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miR-146a Influences Energy Metabolism, Cell Differentiation and Innate Immunity

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Abstract

MicroRNAs play key regulatory roles in many different biological processes, including development, differentiation, homeostasis and inflammation. The latest version of miRBase lists over 1100 distinct microRNA sequences in mice and over 1800 in humans. One pair of mature microRNAs whose 3' regions differ by only 2 nucleotides, *miR-146a* and *miR-146b*, is involved in metabolism, differentiation and immunity. NF-κB directly induces *miR-146a*, while both *miR-146a* and *miR-146b* target NF-κB pathway components interleukin-1 receptor-associated kinase 1 (*Irak1*) and tumor necrosis factor receptor-associated factor-6 (*Traf6*) for repression. Inhibition of *miR-146a* increases glucose-stimulated insulin secretion and promotes differentiation of mouse spermatogonia. Muscle-specific inactivation of mediator complex subunit 1 (*Med1*), another *miR-146a* target, enhances insulin sensitivity and improves glucose tolerance in mice. This review highlights the role of *miR-146a* in metabolic regulation, hematopoietic and spermatogenic differentiation, and induction of the immune response.

Keywords: *miR-146a*; Glucose metabolism; Spermatogonial differentiation; Immune response

Introduction

In mice, the *miR-146a* gene locus encodes a 65-nucleotide (nt) stem loop structure that forms the precursor *miR-146a* molecule. This sequence is 99-nt in humans. *miR-146b*, meanwhile encodes a 109-nt stem loop structure in mice and a 73-nt sequence in humans. Enzymatic processing by Dicer yields 22-nt mature *miR-146a* and *miR-146b* in both species. While the mature forms of *miR-146a* and *miR-146b* differ by only 2 nucleotides, the two genes are located on different chromosomes and have distinct mechanisms of regulation. Initial observations of *miR-146a* were an induction in gene activity following the exposure of human monocytic cells to lipopolysaccharides (LPS), a model for activating the innate immune response [1]. LPS exposure activates Toll-like receptors, which in turn leads to the recruitment and association of IRAK1 and TRAF6, members of the NF-κB pathway. NF-κB binds to and upregulates *miR-146a*, which then targets and represses both *Irak1* and *Traf6* mRNAs to modulate the immune response [1]. *miR-146b* also targets *Irak1* and *Traf6* mRNAs in the NF-κB pathway [1].

Meanwhile, during the differentiation of hematopoietic progenitor cells to megakaryocytes, *miR-146a* is transcriptionally repressed by promyelocytic leukemia zinc finger (PLZF; ZBTB16) [2]. Downregulation of *miR-146a* permits the expression of chemokine (C-X-C motif) receptor 4 (CXCR4), a target of *miR-146a* and an essential protein for megakaryopoiesis [2]. *miR-146a* downregulation also occurs during spermatogonial differentiation, when undifferentiated male germ cells commit to the spermatogenic process [3]. When *miR-146a* is overexpressed in hematopoietic stem cells, which are then transplanted into recipient bone marrow, decreased erythropoiesis and impaired lymphopoiesis result [4]. Indeed, *miR-146a* overexpression promotes myeloid differentiation and macrophage development at the expense of other cell lineages [5].

Studies with the insulin-secreting cell line MIN6B1 show that, in addition to innate immunity and cell differentiation, *miR-146a* functions to modulate glucose metabolism [6]. Inhibition of *miR-146a* in IL-1β-treated cells increases glucose-stimulated insulin secretion and provides protection against cytokine-induced apoptosis [6]. When *Med1*, a *miR-146a* target gene, is genetically ablated in the muscle cells of mice, these animals exhibit significantly lower glucose levels than control animals when administered a glucose tolerance test [7]. Likewise, the *Med1* tissue-specific knockout mice show a greater hypoglycemic response to exogenous insulin than control mice when given an insulin tolerance test [7]. These findings reveal a role for *miR-146a* in regulating metabolic processes within cells and tissues.

Promoter of *miR-146a*

The *miR-146a* gene, located on chromosome 11 in mouse and chromosome 5 in human, contains its own promoter with validated NF-κB and PLZF binding sites [1,2]. Many additional transcription factors are likely to bind sequences within the *miR-146a* promoter. Indeed, *in silico* analysis using MatInspector (Genomatix Software GmbH) predicts 824 putative binding sites between the transcription start site of primary *miR-146a* and 2 kb upstream, including those for retinoic acid receptors (RARα, RARβ, RXR) and DMRT1 (doublesex and mab-3 related transcription factor 1) (unpublished observations, Huszar and Payne). Table 1 lists selected transcription factors predicted to bind to the *miR-146a* promoter. It is clear that both NF-κB and PLZF modulate *miR-146a* through these promoter sequences, resulting in the

C/EBPα	PLZF	RARγ
ETS1	PU.1	RXR
NF-κB	RARα	**DMRT1**

Table 1: Selected transcription factors predicted to bind to the *miR-146a* promoter.

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differential expression of this microRNA and its subsequent influence on energy metabolism, cell differentiation and innate immunity.

Like *miR-146a*, the *miR-146b* gene has its own promoter, which is activated by interleukin (IL)-1β in alveolar epithelial cells and by γ-interferon (IFN-γ) in retinal pigment epithelial cells [8,9]. *miR-146b* is located on chromosome 19 in mouse and chromosome 10 in human. The human *MIR146B* promoter contains a putative signal transducer and activator of transcription 1 (STAT1) binding sequence, although a functional demonstration of STAT1 binding to the *MIR146B* promoter has not yet been shown [9]. Overall, the regulation of *miR-146b* has not been extensively characterized relative to *miR-146a*, and much additional work remains to enable a more accurate and complete comparison between the expression of *miR-146b* and *miR-146a*.

Recently, CpG methylation levels and histone modifications in the *miR-146a* promoter were assessed in 11 cell lines, some of which expressed latent membrane protein 1 (LMP1), a known inducer of *miR-146a* [10,11]. In cells in which *miR-146a* was silent, CpG islands were heavily methylated in two cell lines and moderately methylated in two others. Conversely, in cells actively expressing *miR-146a*, CpG dinucleotides were completely unmethylated [11]. Silent hypermethylated *miR-146a* promoters were also lacking acetylated histones H3 and H4, and H3K4me2, a mark of active chromatin [11]. In contrast, active *miR-146a* associated with H3K4me2 and moderate levels of acetylated H3 and H4. Thus, CpG methylation levels and euchromatic histone modification marks influence the activity of the *miR-146a* promoter, as they do with the promoters of protein coding genes.

Role of *miR-146a* in energy metabolism

The release of insulin from the β-cells of the pancreas is a key step to ensure optimal, steady state levels of glucose in the blood. Susceptibility of these cells to pro-inflammatory cytokines like IL-1β, tumor necrosis factor (TNF)-α and IFN-γ is a major concern, as prolonged exposure can result in cellular damage and death. When MIN6B1 cells are incubated with IL-1β, TNF-α and IFN-γ, *miR-146a* is significantly upregulated in an NF-κB -dependent manner [6]. IL-1β-treated cells in which *miR-146a* activity is blocked exhibit increased insulin secretion and reduced cytokine-induced cell death [6]. Meanwhile, in obese patients *miR-146b* is downregulated in circulating monocytes [12]. Globular adiponectin concentrations regulate *miR-146b* activity, which in turn inhibits NF-κB-mediated inflammation. However, *miR-146b* is not directly involved in insulin signaling; it instead facilitates the anti-inflammatory action of elevated globular adiponectin levels [12].

One individual *miR-146a* target mRNA validated by luciferase assays is *Med1* [3]. Direct binding occurs between *miR-146a* and the 3' untranslated region of *Med1* [3]. When muscle-specific *Med1* knockout mice are generated and subjected to glucose tolerance tests, the mice show reduced glucose levels when compared to controls [7]. These conditional knockout mice also exhibit an increased hypoglycemic response to exogenous insulin. Taken together, these findings reveal that, on the one hand, high levels of *miR-146a* expression in insulin secreting cells adversely affect insulin release and cell survival, while on the other hand, depletion of *miR-146a* target genes like *Med1* results in enhanced insulin sensitivity, improved glucose tolerance, and resistance to high-fat diet-induced obesity [7].

Role of *miR-146a* in cell differentiation

We recently observed that *miR-146a* is significantly down-regulated (~180-fold) when undifferentiated male germ cells commit to differentiate in mice [3]. This differentiation process involves the downregulation of stem cell-associated factors like PLZF, and the upregulation of factors like the Kit receptor (KIT) and stimulated by retinoic acid gene 8 (STRA8). Interestingly, Labbaye et al. [2] demonstrated PLZF binding in the *miR-146a* promoter to repress its activity in differentiating megakaryocytes. As PLZF activates as well as represses target genes, it is possible that PLZF might bind to *miR-146a* in undifferentiated spermatogonia to promote its transcription, and that upon cell differentiation *miR-146a* undergoes downregulation in a PLZF-dependent manner.

In hematopoietic stem cells, the ectopic overexpression of *miR-146a* specifically and selectively promotes the development of monocytes that can mature into macrophages [5]. This occurs at the expense of other hematopoietic cell lineages. When *miR-146a* is overexpressed in megakaryocytes, the ensuing growth, differentiation and maturation of megakaryocytes are impaired [2]. CXCR4 is repressed in these cells when *miR-146a* is overexpressed. Similar results occur when PLZF is silenced. These results collectively show how *miR-146a* influences specific cell fate decisions and lineage specification.

During transforming growth factor beta (TGFβ)-induced intestinal crypt cell differentiation, *miR-146b* is upregulated and targets seven in absentia homolog 2 (SIAH2), an E3 ubiquitin ligase [13]. This repression of SIAH2 results in the expression of SMAD7, which binds to the TGFβ receptor and inhibits its phosphorylation of SMAD2 and SMAD3 [13,14]. The roles of *miR-146b* in cell division and cancer, however, are conflicting. While upregulated *miR-146b* can inhibit the metastasis of gliomas and breast cancer, *miR-146b* overexpression has been detected in acute lymphoblastic leukemia, papillary thyroid carcinoma and lung tumors [15-19]. Further analysis is required to more accurately discern the functional roles of *miR-146b* in cell proliferation, differentiation and transformation.

Role of *miR-146a* in innate immunity

Macrophages, monocytes, natural killer cells and granulocytes, which comprise the innate immune system, serve as the initial line of defense against invading pathogens in an organism. Both the regulation of TNF-α transduction and the establishment of endotoxin tolerance in monocytes are influenced by *miR-146a* [20,21]. This microRNA also appears to function as a negative feedback regulator of inflammatory signaling in endothelial cells [22].

Taganov et al. [1] showed that human monocytic THP-1 cells exposed to LPS activated Toll-like receptor 4 (TLR-4) and induced both *miR-146a* and *miR-146b* as a response. Additional studies have demonstrated that *miR-146a* induction through activated TLR-2, -4, or -5 is a general response in myeloid cells by bacteria or fungi or by exposure to IL-1β or TNF-α [23,24]. These pro-inflammatory cytokines, for example, induce *miR-146a* expression in rheumatoid arthritis synovial tissue [25]. Thus, *miR-146a* is activated by TLR family members and NF-κB, whereby it functions to regulate *Irak1*, *Traf6* and *Nfkb* in order to modulate the immune response to invading pathogens. The regulatory mechanisms involving *miR-146b* and innate immunity are less well understood.

Summary

Like most microRNAs and other non-coding RNA molecules, *miR-146a* regulates many distinct biological processes in different types of cells (Figure 1). Harboring its own promoter, *miR-146a* expression is regulated by transcription factor binding, CpG methylation and histone modification. *miR-146a* influences such distinct cellular events

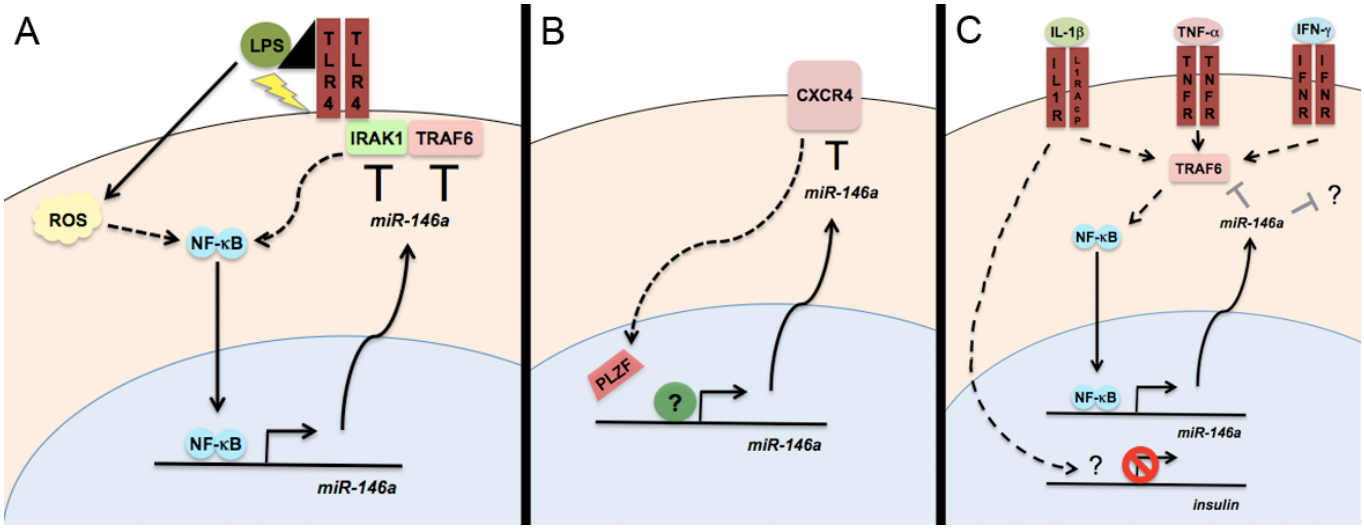

Figure 1: Activities of *miR-146a* in innate immunity, cell differentiation, and energy metabolism. (A) During an innate immune response, LPS activation of Toll-like receptor 4 (TLR4) recruits IRAK1 and TRAF6, which indirectly activate NF-κB. Upon entering the nucleus, NF-κB binds to and activates the transcription of *miR-146a*. *Irak1* and *Traf6* mRNA are then targeted by *miR-146a* for degradation. NF-κB may also be indirectly activated through reactive oxygen species (ROS). (B) In the cell differentiation events of megakaryopoiesis, transcription factor PLZF binds to and represses the *miR-146a* promoter, allowing for the translation of receptor CXCR4. When normal differentiation does not occur, unknown transcription factor(s) activate *miR-146a*, leading to the targeting and degradation of *Cxcr4* mRNA. PLZF is displaced by an unknown mechanism when *miR-146a* is upregulated in these cells. (C) When insulin-secreting cells are exposed to IL-1β, TNF-α IFN-γ receptor-mediated recruitment of TRAF6 leads to the indirect activation of NF-κB and upregulation of *miR-146a*. IL-1β activity also results in the downregulation of the insulin promoter. Not all targets of *miR-146a* are known in these cells. Solid arrow denotes direct path; dashed arrow represents indirect pathway.

such as glucose metabolism, differentiation of hematopoietic and spermatogenic cells, inflammation and immune response activity. Ongoing studies are examining its role with respect to cancer and other diseases. Characterization of additional *miR-146a* target genes proceeds continually, and further insight into the regulation of *miR-146a* activity should provide useful information to the field.

Future directions of *miR-146a* and *miR-146b* research include a greater interrogation of their promoter regions. Specifically, it will be important to know whether PLZF binds to and upregulates *miR-146a* in undifferentiated spermatogonia. Likewise, the potential interaction between STAT1 and *miR-146b* should be tested using multiple epithelial cell lines. Additional analysis of CpG methylation levels and histone modifications in the *miR-146a* promoter should reveal how this microRNA is regulated epigenetically. Additional targets of *miR-146a* and *miR-146b* should also be identified and validated during development, cell differentiation, metabolism, and tumorigenesis. Such analysis will provide greater insight into the biological roles and significance of *miR-146a* and its family member in health and disease.

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