CELLULAR & MOLECULAR BIOLOGY LETTERS
http://www.cmbl.org.pl

Received: 29 August 2012
Final form accepted: 24 October 2012
Published online: 31 October 2012

Volume 18 (2013) pp 47-57
DOI: 10.2478/s11658-012-0037-0
© *2012 by the University of Wrocław, Poland*

Mini review

REGULATION OF ANGIOGENESIS BY HYPOXIA: THE ROLE OF microRNA

PIOTR MADANECKI[1], NIREN KAPOOR[2], ZSUZSA BEBOK[3], RENATA OCHOCKA[1], JAMES F. COLLAWN[3] and RAFAL BARTOSZEWSKI[1,]*
[1]Department of Biology and Pharmaceutical Botany, Medical University of Gdansk, Poland, [2]Department of Neurology, University of Alabama at Birmingham, USA, [3]Department of Cell Biology, Developmental, and Integrative, University of Alabama at Birmingham, USA

Abstract: Understanding the cellular pathways that regulate angiogenesis during hypoxia is a necessary aspect in the development of novel treatments for cardiovascular disorders. Although the pathways of angiogenesis have been extensively studied, there is limited information on the role of miRNAs in this process. miRNAs or their antagomirs could be used in future therapeutic approaches to regulate hypoxia-induced angiogenesis, so it is critical to understand their role in governing angiogenesis during hypoxic conditions. Although hypoxia and ischemia change the expression profile of many miRNAs, a functional role for a limited number of so-called hypoxamiRs has been demonstrated in angiogenesis. Here, we discuss the best examples that illustrate the role of hypoxamiRs in angiogenesis.

Key words: Angiogenesis, Hypoxia, microRNA, miRNA, HypoxamiR, HIF, VEGF

---

* Author for correspondence: Department of Biology and Pharmaceutical Botany, Medical University of Gdansk, Hallera 107, 80-416 Gdansk, Poland. e-mail: rafalbar@gumed.edu.pl, tel: +48 58 349 32 14; fax: +48 58 349 32 11

Abbreviations used: 3'UTR – 3'-untranslated region; CUL2 – cullin 2; DDX6 – member six of the DEAD box protein family; DUSP2 – dual-specificity phosphatase-2; EFNA3 – ephrin-A3; ERK – extracellular signal-regulated kinases; Ets-1 – v-ets erythroblastosis virus E26 oncogene homolog 1; FIH – factor inhibiting HIF-1; HIF – hypoxia-inducible factor; hnRNP L – heterogeneous nuclear ribonucleoprotein L; HRE – hypoxia-response element; miRNA – microRNA; PHD2 – proline-hydroxylase-2; RISC – miRNA-induced silencing complex; SIRi1 – sirtuin; STAT3 – signal transducer and activator of transcription 3; VEGF – vascular endothelial growth factor; VEGFR2 – vascular endothelial growth factor receptor-2; VHL – gene encoding von Hippel-Lindau tumor suppressor protein

INTRODUCTION

Exposure of a cell or an organism to inadequate oxygen levels causes hypoxia and results in global cellular changes in gene expression [1]. Although hypoxia is an integral component of cell physiology in development [2], it is also associated with pathological events such as cardiovascular disorders, inflammation, solid tumors and ischemic disease [3-7]. These pathological events then lead to the restoration of oxygen homeostasis through the activation of repair mechanisms, such as angiogenesis, which is the process of developing new microvessels from pre-existing ones [8]. While post-ischemic tissue revascularization is crucial in neuronal tissues following stroke [9] or in the heart following myocardial infarction [10], the activation of angiogenesis is harmful in other disorders, such as macular degeneration and glaucoma [11] and in many types of cancer [8]. Therefore, there is great interest in using angiogenesis regulation as a possible therapeutic method. Recent studies [12-14] on the role of miRNAs during hypoxia and ischemia have provided a new and interesting link between hypoxia and the regulation of angiogenesis.

miRNAs are 22- to 26-nucleotide, non-coding RNAs that regulate gene expression post-transcriptionally. They act as adaptors for the miRNA-induced silencing complex (RISC) to initiate mRNA decay and thus reduce protein output. Mature miRNAs recognize their target mRNAs through base-pairing interactions between nucleotides numbers 2 and 8 of the miRNA (the seed region) and the complementary nucleotides in the 3'-untranslated region (3'UTR) of the mRNAs [15]. It is estimated that there are more than 1,000 miRNA genes in the human genome, and these could regulate more than one-third of the mRNAs produced [16]. It should be emphasized that many miRNAs are expressed in tissue- and age-specific patterns, suggesting that miRNAs have cell type-specific functions [17, 18], see also [58]. For example, hypoxic conditions in proximal tubule kidney epithelial cells (HK-2) cause the induction of 17 miRNAs and the repression of 7 [19], while in primary fibroblasts only 3 out of 377 miRNAs were repressed during hypoxia [20].

Although hypoxia and ischemia change the expression profiles of many miRNAs [21], a functional role for a limited number of so-called hypoxamiRs [22] in angiogenesis has been demonstrated. We discuss the best examples that illustrate the role of hypoxamiRs in angiogenesis below (Fig. 1) and summarized in Table 1.

HypoxamiRs ASSOCIATED WITH ANGIOGENESIS

HIF-related miRNAs: miR-20a miR-20b, miR-199a, miR-424, miR-130a, miR-130b, miR-155 and miR-210

Hypoxia-inducible factor (HIF) is a key transcription factor in the cellular response to hypoxia HIF is a heterodimeric complex that consists of a hypoxia-inducible, unstable α-subunit and a stable, constitutively expressed β-subunit (also called ARNT1) [23]. Three HIF-α isoforms have been identified in higher metazoans. HIF-1α and HIF-2α share some transcriptional targets and have some

that are unique to each subunit, while HIF-3α has a dominant-negative effect on HIF-dependent gene transcription [24-26].

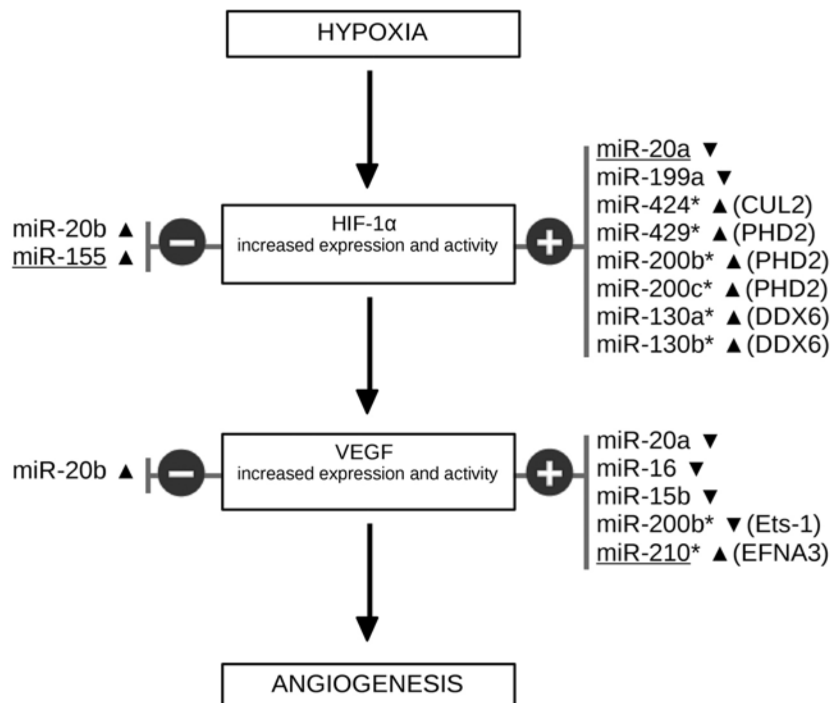

Fig. 1. The influence of hypoxamiRs on hypoxia-induced angiogenesis. During hypoxia, HIF-1α accumulates and is transported to the nucleus, where it can bind to the promoter region of VEGF, termed the hypoxia-response element (HRE), and thereby induce VEGF expression. VEGF is a pivotal angiogenic factor that binds to specialized receptors on the surfaces of endothelial cells and directs them to build new vessels. (▲) induced during hypoxia; (▼) repressed during hypoxia; (+) expression profile changed during hypoxia contributing to increased expression and activity of HIF-1α and/or VEGF; (-) expression profile changed during hypoxia with a negative effect on the expression and activity of HIF-1α and/or VEGF; (*) indirect effects on HIF-1α and/or VEGF, gene symbol in brackets represents direct miRNA target. HypoxamiRs under HIF-1α transcriptional control are underlined.

Under normal oxygen pressure (normoxia), the α-subunit is degraded by the proteasome. It is constitutively targeted for degradation via post-translational modification by proline-hydroxylase-2 (PHD-2) and by von Hippel-Lindau-ubiquitin ligase complexes. Therefore, the HIF-1 complex does not function during normal oxygen pressure [27]. Another protein that contributes to HIF-1 inactivation under normoxic conditions is factor inhibiting HIF-1 (FIH), which also hydroxylates HIF-1 [28]. Since PHD-2 itself is activated by HIF-1, the levels of the HIF-1 complex are regulated via feedback inhibition [29].

HIF is stable during hypoxia because the hydroxylases PHD-2, VHL, and FIH are all inhibited during low oxygen pressure. Once stabilized, the HIF-1 protein can bind to the promoter regions of its target genes, termed hypoxia-response elements (HREs), and thereby induce target gene expression [30, 31]. HIF-dependent transcriptional changes regulate a broad spectrum of cellular functions [23], including angiogenesis. The vascular endothelial growth factor (VEGF) gene is a major transcriptional target [32]. Thus, miRNAs that target HIF are likely to have significant impact on the angiogenesis pathways. It is clear that increasing the vascular network is the primary mechanism for providing oxygen to hypoxic tissues. At present, nine hypoxamiRs that affect HIF expression have been identified: miR-20b, miR-199a, miR-424, miR-130a, miR-130b, miR-200b, miR-200c, miR-429 and miR-155. However, HIF mRNA is a direct target for only three of these miRNAs: miR-20a, miR-20b and miR-199a. Besides being a target of miRNA regulation, HIF is also responsible for the transcription of angiogenic hypoxamiRs such as miR-210 [33]. Each of the HIF-related miRNAs is described in detail below.

miR-20a is downregulated by hypoxia in human nasopharyngeal carcinoma cells (CNE) [34], and it directly targets the 3'UTR of *HIF-1α [35]. Thus,* inhibition of miR-20a production by hypoxia contributes to increased HIF-1α and VEGF protein levels. miR-20a is also upregulated by hypoxia in endometrial stromal cells, where it contributes to the downregulation of dual-specificity phosphatase-2 (DUSP2), leading to prolonged extracellular signal-regulated kinase (ERK) phosphorylation and an increase in the expression of several angiogenic genes [36]. Elevation of miR-20a is upregulated by HIF-1α [36], suggesting the possibility of a negative feedback loop for HIF-1α activity.

miR-20b is upregulated during chemically induced hypoxia ($CoCl_2$) in breast cancer cells (MCF-7), and it targets HIF-1α mRNA [37]. miR-20b also targets the signal transducer and activator of transcription 3 (STAT3) mRNA, and thus affects VEGF expression [37]. A direct interaction between miR-20b and HIF-1α has been also confirmed in H22 cells [35].

miR-199a is downregulated in cardiac myocytes during reduced oxygen pressure and is responsible for accumulation of HIF-1α [38]. Both direct (target site at 3'UTR of HIF-1α mRNA) and indirect interactions were implied in miR-199a-dependent HIF-1α accumulation [38]. To explain the latter, downregulation of miR-199a allows the de-repression of sirtuin (SIRi1), a class III histone deacetylase that downregulates PHD-2, allowing for the stabilization of the HIF-1α protein [38].

miR-424 is induced by hypoxia in endothelial cells, and it targets cullin 2 (CUL2), a scaffolding protein critical to the assembly of the ubiquitin ligase system. Inhibition of this system stabilizes HIF-α isoforms [39]. Furthermore, miR-424 promotes angiogenesis *in vitro* and in mice [39]. The rodent homolog of human miR-424, mu-miR-322, is induced in parallel with HIF-1α in ischemia [39].

miR-130 family (*miR-130a* and *miR-130b*) levels are elevated by hypoxia in human kidney cells (HEK293). Their target is member six of the DEAD box

protein family mRNA (DDX6) [40]. Reduction of DDX6 expression by the miR-130 family enhances the translation of HIF-1α in an internal ribosome entry site element-dependent manner [40].

miR-155 is upregulated by hypoxia in human epithelial colorectal adenocarcinoma cells (Caco2) and in the mouse intestine. It contributes to a decrease in the levels of HIF-1α mRNA and protein, and to a decrease in transcriptional activity [41]. A role for HIF-1α in the induction of miR-155 during hypoxia has been confirmed [41]. Thus, miR-155 induction commits to an isoform-specific negative-feedback loop for HIF-1α activity during prolonged hypoxia [41].

miR-210 is the most consistently and significantly induced miRNA during hypoxia. It is also unique in that it is induced in almost all studied cell lines [22, 42]. The expression of this miRNA is regulated by both HIF-1α [43] and HIF-2α [44]. miR-210 targets the receptor tyrosine kinase ligand ephrin-A3 (EFNA3), which is important for the differentiation of human umbilical vein endothelial cells (HUVEC) under hypoxia and significantly increases the ability of HUVEC to migrate in response to VEGF [25]. However, the specific actions of EFNA3 in angiogenesis require further clarification. Furthermore, overexpression of miR-210 in HUVEC enhances the expression of VEGF and vascular endothelial growth factor receptor-2 (VEGFR2) and thereby promotes angiogenesis [45].

VEGEF-related miRNAs: miR-20a, miR-20b, miR-15b and miR-16

Vascular endothelial growth factor (VEGF) is a pivotal angiogenic factor that binds to specialized receptors on the surfaces of endothelial cells and directs them to build new vessels [46]. Although VEGF expression can be modulated by many factors [47], HIF-dependent VEGF upregulation is accompanied by an increase in VEGF mRNA stability and translation, which are essential for hypoxia-related angiogenesis [48-50]. In spite of the indirect impact of *miR-20a* and *miR-20b* on VEGF levels (through HIF-1α) [35, 37], their functional target sequence on the 3'UTR of VEGF mRNA has been confirmed [34, 35]. *miR-15b* and *miR-16* are sharply downregulated in CNE cells during hypoxia. They also target the 3'UTR of VEGF. However, the direct effect of these miRNAs on endothelial cells has not been determined [34].

Since some miRNAs that have been identified as VEGF regulators (miR-20a and miR-20b) also regulate the expression of other angiogenic factors [34], additional studies are needed to evaluate the significance of the discussed direct and indirect effects on VEGF levels. Recent studies have also established that heterogeneous nuclear ribonucleoprotein L (hnRNP L), which also binds the VEGFA mRNA 3'UTR CA-rich element, prevents miRNA silencing activity during hypoxia [51].

PHD-2 related miRNA: miR-200b, miR-200c, and miR-429

Prolyl hydroxylases (PHDs) catalyze the prolyl hydroxylation of HIF-α subunits, which constitutively targets them for VHL-dependent 26S proteasomal degradation to control HIF levels [52]. PHD enzymes are inhibited during hypoxic conditions, allowing for HIF accumulation and subsequent induction of

angiogenesis [52]. PHD-2 is believed to be the key propyl hydroxylase in controlling HIF-1α during hypoxia [53]. Under normoxic conditions, molecular oxygen, 2-oxoglutarate, iron ions (Fe^{2+}) and ascorbic acid are required to fully activate these enzymes [54]. Additionally, HIF-1 inactivates PHD-2 in a negative feedback manner [29]. Although PHD-2 is inactive during hypoxia, PHD-2 levels are also increased by hypoxia, providing a HIF-1-dependent auto-regulatory mechanism driven by oxygen pressure [55].

miR-200b, miR-200c and *miR-429* levels increase during ischemic preconditioning. These miRNAs target PHD-2 leading to accumulation of HIF-1α and induction of angiogenesis [56]. However, a recent study demonstrated that miR-200b overexpression in human microvascular endothelial cells (HMECs) suppressed the angiogenic response, whereas miR-200b-depleted HMECs exhibited elevated angiogenesis [57]. In HMECs, miR-200b levels were inhibited by hypoxia, and the direct target for this miRNA was v-ets erythroblastosis virus E26 oncogene homolog 1 (Ets-1) mRNA, a crucial angiogenesis-related transcription factor [57]. Thus, hypoxia-induced miR-200b inhibition allows Ets-1 accumulation to promote angiogenesis [57].

CONCLUDING REMARKS

It is clear that understanding the cellular pathways that regulate angiogenesis during hypoxia is necessary in order to develop novel treatments for cardiovascular disorders. Although the pathways of angiogenesis have been extensively studied, there is limited information regarding the role of miRNAs in this process. Considering the fact that miRNAs or their antagomirs could be used in future therapeutic approaches to regulate hypoxia-induced angiogenesis, it is critical to understand the role of miRNAs in governing angiogenesis during hypoxia. Given that tumor growth is critically dependent on the induction of angiogenesis, the therapeutic use of miRNAs and antagomirs to regulate this process is clearly important. That said, this process is complicated and careful consideration should be given to any therapeutic intervention. For example, miRNAs can bind multiple targets and potentially be both positive and negative regulators of gene expression. Thus, miRNAs could cause the opposite biological effect depending on the context, as exemplified by miR-200b [56, 57]. Furthermore, some of the miRNA targets are at the same time miRNA transcriptional activators, e.g. miR-20b, and therefore create complicated regulatory loops that need to be carefully considered [36]. Finally, one has to be aware of the cell- and tissue-specific differences in miRNA expression during hypoxia. Despite these concerns, the very promising reports of hypoxamiRs regulating angiogenic processes show the potential for future therapeutic endeavors. Understanding the role of miRNAs in angiogenesis will remain an active area of research.

Table 1. HypoxamiRs associated with angiogenesis

miRNA	Cell type	Impact of hypoxia on miRNA expression	miRNA target(s) (direct or indirect*)	Putative impact on angiogenesis	References
miR-20a	CNE	Downregulated	HIF-1α VEGF	Antiangiogenic	[34, 35]
miR-20a	Endometriotic stromal cells	Upregulated	DUSP2	Proangiogenic	[36]
miR-20b	Mcf-7 H22	Upregulated	HIF-1α STAT3 VEGF	Antiangiogenic	[34]
miR-199a	Cardiac myocytes	Downregulated	HIF-1α SIRi1/PHD-2*	Antiangiogenic	[38]
miR-424	HUVEC MVEC	Upregulated	CUL2/ HIF-1α*	Proangiogenic	[39]
miR-130a and miR-130b	HEK293	Upregulated	DDX6/ HIF-1α*	Proangiogenic	[40]
miR-155	Caco2	Upregulated	HIF-1α	Antiangiogenic	[41]
miR-210	HUVEC and the majority of the studied cell lines	Upregulated	EFNA3	Proangiogenic	[22, 42]
miR-15b	CNE	Downregulated	VEGF	Antiangiogenic	[34]
miR-16	CNE	Downregulated	VEGF	Antiangiogenic	[34]
miR-200b	HMEC	Downregulated	Ets-1	Antiangiogenic	[57]
miR-200b	Neuro-2a	Upregulated	PHD2	Proangiogenic	[56]
miR-200c	Neuro-2a	Upregulated	PHD2	Proangiogenic	[56]
miR-429	Neuro-2a	Upregulated	PHD2	Proangiogenic	[56]

Acknowledgments. This study was supported by National Science Center OPUS Programme under contract DEC-2011/03/B/NZ3/04387.

REFERENCES

1. Rocha, S. Gene regulation under low oxygen: holding your breath for transcription. **Trends Biochem. Sci.** 32 (2007) 389-397.
2. Guillemin, K. and Krasnow, M.A. The hypoxic response: huffing and HIFing. **Cell** 89 (1997) 9-12.
3. Semenza, G.L. Hypoxia and cancer. **Cancer Metastasis Rev.** 26 (2007) 223-224.
4. Eltzschig, H.K. and Carmeliet, P. Hypoxia and inflammation. **N. Engl. J. Med.** 364 (2011) 656-665.
5. Pierson, D.J. Pathophysiology and clinical effects of chronic hypoxia. **Respir. Care** 45 (2000) 39-51; discussion 51-53.
6. Fulton, A.B., Akula, J.D., Mocko, J.A., Hansen, R.M., Benador, I.Y., Beck, S.C., Fahl, E., Seeliger, M.W., Moskowitz, A. and Harris, M.E. Retinal degenerative and hypoxic ischemic disease. **Doc. Ophthalmol.** 118 (2009) 55-61.
7. Yoshida, Y., Tsunoda, T., Takashima, Y., Fujimoto, T., Doi, K., Sasazuki, T., Kuroki, M., Iwasaki, A. and Shirasawa, S. ZFAT is essential for endothelial cell assembly and the branch point formation of capillary-like structures in an angiogenesis model. **Cell Mol. Biol. Lett.** 15 (2010) 541-550.
8. Tonini, T., Rossi, F. and Claudio, P.P. Molecular basis of angiogenesis and cancer. **Oncogene** 22 (2003) 6549-6556.
9. Beck, H. and Plate, K.H. Angiogenesis after cerebral ischemia. **Acta Neuropathol.** 117 (2009) 481-496.
10. Haider, H., Akbar, S.A. and Ashraf, M. Angiomyogenesis for myocardial repair. **Antioxid. Redox Signal.** 11 (2009) 1929-1944.
11. Shazly, T.A. and Latina, M.A. Neovascular glaucoma: etiology, diagnosis and prognosis. **Semin. Ophthalmol.** 24 (2009) 113-121.
12. Crosby, M.E., Glazer, P.M. and Ivan, M. "Micro"-management of DNA repair genes by hypoxia. **Cell Cycle** 8 (2009) 4009-4010.
13. Gee, H.E., Camps, C., Buffa, F.M., Patiar, S., Winter, S.C., Betts, G., Homer, J., Corbridge, R., Cox, G., West, C.M., Ragoussis, J. and Harris, A.L. hsa-mir-210 is a marker of tumor hypoxia and a prognostic factor in head and neck cancer. **Cancer** 116 (2010) 2148-2158.
14. Pocock, R. Invited review: decoding the microRNA response to hypoxia. **Pflugers Arch.** 461 (2011) 307-315.
15. Bartel, D.P. MicroRNAs: target recognition and regulatory functions. **Cell** 136 (2009) 215-233.
16. Berezikov, E., Guryev, V., Van De Belt, J., Wienholds, E., Plasterk, R.H. and Cuppen, E. Phylogenetic shadowing and computational identification of human microRNA genes. **Cell** 120 (2005) 21-24.
17. Noren Hooten, N., Abdelmohsen, K., Gorospe, M., Ejiogu, N., Zonderman, A.B. and Evans, M.K. microRNA expression patterns reveal differential expression of target genes with age. **PLoS One** 5 (2010) e10724.
18. Ritchie, W., Rajasekhar, M., Flamant, S. and Rasko, J.E. Conserved expression patterns predict microRNA targets. **PLoS Comput. Biol.** 5 (2009) e1000513.

19. Du, R., Sun, W., Xia, L., Zhao, A., Yu, Y., Zhao, L., Wang, H., Huang, C. and Sun, S. Hypoxia-induced down-regulation of microRNA-34a promotes EMT by targeting the notch signaling pathway in tubular epithelial cells. **PLoS One** 7 (2012) e30771.
20. Muth, M., Theophile, K., Hussein, K., Jacobi, C., Kreipe, H. and Bock, O. Hypoxia-induced down-regulation of microRNA-449a/b impairs control over targeted SERPINE1 (PAI-1) mRNA - a mechanism involved in SERPINE1 (PAI-1) overexpression. **J. Transl. Med.** 8 (2010) 33.
21. Kulshreshtha, R., Ferracin, M., Wojcik, S.E., Garzon, R., Alder, H., Agosto-Perez, F.J., Davuluri, R., Liu, C.G., Croce, C.M., Negrini, M., Calin, G.A. and Ivan, M. A microRNA signature of hypoxia. **Mol. Cell. Biol.** 27 (2007) 1859-1867.
22. Chan, S.Y. and Loscalzo, J. MicroRNA-210: a unique and pleiotropic hypoxamir. **Cell Cycle** 9 (2010) 1072-1083.
23. Kaelin, W.G., Jr. and Ratcliffe, P.J. Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. **Mol. Cell** 30 (2008) 393-402.
24. Wang, V., Davis, D.A., Haque, M., Huang, L.E. and Yarchoan, R. Differential gene up-regulation by hypoxia-inducible factor-1alpha and hypoxia-inducible factor-2alpha in HEK293T cells. **Cancer Res.** 65 (2005) 3299-3306.
25. Maynard, M.A., Evans, A.J., Hosomi, T., Hara, S., Jewett, M.A. and Ohh, M. Human HIF-3alpha4 is a dominant-negative regulator of HIF-1 and is down-regulated in renal cell carcinoma. **FASEB J.** 19 (2005) 1396-1406.
26. Li, Q.F., Wang, X.R., Yang, Y.W. and Lin, H. Hypoxia upregulates hypoxia inducible factor (HIF)-3alpha expression in lung epithelial cells: characterization and comparison with HIF-1alpha. **Cell Res.** 16 (2006) 548-558.
27. Mole, D.R., Maxwell, P.H., Pugh, C.W. and Ratcliffe, P.J. Regulation of HIF by the von Hippel-Lindau tumour suppressor: implications for cellular oxygen sensing. **IUBMB Life** 52 (2001) 43-47.
28. Lando, D., Peet, D.J., Whelan, D.A., Gorman, J.J. and Whitelaw, M.L. Asparagine hydroxylation of the HIF transactivation domain a hypoxic switch. **Science** 295 (2002) 858-861.
29. Dery, M.A., Michaud, M.D. and Richard, D.E. Hypoxia-inducible factor 1: regulation by hypoxic and non-hypoxic activators. **Int. J. Biochem. Cell Biol.** 37 (2005) 535-540.
30. Pagé, E.L., Robitaille, G.A., Pouysségur, J. and Richard, D.E. Induction of hypoxia-inducible factor-1α by transcriptional and translational mechanisms. **J. Biol. Chem.** 277 (2002) 48403-48409.
31. Kaluz, S., Kaluzova, M. and Stanbridge, E.J. Regulation of gene expression by hypoxia: integration of the HIF-transduced hypoxic signal at the hypoxia-responsive element. **Clin. Chim. Acta** 395 (2008) 6-13.
32. Pugh, C.W. and Ratcliffe, P.J. Regulation of angiogenesis by hypoxia: role of the HIF system. **Nat. Med.** 9 (2003) 677-684.
33. Huang, X., Ding, L., Bennewith, K.L., Tong, R.T., Welford, S.M., Ang, K.K., Story, M., Le, Q.T. and Giaccia, A.J. Hypoxia-inducible mir-210

regulates normoxic gene expression involved in tumor initiation. **Mol. Cell** 35 (2009) 856-867.

34. Hua, Z., Lv, Q., Ye, W., Wong, C.K., Cai, G., Gu, D., Ji, Y., Zhao, C., Wang, J., Yang, B.B. and Zhang, Y. MiRNA-directed regulation of VEGF and other angiogenic factors under hypoxia. **PLoS One** 1 (2006) e116.
35. Lei, Z., Li, B., Yang, Z., Fang, H., Zhang, G.M., Feng, Z.H. and Huang, B. Regulation of HIF-1alpha and VEGF by miR-20b tunes tumor cells to adapt to the alteration of oxygen concentration. **PLoS One** 4 (2009) e7629.
36. Lin, S.C., Wang, C.C., Wu, M.H., Yang, S.H., Li, Y.H. and Tsai, S.J. Hypoxia-induced microRNA-20a expression increases ERK phosphorylation and angiogenic gene expression in endometriotic stromal cells. **J. Clin. Endocrinol. Metab.** 97 (2012) E1515-1523.
37. Cascio, S., D'andrea, A., Ferla, R., Surmacz, E., Gulotta, E., Amodeo, V., Bazan, V., Gebbia, N. and Russo, A. miR-20b modulates VEGF expression by targeting HIF-1 alpha and STAT3 in MCF-7 breast cancer cells. **J. Cell Physiol.** 224 (2010) 242-249.
38. Rane, S., He, M., Sayed, D., Vashistha, H., Malhotra, A., Sadoshima, J., Vatner, D.E., Vatner, S.F. and Abdellatif, M. Downregulation of miR-199a derepresses hypoxia-inducible factor-1alpha and Sirtuin 1 and recapitulates hypoxia preconditioning in cardiac myocytes. **Circ. Res.** 104 (2009) 879-886.
39. Ghosh, G., Subramanian, I.V., Adhikari, N., Zhang, X., Joshi, H.P., Basi, D., Chandrashekhar, Y.S., Hall, J.L., Roy, S., Zeng, Y. and Ramakrishnan, S. Hypoxia-induced microRNA-424 expression in human endothelial cells regulates HIF-alpha isoforms and promotes angiogenesis. **J. Clin. Invest.** 120 (2010) 4141-4154.
40. Saito, K., Kondo, E. and Matsushita, M. MicroRNA 130 family regulates the hypoxia response signal through the P-body protein DDX6. **Nucleic Acids Res.** 39 (2011) 6086-6099.
41. Bruning, U., Cerone, L., Neufeld, Z., Fitzpatrick, S.F., Cheong, A., Scholz, C.C., Simpson, D.A., Leonard, M.O., Tambuwala, M.M., Cummins, E.P. and Taylor, C.T. MicroRNA-155 promotes resolution of hypoxia-inducible factor 1α activity during prolonged hypoxia. **Mol. Cell Biol.** 31 (2011) 4087-4096.
42. Huang, X., Le, Q.T. and Giaccia, A.J. MiR-210--micromanager of the hypoxia pathway. **Trends Mol. Med.** 16 (2010) 230-237.
43. Camps, C., Buffa, F.M., Colella, S., Moore, J., Sotiriou, C., Sheldon, H., Harris, A.L., Gleadle, J.M. and Ragoussis, J. hsa-miR-210 is induced by hypoxia and is an independent prognostic factor in breast cancer. **Clin. Cancer Res.** 14 (2008) 1340-1348.
44. Zhang, Z., Sun, H., Dai, H., Walsh, R.M., Imakura, M., Schelter, J., Burchard, J., Dai, X., Chang, A.N., Diaz, R.L., Marszalek, J.R., Bartz, S.R., Carleton, M., Cleary, M.A., Linsley, P.S. and Grandori, C. MicroRNA miR-210 modulates cellular response to hypoxia through the MYC antagonist MNT. **Cell Cycle** 8 (2009) 2756-2768.

45. Liu, F., Lou, Y.L., Wu, J., Ruan, Q.F., Xie, A., Guo, F., Cui, S.P., Deng, Z.F. and Wang, Y. Upregulation of MicroRNA-210 regulates renal angiogenesis mediated by activation of VEGF signaling pathway under ischemia/perfusion injury in vivo and in vitro. **Kidney Blood Press. Res.** 35 (2012) 182-191.
46. Goodsell, D.S. The Molecular perspective: VEGF and angiogenesis. **Oncologist** 7 (2002) 569-570.
47. Ferrara, N., Gerber, H.P. and Lecouter, J. The biology of VEGF and its receptors. **Nat. Med.** 9 (2003) 669-676.
48. Oladipupo, S., Hu, S., Kovalski, J., Yao, J., Santeford, A., Sohn, R.E., Shohet, R., Maslov, K., Wang, L.V. and Arbeit, J.M. VEGF is essential for hypoxia-inducible factor-mediated neovascularization but dispensable for endothelial sprouting. **Proc. Nat. Acad. Sci. USA** 108 (2011) 13264-13269.
49. Levy, A.P. Hypoxic regulation of VEGF mRNA stability by RNA-binding proteins. **Trends Cardiovasc. Med.** 8 (1998) 246-250.
50. Ray, P.S., Jia, J., Yao, P., Majumder, M., Hatzoglou, M. and Fox, P.L. A stress-responsive RNA switch regulates VEGFA expression. **Nature** 457 (2009) 915-919.
51. Jafarifar, F., Yao, P., Eswarappa, S.M. and Fox, P.L. Repression of VEGFA by CA-rich element-binding microRNAs is modulated by hnRNP L. **EMBO J.** 30 (2011) 1324-1334.
52. Fandrey, J., Gorr, T.A. and Gassmann, M. Regulating cellular oxygen sensing by hydroxylation. **Cardiovasc Res.** 71 (2006) 642-651.
53. Berra, E., Benizri, E., Ginouves, A., Volmat, V., Roux, D. and Pouyssegur, J. HIF prolyl-hydroxylase 2 is the key oxygen sensor setting low steady-state levels of HIF-1alpha in normoxia. **EMBO J.** 22 (2003) 4082-4090.
54. Appelhoff, R.J., Tian, Y.-M., Raval, R.R., Turley, H., Harris, A.L., Pugh, C.W., Ratcliffe, P.J. and Gleadle, J.M. Differential function of the prolyl hydroxylases PHD1, PHD2, and PHD3 in the regulation of hypoxia-inducible factor. **J. Biol. Chem.** 279 (2004) 38458-38465.
55. Berra, E., Benizri, E., Ginouves, A., Volmat, V., Roux, D. and Pouyssegur, J. HIF prolyl-hydroxylase 2 is the key oxygen sensor setting low steady-state levels of HIF-1[alpha] in normoxia. **EMBO J.** 22 (2003) 4082-4090.
56. Lee, S.-T., Chu, K., Jung, K.-H., Yoon, H.-J., Jeon, D., Kang, K.-M., Park, K.-H., Bae, E.-K., Kim, M., Lee, S.K. and Roh, J.-K. MicroRNAs induced during ischemic preconditioning. **Stroke** 41 (2010) 1646-1651.
57. Chan, Y.C., Khanna, S., Roy, S. and Sen, C.K. miR-200b targets Ets-1 and is down-regulated by hypoxia to induce angiogenic response of endothelial cells. **J. Biol. Chem.** 286 (2011) 2047-2056.
58. Listowski, M.A., Heger, E., Bogusławska, D.M., Machnicka, B., Kuliczkowski, K., Leluk, J. and Sikorski, A.F. microRNAs: fine tuning of erythropoiesis. **Cell. Mol. Biol. Lett.** DOI: 10.2478/s11658-012-0038-z, in press.