Hematopoietic Stem Cells Modulate Macrophage Inflammation Phenotype through MicroRNA-126-dependent Signaling

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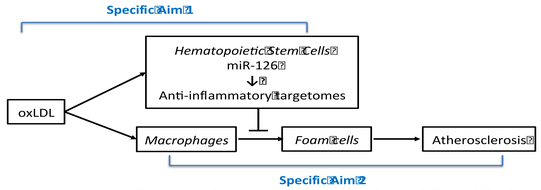
ABSTRACT

Atherosclerosis is a maladaptive inflammatory response from prolonged subendothelial retention of monocyte-derived cells, which differentiate into mononuclear phagocytes that absorb and accumulate lipoproteins to become lipid-laden "foam cells". A potential atherosclerosis therapy currently studied includes hematopoietic stem cells (HSCs), possessing multi-lineage differentiation.



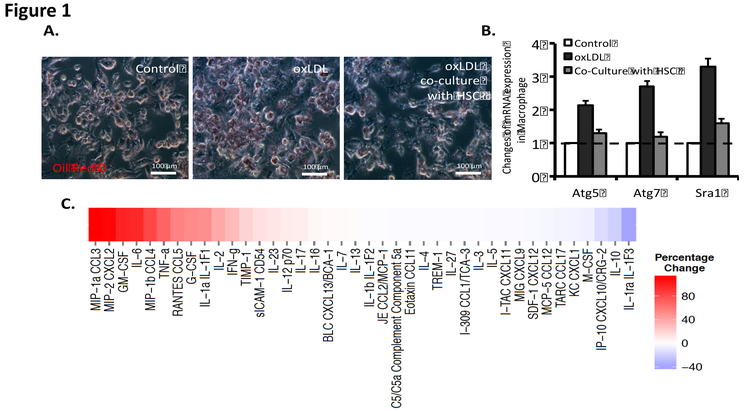
In our experimentation, treating macrophages with oxidized LDL increases *pro-inflammatory* cytokines and their gene expression. In contrast, treating HCSs with oxLDL increases *anti-inflammatory* responses. Co-culture of macrophages with HSCs reduces the ox-LDL-induced foam cell formation. Knockout of miR-126, an HSC regulator and our molecular of interest, abolishes this anti-inflammatory response. To further investigate miR-126 function and its targeted genes, an RNA-seq and RNA differential expression analysis were conducted. Strong evidence suggests a novel potential target gene, SOCS3, that affects PI3k pathways to regulate inflammation. Future work is to elucidate the regulatory mechanism of miR-126, SOCS3 and PI3K as well as analyzing how miR-126 affects the cellular and biochemical composition of mouse blood.

RESULTS

HSCs reduce the oxLDL-induced foam cells formation.

To elucidate how HSCs regulate foam cell formation from macrophages, we treated the mouse macrophage, RAW264, with oxLDL to induce foam cells. Our results showed that oxLDL increased lipid accumulation (Fig. 1A) and macrophage activation-related marker gene expressions. These include autophagy proteins 5 and 7 (Atg5, and Atg7), which control the cholesterol efflux and macrophage scavenger receptor A1 (Sra1) (Fig. 1B). Co-culture of macrophages with HSCs (Figs. 1A and 1B) attenuated such ox-LDL-induced changes. The cytokine array data in Fig. 1C further confirm that oxLDL induces macrophages pro-inflammatory cytokines (e.g., IL-1a and IL-6) and decreased anti-inflammatory cytokines (e.g., IL-10 and IL-1ra).

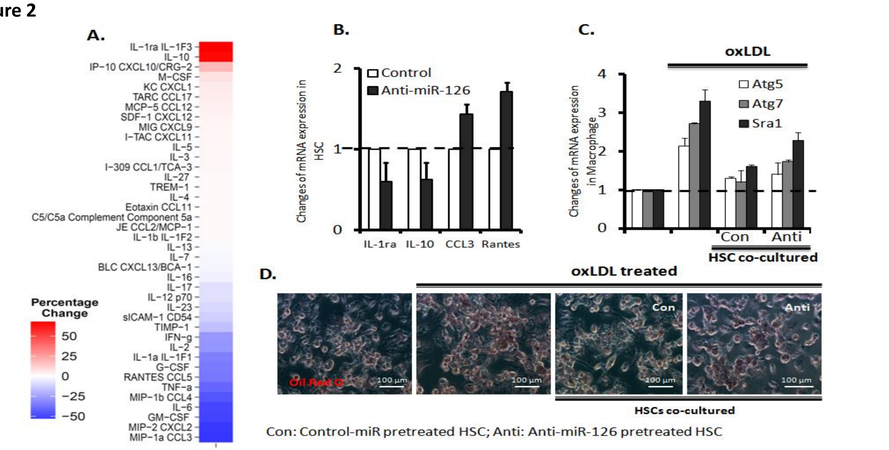
Figure 1



Pretreatment of HSCs with anti-miR-126 resulted in the increases of pro-inflammatory cytokines in HSCs.

In contrast to macrophages, treating HSCs with oxLDL decreased pro-inflammatory cytokines and increased anti-inflammatory cytokines (Fig. 2A). These findings suggest that HSCs function as the immune modulator for macrophage inflammatory cascade through anti-cytokine secretions (Fig. 1). Since miR-126 is a known modulator of vascular function (M. Han, J Zhou), we investigated its role in the HSC modulation of macrophages. MiR-126 knockdown by anti-miR-126 transfection increased pro-inflammatory cytokines (Fig. 2B) in HSCs. We then studied the effects of co-culturing the anti-miR-126-HSCs on oxLDL-treated macrophages. MiR-126 knockdown attenuated the HSCs' anti-foam cell formation functions by increasing the Agt5, Agt7 and Sra1 expressions (Fig. 2C) to enhance lipid accumulation (Fig. 2D).

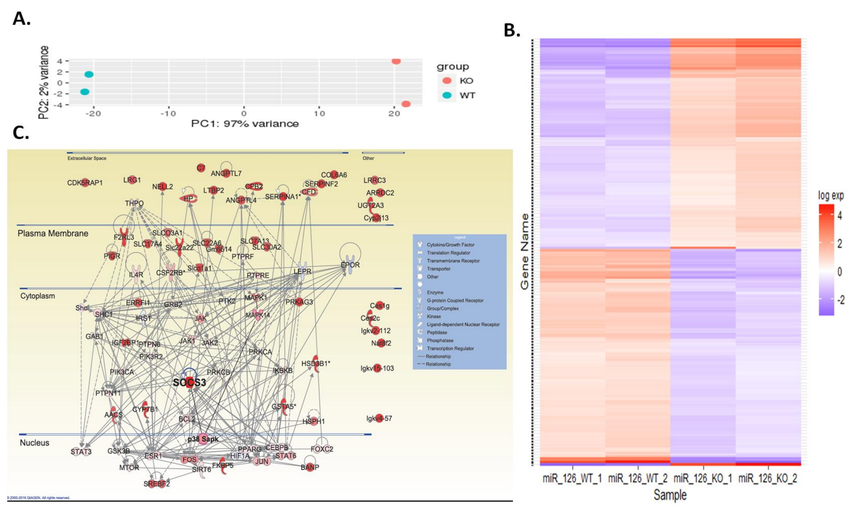
Figure 2



Con: Control-miR pretreated HSC; Anti: Anti-miR-126 pretreated HSC

RNA-seq and differential expression analysis for miR-126 target genes related to inflammation

Figure 3



To further understand the regulatory mechanism and target genes of miR-126, the RNA-seq experiment and differential expression analysis were performed using the bone marrow cells isolated from miR-126 knockout (KO) or control (Ctr) mice. We found that the genetic variance KO and Ctr groups is significant (Fig. 3A). 299 genes are differentially expressed; among which 150 are upregulated and 149 are downregulated in miR-126 KO (Fig. 3B). We used the IPA Pathway Designer Connect tool to summarize all known relationships between the transcriptional regulators and inflammatory factors. with the highlights of statistically significant enrichment of SOCS3 signaling genes (JAK, p38 and GSK). These results suggest that miR-126 KO impairs the immune regulatory function of bone marrow cells, thus contributing to an atherosclerosis phenotype through SOCS3-dependent signaling (Fig. 3C).

METHODS

I. Cell isolation and culture expansion
Mononuclear cells isolated from the mouse bone marrow were used for HSC purification with negative immuno-selection and limiting dilution
Purified mouse HSCs were cultured in RPMI 1640 Medium with 10% FBS, pen/strep, and L-glutamine
Mouse monocytes/macrophages (RAW264.7) were cultured in DMEM with 10% FBS, pen/strep, and L-glutamine

II. HSC/macrophage co-culture and mono-culture experiments
HSCs were cultured w/wo macrophages and treated w/wo oxLDL for 24 hr.
The RNA samples were isolated for gene profiling experiments and qPCR confirmation.
The identified genes were studied to validate the roles of miR-126 in mediating the HSC rescue function on macrophage-foam cell transformation.

III. Profiling of cytokine production by ELISArray
HSCs were pretreated with pre-miR126 or anti-miR126, followed by incubation with oxLDL for 24 hours.
Mouse cytokine Multi-Analyte ELISArray Kit (SABiosciences) was used to measure the cytokine production of IL1A, IL1B, IL2, IL4, IL5, IL6, IL8, IL10, IL12, IL13, IL17A and Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF)
The absorbance levels of the cytokines were determined on a plate reader (Beckman Coulter DTX 880) at 450 nm.

CONCLUSION

- HSCs exhibit anti-inflammatory and anti-activation functions in oxLDL-treated macrophages and inhibit foam cell transformation.
- Inhibition of miR-126 decreases the secretion of atheroprotective cytokines in HSCs.
- HSC inhibits foam cell formation to cause atheroprotection through miR-126-dependent signaling.
- Deletion of miR-126 compromises the immunomodulatory functions of bone marrow cells and contributes to the inflammatory phenotype in a mouse model.
- The present study points to future directions of research on the roles of miR-126 in regulating the immune modulatory functions of HSCs and on the detailed regulatory mechanisms of miR-126, SOCS3 and other potential targets in HSCs.