Life Science Informatics Publications

Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences

Journal Home page http://www.rjlbpcs.com/

RJLBPCS

ISSN 2454-6348

Original Research Article **DOI: 10.26479/2024.1003.04**

VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) UPREGULATION: MICRORNAS (miRNAs)

Mirela Lungu[1], Claudiu N. Lungu[2*], Felicia Mihailuta[3], Maria Andrada Hincu[3]

1. Department of Pediatric and Orthopaedic Surgery, Clinical Country Children Emergency Hospital, Galati, 800010, Romania.

2. Department of Functional and Morphological Science, Faculty of Medicine and Pharmacy, Dunarea de Jos University, 800010 Galati, Romania.

3.University Titu Maiorescu, Faculty of Medine, Bucureşti 031593, Romania.

ABSTRACT: Vascular Endothelial Growth Factor (VEGF) is a signal protein that plays a crucial role in both vasculogenesis (the formation of new blood vessels during embryonic development) and angiogenesis (the growth of blood vessels from pre-existing ones). VEGF upregulation refers to an increase in the expression or activity of VEGF, which can have significant implications in both physiological and pathological processes. In this computational study, microRNAs (miRNA) involved in vascular endothelial growth factor (VEGF) upregulation are presented and characterized computationally. Results show that mic RNA shares a core of critical properties crucial in stimulating angiogenesis. Further studies are needed to identify possible drug targets and clinical implications regarding mic RNA involvement in morphogenesis and angiogenesis.

Keywords: vascular endothelial growth factor, microRNA, molecular descriptors, angiogenesis.

Article History: Received: June 18, 2024; Revised: June 26, 2024; Accepted: June 30, 2024.

Corresponding Author: Claudiu N Lungu

Department of Functional and Morphological Science, Faculty of Medicine and Pharmacy, Dunarea de Jos University, 800010 Galati, Romania

1.INTRODUCTION

Various variables can stimulate the increase in VEGF expression. The leading cause of the increased production of VEGF is hypoxia. Hypoxia-inducible factors (HIFs) are transcription factors that become active and stable in low-oxygen environments, resulting in an elevated production of VEGF.

Peer review under responsibility of Life Science Informatics Publications

HIF-1α and HIF-2α are hydroxylated by prolyl hydroxylase domain (PHD) enzymes, marking them for ubiquitination and proteasomal degradation. Hypoxia inhibits the activity of PHDs due to the lack of oxygen, leading to the stabilization and accumulation of HIF-1α and HIF-2α in the cell[1]. Stabilized HIF-1α and HIF-2α translocate to the nucleus, dimerizing with HIF-1β (ARNT – aryl hydrocarbon receptor nuclear translocator). The HIF complex binds to hypoxia-responsive elements (HREs) in the promoter region of the VEGF gene, activating its transcription and leading to increased VEGF mRNA levels. Hypoxia can also influence the binding of Sp1, a transcription factor, to the VEGF promoter. Sp1 cooperates with HIF-1 to enhance VEGF transcription. NF-κB Pathway: Hypoxia can activate the NF-κB pathway, another transcription factor that can bind to the VEGF promoter and improve its expression. Hypoxia increases the stability of VEGF mRNA by altering the binding of RNA-binding proteins to its 3'-untranslated region (3'-UTR). Proteins such as HuR (Human antigen R) bind to the VEGF mRNA under hypoxic conditions, preventing degradation and ensuring sustained VEGF production. Specific miRNAs are known to be regulated by hypoxia and, in turn, regulate VEGF expression. For instance, hypoxia-induced miRNAs can inhibit the expression of VEGF-targeting miRNAs, leading to increased VEGF mRNA levels and protein synthesis[2]. Hypoxia activates the PI3K/Akt pathway, which can lead to the activation of mTOR (mammalian target of rapamycin). mTOR signaling promotes VEGF expression through various downstream effectors, including enhanced HIF-1α translation. The MAPK/ERK pathway is also activated by hypoxia and contributes to VEGF's transcriptional and post-transcriptional regulation. Hypoxia upregulates VEGF through a multifaceted mechanism involving HIF-1α and HIF-2α stabilization and nuclear translocation, leading to direct transcriptional activation of the VEGF gene. Factors like Sp1 and NF-κB enhance VEGF transcription. Increased mRNA stability through RNA-binding proteins These mechanisms ensure that cells can rapidly respond to hypoxic conditions by promoting angiogenesis, thereby restoring oxygen supply by forming new blood vessels[3,4,5,6,7].

2. MATERIALS AND METHODS

miRNA sequences were obtained from the UniProt database. Double miRNA strains with ß-helix symmetry 3'-5' have been computed. Nucleotide sequences of miR-296, miR-210, miR-155, miR-126, miR-21, and miR-9 have been used to generate the 3D models of the respective structures. The online RNAfold and Biomodel servers were used server was used[8,9]. Finally, some molecular descriptors were computed for each miRNA string.

3. RESULTS AND DISCUSSION

Figure 1 represents the miRNA string resulting from the miRNA nucleotide sequences. Ribbons represent the double-generated strings.

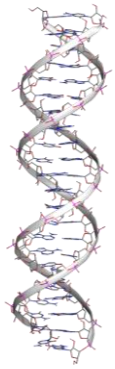
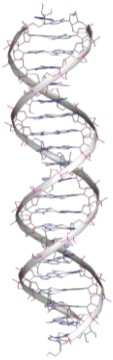
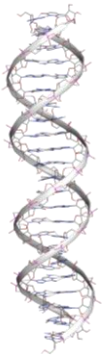
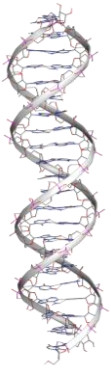
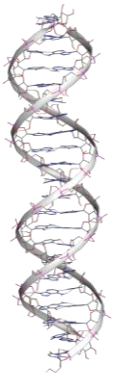
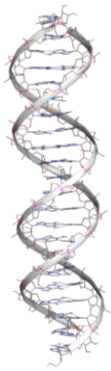
		
miR-296	**miR-210**	**miR-155**
		
miR-126	**miR-21**	**miR-9**

Figure 1: 3D ribbons representation of miRNAs

In Figure 2, the composition of miRNA atoms is shown. The number of carbon, hydrogen, oxygen, and nitrogen atoms are represented using bar charts. The number of oxygen atoms is slightly similar for all six miRNAs. Significant variations are observed in the number of hydrogen atoms.

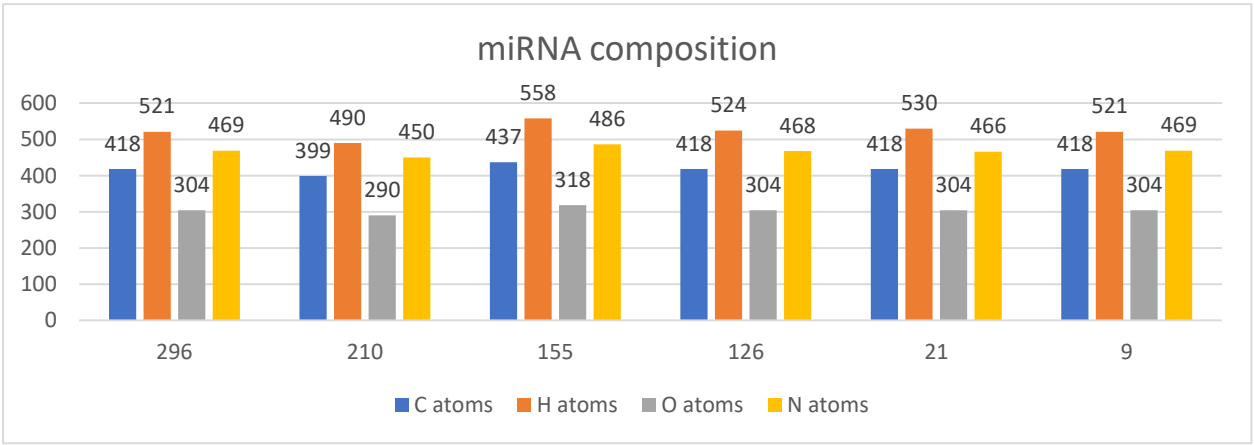


Figure 2: Atom composition of miRNA

In Figure 3, miRNA bounds and heavy atoms are shown. As observed, double bounds are slightly constant across the miRNA series. The number of single bounds presents some variations. For the miRNA 296, 126,21, and 9, respectively, the total bounds are the same (1536).

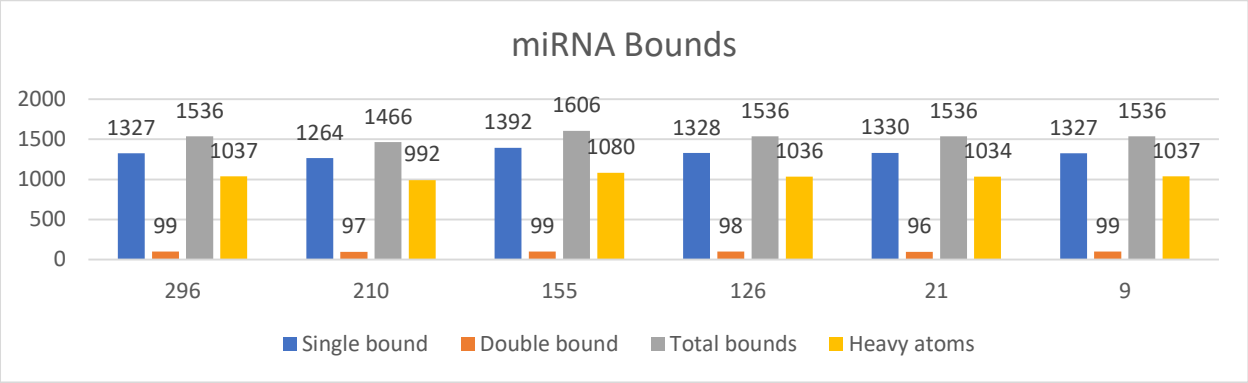


Figure 3: For the miRNA series, the number of single bounds, double bounds, total bounds, and heavy attoms is represented as bar charts.

As seen for the structural data, the miRNAs are similar in structure, but specific variations in the nucleotide sequence make their function specific.

Furthermore, Figure 4 shows miRNA shape, weight, and density. As observed, the weight of each miRNA series member is quite similar. However, the shape and volume are consecutively different.

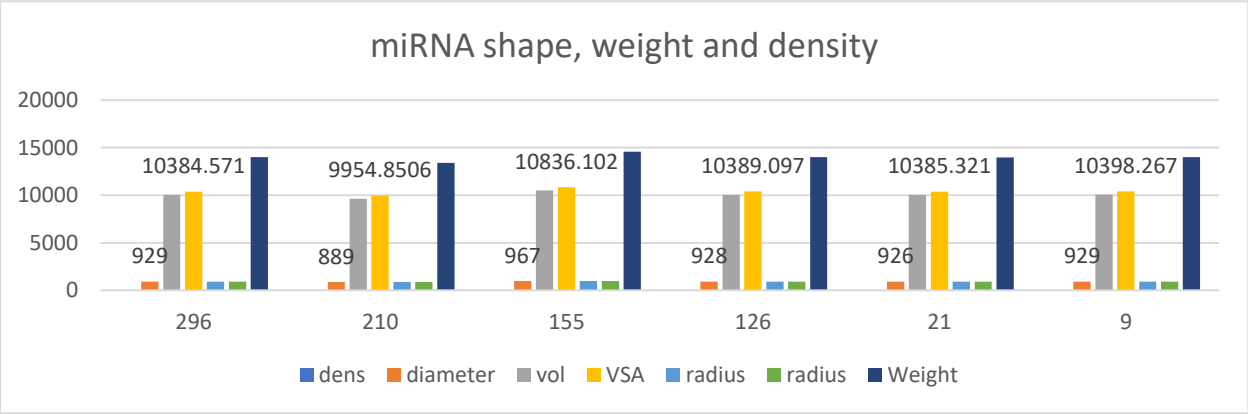


Figure 4: The density, diameter(Å), volume(Å^3), radius(Å), and weight of the miRNA sequences are represented as bar charts.

Figure 5. represents the miRNA Total energy/Solvation energy/SlogPratio. As expected, the miRNA series have slight differences in energy, SlogP, and solvation energy.

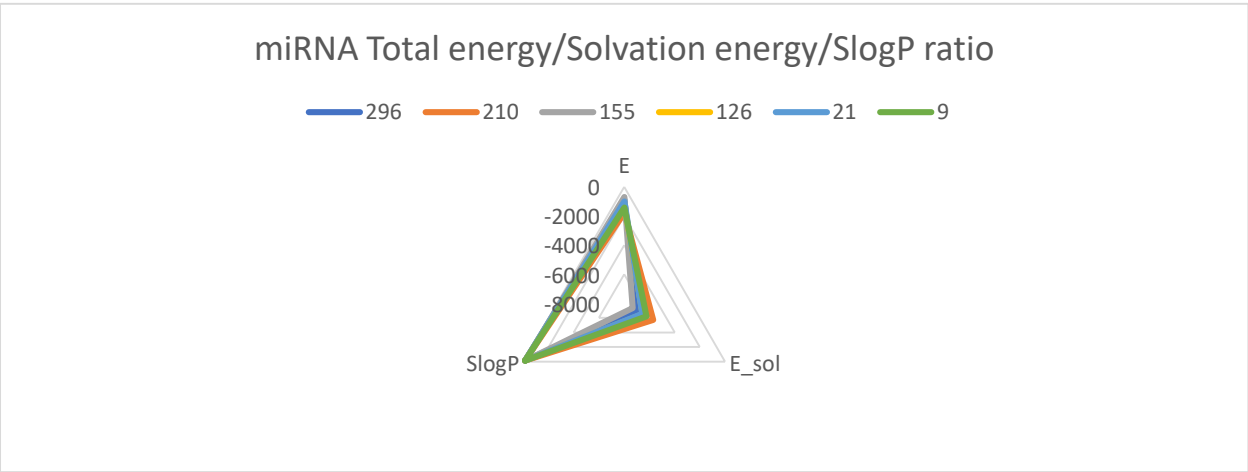


Figure 5: miRNA Total energy/Solvation energy/SlogP ratio.

Regarding the miRNA binding site, the most significant volume is observed at miRNA 126 and 9, with a binding site of 416416 $Å^3$. The minor binding site is observed at miRNA 296(246$Å^3$)(Table 1).

Table 1: Binding sites for the miRNA series

Binding site surface	246$Å^3$	365 $Å^3$	368 $Å^3$	416 $Å^3$	415 $Å^3$	416 $Å^3$
miRNA	296	**210**	**155**	**126**	**21**	**9**

Additional factors, such as Platelet-Derived Growth Factor (PDGF) and Transforming Growth Factor-beta (TGF-β), can promote the synthesis of Vascular Endothelial Growth Factor (VEGF). The pressure exerted on blood artery walls can induce the synthesis of VEGF. Inflammatory cytokines, such as interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-alpha), can increase the production of VEGF. Hypoxia, which refers to insufficient oxygen levels, is a primary factor contributing to the development of VEGF. Cells detect hypoxia and respond by stabilizing and activating hypoxia-inducible factor 1-alpha (HIF-1α). HIF-1α subsequently attaches to the VEGF gene promoter, hence increasing its transcription. Cytokines and growth factors, such as interleukins and platelet-derived growth factors, can induce the expression of VEGF. These chemicals stimulate signaling pathways, including the PI3K/AKT and MAPK/ERK pathways, which increase the transcription of the VEGF gene. Genetic factors, such as variations and mutations, can potentially influence the expression of VEGF. For example, specific cancer-causing viruses such as Kaposi's sarcoma-associated herpesvirus (KSHV) and Epstein-Barr virus (EBV) increase the expression of VEGF to stimulate the formation of new blood vessels and facilitate the growth of tumors[10,11,12]. VEGF is critical for wound healing as it promotes angiogenesis to restore blood supply to the damaged tissue. Physical activity can lead to transient upregulation of VEGF, enhancing blood flow and oxygen delivery to muscles. VEGF forms the placenta, ensuring adequate blood supply to the developing fetus[13,14,15]. Tumors often exploit VEGF to promote angiogenesis, allowing for increased nutrient supply and growth. This process, known as tumor angiogenesis, is a crucial target for anti-cancer therapies. Overexpression of VEGF can lead to pathological neovascularization in the retina, contributing to vision loss. High glucose levels can upregulate VEGF, leading to abnormal blood vessel growth in the retina and subsequent vision problems. VEGF forms new blood vessels in the synovium, exacerbating inflammation and joint damage[16,17,18,19]. Given VEGF's diverse roles in health and disease, modulating its activity has become a significant therapeutic strategy. Anti-VEGF Therapies: Drugs like bevacizumab (Avastin) and ranibizumab (Lucentis) are monoclonal antibodies that inhibit VEGF and are used in treating cancers and eye diseases like AMD and diabetic retinopathy. Gene Therapy: Depending on the clinical context, efforts are being

made to develop gene therapy approaches to enhance or inhibit VEGF expression. VEGF Mimetics and Receptor Agonists: These are being explored to promote angiogenesis in ischemic diseases, such as coronary and peripheral artery disease[20,21]. In addition, microRNAs (miRNAs) are small, non-coding RNAs that regulate gene expression, including genes involved in the vascular endothelial growth factor receptor (VEGFR) pathways. miRNAs typically function by binding to complementary sequences in the 3'-untranslated regions (3'-UTRs) of target mRNAs, leading to mRNA degradation or translational repression. The upregulation of VEGFRs through miRNAs involves intricate mechanisms, including the modulation of both direct and indirect regulatory networks. Specific miRNAs can directly target mRNAs encoding VEGFRs, either enhancing their stability or preventing degradation. Although miRNAs generally act to repress their targets, some can positively influence gene expression under specific contexts or through indirect pathways. It has been reported that miR-296 can upregulate VEGFR2 expression indirectly by downregulating inhibitors of VEGFR2 expression. For example, miR-296 targets hepatocyte growth factor-regulated tyrosine kinase substrate (HGS), a protein involved in the degradation of VEGFR2. By inhibiting HGS, miR-296 promotes VEGFR2 stability and upregulation[22]. miR-296 promotes the stability and upregulation of VEGFR2 by targeting negative regulators of VEGFR2. One key target is hepatocyte growth factor-regulated tyrosine kinase substrate (HGS), which is involved in the degradation of VEGFR2. By inhibiting HGS, miR-296 enhances the stability and expression of VEGFR2, facilitating angiogenesis. Würdinger, T., et al. (2008). This study demonstrated that miR-296 regulates angiogenesis in glioma by targeting HGS, thereby promoting the stability of VEGFR2. The study highlighted the importance of miR-296 in the angiogenic process, showing how its regulation can significantly impact tumor growth and vascularization.[23] Lee, S. H., et al. (2010). This study further explored the role miR-296 in endothelial cells, showing that miR-296 upregulation leads to enhanced angiogenic activity by increasing VEGFR2 levels. The findings provided insights into the potential therapeutic applications of miR-296 modulation in diseases characterized by aberrant angiogenesis. miR-296 plays a crucial role in regulating VEGFR2 by targeting and downregulating inhibitors of VEGFR2 stability, such as HGS. This leads to increased stability and expression of VEGFR2, promoting angiogenesis. The references provided highlight the significance of miR-296 in angiogenesis and its potential as a therapeutic target in diseases involving abnormal blood vessel growth[24]. This sequence is derived from the precursor miRNA (pre-miRNA) hairpin structure of miR-296, which Dicer processes into the mature miRNA. To provide additional context, miR-296-5p is another strand derived from the precursor miRNA, which can also have biological activity. However, miR-296-3p is most commonly associated with regulating angiogenesis and VEGFR pathways[25,26]. miR-126 is known to enhance the expression of VEGFR2 by targeting inhibitors of the VEGF pathway, such as Sprouty-related EVH1 domain-containing protein 1 (SPRED1) and phosphoinositide-3-kinase regulatory subunit 2 (PIK3R2). By

inhibiting these negative regulators, miR-126 facilitates VEGF signaling and promotes angiogenesis[27]. Several miRNAs indirectly upregulate VEGFR expression by targeting negative VEGF signaling pathway regulators. This indirect regulation often involves complex networks of signaling molecules and transcription factors—miR-9 targets multiple negative regulators of VEGF signaling, such as sirtuin 1 (SIRT1). SIRT1 is known to inhibit the HIF-1α pathway. By downregulating SIRT1, miR-9 stabilizes HIF-1α, leading to increased transcription of VEGFR genes and enhanced VEGF signaling[28].miR-21 targets PTEN (phosphatase and tensin homolog), a negative PI3K/Akt pathway regulator. Inhibition of PTEN by miR-21 activates the PI3K/Akt pathway, promoting the expression of VEGFRs and enhancing cell survival and angiogenesis[29]. miRNAs can also regulate upstream signaling pathways that affect the expression and activity of VEGFRs.miR-155 can upregulate VEGFR expression by targeting SH2 domain-containing inositol 5'-phosphatase 1 (SHIP1), another negative regulator of the PI3K/Akt pathway. By inhibiting SHIP1, miR-155 enhances PI3K/Akt signaling, increasing VEGFR expression and angiogenic responses. Under hypoxic conditions, miR-210 is upregulated and targets several mitochondrial metabolism and angiogenesis genes. miR-210 can promote VEGFR expression by stabilizing HIF-1α, enhancing the transcription of VEGFRs and other hypoxia-responsive genes. miRNAs regulate VEGFR upregulation through various mechanisms, including direct targeting of VEGFR mRNA: Stabilizing or preventing the degradation of VEGFR mRNA. Targeting negative regulators: Inhibiting proteins that negatively regulate VEGF signaling pathways, thereby promoting VEGFR expression. Modulating upstream pathways: Influencing signaling pathways that control VEGFR expression and activity. These mechanisms highlight the intricate role of miRNAs in fine-tuning VEGF signaling and promoting angiogenesis, which is crucial for tissue repair, cancer progression, and response to hypoxia[30].

4. CONCLUSION

VEGFR can be upregulated by miRNA. The miRNA structure shows slight differences. Those specific structural architectures can explain their different interactions with distinct molecules. Energetically, miRNA shows a particular profile. Also, the miRNA Total energy/Solvation energy/SlogP ratios show a similar profile, suggesting that miRNA can target the same pathway. Further studies are needed to develop effective therapies based on miRNAs.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals or humans were used for the studies that are based on this research.

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

None.

ACKNOWLEDGEMENT

The authors contributed equally to this manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

REFERENCES

1. R Liu, C-M Liu, L-L Cui, L Zhou, N Li, X-D Wei. Expression and significance of MiR-126 and VEGF in proliferative diabetic retinopathy. Eur Rev Med Pharmacol Sci. 2019;23(15):6387-6393.
2. Yajun Guo, Miaomiao Liu , Jos Dorian Lawson Mfoundou, Xinrong Wang. Expression and distribution patterns of VEGF, TGF-β1 and HIF-1α in the ovarian follicles of Tibetan sheep. Vet med Sci. 2022;8(5):2223-2229.
3. I Sae Byeol Choi 1, Jong Bae Park, Tae-Jin Song, Sang Yong Choi. Molecular mechanism of HIF-1-independent VEGF expression in a hepatocellular carcinoma cell line.Int J Mol Med. 2011;28(3):449-54.
4. Y Jing Fang , Chang Xia, Zongxian Cao, Jenny Z Zheng, Eddie Reed, Bing-Hua Jiang. Apigenin inhibits VEGF and HIF-1 expression via PI3K/AKT/p70S6K1 and HDM2/p53 pathways. FASEB J. 2005;19(3):342-53.
5. Y Mou Yueyang , Hu Yaqin, Xue Guolian, Zhao Wenjian, Jiao Yang , Li Chen , Cao Haiyan , Chao Min , Deng Jianping , Dai Penggao , Zhu Hongli , Wang Liang . Glioma angiogenesis is boosted by ELK3 activating the HIF-1. BMC Cancer. 2023;23(1):662.
6. Sandra Cascio , Aleco D'Andrea, Rita Ferla, Eva Surmacz, Eliana Gulotta, Valeria Amodeo, Viviana Bazan, Nicola Gebbia, Antonio Russo. miR-20b modulates VEGF expression by targeting HIF-1 alpha and STAT3 in MCF-7 breast cancer cells. J Cell Physiol. 2010 ;224(1):242-9.
7. Ronny Lorenz, Dominik Luntzer , Ivo L Hofacker , Peter F Stadler , Michael T Wolfinger. SHAPE directed RNA folding. Bioinformatics. 2016;32(1):145-7.
8. Patricia Mondelo-Macía , Luis León-Mateos , David Bernardo, Roberto Díaz-Peña. Top-of-the-art cytometry as a novel tool to aid in lung cancer immunotherapy. Transl Lung Cancer Res. 2023;12(5):957-961.
9. Hachana, S., Fontaine, O., Sapieha, P., et al. The effects of anti-VEGF and kinin B1 receptor blockade on retinal inflammation in laser-induced choroidal neovascularization. British Journal of Pharmacology. 2020;177(9), 1949–1966.
10. Calvo, P. M., Hernández, R. G., Pastor, A. M. VEGF and neuronal survival. Neuroscientist. 2024; 30(1):71-86

11. Songyi Han , Jeongsoon Huh , Wooseong Kim , Seongkeun Jeong , Do Sik Min, Yunjin Jung. Phospholipase D activates HIF-1-VEGF pathway via phosphatidic acid. Exp Mol Med. 2014; 19;46(12):e126.
12. Ling Zhang, Wei Ge, Ke Hu, YanYan Zhang, ChangHu Li, XiMing Xu, Du He, ZhenYu Zhao, JinZhong Zhang, FangFang Jie, Yu Chen, YongFa Zheng. Endostar down-regulates HIF-1 and VEGF expression and enhances the radioresponse to human lung adenocarcinoma cancer cells. Mol Biol Rep. 2012;39(1):89-95.
13. Zhang, Y., Zhang, Y. Y., Pan, Z. W. GDF11 promotes wound healing in diabetic mice via stimulating HIF-1α-VEGF/SDF-1α-mediated endothelial progenitor cell mobilization and neovascularization. Acta Pharmacologica Sinica. 2023;44(5), 999–1013.
14. Girriso F Benderro , Xiaoyan Sun, Youzhi Kuang, Joseph C Lamanna. Decreased VEGF expression and microvascular density, but increased HIF-1 and 2α accumulation and EPO expression in chronic moderate hyperoxia in the mouse brain. Brain Res. 2012; 30:1471:46-55.
15. Lin Liu , Dan Zhu , Wenzhi Ding , Ting Zhang , Xiaoyun Ma , Jun Zou. MiRNA-21-HIF-1α-VEGF Axis Is Associated with Myopic Choroidal Neovascularization in Guinea Pigs.Ophtalmic Res. 2022;65(5):493-505.
16. Ruibin Zhao , Lijuan Qian , Li Jiang. miRNA-dependent cross-talk between VEGF and Ang-2 in hypoxia-induced microvascular dysfunction. Biochem Biophys Res Commun. 2014; 26;452(3):428-35.
17. Dina Sabry , Sahar E M El-Deek , Moataz Maher , Mona A H El-Baz , Hala M El-Bader , Eman Amer , Elham A Hassan , Wael Fathy , Heba E M El-Deek . Role of miRNA-210, miRNA-21 and miRNA-126 as diagnostic biomarkers in colorectal carcinoma: impact of HIF-1α-VEGF signaling pathway. Mol Cell Biochem. 2019;454(1-2):177-189.
18. Alireza Saati-Zarei , Arsalan Damirchi , Seyed Mohammad Taghi Razavi Tousi, Parvin Babaei. Myocardial angiogenesis induced by concurrent vitamin D supplementation and aerobic-resistance training is mediated by inhibiting miRNA-15a, and miRNA-146a and upregulating VEGF/PI3K/eNOS signaling pathway. Pflugers Arch. 2023;475(4):541-555.
19. Asawari Korde , Maria Haslip , Prachi Pednekar , Alamzeb Khan , Maurizio Chioccioli, Sameet Mehta , Francesc Lopez-Giraldez , Santos Bermejo, Mauricio Rojas , Charles Dela Cruz , Michael A Matthay , Jordan S Pober , Richard W Pierce , Shervin S Takyar. MicroRNA-1 protects the endothelium in acute lung injury. JCI Insight. 2023;22;8(18):e164816.
20. Andriana I Papaioannou, Konstantinos Kostikas,Panagoula Kollia, Konstantinos I Gourgoulianis Clinical implications for Vascular Endothelial Growth Factor in the lung: friend or foe? Respiratory Research. 2006;7(1):128.
21. Wang Y. MicroRNA-296 promotes tumorigenesis by increasing cell invasion and proliferation. Cell Research, 2010;20(12), 1625-1633.

22. Würdinger, T., Tannous, B. A., Saydam, O., Skog, J., Grau, S., Beaulieu, R., ... & Breakefield, X. O. miR-296 regulates growth factor receptor overexpression in angiogenic endothelial cells. Cancer Cell. 14(5), 2008;382-393.
23. Lee, S. H., & Lee, J. G. Regulation of endothelial function by microRNAs: new perspectives. Endocrinology and Metabolism.2010; 25(1), 2-10.
24. Würdinger, T. miR-296 regulates growth factor receptor overexpression in angiogenic endothelial cells. Cancer Cell.2008;14(5), 382-393.
25. Lee, S. H., & Lee, J. G.. Regulation of endothelial function by microRNAs: new perspectives. Endocrinology and Metabolism.2010; 25(1), 2-10.
26. Fish, J. E., & Srivastava, D. MicroRNAs: Opening a new vein in angiogenesis research. Science Signaling. 2009;2(52), pe1.
27. Sun, X., He, S., & Wara, A. K. M. Systemic delivery of microRNA-1 inhibitors using targeted nanoparticles inhibits myocardial infarction. Nature Communications. 2012;3;1192.
28. Sabatel, C., Malvaux, L., Bovy, N., et al.. MicroRNA-21 exhibits antiangiogenic function by targeting RhoB expression in endothelial cells. PLoS One.2011;6(2), e16979.
29. Zhang, W., & Xiao. MicroRNAs regulate the immune response against infections. Journal of Genetics and Genomics.2014; 41(9), 499-510.
30. Huang, X., Le, Q.-T., & Giaccia, A. J. MiR-210—micromanager of the hypoxia pathway. Trends in Molecular Medicine.2010;16(5), 230-237.