

CAENORHABDITIS ELEGANS AND ANGIOGENESIS

İbrahim Kılıççalan¹, Abdalbaki Erkovan¹, Emine Şen²

¹Gulhane School of Medicine, University of Health Sciences, Ankara, TURKEY

²Department of Biochemistry, Faculty of Pharmacy, Altınbaş University, Istanbul, TURKEY

Abstract: Angiogenesis is sprouting of new capillaries from already existing ones. It is a dynamic process that can be seen in every phase of human life. It is among the dynamic mechanisms of both physiological and pathological processes. Vascular endothelial growth factor is one of the many molecules that play a role in angiogenesis. Vascular endothelial growth factor is released specifically to the endothelium. It regulates mitogenesis, vascular tone, vascular permeability and vasodilatation in the vascular endothelium. *Caenorhabditis elegans* is a nematode used to detect and screen the developmental processes and genetic mutations. It is appropriate to study at the organism level to isolate cells and to demonstrate intercellular interactions *in vivo*. Polyvinyl fluoride-1 is a molecule that plays a role in the neural development of *Caenorhabditis elegans*. In addition, the polyvinyl fluoride-1 molecule is told to be effective in angiogenesis. Studies have shown that polyvinyl fluoride-1 binds to vascular endothelial growth factor receptor-1 and vascular endothelial growth factor receptor-2, but not to vascular endothelial growth factor receptor-3 and platelet derived growth factor receptor β . In the research of human umbilical vein endothelial lines, it was observed that polyvinyl fluoride-1 induced angiogenesis and vascular tube formation. These results suggest that *Caenorhabditis elegans* may have a very important role in vascular endothelial growth factor studies. *Caenorhabditis elegans* model is used in many scientific areas such as aging, nervous system and genetic changes. However, only a few laboratories around the world studied the *Caenorhabditis elegans* angiogenesis model. Besides, this model is not currently used in Turkey. This provides a great advantage in terms of the utilization of this model in angiogenesis.

Keywords: *Caenorhabditis elegans*, vascular endothelial growth factors, VEGFR-1, VEGFR-2

INTRODUCTION

Angiogenesis

Angiogenesis is sprouting of new vessels from mature endothelial cells. This process is a condition that may occur in every phase of human life in both physiological-pathological processes in the body. Physiologically, all tissues need capillaries for diffusion of nutrients and metabolites. To maintain this, the capillaries should be formed from the main vessels. Changes in angiogenesis and capillaries are determined by metabolic activity. Oxygen plays a vital role in these processes (1).

Studies on angiogenesis began in 1971 with the hypothesis that angiogenesis might be the reason for tumor growth (Figure 1) (2). After this hypothesis, ideas such as stimulation of angiogenesis may be used therapeutically in ischemic heart diseases, peripheral artery diseases, and wound recoveries have been thought. In addition to this, there has been a supposal that inhibition or reduction of angiogenesis may be used therapeutically in cancer, ophthalmic diseases, rheumatoid diseases, and other diseases (1). Apart from these pathological conditions, capillaries develop or regress in

healthy tissues according to functional needs. In other words, physiologically, stimulation and inhibition of angiogenesis occur in the body.

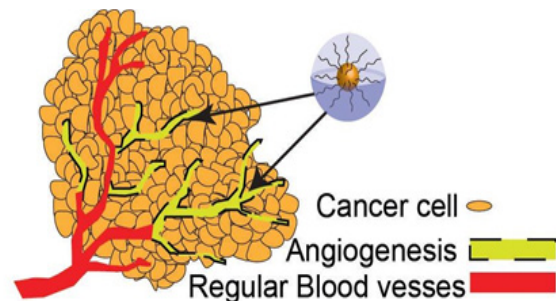


Figure 1: An illustration of angiogenesis on a tumor cell (3).

Various molecules play a role in the regulation of angiogenesis in the body: Fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), angiostatin, thrombostatin etc. (4). Angiogenesis is stimulated and inhibited by such factors.

Vascular Endothelial Growth Factor

Vascular endothelial growth factor is a molecule that regulates mitogenesis, vascular tone, vascular permeability and vasodilation in the vascular endothelium (5). Members of the VEGF family and VEGF receptors play a critical role in physiological and pathological angiogenesis (6). VEGF is a strong angiogenic factor and it is upregulated in many tumor tissues (7). VEGF and VEGF receptors are expressed from many cells, especially endothelial cells and tumor cells (8).

Glycoproteins which form the VEGF family, show their effects on cell membrane receptors (6). These receptors that VEGF family affects are called vascular endothelial growth factor receptors (VEGFRs). VEGFRs are structurally tyrosine kinase receptors (8). VEGF receptors are similar to platelet-derived growth factor receptor (PDGFR). All subtypes of the VEGFR family have seven immunoglobulin-like domains in the extracellular medium and a tyrosine kinase domain in the intracellular medium (6).

The VEGF family consists of five members that have a homodimeric structure: VEGF-A, VEGF-B, VEGF-C, and VEGF-D and Placental growth factor (PlGF). VEGF-A plays a regulatory role in angiogenesis, progression vasculogenesis and differentiation of progenitor endothelial cells. VEGF-B and PlGF induce angiogenesis in ordinary tissues. However, increased PlGF production promotes the development of pathological angiogenesis in tumor and inflammatory lesions. The effects of VEGF-B and PlGF on angiogenesis are weaker than VEGF-A. VEGF-C plays a role in embryonic lymphangiogenesis (6). VEGF-D has mitogenic effects for endothelial cells. VEGF-D is a glycoprotein that stimulates remodeling of the blood vessels and lymphatic vessels (9).

VEGFR family consists of three members: VEGFR-1, VEGFR-2, and VEGFR-3. Vascular endothelial cells, hematopoietic stem cells, some tumor cells, monocytes and macrophages express VEGFR-1 (Figure 2). VEGFR-2 is expressed from vascular endothelial cells, hematopoietic stem cells and some tumor cells. VEGFR-3 is specifically expressed from lymphatic endothelial cells in the human body (6). VEGF types show their effects by binding to VEGFRs. The level of binding varies according to the types of VEGF and VEGFRs. VEGF-A binds to VEGFR-1 and VEGFR-2 among VEGFRs. VEGF-A shows higher affinity to VEGFR-1 than to VEGFR-2. In addition, the tyrosine ki-

nase activity of VEGFR-2 is higher than VEGFR-1 (8). The pro-angiogenic signal occurs mainly via VEGFR-2 (8). VEGF-B and PlGF only bind to VEGFR-1 among VEGFRs (6). VEGF-C and VEGF-D bind to VEGFR-2 and VEGFR-3, but not to VEGFR-1 (10).

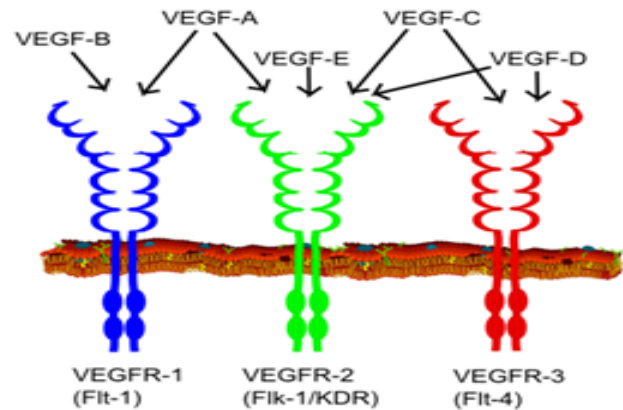


Figure 2: VEGF family members and their receptors (11).

Caenorhabditis Elegans

Caenorhabditis elegans (*C. elegans*) is a small, non-pathogenic nematode found in nature in its free form. Larvae are 0.25 millimeters long, adults are 1 millimeter long (12). *C. elegans* are transparent and have individual cells. Their subcellular details can easily be observed. *C. elegans*' proteins can be labeled using fluorescent proteins (12). Thus, advanced details can be obtained. Fluorescent proteins can also be used to characterize developmental processes and mutations that effect cell function, to isolate cells, and to characterize intracellular and extracellular protein interactions in vivo.

Caenorhabditis elegans' life cycle is really fast. The time from the egg to the non-egg-producing adult is 3 days at 25 °C. *C. elegans* consists male or hermaphrodite sex characteristics. Male *C. elegans* occur in less than 0.2%. Men are initially observed as a self-fertilizing hermaphrodite. Because of all these features, *C. elegans* is an important model for eukaryotic genetic studies. At the same time, *C. elegans* is the first multicellular organism known to hold a full genome sequence; thus allowing the molecular identification of many primary genes in genetic, developmental and biological processes of the cell and the discovery of new key molecules (12).

C. elegans' genome and 60-80% of human genes are found to be orthologue (12). In addition, in the *C. elegans*' genome, 40% of genes that are associated with human diseases have orthologs. Therefore, the discoveries in *C. elegans* are valuable for the study of human health and diseases.

Growth and Maintenance

Caenorhabditis elegans can easily be isolated from rotten vegetables and fruits that are rich in bacterial food sources. In the laboratory, *C. elegans* are grown in agar plates containing *Escherichia coli* (*E. coli*). After *C. elegans* consume the bacteria in the agar, they pass into the dauer form and survive for at least one month. This period can be up to 6 months at 15°C. The *C. elegans* in the dauer form do not need continuous feeding. When healthy, developing animals are needed in the laboratory a piece of old plaque agar is transferred to a new plate containing bacteria, then *C. elegans* return to L4 larvae form from the dauer form and maintain their normal developmental processes (12).

Caenorhabditis elegans is suitable for experimental usage because it can be reused after freezing and to produce new *C. elegans* from a single hermaphrodite. *C. elegans* can be grown at temperatures between 12°C to 25°C. A 10°C increase accelerates the growth twice (12). Development at different temperatures makes it possible to follow the growth rate in *C. elegans*. It also helps in the isolation and utilization of temperature-sensitive mutants (12). *C. elegans* is an easy and cost-effective experimental animal.

Sexual Forms

Caenorhabditis elegans has two forms of wild type: hermaphrodite and male (Figure 3). Hermaphrodite *C. elegans*' gonads produce amoeboid sperms which first stand in L4 staged spermatheca (12). Then, in the period close to adulthood, the ovary changes the fate of the egg cell to produce larger oocytes (12). Normally, hermaphrodites produce sperm a certain time before producing oocytes. The hermaphrodites store the sperm they produce before producing oocytes and normally fertilize these sperms with their newly produced oocytes. About 300 new eggs occur here. If hermaphrodites mate with men, they can produce 1000 eggs. This shows that sperm produced by hermaphrodite is a limited factor in self-fertilization (12). The chromosomes of the hermaphrodite and male *C. elegans* are diploid and contain five autosomal chromosomes (12). The sexes differ in terms of sex chromosome. Hermaphrodites

have two X chromosomes, while males have one X chromosome (Hermaphrodite: XX, Male: XO) (12). Most of the eggs formed by hermaphrodites are hermaphrodites. Only 0.1-0.2% of the eggs form male *C. elegans*.

Male *C. elegans* is fundamental because it allows the exchange of materials necessary to achieve genetic diversity and gene mapping (12).

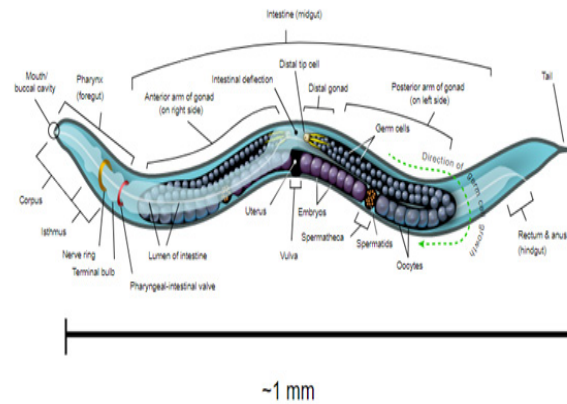


Figure 3: *Caenorhabditis elegans* hermaphrodite adult (13).

Role of *Caenorhabditis elegans* in Genetic Studies
Caenorhabditis elegans is one of the most important and useful experimental animals for genetic studies. One of the major reasons for the use of *C. elegans* in genetic studies is that it is a model suitable for genetic manipulation. In addition, *C. elegans* has many advantages in terms of genetic use. One of the advantages of the usage of *C. elegans* is that they show genetically rapid development. Mutant homozygotes can be detected in two weeks after mutagenesis.

Polyvinyl Fluoride-1

Polyvinyl fluoride -1 (PVF-1) is a polymer expressed from the region of the gene in *Drosophila melanogaster*, *Caenorhabditis remanei*, *Caenorhabditis briggsae* and *Caenorhabditis elegans*. The protein it encodes is called PVF-1. PVF-1 protein is similar to VEGF-A, VEGF-B and PIGF molecules in the molecular structure (14).

Polyvinyl fluoride-1 molecule acts on VEGFR 1-4 receptors in *C. elegans* (15). Further studies have shown that PVF-1 binds to VEGFR-1 and VEGFR-2 but not to VEGFR-3 and PDGF receptor β (14). This results in PVF-1's VEGF A and PIGF is closer to the suggestion.

These molecules interact with PVF-1, thus play a role in both angiogenesis and neural development (14).

The relation between Polyvinyl Fluoride-1 and Angiogenesis

Polyvinyl fluoride-1, which is a molecule encoded from PVF-1 gene region in *C. elegans* has an important role for ray formation. Molecular studies show that; PVF-1 molecule binds to VEGFR-1 and VEGFR-2.

Polyvinyl fluoride-1 induces angiogenesis and capillary tube formation in human umbilical vein endothelial cells (HUVECs) (15). These results suggest that *C. elegans* may have a very important place in VEGF studies.

DISCUSSION

Angiogenesis is in the pathological and physiological processes in our body. This situation shows the importance of angiogenesis in our life cycle. Angiogenesis is the necessary mechanism for the human body. If the production of molecules that lead to angiogenesis is inhibited or the receptors to which the molecules bind are blocked, the formation of angiogenesis is prevented and tumor angiogenesis is inhibited. In this way, the tumor tissue cannot be fed, the vessels required for metastasis cannot be obtained and the body is minimized for damage. Also, tumor angiogenesis is induced to increase the likelihood of tumor-specific drugs. In this way, the drug dose to be given to the patient is reduced, the cost of the drug is decreased and the patient takes a minimum level of medication. These two opposite situations are important for understanding the importance of the balance in angiogenesis.

Many molecules play an important role in angiogenesis that is so critical for our body. VEGF comes at the beginning of these molecules. VEGF is a molecule that regulates mitogenesis, vascular tone, vascular permeability and vasodilation in the vascular endothelium, which is specific to the endothelium (5). VEGF has five different subtypes; VEGF-A, VEGF-B, VEGF-C, VEGF-D and placental growth factor (PlGF) (6). VEGF-A plays a regulatory role in the progression of vasculogenesis, angiogenesis, and differentiation of progenitor endothelial cells (6). VEGF-B and PlGF induce angiogenesis in ordinary tissues (6). However, increased PlGF production promotes the development

of pathological angiogenesis in tumor and inflammatory lesions (6). The effect of VEGF-B and PlGF on angiogenesis are weaker than VEGF-A. VEGF-C plays a role in embryonic lymphangiogenesis (6). VEGF-D is a mitogen for endothelial cells VEGF-D is a glycoprotein that stimulates the remodeling of blood vessels and lymphatic vessels. (9) VEGF shows the effect on the VEGF receptor, which is a tyrosine kinase receptor on the cell surface. There are three subtypes of VEGFR: VEGFR-1, VEGFR-2, and VEGFR-3. Vascular endothelial cells and hematopoietic stem cells express VEGFR-1 and VEGFR-2 in physiological conditions (6). VEGFR-1 and VEGFR-2 are also expressed in some tumor cells pathologically (6). Furthermore, monocytes, and macrophages express VEGFR-1 (6). The kinase activity of VEGFR-1 is very low compared to VEGFR-2 (8). VEGFR-3 is usually expressed from lymphatic endothelial cells in the body (5).

The future of angiogenesis can be changed using VEGF agonists and antagonists. This situation provides the ability to stop the development of many cancers and diseases, regression of diseases and full cure.

CONCLUSION

Caenorhabditis elegans is a good and useful model for the detection of VEGF agonists and antagonists. The PVF-1 molecule, which is responsible for the formation of the first of the rays at the tail of male *C. elegans*, resembles VEGF-A, VEGF-B and PlGF in the molecular structure (14). Studies have shown that PVF-1 binds to VEGFR-1 and VEGFR-2 (14). This suggests that *C. elegans* is a good, useful, inexpensive, simple model for the detection of VEGF agonists and antagonists. Inhibition of angiogenesis, which plays an important role in the pathogenesis of important diseases, with the molecules to be discovered will prevent the progression of many diseases and prevent further discomfort. It will also increase the diversity of treatment options in tumors and many other diseases.

Ethics Committee Approval: N/A

Informed Consent: N/A

Conflict of Interest: The authors declared no conflict of interest.

Author contributions: Concept: AE, İK. Design: AE, İK. Supervision: EŞ, AE, İK. Resources: EŞ, AE, İK. Materials: AE, İK. Data collection and/or processing: AE,

İK. Analysis and/or Interpretation: AE, İK. Literature Search: EŞ, AE, İK. Writing Manuscript: AE, İK. Critical Review: EŞ, AE, İK.

Financial disclosure: The authors declared that this study received no financial support.

Acknowledgements: We thank Prof. Abdullah Olgun from Istinye University Faculty of Pharmacy for his support.

REFERENCES

- Adair TH, Montani JP. Overview of Angiogenesis. *Angiogenesis* (online) 2010 (cited 2019 Jan 5). Available from: URL: <https://www.ncbi.nlm.nih.gov/books/NBK53238/>.
- Folkman J. Tumor angiogenesis: therapeutic implications. *N Engl J Med* 1971;285(21):1182-8.
- Rao DA, Mishra G, Doddapaneni BS et al. Combinatorial polymeric conjugated micelles with dual cytotoxic and antiangiogenic effects for the treatment of ovarian cancer. *Chemistry of Materials* 2016;28:6068-79.
- Adams RH, Alitalo K. Molecular regulation of angiogenesis and lymphangiogenesis. *Nature Reviews Molecular Cell Biology* 2007;8(6):464-78.
- Ng YS, Krilleke D, Shima DT. VEGF function in vascular pathogenesis. *Experimental Cell Research* 2006;312(5):527-37.
- Takahashi H, Shibuya M. The vascular endothelial growth factor (VEGF)/ VEGF receptor system and its role under physiological and pathological conditions. *Clinical Science* 2005;109(3):227-41.
- Duffy AM, Bouchier-Hayes DJ, Harmey JH. Vascular endothelial growth factor (VEGF) and its role in non-endothelial cells: autocrine signalling by VEGF. *Madame Curie Bioscience Database* 2000-2013. Available from: URL: <https://www.ncbi.nlm.nih.gov/books/NBK6482/>.
- Rahimi N. Vascular endothelial growth factor receptors: molecular mechanisms of activation and therapeutic potentials. *Exp Eye Res* 2006;83(5):1005-16.
- Stacker SA, Achen MG. Emerging roles for VEGF-D in human disease. *Biomolecules* 2018;8(1):1.
- Lange C, Storkebaum E, Almodovar CR et al. Vascular endothelial growth factor: a neurovascular target in neurological diseases. *Nature Reviews Neurology* 2016;12:439-54.
- Hägström M. Interactions of VEGF ligands and VEGF receptors (online) 2014 (cited 2019 Jan 5). Available from: URL: https://en.wikiversity.org/wiki/WikiJournal_of_Medicine/Medical_gallery_of_Mikael_H%C3%A4gg_tr%C3%B6m_2014#/media/File:VEGF_receptors.png.
- Corsi AK, Wightman B, Chalfie M. A transparent window into biology: a primer on caenorhabditis elegans (online) 2015 (cited 2019 Jan 5). Available from: URL: http://www.wormbook.org/chapters/www_celegansintro/celegansintro.pdf.
- Schroeder KD. *Caenorhabditis elegans hermaphrodite adult* (online) 2013 (cited 2019 Jan 5). Available from: URL: <https://commons.wikimedia.org/w/index.php?curid=26958836>.
- Tarsitano M, De Falco S, Colonna V et al. The *C. elegans* pvf-1 gene encodes a PDGF/VEGF-like factor able to bind mammalian VEGF receptors and to induce angiogenesis. *FASEB J* 2006;20(1):227-33.
- Dalpe G, Tarsitano M, Persico MG et al. *C. elegans* PVF-1 inhibits permissive UNC-40 signalling through CED-10 GTPase to position the male ray 1 sensillum. *Development* 2013;140(19):4020-30.