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-xB 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-xB pathway. NF-xB 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-xB 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 spermatogenic 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 MIN6βI 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-xB and PLZF binding sites [1,2]. Many predicted 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 (doublsex 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-xB and PLZF modulate miR-146a through these promoter sequences, resulting in the

| C/EBPα | PLZF | RARγ |
|--------|------|------|
| ETS1   | PU.1 | RRX  |
| NF-xB  | 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 euchromatin 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 spermatagonia 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 Nfkβ 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
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 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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