The Role of Vascular Endothelial Growth Factor (VEGF) in Tumour Angiogenesis and Anti-Angiogenic Therapeutic Methods to Treat Cancer and Various Retinal Diseases

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Abstract

Tumour cells have a high oxygen and nutrient demand, and thus require a constant blood supply to survive and proliferate. As a result, they secrete abnormally high amounts of a cytokine called Vascular Endothelial Growth Factor (VEGF) to stimulate the formation of new blood vessels from pre-existing ones - a process known as angiogenesis – to acquire an adequate blood supply. This process involves a signaling pathway that begins with VEGF molecules binding to VEGF Receptors (VEGFR) on vascular endothelial cells in blood vessels, which leads to conformational changes in the receptors, then intracellular signal transduction by second messengers, and finally, the activation of the gene that is responsible for blood vessel growth. This vasculature that forms around malignant tumours not only allows for the expansion of the tumour tissue, but, even more concerningly, can lead to metastasis. Therefore, tumour angiogenesis plays a crucial role in the spread of cancer throughout the body, which could kill a patient within months. However, the special characteristics of the molecular mechanisms underlying this signaling pathway offers unique therapeutic opportunities, which is why many scientists today are researching and developing potential treatments to inhibit tumour angiogenesis by targeting certain checkpoints in the pathway. By inhibiting malignant tumour cells from receiving an adequate blood supply, it will be possible to prevent further cancer growth, proliferation, and metastasis from occurring. This same angiogenic pathway can also result in ocular angiogenesis which is responsible for the cause and progression of many retinal diseases, in which high vascular permeability is particularly an issue of concern.

Keywords: vascular endothelial growth factor (VEGF), angiogenesis, tumour, metastasis, phosphorylation, neovascularization, retinal disease

Introduction

Angiogenesis is a normal and essential physiological process regulated by VEGF that occurs in all humans from embryonic development to wound healing in adults. However, VEGF is also a key mediator of angiogenesis in cancer, in which it is upregulated by oncogene expression, various growth factors, and hypoxia (1). As a result of increased VEGF production by malignant cells, a vasculature forms around the tumour to meet the cells' oxygen and nutrient demands. Tumours at first exploit existing blood vessels within an organ for their blood supply, however, their vasculature soon destabilizes due to the release of angiopoietin-2 by vascular endothelial cells (2). This loss of vascular structural and functional integrity is caused by morphological

abnormalities in the blood vessels which causes them to become leaky and hemorrhagic, meaning that the tumour vasculature is suboptimal, thus resulting in hypoxia (1,2). The resultant hypoxia leads to the selection of more aggressive tumour cells primarily due to an increase in the levels of an oxygen-sensitive transcriptional activator known as hypoxia-inducible factor-1 (HIF-1), thereby upregulating the phenotypic expression of VEGF (25). This increased VEGF production can result in an exponential growth in the tumour's size, proliferation, and metastatic potential, which is a highly concerning issue among patients who are suffering from cancer. The unique nature of the VEGF molecular signaling pathway, however, offers a plethora of opportunities for the development of novel therapeutics to treat or possibly even eradicate certain types of cancer. Therapeutics that aim to inhibit several VEGF signaling components have been developed to halt angiogenesis in diseases that involve tissue growth and inflammation, such as cancer (4). The ultimate goal with developing anti-angiogenesis treatments is essentially to deprive solid tumours of the oxygen and nutrients they require to survive and proliferate so that they die. This article discusses the VEGF signaling pathway mechanism and its role in diseases such as cancer, anti-angiogenic therapeutic techniques that have been investigated by scientists and their inhibitory mechanisms of action in the VEGF signaling pathway, as well as some effective anti-angiogenic drugs that have been developed to date. Irregular angiogenesis due to abnormal VEGF production can also contribute to the progression of retinal diseases such as wet age-related macular degeneration and macular edema, which will be further discussed in the article.

VEGF Molecular Signaling Pathway Mechanism

Vascular endothelial growth factor-A (VEGF-A) is essential for endothelial cell functions associated with angiogenesis (3). The signaling pathway begins with the binding of VEGF Receptors (VEGFR) to the VEGF cytokine to form a homodimer, a process known as Dimerization (6). The binding of a VEGF molecule to two VEGFR molecules induces autophosphorylation of the intracellular domains of the receptors, leading to signal transduction (5,6). In this section, we will be investigating the most prominent ligand-receptor complex in the VEGF System: VEGF-A/VEGFR2. VEGFR2, the major signal transducer for angiogenesis, is involved in the phospholipase C-γ-protein kinase C-mitogen-activated protein kinase (PLC-γ-PKC-MAPK) pathway, which is the pathway of interest that will be explained in this section (7). In this system, the VEGF-A molecule binds to and dimerizes VEGFR-1 and VEGFR-2, and signal transduction networks initiated by the VEGF-A/VEGFR2 complex promote angiogenesis, vascular permeability, as well as endothelial cell survival, migration, and proliferation (3,4,5). The dimerization of VEGFR-1 and VEGFR-2 as well as the autophosphorylation between the receptors' intracellular kinase domains is followed by the activation of the VEGF Receptor Tyrosine Kinase (RTK) which induces the phosphorylation of an effector known as phospholipase C - gamma (PLC-γ) (4,8). After that, PLC-γ initiates a downstream intracellular signaling cascade that is facilitated by the sequential phosphorylation of a series of proteins called second messengers (8). The intracellular signal transduction process is regulated by several kinases of the MAPK pathway which phosphorylate and activate the downstream second messenger proteins, following the order: Ras-Raf-MEK-ERK (9,10). The Ras/Raf/MAPK (MEK)/ERK pathway is the most important signaling cascade among all MAPK signal transduction pathways and plays a paramount role in the survival and development of malignant

tumour cells (9). In the last step of the PLC-γ-PKC-MAPK pathway, activated ERK 1/2 phosphorylates another kinase called RSK and both molecules travel to the nucleus of the vascular endothelial cell to activate multiple transcription factors which leads to effector protein synthesis, thus promoting cell proliferation and survival, and ultimately resulting in angiogenesis (11). Therefore, it can be observed through the PLC-γ-PKC-MAPK pathway that the hyperactivation of the expression of ERK 1/2 plays a crucial role in the development and progression of cancer through the promotion of rapid – and most of all aberrant – tumour neovascularization (12).

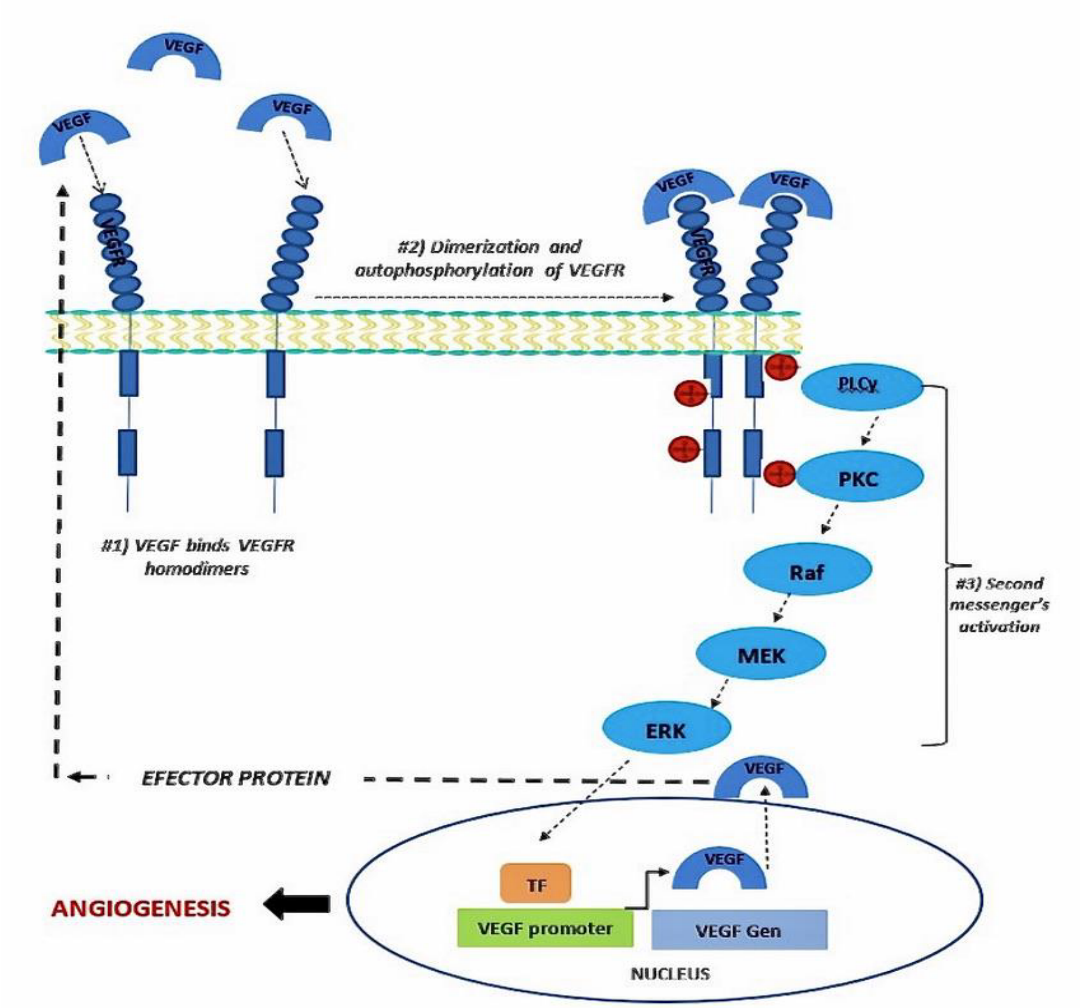

Figure 1: The PLC-γ-PKC-MAPK Pathway (simplified diagram)

Role of VEGF in Retinal Diseases

Levels of VEGF-A have been shown to be elevated in the vitreous humour of patients with angiogenesis-associated ocular disorders such as neovascular (wet) age-related macular degeneration (AMD) as a result of choroidal neovascularization (CNV), macular edema (buildup of fluid such as blood in the macula), and retinal vein occlusion (16). CNV in AMD can be induced by several factors, such as the accumulation of lipids as metabolic by-products,

oxidative stress, abnormal interstitial pressure within Bruch's membrane (located between the retinal pigment epithelium [RPE] and the choroidal capillaries), and hypoxia as a result of a reduction in choriocapillaris blood flow (16,26). In response to metabolic stress, the RPE tissue produces various factors such as VEGF which induce the proliferation of CNV (27). VEGF is also a powerful agonist of vascular permeability, which causes vascular leakage and therefore macular edema (28). Proinflammatory cytokines are also secreted, which contribute to macular edema and diabetic retinopathy as they cause the breakdown of the blood-retinal barrier (35). Consequently, inhibiting VEGF activity is central to the treatment of wet AMD and macular edema, as well as the prevention of progressive capillary nonperfusion (lack of blood flow to parts of the retina which can potentially cause blindness), especially in conditions such as proliferative diabetic retinopathy and retinal vein occlusion (34).

Anti-Angiogenic Therapeutic Techniques & Pharmacologic Agents

1) Anti-VEGF Monoclonal Antibody Treatment

In this anti-angiogenic therapeutic technique, anti-VEGF monoclonal antibodies are developed that bind to the VEGF molecules and prevent them from binding to the VEGF receptor tyrosine kinases, essentially preventing the growth factor signal from reaching the cell, and thus inhibiting the signaling pathway that eventually leads to angiogenesis.

Bevacizumab: Clinical trials assessing the efficacy of VEGF inhibitors are currently taking place in a variety of malignancies, and recently, a humanized anti-VEGF-A monoclonal antibody called Bevacizumab (sold under the brand name, Avastin®) has been approved by the FDA as a first-line therapeutic for metastatic colorectal cancer in combination with chemotherapy (13).

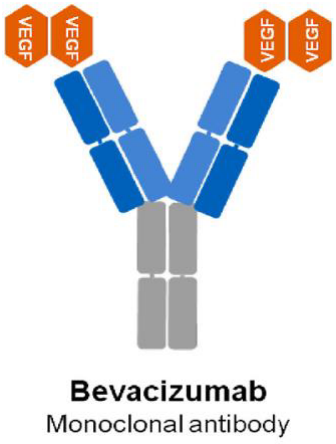

Figure 2: Structure of Bevacizumab

Figure 3: Avastin medication

Bevacizumab Mechanism of Action: Bevacizumab acts by selectively and extracellularly binding to circulating VEGF-A cytokines, thereby inhibiting the binding of VEGF-A to its receptors, VEGFR1 and VEGFR2, on the surface of vascular endothelial cells, and this inhibition

leads to a significant reduction in the microvascular growth of tumour blood vessels, thus limiting and normalizing the blood supply to tumour tissues, allowing them to be cured more easily using more traditional methods such as chemotherapy (14). These effects also lower the interstitial pressure of solid tumour tissue by decreasing vascular permeability, may potentially increase the delivery of chemotherapeutic agents such as chemotherapy drugs to the tumour cells, promote the apoptosis of tumour cells, and most importantly, prevent metastasis from occurring (14,15).

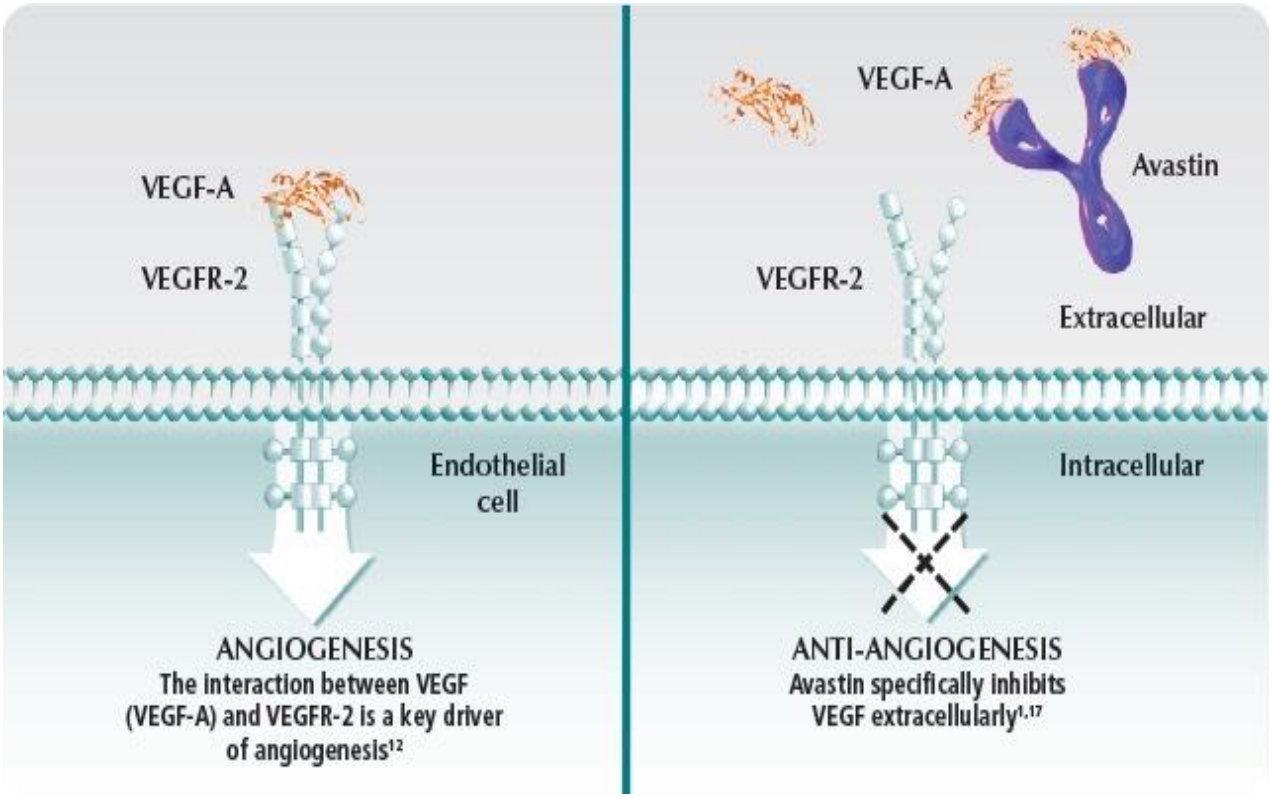

Figure 4: Bevacizumab mechanism of action

Ranibizumab: Sold under the brand name, Lucentis®, Ranibizumab is a recombinant humanized IgG1 monoclonal antibody fragment that binds with high affinity to all active isoforms of VEGF-A and inhibits the cytokine from binding to its receptors on the surface of vascular endothelial cells – very similar to how Bevacizumab functions – thus preventing the neovascularization and vascular leakage that contribute to the progression of retinal diseases (16, 19). Ranibizumab is commonly used to treat visual impairments involving aberrant angiogenesis in the retina such as neovascular AMD, macular edema following retinal vein occlusion, diabetic macular edema, myopic choroidal neovascularization, and diabetic retinopathy (17). These retinal conditions occur due to the secretion of high levels of VEGF by photoreceptor cells which in turn leads to the angiogenesis of retinal blood vessels with high vascular permeability, thus causing leakage of blood in the retina or RPE, and ultimately resulting in the disruption or loss of vision. VEGF-A – which binds to the extracellular ligand-binding domains of two receptor tyrosine kinases, VEGFR1 and VEGFR2 – is currently Ranibizumab's major target of inhibition for the treatment of retinal diseases by the suppression of aberrant angiogenesis (16). Each

molecule of Ranibizumab has only one VEGF paratope due to the fact that it is a monoclonal antibody fragment, meaning that two Ranibizumab molecules are required to bind a VEGF dimer on the surface of a vascular endothelial cell, while each Bevacizumab molecule – which is a full-length monoclonal antibody – has two VEGF paratopes (16,18).

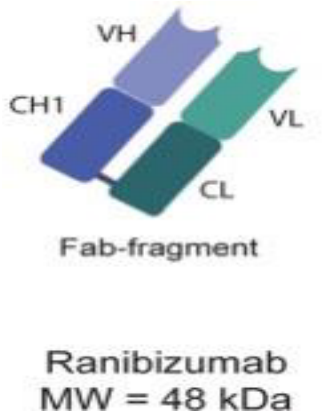

Figure 5: Structure of Ranibizumab

Figure 6: Lucentis medication (for intravitreal injection)

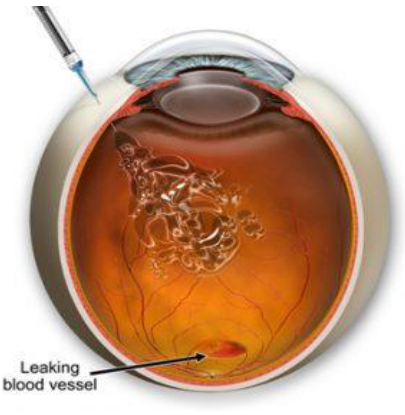

Figure 7: Intravitreal injection of Ranibizumab to treat macular edema

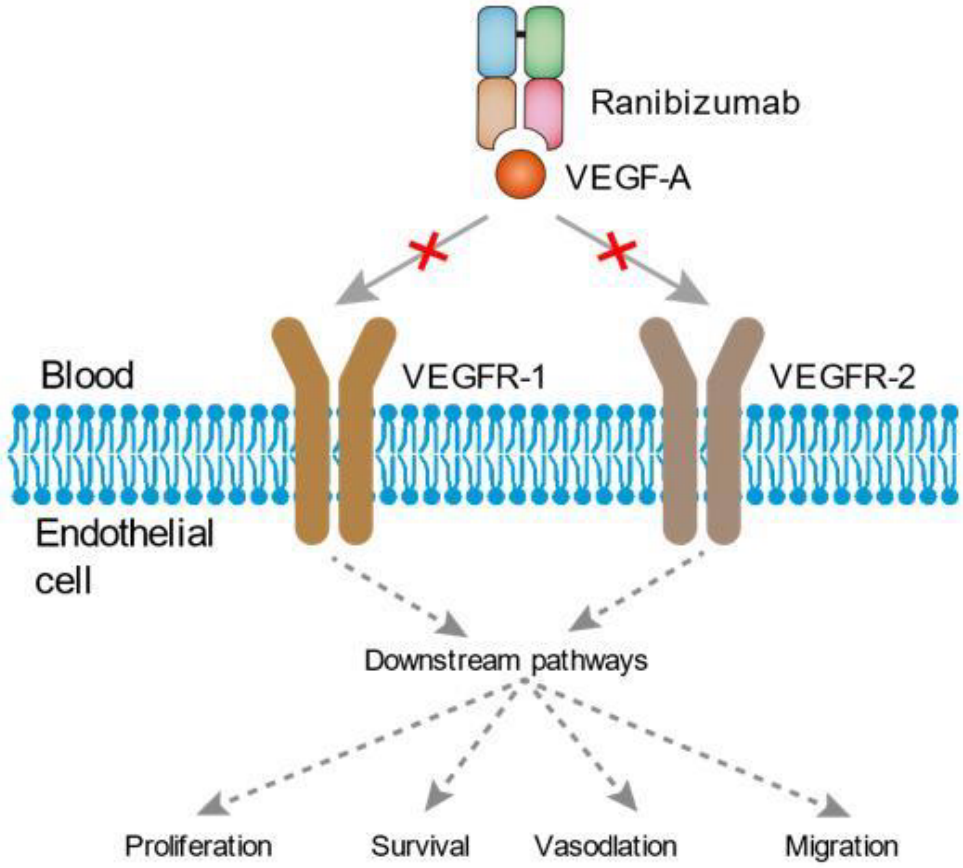

Figure 8: Ranibizumab mechanism of action

Aflibercept: Sold under the brand names, Eylea® and Zaltrap®, Aflibercept is a soluble decoy receptor that binds to VEGF-A, VEGF-B and placental growth factor (PlGF) with higher affinity than the cytokines' natural receptors (20,23). In one experimental model, Aflibercept's equilibrium dissociation constant, K_d (inversely related to binding affinity), for VEGF-A_{165} was 0.49 pM, compared with 9.33 pM and 88.8 pM for VEGFR1 and VEGFR2, respectively (20). It is called a decoy receptor because VEGF-A mistakenly binds to Aflibercept instead of binding to its natural receptors, thus suppressing their activation and preventing the subsequent development of angiogenesis (20). Structurally speaking, Aflibercept is a humanized recombinant fusion protein that consists of an IgG backbone fused to extracellular domain sequences of the receptors, VEGFR1 and VEGFR2 (20,22,23). The therapeutic is currently used to treat retinal diseases – including diabetic retinopathy, macular edema following retinal vein occlusion, diabetic macular edema, and most prominently, neovascular AMD – by preventing highly permeable blood vessels from forming underneath the retina, a condition that can leak blood and impede vision (20,21,22). Aflibercept has also been shown to be effective against metastatic colorectal cancer when combined with cytotoxic chemotherapy (24).

Figure 9: Eylea medication (for intravitreal injection)

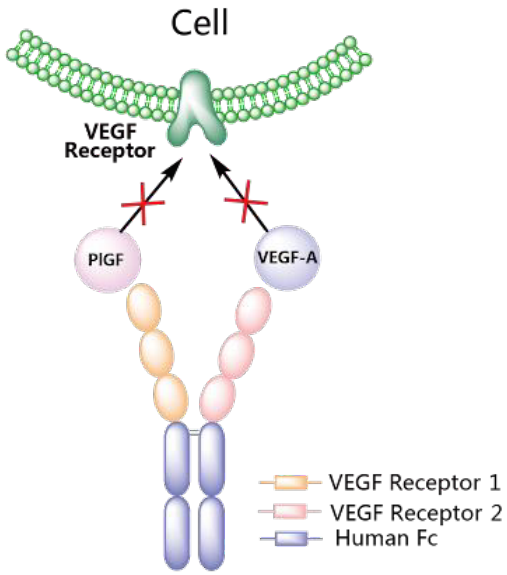

Figure 10: Aflibercept structure and mechanism of action

2) Tyrosine Kinase Inhibitors

Tyrosine kinases are cellular signaling proteins that mediate a variety of biological activities such as cell proliferation and migration, and in angiogenesis, multiple kinases are involved including the receptor tyrosine kinase, VEGFR (29,33). Anti-angiogenic tyrosine kinase inhibitors (TKIs) are anticancer pharmacologic agents that suppress the signal transduction pathways of protein kinases by several modes of inhibition (30). Three anti-angiogenic TKIs with differential binding capacities to angiogenic tyrosine kinases were recently approved for the treatment of patients with advanced cancer: sunitinib, sorafenib and pazopanib (31). The

inhibitors all follow the same fundamental mechanism of action: they competitively bind to the catalytic binding site of tyrosine kinases and inhibit phosphorylation – the addition of phosphate groups after their extraction from ATP molecules to intracellular kinase domains on receptor tyrosine kinases to activate the signal transduction pathway that leads to the transcription of angiogenic factors – thereby suppressing angiogenesis (30,31,32).

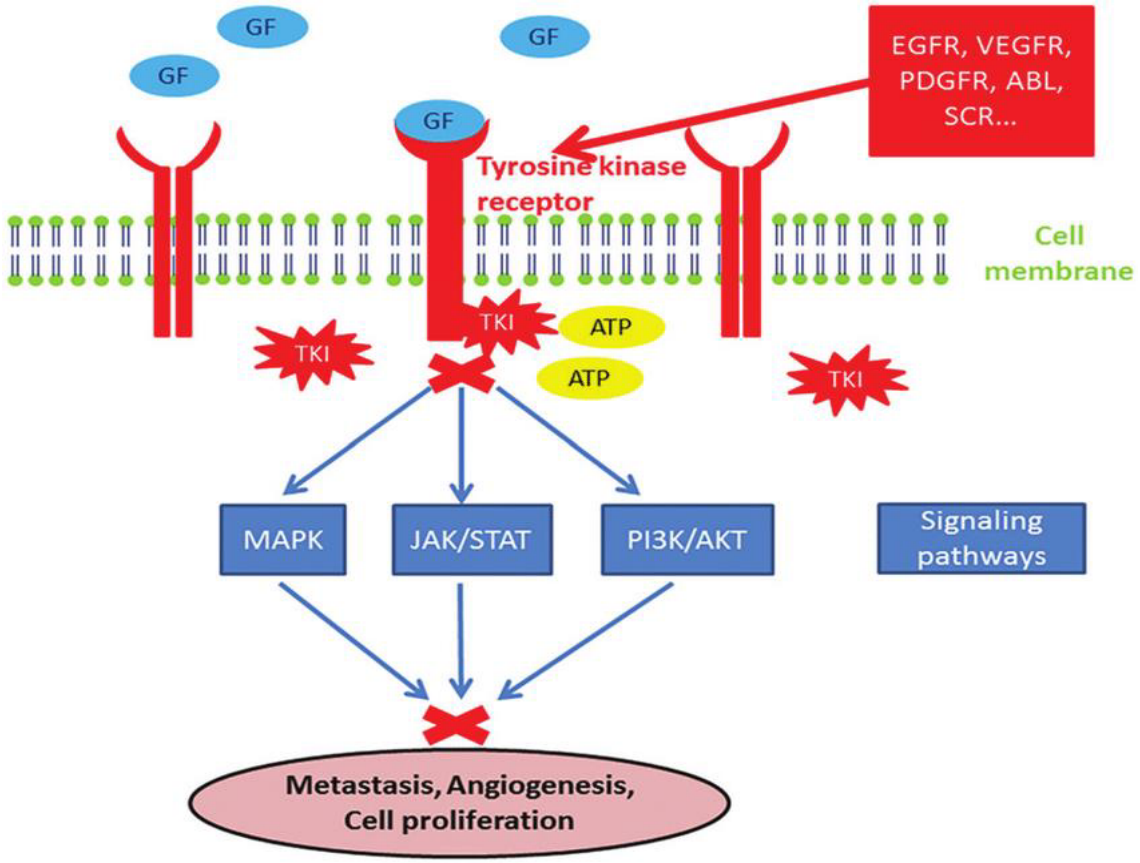

Figure 11: Tyrosine kinase inhibitor mechanism of action

Conclusion

Tumour neovascularization as a result of angiogenesis is a crucial event in the progression and spread of cancer throughout the body. Malignant tumour cells 'turn off' their apoptosis genes, express their oncogenes (which causes abnormal cell growth and proliferation), and secrete abnormally high loads of VEGF and other factors to promote further angiogenesis and increase their blood supply. As such aggressive cells, tumours require a vasculature exceeding that of healthy cells and tissues around the body, which could have serious consequences for the patient, as it could disrupt certain physiological functions and systems in the body, and worst of all, result in metastasis. Therefore, it is of utmost importance to develop therapeutics to inhibit tumour angiogenesis from occurring in order to prevent malignant tumour cells from acquiring any blood supply, therefore depriving them from the oxygen and nutrients they require to

survive. Anti-VEGF monoclonal antibody treatments and tyrosine kinase inhibitors have evidently shown to be promising anti-angiogenic therapies to treat cancer and various retinal diseases, but more research must be performed to enhance the efficacy of these treatments, eliminate their adverse side effects, and investigate which method of administration is best. Furthermore, medical research into other potential anti-angiogenic therapeutic methods – such as the development of antibodies that block VEGFRs, the inhibition of second messengers, or the suppression of VEGF gene expression – can offer an endless array of opportunities that will allow us to develop more novel, life-saving therapeutics against angiogenesis in a variety of cancers and retinal diseases.

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