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Review

Hypoxia-regulated microRNAs in human cancer

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Hypoxia plays an important role in the tumor microenvironment by allowing the development and maintenance of cancer cells, but the regulatory mechanisms by which tumor cells adapt to hypoxic conditions are not yet well understood. MicroRNAs are recognized as a new class of master regulators that control gene expression and are responsible for many normal and pathological cellular processes. Studies have shown that hypoxia inducible factor 1 (HIF1) regulates a panel of microRNAs, whereas some of microRNAs target HIF1. The interaction between microRNAs and HIF1 can account for many vital events relevant to tumorigenesis, such as angiogenesis, metabolism, apoptosis, cell cycle regulation, proliferation, metastasis, and resistance to anticancer therapy. This review will summarize recent findings on the roles of hypoxia and microRNAs in human cancer and illustrate the machinery by which microRNAs interact with hypoxia in tumor cells. It is expected to update our knowledge about the regulatory roles of microRNAs in regulating tumor microenvironments and thus benefit the development of new anticancer drugs.

Keywords: microRNA; hypoxia; HIF1; human cancer; angiogenesis; apoptosis; cell cycle; cancer metastasis; chemoresistance; radioresistance

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Introduction

As the oxygen (O_2) concentration is significantly lower in tumor tissue than in the surrounding normal tissue, hypoxia has been a focus of attention because of its important role in maintaining tumor microenvironments[1]. For instance, the average O_2 pressure in breast tumors is approximately 10 mmHg, whereas normal breast tissue has an O_2 pressure of more than 60 mmHg[2]. In general, hypoxia regulates tumor-cell phenotypes by altering genes that are sensitive to O_2 pressure. Hypoxia-inducible factor-1 (HIF1) is one of the most studied genes that play a vital role in the process of hypoxia[3]. As a transcription factor, HIF1 can be induced by low O_2 pressure and can subsequently influence the expression of a number of genes via transcriptional regulation. Thus, aside from the disruption of normal cellular metabolic processes, low O_2 results in dysregulation of hypoxia-related genes, such as HIF1. This dysregulation can account for many oncogenic phenotypes, including tumor cell transformation, invasion, metastasis, and resistance to chemotherapy and radiation therapy[3].

MicroRNAs (miRNAs) are a class of endogenous, 18–24 nucleotide, non-protein-coding small RNA molecules that regulate eukaryotic gene expression at the post-transcriptional and translational levels. They are involved in a wide variety of normal and pathological cellular processes, such as cell death, neuronal patterning, development, metabolism, and oncogenesis[4]. Numerous studies show that miRNAs with oncogenic and tumor suppressive signatures are correlated with various types of human cancer; however, the mechanistic role of miRNAs in malignant diseases is not yet completely understood. In particular, we lack a clear understanding of how miRNAs interact with conditions in the tumor microenvironment, such as hypoxia. Recently, a few reports revealed that miRNAs were associated with several key signaling pathways that respond to hypoxia and played important roles in hypoxic adaptation. Here, we will summarize these novel discoveries to illustrate the interaction between miRNAs and hypoxia in order to better understand the role of this interaction in tumorigenesis.

Hypoxia regulates the expression of miRNAs

Previous studies have discovered a number of miRNAs that are differentially expressed in response to hypoxia (hypoxia-regulated miRNAs; HRMs) by using the microarray method. For instance, miR-210, -155, -372/373, and -10b were found to be up-regulated[5–11], whereas miR-20b and miR-200b were found to be down-regulated, in response to hypoxia[12, 13]. Surprisingly, most of the HRMs apparently lack consistent phenotypes across studies. The exception to this rule appears to be a "master" HRM, miR-210, a finding that has been confirmed by

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various groups[14]. This lack of consistency may be attributable to a combination of technical variables, including the sensitivity of screening methods, the duration and severity of oxygen deprivation, and the cellular context[15]. However, in addition to the large variety of miRNA profiling platforms, we think that the lack of appropriate normalization methods specific to miRNAs is also of significance when calibrating variations in measurements of expression[16–19]. Most of the methods currently used for miRNA analysis were derived from established normalization methods based on the assumption that the total number of arrayed probes is large enough (>5000). To date, only approximately 2000 human miRNAs have been named; obviously, these established methods cannot well meet the needs of miRNA profiling analysis. Therefore, in addition to calling for novel specific normalization methods, it is extremely important to functionally validate candidate HRMs that are identified from array data.

Similar to protein encoding genes, miRNAs are transcribed from miRNA genes via classical transcription machinery that involves RNA polymerase. Thus, transcription factors play crucial roles in regulating the expression of miRNAs. For instance, c-Myc and E2F can transcriptionally up-regulate miR-17-92 and significantly influence tumor transformation mediated by miR-17-92[20, 21]. Since Kulshreshtha *et al* first reported in 2007 that the expression of an miRNA panel could be induced by hypoxia[22], numerous additional studies of HRMs have emerged. Table 1 summarizes most of the HRMs that have been reported to date. Hypoxia response elements (HRE) contained in the promoter regions of HRM genes can be bound by the α and β subunits of HIF1, and hypoxia can improve the affinity of such a complex thereby promoting the transcription of HRMs. Many HRMs, such as miR-210, -155, and -373, have been demonstrated to contain HREs by which HIF1 regulates the expression of these HRMs[5, 6, 14].

Table 1. Differentially expressed miRNAs in response to hypoxia.

Upregulated miRNAs	Downregulated miRNAs
miR-10b, miR-103, miR-107, miR-125a, miR-152, miR-155, miR-181b, miR-188, miR-191, miR-193b, miR-203, miR-205, miR-206, miR-21, miR-210, miR-213, miR-224, miR-23, miR-24, miR-26, miR-27, miR-30a-5p, miR-30c, miR-30d, miR-322, miR-333, miR-335, miR-339, miR-373, miR-451, miR-491, miR-497, miR-512-5p, miR-562, miR-93	let-7f, miR-128b, miR-150, miR-159, miR-17-92, miR-181d, miR-196a, miR-196b, miR-199a, miR-199b, miR-200a, miR-200b, miR-20b, miR-22, miR-25, miR-30, miR-424, miR-449, miR-489

Many transcription factors, such as TWIST, PPARγ, and GATA1, can be regulated by HIF1 at the transcriptional level[23–25]. As a result, HIF1 can be involved in the regulation of miRNA expression by influencing these transcription factors. For example, when HIF1 is stabilized by insufficient oxygen levels, TWIST can be induced to up-regulate miR-10b[8]. MiR-10b is a well-documented oncogenic miRNA that mediate the metastasis of various human cancers[26–28]. Therefore, HRM expression induced by HIF1 may account for the mechanism by which hypoxia facilities tumor progression. Intriguingly, studies also reported that HIF1 had been associated with down-regulation of miRNAs. The miR-17-92 cluster was reported to be reduced in hypoxia-treated cells containing wild-type p53, but not in p53-deficient cells[29]. As shown in this study, p53 directly repressed the expression of miR-17-92 through the c-Myc independent transcriptional regulation, which was thought to be responsible for hypoxia-induced apoptosis[29]. In contrast, Lei *et al* found that the knockdown of HIF1 resulting in the increase of miR-20b[13], while Chan *et al* reported that hypoxia led to the reduction of miR-200b[12].

In addition to HIF1, other genes and signaling pathways may also contribute to the adaptation of tumor cells to hypoxia. For example, oxygen deprivation could promote the induction of miR-21 via an Akt2-dependent process. The hypoxia-generated signals transduced by Akt2 have been reported to increase the activity of NF-κB and CREB, which were able to transcriptionally up-regulate the expression of miR-21[30]. Hypoxia was also involved in the biogenesis of miRNA. The protein Ago2 is a crucial component of the RNA-induced silencing complex (RISC), and the hydroxylation of Ago2 is a key step for the assembly of Ago2 to heat shock protein 90 (Hsp90) in RISC[31]. Previous studies have shown that hypoxia was able to increase the level of type 1 collagen prolyl-4-hydroxylase[C-P4H(I)], which could lead to prolyl-hydroxylation and accumulation of Ago2, thus increasing the endonuclease activity of Ago2 through either the HIF1 independent or dependent pathways[31, 32]. These mechanisms are summarized in Figure 1.

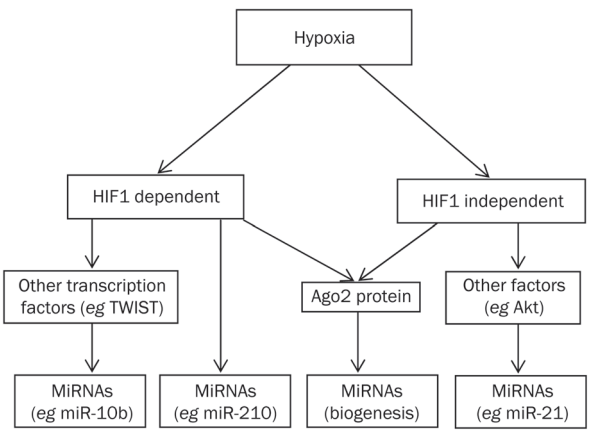

Figure 1. The machinery that hypoxia regulates miRNA expression.

miRNAs regulate HIF1

Thanks to the signature of regulating hundreds of target genes simultaneously, miRNAs are capable of repressing the expression of genes associated with hypoxia. For example, Kelly *et al* found that hypoxia-induced miR-210 could repress glycerol-3-

phosphate dehydrogenase 1-like (GPD1L), which, in turn, stabilized HIF1α by reducing hyperhydroxylation[33]. Similarly, cullin 2 (CUL2), a scaffolding protein critical to the assembly of the ubiquitin ligase system, was able to be suppressed by miR-424[34]. Given that hypoxia can induce miR-424 in human endothelial cells, the decline of CUL2 potentially stabilized HIF1α[34]. Therefore, when low O_2 induces the expression of HRMs, genes targeted by these miRNAs stabilize HIF1 by forming positive-feedback loops. Moreover, some HRMs are also involved in the destabilization of HIF1. The down-regulation of miR-20b, miR-199, and miR-17-92 by hypoxia stabilized HIF1 because these HRMs were able to repress the expression of HIF1 through direct targeting[13, 29, 35, 36]. In addition, Brunning *et al* reported that the hypoxic induction of miR-155 could negatively influence the stability and activity of HIF1α in *in vitro* and *in vivo* models[6]. Some non-HRMs, such as miR-519c and miR-107, were reported to target HIF1α[37] and HIF1β[38], respectively. Although HIF2 is another important isoform of HIF that has been intensively studied in hypoxia, there are few studies reporting associations between HIF2 and miRNAs. As such, we only summarize the regulation of HIF1 by miRNAs in Figure 2.

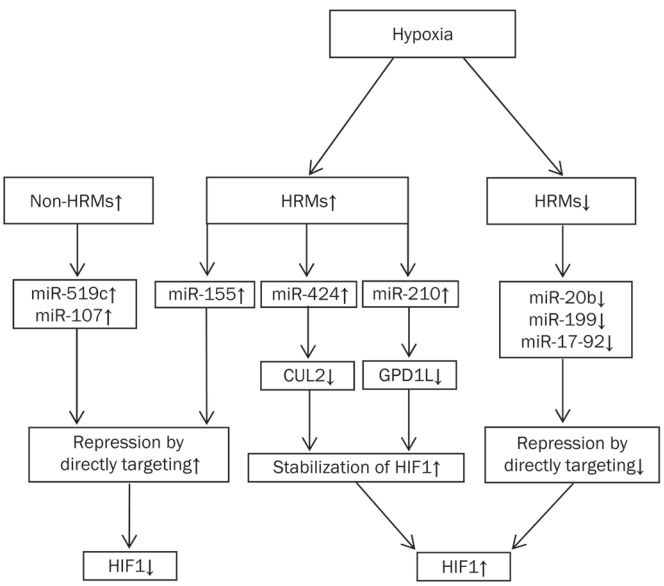

Figure 2. The machinery that miRNA regulates HIF1.

The roles of HRMs in human cancer

Angiogenesis

Angiogenesis is a highly coordinated process of tissue remodeling that leads to the formation of new blood vessels[39]. Hypoxic regions modulate the induction of angiogenesis via the regulation of pro- and anti-angiogenic factors[40, 41]. When cells are subjected to hypoxia, HIF1 up-regulates a variety of angiogeneic growth factors via transcriptional modulation, including vascular endothelial growth factor (VEGF)[42], angiopoietin 2[43], stromal-derived factor 1[44], and stem cell factor[45]. When these factors bind to specific receptors that are expressed on the surface of vascular endothelial and smooth muscle cells, angiogenic budding of new capillaries from existing vessels is initiated. As angiogenesis is important for tumor growth and metastasis, we will summarize the recent studies that have revealed an additional layer of angiogenic regulation via the action of specific HRMs.

The miRNA miR-210 was reported to target the receptor tyrosine kinase ligand Ephrin-A3 (EFNA3) and enhance the differentiation of human umbilical vein endothelial cells (HUVEC)[46]. When induced by hypoxia, miR-424 was able to promote angiogenesis through targeting CUL2, a scaffolding protein critical to the assembly of the ubiquitin ligase system described earlier. This process stabilizes HIF1α and allows it to transcriptionally activate VEGF[34]. MiR-20b has been shown to negatively regulate angiogenesis by targeting VEGF and HIF1α[13, 47]. In low-oxygen conditions, miR-20b could be down-regulated by HIF1α and attenuate the inhibitory effect on VEGF and HIF1α. The regulatory interactions among miR-20b, HIF1α, and VEGF thereby enabled tumor cells to adapt to different oxygen concentrations[13, 47]. In addition to the inhibitory effects of miR-519c on angiogenesis through the direct targeting of HIF1α[37], miR-21 was demonstrated to induce tumor angiogenesis by targeting PTEN and activating the Akt and ERK1/2 signaling pathways, which led to elevation of HIF1α and VEGF[48]. Delivery of the miR-200b mimics in human microvascular endothelial cells (HMECs) suppressed the angiogenic response, whereas miR-200b-depleted HMECs exhibited elevated angiogenesis *in vitro*, as evidenced by the matrigel tube formation and cell migration assays. Both oxygen deprivation and HIF1 stabilization could inhibit miR-200b expression[12]. In addition, down-regulation of miR-107 was found to promote tumor angiogenesis in hypoxic conditions, which may be attributed to the reduced inhibition of HIF1β by miR-107[38].

Metabolism

When oxygen levels are insufficient, a cell's metabolism shifts from mitochondrial oxidative phosphorylation to glycolysis. As such, HIF1 may be involved in the induction of kinases and enzymes that are necessary for this adaptation[49]. Recently, several groups have demonstrated that miR-210 contributes to this metabolic shift by repressing several steps that occur during mitochondrial metabolism, particularly the electron transport chain (ETC) complexes[50–53]. The iron-sulfur cluster scaffold homolog and cytochrome c oxidase assembly factor (COX10) proteins that repress mitochondrial respiration are both targeted by miR-210. Additionally, miR-210 can target NADH dehydrogenase (ubiquinone) 1 alpha subcomplex 4 (NDUFA4)[54], succinate dehydrogenase complex[55], and GPD1-L[52], which play important roles in cell metabolism.

Apoptosis

Fasanaro *et al* reported that inhibition of miR-210 induces endothelial cell apoptosis in both normoxic and hypoxic conditions by targeting Ephrin-A3[47]. Additionally, Kim

et al showed that ischemic preconditioning could augment survival of mesenchymal stem cells through miR-210 expression by targeting the apoptotic component CASP8AP2[56]. Recently, miR-21 was found to be an anti-apoptotic miRNA under hypoxic conditions[57], where it exhibited a protective effect against hypoxia/reoxygenation-induced cell apoptosis through targeting programmed cell death 4 (PDCD4) in cultured cardiac myocytes. This protective effect was further confirmed in rat hearts after ischemia/reperfusion injury *in vivo*[57]. Another study also suggested that miR-21 might regulate apoptosis though targeting PTEN and Fas ligand (FasL)[58].

Cell cycle and proliferation

The HRM miR-210 was recently demonstrated to inhibit the expression of E2F3 and MNT[54, 59]. E2F3 belongs to the E2F family that control cell cycle progression via influencing genes required for DNA synthesis at G_1/S phase[54]. MNT is a known antagonist of c-Myc and involved in regulation of cell cycle and proliferation. Induction of miR-210 and subsequent MNT repression could accelerate the G_1/S transition and promote tumor cell proliferation[59]. Therefore, induction of miR-210 by hypoxia may be involved in these essential cellular processes in tumor cells.

Cancer metastasis

Ying *et al* reported that miR-210 was induced by hypoxia in hepatocellular carcinoma (HCC) cells and could promote hypoxia-induced HCC cell metastasis[60]. Vacuole membrane protein 1 (VMP1) was identified as the direct target of miR-210, and down-regulation of VMP1 by hypoxia was involved the mobility of HCC cells[60]. Chen *et al* found that miR-103/107 expression was elevated by hypoxia in colon tumor cells and substantially repressed the tumor metastasis suppressor death-associated protein kinase (DAPK) and Kruppel-like factor 4 (KLF4)[61]. Loayza-Puch *et al* showed that miR-372/373 could be up-regulated by hypoxia through the transcriptional regulation of HIF1α and TWIST, while miR-21 was up-regulated by RAS/ERK signaling[11]. The induction of these HRMs sequentially decreased the expression of the membrane-anchored metalloproteinase regulator RECK, which was recognized as a suppressor of tumor metastasis[11].

Cancer therapy

Hypoxia has been reported to be correlated with chemoresistance and radioresistance in anticancer therapy[62, 63]. Although it has been studied for decades, the mechanism by which cancer cells are resistant to anticancer therapy during hypoxic conditions is still not completely understood. Zhu *et al* reported that the multidrug resistance gene MDR1 could be induced in HIF1 transfected human hepatoma HepG2 cells, which consequently became more resistant to 5-FU treatment than the control cells[64]. A recent study revealed that suppression of the "master" HRM, miR-210, by the antisense method, was able to significantly sensitize human hepatoma cells to radiation by inhibiting proliferation and promoting apoptosis[65]. Therefore, HRMs could be responsible for tumor cell chemoresistance and radioresistance and have the potential to serve as novel biomarkers and therapeutic targets for future anticancer therapy.

Summary and perspective

Even though a number of studies on hypoxia and human cancer have been published, the physiological and pathophysiological regulation of hypoxia is still poorly understood. Research on miRNAs promises to shed light on the mechanism that underlies the regulation of hypoxia, for two reasons. First, miRNAs can rapidly respond to stress caused by hypoxia by post-transcriptional/translational modulation at the cellular level. Second, miRNAs are capable of regulating numerous genes and influencing multiple components of a signaling pathway simultaneously. As such, it is important to understand the function and regulatory basis of HRMs in response to hypoxia. In our opinion, future studies on HRMs will continue to focus on the following directions. (1) The discovery of novel HRMs; (2) The validation of newly discovered HRMs and their functions during hypoxic conditions; (3) The identification of HRM target genes; (4) The development of novel therapeutic and preventive drugs that target HRMs; and (5) The study of the translational potential of HRMs in treating cancer patients. To complement previously published review articles, here we summarize the latest findings on hypoxia-regulated miRNAs in human cancers. We expect that this review will be able to further our understanding of the regulatory role played by miRNAs in the tumor microenvironment and will benefit the development of new anticancer drugs.

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