A MicroRNA Circuitry Links Macrophage Polarization to Metabolic Homeostasis

Running title: Meng et al.; miR-223 regulates macrophage polarization

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Macrophages play an important role in tissue repair and remodeling and innate immune response. Tissue macrophages are highly heterogeneous and can undergo two distinct programs of functional specification termed classical (M1) and alternative (M2) activation\(^1,2\). In response to signals elicited by bacterial infections, such as lipopolysaccharide (LPS) and interferon-\(\gamma\) (IFN-\(\gamma\)), macrophages adopt a pro-inflammatory phenotype and contribute to defense against invading pathogens through phagocytosis, bactericidal activity, and the secretion of pro-inflammatory cytokines and chemokines. In contrast, interleukin-4 (IL-4) and IL-13 promote alternative activation of macrophages that favors tissue remodeling and repair, parasite elimination, and tumor progression. At the molecular level, M1 and M2 macrophages express unique cell surface markers and secrete distinct sets of effector molecules. Classically activated macrophages secrete pro-inflammatory cytokines, such as tumor necrosis factor \(\alpha\) (TNF\(\alpha\)), IL-1\(\beta\), and IL-6, and produces reactive oxygen species and nitric oxide, whereas alternatively activated macrophages preferentially synthesize anti-inflammatory cytokines such as IL-10 and have subdued pro-inflammatory cytokine gene expression. A balance between classical and alternative macrophage activation serves to maintain tissue homeostasis and host defense.

Chronic low-grade inflammation in metabolic tissues is now recognized as a central feature of obesity that exacerbates the progression of insulin resistance\(^1,3-5\). The inflammatory state in obesity is distinct from classical inflammation activated by pathogens in that it is engaged in response to nutrient excess and relatively chronic in nature. The pathological expansion of adipose tissue in obesity leads to elevated production of pro-inflammatory cytokines, increased infiltration of immune cells, particularly adipose tissue macrophages (ATMs), and the formation of crown-like structures, where dead adipocytes and adipocyte remnants are surrounded by phagocytic macrophages\(^6,7\). Remarkably, while resident ATMs in
lean subjects are primarily alternatively activated M2 macrophages, high-fat feeding induces a phenotypic switch in macrophage polarization toward a pro-inflammatory type in mouse adipose tissue\textsuperscript{8}. In addition, chronic inflammation leads to extensive and potentially pathogenic remodeling of adipose tissue in obesity\textsuperscript{9}. The relationship between macrophage polarization and insulin sensitivity extends beyond simple correlation. In fact, mouse models with defective pro-inflammatory cytokine signaling tend to have improved glucose homeostasis and insulin sensitivity following diet-induced obesity\textsuperscript{1,3,5}. On the contrary, genetic and pharmacological manipulations that impair macrophage alternative activation exacerbate obesity-induced insulin resistance and associated metabolic disorders, such as hepatic steatosis. Anti-inflammation therapies have demonstrated promising effects in lowering blood glucose in recent clinical studies\textsuperscript{1,3}.

How is macrophage polarization fine-tuned in metabolic tissues? One of the most striking aspects of nutrient excess-induced inflammation is that certain nutrients are capable of directly engaging inflammatory signaling pathways. Saturated fatty acids bind to toll-like receptor (TLR) and activate pro-inflammatory response whereas polyunsaturated fatty acids elicit beneficial metabolic effects in part through repressing inflammatory signaling\textsuperscript{10,11}. Chronic nutrient excess also promotes ER stress and stimulates JNK/IKK signaling, leading to NF-κB activation and the induction of pro-inflammatory gene expression\textsuperscript{12}. Recent studies have identified several transcription factors and coactivators that regulate macrophage alternative activation. STAT6 is a transcription factor downstream of IL-4 receptor signaling that plays a crucial role in specifying the alternatively activated phenotype\textsuperscript{13}. STAT6 induces the expression of transcriptional coactivator PGC-1β, which promotes mitochondrial oxidative metabolism, a key feature of alternatively activated macrophages. Nuclear hormone receptors PPARγ and PPARδ appear to
be indispensable for sustaining alternative activation state\(^1\). Interestingly, Kruppel-like factor 4 (KLF4) promotes M2 macrophage polarization through a dual mechanism: activation of M2 genetic program cooperatively with STAT6 and active repression of M1 macrophage gene expression\(^14\).

In this issue of *Circulation*, Zhou and colleagues identified microRNA-223 (miR-223) as an important regulator of macrophage polarization and provided evidence that supports the functional significance of this new pathway in metabolic homeostasis\(^15\) (Figure 1). MicroRNAs (miRNAs) are short non-coding RNAs that are approximately 22-nucleotide in length and modulate gene expression through binding to target mRNAs\(^16\). The pairing of miRNA to its target mRNAs typically result in their degradation and/or repression of translation. Accumulating evidence has demonstrated that miRNAs represent an important layer of gene regulation in the context of metabolic homeostasis\(^17\). For example, miR-33a and miR-33b, whose host genes are SREBF2 and SREBF1, respectively, are critical regulators of cholesterol metabolism\(^18\), whereas miR-103 and miR-107 regulate insulin sensitivity and glucose homeostasis by modulating caveolin-1 in adipocytes\(^19\). Previous studies have implicated different miRNA members in the regulation of innate and adaptive immune responses as well as immune cell differentiation\(^20\). However, a role for miRNA in macrophage polarization has not been explored.

MiR-223 is highly enriched in bone marrow and macrophages isolated from adipose tissue, whereas its expression in adipocytes is relatively low\(^15\). In cultured bone marrow derived macrophages (BMDMs), miR-223 expression was dramatically induced in response to IL-4 but significantly repressed by LPS treatments, suggesting that miR-223 may regulate certain aspects of macrophage activation. In support of this, miR-223 deficient macrophages elicited a heightened response to LPS in the induction of TNF\(\alpha\) gene expression. In contrast, the
expression of Arginase 1 (Arg1), a marker for M2 macrophages, was blunted in the absence of miR-223. Consistently, the expression of pro-inflammatory cytokines, such as TNFα, IL-6, and IL-1β, in miR-223 null adipose tissue was also elevated. These studies demonstrate that miR-223 likely exerts cell-autonomous effects on macrophage polarization. In vivo metabolic analyses revealed that miR-223 deficient mice developed more severe insulin resistance following high-fat feeding that was accompanied with adipose tissue inflammation. Compared to control, ATMs from miR-223 null mice adopted a more pro-inflammatory profile. Given that miR-223 is highly enriched in myeloid cells, the author transplanted syngeneic wild type mice with bone marrow cells isolated from miR-223 deficient mice. Myeloid-specific miR-223 deficiency recapitulated insulin resistance and glucose intolerance observed in miR-223 null mice, suggesting that the metabolic actions of miR-223 are largely attributed to its function in the hematopoietic lineages.

As miR-223 expression is highly responsive to IL-4 and LPS, it could be predicted that its abundance in adipose tissues is likely regulated by diet-induced obesity, which causes a switch from M2 to M1 polarization. Paradoxically, Zhuang et al. found that the expression of miR-223 in adipose tissue remains similar between lean and obese groups. Previous studies have demonstrated that miR-223 is also expressed in other cell types, such as granulocytes. As such, it cannot be ruled out that changes in the abundance of other immune cells within adipose tissue may mask the regulation of miR-223 in ATMs in obesity. At the molecular level, a major target of miR-223 in macrophages is Pknox1, which itself favors the classical activation pathway. In fact, RNAi knockdown of Pknox1 blunted the induction of pro-inflammatory cytokine IL-1β expression by LPS while augmenting Arg1 expression in macrophages. Ectopic overexpression of Pknox1 promotes polarization toward pro-inflammatory phenotype. An important question that remains unanswered is how the miR-223/Pknox1 pathway interacts with
known regulatory pathways that control macrophage activation. In the context of granulocyte differentiation, the expression of miR-223 is controlled by antagonistic action of two transcriptional factors, NFIA and C/EBPα21. Interestingly, the expression of NFIA is negatively regulated by miR-223, illustrating the ability of miR-223 to form regulatory circuitry with other transcription factors. Elucidating the exact mechanisms of the crosstalk between miR-223 and PPARs, PGC-1β, and KLF4 may shed new light on the control of macrophage polarization and its role in metabolic homeostasis.

Of note, recent studies found that certain miRNAs can be secreted into circulation in the form of microvesicles or lipoprotein particles or in complex with Argonaute proteins22-24. In fact, several disease conditions, including cancer, type 2 diabetes, and cardiovascular diseases, are associated with unique expression signatures of plasma miRNAs, suggesting that circulating miRNAs could serve as novel diagnostic biomarkers. In addition, the presence of relatively stable miRNAs in circulation supports an emerging role of miRNAs as potential signaling molecules17.

Interestingly, recent work showed that plasma levels of five miRNAs, including miR-223, were significantly decreased in patients with type 2 diabetes25. In the current study, the authors showed that insulin-stimulated AKT phosphorylation in adipocytes is attenuated in the presence of miR-223 null BMDM co-culture. While increased expression of pro-inflammatory cytokines in miR-223 deficiency provides a plausible explanation, an alternative interpretation of these findings is that miR-223 secreted from the BMDM may directly target adipocytes and modulate their insulin sensitivity. To extend this scenario in vivo, it is possible that miR-223 secreted by macrophages may target distal metabolic tissues and exert its effects on energy metabolism in a non-cell autonomous manner. As such, decreased plasma levels of miR-223 in
type 2 diabetes and in miR-223 deficient mice may not only affect macrophage polarization, but also regulate metabolic signaling in other tissues. Future experiments are needed to elucidate these intriguing possibilities.

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**References:**


**Figure Legend:**

**Figure 1.** Regulation of macrophage polarization and metabolic homeostasis by miR-223. MiR-223 targets Pknox1 through binding to its 3’ UTR and promotes the alternative (M2) activation pathway. Mice lacking miR-223 have elevated classically (M1) activated macrophages and develop more severe insulin resistance following high-fat feeding. MiR-223 may modulate systemic energy metabolism through its cell-autonomous actions in macrophages and potentially by targeting metabolic tissues following its secretion into circulation.
miR-223

M2 macrophage

Anti-inflammatory cytokines

M1 macrophage

Pro-inflammatory cytokines

Insulin sensitive

Insulin resistant