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ANGIOGENESIS AND CANCER THERAPY

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ABSTRACT: Cancer spreads by metastasis which is the ability of cancer cells to penetrate into lymphatic and blood vessels, circulate through the bloodstream, and then invade and grow in normal tissues elsewhere. It is this ability to spread to other tissues and organs that makes cancer a potentially life-threatening disease. Cancer researchers involve in the study of the conditions necessary for cancer metastasis have discovered that, the most critical event required is the growth of a new network of blood vessels. This process of forming new blood vessels is termed angiogenesis. Many angiogenic inhibitors have been identified and used for therapeutic purposes but have not proved very beneficial in terms of long-term survival. This could be due to the non-specific nature of these inhibitors which accounts for their high toxic levels. We believe the way forward is to identify angiogenic inhibitors which are specific to vascular endothelial growth factor (VEGF) or their receptors (VEGFR-1, VEGFR-2, VEGFR-3). These target specific inhibitors of angiogenesis which come with minimum toxic levels could be explored to develop effective cancer therapy.

INTRODUCTION: Angiogenesis plays a very critical role in the development of cancer. Cancer spreads by metastasis which is the ability of cancer cells to penetrate into lymphatic and blood vessels, circulate through the bloodstream, and then invade and grow in normal tissues elsewhere. It is this ability to spread to other tissues and organs that makes cancer a potentially life-threatening disease [1]. Cancer researchers involve in the study of the conditions necessary for cancer metastasis have discovered that one of the critical events required is the growth of a new network of blood vessels. This process of forming new blood vessels is termed angiogenesis [2].

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The tumor cells produce or cause nearby cells to produce growth factors that stimulate the formation of this new blood vessels [3]. One of the well-studied angiogenesis factors which is very critical in the proliferation and survival of tumor cells and in the neovascularization is the vascular endothelial derived growth factor (VEGF) family and the associated receptors, especially receptor 2 (VEGFR-2) [4]. Studies have shown that the blood vessels created in response to the presence of tumor cell are not exactly the same as normal blood vessels and are also less organized and leakier than normal vessels [5]. The inhibition of VEGFR-2 activity and its downstream signaling are therefore important targets for cancer therapy.

Methodology: This review was done by compiling references from major databases like PubMed, Science Direct, Google scholar, Scopus, Online journals, Open J Gate, etc.

We also used data and information on ongoing studies in our laboratory on angiogenesis.

Normal Angiogenesis: Angiogenesis is the process of new blood vessel formation from pre-existing vasculatures. It plays an essential role in development, normal tissue growth, wound healing and the female reproductive cycle (placental development, ovulation, corpus luteum) [6]. A developing child in the mother's womb must create the network of arteries, veins, and capillaries that are found in the human body. This process of vasculogenesis creates the primary network of vascular endothelial cells that become major blood vessels.

Angiogenesis then remodels this network into capillaries that forms the child's circulatory system. In women, angiogenesis is active a few days each month as new blood vessels lines the uterus during the menstrual cycle. Also, angiogenesis is necessary for the repair or regeneration of tissue during wound healing [7]. Angiogenesis is therefore a normal process that occurs in healthy cells for special purposes but tumor cells are also able to explore this to support their perpetual growth [2].

Angiogenesis in Cancer: Although tumor cells are abnormal, they still require oxygen and nutrients to survive [7]. The development of blood vessels is therefore an essential step in the growth of a tumor. Solid tumors smaller than 1 to 2 cubic millimeters are usually vascularised, because to spread efficiently, they need to be supplied by blood vessels that bring oxygen and nutrients and remove metabolic waste. Beyond the critical volume of 2 cubic millimeters, oxygen and essential nutrients have difficulty diffusing to the cells in the center of the tumor [3]. This is because the area around the cells in a tumor starts to get too far from the blood vessels. This results in a state of cellular hypoxia which triggers changes in the behavior of the tumor cells.

The state of cellular hypoxia marks the start of tumoral angiogenesis. Studies have shown that, the tumor cells produce or cause nearby cells to produce growth factors that stimulate the formation of blood vessels [5]. The development of new blood vessels is an important process and stage in tumors progression. This process favors the transition from hyperplasia to neoplasia. This neovascularization also influences the spread of tumor cells in the entire body and eventually leading to metastasis formation [6]. Tumor cells that do not produce or cause nearby cells to produce angiogenesis factors cannot progress.

Vascular Endothelial Growth factor: Many different proteins have been identified as angiogenic activators (angiogenesis factors), including vascular endothelial growth factor (VEGF) family, basic fibroblast growth factor (bFGF), angiogenin, transforming growth factor (TGF-α, TFG-β), tumor necrosis factor (TNF-α), platelet-derived endothelial growth factor, granulocyte colony-stimulating factor, placental growth factor, interleukin-8, hepatocyte growth factor, and epidermal growth factor [8].

Various studies have proved that the endothelial growth factor and their associated receptor 2 (VEGFR-2) are critical in the proliferation and survival of various cancers through the regulation of neovascularization[1, 9]. The VEGF family (**Fig. 1**) is currently made up of the following members; VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F and P1GF. All of them have a common VEGF homology domain [10]. Vascular endothelial growth factor (VEGF-A) is the key molecule for angiogenesis and for the survival of the endothelium. It is a specific endothelial cell mitogen and a strong vascular permeability factor (VPF) and it is secreted as homodimer of 45kDa by many different cell types [11].

Many different splice variants of VEGF-A have been described by researchers, but VEGF 165 is the most predominant protein and anchors with its heparin binding domain to extracellular matrix and to heparin sulfate [12]. Within the VEGF family, some are involved in the proliferation of blood vessels as has already been pointed out in the case of VEGF-A, whereas others are also involved in lymphangiogenesis [13]. VEGF-A, VEGF-B, VEGF-C and VEGF-E act on their respective receptors to cause proliferation of blood vessels, whereas VEGF-C and VEGF-D are involved in lymphangiogenesis [8]. As shown in **Fig. 2**, the following three receptors have been identified for the VEGF family; VEGFR-1 (Flt-1), VEGFR-2 (KDR) and VEGFR-3 (Flt-4). VEGF-A signals through two receptors; VEGFR-1 and VEGFR-2. These receptors are expressed predominantly but not exclusively on vascular endothelial cells [11].

VEGFR-2 is known to be the principal signaling receptor for vascular endothelial cells, whereas VEGFR-1 plays important role in migration and possibly function as a decoy receptor to regulate the bioavailability of VEGF-A in a tissue [14]. VEGFR-3 is reported to be predominately expressed on lymphatic vessels during development and therefore play important role in lymphangiogenesis [14].

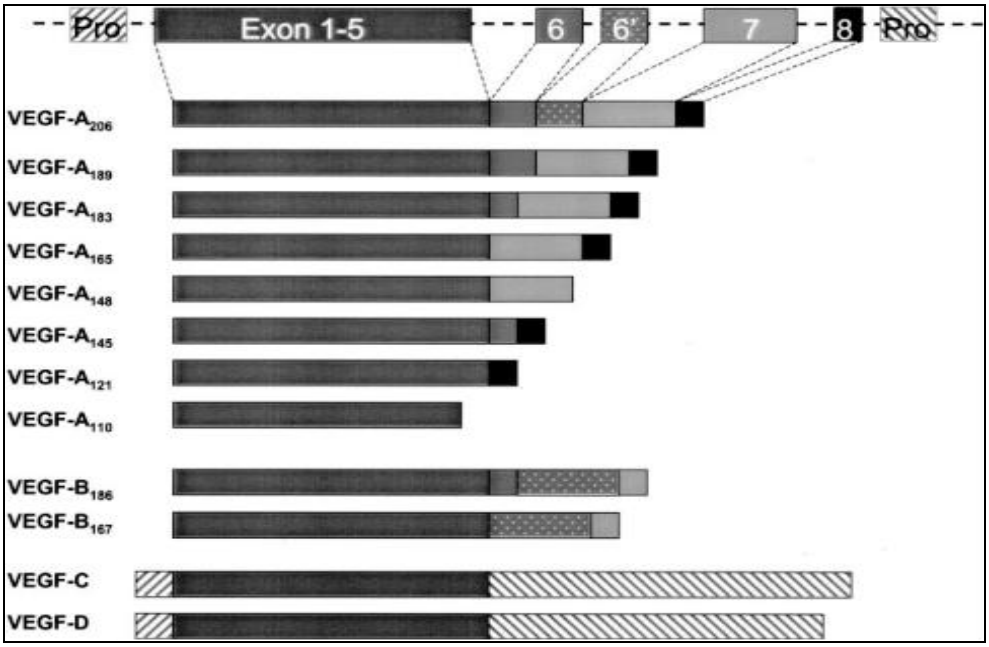

FIG 1: GENE STRUCTURE OF VEGF-A, VEGF-B, VEGF-C, AND VEGF-D. The VEGF-A gene consists of eight exons that give rise to seven isoforms of 121, 145, 148, 165, 183, 189, and 206 amino acids through differential splicing. An additional VEGF-A isoform of 110 amino acids results from proteolytic cleavage. VEGF-B exists as two isoforms of 167 and 186 amino acids. VEGF-C and VEGF-D are proteolytically released from their respective proproteins. All VEGF family members share a highly preserved VEGF homology domain, encoded by exons 1 to 5 [10].

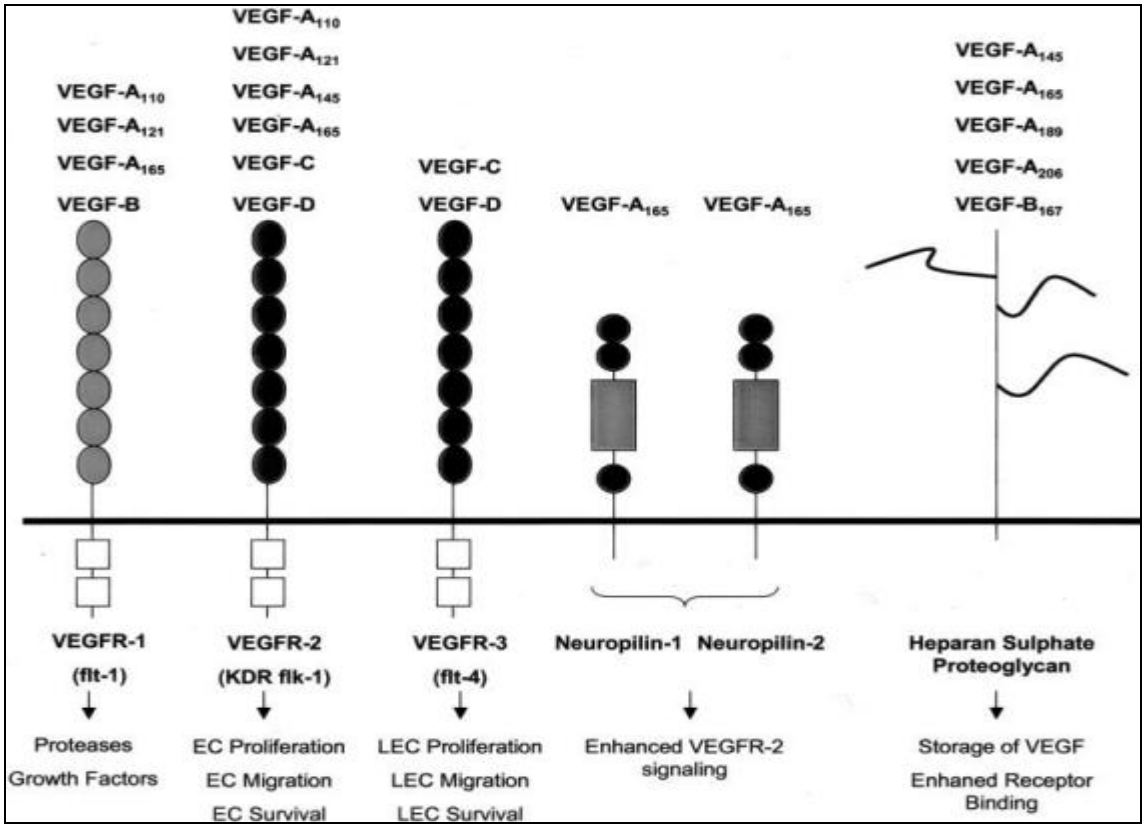

FIG. 2: THE VEGF RECEPTOR FAMILY. The three signaling tyrosine kinase receptors VEGFR-1 (flt-1), VEGFR-2 (KDR flk-1), and VEGFR-3 (flt-4) consist of seven immunoglobulin-like structures in the extracellular domain, a single transmembrane region, and a consensus tyrosine kinase domain interrupted by a kinase insert domain. The accessory receptors neuropilin 1 and neuropilin 2 possess no kinase activity of their own but enhance VEGFR-2 signaling. The heparan sulfate proteoglycans not only enhance VEGFR binding but also serve as a reserve of VEGF [10].

Mechanism of the Tumoral Angiogenic factors: Certain cytokines and some other growth factors have been implicated in the expression of VEGF in cancerous tissues and their adjacent stroma. This is an important step in neovascularization [15]. Hypoxia resulting from the increase in the distance between the growing tumor cells and the capillaries carrying oxygen and nutrients induces the expression of VEGF and its receptor through hypoxia-inducible factors-1α (HIF-1α) [16]. Cancer cells feed on the new blood vessels by producing VEGF and then secret it into the nearby tissues.

The cancer cells bind to the receptors on the endothelial cells when they encounter endothelial cells. This process activates the relay proteins that transmit a signal into the nucleus of the endothelial cell [8]. This nuclear signal activates a group of genes to produce materials that are needed for new endothelial cell growth. Endothelial cells activated by VEGF produce matrix metalloproteinases (MMPs). These break down the extracellular matrix which fills the spaces between cells with protein and polysaccharides. This allows the endothelial cells to migrate [17]. The cells begin to divide as they migrate into the nearby tissues. They then organize into hollow tubes that evolve and develop gradually into a mature network of blood vessels with the help of an adhesion factor, such as integrin α or β. Angiotensin-1, -2, and their receptor Tie-2 stabilizes and regulate vascular growth [8, 17].

Vascular Endothelial Growth Factor (VEGF) as a Prognostic factor of Angiogenesis: Several studies have clearly shown that angiogenic factors play an important role in the growth and spread of tumors however an adequate assessment and measurement of tumor angiogenic activity has not been established and therefore no simple reliable methods are available[8, 10]. A lot of research groups have attempted quantifying angiogenesis and providing prognostic information by measuring tumor microvessel density, intratumoral VEGF determination or monitoring circulating VEGF [10], [18].

Immunohistochemical examination of microvessel density is believed to be a marker representing the effect of angiogenesis and often correlates with intratumoral VEGF mRNA levels [10]. The presence of microvessels reflects the potential for invasion and metastasis; this is because newly generated microvessels are an initial target of cancer cell invasion. As reported elsewhere [19-22], many researchers have examined the prognostic value of microvessel density in prostate [19], colorectal [20], breast [21], lung [22] and other common cancers. Although most of these studies were able to establish a positive correlation between microvessel density and tumor cell recurrence, some have also reported negative correlation between microvessel density and tumor development [23]. This therefore shows that although, microvessel density is a useful maker for prognostic it cannot be relied upon completely for therapeutic purposes [23].

According to reports elsewhere [10], intratumoral vascular endothelial growth factor can also be measured using immunohistochemistry, quantitative immunoassays, RT-PCR or western blotting. Although recent studies [24] have shown that the quantity of VEGF-A expressed by tumor cells affect clinical outcome, none of the available techniques employed in quantifying tumor VEGF-A in solid tumors are routine and therefore not certain if such techniques were widely undertaken that they would be clinically useful or cost-effective to predict outcomes in individual patients [18].

In the case of monitoring circulating vascular endothelial growth factor, studies by researchers have revealed that the level of VEGF-A circulation is a useful marker for tumor status and prognosis in most types of human cancer[25, 26, and 27]. A high level of VEGF-A in cancer patients serum is generally considered as unfavorable clinical parameter which indicates disease progression, lack of response to therapy and possible poor survival. Increased levels of VEGF in serum could therefore be clinically useful in predicting tumor progression, recurrence or metastatic spread in cancer patients [18]. However, the VEGF-A serum measurement cannot be used as a screening tool because VEGF-A serum measurement is not efficient at the early stages of the disease [10]. This is because the serum level of VEGF-A is usually low at the early stages of the disease.

Angiogenesis Inhibitors for Cancer: There are currently thirteen approved anti-cancer therapy in USA in particular and the world at large with recognized antiangiogenic properties in oncology [28]. These agents, who interrupt critical cell signaling pathways involved in tumor angiogenesis and growth, comprise three primary categories.

These are; monoclonal antibodies directed against specific proangiogenic growth factors and/or their receptors, small molecule tyrosine kinase inhibitors (TKIs) of multiple proangiogenic growth factor receptors, and inhibitors of mTOR (mammalian target of rapamycin) [28, 29]. In addition, at least two other approved angiogenic agents may indirectly inhibit angiogenesis through mechanisms that are not completely understood [28, 29].

A humanized monoclonal antibody such as bevacizumab (Avastin) and aflibercept are capable of binding to biologically active forms of vascular endothelial growth factor (VEGF) and therefore prevents its interaction with VEGF receptors (VEGFR-1 and VEGFR-2), thereby inhibiting endothelial cell proliferation and angiogenesis [29-31]. Seven small molecule tyrosine kinase inhibitors (TKIs) with antiangiogenic activity are currently approved as anticancer therapies. These are axitinib (Inlyta), cabozantinib (Cometriq), pazopanib (Votrient), regorafenib (Stivarga), soafenib (Nexavar), sunitinib (Sutent), and vandetanib (Caprelsa) [30].

These inhibitors target VEGF receptors (VEGFR-1, VEGFR-2 and VEGFR-3). Also two mammalian target of rapamycin (mTOR) inhibitors; temsirolimus (Torisel) and everolimus (Afinitor) have been approved currently for anti-cancer therapy [28-30]. Other known antiangiogenic agents are; Interferon alpha, Lenalidomide, Thalidomide, and rhEndostatin. Interferon alpha is a pharmacological version of an endogenous cytokine with antiagiogenic activity, whereas rhEndostatin which is only available in China is an endogenous angiogenesis inhibitor and recombinant protein that blocks VEGF-induced tyrosine phosphorylation of KDR-Flk-1 in endothelial cells, and down regulates MMP-2/9 [28, 29]. Lenalidomide and Thalidomide on other hand possess immunomodulatory, anti-inflammatory and antiangiogenic properties, although the exact mechanisms of action are not fully understood [28-30].

Angiogenesis and Cancer Therapy: Angiogenesis plays a very important role in the growth of a tumor cell. This is because the tumor cell just like normal cells requires constant supply of oxygen and nutrients to survive and progress to infect other organs. The mechanism of tumoral angiogenesis has been well studied and all the parameters involved identified by researchers.

This background knowledge of tumoral angiogenesis has created the right platform for scientists to come out with therapeutic methods that target the inhibition of tumoral angiogenesis. An effective method that inhibits tumoral angiogenesis will cut supply of oxygen and nutrients to tumor cells and therefore prevent their growth and metastasis. As mentioned above, Researchers have been able to identified inhibitors to tumoral angiogenesis [30]. They have even gone further by using these inhibitors for therapeutic purposes [28]. The interest of our laboratory currently is on monoclonal antibody therapy, because we believe it is associated with little toxicity.

The vast majority of clinical competitors for Avastin in oncology are small molecule VEGFR inhibitors which have multiple intracellular targets accounting for some of the additional observable toxicities not directly related to VEGFR-2 inhibition [32]. More than 30 small molecule VEGFR inhibitors including selective, dual and multityrosine kinase inhibitors are in development and 27 of them are in clinical stages [32]. Although an overall survival advantage was seen in non-small cell lung cancer when combined with chemotherapy, Avastin did not extend survival in the other disease settings and was approved based on improvement in progression-free survival or in objective response rate [33-35].

Moreover, the nonspecific inhibition of Avastin on VEGF induced downstream pathways that brought about some side effects such as hypertension and endothelial bleeding[36]. However we believe the development of novel monoclonal antibodies particularly against VEGFR-2 will offer a promising solution to cancer therapies. Our laboratory has since produced scFv which has demonstrated potent anti-angiogenic activities in both proliferation and in migration of human umbilical vein endothelial cells (HUVECs). We are still conducting further studies on it and we believe it could likely serve as therapeutic antibody cancer therapy with minimum toxicity [1].

CONCLUSION: Successful inhibition of tumoral angiogenesis is an important step towards cancer therapy discovery, knowing very well that it plays a major role in tumor growth and metastasis. Tumor cells which are not able to stimulate angiogenesis stop growing, when they reach a certain size and therefore cannot develop to the dreadful metastatic stage.

Many researchers of cancer therapy consider inhibition of angiogenesis an important area to focus, when it comes to cancer therapy knowing the prospects it holds. This has informed the recent interest of many researchers in this area of research. Many inhibitors of angiogenesis have been identified by researchers and are being used for therapeutic purposes and also some are at the developing and clinical stages.

Our laboratory just like other laboratories with interest in identifying angiogenesis inhibitors which could become a possible agent for cancer therapy is also working fervently on this research area. We strongly believe that, the target now should be on inhibitor with minimal toxic levels. That is the concentration should be on inhibitors that target VEGF or their receptors (VEGFR-1, VEGF-2, VEGFR-3) specifically.

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Competing Interests: Authors disclose no potential conflicts of interest.

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