Biochemistry and Biophysics Reports 3 (2015) 202–206

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

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

Micro-RNAs and macrophage diversity in atherosclerosis: New players, new challenges…new opportunities for therapeutic intervention?

Guillermo Vazquez*

Department of Physiology and Pharmacology, Center for Diabetes and Endocrine Research, Center for Hypertension and Personalized Medicine, University of Toledo College of Medicine and Life Sciences, Health Science Campus, 3000 Transverse Drive, Toledo, OH 43614, USA

ARTICLE INFO

Article history:
Received 12 June 2015
Received in revised form
21 July 2015
Accepted 12 August 2015
Available online 13 August 2015

Keywords:
Macrophage diversity
Micro-RNAs
Atherosclerosis

ABSTRACT

Efforts in experimental therapeutics of atherosclerosis are mostly focused on identifying candidate targets that can be exploited in developing new strategies to reduce plaque progression, induce its regression and/or improve stability of advanced lesions. Plaque macrophages are central players in all these processes, and consequently a significant amount of research is devoted to understanding mechanisms that regulate, for instance, macrophage apoptosis, necrosis or migration. Macrophage diversity is a key feature of the macrophage population in the plaque and can impact many aspects of lesion development. Thus, searching for molecular entities that contribute to atherorelevant functions of a specific macrophage type but not others may lead to identification of targets that can be exploited in phenotype selective modulation of the lesional macrophage. This however, remains an unmet goal. In recent years several studies have revealed critical functions of micro-RNAs (miRs) in mechanisms of macrophage polarization, and a number of miRs have emerged as being specific of distinctive macrophage subsets. Not only can these miRs represent the first step towards recognition of phenotype specific targets, but they may also pave the way to reveal novel atherorelevant pathways within macrophage subsets. This article discusses some of these recent findings, speculates on their potential relevance to atherosclerosis and elaborates on the prospective use of miRs to affect the function of plaque macrophages in a phenotype selective manner.

Contents

1. Introduction

Atherosclerosis, the major cause of coronary artery disease, is a chronic arterial disease dominated by a maladaptive inflammatory response [1,2]. Clinical manifestations of atherosclerosis, which directly relate to the molecular and cellular features of the plaque, go from ischemic symptoms due to lesions causing critical arterial stenosis, to acute thromboembolic events that follow plaque rupture [3,4]. The development of atherosclerotic lesions implies a complex intermingling among endothelial and smooth muscle cells, macrophages, and cytokines and inflammatory mediators that lodge in the lesion setting. From a therapeutic perspective, desirable goals in atherosclerosis include reducing plaque progression, improving stability of advanced lesions and/or inducing

* Correspondence to: 3000 Transverse Drive, UTHSC Mail stop 1008, Toledo, OH 43614 USA. Fax: +419 383 2871.
E-mail address: Guillermo.Vazquez@utoledo.edu

http://dx.doi.org/10.1016/j.bbrep.2015.08.009

plaque regression. Plaque macrophages are major protagonists in all these processes. For instance, the balance between accumulation of apoptotic macrophages and their clearance from the lesion through efferocytosis modulates cellularity of early lesions and necrosis and stability of advanced plaques [5,6], and macrophage egress from the lesion is a major determinant of plaque regression [7,8]. Despite the progress made in identifying mechanisms associated to these processes, the potential applicability to human pathology of strategies aimed at manipulating macrophage apoptosis and/or migration is still a matter of debate. An often ignored aspect is the fact that the macrophage population that inhabits in the atherosclerotic lesion is composed of phenotypically and functionally distinct subsets. Although in recent years this concept has to some extent re-gained attention in the field, experimental work examining distinct effects of individual macrophage subsets in plaque composition, progression and stability appears sporadically in the literature and with little or no therapeutic perspective [9,10]. Acknowledging the distinctive impact of macrophage diversity on plaque characteristics is of most importance, as it may lead to some definitions to develop alternative therapeutic strategies. The identification of molecular entities that specifically contribute to atherorelevant functions of a particular macrophage type but not others remains an as yet unmet goal. Filling this gap of knowledge may drive drug development toward targets to modulate detrimental phenotype selective functions while minimally interfering with those of the desirable types.

Evidence accumulated in recent years revealed key roles of micro-RNAs (miRs) in macrophage functions that are of relevance to atherosclerosis, in particular in the regulation of components of the reverse cholesterol transport system (RCT) [11,12]. Also, a number of recent reports, although not aimed at studying atherosclerosis, underscored critical functions of miRs in mechanisms of macrophage polarization (see Section 3 below). This is of upmost importance, as phenotype selective miRs may lead the way towards identification of phenotype specific targets, or to becoming target candidates themselves. Here I discuss some recent salient findings regarding miRs and macrophage diversity, and speculate on their potential relevance to atherosclerosis and the prospective use of these miRs to manipulate plaque macrophage functions in a phenotype selective manner.

2. Macrophage diversity in atherosclerosis

The presence of distinctive macrophage subsets in atherosclerotic lesions of humans and animal models of the disease is well documented [13–15]. In brief, the M1 or inflammatory, or classically activated, and the M2 or anti-inflammatory, or alternatively activated types, dominate in atherosclerosis [9,10,16]. *In vitro*, naïve macrophages can be induced to differentiate to the M1 type with interferon γ (IFNγ) and/or lipopolysaccharide (LPS), whereas interleukins 4 or 13 (IL4, IL13) promote M2 differentiation [15,17]. Macrophages grown in granulocyte-macrophage colony stimulating factor-1 (GM-CSF1) or in macrophage colony stimulating factor-1 (M-CSF1) are sometimes regarded as, respectively, M1 or M2, but there is insufficient evidence to link these phenotypes to GM-CSF1- or M-CSF1-derived cells (see also [18]). Unlike the more controlled *in vitro* setting however, assigning phenotypes to plaque macrophages, which are exposed to a myriad of stimuli of varying proportions, is not trivial. Indeed, evidence for fully divergent phenotypes in atherosclerotic plaques is still lacking. The study of the transcriptome of plaque macrophages has advanced enormously with the use of highly sensitive protocols for quantitative real-time polymerase chain reaction (qRT-PCR) that can be applied to macrophages isolated from lesions by laser capture microdissection (LCM) [19,20]. As this approach spreads, we should expect better comparisons between the marker signature of *in vitro* M1 and M2 macrophages and that of LCM captured lesional cells. This can also provide a robust profiling of macrophage markers somewhat reflective of the perturbations these cells encountered *in vivo*, something difficult, if not impossible to recapitulate *in vitro*.

During atherosclerosis the relative abundance of M1 and M2 macrophages (M1/M2 ratio) varies with lesion stage and in general increases as the lesion progresses. But these types co-exist throughout plaque development, intermediate phenotypes exist and phenotype inter-conversion can occur [15,16]. The M2 macrophages play a key role in regression of atherosclerosis. Indeed, plaque regression is often accompanied by a decrease in M1/M2 ratios [21,22]. Augmented macrophage egress from advanced plaques with concomitant enrichment in M2 cells was found to correlate with plaque regression in atherosclerotic mice subjected to lipid lowering interventions [21]. Notably, endoplasmic reticulum (ER) stress, a major pro-apoptotic mechanism in lesional macrophages [23,24], is required for differentiation of M2 macrophages, to the extent that suppression of ER stress shifts M2's towards the M1 phenotype [25]. Intuitively, this suggests that interventions targeting components of the unfolded protein response (UPR) as a strategy to reduce macrophage apoptosis but with no discrimination of phenotypes, may favor M2-to-M1 conversion, with a subsequent negative effect on plaque characteristics. Hence, manipulation of macrophage apoptosis and/or egress from plaques in a phenotype selective manner may be an attractive approach to improve stability and/or favor regression of established lesions. Molecular elements that contribute to these processes in a subset selective fashion have not been systematically investigated. Recent work from our group shows that in M1, but not M2 macrophages, genetic or pharmacological inhibition of the calcium-permeable cation channel Transient Receptor Potential Canonical 3 (TRPC3) impairs activation of the UPR and reduces ER stress-induced apoptosis, despite this channel being expressed and functional in both macrophage subsets [26,27]. Although awaiting *in vivo* validation, these findings reveal TRPC3 as a molecular element whose function selectively affects a specific macrophage type, and suggest that targeting TRPC3, rather than its downstream effectors, may be a way to modulate macrophages in a phenotype selective manner. This brings about an important new concept, *i.e.*, *modulating* macrophage functions in a phenotype selective way, rather than *targeting* macrophages in a phenotype selective manner. The latter implies the targeting of molecules uniquely expressed in, for instance, M1 cells but not in the M2's. This is currently unattainable as most signaling molecules and phenotypic markers are to some extent expressed in all macrophage types. In contrary, affecting macrophage functions in a phenotype selective manner refers to the recognition of molecules that, as seems to be the case for the pro-apoptotic role of TRPC3 in M1 macrophages, serve to a specific function in one macrophage type but not in the other, regardless of such molecules being expressed in both subsets.

TRPC3 is ubiquitously expressed [28] and this may, *a priori*, be envisaged as a limitation for selective targeting. In fact, this is a concern with most signaling molecules so far identified in macrophages as of potential therapeutic use in atherosclerosis. Importantly, macrophage-targeted drug delivery is now possible through nanoparticle delivery systems [29,30], and this has been shown to represent a viable approach to target plaque macrophages [31].

3. Micro-RNAs, macrophage diversity and atherosclerosis

Micro-RNAs (miRs) are a large family of small (~22 nucleotides) non-coding RNA molecules that regulate gene expression

post-transcriptionally by binding to the 3'-untranslated region (3'UTR) of target mRNAs and repress their expression [32].

There is compelling evidence supporting critical roles of miRs in regulation of RCT [11,12,33,34]. An important discovery was the demonstration that short-term antagonism of miR-33, a potent repressor of ATP-binding cassette transporter-1 (ABCA1) in mice with established atherosclerosis promotes plaque regression and improves plaque stability [35]. More recently however, studies of long-term inhibition of miR-33 in non-atherosclerotic mice showed deleterious effects such as mild hepatic steatosis and hypertriglyceridemia, raising concerns on the therapeutic potential of miR-33 in atherosclerosis [36]. Despite this, the studies on miR-33 in mouse models of atherosclerosis demonstrated that manipulation of miRs in plaque macrophages is feasible and provided proof of principle on the potential therapeutic use of anti-miRs in atherosclerosis. Unlike the well-recognized impact of miRs on RCT, their effects on mechanisms of macrophage polarization are just beginning to be appreciated. Studies using microarray analysis to examine expression profiles of miRs in bone marrow-derived macrophages from Balb/c mice showed that miR-181a, miR-155-5p, miR-204-5p, miR-451 and miR-127-3p were increased in M1 macrophages – induced with LPS plus IFNγ – compared to M2's – induced with IL4 [37]. In contrary, the expression of miR-125b-5p, miR-146a-3p, miR-143-3p and miR-145-5p was higher in M2 compared to the M1 macrophages [37]. This was confirmed by qRT-PCR, and analysis of their potential biological relevance by functional categorization showed association of miR-181a, miR-155-5p, miR-204-5p, and miR-146a-3p with apoptosis. Potential targets for these miRs were however not explored, and thus it remains to be determined whether upregulation of any of these miRs truly affects apoptosis of M1 or M2 cells.

Studies in bone marrow-derived macrophages from C57BL/6 mice grown in GM-CSF1 or M-CSF1, revealed interesting aspects of the biological relevance of miR-125a-5p in M2 macrophages [38]. In these cells stimulation of Toll-like receptors (TLR) 2 and 4, but not TLR3, favored expression of miR-125a-5p. Of importance, miR-125b-5p, which has the same seed sequence as miR-125a-5p, was downregulated by TLR4 stimulation [38], probably illustrating the fact that sequence context outside the seed region also affects the binding of miRs to their targets and their expression [39]. Notably, in macrophages overexpressing miR-125a-5p, LPS treatment favored M2 differentiation, whereas knockdown of miR-125a-5p promoted M1 differentiation [38]. TLR2 and TLR4 are key in mechanisms of ER stress-induced apoptosis in macrophages [40]. Hence, it is possible that upregulation of miR-125a-5p under conditions of ER stress contributes to modulation of downstream apoptotic pathways. Considering the strong effect of miR-125a-5p in suppressing the M1 phenotype while favoring M2 activation, and the permissive effect of ER stress on differentiation of M2 macrophages, the question raises whether miR-125a-5p modulates mechanisms that drive the M2 phenotype when macrophages undergo ER stress. *In vivo* studies examining the impact of miR-125a-5p mimics on plaque necrosis and regression and how these correlate with M1/M2 ratios may bring answers to this question. Findings on the expression of miR-125a-5p in polarized human macrophages contrast with those in mice. Eigsti et al. [41] found that miR-125a-5p was downregulated by both interferons (M1 inducers) and IL4 (M2 inducer) during the transition monocyte-to-macrophage. Accumulation of miR-146a and miR-155 was increased by TLR activation whereas that of miR-193b and miR-222 was induced by IL4 [41]. Although species differences – mouse in [38] *vs.* human in [41] – may account for differential expression of a particular miR, these findings also suggest that the miR signature of monocytes is finely tuned during differentiation to macrophages and strongly influenced by environmental cues.

It has been found that early lesions in Apoe knockout mice have high levels of the macrophage-derived miR-342-5p and miR-155 [42]. MiR-342-5p targets the 3'UTR of Akt1, leading to augmented production of inflammatory mediators by macrophages through a mechanisms that also involves upregulation of miR-155. This correlated with lesion progression, and local or systemic administration of an antagomir against miR-342-5p reduced plaque growth [42]. The phosphatidylinositol-3-kinase (PI3K)/AKT axis is key to macrophage survival [43,44] and also seems required for proper migration signaling downstream the chemokine receptor CCR7 [45]. Hence, the beneficial impact of miR-342-5p antagonism in early lesions may to some extent be related to increased macrophage survival and enhanced migration of surviving cells. Plaque size reduction in the face of increased macrophage survival seems difficult to reconcile with the idea that reduced macrophage apoptosis in early plaques leads to increased cellularity [6,46]. However, we and others [17,26] have shown that in early lesions of Apoe knockout mice M1 macrophages make up for a small portion of the total macrophage population. Therefore, it is conceivable that in early stages the benefit of higher migration capacity of macrophages with reduced expression of miR-342-5p prevails over any detrimental effect due to increased survival. Three studies reported upregulation of miR-155 in M1 macrophages [37,41,42]. Interestingly, miR-155 was found to be upregulated in aortic plaques of Apoe knockout mice fed a high fat diet and in carotid plaques of Apoe knockout mice with partial carotid artery ligation [47]. In the latter model, hematopoietic deficiency of miR-155 results in reduced plaque burden and cellularity [47]. This is in part due to loss of miR-155-mediated repression of B-cell leukemia/lymphoma 6 (BCL6), a transcription factor that attenuates Nuclear Factor kappa B pro-inflammatory signaling [47]. The role of miR-155 in atherosclerosis was further investigated in recent studies from the same laboratory. Using Apoe knockout mice fed an atherogenic diet for twelve and twenty four weeks to induce, respectively, development of early and advanced lesions, Wei et al. [48] examined the impact of global- and hematopoietic-deficiency of miR-155 on the characteristics of atherosclerotic lesions. Lack of miR-155 resulted in early plaques of bigger size and cellularity compared to control animals [48]. Notably, CSF1-dependent macrophage proliferation *in vitro* was markedly reduced by miR-155, suggesting that the increased in lesion volume and cellularity in the miR-155 knockout mice was related to the impact of this miR on macrophage proliferation. Contrarily, advanced plaques of Apoe knockout mice benefited from miR-155 deficiency, as manifested by a reduction in both apoptotic cell number and areas of necrosis [48]. This is part explained by an inhibitory action of miR-155 on BCL6-dependent regulation of efferocytosis, which is critical in preventing necrotic core growth in advanced plaques [6,49]. Altogether, these studies illustrate opposite roles of macrophage miR-155 in early *vs.* advanced atherosclerosis, highlighting the importance of longitudinal *in vivo* studies to better define the impact of a particular miR in macrophage functions in the plaque.

There are discordant findings on the effects of miR-21 in macrophage polarization. Caescu et al. [50] showed that treatment of macrophages with CSF1 upregulates miR-21 resulting in a robust anti-inflammatory effect which favors M2 differentiation [50]. This is in agreement with the concept of CSF1 receptor signaling being essential for M2 differentiation and inhibition of the M1 phenotype [51]. Wang et al. [52] found that in thioglycollate-elicited peritoneal macrophages miR-21 is downregulated by activation of a prostaglandin E_2 (PGE_2)/cyclic AMP pathway which results in enhanced expression of M2- but not M1-related genes. Accordingly, peritoneal macrophages from miR-21 deficient mice were predominantly of the M2 type [52]. A potential explanation for the discordant findings from these two studies is that a different baseline repertoire of miRs in these distinct macrophage populations – cell lines [53] *vs.* elicited peritoneal macrophages

Table 1
This table summarizes some of the most salient recent findings regarding differential expression of miRs in the M1 and/or M2 macrophage subtypes (see text for discussion). In most instances, the impact of such differential expression in atherosclerosis has not been evaluated (indicated as "n.d."); this author's speculations on potential roles in atherogenesis are indicated and followed by a question mark. When specified in the original publication, the phenotype inducing agent is indicated in parenthesis.

miR	Species	Macrophage type	Effect on atherosclerosis	Reference
181a, 155-5p, 204-5p, 451, 127-3p	Mouse	M1(LPS, IFNg) > M2(IL4)	n.d.	[37]
125b-5p, 46a-3p, 143-3p, 145-5p	Mouse	M2(IL4) > M1(LPS, IFNg)	n.d.	[37]
125a-5p	Mouse	M2(GM-CSF1/M-CSF1)	Promotion of M2 phenotype under ER stress?	[38]
146a, 155	Human	M1(LPS) > M2(IL4)	n.d.	[41]
193b, 222	Human	M2(IL4) > M1(LPS)	n.d.	[41]
155	Mouse	M1 > M2	Promotes NFkB signaling; differential effect on plaque size and cellularity (early *vs.* advanced plaques)	[37],[41],[42],[47],[48]
21	Mouse	M2(CSF1) > M1	Plaque regression?	[48]
21	Mouse	M1 > M2 (elicited peritoneal)	n.d.	[50]
223	Mouse	M2(IL4) > M1	n.d.	[53]

[52] – may provide alternative signaling platforms that differentially affect regulation of miR-21 expression. The findings discussed in this section are summarized in Table 1.

4. Novel targeting venues at the expense of added complexity: *quo vadis*?

Examination of the transcriptome of plaque macrophages by combining LCM with microarray or RNA-seq analysis, has generated valuable information on the expression signature of miRs and how these compare to the markers profile of *in vitro* generated M1 and M2 macrophages. Notably, some information on the transcriptome of plaque macrophages has found good correlation with what one would expect from *in vitro* M1 and M2 cells [21,35,54]. Comparative microarray and RNA-seq transcriptome analysis like those performed in some of the studies above, renders a high number of miRs whose expression is changed under specific experimental conditions. Validation of these changes by qRT-PCR narrows the number of candidates for further functional validation. However, most miRs have hundreds, if not thousand potential targets, and a combination of technical, time and cost limitations often leads to selection, with not little bias, of a few targets for further investigation. This approach can impose, *a priori*, significant risks with *in vivo* validation of miR mimics –agomirs – or antagonists –antagomirs. Whereas a particular miR may have a strong repressing effect on, for instance, a target whose expression is desirable in the lesion setting –*v.g.*, macrophage CCR7-, antagonizing this miR to promote advantageous pathways may concomitantly lead to upregulation of non-desirable genes. This calls for a rationale selection of targetable miRs. In this regard, it is vital to gather minimal knowledge of functional categories of additional targets whose de-repression – when antagomirs are used – or downregulation – when agomirs are used – might negatively impact the outcome of the targeting strategy. An extra level of complexity is added by the discovery that macrophage-derived microvesicles, or exosomes, contain functional miRs that can be delivered to target cells, including other macrophages and monocytes [55–57]. In this scenario, dominating miRs in a particular macrophage subset could be delivered to newly recruited monocytes or to neighbor macrophages to "instigate" specific cellular responses or to induce differentiation into a particular phenotype. For example, miR-223, which is specific of IL4-induced M2 macrophages, has been shown to be microvesicle-transferred into naïve cells and able to promote M2-like differentiation [55]. Hence, characterization of molecular components of this novel mechanism of "miR-paracrine signaling" may lead to identification of new targets to regulate macrophage diversity *in situ* in the atherosclerotic plaque. Irrespectively of the potential use of miRs as therapeutic targets, another question arises, whether macrophage subsets in the plaque could be identified on the basis of their miR expression signature. In this regard, the same considerations discussed above (see Section 2) apply. Most of the evidence on miR expression in macrophage subtypes derives from *in vitro* studies under controlled differentiating conditions, but little is known about the miR signature of plaque macrophages, whose differentiation programs are modulated by a myriad of stimuli in the plaque microenvironment. Again, better definitions should derive from the growing number of studies aimed at characterizing the transcriptome of plaque macrophages [19,20]. If anything, it would not be surprising that, similar to the situation with canonical markers of M1 and M2 macrophages (see Section 2 above), fully divergent phenotypes in plaques could not be defined based on absolute presence or absence of a particular set of miRs, and that relative proportions of specific miRs would be more informative instead.

It is obvious that we are at the embryonic stage of our understanding of the mechanisms of miR-dependent regulation of macrophage differentiation. Defining which miRs are relevant to the many macrophage functions in atherosclerosis and narrowing their targets by systematic *in vitro* and *in vivo* validation, will reveal potential therapeutic targets and hopefully novel, previously unforeseen atherorelevant pathways.

Acknowledgment

This work was supported by NIH Grant R01HL111877-04 (G.V.).

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bbrep.2015.08.009.

References

[1] P. Libby, Inflammation in atherosclerosis, Arterioscler. Thromb. Vasc. Biol. 32 (2012) 2045–2051.
[2] H. Lu, A. Daugherty, Atherosclerosis: cell biology and lipoproteins, Curr. Opin. Lipidol. 25 (2014) 157–158.
[3] E. Falk, M. Nakano, J.F. Bentzon, A.V. Finn, R. Virmani, Update on acute coronary syndromes: the pathologists' view, Eur. Heart J. 34 (2013) 719–728.
[4] S.M. Schwartz, Z.S. Galis, M.E. Rosenfeld, E. Falk, Plaque rupture in humans and mice, Arterioscler. thromb. Vasc. Biol. 27 (2007) 705–713.
[5] I. Tabas, Macrophage death and defective inflammation resolution in atherosclerosis, Nat. Rev. Immunol. 10 (2010) 36–46.

[6] E.L. Gautier, T. Huby, J.L. Witztum, B. Ouzilleau, E.R. Miller, F. Saint-Charles, P. Aucouturier, M.J. Chapman, P. Lesnik, Macrophage apoptosis exerts divergent effects on atherogenesis as a function of lesion stage, Circulation 119 (2009) 1795–1804.
[7] G.J. Randolph, Mechanisms that regulate macrophage burden in atherosclerosis, Circ. Res. 114 (2014) 1757–1771.
[8] J.E. Feig, Regression of atherosclerosis: insights from animal and clinical studies, Ann. Glob. Health 80 (2014) 13–23.
[9] K.J. Moore, F.J. Sheedy, E.A. Fisher, Macrophages in atherosclerosis: a dynamic balance, Nat. Rev. Immunol. 13 (2013) 709–721.
[10] J.L. Johnson, A.C. Newby, Macrophage heterogeneity in atherosclerotic plaques, Curr. Opin. Lipidol. 20 (2009) 370–378.
[11] A. Canfran-Duque, C.M. Ramirez, L. Goedeke, C.S. Lin, C. Fernandez-Hernando, MicroRNAs and HDL life cycle, Cardiovasc. Res. 103 (2014) 414–422.
[12] K.J. Rayner, K.J. Moore, MicroRNA control of high-density lipoprotein metabolism and function, Circ. Res. 114 (2014) 183–192.
[13] E.L. Gautier, L. Yvan-Charvet, Understanding macrophage diversity at the ontogenic and transcriptomic levels, Immunol. Rev. 262 (2014) 85–95.
[14] A. Mantovani, C. Garlanda, M. Locati, Macrophage Diversity and Polarization in Atherosclerosis, Arterioscler. Thromb. Vasc. Biol. 29 (2009) 1419–1423.
[15] A. Sica, A. Mantovani, Macrophage plasticity and polarization: in vivo veritas, J. Clin. Investig. 122 (2012) 787–795.
[16] S. Gordon, A. Mantovani, Diversity and plasticity of mononuclear phagocytes, Eur. J. Immunol. 41 (2011) 2470–2472.
[17] J. Khallou-Laschet, A. Varthaman, G. Fornasa, C. Compain, A.-T. Gaston, M. Clement, M.l. Dussiot, O. Levillain, S.p. Graff-Dubois, A. Nicoletti, G. Caligiuri, Macrophage Plasticity in Experimental Atherosclerosis, PloS One 5 (2010) e8852.
[18] P.J. Murray, J.E. Allen, S.K. Biswas, E.A. Fisher, D.W. Gilroy, S. Goerdt, S. Gordon, J.A. Hamilton, L.B. Ivashkiv, T. Lawrence, M. Locati, A. Mantovani, F.O. Martinez, J.L. Mege, D.M. Mosser, G. Natoli, J.P. Saeij, J.L. Schultze, K.A. Shirey, A. Sica, J. Suttles, I. Udalova, J.A. van Ginderachter, S.N. Vogel, T.A. Wynn, Macrophage activation and polarization: nomenclature and experimental guidelines, Immunity 41 (2014) 14–20.
[19] J.E. Feig, E.A. Fisher, Laser capture microdissection for analysis of macrophage gene expression from atherosclerotic lesions, Methods Mol. Biol. (Clifton, N.J.) 1027 (2013) 123–135.
[20] E. Trogan, R.P. Choudhury, H.M. Dansky, J.X. Rong, J.L. Breslow, E.A. Fisher, Laser capture microdissection analysis of gene expression in macrophages from atherosclerotic lesions of apolipoprotein E-deficient mice, Proc. Natl. Acad. Sci. 99 (2002) 2234–2239.
[21] J.E. Feig, S. Parathath, J.X. Rong, S.L. Mick, Y. Vengrenyuk, L. Grauer, S.G. Young, E.A. Fisher, Reversal of hyperlipidemia with a genetic switch favorably affects the content and inflammatory state of macrophages in atherosclerotic plaques, Circulation 123 (2011) 989–998.
[22] C. Martel, W. Li, B. Fulp, A.M. Platt, E.L. Gautier, M. Westerterp, R. Bittman, A. R. Tall, S.-H. Chen, M.J. Thomas, D. Kreisel, M.A. Swartz, M.G. Sorci-Thomas, G. J. Randolph, Lymphatic vasculature mediates macrophage reverse cholesterol transport in mice, J. Clin. Investig. 123 (2013) 1571–1579.
[23] C.M. Scull, I. Tabas, Mechanisms of ER stress-induced apoptosis in atherosclerosis, Arterioscler. Thromb. Vasc. Biol. 31 (2011) 2792–2797.
[24] A.X. Zhou, I. Tabas, The UPR in atherosclerosis, Semin. Immunopathol. 35 (2013) 321–332.
[25] J. Oh, A.E. Riek, S. Weng, M. Petty, D. Kim, M. Colonna, M. Cella, C. Bernal-Mizrachi, Endoplasmic reticulum stress controls M2 macrophage differentiation and foam cell formation, J. Biol. Chem. 287 (2012) 11629–11641.
[26] J.Y. Tano, S. Solanki, R.H. Lee, K. Smedlund, L. Birnbaumer, G. Vazquez, Bone marrow deficiency of TRPC3 channel reduces early lesion burden and necrotic core of advanced plaques in a mouse model of atherosclerosis, Cardiovasc. Res. 101 (2014) 138–144.
[27] S. Solanki, P.R. Dube, J.Y. Tano, L. Birnbaumer, G. Vazquez, Reduced endoplasmic reticulum stress-induced apoptosis and impaired unfolded protein response in TRPC3-deficient M1 macrophages, Am. J. Physiol. Cell Physiol. 307 (2014) C521–C531.
[28] Y. Liao, J. Abramowitz, L. Birnbaumer, The TRPC family of TRP channels: roles inferred (mostly) from knockout mice and relationship to ORAI proteins, Handb. Exp. Pharmacol. 223 (2014) 1055–1075.
[29] A. Singh, M. Talekar, A. Raikar, M. Amiji, Macrophage-targeted delivery systems for nucleic acid therapy of inflammatory diseases, J. Control. Release: Off. J. Control. Release Soc. 190 (2014) 515–530.
[30] S. Jain, M. Amiji, Macrophage-targeted nanoparticle delivery systems, in: S. Svenson, R.K. Prud'homme (Eds.), Multifunctional Nanoparticles for Drug Delivery Applications, Springer US, Boston MA, 2012, pp. 47–83.
[31] G. Fredman, N. Kamaly, S. Spolitu, J. Milton, D. Ghorpade, R. Chiasson, G. Kuriakose, M. Perretti, O. Farokhzad, I. Tabas, Targeted nanoparticles containing the proresolving peptide Ac2-26 protect against advanced atherosclerosis in hypercholesterolemic mice, Sci. Transl. Med. 7 (2015) 275ra220.
[32] T. Dalmay, Mechanism of miRNA-mediated repression of mRNA translation, Essays Biochem. 54 (2013) 29–38.
[33] E.J. Hennessy, K.J. Moore, Using microRNA as an alternative treatment for hyperlipidemia and cardiovascular disease: cardio-miRs in the pipeline, J. Cardiovasc. Pharmacol. 62 (2013) 247–254.
[34] J.F. Aranda, J. Madrigal-Matute, N. Rotllan, C. Fernández-Hernando, MicroRNA modulation of lipid metabolism and oxidative stress in cardiometabolic diseases, Free Radic. Biol. Med. 64 (2013) 31–39.
[35] K.J. Rayner, F.J. Sheedy, C.C. Esau, F.N. Hussain, R.E. Temel, S. Parathath, J.M. van Gils, A.J. Rayner, A.N. Chang, Y. Suarez, C. Fernandez-Hernando, E.A. Fisher, K. J. Moore, Antagonism of miR-33 in mice promotes reverse cholesterol transport and regression of atherosclerosis, J. Clin. Investig. 121 (2011) 2921–2931.
[36] L. Goedeke, A. Salerno, C.M. Ramirez, L. Guo, R.M. Allen, X. Yin, S.R. Langley, C. Esau, A. Wanschel, E.A. Fisher, Y. Suarez, A. Baldan, M. Mayr, C. Fernandez-Hernando, Long-term therapeutic silencing of miR-33 increases circulating triglyceride levels and hepatic lipid accumulation in mice, EMBO Mol. Med. 6 (2014) 1133–1141.
[37] Y. Zhang, M. Zhang, M. Zhong, Q. Suo, K. Lv, Expression profiles of miRNAs in polarized macrophages, Int. J. Mol. Med. 31 (2013) 797–802.
[38] S. Banerjee, H. Cui, N. Xie, Z. Tan, S. Yang, M. Icyuz, V.J. Thannickal, E. Abraham, G. Liu, miR-125a-5p regulates differential activation of macrophages and inflammation, J. Biol. Chem. 288 (2013) 35428–35436.
[39] G. Sun, H. Li, J.J. Rossi, Sequence context outside the target region influences the effectiveness of miR-223 target sites in the RhoB 3'UTR, Nucleic Acids Res. 38 (2010) 239–252.
[40] T.A. Seimon, A. Obstfeld, K.J. Moore, D.T. Golenbock, I. Tabas, Combinatorial pattern recognition receptor signaling alters the balance of life and death in macrophages, Proc. Natl. Acad. Sci. 103 (2006) 19794–19799.
[41] R.L. Eigsti, B. Sudan, M.E. Wilson, J.W. Graff, Regulation of activation-associated microRNA accumulation rates during monocyte-to-macrophage differentiation, J. Biol. Chem. 289 (2014) 28433–28447.
[42] Y. Wei, M. Nazari-Jahantigh, L. Chan, M. Zhu, K. Heyll, J. Corbalan-Campos, P. Hartmann, A. Thiemann, C. Weber, A. Schober, The microRNA-342-5p fosters inflammatory macrophage activation through an Akt1- and microRNA-155-dependent pathway during atherosclerosis, Circulation 127 (2013) 1609–1619.
[43] J.Y. Tano, R.H. Lee, G. Vazquez, Involvement of calmodulin and calmodulin kinase II in tumor necrosis factor alpha-induced survival of bone marrow derived macrophages, Biochem. Biophys. Res. Commun. 427 (2012) 178–184.
[44] P.G. Yancey, J. Blakemore, L. Ding, D. Fan, C.D. Overton, Y. Zhang, M.F. Linton, S. Fazio, Macrophage LRP-1 Controls Plaque Cellularity by Regulating Efferocytosis and Akt Activation, Arterioscler. Thromb. Vasc. Biol. 30 (2010) 787–795.
[45] W. Xuan, Q. Qu, B. Zheng, S. Xiong, G.H. Fan, The chemotaxis of M1 and M2 macrophages is regulated by different chemokines, J. Leukoc. Biol. 97 (2015) 61–69.
[46] V.R. Babaev, J.D. Chew, L. Ding, S. Davis, M.D. Breyer, R.M. Breyer, J.A. Oates, S. Fazio, M.F. Linton, Macrophage EP4 Deficiency Increases Apoptosis and Suppresses Early Atherosclerosis, Cell Metab. 8 (2008) 492–501.
[47] M. Nazari-Jahantigh, Y. Wei, H. Noels, S. Akhtar, Z. Zhou, R.R. Koenen, K. Heyll, F. Gremse, F. Kiessling, J. Grommes, C. Weber, A. Schober, MicroRNA-155 promotes atherosclerosis by repressing Bcl6 in macrophages, J. Clin. Investig. 122 (2012) 4190–4202.
[48] Y. Wei, M. Zhu, J. Corbalan-Campos, K. Heyll, C. Weber, A. Schober, Regulation of Csf1r and Bcl6 in macrophages mediates the stage-specific effects of microRNA-155 on atherosclerosis, Arterioscler. Thromb. Vasc. Biol. 35 (2015) 796–803.
[49] S. Li, Y. Sun, C.-P. Liang, E.B. Thorp, S. Han, A.W. Jehle, V. Saraswathi, B. Pridgen, J.E. Kanter, R. Li, C.L. Welch, A.H. Hasty, K.E. Bornfeldt, J.L. Breslow, I. Tabas, A. R. Tall, Defective phagocytosis of apoptotic cells by macrophages in atherosclerotic lesions of ob/ob mice and reversal by a fish oil diet, Circ. Res. 105 (2009) 1072–1082.
[50] C.I. Caescu, X. Guo, L. Tesfa, T.D. Bhagat, A. Verma, D. Zheng, E.R. Stanley, Colony stimulating factor-1 receptor signaling networks inhibit mouse macrophage inflammatory responses by induction of microRNA-21, Blood 125 (2015) e1–13.
[51] J.A. Hamilton, Colony-stimulating factors in inflammation and autoimmunity, Nat. Rev. Immunol. 8 (2008) 533–544.
[52] Z. Wang, S. Brandt, A. Medeiros, S. Wang, H. Wu, A. Dent, C.H. Serezani, MicroRNA 21 is a homeostatic regulator of macrophage polarization and prevents prostaglandin E2-mediated M2 generation, PloS One 10 (2015) e0115855.
[53] C.I. Caescu, X. Guo, L. Tesfa, T.D. Bhagat, A. Verma, D. Zheng, E.R. Stanley, Colony stimulating factor-1 receptor signaling networks inhibit mouse macrophage inflammatory responses by induction of microRNA, Blood 21 (125) (2015) e1–e13.
[54] A. Wanschel, T. Seibert, B. Hewing, B. Ramkhelawon, T.D. Ray, J.M. van Gils, K. J. Rayner, J.E. Feig, E.R. O'Brien, E.A. Fisher, K.J. Moore, Neuroimmune guidance cue Semaphorin 3E is expressed in atherosclerotic plaques and regulates macrophage retention, Arterioscler. Thromb. Vasc. Biol. 33 (2013) 886–893.
[55] N. Ismail, Y. Wang, D. Dakhlallah, L. Moldovan, K. Agarwal, K. Batte, P. Shah, J. Wisler, T.D. Eubank, S. Tridandapani, M.E. Paulaitis, M.G. Piper, C.B. Marsh, Macrophage microvesicles induce macrophage differentiation and miR-223 transfer, Blood 121 (2013) 984–995.
[56] M. Hulsmans, P. Holvoet, MicroRNA-containing microvesicles regulating inflammation in association with atherosclerotic disease, Cardiovasc. Res. 100 (2013) 7–18.
[57] M. Yang, J. Chen, F. Su, B. Yu, F. Su, L. Lin, Y. Liu, J.D. Huang, E. Song, Microvesicles secreted by macrophages shuttle invasion-potentiating microRNAs into breast cancer cells, Mol. Cancer 10 (2011) 117.