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FORUM EDITORIAL

MicroRNAs in Hypoxia Response

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Abstract

Among the complex and articulated molecular mechanisms activated by hypoxia, microRNAs play a central role. Specifically, this Forum is dedicated to hypoxamiRs, defined as microRNAs that not only are directly regulated by hypoxia but also regulate cell responses to decreased oxygen tension. We assembled a collection of reviews and research communications by authoritative leaders in a variety of disciplines, describing hypoxamiRs from different angles. Fundamental technical issues as well as hypoxamiR impact on general events, such as metabolism and development, are critically reviewed. The regulation and the role of hypoxamiRs in cardiovascular diseases and in cancer are also addressed. An increased understanding of the function of hypoxamiR in gene regulatory networks of great physiopathological relevance may allow the development of innovative therapeutic approaches for all ischemic diseases. *Antioxid. Redox Signal.* 21, 1164–1166.

MicroRNA Biogenesis and Function

In the last few years, tens of thousands of noncoding RNAs have been found to be transcribed from the mammalian genome and, although they are not translated into proteins, some of them have been found to have biological functions. However, no function has been assigned so far to most noncoding RNAs. One exception is represented by a family of short noncoding RNAs named microRNAs (miRNAs) [for extensive review, see Carthew and Sontheimer (2)]. miRNAs are endogenous short RNAs about 22 nucleotides long, which were first discovered in 1993 in *Caenorhabditis elegans*. However, miRNAs were not recognized as a distinct class of biological regulators with conserved functions until the early 2000s. Since then, miRNAs received an ever increasing attention and are now commonly recognized as crucial post-transcriptional gene expression regulators. So far, more than 2500 miRNAs have been identified in humans (www.mirbase.org/) and they are predicted to regulate at least half of all the protein-encoding genes.

MiRNA biogenesis, summarized in Figure 1, is driven in most cases by RNA polymerase II that catalyzes the synthesis of a long primary transcript (pri-miRNA), typically containing both the cap structure and poly-A tail. Next, pri-miRNAs are processed by the nuclear RNase III Drosha, forming a 60–70 nucleotide hairpin-shaped intermediate, named ''precursor miRNA'' (pre-miRNA). This precursor is then exported out of the nucleus and cleaved by the cytoplasmic RNase III Dicer into a short miRNA duplex. One strand of this short-lived duplex is degraded, while the other strand is retained as mature miRNA, which is integrated into the effector complex named RNA-induced silencing complex (RISC).

The mature miRNA allows the RISC to recognize the target mRNA by sequence complementarity, often found in the 3′-untranslated region. However, target sequences can also be in the coding region or in the 5′-untranslated region. This leads in most circumstances to degradation and/or translational inhibition of the target mRNAs. Target identification algorithms predict that thousands of protein-coding mRNAs are regulated *via* such mechanisms. Given that a single miRNA can target up to 200 mRNAs and a single mRNA often has several miRNA recognition sequences, miRNAs are involved in virtually all known biological processes. This multiplicity of targets poses a major challenge for the miRNA researchers, in particular, when it comes to the identification of a biologically meaningful target.

Critical Issues in Hypoxia Response

Hypoxia is a fundamental physiological stimulus that triggers both adaptive (homeostatic) and maladaptive (pathological) responses [for extensive review, see Prabhakar and Semenza (5)]. Hypoxia is commonly defined as a reduction in

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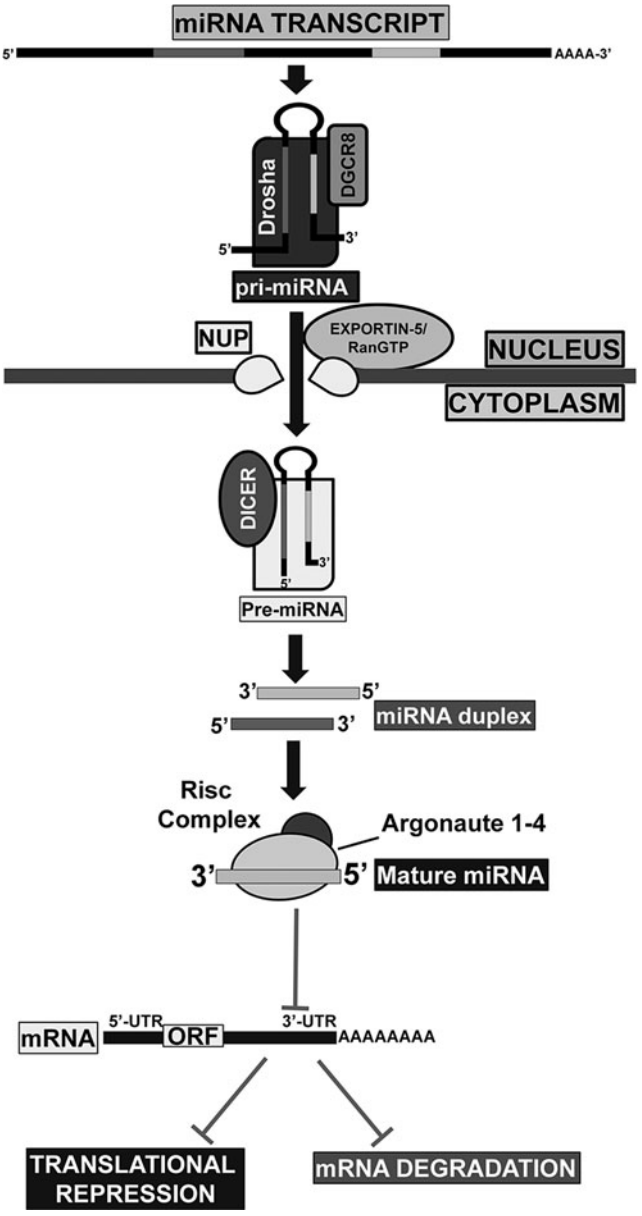

FIG. 1. MicroRNA precursor is matured by the subsequent action of Drosha/DGCR8 and Dicer nucleases. Next, the mature product is loaded on the Argonaute-containing effector complex (RISC), inhibiting the target mRNA. RISC, RNA-induced silencing complex.

oxygen availability in one site or condition compared with another; as such, it is a highly relative term. Hypoxia may involve the entire organism, as in anemic patients or upon severe blood loss or when the subject ascends to high altitude. It can be restricted to certain areas within a specific organ, as it happens not only in many ischemic cardiovascular diseases such as myocardial infarction or stroke but also in the inner regions of most solid tumors. Finally, it can be limited to certain cells within a tissue; as a general rule, cells closer to the arteries are exposed to higher oxygen concentrations compared with cells close to the veins.

The duration of hypoxia can be acute (ranging from seconds to minutes) or chronic (hours to days). At the molecular level, the responses deployed are very different: acute hypoxia induces fast but rapidly vanishing responses, which are often due to the modification of existing proteins. Conversely, chronic hypoxia induces delayed but more prolonged responses, based on the modifications of gene expression.

Hypoxia triggers a variety of adaptations, all aimed at limiting the damage inflicted by low oxygen and restoring a normal oxygen tension. The nature of the responses varies according to the type of hypoxic stimulus and its duration.

Systemic hypoxia increases blood pressure and breathing within seconds after its onset by means of neural reflexes, which arise in the carotid body, specialized sensory organs for monitoring arterial blood oxygen.

In addition, the reduced oxygen-carrying capacity of the blood, for instance, upon severe bleeding, leads to systemic hypoxia. This is sensed by the kidney and liver, triggering their production of erythropoietin (EPO). EPO, in turn, stimulates bone marrow erythropoiesis, eventually increasing the blood oxygen transport capacity.

A more local response is deployed by tissues exposed to hypoxia, for instance, due to a thromboembolic event or for the rapid cell growth occurring during the development of a cancer mass. Hypoxic tissues express high levels of angiogenic growth factors, such as vascular endothelial growth factor (VEGF) and angiopoietin (ANGPT) 1 and 2, which stimulate the growth of new blood vessels from the pre-existing vessels, alleviating the hypoxia.

Hypoxia also induces important changes at the cellular level. Decreased oxygen availability induces a shift from oxidative to glycolytic metabolism. To compensate for the greatly reduced efficiency of ATP generation associated with this metabolic switch, the transcription of the genes encoding glucose transporters and glycolytic enzymes is also induced, thereby increasing flux through the pathway.

It is often assumed that the switch from oxidative to glycolytic metabolism in cells exposed to hypoxia is because oxygen is a limiting substrate for cellular respiration (and thus for ATP synthesis) under these conditions. However, this is not the only reason and possibly not even the most important. Indeed, mitochondria are a main source of reactive oxygen species (ROS) within mammalian cells, and this is paradoxically more true when oxygen tension is low. Thus, the switch from oxidative to glycolytic metabolism is required to maintain redox homeostasis. Accordingly, genetic mouse models (*i.e.*, *Hif1a* ko) have shown that, in fibroblasts maintaining high oxidative phosphorylation levels under hypoxic conditions, normal ATP levels are present, but at the cost of generating toxic amounts of ROS.

HypoxamiRs

Recent evidence shows that, among the articulated molecular mechanisms triggered by hypoxia, miRNAs play a pivotal role. In particular, this Forum is dedicated to hypoxamiR, defined as miRNAs that not only are directly regulated by hypoxia but also regulate cell responses to decreased oxygen tension.

We assembled a collection of reviews by leaders in a variety of disciplines, exploring the topic from different angles. Hopefully, this will contribute to a profitable cultural exchange between different thematic areas. Two Original Research Communications provide examples of the relevance of hypoxamiR for cardiovascular diseases.

The first fundamental step to understand miRNA involvement in any event is obtaining a picture as accurate and complete as possible of the miRNAs expressed and modulated by the relevant stimulus, in our case hypoxia. Thus, methods of detecting and quantifying miRNAs have been actively studied. The review of Radovich and Ragoussis (this Forum) describes both classical methods of miRNA detection, quantitative real-time PCR and microarray, as well as the newest method, next-generation sequencing, discussing the pros and cons of each and the technical challenges presented by each method.

Another general aspect of fundamental importance is the identification of the transcripts targeted by the miRNA of interest. This is particularly true since miRNAs have no protein coding potential and considering the complexity of the molecular rules governing miRNA/target transcript pairing. The review of Bertrand Mari *et al.* (Bertero *et al.*, this Forum) describes the main *in silico* and experimental approaches used to identify the multiplicity of hypoxamiR targets and presents new methodologies for future investigations.

As previously mentioned, hypoxia is a fundamental physiological stimulus that triggers both adaptive and maladaptive responses in a variety of physiopathologically relevant situations. The review of Joseph Loscalzo *et al.* (Cottrill *et al.*, this Forum) will focus on the metabolic changes induced by hypoxia, that is, the shift toward anaerobic metabolism that persists even after normal oxygen levels have been restored. Indeed, many newly discovered targets of hypoxamiR converge on pathways known to be involved in this pathological phenomenon and the apoptosis-resistant phenotype associated with it.

Finally, the reviews of Fabio Martelli *et al.* (Greco *et al.*, this Forum) and Mircea Ivan *et al.* (Gee *et al.*, this Forum) will address the regulation and the role of hypoxamiRs in cardiovascular ischemic diseases and in cancer, respectively. Given the impact of these diseases for the morbidity and mortality in western countries and worldwide, these topics are of obvious relevance.

While most hypoxamiRs are modulated by decreased oxygen tension in a tissue-restricted manner, one hypoxamiR, miR-210, seems to have a particularly pervasive role, since it is induced in virtually all ischemic diseases and solid tumors tested so far (3). In this Forum, miR-210 regulation and function are comprehensively described, highlighting all the complexity. For instance, Zaccagnini *et al.* (this Forum) investigated the role of miR-210 in acute hindlimb ischemia. In ischemic muscles, the inhibition of miR-210 increases redox stress, further increasing both apoptosis and necrosis and decreasing blood perfusion. Accordingly, miR-210 also displays an antiapoptotic and proangiogenic function when overexpressed in infarcted hearts (4). However, miR-210-specific function may change according to the milieu. For instance, miR-210 attenuates keratinocyte proliferation and impairs closure in a murine model of ischemic wounds (1). Thus, the role of miR-210 requires experimental investigation for each physiopathological context.

Despite the pervasive role of miR-210, other hypoxamiRs are also important in cardiovascular diseases. For instance, Fiedler *et al.* (this Forum) identified a crucial role of miR-204 in the regulation of smooth muscle cell proliferation, contributing to loss of vascularization.

An increased understanding of the function of hypoxamiRs in gene regulatory networks associated with cardiovascular and neoplastic diseases will allow the development of innovative therapeutic approaches. Indeed, the highly synergistic functions of miRNA may make them ideal therapeutic targets. The use of antisense inhibitors is currently being considered in diseases in which hypoxia and metabolic deregulation play a pathogenetic role. Furthermore, exploration of pleiotropic hypoxamiR functions will likely continue to offer a unique insight into the target gene regulatory networks, likely allowing the identification of novel molecular mechanisms of disease.

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Abbreviations Used

ANGPT = angiopoietin
EPO = erythropoietin
miRNAs = microRNAs
PCR = polymerase chain reaction
pre-miRNA = precursor miRNA
RISC = RNA-induced silencing complex
ROS = reactive oxygen species
VEGF = vascular endothelial growth factor