Physiology of VEGF gene and VEGFR

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Abstract:

Angiogenesis is an important component of many physiological processes, such as the female sexual cycle, placenta formation, the processes of growth and differentiation of tissues, and reparative processes including wound healing, fracture repair, and liver regeneration. The formation of new blood vessels during angiogenesis and vasculogenesis allows the growth and functioning of multicellular organisms. Pathological angiogenesis most commonly occurs in ischaemic, inflammatory and neoplastic diseases. Conditions in the pathogenesis of which angiogenesis plays an important role are sometimes labelled angiogenic diseases. To date, a number of pro-and anti-angiogenic factors have been defined. VEGF is the only specific mitogen for endothelial cells. It stimulates their growth and inhibits apoptosis, increases vascular permeability in many tissues, promotes vasculogenesis and angiogenesis. VEGF signalling activity in relation to the cell is dependent on having its specific membrane receptors (Flt-1, KDR, Flt-4). Angiogenesis plays a protective role in ischaemic heart disease and myocardial infarction.

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Introduction:

Angiogenesis refers to the biological mechanism through which new blood vessels arise from existing ones. This process plays a crucial role in various physiological functions as well as in several pathological conditions (1).

The vascular system constitutes a vital element of the bone marrow microenvironment, facilitating the transport of nutrients and metabolic waste while ensuring the proper functioning of hematopoiesis (2). Angiogenesis is a multifaceted process that involves the proliferation, migration, and branching of vascular endothelial cells, leading to the creation of new microvessels from preexisting ones in response to a range of stimuli (3).

The rapid division and proliferation of tumor cells demand substantial nutrient supplies, which are delivered via blood vessels. Consequently, the survival, invasion, and metastasis of these tumor cells necessitate the development and growth of new blood vessels (4). In solid tumors, endothelial cells within the proliferating blood vessels exhibit elevated levels of vascular

endothelial growth factor (VEGF) and its receptor, vascular endothelial growth factor receptor (VEGFR) **(4).**

In the context of hematologic malignancies, VEGF promotes mitotic activity, stimulates growth, survival, and migration, and enhances the self-renewal capabilities of leukemia progenitor cells **(5).**

Vascular endothelial growth factor (VEGF) serves as the primary direct stimulator of angiogenesis, influencing various endothelial cell functions such as mitogenesis, permeability, vascular tone, and the production of vasoactive molecules **(6).** The VEGF family comprises five glycoproteins, namely VEGFA, VEGFB, VEGFC, VEGFD (also known as FIGF), and placental growth factor (PlGF, also referred to as PGF) **(7)**.

The human VEGF gene family is situated on the short arm of chromosome 6 and includes five members: VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor **(8).**

The expression levels of VEGF and its receptors are recognized as indicators of angiogenesis in solid tumors. Additionally, various types of leukemia, similar to solid tumors, have demonstrated elevated microvessel density (MVD) in bone marrow **(9)**. While naturally occurring VEGFA and PlGF heterodimers exist, the members of the VEGF family are predominantly homodimeric polypeptides **(10).**

Among the VEGF family, VEGFA (commonly known as VEGF) is the most extensively characterized. It is expressed in several isoforms resulting from alternative splicing, producing mature proteins of 121, 165, 189, and 206 amino acids. The VEGF165 isoform is the most prevalent and is frequently overexpressed in various human tumors **(11).**

VEGFs exert their functions by binding to three structurally related receptor tyrosine kinases: VEGFR1 (FLT1), VEGFR2 (KDR, FLK1), and VEGFR3 (FLT4). These receptors consist of an extracellular ligand-binding region with an immunoglobulin-like domain, a transmembrane domain, and a tyrosine kinase (TK) domain within the cytoplasmic region. The interaction between VEGFs and VEGFRs results in the phosphorylation of distinct tyrosine residues across various intracellular domains of the different VEGFRs **(12).**

The expression patterns of these receptors are notably overlapping yet distinct. Generally, VEGFR1 is found in monocytes and macrophages, while VEGFR2 is present in vascular endothelial cells and VEGFR3 in lymphoid endothelial cells. However, the distribution of VEGF receptors is broader than previously anticipated **(13).**

Three structurally analogous type III receptor tyrosine kinases, namely VEGFR1 (also referred to as Flt-1), VEGFR2 (known as KDR), and VEGFR3 (identified as FLT4), become activated upon the binding of VEGF ligands. Leukemia cells frequently express one or both of the primary VEGF receptor tyrosine kinases, specifically the c-fms-like tyrosine kinase (Flt-1) and the kinase domain receptor (KDR), and they are capable of producing and secreting VEGF **(14).**

Regarding other receptor tyrosine kinases, the binding of VEGF to its receptor induces either homodimerization or heterodimerization of the receptors, followed by autophosphorylation at specific tyrosine residues. This process initiates an intracellular signaling cascade facilitated by various effectors that can recognize and bind to the phosphorylated tyrosine residues of the

activated receptors. These interactions are mediated by Src homology 2 (SH2) domains, phosphotyrosine-binding domains, and other components of the signaling proteins **(15).**

There is substantial evidence indicating that VEGFR2 serves as the primary mediator of VEGF-induced responses in endothelial cells, playing a vital role as a signal transducer in both physiological and pathological angiogenesis. The signaling pathways associated with VEGFR2 are relatively well characterized. In human VEGFR2, the principal autophosphorylation site activated by VEGF binding is Y1175, which functions as a docking site for phospholipase C-gamma. This interaction indirectly facilitates the activation of the mitogen-activated protein kinase pathway, thereby influencing cell proliferation. Furthermore, Y1175 also acts as a binding site for other adaptor molecules, including Src homology 2 (SH2) domain-containing protein adaptor protein B (Shb) **(16).**

The interaction between Shb and phosphorylated Y1175 of VEGFR2 is essential for the VEGF-dependent activation of the phosphatidylinositol 3-kinase/Akt anti-apoptotic pathway. Another significant autophosphorylation site in human VEGFR2 is Y1214, which plays a role in the activation of Cdc42 and p38 mitogen-activated protein kinase **(17).**

Autocrine and paracrine stimulation by VEGF leads to a mitogenic response in hematologic malignancies, particularly enhancing the self-renewal of leukemia progenitors **(18).** The critical function of autocrine VEGF loops in hematological malignancies is supported by the co-expression of both VEGF and its receptors in conditions such as leukemia, lymphoma, and multiple myeloma, along with their direct effects on tumor cell survival, migration, and proliferation. Additionally, it has been demonstrated that autocrine activation occurs in the expression of VEGF and other pro-angiogenic factors, such as tissue factor, in acute myeloid leukemia (AML) blasts **(19).**

VEGF-A, VEGF-C, VEGFR-2, and VEGFR-3 are found to be overexpressed in acute myeloid leukemia (AML). The human vascular endothelial growth factor (VEGF) family includes VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placenta growth factor (PlGF) **(20).**

Various isoforms of VEGF-A and VEGF-B arise from alternative splicing, while different forms of VEGF-C and VEGF-D result from proteolytic processing. Currently, there is no evidence indicating abnormal expression levels of VEGF-B and/or VEGF-D in AML **(21).**

The most extensively studied and likely the most significant members of the VEGF family in the context of AML are VEGF-A and VEGF-C. VEGF ligands have the ability to bind to one or more VEGF receptors, namely VEGFR-1, VEGFR-2 (also known as kinase insert domain receptor, KDR), and VEGFR-3 (also referred to as fms-related tyrosine kinase-4, FLT-4). Isoforms of VEGF-A can interact with either VEGFR-1 or VEGFR-2. The specificity of receptor binding for VEGF-C is regulated by its proteolytic processing (**14**).

VEGFR-2 shows a preference for the fully processed mature form of VEGF-C, whereas VEGFR-3 is capable of binding to both processed and non-processed forms **(22).**

Research indicates that approximately 85% of bone marrow biopsies from AML patients exhibit elevated levels of VEGF-A protein expression when compared to normal bone marrow (NBM) **(23).** The highest levels of VEGF-A mRNA expression are observed in patients with t(8;21) and

t(15;17) translocations, while VEGF-C protein expression is significantly increased in all AML bone marrow samples relative to NBM controls **(24).**

In addition to the increased expression of VEGF ligands, VEGF receptors are also prominently expressed across various subgroups of patients with acute myeloid leukemia (AML). The expression levels of VEGFR-1 protein were found to be comparable between AML bone marrow biopsies and normal bone marrow (NBM) controls **(25).**

Notably, when comparing bone marrow samples from AML patients with the t(15;17) translocation to those with other cytogenetic abnormalities, it was observed that the former group exhibited significantly elevated levels of VEGFR-1 mRNA expression **(25).**

Furthermore, the overall levels of VEGFR-2 protein are markedly higher in the bone marrow of AML patients in comparison to NBM. The highest levels of VEGFR-2 mRNA expression were identified in samples from AML patients with the t(8;21) translocation, surpassing those of other cytogenetic subgroups **(26).**

Additionally, membrane-restricted VEGFR-2 protein expression levels were noted, with 88% of AML bone marrow samples demonstrating significantly elevated membrane VEGFR-2 expression across all cytogenetic variations, while MLL-rearranged AML samples exhibited the highest levels of membrane VEGFR-2 expression. The overall expression levels of VEGFR-3 protein were also found to be significantly increased in bone marrow biopsies from AML patients **(27)**

VEGF-A and VEGF-C ligands are found to be overexpressed in the bone marrow of patients with acute myeloid leukemia (AML), alongside an increased expression of VEGFR-2 and VEGFR-3. The presence of VEGF-A and VEGF-C proteins is noted in the cytoplasm of AML blast cells, and these proteins may also be secreted **(14).**

Total expression of VEGFR-2 is observed on the membranes of AML blast cells, as well as in the cytoplasm and nucleus. The activated form of VEGFR-2 is specifically located in both the cytoplasm and nucleus of these cells, while VEGFR-3 is predominantly expressed in the cytoplasm. The binding of VEGF to its receptors can trigger activation through both internal and external signaling mechanisms. AML cells produce VEGF-A, which can bind to and activate VEGFR-2 **(14).**

VEGF-C is responsible for inducing the phosphorylation of both VEGFR-2 and VEGFR-3 in AML cells. The expression patterns of these VEGF receptors indicate that autocrine signaling in AML blasts may be modulated through internal signaling loops involving VEGFR-2 and VEGFR-3 (intrinsic signaling pathway), whereas the external signaling pathway appears to be primarily restricted to VEGFR-**2 (28**).

VEGF-A and VEGF-C serve as negative prognostic indicators in acute myeloid leukemia (AML). The overexpression of VEGF-A and VEGF-C holds significant prognostic implications for AML. Elevated plasma levels of VEGF-A correlate with diminished rates of complete remission and lower survival outcomes among AML patients. The variability observed in VEGF-A expression among these patients may be influenced by factors such as alternative exon splicing, single-nucleotide polymorphisms (SNPs), or genetic mutations **(29).**

VEGF-A can undergo alternative splicing, resulting in various isoforms, including VEGF121, VEGF145, VEGF148, VEGF165, VEGF183, VEGF189, and VEGF206. These isoforms exhibit distinct functionalities, particularly in their interactions with the extracellular matrix (ECM). Notably, a significant co-expression of VEGF121, VEGF165, VEGF183, and VEGF189 has been observed in AML cases **(30).**

References:

1. Pezzella, F., & Kerbel, R. S. (2022). On coalescent angiogenesis and the remarkable flexibility of blood vessels. Angiogenesis, 25(1), 1–3. https://doi.org/10.1007/s10456-021-09825-2
2. Singh, P., Mohammad, K. S., & Pelus, L. M. (2020). CXCR4 expression in the bone marrow microenvironment is required for hematopoietic stem and progenitor cell maintenance and early hematopoietic regeneration after myeloablation. Stem Cells, 38(7), 849–859. https://doi.org/10.1002/stem.3174
3. Shiiya, T., & Hirashima, M. (2023). From lymphatic endothelial cell migration to formation of tubular Lymphatic Vascular Network. Frontiers in Physiology, 14. https://doi.org/10.3389/fphys.2023.1124696
4. Ribatti, D. (2021). Tumor blood vessels and tumor endothelial cells. *Tumor Microenvironment Regulation of Tumor Expansion*, 51–60. https://doi.org/10.1016/b978-0-12-822803-6.00005-3
5. Swoboda, D. M., & Sweet, K. (2020). Acute myeloid leukemia. *Handbook of Hematologic Malignancies*. https://doi.org/10.1891/9780826149770.0017
6. Salajegheh, A. (2016). Vascular Endothelial Growth Factor (VEGF). Angiogenesis in Health, Disease and Malignancy, 363–374. https://doi.org/10.1007/978-3-319-28140-7_58
7. Pamies Corts, A., Llop, D., Ibarretxe, D., Taverner, D., Rosales, R., Plana, N., Masana, L., Vallve, J. C., & Paredes, S. (2023). AB0053 angiopoietin-2, VEGFA, VEGFC, VEGFD, EGF, PLGF and HB-EGF are growth factors associated with subclinical arteriosclerosis in a cohort of patients with rheumatoid arthritis. *Scientific Abstracts*. https://doi.org/10.1136/annrheumdis-2023-eular.4465
8. Goodsell, D. S. (2022). Vascular Endothelial Growth Factor (VEGF) and angiogenesis. *RCSB Protein Data Bank*. https://doi.org/10.2210/rcsb_pdb/mom_2022_3
9. Surov, A., & Wienke, A. (2021). Associations between FDG PET and expression of VEGF and microvessel density in different solid tumors: A meta-analysis. Academic Radiology, 28(4). https://doi.org/10.1016/j.acra.2020.02.030
10. SENNLAUB, F. (2014). VEGF family members (alternatively, molecular identities of anti-VEGF agents). *Acta Ophthalmologica*, *92*(s253), 0–0. https://doi.org/10.1111/j.1755-3768.2014.3211.x
11. Tao, P., Wen, H., Yang, B., Zhang, A., Wu, X., & Li, Q. (2017). Mir-144 inhibits growth and metastasis of cervical cancer cells by targeting VEGFA and VEGFC. *Experimental and Therapeutic Medicine*. https://doi.org/10.3892/etm.2017.5392
12. Fathi, Z., Boojar, M. M., Sajedi, R. H., Dehnavi, E., & Jahanafrooz, Z. (2024). Expression of VEGFR2 ligand binding domain in Pichia PinkTM 4 cells and evaluation of its interactions with VEGF-A165 receptor binding domain. *Molecular Biotechnology*.

https://doi.org/10.1007/s12033-024-01057-1

13. Sarabipour, S., Ballmer-Hofer, K., & Hristova, K. (2016). VEGFR-2 conformational switch in response to ligand binding. Elife, 5, e13876.
14. Stevens, M., & Oltean, S. (2019). Modulation of receptor tyrosine kinase activity through alternative splicing of ligands and receptors in the VEGF-A/VEGFR AXIS. *Cells*, *8*(4), 288. https://doi.org/10.3390/cells8040288
15. Jadwin, J. A., Oh, D., Curran, T. G., Ogiue-Ikeda, M., Jia, L., White, F. M., Machida, K., Yu, J., & Mayer, B. J. (2016). Time-resolved multimodal analysis of src homology 2 (SH2) domain binding in signaling by receptor tyrosine kinases. *eLife*, *5*. https://doi.org/10.7554/elife.11835
16. Zhang, Y., Zhang, H., Han, Z., Wang, X., Li, X., Yuan, P., Ji, S., & Liu, Q. (2022). A-kinase interacting protein 1 regulates the cell proliferation, invasion, migration and angiogenesis of clear cell renal cell carcinoma cells and affects the ERK/c-myc signaling pathway by binding to RAC1. *Experimental and Therapeutic Medicine*, *24*(3). https://doi.org/10.3892/etm.2022.11489
17. Kim, M.-J., Byun, J.-Y., Yun, C.-H., Park, I.-C., Lee, K.-H., & Lee, S.-J. (2023). *Supplementary Figures S1-S4 from c-Src-P38 Mitogen-Activated Protein Kinase Signaling Is Required for AKT Activation in Response to Ionizing Radiation*. https://doi.org/10.1158/1541-7786.22517040
18. Zhao Yan, Z. Y. (2015). Effects of resveratrol and vitamin C on the self-renewal capacity of blast progenitors in hematologic malignancies. *Journal of Leukemia*, *s1*. https://doi.org/10.4172/2329-6917.s1-006
19. Han, W., Carpenter, R. L., & Lo, H.-W. (2013). TGLI1 upregulates expression of VEGFR2 and VEGF-A, leading to a robust VEGF-VEGFR2 autocrine loop and cancer cell growth. *Cancer Hallmarks*, *1*(1), 28–37. https://doi.org/10.1166/ch.2013.1006
20. Pang, V., Bates, D. O., & Leach, L. (2017). Competitive interplay between VEGF-A165A, VEGF-A165B, PLGF and VE-cadherin in the human feto-placental endothelial barrier. *Placenta*, *57*, 313. https://doi.org/10.1016/j.placenta.2017.07.281
21. PENG, J., PAN, K., LIU, Z.-R., QIN, Y.-H., & PENG, Q.-H. (2018). Effects of Shuang Dan Ming Mu capsule on expression of VEGF-A, VEGF-B, VEGF-C and the VEGF receptor, FLK-1, in diabetic retinopathy rats. *Digital Chinese Medicine*, *1*(3), 228–238. https://doi.org/10.1016/s2589-3777(19)30030-8
22. Assareh, E., Mehrnejad, F., Mansouri, K., Esmaeili Rastaghi, A. R., Naderi-Manesh, H., & Asghari, S. M. (2019). A cyclic peptide reproducing the α1 helix of VEGF-B binds to VEGFR-1 and VEGFR-2 and inhibits angiogenesis and tumor growth. *Biochemical Journal*, *476*(4), 645–663. https://doi.org/10.1042/bcj20180823
23. Medinger, M., Tichelli, A., Bucher, C., Halter, J., Dirnhofer, S., Rovo, A., Passweg, J., & Tzankov, A. (2012). GVHD after Allogeneic Haematopoietic SCT for AML: Angiogenesis, vascular endothelial growth factor and VEGF receptor expression in the BM. *Bone Marrow Transplantation*, *48*(5), 715–721. https://doi.org/10.1038/bmt.2012.200

24. Andrews, C. N., Murphy, K., Moran, S., Thornton, P., Harten, N., & Murphy, P. T. (2014). Prognostic significance of WT1 and prame gene expression in bone marrow samples of MDS and AML patients treated with azacytidine. *Blood*, *124*(21), 5599–5599. https://doi.org/10.1182/blood.v124.21.5599.5599

25. Minna, S. (2017). *Expression Profile of VEGF, VEGF-C and VEGF-D and Related Receptors in Epithelial Ovarian Tumors and Distant Metastasis*. https://doi.org/10.26226/morressier.597eedbdd462b80296ca1a20

26. Moonesi, M., Omran, H. M., Khosravi, S. Z., Ramshe, S. M., Farsani, M. allahbakhshian, Solali, S., & Hagh, M. F. (2023). *Comparison of the Relative Expression of the IGF Gene Family in the Bone Marrow of Patients with AML Compared with Normal Individuals*. https://doi.org/10.21203/rs.3.rs-2989442/v1

27. Qazi, S., & Uckun, F. M. (2022). Augmented expression of the IL3RA/CD123 gene in MLL/KMT2A-rearranged pediatric AML and infant all. Onco, 2(3), 245–263. https://doi.org/10.3390/onco2030014

28. Zhang, Yue, Ding, X., Miao, C., & Chen, J. (2019). *Propofol Attenuated the Effect of TNF-α on Occludin Expression by Inhibiting HIF-1α/VEGF/VEGFR-2/Erk Signaling Pathway in HCMEC/D3 Cells*. https://doi.org/10.21203/rs.2.409/v5

29. Sertoglu, E., yücel, çiğdem, Omma, A., Hayran, Y., Colak, S., CAN SANDIKÇI, S., Durukan, A., & Ozgurtas, T. (2021). Determination of serum vascular endothelial growth factor (VEGF) and VEGF receptor levels with VEGF gene polymorphisms in patients with Behçet's uveitis. *Advances in Clinical and Experimental Medicine*, *31*(3), 231–240. https://doi.org/10.17219/acem/143586

30. Ceylan, H. K., Tayhan, S. E., & Gökçe, İ. (2020). *Expression and Characterization of Functional Human Vascular Endothelial Growth Factor (VEGF165) in Kluyveromyces Lactis*. https://doi.org/10.21203/rs.3.rs-121863/v1