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MicroRNAs Regulation of Tumor Angiogenesis

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1. Introduction

In the 1970s, Dr. Judah Folkman proposed that many solid tumors require angiogenesis to grow beyond 2 mm in size (Folkman, 1971). His theory held that solid tumors could grow without additional blood vessels up to 1-2 mm, obtaining oxygen and nutrients by diffusion. However, these tumors could undergo an angiogenic switch, releasing substances that would induce new capillary sproutings from existing blood vessels, which would increase oxygen and nutrient delivery to the tumor, permitting tumor expansion. Since Dr. Folkman's hypothesis, assays for angiogenesis have been developed, new angiogenic molecules such as VEGF and VEGF receptors have been discovered, endogenous inhibitors of angiogenesis such as angiostatin have been defined, and anti-angiogenic drugs have been developed to treat cancers (Ferrara, 2002; Ferrara and Kerbel, 2005; Kerbel, 2008). However, the biology of human tumor angiogenesis is poorly understood, and the molecular mechanisms that modulate the angiogenic switch are not well characterized.

MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression in a post-transcriptional manner (Bartel, 2004). miRNAs regulate physiological processes such as cell proliferation, apoptosis, and differentiation (Ambros, 2004; Bartel, 2004; He and Hannon, 2004). However, miRNAs are also involved in pathophysiological processes such as oncogenesis (Croce, 2008, 2009). Recent studies unveiled new functions of miRNAs that control angiogenesis and those miRNAs are thought to be new tools for manipulating tumor angiogenic balance. In this chapter, I summarize how a set of miRNAs can regulate tumor angiogenesis, reviewing the current findings and our data.

I first describe the biogenesis and function of miRNAs and briefly review general roles of miRNAs in cancer. Next I classify a set of miRNAs that positively or negatively regulate tumor angiogenesis and add current progress in this area. Lastly I focus on hypoxia inducible factors as key molecule of hypoxia signaling and tumor angiogenesis in a view of regulation by miRNAs.

2. microRNAs (miRNAs)

Non-coding RNA (ncRNA), lin-4 and lin-14, were first identified to regulate larval developmental timing in *C. elegans* in 1993 (Lee et al., 1993; Wightman et al., 1993). By now, thousands of miRNAs have been discovered in many organisms and mammalian miRNAs are predicted to alter the activity of about 50% of all protein coding genes (Filipowicz et al., 2008). miRNAs control gene expression by destabilizing or inhibiting transcription,

involving in a variety of biological processes. According to mapping of 186 miRNAs and comparison of locations of previous reported nonrandom genetic alterations, Calin et al. discovered that miRNA genes are frequently (more than 50%) located at fragile sites, in minimal regions of loss of heterozygosity, in minimal regions of amplification or in common breakpoint regions (Calin et al., 2004b). As the diverse functions of miRNAs are further studied, our understanding of the molecular pathways of cancers becomes more complicated (Nana-Sinkam and Croce).

2.1 miRNA biogenesis

Figure 1 illustrates intergenic miRNA biogenesis. First mRNAs are transcribed into primary miRNAs (pri-miRNAs) by RNA polymerase II. These long pri-miRNAs contain stem-loop hairpin structures, which are cleaved into smaller pieces (pre-miRNA) by RNase III Drosha and DiGeorge syndrome critical region 8 (DGCR8) complex. Pre-miRNA is transported from nucleus into cytoplasm by exportin 5 and then diced into double stranded mature miRNAs by Dicer and TAR RNA-binding protein 2 (TRBP) complex. Both or one strand of single mature miRNAs is incorporated into Argonaute containing RNA-induced silencing complex (RISC), which bind to target mRNAs and dampen their expression.

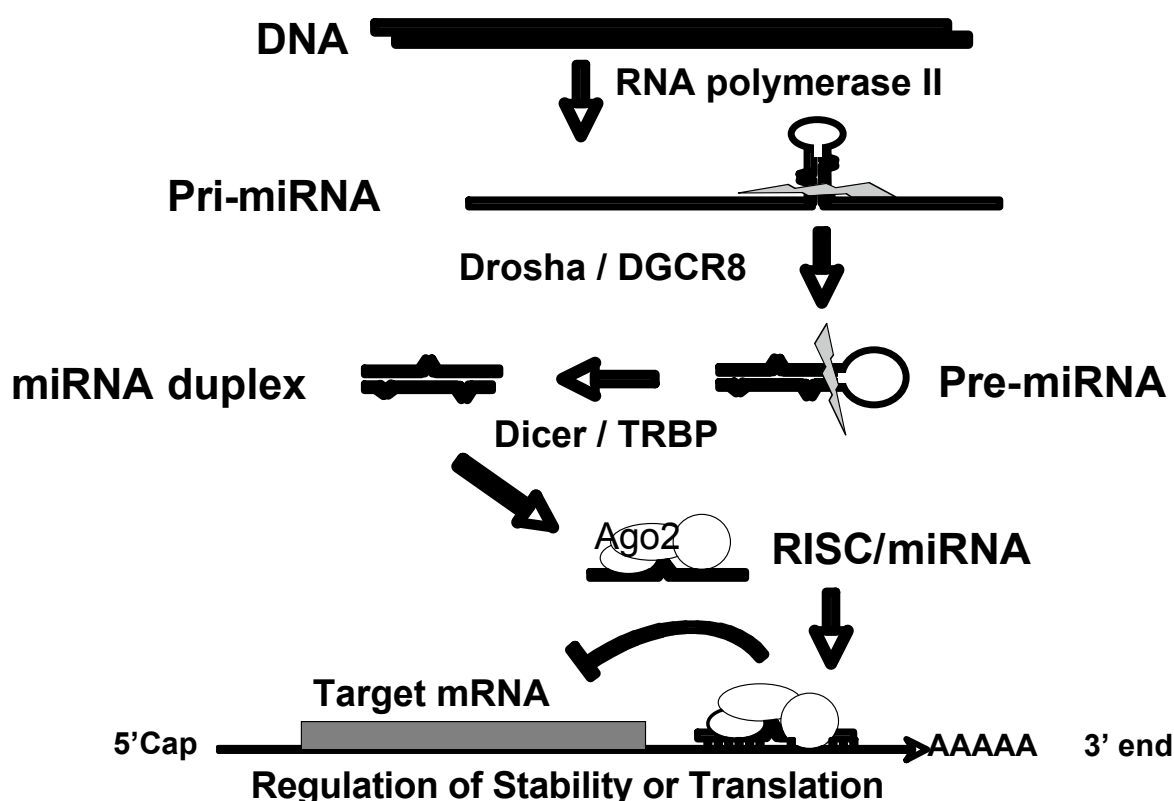


Fig. 1. miRNA biogenesis. DNA is transcribed into a primary micro-RNA (pri-miR). The pri-miR is processed into a 70 nt precursor micro-RNA (pre-miR). The pre-miR is processed into a 22 nt long micro-RNA (miRNA). A single strand of the mature miRNA is loaded onto a nucleoprotein RISC complex that silences target messenger RNAs.

MiRNAs suppress target mRNA expression in two ways, Argonaute-catalyzed cleavage of mRNA or mRNA destabilization (Filipowicz et al., 2008). If the seed sequence of a particular

miRNA perfectly matches the sequence of a target mRNA, then the target mRNA is degraded. Sequence identity between micro-RNA and mRNA often occur in plants. In mammalian cells, there are very few reports about miRNA cleavage of target mRNA (Davis et al., 2005; Jones-Rhoades et al., 2006). Instead, the seed sequence of miRNA usually has homology but not identity to target mRNA in mammalian cells. This imperfect match represses translation by decreasing mRNA stability. The match sequences of 2nd to 6th nucleotide position in 5' end of miRNAs (seed sequence) are sufficient to suppress gene expressions. Some miRNAs suppress target mRNAs expression unless the seed sequence does not completely match to target mRNA target (Bartel, 2009; Shin et al.). Moreover miRNAs can reduce not only the expressions of protein from the target mRNAs, but also the levels of their target transcripts (Lim et al., 2005).

To accomplish miRNA searches, accurate methods for target prediction are required. There are many miRNA target prediction programs available on-line (Hofacker, 2007; Sethupathy et al., 2006). These prediction systems depend on algorithms of miRNA and mRNA (especially region of seed sequence) and evolutionarily conservation. Targetscan, miRanda, and PicTar are commonly used tools for target prediction. Those programs provided a lot of information for potential target genes, but the false positive rate were calculated about 24-70% (Thomson et al.). To avoid false positive target genes, overlapped targets from those three prediction programs are generally chosen as more convincing potential target genes.

2.2 miRNAs function in cancer

Genomic alterations of miRNAs are associated with human cancers (Calin et al., 2004a; Iorio et al., 2005). MiR-15a and miR-16a are frequently downregulated in patients with B cell chronic lymphocytic leukemia (Calin et al., 2004b). Lu et al. performed miRNA expression profiling of 334 human cancer samples and showed the feasibility of classification tumor types and stages on miRNA profiles (Lu et al., 2005). This suggested that monitoring the expression of miRNAs in human cancer is very informative for cancer diagnosis. There have been many studies focusing on this diagnostic role of miRNAs and on the association between cancer specific miRNAs and their function. Let-7 family members regulate the human RAS oncogene; and human lung tumor tissues displayed reduced levels of let-7 and increased levels of RAS protein compared to normal lung tissues, suggesting that let-7 might control oncogenesis in humans (Johnson et al., 2005). Furthermore, p53 upregulated the expression of miR-34 family members, which functions as a tumor suppressor (Chang et al., 2007; He et al., 2007; Raver-Shapira et al., 2007).

3. miRNAs and tumor angiogenesis

Distinct miRNAs regulate tumor angiogenesis in diverse pathways. These miRNAs that regulate angiogenesis are classified into three groups; (1) miRNAs that are not altered under hypoxic condition, (2) hypoxia regulated miRNAs, and (3) miRNAs that regulate HIF-1 signaling molecules. I address functions of each miRNA and discuss two types of angiogenesis miRNAs; pro-angiogenic miRNAs and anti-angiogenic miRNAs (Figure 2).

3.1 Hypoxia independent miRNAs

3.1.1 miR-126

Several studies of miRNA profiling revealed that miR-126 is expressed specifically in endothelial cells (Harris et al., 2008; Kuehbach et al., 2007). MiR-126 is encoded on an

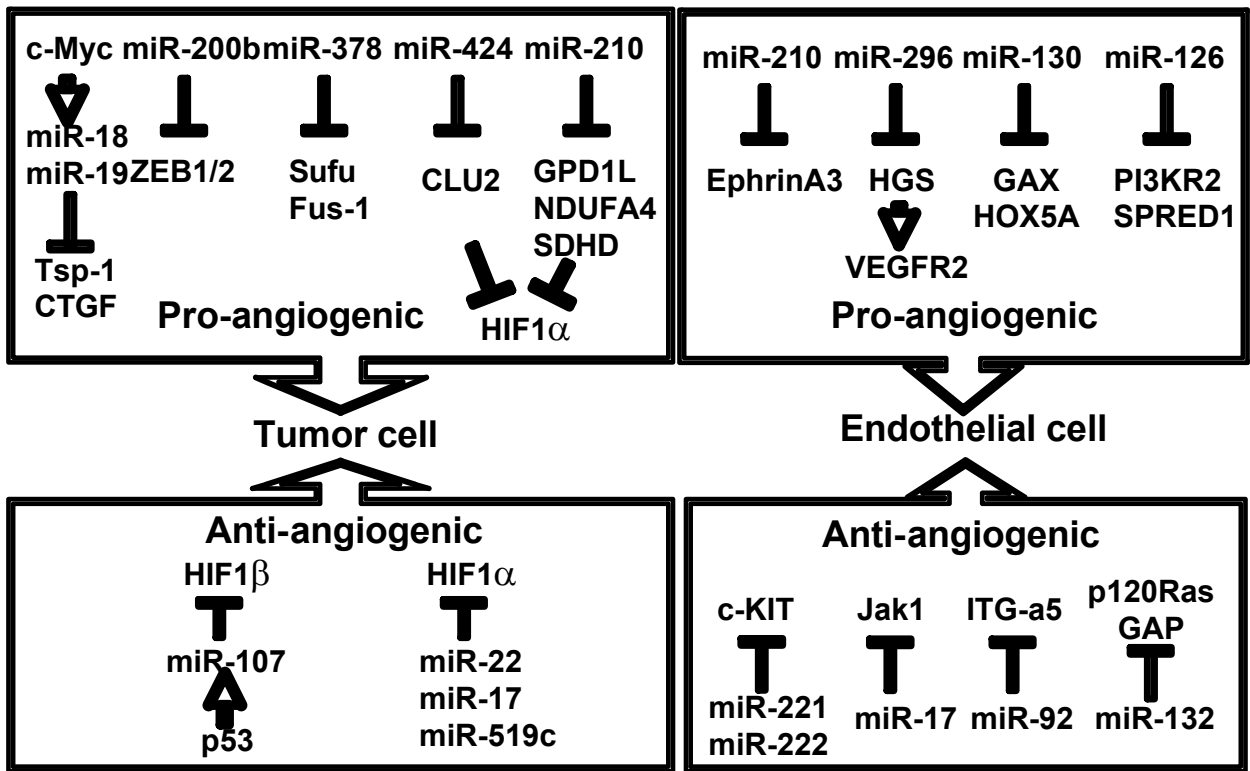


Fig. 2. Role of miRNAs in tumor angiogenesis. Different miRNAs contribute to angiogenic response in tumor cells and endothelial cells.

intron of *Egfl7* gene and *Egfl7* knockout mice displays vascular abnormalities (Schmidt et al., 2007). Endothelial outgrowth was impaired in aortic rings from *miR-126*^{-/-} mice and endothelial cells from *miR-126*^{-/-} mice diminished angiogenic response to FGF-2, suggesting that miR-126 knockout endothelial cells are defective in angiogenesis (Fish et al., 2008; Wang et al., 2008). Moreover Sprouty-related EVH domain-containing protein (Spred-1) and PI3K regulatory subunit 2 (PIK3R2) were defined as target gene of miR-126. Therefore, pro-angiogenic activity of miR-126 is mediated via the increase of MAP kinase and PI3K signaling in response to angiogenic growth factors. In cancer cells, miR-126 behaves as an anti-tumor miRNA. MiR-126 inhibited metastasis in human breast cancer and tumorigenesis in colon cancer and lung cancer (Crawford et al., 2008; Guo et al., 2008; Tavazoie et al., 2008), but the non-cell-autonomous effects of miR-126 in tumor on angiogenesis are still unclear.

3.1.2 miR-132

MiR-132 has been recognized as an angiogenic growth factor inducible miRNA in endothelial cells. In normal endothelial cells, the level of miR-132 is very low, but miR-132 is highly expressed in human tumors and hemangiomas. VEGF and bFGF or conditioned media from tumors activated cAMP-response element binding protein (CREB) and increased miR-132 levels in various types of endothelial cells (Anand et al.; Lagos et al.). Interestingly neurotrophic growth factors also induced miR-132, promoting dendritic growth and spine formation in neuronal culture (Magill et al.). MiR-132 facilitated pathological angiogenesis by suppressing p120RasGAP, a molecular brake for RAS. Knockdown of miR-132 decreased angiogenesis in tumor models (Anand et al.). Moreover, endothelial cells from several pathological human and mouse tissues including tumors had a dramatic

decrease in p120RasGAP expression and a corresponding increase in miR-132 level (Anand and Cheresch). These findings suggest that miR-132 controls an angiogenic switch in tumor cells.

3.1.3 miR-296

The upregulation of growth factor receptors on endothelial cells is an important step in angiogenesis. Angiogenic growth factors increased the expression of miR-296 in human brain microvascular endothelial cells and knockdown of miR-296 inhibited endothelial cell migration and tube formation by modulating hepatocyte growth factor-regulated tyrosine kinase substrate (HGS) (Wurdinger et al., 2008). HGS mediated the sorting of growth factor receptors (PDGFR- β and VEGFR2) to lysosomes to degrade them (Ewan et al., 2006; Takata et al., 2000). Inhibition of miR-296 showed significant decrease glioma angiogenesis in vivo, suggesting that miR-296 acts as a pro-angiogenic miRNA in endothelial cells.

Loss of miR-296 expression was observed during tumor progression in human cancers (Hong et al.). One of potential target proteins was Scribble (Scrib) and overexpression of Scrib in human mammary epithelial cells (HMEC) inhibited cell migration and invasion (Vaira et al.). The levels of Scrib were upregulated in primary lesions and distant metastases of liver, colon, lung, breast and stomach cancer, but the exact function in tumors has been not determined. Another target, high-motility group AT-hook gene 1 (HMGA1) was identified in prostate cancer (Wei et al.). HMGA1 expression was associated with high-grade prostate cancer and proliferative and metastatic potential in vitro (Diana et al., 2005). These findings indicate that inhibition of miR-296 cell-autonomously downregulates tumor angiogenesis.

3.1.4 miR-378

MiR-378 enhanced cell survival and promotes tumor growth and angiogenesis. Knockdown of miR-378 decreased cell survival in U87 glioma cells. Injection of miR-378 overexpressed U87 cells into nude mice increased tumor volume and elevated the size of blood vessels (Lee et al., 2007). At least two potential targets of miR-378 were identified; suppressor of fused (Sufu) and tumor suppressor candidate 2 (Fus-1). Sufu is a negative regulator of sonic hedgehog signaling pathway (Yue et al., 2009). Sonic hedgehog pathway is a crucial regulator of angiogenesis and metastasis and increases angiogenic factors such as angiopoietin-1 and angiopoietin-2 (Pola et al., 2001). Sonic hedgehog promotes capillary morphogenesis, induced endothelial cell migration, and increased the expression of MMP-9 and osteopontin in endothelial cells via Rho kinase (Renault et al.). These data suggested that miR-378 might control not only angiogenic potential in tumor cells but also angiogenic activity in endothelial cells.

3.1.5 miR-17-92 in tumors

The miR-17-92 cluster is a polycistronic miRNA gene, containing six mature miRNAs (miR-17, miR-18a, miR-19a, miR-19b-1, miR-20a, and miR-92) on *c13orf25* transcript (He et al., 2005; Tanzer and Stadler, 2004). These six miRNAs and two miR-17-92 cluster paralogs (miR-106a-363 and miR-106b-25) are categorized into four families by their seed sequences; (1) miR-17 family (miR-17, -20a, -20b, -106a, -106b, and -93), (2) miR-18 family (miR-18a and miR-18b), (3) miR-19 family (miR-19a, -19b-1, and -19b-2), and (4) miR-92 family (miR-92a-1, -92a-2, -383, and -25) (Figure 3A).

The transcripts of region containing miR-17-92 precursor were elevated n various cancers, such as B-cell lymphoma, multiple myeloma, thyroid cancer, and lung cancer (Hayashita et al., 2005; Inomata et al., 2009; Pichiorri et al., 2008; Takakura et al., 2008). This elevation of miR-17-92 contributes to tumor growth partially by enhancing tumor angiogenesis (Dews et al., 2006).

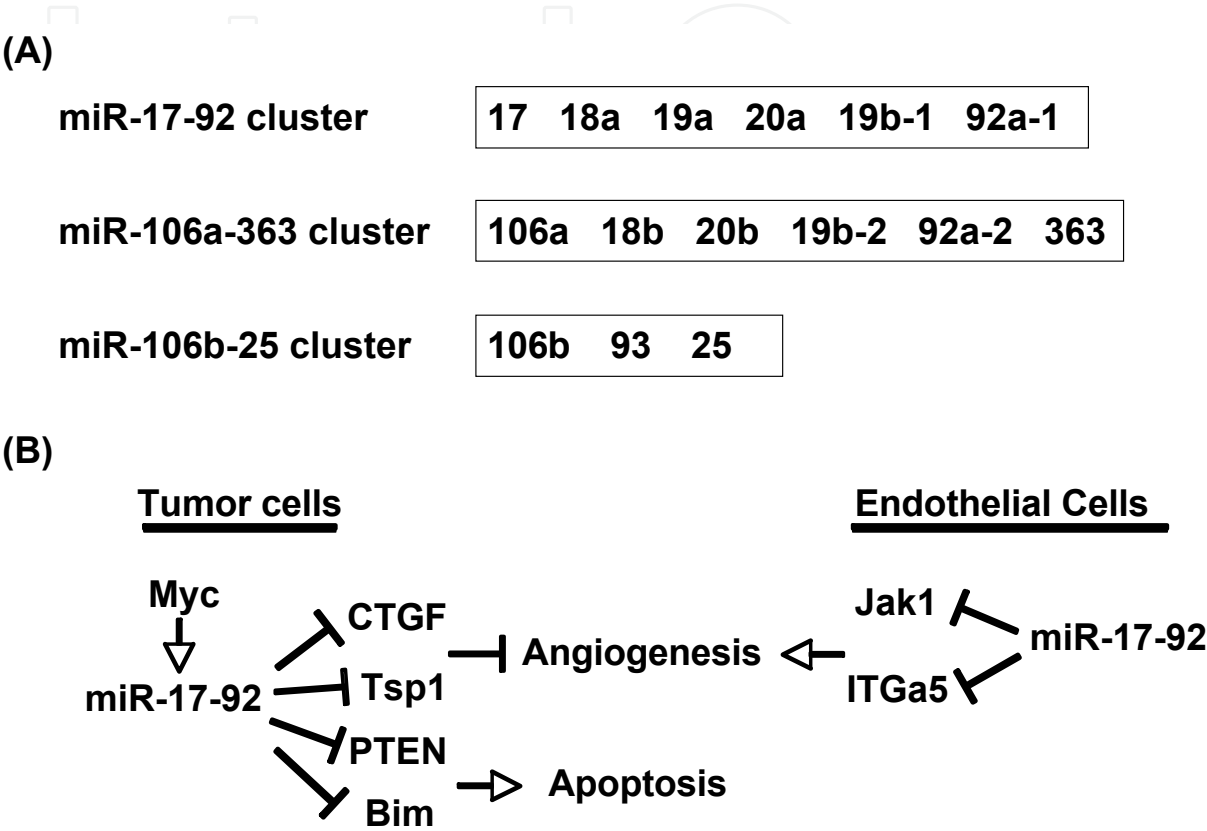


Fig. 3. The pleiotropic functions of miR-17-92 cluster. (A) Primary transcripts of miR-17-92 cluster and its paralogs. (B) Mir-17-92 can promote proliferation and increase angiogenesis in cancer cells. In endothelial cells, miR-17-92 inhibits angiogenesis.

miR-17-92 cluster has been identified as a direct transcriptional target of c-Myc by using a spotted oligonucleotide array (O'Donnell et al., 2005). c-Myc is a helix-loop-helix oncogenic transcription factor that regulates cell proliferation and transformation and activated in many human cancers (Dang, 1999). Myc binds to E-box element with Max, driving cell cycle progression. Myc also negatively regulates some gene transcriptions with Miz-1. Dews et al. have shown that Myc-overexpressing tumors possessed more robust neovascularization and miR-17-92 mediated tumor angiogenesis by Myc (Dews et al., 2006). They also found that miR-17-92 targeted two anti-angiogenic factors, thrombospondin-1 (TSP-1) and connective tissue growth factor (CTGF) expression in RasGfpMyc colonocytes. Another transcription factor that regulates miR-17-92 cluster has been reported. E2F1 and E2F3 also directly activated miR-17-92 transcription as well as turns on the progression of the cell cycle (Sylvestre et al., 2007; Woods et al., 2007). MiR-17 and miR-20 inhibited the expression of E2F1, E2F2, and E2F3 (O'Donnell et al., 2005). These data suggested a negative feedback loop between miR-17-92 cluster, E2F, and Myc.

The regulation and function of miR-17-92 have become more complicated. Each miRNAs in this cluster works cooperatively and independently. Each individual miRNA has different target genes because of their own seed sequences. For example, miR-19, which is thought to be a major oncogenic component of miR-17-92 cluster, suppressed PTEN expression (Olive et al., 2009). In the E μ -myc model of Burkitt's lymphoma, miR-19 was necessary and sufficient for miR-17-92 to promote c-Myc-induced B lymphomagenesis. MiR-17, miR-20, and miR106b regulate cell cycle progression through the inhibition of cyclin dependent kinase inhibitor, p21 (Ivanovska et al., 2008).

3.1.6 miR-17-92 in endothelial cells

The miR-17-92 cluster is highly expressed in endothelial cells (Kuehbachner et al., 2007). Overexpression of miR-92a inhibited angiogenesis in endothelial cells in part by repressing integrin $\alpha 5$ (ITGa5). Inhibition of miR-92a in a mouse model of hind limb ischemia augmented neovascularization and functional recovery of damaged tissue (Bonauer et al., 2009). The same research group has explored the role of the other miRNAs of miR-17-92 cluster in endothelial cells as well (Doebele et al.). Overexpression of all individual members of miR-17-92 cluster (miR-17, miR-18a, miR-19a, and miR-20a) inhibited endothelial proliferation and migration in vitro, in contrast, and individual knockdown enhanced migration and proliferation. MiR-17 and miR-20 were particularly potent inhibitors of endothelial migration, and their effects were mediated in part through the tyrosine kinase Jak1 (Doebele et al.).

These data indicate that individual miRNAs in miR-17-92 cluster suppress angiogenesis of normal endothelial cells in vivo. What about their effect upon angiogenesis of endothelial cells in tumors in vivo? Although inhibitors of miR-17 and miR-20 increased vascularization of Matrigel plugs in mice, but these same inhibitors did not affect tumor angiogenesis (Doebele et al.). These results show that specific miRNAs can regulate angiogenesis in one context (Matrigel plugs) but have no effect upon angiogenesis in another context (tumor angiogenesis). One possible explanation is that different sets of genes modulate angiogenesis in different physiological situations (Figure 3B). Suarez et al. showed that reduction of miRNAs by knockdown of Dicer in endothelial cells impaired angiogenic response and reconstitution of individual miRNAs in miR-17-92 cluster rescued angiogenic inhibition (Suarez et al., 2008). These results show that endothelial miRNAs are important for angiogenesis.

In summary, contradictory data show that miR-17 inhibits angiogenesis following hind limb ischemia, but all miRNAs together promote angiogenesis in hind limb ischemia. The individual contribution to angiogenesis of distinct miRNA inside endothelial cells is not fully known.

3.1.7 miR-221/222

miR-221 and miR-222 are abundant in endothelial cells, and they affect angiogenic activity of stem cell factor (SCF) by modulating the level of its receptor c-Kit (Poliseno et al., 2006). miR-221 strongly upregulated GAX expression by inhibiting ZEB2, a modulator of the epithelial-mesenchymal transition (Chen et al.). GAX is a regulatory gene controlling the angiogenic phenotype in endothelial cells. In several solid tumors, miR-221/222 is highly expressed (le Sage et al., 2007; Medina et al., 2008) and regulated by proto-oncogene ETS-1, involving in the pathogenesis of cancers (Mattia et al.). In melanoma progression, ETS-1 is directly targeted by miR-222, but not by miR-221, which makes a complex ETS-1/miR-222 co-regulatory loop. miR-222 might inhibit angiogenesis by repressing Ets signaling in endothelial cells.

3.2 Hypoxia-regulated miRNAs

3.2.1 miR-210

Hypoxia promotes tumor angiogenesis. Hypoxia induced miRNAs are identified both in cancer and endothelial cells. Hypoxia dramatically increased miR-210 expression (Huang et al., 2009). The expression of miR-210 is higher in many solid tumors and correlates with poor survival in cancer patients (Greither et al.; Huang et al., 2009; Puissegur et al.). Overexpression of miR-210 in endothelial cells stimulated angiogenesis and migration and inhibition of miR-210 decreased hypoxia induced tube formation of endothelial cells (Fasanaro et al., 2008). MiR-210 targeted the receptor tyrosine kinase Ephrin-A3 that involves in vascular remodeling. Glycerol-3-phosphate dehydrogenase (GPD1L) was identified as another direct target of miR-210 in cancer cells (Kelly et al.). GPD1L is linked to the stability of HIF-1 α protein through the activity of prolyl hydroxylases (PHDs). Hypoxia triggered accumulation of miR-210 and increased miR-210 inactivates PHDs by decreasing GPD1L protein, which blocked HIF-1 α degradation. MiR-210 also targeted specific mitochondrial components with consequences on the regulation of cell death and survival and the modulation of HIF-1 activity (Puissegur et al.). Overexpression of miR-210 directly repressed two members of electron transport chain (ETC) complexes: NADH dehydrogenase (ubiquinone) 1 alpha subcomplex 4 (NDUFA4), a subunit of ETC complex I, and succinate dehydrogenase complex, subunit D (SDHD), a subunit of the ETC complex II and induced mitochondrial dysfunctions, which accumulated succinate, inhibiting PHDs activity, then increasing HIF-1 α . These two models represent a positive feedback loop. These data suggest that miR-210 has pro-angiogenic activity. This hypoxia-induced miRNA could be one of promising tumor markers.

3.2.2 miR-424

Hypoxia increased miR-424 level in endothelial cells and in ischemic tissues undergoing vascular remodeling and angiogenesis. Increased level of miR-424 led to degradation of an ubiquitin ligase scaffold protein cullin-2 (CLU2), which prevented HIF-1 α downregulation by destabilizing the E3-ligase assembly (Ghosh et al., 2010). The expression of miR-424 was regulated by C/EBP- α /RUNX-1-mediated transactivation of PU.1. This finding indicates that miR-424 acts as a pro-angiogenic factor.

3.2.3 miR-200b

MiR-200b, one of five members of miR-200 family inhibited cell migration and epithelial-mesenchymal transition (EMT) in epithelial cancer cells by modulating the expression of zinc finger E-box-binding homeobox 1 and 2 (ZEB1 and ZEB2) (Burk et al., 2008; Park et al., 2008). The miR-200 family is regulated by transforming growth factor β 1 (TGF- β 1) and platelet-derived growth factor (PDGF) (Gregory et al., 2008). MiR-200b is inducible by hypoxia and down-regulates v-ets erythroblastosis virus E26 oncogene homolog 1 (ETS-1), promoting angiogenesis activity in endothelial cells (Chan et al., 2011).

3.3 HIF-1 and miRNAs

Continued tumor growth depends upon obtaining oxygen and nutrients. As tumor grows, the center of tumor becomes hypoxic, causing an angiogenic switch (Kaelin, 2005). In the presence of oxygen, prolyl hydroxylase domain protein 2 (PHD2 or EGLN1) hydroxylates prolyl residues on hypoxia-inducible factor-1 alpha (HIF-1 α) (Semenza, 2007). After prolyl

hydroxylation, HIF-1 α binds to a complex containing von Hippel-Lindau (VHL) which leads to HIF-1 α degradation. However, under hypoxic conditions, HIF-1 α is not prolyl hydroxylated. Instead, HIF-1 α interacts with its partner HIF-1 β , and the HIF-1 complex translocates to the nucleus, binds to hypoxia responsive element (HRE), and regulates transcription of hundreds of genes. Genes transactivated by HIF-1 help to adapt the cells to low oxygen levels, controlling erythropoiesis, glycolysis, and angiogenesis (Harris, 2002; Semenza, 2003). Interest in the role of HIF-1 in cancer has been growing and HIF-1 is thought to be a therapeutic target of cancer progression (Semenza, 2003). Therefore we demonstrate a set of miRNAs that affect HIF-1 complex (Figure 4).

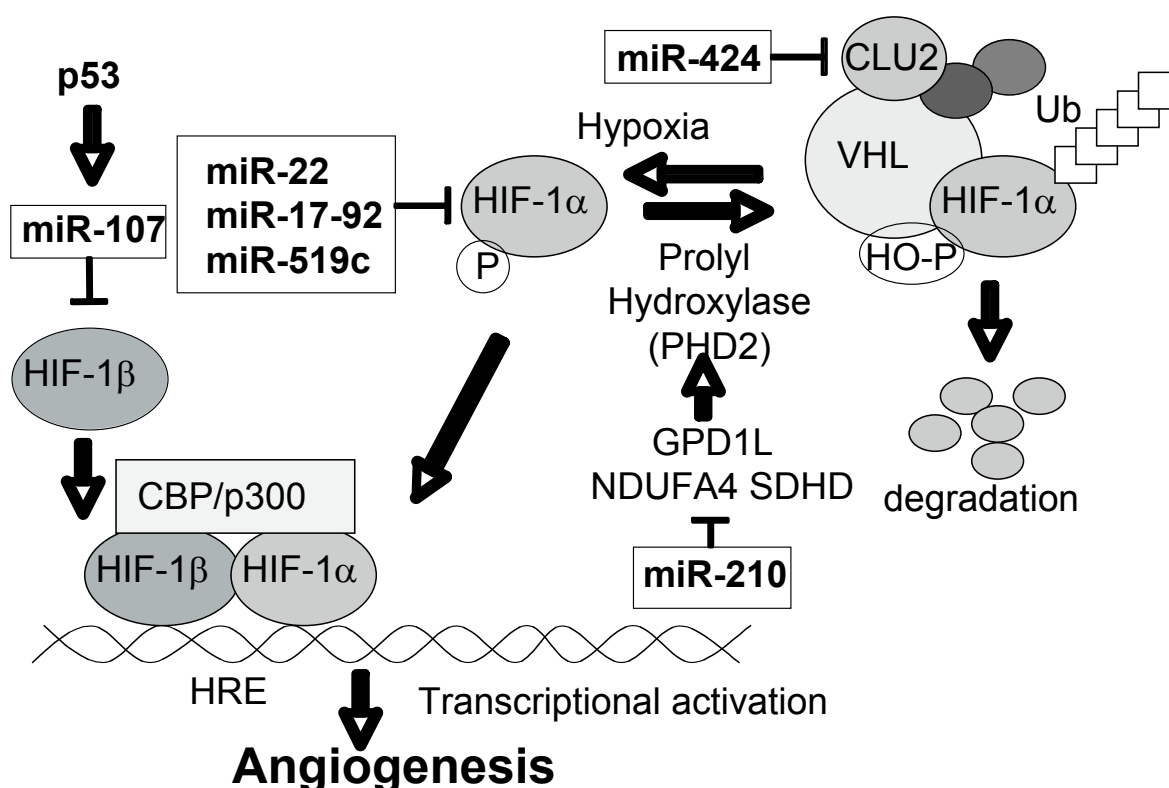


Fig. 4. Regulation of hypoxia signaling via miRNAs. Normally prolyl hydroxylase (PHD) hydroxylates HIF-1 α on prolyl residues, marking HIF-1 α for destruction through pVHL. However, during hypoxia, HIF-1 α is not hydroxylated, and instead HIF-1 α binds to HIF-1 β , translocates to the nucleus, and activates gene transcription. A set of miRNAs regulates hypoxia signaling.

3.3.1 HIF-1 α

HIF-1 α expression is associated with poor prognosis in a variety of cancer such as breast, ovary, and brain cancer (Semenza, 2003). There are several miRNAs that regulate HIF-1 α . First HIF-1 α was reported as the target of miR-17-92 miRNA cluster in lung cancer cells (Taguchi et al., 2008). Taguchi et al. performed proteomic comparison between a miR-17-92-transfected clone and an empty vector-transfected clone from normal human bronchial epithelial cell line and then found that miR-17-92 targets HIF-1 α . MiR-20b, located on 106a-

363 cluster, a member of miR-17-92 family, was also shown to regulate HIF-1 α expression (Lei et al., 2009). The inhibition of miR-20b increased HIF-1 α protein and VEGF in normoxic tumor cells. In contrast, the increase of miR-20b in hypoxic tumor cells decreased the expression of HIF-1 α and VEGF. HIF-1 α , VEGF, and STAT3 are identified to be direct targets of miR-20b (Cascio et al.; Lei et al., 2009). As describing above, the miR-17-92 family has a complicated network to control tumor angiogenesis.

MiR-519c reduced tumor angiogenesis through direct binding to the HIF-1 α 3' UTR (Cha et al.). Mice injected with lung adenocarcinoma cells overexpressing miR-519c suppressed tumor angiogenesis, growth, and metastasis. Interestingly hepatocyte growth factor (HGF) suppressed miR-519c expression. The expression of miR-519c was closely correlated with tumor progression in human lung cancer tissues.

We found that miR-22 regulated HIF-1 α in HCT116, human colon cancer cells (Yamakuchi et al.). Overexpression of miR-22 decreased transactivation of the 3'UTR of HIF-1 α . In contrast, miR-22 did not alter transactivation of HIF-1 α when the miR-22 binding site within the 3' UTR of HIF-1 α is mutated. Thus miR-22 directly regulates expression of HIF-1 α . The function of miR-22 varies between different cancer types. The human miR-22 gene is located within a loss of heterozygosity region (LOH) in several cancer cells (Calin et al., 2004b; Xiong et al.). Ectopic expression of miR-22 suppressed proliferation in breast cancer cells (Xiong et al.). In contrast, knockdown of miR-22 increases apoptosis in human bronchial epithelial cells (Liu et al.). We found that miR-22 downregulated VEGF secretion and inhibited endothelial cell migration, suggesting that miR-22 acts as an anti-angiogenesis factor in colon cancer cell lines. Moreover the expression of miR-22 is inversely correlated to the level of VEGF in human colon cancer (Yamakuchi et al.), supporting the idea that miR-22 has an anti-angiogenic effect.

3.3.2 HIF-1 β

We recently reported that miR-107 regulated tumor angiogenesis in colon cancer (Yamakuchi et al.). First we performed miRNAs profiling to identify miRNAs expressed in human colon cancer samples and found several miRNAs that are highly expressed in human colon cancer specimen such as miR-923, miR-1826, and miR-1915. The tumor suppressor gene p53 is mutated in more than half of all human cancer (Vogelstein and Kinzler, 2004). Since anti-angiogenic therapy is more effective in tumors with wild-type p53 than in tumors with mutant p53, p53 plays an important role in the regulation of tumor angiogenesis. Therefore we selected miRNAs regulated by p53. In the colon cancer cell line HCT116, we've identified several miRNAs induced by p53 (Chang et al., 2007). Furthermore we searched for miRNAs that can target HIF-1 signaling genes using prediction programs described above. Finally we've chosen miR-23a, miR-26, miR-103, and miR-107 as candidate regulators of tumor angiogenesis. Among these miRNAs, only miR-107 could regulate hypoxia signaling and was regulated by p53 in vitro. HIF-1 β was shown to be a target gene of miR-107 by Western blotting and reporter gene assay. HIF-1 β (also called ARNT, dioxin receptor) is a basic helix-loop-helix transcription factor and a counterpart of HIF-1 α (Fukunaga et al., 1995; Reisz-Porszasz et al., 1994). Homozygous knockout of HIF-1 β in mice was lethal because of poor placentation and decreased branching of the placental vasculature (Kozak et al., 1997; Maltepe et al., 1997). One of the interesting points in our findings is the regulation of HIF-1 β . HIF-1 β has been thought to be constitutively expressed in many cells (Wang et al., 1995). Our data indicated that miR-107 modulated the expression of HIF-1 β , following tumor angiogenesis.

miR-107 controlled tumor angiogenesis through suppressing HIF-1 β in vivo. HCT116 tumor cells overexpressing miR-107 subcutaneously implanted in nude mice grew slower than control cells. To exclude the effect of p53, p53 genetically knockout HCT116 cells were used. Injection of p53 knockout HCT116 cells obtained bigger tumor compared to p53 wild type HCT116 cells. When p53 knockout HCT116 cells with overexpressing miR-107 were injected, the size of tumor and the number of vessels in tumor were significantly decreased compared to p53 knockout HCT116 cells transduced with scramble miRNA.

In human colon cancer, there was a negative correlation between the expression of miR-107 and VEGF level. Data from both clinical studies and experimental models suggested that miR-107 is an anti-angiogenic miRNA.

3.3.3 HIF-2 α and HIF-3 α

HIF-2 α and HIF-3 α has been identified as partners with HIF-1 β . HIF-2 α is closely related to HIF-1 α , and both activate HRE-dependent gene transcription (Wenger, 2002). HIF-3 α lacks structures for transactivation existed in the C-termini of HIF-1 α and HIF-2 α . HIF-2 α might contribute to tumor angiogenesis in some cells (Burkitt et al., 2009; Favier et al., 2007; Giatromanolaki et al., 2006). However regulation of HIF-2 α or HIF-3 α by miRNAs has not reported yet.

4. Conclusion

Tumor angiogenesis is a complex process, consisting of a balance between pro-angiogenic factors and anti-angiogenic factors. Since Dr. Folkman generated the hypothesis that tumors will die without an adequate blood supply, anti-angiogenic therapies for cancer patients have been rapidly developed. Research into miRNA has produced new insights into tumorigenesis. New data on regulation of tumor angiogenesis by miRNAs suggest that miRNAs are promising therapeutic molecules to prevent and treat cancer.

5. Acknowledgment

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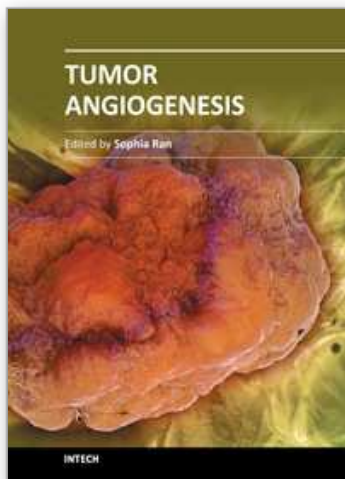
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Tumor angiogenesis is the main process responsible for the formation of new blood vessels that promote tumor growth and metastasis. This process is driven by potent pro-angiogenic factors that are predominant in the tumor environment and are produced by both malignant cells and the host cells recruited to the tumor site. Tumor environment is characterized by the imbalance between pro-angiogenic and anti-angiogenic factors, which drives the construction of numerous but structurally defective vessels. These poorly perfused and abnormal vessels significantly contribute to the tumor pathology not only by supporting the expansion of the tumor mass but also by promoting chronic inflammation, enhancing thrombosis, impeding drug delivery, and disseminating tumor cells. These problems associated with tumor vasculature continue to attract great attention of scientists and clinicians interested in advancing the understanding of tumor biology and development of new drugs. This book compiles a series of reviews that cover a broad spectrum of current topics related to the pathology of tumor blood vessels including mechanisms inducing new vessels, identification of new targets for inhibition of tumor angiogenesis, and potential clinical use of known and novel anti-angiogenic therapies. The book provides an update on tumor angiogenesis that could be useful for oncologists, cancer researchers and biologists with interests in vascular and endothelial cell behavior in the context of cancer.

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