisms that may down-regulate miR-124 expression in myeloid cells of HCV-infected patients. As bioinformatic analysis revealed that STAT-3 has a binding site in the miR-124 promoter region, and previous studies indicated that STAT-3 can regulate miRNA gene expression in chronic lymphocytic leukaemia cells,[35] whereas miR-124 can inhibit STAT-3 to suppress the development of ulcerative colitis or colon cancer,[32,33] we next investigated whether STAT-3 regulates miR-124 expression in myeloid cells during HCV infection. To this end, we measured protein levels of pSTAT-3 in myeloid cells from HCV-infected patients and healthy participants. As shown in Fig. 2(a), pSTAT-3 was significantly up-regulated in $CD33^+$ myeloid cells from HCV-infected patients compared with healthy participants. Notably, siRNA-mediated silencing of STAT-3 expression significantly reduced its protein levels (Fig. 2b, left panel), and significantly increased the miR-124 expression (Fig. 2b, right panel).

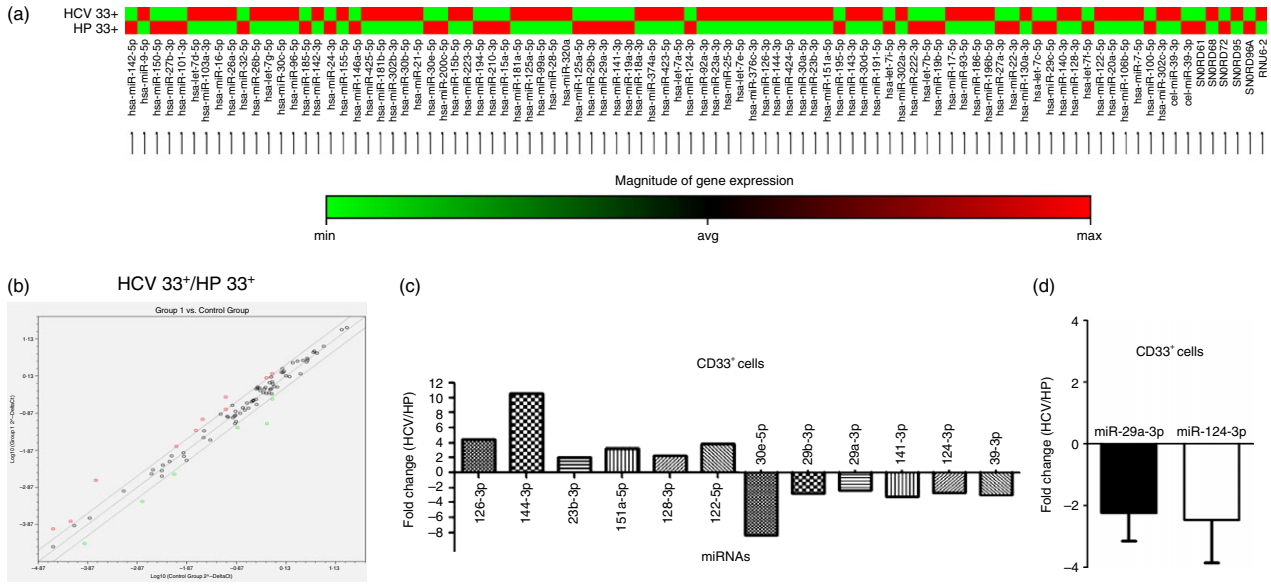

Figure 1. Analysis of microRNA (miRNA) expression array in $CD33^+$ myeloid cells from hepatitis C virus (HCV) -infected patients versus healthy participants (HP). (a) miRNA array heat map. Each column represents results of differentially expressed miRNAs in $CD33^+$ myeloid cells from six HCV-infected patients and six healthy participants, respectively. Each row corresponds to a single miRNA probe. Colour indicates the level of miRNA expression (red: high level, green: low level). (b) miRNA array scatter plot. The intensity of each miRNA expressed as log-base 10 in the two groups (*x*-axis: healthy participants; *y*-axis: HCV-infected patients). The middle line indicates a fold-change ($2^{-\Delta\Delta ct}$) of 1. The top and the bottom lines indicate the desired twofold change in miRNA threshold. The red points above the top line represent up-regulated miRNAs. The green points below the bottom line represent down-regulated miRNAs. (c) Summary data of those significantly up-regulated and down-regulated miRNAs in HCV-infected patients versus healthy participants. (d) Real-time PCR analysis of miR-29a-3p and miR-124-3p expression (mean ± SEM) in $CD33^+$ myeloid cells from six HCV-infected patients versus six healthy participants. Quantitative PCR were performed in triplicate with SNORD61 as internal control. [Colour figure can be viewed at wileyonlinelibrary.com]

Regulation of miRNA expression occurs both transcriptionally and post-transcriptionally.[36] Although transcriptional repression by STAT-3 may contribute to the inhibition of miR-124 expression, we hypothesized that post-transcriptional regulation might also be involved in the decline of miR-124 that was observed in myeloid cells from HCV infection. Indeed, it has been shown that the 3′ to 5′ exoribonuclease ERI-1 may directly degrade precursor or mature miRNAs through its ribonuclease activity. Both natural killer and T cells deficient in ERI-1 display a global, sequence-independent increase in miRNA abundance.[37] To investigate whether ERI-1 might be involved in the down-regulation of miR-124 levels in myeloid cells of HCV-infected patients, we determined the protein levels of ERI-1 in $CD33^+$ myeloid cells from HCV-infected individuals and age-matched healthy participants. As shown in Fig. 2(c), ERI-1 was significantly up-regulated in $CD33^+$ myeloid cells from chronically HCV-infected patients compared with healthy participants. The siRNA-mediated silencing of ERI-1 expression significantly reduced its levels in $CD33^+$ myeloid cells from HCV-infected patients (Fig. 2d, left panel). However, silencing of ERI-1 expression only moderately increased the level of miR-124 in myeloid cells from HCV-infected patients (Fig. 2d, right panel; $P = 0{\cdot}0572$). Taken together, these results suggest that overexpression of STAT-3 plays a major role in the down-regulation of miR-124 expression in myeloid cells during HCV infection; whereas ERI-1 expression may also contribute, to a lesser extent, to the down-regulation of miR-124 expression.

HCV-induced decline in miR-124 expression regulates inhibitory molecules in myeloid cells and promotes $Foxp3^+$ Treg cell expansion during viral infection

We have recently shown that HCV induces the expansion of MDSCs that express high levels of IL-10, TGF-β and pSTAT-3.[19] We next examined whether the HCV-induced decline in miR-124 or miR-29a expression plays a role in up-regulation of these proteins. Because of low transfection efficiency in human myeloid cells, we used the Amaxa's Nucleofector System to transfect GFP and control vectors, and achieved a transfection efficiency of up to 60% in $CD33^+$ myeloid cells (data not shown). When we transfected a miR-124 mimic in $CD33^+$ myeloid cells, miR-124 expression was increased several fold over the negative control transfection (Fig. 3a). To investigate whether the decline in miR-124 expression is involved in the expansion and suppressive functions of myeloid cells

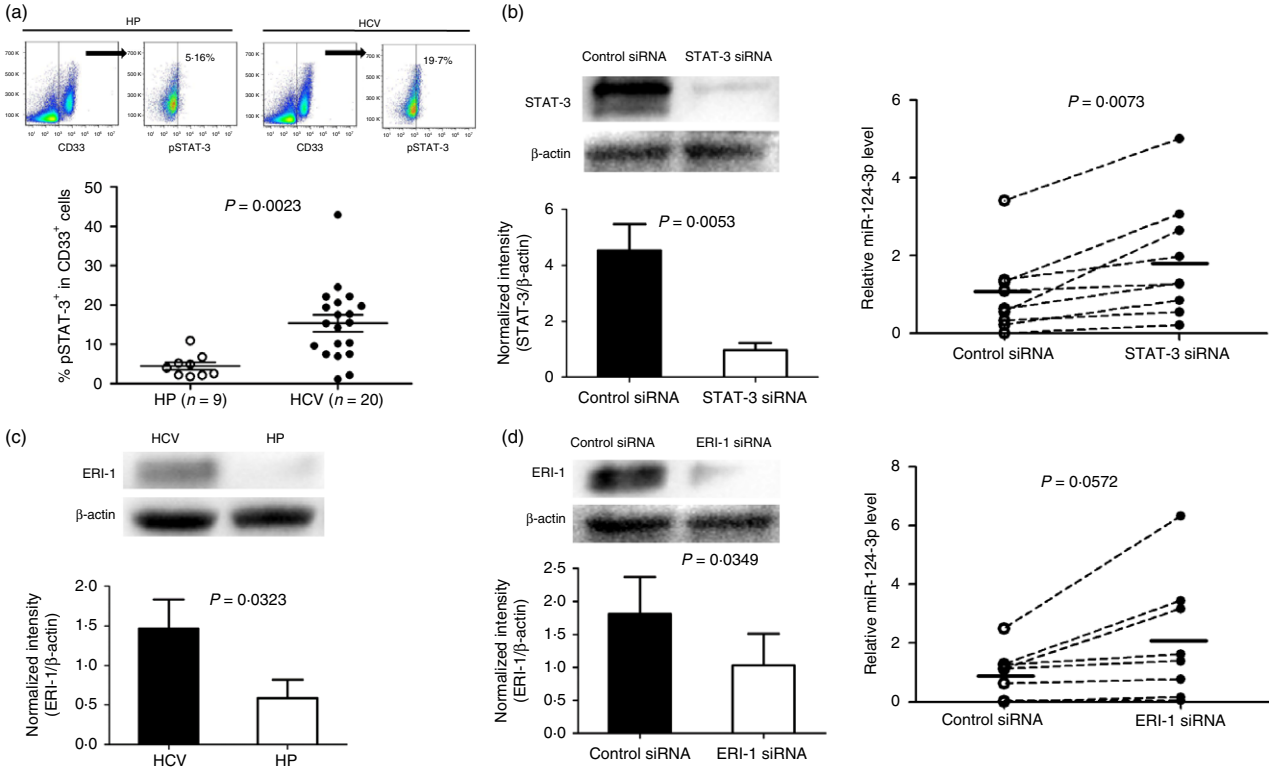

Figure 2. Characterization of the mechanisms leading to the decline of miR-124 in myeloid cells of hepatitis C virus (HCV) -infected patients. (a) Representative dot plots analysed by flow cytometry and summary data for the expression of phosphorylated signal transducer and activator of transcription 3 (pSTAT-3) in $CD33^+$ myeloid cells from peripheral blood of 20 HCV-infected patients versus nine healthy participants (HP). (b) Left panel: Representative Western blot imaging of STAT-3 expression in myeloid cells transfected by STAT-3 small interfering RNA (siRNA) or control siRNA, and summary data of normalized STAT-3/β-actin intensity in myeloid cells from seven HCV-infected patients. Right panel: summary data for miR-124 expression in nine HCV myeloid cells transfected with STAT-3 siRNA or control siRNA. (c) Representative Western blot imaging of ERI-1 expression and summary data of normalized ERI-1/β-actin intensity in myeloid cells isolated from eight HCV-infected patients versus eight healthy participants (HP). (d) Left panel: Representative Western blot imaging of ERI-1 expression in myeloid cells transfected by ERI-1 siRNA or control siRNA and summary data of normalized ERI-1/β-actin intensity in myeloid cells from five HCV-infected patients. Right panel: summary data for miR-124 expression in eight HCV myeloid cells transfected with ERI-1 siRNA or control siRNA. The *P*-values of the statistical analysis for the experiments are shown above on each panel. [Colour figure can be viewed at wileyonlinelibrary.com]

in the setting of HCV infection, we transfected $CD33^+$ myeloid cells derived from HCV-infected patients with miR-124 mimic or negative control mimic, and stimulated the cells with LPS/R848, followed by flow cytometric analysis of MDSC frequencies and IL-10, TGF-β or pSTAT-3 expression. Phenotypically, short-term transfection of HCV $CD33^+$ myeloid cells with either miR-124 mimic or miR-29a mimic did not alter the differentiation or maturation of myeloid cells, as demonstrated by equal expression levels of the HLA-DR marker on these cells (data not shown). Functionally, however, the elevated levels of IL-10, TGF-β, STAT-3 and pSTAT-3 proteins in MDSCs observed during chronic HCV infection[19] were reduced when cells were transfected with the miR-124 mimic (Fig. 3b–e) but not the miR-29a mimic (data not shown). These results suggest that miR-124, but not miR-29a, is involved in the regulation of pSTAT-3, IL-10 and TGF-β expressions in MDSCs during HCV infection.

We have recently shown that expansion of MDSCs contributed to the generation or maintenance of Treg cells during HCV infection.[19] Depletion of $CD33^+$ myeloid cells from PBMCs significantly reduced the Treg cell numbers that were developed during chronic HCV infection.[19] We further investigated whether the inhibition of miR-124 expression in myeloid cells affects the development of Treg cells in the setting of HCV infection. To this end, $CD33^+$ myeloid cells were transfected with miR-124 mimic or negative control and then co-cultured with healthy $CD4^+$ T cells in the presence of T-cell receptor stimulation ligands for 5 days. As shown in Fig. 3(f), flow cytometric analysis showed a marked decrease in the differentiation of $CD4^+$ $CD25^+$ $Foxp3^+$ Treg cells when myeloid cells from HCV-infected patients that were transfected with the miR-124 mimic were incubated with healthy $CD4^+$ T cells compared with those transfected with the negative control. Taken together, our results

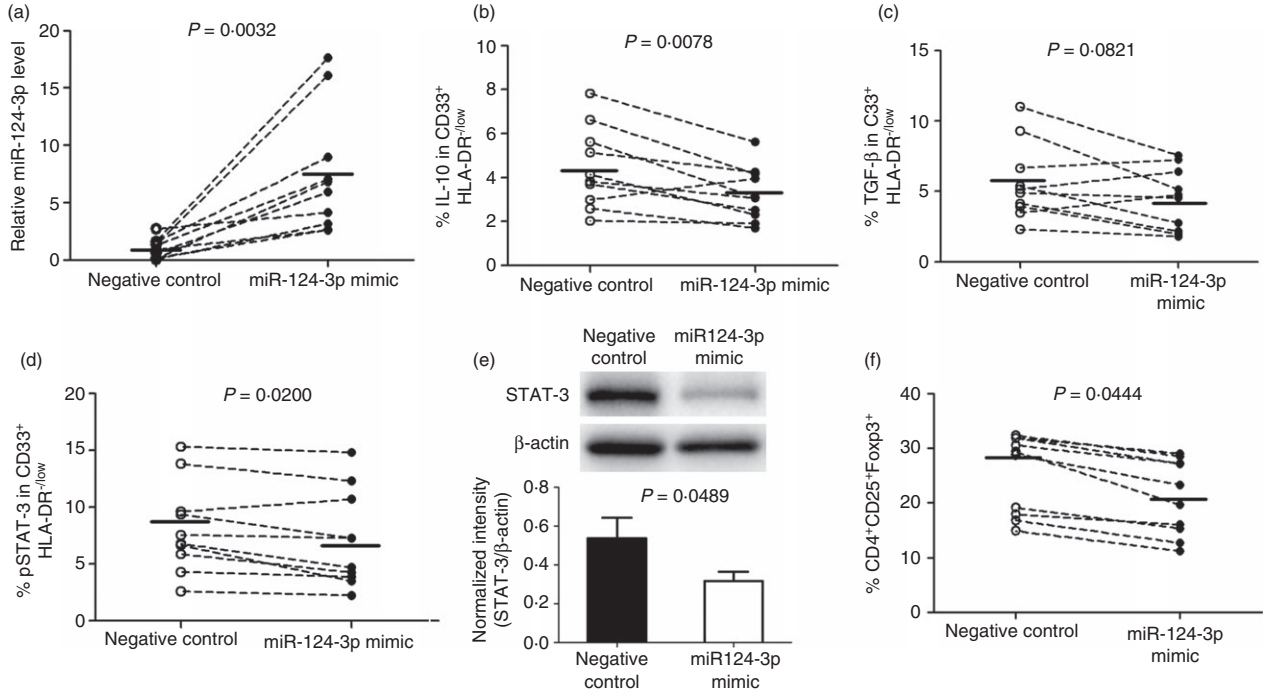

Figure 3. Reconstitution of miR-124 restores the dysregulated mediators in myeloid cells for regulatory T (Treg) cell differentiation. (a) MicroRNA (miRNA) transfection efficiency. Real-time PCR analysis of miR-124 expression in $CD33^+$ myeloid cells from 10 hepatitis C virus (HCV) -infected patients transfected with miR-124 mimic or negative control. Quantitative PCR were performed in triplicate with SNORD61 as internal control. (b–d) Functional analysis of myeloid-derived suppressor cells (MDSCs) following miR-124 transfection. $CD33^+$ myeloid cells isolated from HCV-infected patients were transfected with miR-124 mimic or negative control for 24 hr, stimulated with Toll-like receptor ligand lipopolysaccharide (LPS)/R848 for 6 hr, and then immune-stained for interleukin-10 (IL-10), transforming growth factor-β (TGF-β) and phosphorylated signal transducer and activator of transcription 3 (pSTAT-3) expression in $CD33^+$ $HLA\text{-}DR^{-/low}$ cells, followed by flow cytometry analysis. Results are shown by repeated experiments using cells from 10 HCV-infected patients. (e) Representative Western blot imaging of total STAT-3 protein expression, and summary data of normalized STAT-3/β-actin intensity in myeloid cells isolated from five HCV-infected patients following transfection by miR124 mimic or negative control for 48 hr. (f) miRNA-regulated MDSCs control $Foxp3^+$ Treg cell development. $CD33^+$ cells isolated from HCV-infected patients were transfected with miR-124 mimic or negative control and then co-cultured with healthy $CD4^+$ T cells in the presence of TCR stimulation for 5 days, $CD4^+$ $CD25^+$ $Foxp3^+$ Treg cell frequencies were analysed and results from 10 participants were shown. *P* values are shown in each figure.

suggest that the decline in miR-124 expression in myeloid cells is involved in the regulation of STAT-3 phosphorylation and the production of suppressive cytokines in MDSCs during HCV infection, which leads to Treg cell development.

Discussion

The MDSCs in tumour progression and antitumour responses have been well-studied; however, the significance of MDSCs in viral persistence is less well understood. Although dysregulated myelopoiesis by inflammatory cytokines such as IL-6, IL-10 and TGF-β may partially explain the expansion of MDSCs that exert suppressive activities during viral infection, the underlying mechanism for this inflammation-driven development and expansion of MDSCs during HCV infection remains elusive. Whether miRNAs are involved in the aberrant myelopoiesis and their suppressive activities during chronic viral infection remains unclear. Previous studies have shown that HCV core-treated $CD33^+$ myeloid cells exhibit a $CD14^+$ $CD11b^+$ $HLA\text{-}DR^{-/low}$ phenotype, similar to MDSCs, with up-regulation of $p47^{phox}$, a component of the NOX2 complex, which is critical for ROS production.[26] In addition, STAT-3 has been shown to directly regulate the production of immunosuppressive molecules, such as IL-6, IL-10, IL-4Rα and $p47^{phox}$.[38–40] This study reveals that during HCV infection: (i) miR-124 is down-regulated in myeloid cells during HCV infection; (ii) HCV-induced overexpression of STAT-3 plays a major role in the decline of miR-124 in myeloid cells; and (iii) the decline of miR-124 results in up-regulation pSTAT-3, IL-10 and TGF-β expression in myeloid cells, which in turn promotes T-cell differentiation including $Foxp3^+$ Treg cell expansion. Hence, this study provides evidence of the underlying mechanism of the MDSC-mediated immune suppression observed in HCV-infected patients.

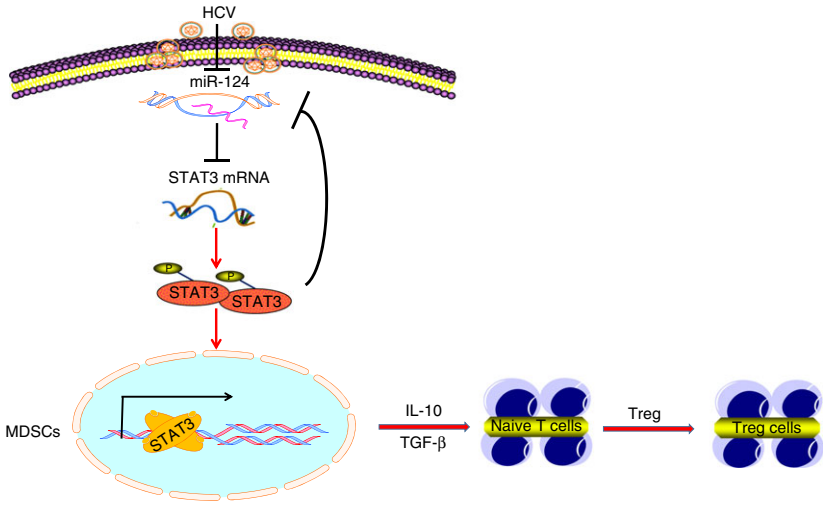

Figure 4. Schematic model for hepatitis C virus (HCV) -induced myeloid-derived suppressor cell (MDSC) control regulatory T (Treg) cell differentiation via microRNA (miRNA) -mediated signal transducer and activator of transcription 3 (STAT-3) signalling. HCV infection deregulates miR-124 expression in MDSCs, which in turn, alters STAT-3 and regulatory cytokine [interleukin-10 (IL-10) and transforming growth factor-β (TGF-β)] expression. Interestingly, STAT-3 can reciprocally regulate miR-124 expression in myeloid cells. The reciprocal regulation of miR-124 and STAT-3 in MDSCs promotes Foxp3$^+$ Treg cell development. Therefore, reconstitution of miR-124 and/or inhibition of STAT-3 may provide a novel approach for HCV immunotherapy. [Colour figure can be viewed at wileyonlinelibrary.com]

Growing evidence implicates miRNAs in myeloid cell biology, including lineage development and suppressive functions.[30] For example, miR-21, miR-29a and miR-196b have been shown to induce myeloid progenitor expansion, whereas miR-223 has been shown to promote granulopoiesis, and miR-17, miR-20a and miR-106a have been implicated in blast proliferation and inhibit monocytic differentiation and maturation of human CD34$^+$ haematopoietic progenitor cells.[41–44] Given that dysregulated myeloid differentiation and maturation processes result in MDSC generation and expansion, it is possible that aberrant expression of miRNAs contributes to MDSC expansion under pathological conditions.[4,5] Although we have shown a decline in miR-124 expression and overexpression of pSTAT-3 in MDSCs derived from HCV-infected patients, little is known about the factors that regulate miR-124 and/or STAT-3 expression in MDSCs. MicroRNA biogenesis is regulated at the transcriptional level by cell-specific transcription factors and/or at the post-transcriptional level by proteins that control miRNA degradation; notably, these two regulatory processes can be affected by inflammatory signals derived from infection or cancer.[4,5] Indeed, it has been reported that HCV core protein suppresses miR-124 by way of induction of DNMT1, and overexpression of miR-124 suppressed cholangiocarcinoma cell migration and invasion by targeting SMYD3 as well as c-Myc and MMP9.[45] Our results showed that miR-124 was down-regulated, whereas phosphorylated (active) STAT-3 was up-regulated in MDSCs during chronic HCV infection, suggesting that they may negatively regulate each other in myeloid cells during HCV infection. Indeed, reconstitution of miR-124 in myeloid cells reduced the elevated levels of pSTAT-3 and total STAT-3 protein expression, which in turn reduced IL-10 and TGF-β expression in CD33$^+$ MDSCs, and subsequently enhanced CD4 T-cell differentiation into Treg cells. Based on these findings, we propose a model (depicted in Fig. 4) that represents a novel mechanism by which HCV modulates the suppressive activities of MDSCs through an miR-124-regulated STAT-3 pathway during chronic viral infection. These findings show a reciprocal relationship between miR-124 and STAT-3 regulation in controlling CD33$^+$ MDSC expansion and activities and suggest that miR-124 and/or STAT-3 inhibition may provide therapeutic tools for infection-associated expansion of MDSCs and Treg cells during chronic viral infection.

In summary, this study shows, for the first time, that HCV-induced MDSCs control T-cell differentiation through the miR-124-regulated STAT-3 pathway, which sheds new light on the features of MDSC biology and provides a novel mechanism for the suppression of T-cell functions during chronic HCV infection. Hence, targeting miR-124 and/or STAT3 to correct the aberrant MDSC and/or Treg cell expansion may be a promising strategy for immunotherapy to treat human viral diseases.

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Disclosures

The authors declare no competing interest of this work.

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