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Review

OxymiRs in cutaneous development, wound repair and regeneration

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ABSTRACT

The state of tissue oxygenation is widely recognized as a major microenvironmental cue that is known to regulate the expression of coding genes. Recent works have extended that knowledge to demonstrate that the state of tissue oxygenation may potently regulate the expression of microRNAs (miRs). Collectively, such miRs that are implicated in defining biological outcomes in response to a change in the state of tissue oxygenation may be referred to as oxymiRs. Broadly, oxymiRs may be categorized into three groups: (A) the existence (expression and/or turnover) of which is directly influenced by changes in the state of tissue oxygenation; (B) the existence of which is indirectly (*e.g.* oxygen-sensitive proteins, metabolites, pH, *etc.*) influenced by changes in the state of tissue oxygenation; and (C) those that modify biological outcomes to changes in the state of tissue oxygenation by targeting oxygen sensing pathways. This work represents the first review of how oxymiRs may regulate development, repair and regeneration. Currently known oxymiRs may affect the functioning of a large number of coding genes which have hitherto fore never been linked to oxygen sensing. Many of such target genes have been validated and that number is steadily growing. Taken together, our understanding of oxymiRs has vastly expanded the implications of changes in the state of tissue oxygenation. This emerging paradigm has major implications in untangling the complexities underlying diseases associated with ischemia and related hypoxic insult such as chronic wounds.

Contents

1. Introduction

MicroRNAs (miRs) are a class of ~21–23 nucleotide long non-coding RNAs (ncRNAs). The human genome is estimated to encode over a 1000 miRs which in turn regulates the functionality of the majority of protein-coding human genes. miRs bind to their target mRNA transcripts by partial sequence complementarity. Such RNA–RNA interaction is usually implicated in post-transcriptional gene silencing by causing translational repression or degradation of the coding target mRNA. The state of tissue oxygenation is widely recognized as a major microenvironmental cue that regulates the expression of coding genes. Recent works have extended that knowledge to demonstrate that the state of tissue oxygenation may

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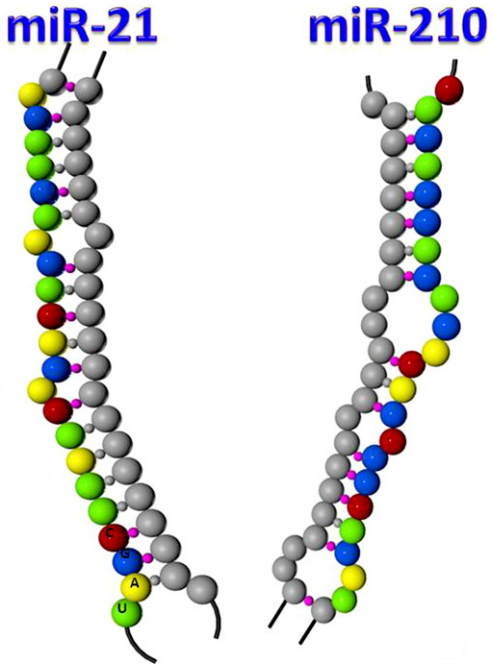

Fig. 1. Human miR-210 and miR-21 stem loops: two major oxymiRs. Art developed based on miRNAMap (http://mirnamap.mbc.nctu.edu.tw). The stem loops for each miR is highlighted.

potently regulate the expression of miRs [1–7]. Hypoxia-sensitive miRs are collectively referred to as hypoxamirs. The presence of hyperoxia-inducible miRs has now made it clear that the miR turnover machinery is sensitive to changes in the state of tissue oxygenation in both directions [7,8]. Furthermore, there are miRs that modify the biological responses to changes in the state of tissue oxygenation. Expression of these miRs may not be directly regulated by changes in tissue oxygenation but these miRs silence mediators of the oxygen-sensing pathways. Collectively, such miRs that are implicated in defining biological outcomes in response to a change in the state of tissue oxygenation may be referred to as *oxymiRs* (Fig. 1). Advances in our understanding of the significance of oxymiRs add a new dimension of sophistication to how the state of tissue oxygenation may regulate coding genes. We now know that oxygen-sensitive coding genes are not simply limited to those with oxygen-sensitive transcriptional control. A much larger subset of the human genome may be regulated by the state of tissue oxygenation through post-transcriptional gene silencing executed by oxymiRs. On one hand, oxygen-sensitive transcription regulatory pathways may control the expression of both mRNAs as well as miRs. On the other hand, oxygen-sensitive miRs may cause post transcriptional silencing of a large number of target mRNAs most of which were hitherto not known to be oxygen-sensitive. Introduction of the concept of oxymiRs substantially adds to the overall biological significance of tissue oxygenation as it relates to regulating gene function and downstream biological outcomes. Change in tissue oxygenation is recognized as key drivers of embryonic development and tissue repair (Fig. 2). In addition, stem cell biology is highly oxygen sensitive. In this work, we maintain focus on the skin as an organ and draw an outline of the expanded potential significance of changes in tissue oxygenation in light of the emergent knowledge on oxymiRs.

2. Molecular aspects of oxygen sensing

Under conditions of oxygen limitation, the respiratory chain is limited by the unavailability of oxygen as the final electron

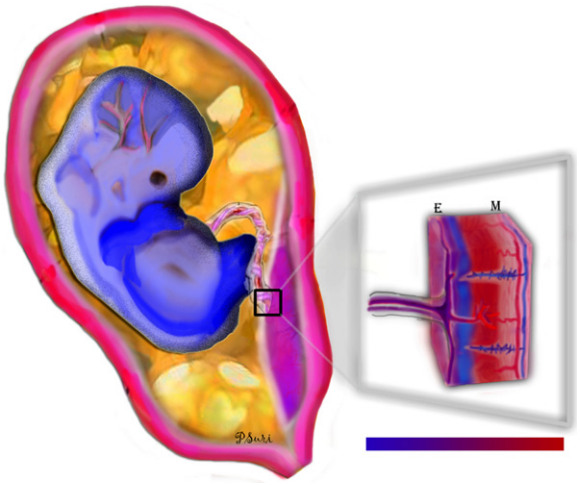

Fig. 2. Hypoxia: a major factor regulating embryonic development as well as wound healing.

acceptor. In order to support survival, cells switch from aerobic to anaerobic metabolism. This was first described as the *Pasteur Effect* which was based on the observation that oxygen-starved yeast markedly increased carbohydrate consumption. The switch from aerobic to anaerobic metabolism is enacted by metabolites acting on enzymes of the glycolytic pathway. Poor oxygen availability trigger austerity measures aimed at energy conservation [9,10]. Non-essential energy consuming functions are suspended to support cell survival. As part of ensuring cell survival hypoxia induces glycolytic enzymes and glucose transporters that are optimized to function better under hypoxia [11]. One such hypoxia-induced systemic survival response is represented by the induction of glycoprotein hormone erythropoietin (EPO). EPO stimulates red cell blood cell and hemoglobin production strengthening blood-borne oxygen delivery to tissues. The study of EPO reporter genes to explain the underlying hypoxia-dependent mechanisms of induction led to the discovery of hypoxia-inducible factor-1 (HIF-1). HIF-1 was recognized as a hypoxia-inducible transcription factor. Today, HIF-1 has emerged as a global regulator of hypoxic gene expression [12]. More recently, HIF has been directly implicated as a transcription factor that drives the expression of hypoxamirs [13,14]. Rapid and reversible regulation of gene expression by hypoxamirs tune metabolic networks with precision and control. In this way, hypoxamirs may function as molecular rheostats facilitating survival and adaptation to hypoxic conditions [15].

2.1. OxymiRs: miRs responsible for biological response to changes in tissue oxygenation

Broadly, oxymiRs may be categorized into three groups (Fig. 3): (A) the existence (expression and/or turnover) of which is directly influenced by changes in the state of tissue oxygenation; (B) the existence of which is indirectly (*e.g.* oxygen-sensitive proteins, metabolites, pH, *etc.*) influenced by changes in the state of tissue oxygenation; and (C) those that modify biological outcomes to changes in the state of tissue oxygenation by targeting mediators of oxygen sensing pathways (Table 1). That changes in oxygenation state may influence the expression of miRs was initially observed when cancer and transformed cell lines were exposed to hypoxia and differentially expressed miRs were cataloged [6,44,48,102,103]. These hypoxamirs, which would fall under *group A* oxymiR, were noted to be highly abundant in human tumors. Profiling studies have led to the recognition of hypoxia inducible miRs including miR-23, -24, -26, -27, -103, -107, -181, -210, and -213 [44]. Among the hypoxia-sensitive miRs

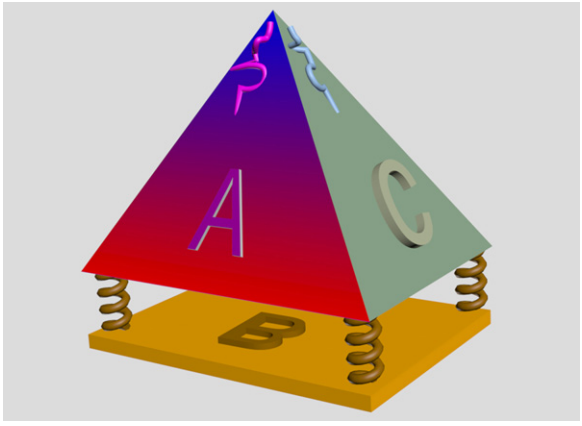

Fig. 3. OxymiRs: broadly categorized into three groups. *Group A*: the existence (expression and/or turnover) of which is directly influenced by changes in the state of tissue oxygenation; *Group B*: the existence of which is indirectly (*e.g.* oxygen-sensitive proteins, metabolites, pH, *etc.*) influenced by changes in the state of tissue oxygenation; and *Group C*: those that modify biological outcomes to changes in the state of tissue oxygenation by targeting oxygen sensing pathways.

only four, miR-210, miR-30b, miR-93 and miR-181b, were common across studies hypoxamirs are likely to be cell-type specific. SOLiD sequencing of small RNAs obtained from human endothelial cells exposed to hypoxia or normoxia identified two novel miRNAs which were down-regulated by hypoxia, while miR-210 was significantly induced [39]. Currently held as the master hypoxamir [1], miR-210 emerged as the first miR the expression of which is directly regulated by HIF1alpha as transcription factor [13]. Other miRs in this category include miR-373 [14]. Examples of *group B* oxymiRs would include miRs sensitive to anaerobic metabolites such as lactate or to oxidative stress caused by say hyperoxic insult. miRs are now recognized to be integral to the classical Warburg effect or aerobic glycolysis, a hallmark of cancer cells [104]. Oxidative stress responsive miRs regulate glycolysis in diverse biological system [105]. *Group C* oxymiRs are exemplified by say miRs which are now known to target and silence HIF-1. In this way, miRs may regulate hypoxia response as a whole. For example, in cancer cells miR-17-92 cluster silences HIF-1alpha *via* a c-myc dependent pathway [17]. In cardiac myocytes, miR-199a silences HIF-1alpha. Downregulation of miR-199a de-repressed HIF1alpha and enabled hypoxia preconditioning [63]. miRs may regulate HIF-1alpha function *via* hypoxia-independent mechanisms as well. For example, miR-519c can bind to the 3′UTR and functions as a hypoxia-independent silencer of HIF-1a [101]. In chronic lymphocytic leukemia B cells, miR-92-1 silences the von Hippel-Lindau protein (pVHL) enhancing HIF-response and VEGF expression [106]. In cancer cells, miR-20b silences HIF1alpha as well as its downstream product VEGF. miR-20b directly target the 3′UTR of *Hif1a* and *Vegfa*. Interestingly, forced overexpression of HIF-1alpha in normoxic tumor cells repressed miR-20b pointing towards a close bidirectional circuitry that enables amplification of hypoxia response in a setting where stabilized HIF-1alpha drives its own gene expression by downregulating miR-20b [22,23]. Other modifiers of HIF-1 response include miR-31. miR-31 targets the 3′UTR of factor-inhibiting hypoxia-inducible factor (FIH). FIH is known to inhibit the ability of HIF to act as a transcriptional regulator under normoxic conditions. Thus, as silencer of an inhibitor, miR-31 supports HIF function [107]. Repressed by hypoxia, miR-135a and miR-199a-5p are directly implicated in the induction of 5-lipoxygenase activating protein in response to lowered state of oxygenation. Thus, these miRs directly contribute to hypoxia-induced leukotriene formation [108].

Table 1
OxymiRs: a steadily growing list recognizing the global impact of tissue oxygenation on biological regulatory networks.

miR-16	[16]
(miR-17-92)	[17]
miR-20a	[18–21]
(miR-20b)	[22,23]
miR-21	[24–41]
(miR-22)	[42]
miR-23b	[43]
miR-26	[44]
miR-27	[45]
(miR-31)+	[46]
miR34a	[47]
miR-93	[48]
miR-101	[49]
miR-107	[44]
(miR-107)	[50]
(miR128)	[51]
(miR-130)+	[52]
(miR-138)	[53]
miR-145	[54]
miR-155	[29,55–62]
miR-199a	[20,63–66]
miR-200b	[67,68]
(miR-200b)	
miR-205	[69]
miR-210	[1,2,4,13,27,70–93]
miR-328	[94]
miR-373	[14,33,91,95]
miR-375	[96]
miR-424	[48,97,98]
miR-484	[99]
miR-495	[100]
(miR-519c)	[101]
Hyperoxamirs	[7,8]

Hypoxamir component of oxymiRs are listed individually. miRs in parentheses target HIF signaling. miRs in parentheses marked with a plus sign target elements of the HIF signaling pathway such that they support HIF function. Italicized miRs are down-regulated by hypoxia. Non-italicized miRs are upregulated in response to hypoxia. Hyperoxamir studies are few and therefore lumped at the end.

3. Development

The changing oxygen tensions *in utero* help develop the mammalian conceptus. It is long known that changes in tissue oxygenation during the course of embryonic development are strategic and serve as a key driver of the overall development process [109–111]. Both cleavage and post-compaction stages of development are highly sensitive to the state of tissue oxygenation [112]. During the course of development, exposure of embryos to oxygen is minimized such that the state of oxygenation is just sufficient to sustain life. Thus, compared to the maternal tissue, embryos and fetuses are hypoxic (Fig. 2). The trophoblast shell excludes entry of maternal blood protecting the developing embryo from oxygen toxicity as could be posed by normoxic maternal blood. Temporarily, embryos may have to rely on anaerobic energy. While such oxygen minimized state averts the risk of oxygen toxicity [113–115], any further worsening of the state of oxygenation poses serious threat to the health of the developing embryo [116,117]. The state of tissue oxygenation of the developing embryo, like for other scenarios, depend on the balance between oxygen supply and utilization. In humans, the heart is the first organ to become functionally active in the developing embryo. As early as in the 4th week of gestation, beating of the heart starts. Within a few days of that, circulation of blood is enabled to provide blood borne factors such as oxygen to the rapidly growing embryo. Expressed per unit of body weight, oxygen uptake markedly increases starting on the third day after fertilization. The development sequel is designed to provide the lowest required state of oxygenation throughout prenatal stages. At birth, oxygen uptake increases marginally. Lactate serves as the primary

metabolic fuel for the brain during the early neonatal period [118]. In the old adult, slowing down of oxygen uptake per unit weight resembles that of the newly fertilized zygote [119]. Fluxes of tissue oxygenation within the hypoxic range is a quintessence driver of fetal development [120]. The low state of fetal tissue oxygenation is known to activate hypoxia-inducible factor-1 (HIF-1), which in turn specifically induces expression of a variety of genes that help execute successful development. Fetal hypoxia is known to induce a number of biophysical, cardiovascular, endocrine, metabolic and cell signaling responses that are required to support fetal development in the intrauterine environment [121,122]. At the stage of pre-implantation embryo development, even transient disruption of the hypoxic *milieu* may adversely influence embryo development [123]. Fetal carbohydrate metabolism is highly sensitive to such disruption in tissue oxygenation [123]. Indeed, improved *in vitro* fertilization outcomes have been obtained by culturing human embryo under low oxygen tension (5% O_2) [124–126].

4. OxymiRs in skin development

Skin morphogenesis is governed by a discrete sets of differentially expressed miRs [127]. The severe pathological phenotype of mice deficient in key enzymes of the miR biogenesis pathway in the skin argues in favor of essential functions of miRs in skin development. Deep sequencing analysis of miR depletion in both Dicer- and DGCR8-null skin demonstrated that the most abundantly expressed skin miRs are dependent on both Dicer and DGCR8 pathways of miR biogenesis [128]. Skin development is tightly regulated by post-translational gene silencing executed by miR–mRNA regulatory networks [129]. On one hand, miRs regulate the expression of cell type-specific master transcription regulators implicated in skin development and homeostasis. On the other hand, miRs regulate the effects of these developmental pathways by targeting downstream signaling mediators. The embryonic epidermis originates as a single layer of multipotent epithelial cells which progresses in development through stratification and differentiation. While a causal relationship remains to be established, the master hypoxamir-210 is known to be responsive to human keratinocyte differentiation both *in vitro* and *in vivo* [130].

SMADs antagonize the transforming growth factor-beta (TGF-beta) superfamily including TGF-beta, Activin, and bone morphogenetic proteins (BMPs). These three signaling pathways play important roles in skin development [131]. OxymiR-210 is known to silence the Activin A receptor type 1B (AcvR1b) gene, a member of the TGF family of receptors [132]. Bone morphogenetic proteins regulate embryonic development and postnatal life. In developing and postnatal skin, BMPs play a key role in driving cell proliferation and differentiation in the epidermis and in the hair follicle. BMP signaling is essential in the control of cell differentiation and apoptosis in developing epidermis [133]. Elevated miR-21 may bolster HIF-signaling by increasing the expression of HIF-1alpha and VEGF [34]. OxymiR-21 represents an important downstream component of BMP signaling in epidermal keratinocytes. miR-21 is expressed in the epidermis and hair follicle epithelium in normal mouse skin. BMP4 inhibits miR-21 expression in keratinocytes. In transgenic mice overexpressing the BMP antagonist noggin under control of the K14 promoter, miR-21 expression was markedly higher. Transfection of keratinocytes with miR-21 mimic identified two groups of the BMP target genes, which were differentially regulated by miR-21. These include BMP-dependent tumor-suppressor genes (Pten, Pdcd4, Timp3 and Tpm1) negatively regulated by miR-21, as well as miR-21-independent Id1, Id2, Id3 and Msx2 that predominantly mediate the effects of BMPs on cell differentiation [134].

5. OxymiRs in skin repair

The basal proliferating compartment of the skin hosting epidermal stem cells is the primary driver of skin renewal which is responsible for tissue homeostasis and repair. miRs play a central role in the formation of epidermis and skin appendages, in particular, at the interface between stemness and differentiation [135]. Cutaneous wound healing involves changes in the expression of specific miRNA at specific phases of wound healing [136] which in turn influences healing outcomes [137]. miR-200b is an oxymiR because in human microvascular endothelial cells it is repressible by hypoxia [67,68]. In dermal microvascular cells, miR200b silences Ets-1, VEGFR2 and GATA2 to silence angiogenesis in the mature skin. Upon injury, hypoxia-represses miR-200b to switch on wound angiogenesis. In mice with diabetes mellitus, excessive tumor necrosis factor-alpha disrupts wound-induced repression of miR-200b blunting wound angiogenesis [68].

p63 is a master transcription regulator that is abundant in the basal proliferative compartment of the epidermis and its expression is directly associated with the and regenerative capacity of keratinocytes [138]. p63 maintains keratinocyte cell cycle progression by directly repressing oxymiR-34a and miR-34c [139]. Thus, specific miR-34 family members have a significant role downstream of p63 in controlling epidermal cell proliferation. miR-34a is repressed by mild hypoxia and could promote epithelial-mesenchymal transition (EMT). During EMT epithelial cells lose their epithelial characteristics and acquire a mesenchymal-like phenotype. In early embryogenesis EMT is implicated in migration and transient dedifferentiation of embryonic epithelial cells, gastrulation and the migration of neural crest cells. During cutaneous wound re-epithelialization, cell migration and the reduced intercellular adhesion of keratinocytes recapitulate several aspects of EMT [140].

The two most studied oxymiRs are represented by miR-210 and miR-21 (Fig. 1). In the following segment, we direct the spotlight on the functional significance of these two oxymiRs.

6. OxymiRs miR-210 and miR21

6.1. Development

Implantation of the blastocyst embryo to the wall of the uterus enables the fetus to receive oxygen and nutrients from the mother. Regulated by active blastocysts miR-21 is highly expressed in the subluminal stromal cells at the implantation site. Current evidence supports that miR-21 plays a central supporting role during embryo implantation [141]. Deep sequencing studies revealed that although the number of miR-21 reference sequence under activation was slightly lower than that under delayed implantation, the total level of miR-21 under activation was higher than that under delayed implantation [142]. Maternal cigarette smoking may down-regulate placental miR-21 expression possibly complicating fetal development [143]. Interestingly, miR-21 expression is markedly reduced in infants with the lowest birthweights [144]. During the onset of development, oogenesis, populations of both maternal RNA and protein are accumulated as the oocyte grows and matures. The entry of sperm into the ovulated oocyte triggers the embryogenesis phase of the developmental program. Thus, the earliest stages of embryogenesis are regulated oocytes charged with maternally inherited components. As development progresses maternally inherited components wither and early embryogenesis becomes dependent on the embryonic genome. miR-21 is known to be implicated in such maternal-to-embryonic transition. During the development of 1–8 cell stage, the expression of mature miR-21 steadily increases suggesting potential involvement of this

oxymiR during early development [145]. In vertebrates, neural crest cells are a transient, multipotent, migratory cell population that gives rise to a diverse cell lineage including melanocytes, craniofacial cartilage and bone, smooth muscle, peripheral and enteric neurons and glia. In neural crest cells, miR-21 silences Sprouty 2, an inhibitor of Erk1/2 signaling, and results in loss of function of the miR biogenesis enzyme Dicer. In this way, miR-21 may regulate neural crest cell survival, migration, and patterning in craniofacial and cardiovascular development [146]. Morphogenesis is a fundamental aspect of developmental biology that causes an organism to develop its shape. Branching morphogenesis is responsible for the creation of branched structures in the body. Branching morphogenesis in murine submandibular glands is regulated by many biological processes through interactions between the epithelium and the mesenchyme. In the mesenchyme, miR-21 has been implicated in branching morphogenesis through silencing of messenger RNAs for Reck and Pdcd4 [147]. Evidence on the role of miR-210 in development is relatively scanty. Hypoxamir-210 is known to be implicated in the pathophysiology of gestational hypertension or preeclampsia [148–152]. miR-210 silences iron-sulfur cluster scaffold homologue in human trophoblast cell lines disrupting iron metabolism associated with defective placentation [87].

6.2. Stem cell biology

Committing human embryonic stem cells to defined cell lineages is a critical requirement for evaluating their potential in regenerative medicine. miR-210 [1] is a hallmark of commitment of human embryonic stem cells to vascular endothelial cells [153]. Recent studies indicate pro-angiogenic properties of endothelial miR-210 [154]. Differentiation and proliferation of hematopoietic stem cells to mature red blood cells represents erythropoiesis. During erythropoiesis, globin gene expression is regulated by a sophisticated network of factors. miR-210 has been directly implicated in increased expression of gamma-globin genes in differentiating erythroid cells [155,156]. Under conditions of hypoxia, miR-210 supports stem cell survival [157]. Survival of mesenchymal stem cells under conditions of anoxic stress was improved by episodes of ischemic pre-conditioning by a miR-210 dependent mechanism. Inhibition of miR-210 abrogated such cytoprotection pointing towards miR-210 as a stem cell survival factor in the heart. In this case, miR-210 promoted stem cell survival by targeting caspase-8-associated protein 2 (CASP8AP2) [78], or its human homologue FLICE-associated protein homolog (FLASH), a protein that facilitates Fas-induced apoptosis. More recent studies recognize the cardioprotective properties of miR-210 against anoxia-reoxygenation [158]. Direct transfer of miR-210 from mesenchymal stem cells to host cardiomyocytes seemed to improve functional recovery of the ischemic heart [159]. Mild hypoxia supports the survival and proliferation of neural progenitor cells. Consistent with findings in cardiac mesenchymal stem cells, miR-210 expression seems to support survival of neural progenitor cells. In these cells, under hypoxic conditions miR-210 expression was directly induced by a HIF1alpha-dependent pathway [160].

miR-21 regulates self-renewal of embryonic stem cells. Maintenance of pluripotency under continued proliferation is a key requirement for stem cell renewal. Proliferation of embryonic stem cells, previously thought to be constitutive, is now known to be regulated by several factors. In murine embryonic stem cells, the neuronal repressor REST maintains self-renewal and pluripotency by suppressing miR-21. Thus, miR-21 is a REST-regulated miR that specifically suppresses the self-renewal of mouse embryonic stem cells [161]. Residing within a niche microenvironment in the testes, spermatogonial stem cells represent the foundation of spermatogenesis regulating self-renewal, pluripotency, quiescence and ability to differentiate. Transient inhibition of miR-21 in germ cell cultures enriched from spermatogonial stem cells increased the number of germ cells undergoing apoptosis and significantly reduced the number of donor-derived colonies of spermatogenesis. In these cells, miR-21 is regulated by the transcription factor ETV5, known to be critical for self-renewal of spermatogonial stem cells [162]. The adipose tissue is an abundant source for mesenchymal stem cell supply for regenerative medicine. Through its effect on TGF-beta signaling miR-21 plays a key role in adipogenic differentiation of human adipose tissue-derived mesenchymal stem cells [163]. miR-21 level tightly correlated with the adipocyte number in the white adipose tissue of obese mice providing novel testable hypothesis explaining the mechanisms of obesity [164]. The Notch signaling pathway is critically involved in stem-cell maintenance with various capacities to induce proliferation or differentiation. Interestingly, elevated Notch-1 levels lead to increased expression of miR-21 hinting at the possible significance of miR-21 in stem cell maintenance [165]. Comparable to miR-210, miR-21 also serves as a survival factor of mesenchymal stem cells [157]. In the liver, miR-21 enables rapid hepatocyte proliferation during organ regeneration by accelerating the translation of cyclin D1 [166].

6.3. Wound healing

Despite having favorable effects of stem cell biology, angiogenesis and insults involving mild (compared to near-anoxic insult associated with infected ischemic skin wounds [167]) hypoxic insult, miR-210 stalls the healing of ischemic open wounds [1,93]. The recognition that miRs may have cell and organ specific effects is on the rise [97,168–172]. Compelling support of this notion is provided by the observation that individual miRs can be heterogeneous in length and/or sequence. These variants, isomiRs, can be expressed in a cell-specific manner. Some isomiRs may affect target selection, miRNA stability, or loading into the RNA-induced silencing complex thus affecting the overall post-transcriptional gene silencing process [173]. In keratinocytes, miR-210 arrests cell growth by silencing E2F3 [93]. Growth arrest by miR-210 has been consistently reported in other cell types as well including human embryonic kidney [89] and ovarian cancer cells [13]. Emerging evidence supports that miR-210 also inhibits cell proliferation by a FGFRL1-dependent mechanism. FGFRL1 signaling is known to support cell proliferation by facilitating cell cycle progression [174]. Over-expression of FGFRL1 significantly rescued the growth inhibitory effect of miR-210 *in vitro* [174] and *in vivo* [77], establishing that miR-210 inhibits cell proliferation by a FGFRL1-dependent mechanism. miR-210 also stalls cell proliferation by silencing homeobox genes. Over-expression of HOXA1 induces activation of p44/42 MAP kinase leading to increased cell proliferation [66]. Because over-expression of HOXA1 reverses the growth inhibitory effect of miR-210 [40] it is plausible that the miR-210-dependent growth inhibitory effect is, at least partially, due to direct silencing of HOXA1 [175]. The keratinocyte growth arrest property of miR-210 stalls wound re-epithelialization [93]. The wound tissue, especially one complicated by sustained inflammation and build-up of reactive oxygen species, is expected to suffer from DNA damage that needs to be repaired during the healing process. miR-210 opposes DNA repair by silencing repair enzymes such as RAD52 [95].

Wound healing is a energy demanding process that relies on mitochondrial oxidative metabolism [167]. Another property of miR-210 that is in conflict with wound healing is its repression of mitochondrial metabolism. miR-210 is known to silence several proteins required for the normal functioning of the tricarboxylic acid cycle. miR-210 delivery inhibited mitochondrial energy

production [75], lowered oxygen consumption [75], elevated lactate levels [82,83], lowered mitochondrial membrane potential [90] and compromised mitochondrial structural integrity [90]. These findings are consistent with a more recent report demonstrating mitochondrial dysfunction following miR-210 overexpression [176]. Fe—S cluster is responsible for the catalytic function of aconitase, a stereo-specific isomerization of citrate to isocitrate which fuels the TCA-cycle. miR-210 silences the Fe—S cluster assembly homologue (ISCU) 1/2 [75,82]. In addition, miR-210 regulates cytochrome *c* oxidase assembly protein (COX10) [82] and succinate dehydrogenase subunit D (SDHD) [90], slowing down mitochondrial respiration. Oxygen conservation following severe hypoxic insult is a well known austerity measure aimed at affected cells living longer in a somewhat suspended animation mode. Such response does not favor active tissue regrowth as would be necessary for wound healing. The observation that miR-210 remains elevated even after when normoxia is restored or a day [72,177] suggests that miR-210 induces a long-lasting inhibitory effect on mitochondrial metabolism even in the presence of sufficient oxygen. Inhibition of miR-210 in the ischemic wound may therefore prove to be an effective strategy to re-establish normal mitochondrial respiration and resume the healing process.

Like miR-210, miR-21 elevation has been observed to delay cutaneous wound healing [137]. The study of clinically presented venous ulcers, a type of chronic non-healing wound, identified miR-21 as suppressor of wound epithelialization. Here, miR-21 silenced early growth response factor 3 (EGR3) as well as the leptin receptor (LepR) [137]. In keratinocytes, miR-21 is TGFbeta-inducible and accounts for its growth arrest properties [178]. Although reports on the effect of miR-21 on wound epithelialization remain somewhat conflicting [179], the body of literature on the role of miR-21 in fibrosis is more decisive. miR-21 has emerged to be a major driver of fibroblast biology [180]. Elevated miR-21 causes fibroblast dysfunction leading to fibrosis outcome. First evidence on this was reported by our laboratory describing changes in miR expression in response to ischemia-reperfusion in the murine heart, demonstrating that miR-21 regulates matrix metalloprotease-2 (MMP-2) expression in cardiac fibroblasts of the infarct zone *via* a phosphatase and tensin homologue (PTEN) pathway [181]. The observation that miR-21 silences PTEN has additional implications. Endothelial-to-mesenchymal transition (EndMT) is emerging as a significant contributor to transforming growth factor-β (TGF-β) dependent fibrosis. miR-21 contributes to, at least in part, TGF-β-mediated EndMT *via* silencing of PTEN [182]. In the skin, abundance of miR-21 correlated with fibrosis associated with systemic sclerosis [183].

7. Conclusion

Changes in the state of tissue oxygenation are commonly noted during development as well as during conditions such as wound healing where vascular supply to the tissue is disrupted (Fig. 4). From a systemic to molecular level, the body is supported by a range of networked oxygen sensors that reads changes in the flux of tissue oxygenation as a cue and mounts biological responses. Our understanding of this process of oxygen sensing and related biological response has been substantially enhanced by the recognition that the expression of small non-coding genes such as miRs may be oxygen-sensitive. Both hypoxia as well as hyperoxia-responsive miRs has been reported giving rise to a broad class of miRs which may be broadly referred to as oxymiRs. This work categorizes oxymiRs into three defined groups. Which oxymiR gets induced in response to a given situation is not only decided by the state and flux of tissue oxygenation but also on co-cues elicited by the specific biological condition. The number of miRs represented by these three groups is rapidly growing. According to computational predictions currently known oxymiRs may affect the functioning of a large number of coding genes which have hitherto fore never been linked to oxygen sensing or responsiveness. Many of such target genes have been validated and that number is steadily growing. Taken together, the concept of oxymiRs has vastly expanded the implications of changes in the state of tissue oxygenation. This emerging paradigm has major implications in untangling the complexities underlying diseases associated with ischemia and related hypoxic insult such as chronic wounds.

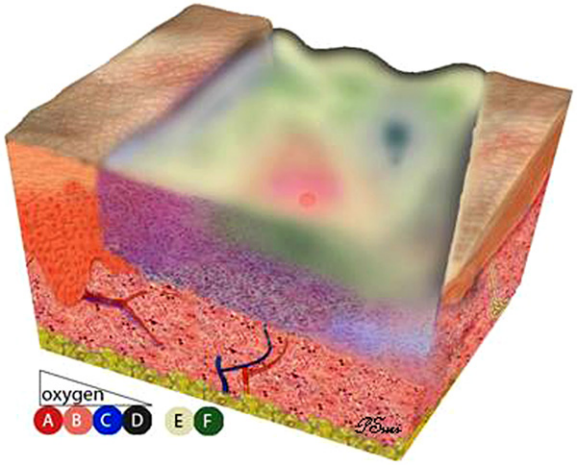

Fig. 4. Pockets of tissue oxygenation at the cutaneous wound site. Shade of red or pink represents oxygenated tissue (A and B). Shade of black (anoxia) or blue represents graded hypoxia (C and D). Tissue around each blood vessel is dark pink in shade representing regions that are well oxygenated (oxygen-rich pockets). Bacteria and bacterial infection are presented by shades of yellow and green (E and F) on the surface of the open wound.

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References

[1] Chan YC, Banerjee J, Choi SY, Sen CK. miR-210: the master hypoxamir. Microcirculation 2012;19:215–23.
[2] Devlin C, Greco S, Martelli F. Ivan M. miR-210: More than a silent player in hypoxia. IUBMB Life 2011;63:94–100.
[3] McCormick R, Buffa FM, Ragoussis J, Harris AL. The role of hypoxia regulated microRNAs in cancer. Current Topics in Microbiology and Immunology 2010;345:47–70.
[4] Huang X, Le QT, Giaccia AJ. miR-210—micromanager of the hypoxia pathway. Trends in Molecular Medicine 2010;16:230–7.
[5] Ivan M, Harris AL, Martelli F, Kulshreshtha R. Hypoxia response and microRNAs: no longer two separate worlds. Journal of Cellular and Molecular Medicine 2008;12:1426–31.
[6] Kulshreshtha R, Ferracin M, Negrini M, Calin GA, Davuluri RV, Ivan M. Regulation of microRNA expression: the hypoxic component. Cell Cycle 2007;6:1426–31.
[7] Dong J, Carey WA, Abel S, Collura C, Jiang G, Tomaszek S, et al. microRNA–mRNA interactions in a murine model of hyperoxia-induced bronchopulmonary dysplasia. BMC Genomics 2012;13:204.
[8] Zhang X, Peng W, Zhang S, Wang C, He X, Zhang Z, et al. MicroRNA expression profile in hyperoxia-exposed newborn mice during the development of bronchopulmonary dysplasia. Respiratory Care 2011;56:1009–15.
[9] Aragones J, Fraisl P, Baes M, Carmeliet P. Oxygen sensors at the crossroad of metabolism. Cell Metabolism 2009;9:11–22.
[10] Boutilier RG, St-Pierre J. Surviving hypoxia without really dying. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology 2000;126:481–90.
[11] Fandrey J. Hypoxia-inducible gene expression. Respiration Physiology 1995;101:1–10.

[12] Semenza GL. Hypoxia-inducible factors in physiology and medicine. Cell 2012;148:399–408.
[13] Giannakakis A, Sandaltzopoulos R, Greshock J, Liang S, Huang J, Hasegawa K, et al. miR-210 links hypoxia with cell cycle regulation and is deleted in human epithelial ovarian cancer. Cancer Biology and Therapy 2008;7:255–64.
[14] Crosby ME, Devlin CM, Glazer PM, Calin GA, Ivan M. Emerging roles of microRNAs in the molecular responses to hypoxia. Current Pharmaceutical Design 2009;15:3861–6.
[15] Pocock R. Invited review: decoding the microRNA response to hypoxia. Pflugers Archiv 2011;461:307–15.
[16] Dejean E, Renalier MH, Foisseau M, Agirre X, Joseph N, de Paiva GR, et al. Hypoxia-microRNA-16 downregulation induces VEGF expression in anaplastic lymphoma kinase (ALK)-positive anaplastic large-cell lymphomas. Leukemia 2011;25:1882–90.
[17] Taguchi A, Yanagisawa K, Tanaka M, Cao K, Matsuyama Y, Goto H, et al. Identification of hypoxia-inducible factor-1 alpha as a novel target for miR-17-92 microRNA cluster. Cancer Research 2008;68:5540–5.
[18] Brock M, Samillan VJ, Trenkmann M, Schwarzwald C, Ulrich S, Gay RE, et al. AntagomiR directed against miR-20a restores functional BMPR2 signalling and prevents vascular remodelling in hypoxia-induced pulmonary hypertension. European Heart Journal 2012 [Epub ahead of print].
[19] Frank D, Gantenberg J, Boomgaarden I, Kuhn C, Will R, Jarr KU, et al. MicroRNA-20a inhibits stress-induced cardiomyocyte apoptosis involving its novel target Egln3/PHD3. Journal of Molecular and Cellular Cardiology 2012;52:711–7.
[20] Kang SG, Lee WH, Lee YH, Lee YS, Kim SG. Hypoxia-inducible factor-1alpha inhibition by a pyrrolopyrazine metabolite of oltipraz as a consequence of microRNAs 199a-5p and 20a induction. Carcinogenesis 2012;33:661–9.
[21] Lin SC, Wang CC, Wu MH, Yang SH, Li YH, Tsai SJ. Hypoxia-induced microRNA-20a expression increases ERK phosphorylation and angiogenic gene expression in endometriotic stromal cells. Journal of Clinical Endocrinology and Metabolism 2012;97(8):E1515–23.
[22] Lei Z, Li B, Yang Z, Fang H, Zhang GM, Feng ZH, et al. Regulation of HIF-1alpha and VEGF by miR-20b tunes tumor cells to adapt to the alteration of oxygen concentration. PLoS ONE 2009;4:e7629.
[23] Cascio S, D'Andrea A, Ferla R, Surmacz E, Gulotta E, Amodeo V, et al. miR-20b modulates VEGF expression by targeting HIF-1 alpha and STAT3 in MCF-7 breast cancer cells. Journal of Cellular Physiology 2010;224:242–9.
[24] Dong S, Cheng Y, Yang J, Li J, Liu X, Wang X, et al. MicroRNA expression signature and the role of microRNA-21 in the early phase of acute myocardial infarction. Journal of Biological Chemistry 2009;284:29514–25.
[25] Caruso P, MacLean MR, Khanin R, McClure J, Soon E, Southgate M, et al. Dynamic changes in lung microRNA profiles during the development of pulmonary hypertension due to chronic hypoxia and monocrotaline. Arteriosclerosis, Thrombosis, and Vascular Biology 2010;30:716–23.
[26] Cheng Y, Zhu P, Yang J, Liu X, Dong S, Wang X, et al. Ischaemic preconditioning-regulated miR-21 protects heart against ischaemia/reperfusion injury via anti-apoptosis through its target PDCD4. Cardiovascular Research 2010;87:431–9.
[27] Gee HE, Camps C, Buffa FM, Patiar S, Winter SC, Betts G, et al. hsa-mir-210 is a marker of tumor hypoxia and a prognostic factor in head and neck cancer. Cancer 2010;116:2148–58.
[28] Loayza-Puch F, Yoshida Y, Matsuzaki T, Takahashi C, Kitayama H, Noda M. Hypoxia and RAS-signaling pathways converge on, and cooperatively down-regulate, the RECK tumor-suppressor protein through microRNAs. Oncogene 2010;29:2638–48.
[29] Neal CS, Michael MZ, Rawlings LH, Van der Hoek MB, Gleadle JM. The VHL-dependent regulation of microRNAs in renal cancer. BMC Medicine 2010;8:64.
[30] Sarkar J, Gou D, Turaka P, Viktorova E, Ramchandran R, Raj JU. MicroRNA-21 plays a role in hypoxia-mediated pulmonary artery smooth muscle cell proliferation and migration. American Journal of Physiology – Lung Cellular and Molecular Physiology 2010;299:L861–71.
[31] Sayed D, He M, Hong C, Gao S, Rane S, Yang Z, et al. microRNA-21 is a downstream effector of AKT that mediates its antiapoptotic effects via suppression of Fas ligand. Journal of Biological Chemistry 2010;285:20281–90.
[32] Baggish AL, Hale A, Weiner RB, Lewis GD, Systrom D, Wang F, et al. Dynamic regulation of circulating microRNA during acute exhaustive exercise and sustained aerobic exercise training. Journal of Physiology 2011;589:3983–94.
[33] Gorospe M, Tominaga K, Wu X, Fahling M, Ivan M. Post-transcriptional control of the hypoxic response by RNA-binding proteins and microRNAs. Frontiers in Molecular Neuroscience 2011;4:7.
[34] Liu LZ, Li C, Chen Q, Jing Y, Carpenter R, Jiang Y, et al. miR-21 induced angiogenesis through AKT and ERK activation and HIF-1alpha expression. PLoS ONE 2011;6:e19139.
[35] Polytarchou C, Iliopoulos D, Hatziapostolou M, Kottakis F, Maroulakou I, Struhl K, et al. Akt2 regulates all Akt isoforms and promotes resistance to hypoxia through induction of miR-21 upon oxygen deprivation. Cancer Research 2011;71:4720–31.
[36] Wang M, Li W, Chang GQ, Ye CS, Ou JS, Li XX, et al. microRNA-21 regulates vascular smooth muscle cell function via targeting tropomyosin 1 in arteriosclerosis obliterans of lower extremities. Arteriosclerosis, Thrombosis, and Vascular Biology 2011;31:2044–53.
[37] Parikh VN, Jin RC, Rabello S, Gulbahce N, White K, Hale A, et al. MicroRNA-21 integrates pathogenic signaling to control pulmonary hypertension: results of a network bioinformatics approach. Circulation 2012;125:1520–32.
[38] Pullamsetti SS, Doebele C, Fischer A, Savai R, Kojonazarov B, Dahal BK, et al. Inhibition of microRNA-17 improves lung and heart function in experimental pulmonary hypertension. American Journal of Respiratory and Critical Care Medicine 2012;185:409–19.
[39] Voellenkle C, Rooij J, Guffanti A, Brini E, Fasanaro P, Isaia E, et al. Deep-sequencing of endothelial cells exposed to hypoxia reveals the complexity of known and novel microRNAs. RNA 2012;18:472–84.
[40] Xu X, Kriegel AJ, Liu Y, Usa K, Mladinov D, Liu H, et al. Delayed ischemic preconditioning contributes to renal protection by upregulation of miR-21. Kidney International 2012.
[41] Yang S, Banerjee S, Freitas A, Cui H, Xie N, Abraham E, et al. miR-21 regulates chronic hypoxia-induced pulmonary vascular remodeling. American Journal of Physiology – Lung Cellular and Molecular Physiology 2012;302:L521–9.
[42] Yamakuchi M, Yagi S, Ito T, Lowenstein CJ. microRNA-22 regulates hypoxia signaling in colon cancer cells. PLoS ONE 2011;6:e20291.
[43] He HC, Zhu JG, Chen XB, Chen SM, Han ZD, Dai QS, et al. microRNA-23b downregulates peroxiredoxin III in human prostate cancer. FEBS Letters 2012;586:2451–8.
[44] Kulshreshtha R, Ferracin M, Wojcik SE, Garzon R, Alder H, Agosto-Perez FJ, et al. A microRNA signature of hypoxia. Molecular and Cellular Biology 2007;27:1859–67.
[45] Lin Q, Gao Z, Alarcon RM, Ye J, Yun Z. A role of miR-27 in the regulation of adipogenesis. FEBS Journal 2009;276:2348–58.
[46] Peng H, Hamanaka RB, Katsnelson J, Hao LL, Yang W, Chandel NS, et al. microRNA-31 targets FIH-1 to positively regulate corneal epithelial glycogen metabolism. FASEB Journal 2012;26(8):3140–7.
[47] Du R, Sun W, Xia L, Zhao A, Yu Y, Zhao L, et al. Hypoxia-induced down-regulation of microRNA-34a promotes EMT by targeting the Notch signaling pathway in tubular epithelial cells. PLoS ONE 2012;7:e30771.
[48] Donker RB, Mouillet JF, Nelson DM, Sadovsky Y. The expression of Argonaute2 and related microRNA biogenesis proteins in normal and hypoxic trophoblasts. Molecular Human Reproduction 2007;13:273–9.
[49] Cao P, Deng Z, Wan M, Huang W, Cramer SD, Xu J, et al. MicroRNA-101 negatively regulates Ezh2 and its expression is modulated by androgen receptor and HIF-1alpha/HIF-1beta. Molecular Cancer 2010;9:108.
[50] Yamakuchi M, Lotterman CD, Bao C, Hruban RH, Karim B, Mendell JT, et al. P53-induced microRNA-107 inhibits HIF-1 and tumor angiogenesis. Proceedings of the National Academy of Sciences of the United States of America 2010;107:6334–9.
[51] Shi ZM, Wang J, Yan Z, You YP, Li CY, Qian X, et al. MiR-128 inhibits tumor growth and angiogenesis by targeting p70S6K1. PLoS ONE 2012;7:e32709.
[52] Saito K, Kondo E, Matsushita M. microRNA 130 family regulates the hypoxia response signal through the P-body protein DDX6. Nucleic Acids Research 2011;39:6086–99.
[53] Song T, Zhang X, Wang C, Wu Y, Cai W, Gao J, et al. MiR-138 suppresses expression of hypoxia-inducible factor 1alpha (HIF-1alpha) in clear cell renal cell carcinoma 786-O cells. Asian Pacific Journal of Cancer Prevention 2011;12:1307–11.
[54] Caruso P, Dempsie Y, Stevens HC, McDonald RA, Long L, Lu R, et al. A role for miR-145 in pulmonary arterial hypertension: evidence from mouse models and patient samples. Circulation Research 2012;111:290–300.
[55] Dunwoodie SL. The role of hypoxia in development of the Mammalian embryo. Developmental Cell 2009;17:755–73.
[56] Yeligar S, Tsukamoto H, Kalra VK. Ethanol-induced expression of ET-1 and ET-BR in liver sinusoidal endothelial cells and human endothelial cells involves hypoxia-inducible factor-1alpha and microrNA-199. Journal of Immunology 2009;183:5232–43.
[57] Juan D, Alexe G, Antes T, Liu H, Madabhushi A, Delisi C, et al. Identification of a microRNA panel for clear-cell kidney cancer. Urology 2010;75:835–41.
[58] Jung M, Schaefer A, Steiner I, Kempkensteffen C, Stephan C, Erbersdobler A, et al. Robust microRNA stability in degraded RNA preparations from human tissue and cell samples. Clinical Chemistry 2010;56:998–1006.
[59] Babar IA, Czochor J, Steinmetz A, Weidhaas JB, Glazer PM, Slack FJ. Inhibition of hypoxia-induced miR-155 radiosensitizes hypoxic lung cancer cells. Cancer Biology and Therapy 2011;12:908–14.
[60] Bruning U, Cerone L, Neufeld Z, Fitzpatrick SF, Cheong A, Scholz CC, et al. MicroRNA-155 promotes resolution of hypoxia-inducible factor 1alpha activity during prolonged hypoxia. Molecular and Cellular Biology 2011;31:4087–96.
[61] Lages E, Guttin A, El Atifi M, Ramus C, Ipas H, Dupre I, et al. MicroRNA and target protein patterns reveal physiopathological features of glioma subtypes. PLoS ONE 2011;6:e20600.
[62] Rink C, Khanna S. MicroRNA in ischemic stroke etiology and pathology. Physiological Genomics 2011;43:521–8.
[63] Rane S, He M, Sayed D, Vashistha H, Malhotra A, Sadoshima J, et al. Downregulation of miR-199a derepresses hypoxia-inducible factor-1alpha and Sirtuin 1 and recapitulates hypoxia preconditioning in cardiac myocytes. Circulation Research 2009;104:879–86.
[64] Duan Q, Wang X, Gong W, Ni L, Chen C, He X, et al. ER stress negatively modulates the expression of the miR-199a/214 cluster to regulates tumor survival and progression in human hepatocellular cancer. PLoS ONE 2012;7:e31518.

[65] Jia XQ, Cheng HQ, Qian X, Bian CX, Shi ZM, Zhang JP, et al. Lentivirus-mediated overexpression of microRNA-199a inhibits cell proliferation of human hepatocellular carcinoma. Cell Biochemistry and Biophysics 2012;62: 237–44.
[66] Mizuno S, Bogaard HJ, Gomez-Arroyo J, Alhussaini A, Kraskauskas D, Cool CD, et al. MicroRNA-199a-5p is associated with hypoxia inducible factor-1alpha expression in the lung from COPD patients. Chest 2012.
[67] Chan YC, Khanna S, Roy S, Sen CK. miR-200b targets Ets-1 and is down-regulated by hypoxia to induce angiogenic response of endothelial cells. Journal of Biological Chemistry 2011;286:2047–56.
[68] Chan YC, Roy S, Khanna S, Sen CK. Downregulation of endothelial microRNA-200b supports cutaneous wound angiogenesis by desilencing GATA binding protein 2 and vascular endothelial growth factor receptor 2. Arteriosclerosis, Thrombosis, and Vascular Biology 2012;32:1372–82.
[69] Mouillet JF, Chu T, Nelson DM, Mishima T, Sadovsky Y. MiR-205 silences MED1 in hypoxic primary human trophoblasts. FASEB Journal 2010;24:2030–9.
[70] Camps C, Buffa FM, Colella S, Moore J, Sotiriou C, Sheldon H, et al. hsa-miR-210 is induced by hypoxia and is an independent prognostic factor in breast cancer. Clinical Cancer Research 2008;14:1340–8.
[71] Corn PG. Hypoxic regulation of miR-210: shrinking targets expand HIF-1's influence. Cancer Biology and Therapy 2008;7:265–7.
[72] Fasanaro P, D'Alessandra Y, Di Stefano V, Melchionna R, Romani S, Pompilio G, et al. MicroRNA-210 modulates endothelial cell response to hypoxia and inhibits the receptor tyrosine kinase ligand Ephrin-A3. Journal of Biological Chemistry 2008;283:15878–83.
[73] Galanis A, Pappa A, Giannakakis A, Lanitis E, Dangaj D, Sandaltzopoulos R. Reactive oxygen species and HIF-1 signalling in cancer. Cancer Letters 2008;266:12–20.
[74] Pulkkinen K, Malm T, Turunen M, Koistinaho J, Yla-Herttuala S. Hypoxia induces microRNA miR-210 in vitro and in vivo ephrin-A3 and neuronal pentraxin 1 are potentially regulated by miR-210. FEBS Letters 2008;582:2397–401.
[75] Chan SY, Zhang YY, Hemann C, Mahoney CE, Zweier JL, Loscalzo J. MicroRNA-210 controls mitochondrial metabolism during hypoxia by repressing the iron-sulfur cluster assembly proteins ISCU1/2. Cell Metabolism 2009;10:273–84.
[76] Fasanaro P, Greco S, Lorenzi M, Pescatori M, Brioschi M, Kulshreshtha R, et al. An integrated approach for experimental target identification of hypoxia-induced miR-210. Journal of Biological Chemistry 2009;284:35134–43.
[77] Huang X, Ding L, Bennewith KL, Tong RT, Welford SM, Ang KK, et al. Hypoxia-inducible mir-210 regulates normoxic gene expression involved in tumor initiation. Molecular Cell 2009;35:856–67.
[78] Kim HW, Haider HK, Jiang S, Ashraf M. Ischemic preconditioning augments survival of stem cells via miR-210 expression by targeting caspase-8-associated protein 2. Journal of Biological Chemistry 2009;284:33161–8.
[79] Mathew LK. Simon MC. mir-210: a sensor for hypoxic stress during tumorigenesis. Molecular Cell 2009;35:737–8.
[80] Zhang Z, Sun H, Dai H, Walsh RM, Imakura M, Schelter J, et al. MicroRNA miR-210 modulates cellular response to hypoxia through the MYC antagonist MNT. Cell Cycle 2009;8:2756–68.
[81] Chan SY, Loscalzo J. MicroRNA-210: a unique and pleiotropic hypoxamir. Cell Cycle 2010;9:1072–83.
[82] Chen Z, Li Y, Zhang H, Huang P, Luthra R. Hypoxia-regulated microRNA-210 modulates mitochondrial function and decreases ISCU and COX10 expression. Oncogene 2010;29:4362–8.
[83] Favaro E, Ramachandran A, McCormick R, Gee H, Blancher C, Crosby M, et al. MicroRNA-210 regulates mitochondrial free radical response to hypoxia and krebs cycle in cancer cells by targeting iron sulfur cluster protein ISCU. PLoS ONE 2010;5:e10345.
[84] Ho AS, Huang X, Cao H, Christman-Skieller C, Bennewith K, Le QT, et al. Circulating miR-210 as a novel hypoxia marker in pancreatic cancer. Translational Oncology 2010;3:109–13.
[85] Hu S, Huang M, Li Z, Jia F, Ghosh Z, Lijkwan MA, et al. MicroRNA-210 as a novel therapy for treatment of ischemic heart disease. Circulation 2010;122:S124–31.
[86] Kelly TJ, Souza AL, Clish CB, Puigserver P. A hypoxia-induced positive feedback loop promotes hypoxia-inducible factor 1alpha stability through miR-210 suppression of glycerol-3-phosphate dehydrogenase 1-like. Molecular and Cellular Biology 2011;31:2696–706.
[87] Lee DC, Romero R, Kim JS, Tarca AL, Montenegro D, Pineles BL, et al. miR-210 targets iron-sulfur cluster scaffold homologue in human trophoblast cell lines: siderosis of interstitial trophoblasts as a novel pathology of preterm preeclampsia and small-for-gestational-age pregnancies. American Journal of Pathology 2011;179:590–602.
[88] Mutharasan RK, Nagpal V, Ichikawa Y. Ardehali H. microRNA-210 is upregulated in hypoxic cardiomyocytes through Akt- and p53-dependent pathways and exerts cytoprotective effects. American Journal of Physiology – Heart and Circulatory Physiology 2011;301:H1519–30.
[89] Nakada C, Tsukamoto Y, Matsuura K, Nguyen TL, Hijiya N, Uchida T, et al. Overexpression of miR-210, a downstream target of HIF1alpha, causes centrosome amplification in renal carcinoma cells. Journal of Pathology 2011;224: 280–8.
[90] Puissegur MP, Mazure NM, Bertero T, Pradelli L, Grosso S, Robbe-Sermesant K, et al. miR-210 is overexpressed in late stages of lung cancer and mediates mitochondrial alterations associated with modulation of HIF-1 activity. Cell Death and Differentiation 2011;18:465–78.
[91] Quero L, Dubois L, Lieuwes NG, Hennequin C, Lambin P. miR-210 as a marker of chronic hypoxia, but not a therapeutic target in prostate cancer. Radiotherapy and Oncology 2011;101:203–8.
[92] Zeng L, Liu J, Wang Y, Wang L, Weng S, Tang Y, et al. MicroRNA-210 as a novel blood biomarker in acute cerebral ischemia. Frontiers in Bioscience (Elite Edition) 2011;3:1265–72.
[93] Biswas S, Roy S, Banerjee J, Hussain SR, Khanna S, Meenakshisundaram G, et al. Hypoxia inducible microRNA 210 attenuates keratinocyte proliferation and impairs closure in a murine model of ischemic wounds. Proceedings of the National Academy of Sciences of the United States of America 2010;107:6976–81.
[94] Guo L, Qiu Z, Wei L, Yu X, Gao X, Jiang S, et al. The microRNA-328 regulates hypoxic pulmonary hypertension by targeting at insulin growth factor 1 receptor and L-type calcium channel-alpha1C. Hypertension 2012;59:1006–13.
[95] Crosby ME, Kulshreshtha R, Ivan M, Glazer PM. MicroRNA regulation of DNA repair gene expression in hypoxic stress. Cancer Research 2009;69: 1221–9.
[96] Chang Y, Yan W, He X, Zhang L, Li C, Huang H, et al. miR-375 inhibits autophagy and reduces viability of hepatocellular carcinoma cells under hypoxic conditions. Gastroenterology 2012;143:177–87, e8.
[97] Kawahigashi Y, Mishima T, Mizuguchi Y, Arima Y, Yokomuro S, Kanda T, et al. MicroRNA profiling of human intrahepatic cholangiocarcinoma cell lines reveals biliary epithelial cell-specific microRNAs. Journal of Nippon Medical School 2009;76:188–97.
[98] Ghosh G, Subramanian IV, Adhikari N, Zhang X, Joshi HP, Basi D, et al. Hypoxia-induced microRNA-424 expression in human endothelial cells regulates HIF-alpha isoforms and promotes angiogenesis. Journal of Clinical Investigation 2010;120:4141–54.
[99] Wang K, Long B, Jiao JQ, Wang JX, Liu JP, Li Q, et al. miR-484 regulates mitochondrial network through targeting Fis1. Nature Communications 2012;3:781.
[100] Hwang-Verslues WW, Chang PH, Wei PC, Yang CY, Huang CK, Kuo WH, et al. miR-495 is upregulated by E12/E47 in breast cancer stem cells, and promotes oncogenesis and hypoxia resistance via downregulation of E-cadherin and REDD1. Oncogene 2011;30:2463–74.
[101] Cha ST, Chen PS, Johansson G, Chu CY, Wang MY, Jeng YM, et al. MicroRNA-519c suppresses hypoxia-inducible factor-1alpha expression and tumor angiogenesis. Cancer Research 2010;70:2675–85.
[102] Hebert C, Norris K, Scheper MA, Nikitakis N, Sauk JJ. High mobility group A2 is a target for miRNA-98 in head and neck squamous cell carcinoma. Mol Cancer 2007;6:5.
[103] Hua Z, Lv Q, Ye W, Wong CK, Cai G, Gu D, et al. MiRNA-directed regulation of VEGF and other angiogenic factors under hypoxia. PLoS ONE 2006;1: e116.
[104] Gao P, Sun L, He X, Cao Y, F Zhang H. MicroRNAs the Warburg effect: new players in an old arena. Current Gene Therapy 2012;12:285–91.
[105] Tang H, Lee M, Sharpe O, Salamone L, Noonan EJ, Hoang CD, et al. Oxidative stress-responsive microRNA-320 regulates glycolysis in diverse biological systems. FASEB Journal 2012.
[106] Ghosh AK, Shanafelt TD, Cimmino A, Taccioli C, Volinia S, Liu CG, et al. Aberrant regulation of pVHL levels by microRNA promotes the HIF/VEGF axis in CLL B cells. Blood 2009;113:5568–74.
[107] Liu CJ, Tsai MM, Hung PS, Kao SY, Liu TY, Wu KJ, et al. miR-31 ablates expression of the HIF regulatory factor FIH to activate the HIF pathway in head and neck carcinoma. Cancer Research 2010;70:1635–44.
[108] Gonsalves CS, Kalra VK. Hypoxia-mediated expression of 5-lipoxygenase-activating protein involves HIF-1alpha and NF-kappaB and microRNAs 135a and 199a-5p. Journal of Immunology 2010;184:3878–88.
[109] Bartels H, Baumann F. Metabolic rate of early embryos (4–22 somites) at varying oxygen pressures. Respiration Physiology 1972;16:1–15.
[110] New DA, Coppola PT. Effects of different oxygen concentrations on the development of rat embryos in culture. Journal of Reproduction and Fertility 1970;21:109–18.
[111] Auerbach S, Brinster RL. Effect of oxygen concentration on the development of two-cell mouse embryos. Nature 1968;217:465–6.
[112] Wale PL, Gardner DK. Time-lapse analysis of mouse embryo development in oxygen gradients. Reproductive Biomedicine Online 2010;21:402–10.
[113] Umaoka Y, Noda Y, Narimoto K, Mori T. Effects of oxygen toxicity on early development of mouse embryos. Molecular Reproduction and Development 1992;31:28–33.
[114] Sapunar D, Saraga-Babic M, Peruzovic M, Marusic M. Effects of hyperbaric oxygen on rat embryos. Biology of the Neonate 1993;63:360–9.
[115] Ishibashi M, Akazawa S, Sakamaki H, Matsumoto K, Yamasaki H, Yamaguchi Y, et al. Oxygen-induced embryopathy and the significance of glutathione-dependent antioxidant system in the rat embryo during early organogenesis. Free Radical Biology and Medicine 1997;22:447–54.
[116] Kingdom JC, Kaufmann P. Oxygen and placental villous development: origins of fetal hypoxia. Placenta 1997;18:613–21 [discussion 23-6].
[117] Nordstrom L, Arulkumaran S. Intrapartum fetal hypoxia and biochemical markers: a review. Obstetrical and Gynecological Survey 1998;53: 645–57.
[118] Medina JM. The role of lactate as an energy substrate for the brain during the early neonatal period. Biology of the Neonate 1985;48:237–44.
[119] Adolph EF. Uptakes and uses of oxygen, from gametes to maturity: an overview. Respiration Physiology 1983;53:135–60.

[120] Semenza GL, Agani F, Iyer N, Kotch L, Laughner E, Leung S, et al. Regulation of cardiovascular development and physiology by hypoxia-inducible factor 1. Annals of the New York Academy of Sciences 1999;874: 262–8.
[121] Hooper SB. Fetal metabolic responses to hypoxia. Reproduction, Fertility, and Development 1995;7:527–38.
[122] Simon MC, Keith B. The role of oxygen availability in embryonic development and stem cell function. Nature Reviews Molecular Cell Biology 2008;9:285–96.
[123] Wale PL, Gardner DK. Oxygen regulates amino acid turnover and carbohydrate uptake during the preimplantation period of mouse embryo development. Biology of Reproduction 2012;87:1–8.
[124] Noda Y, Goto Y, Umaoka Y, Shiotani M, Nakayama T, Mori T. Culture of human embryos in alpha modification of Eagle's medium under low oxygen tension and low illumination. Fertility and Sterility 1994;62:1022–7.
[125] Catt JW, Henman M. Toxic effects of oxygen on human embryo development. Human Reproduction 2000;15(Suppl. 2):199–206.
[126] Bontekoe S, Mantikou E, van Wely M, Seshadri S, Repping S, Mastenbroek S. Low oxygen concentrations for embryo culture in assisted reproductive technologies. Cochrane Database of Systematic Reviews 2012;7:CD008950.
[127] Yi R, O'Carroll D, Pasolli HA, Zhang Z, Dietrich FS, Tarakhovsky A, et al. Morphogenesis in skin is governed by discrete sets of differentially expressed microRNAs. Nature Genetics 2006;38:356–62.
[128] Yi R, Pasolli HA, Landthaler M, Hafner M, Ojo T, Sheridan R, et al. DGCR8-dependent microRNA biogenesis is essential for skin development. Proceedings of the National Academy of Sciences of the United States of America 2009;106:498–502.
[129] Botchkareva NV. MicroRNA/mRNA regulatory networks in the control of skin development and regeneration. Cell Cycle 2012;11:468–74.
[130] Hildebrand J, Rutze M, Walz N, Gallinat S, Wenck H, Deppert W, et al. A comprehensive analysis of microRNA expression during human keratinocyte differentiation in vitro and in vivo. Journal of Investigative Dermatology 2011;131:20–9.
[131] Owens P, Han G, Li AG, Wang XJ. The role of Smads in skin development. Journal of Investigative Dermatology 2008;128:783–90.
[132] Mizuno Y, Tokuzawa Y, Ninomiya Y, Yagi K, Yatsuka-Kanesaki Y, Suda T, et al. miR-210 promotes osteoblastic differentiation through inhibition of AcvR1b. FEBS Letters 2009;583:2263–8.
[133] Botchkarev VA, Sharov AA. BMP signaling in the control of skin development and hair follicle growth. Differentiation 2004;72:512–26.
[134] Ahmed MI, Mardaryev AN, Lewis CJ, Sharov AA, Botchkareva NV. MicroRNA-21 is an important downstream component of BMP signalling in epidermal keratinocytes. Journal of Cell Science 2011;124:3399–404.
[135] Aberdam D, Candi E, Knight RA, Melino G. miRNAs, 'stemness' and skin. Trends in Biochemical Sciences 2008;33:583–91.
[136] Shilo S, Roy S, Khanna S, Sen CK. MicroRNA in cutaneous wound healing: a new paradigm. DNA and Cell Biology 2007;26:227–37.
[137] Pastar I, Khan AA, Stojadinovic O, Lebrun EA, Medina MC, Brem H, et al. Induction of specific micro Ribonucleic acids (miRNAs) inhibits cutaneous wound healing. Journal of Biological Chemistry 2012;287:29324–35.
[138] Koster MI. p63 in skin development and ectodermal dysplasias. Journal of Investigative Dermatology 2010;130:2352–8.
[139] Antonini D, Russo MT, De Rosa L, Gorrese M, Del Vecchio L, Missero C. Transcriptional repression of miR-34 family contributes to p63-mediated cell cycle progression in epidermal cells. Journal of Investigative Dermatology 2010;130:1249–57.
[140] Nakamura M, Tokura Y. Epithelial-mesenchymal transition in the skin. Journal of Dermatological Science 2011;61:7–13.
[141] Hu SJ, Ren G, Liu JL, Zhao ZA, Yu YS, Su RW, et al. MicroRNA expression and regulation in mouse uterus during embryo implantation. Journal of Biological Chemistry 2008;283:23473–84.
[142] Su RW, Lei W, Liu JL, Zhang ZR, Jia B, Feng XH, et al. The integrative analysis of microRNA and mRNA expression in mouse uterus under delayed implantation and activation. PLoS ONE 2010;5:e15513.
[143] Maccani MA, Avissar-Whiting M, Banister CE, McGonnigal B, Padbury JF, Marsit CJ. Maternal cigarette smoking during pregnancy is associated with downregulation of miR-16, miR-21, and miR-146a in the placenta. Epigenetics 2010;5:583–9.
[144] Maccani MA, Padbury JF, Marsit CJ. miR-16 and miR-21 expression in the placenta is associated with fetal growth. PLoS ONE 2011;6:e21210.
[145] Mondou E, Dufort I, Gohin M, Fournier E, Sirard MA. Analysis of microRNAs and their precursors in bovine early embryonic development. Molecular Human Reproduction 2012;18:425–34.
[146] Huang ZP, Chen JF, Regan JN, Maguire CT, Tang RH, Dong XR, et al. Loss of microRNAs in neural crest leads to cardiovascular syndromes resembling human congenital heart defects. Arteriosclerosis, Thrombosis, and Vascular Biology 2010;30:2575–86.
[147] Hayashi T, Koyama N, Azuma Y, Kashimata M. Mesenchymal miR-21 regulates branching morphogenesis in murine submandibular gland in vitro. Developmental Biology 2011;352:299–307.
[148] Pineles BL, Romero R, Montenegro D, Tarca AL, Han YM, Kim YM, et al. Distinct subsets of microRNAs are expressed differentially in the human placentas of patients with preeclampsia. American Journal of Obstetrics and Gynecology 2007;196. p. 261 e1–261 e6.
[149] Zhu XM, Han T, Sargent IL, Yin GW, Yao YQ. Differential expression profile of microRNAs in human placentas from preeclamptic pregnancies vs. normal pregnancies. American Journal of Obstetrics and Gynecology 2009;200. p. 661 e1–661 e7.
[150] Mayor-Lynn K, Toloubeydokhti T, Cruz AC, Chegini N. Expression profile of microRNAs and mRNAs in human placentas from pregnancies complicated by preeclampsia and preterm labor. Reproductive Sciences 2011;18: 46–56.
[151] Enquobahrie DA, Abetew DF, Sorensen TK, Willoughby D, Chidambaram K, Williams MA. Placental microRNA expression in pregnancies complicated by preeclampsia. American Journal of Obstetrics and Gynecology 2011;204. p. 178 e12–178 e21.
[152] Zhang Y, Fei M, Xue G, Zhou Q, Jia Y, Li L, et al. Elevated levels of hypoxia-inducible microRNA-210 in pre-eclampsia: new insights into molecular mechanisms for the disease. Journal of Cellular and Molecular Medicine 2012;16:249–59.
[153] Kane NM, Meloni M, Spencer HL, Craig MA, Strehl R, Milligan G, et al. Derivation of endothelial cells from human embryonic stem cells by directed differentiation: analysis of microRNA and angiogenesis in vitro and in vivo. Arteriosclerosis, Thrombosis, and Vascular Biology 2010;30: 1389–97.
[154] Alaiti MA, Ishikawa M, Masuda H, Simon DI, Jain MK, Asahara T, et al. Up-regulation of miR-210 by vascular endothelial growth factor in ex vivo expanded CD34+ cells enhances cell-mediated angiogenesis. Journal of Cellular and Molecular Medicine 2012;16:2413–21.
[155] Bianchi N, Zuccato C, Lampronti I, Borgatti M, Gambari R. Expression of miR-210 during erythroid differentiation and induction of gamma-globin gene expression. BMB Reports 2009;42:493–9.
[156] Bianchi N, Zuccato C, Finotti A, Lampronti I, Borgatti M, Gambari R. Involvement of miRNA in erythroid differentiation. Epigenomics 2012;4: 51–65.
[157] Nie Y, Han BM, Liu XB, Yang JJ, Wang F, Cong XF, et al. Identification of MicroRNAs involved in hypoxia- and serum deprivation-induced apoptosis in mesenchymal stem cells. International Journal of Biological Sciences 2011;7:762–8.
[158] Kim HW, Mallick F, Durrani S, Ashraf M, Jiang S, Haider KH. Concomitant activation of miR-107/PDCD10 and hypoxamir-210/Casp8ap2 and their role in cytoprotection during ischemic preconditioning of stem cells. Antioxidants and Redox Signalling 2012;17:1053–65.
[159] Kim HW, Jiang S, Ashraf M, Haider KH. Stem cell-based delivery of Hypoxamir-210 to the infarcted heart: implications on stem cell survival and preservation of infarcted heart function. Journal of Molecular and Medicine (Berlin) 2012;90:997–1010.
[160] Liu ZH, Yang G, Zhao T, Cao GJ, Xiong L, Xia W, et al. Small ncRNA expression and regulation under hypoxia in neural progenitor cells. Cellular and Molecular Neurobiology 2011;31:1–5.
[161] Singh SK, Kagalwala MN, Parker-Thornburg J, Adams H, Majumder S. REST maintains self-renewal and pluripotency of embryonic stem cells. Nature 2008;453:223–7.
[162] Niu Z, Goodyear SM, Rao S, Wu X, Tobias JW, Avarbock MR, et al. MicroRNA-21 regulates the self-renewal of mouse spermatogonial stem cells. Proceedings of the National Academy of Sciences of the United States of America 2011;108:12740–5.
[163] Kim YJ, Hwang SJ, Bae YC, Jung JS. MiR-21 regulates adipogenic differentiation through the modulation of TGF-beta signaling in mesenchymal stem cells derived from human adipose tissue. Stem Cells 2009;27:3093–102.
[164] Kim YJ, Hwang SH, Cho HH, Shin KK, Bae YC, Jung JS. MicroRNA 21 regulates the proliferation of human adipose tissue-derived mesenchymal stem cells and high-fat diet-induced obesity alters microRNA 21 expression in white adipose tissues. Journal of Cellular Physiology 2012;227:183–93.
[165] Bao B, Wang Z, Ali S, Kong D, Li Y, Ahmad A, et al. Notch-1 induces epithelial-mesenchymal transition consistent with cancer stem cell phenotype in pancreatic cancer cells. Cancer Letters 2011;307:26–36.
[166] Ng R, Song G, Roll GR, Frandsen NM, Willenbring H. A microRNA-21 surge facilitates rapid cyclin D1 translation and cell cycle progression in mouse liver regeneration. Journal of Clinical Investigation 2012;122: 1097–108.
[167] Sen CK. Wound healing essentials: let there be oxygen. Wound Repair and Regeneration 2009;17:1–18.
[168] Liu X, Cheng Y, Yang J, Xu L, Zhang C. Cell-specific effects of miR-221/222 in vessels: molecular mechanism and therapeutic application. Journal of Molecular and Cellular Cardiology 2012;52:245–55.
[169] Pullen TJ, da Silva Xavier G, Kelsey G, Rutter GA. miR-29a and miR-29b contribute to pancreatic beta-cell-specific silencing of monocarboxylate transporter 1 (Mct1). Molecular and Cellular Biology 2011;31: 3182–94.
[170] Schneider M, Andersen DC, Silahtaroglu A, Lyngbaek S, Kauppinen S, Hansen JL, et al. Cell-specific detection of microRNA expression during cardiomyogenesis by combined in situ hybridization and immunohistochemistry. Journal of Molecular Histology 2011;42:289–99.
[171] Calissano M, Latchman DS. Cell-specific regulation of the pro-survival Brn-3b transcription factor by microRNAs. Molecular and Cellular Neurosciences 2010;45:317–23.
[172] Judson RL, Babiarz JE, Venere M, Blelloch R. Embryonic stem cell-specific microRNAs promote induced pluripotency. Nature Biotechnology 2009;27:459–61.
[173] Neilsen CT, Goodall GJ, Bracken CP. IsomiRs—the overlooked repertoire in the dynamic microRNAome. Trends in Genetics 2012;28:544–9.

[174] Tsuchiya S, Fujiwara T, Sato F, Shimada Y, Tanaka E, Sakai Y, et al. MicroRNA-210 regulates cancer cell proliferation through targeting fibroblast growth factor receptor-like 1 (FGFRL1). Journal of Biological Chemistry 2011;286:420–8.
[175] Mohankumar KM, Xu XQ, Zhu T, Kannan N, Miller LD, Liu ET, et al. HOXA1-stimulated oncogenicity is mediated by selective upregulation of components of the p44/42 MAP kinase pathway in human mammary carcinoma cells. Oncogene 2007;26:3998–4008.
[176] Muralimanoharan S, Maloyan A, Mele J, Guo C, Myatt LG, Myatt L. MIR-210 modulates mitochondrial respiration in placenta with preeclampsia. Placenta 2012.
[177] Fasanaro P, Greco S, Ivan M, Capogrossi MC, Martelli F. microRNA: emerging therapeutic targets in acute ischemic diseases. Pharmacology and Therapeutics 2010;125:92–104.
[178] Wang T, Zhang L, Shi C, Sun H, Wang J, Li R, et al. TGF-beta-induced miR-21 negatively regulates the antiproliferative activity but has no effect on EMT of TGF-beta in HaCaT cells. International Journal of Biochemistry and Cell Biology 2012;44:366–76.
[179] Yang X, Wang J, Guo SL, Fan KJ, Li J, Wang YL, et al. miR-21 promotes keratinocyte migration and re-epithelialization during wound healing. International Journal of Biological Sciences 2011;7:685–90.
[180] Sen CK, Roy S. MicroRNA 21 in tissue injury and inflammation. Cardiovascular Research 2012;96:230–3.
[181] Roy S, Khanna S, Hussain SR, Biswas S, Azad A, Rink C, et al. MicroRNA expression in response to murine myocardial infarction: miR-21 regulates fibroblast metalloprotease-2 via phosphatase and tensin homologue. Cardiovascular Research 2009;82:21–9.
[182] Kumarswamy R, Volkmann I, Jazbutyte V, Dangwal S, Park DH, Thum T. Transforming growth factor-beta-induced endothelial-to-mesenchymal transition is partly mediated by microRNA-21. Arteriosclerosis, Thrombosis, and Vascular Biology 2012;32:361–9.
[183] Zhu H, Li Y, Qu S, Luo H, Zhou Y, Wang Y, et al. MicroRNA expression abnormalities in limited cutaneous scleroderma and diffuse cutaneous scleroderma. Journal of Clinical Immunology 2012;32:514–22.