International Journal of Pharmaceutical and Bio-Medical Science

ISSN(print): 2767-827X, ISSN(online): 2767-830X

Volume 04 Issue 04 April 2024

Page No: 407-415

DOI: https://doi.org/10.47191/ijpbms/v4-i4-23, Impact Factor: 7.792

Vascular Endothelial Growth Factor (VEGF) and Its Role in the Progression and Development of Breast Tumor

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ABSTRACT

Breast cancer is the most common type of cancer in women and is widely known. Angiogenesis is the process through which new blood vessels develop from the body's current vascular system. It is essential for tumor growth, invasiveness, and metastasis, and so plays a pivotal role in the development of carcinoma. Proteolytic and proangiogenic catalyst activators and inhibitors regulate angiogenesis in a hierarchical fashion. The angiogenic process is largely under the direction of VEGF. In a select number of malignant tumors, the VEGF gene is overexpressed. The function of VEGF in angiogenesis has been the subject of intensive study in recent years. In breast cancer patients, VEGF plasma levels are highly predictive of tumor growth and survival. Several VEGF gene polymorphisms, have been identified to affect gene expression level in prior investigations. Epidemiological studies have linked polymorphisms in the VEGF gene to altered cancer risk, tumor growth, and metastasis. Previous research on VEGF polymorphism to evaluate the association between genes and breast cancer susceptibility was scant. The current review discusses the role of VEGF in the progression of breast cancer in addition to its promising usage as a predictive marker for breast cancer.

KEYWORDS: Angiogenesis, Breast Tumor, Vascular Endothelial Growth Factor, +405G/C (rs2010963), -2578C/A (rs699947).

ARTICLE DETAILS

Published On:
30 April 2024

Available on:
https://ijpbms.com/

INTRODUCTION

The Vascular Endothelial Growth Factor (VEGF) family of angiogenic and vasculogenic growth factors is extremely powerful. The effects of VEGF are not exclusive to the cardiovascular system. Bone and blood cell production and other tissue remodeling also fall within this category. Numerous cell types, including tumor cells, endothelial cells, macrophages, platelets, and keratinocytes, secrete VEGF for these reasons (1).

Over the past two decades, scientists have thoroughly described the vascular endothelial growth factor (VEGF) family, which makes up the most crucial signaling pathway in angiogenesis (2). There are seven known members of this family: vascular endothelial growth factor (VEGF)-A, -B, -C, -D, -E, -F, and -1 and -2 of placental growth factor (PIGF). In specifically, vascular endothelial growth factor-A (or VEGF-A) is the key player in angiogenesis. The VEGF homology domain is shared by all family members. Each monomer contains a conserved central four-stranded -sheet that dimerizes in an antiparallel, side-by-side orientation. This core area is made up of a cystine knot motif, with eight invariant cysteine residues implicated in inter- and intra-molecular disulfide connections at one end of the molecule(3).

The adult heart, adrenal gland, kidney, and lung have the highest VEGF-A mRNA levels of any normal tissue. Liver, spleen, and gastric mucosa all have lower VEGF-A transcript levels, although they are still detectable. There are also at least eight distinct homodimeric isoforms of VEGF-A. There are a wide variety of monomer compositions, with amino acid counts ranging from 110 to 206. The several VEGF isoforms are produced from the same gene by means of alternative mRNA splicing. Multiple physiological roles for VEGF isoforms are suggested by the strikingly diverse

secretion patterns of the resulting four polypeptides. Although all members of this family have an identical signal sequence, the smaller members (110 -165) are released by cells and may act as paracrine, whilst the large ones are mainly cell attached and may act as autocrine (4).

Figure 1 shows that VEGF-A, VEGF-B, VEGF-C, VEGF-D, PlGF (placental growth factor), VEGF-E (Orf-VEGF), and svVEGF from Trimeresurus flavoviridis all belong to the VEGF family. Humans, like other mammals, contain five of the seven genes that make up the VEGF family. Co-receptors neuropilin1 (NRP-1) and neuropilin2 (NRP-2) vascular endothelial growth factor receptor 1 (VEGFR-1), also known as fms-like tyrosine kinase-1 (FLT-1) and kinase insert domain receptor (KDR) in humans and fetal liver kinase (FLk) in mice. Significant functions for VEGF-A and its receptors VEGFR-1 and VEGFR-2 can be found in both normal and abnormal angiogenesis, including tumor angiogenesis (5, 6).

The first VEGF identified was vascular endothelial growth factor -A, commonly known as the vascular permeability factor (VPF). It acts specifically on endothelial cells as a mitogen. The p21.3 region of chromosome 6 encodes the VEGF-A gene, which consists of 8 exons and 7 introns (7).

Figure (2) depicts the five distinct isoforms of VEGF-A that emerge from alternative exon splicing; these are VEGF-A(121), VEGF-A(145), VEGF-A(165), VEGF-A(189), and VEGF-A(206), with 121, 145, 165, 189, and 206 amino acids, respectively (7). Two similar receptor tyrosine kinases, FLT-1 and KDR (9). are expressed on vascular endothelial cells and bind VEGF-A with great affinity. There are eight exons in the VEGF-A gene. The number of amino acids (aa) in the various isoforms produced by alternative mRNA splicing varies widely. The human endometrium is the primary tissue for the expression of VEGF-A splice variants VEGF-A121 and VEGF-A165 (10).

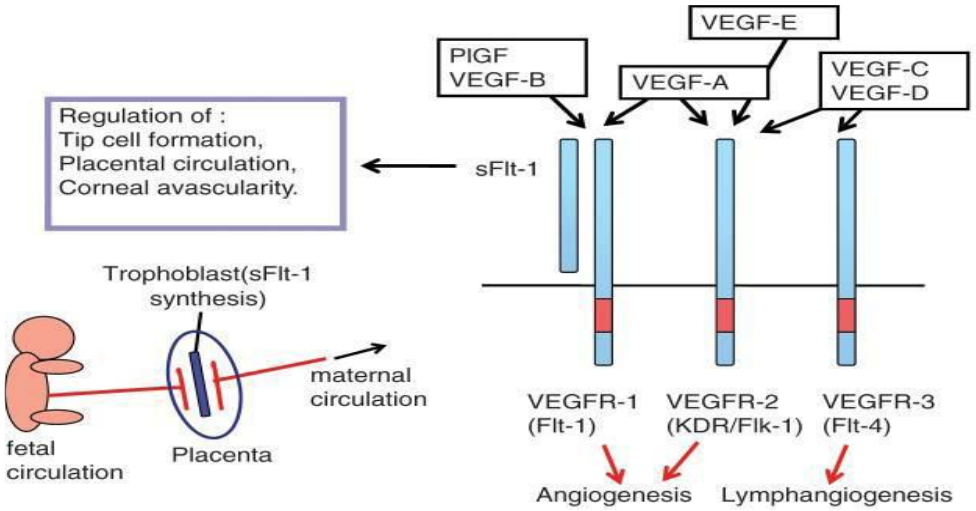


Figure (1): VEGF and VEGFR system (8)

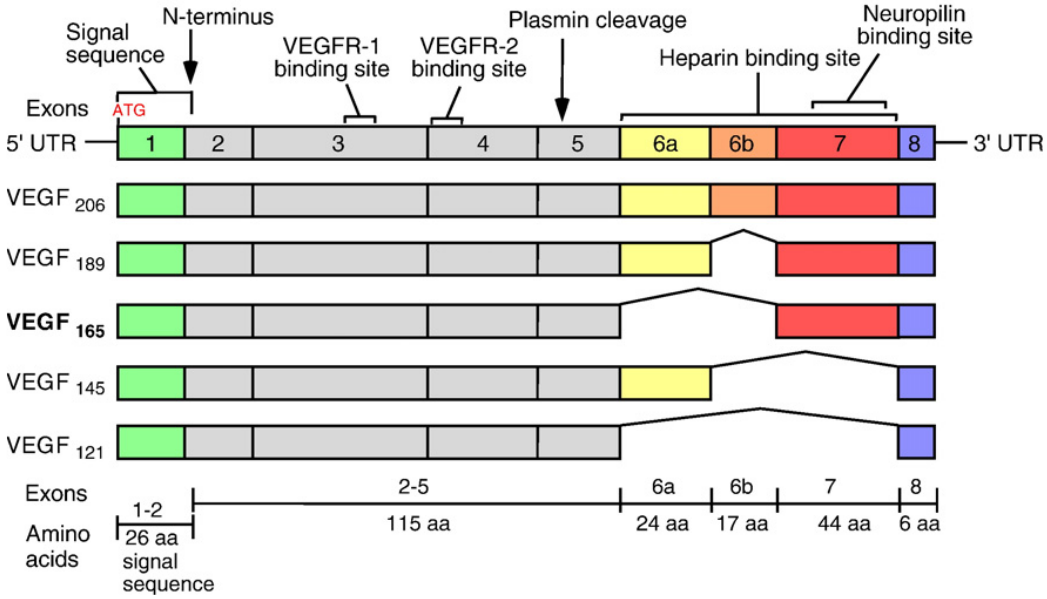


Figure (2): Exon structure of human VEGF-A mRNA splice variants. aa: amino acid, UTR: Untranslated region (11).

The molecular weight of the dimeric glycoprotein vascular endothelial growth factor -A is 33-42 k. Hypoxia, growth factors, nitric oxide, p53 mutations, thyroid-stimulating hormone, and tumor promoters are just some of the situations that control its gene expression. The adrenal gland, lungs, kidneys, and heart might all have more VEGF-A mRNA than other organs (7). Although VEGF-A is expressed at low levels in many human and animal tissues, it is produced at high levels in the places where angiogenesis is necessary. These include fetal tissue, the placenta, the corpus luteum, and the vast majority of human malignancies (12).

VEGF and its major characteristics

The coordinated interactions between activators and inhibitors of angiogenesis serve to govern the process of angiogenesis on both a spatial and a temporal scale. VEGF, or vascular endothelial growth factor, is a powerful pro-angiogenic factor that not only plays a crucial role in the development of embryos, but also in adult organisms (13). In particular, hypoxia is not the only factor that activates VEGF; cytokines, hormones like testosterone and progesterone, and transcription factors like c-Fos also play a role in the development of new blood vessels. Because of the abundance of surface VEGF receptors (VEGFRs) on endothelial cells, VEGF can trigger a variety of responses from these cells, including proliferation, migration, and a dramatic, biphasic increase in permeability that is selective for small and medium-sized molecules. The diverse locations of the tyrosine kinase receptors VEGFR-1, -2, and -3 provide an explanation for the wide range of VEGF's cellular effects. VEGFR-1 is expressed by endothelial cells, while VEGFR-2 and VEGFR-3 are expressed by cells with plasma membranes (14).

While lymphatic endothelial cells produce a significant amount of VEGFR-3, VEGRF-1 and -2 are detected not just on endothelial cells but also on other cell types such as hepatocytes, neurons, pigment epithelium cells, hematopoietic stem cells, mast cells, osteoblasts, retinal and many more cell types. VEGFR-3 is the only member of its family that is primarily expressed on lymphatic endothelial cells. Endothelial cells not only produce the VEGF receptor isoforms NRP-1 and NRP-2 but also express the neuropilin-1 and neuropilin-2 proteins themselves. On neural cells, the protein known as neuropilin was first discovered to function as a receptor for a neuronal guidance mediator family known as class 3 semaphorin/collapsin. The existence of multiple subtypes, such as placental growth factor (PlGF) and VEGF-A, B, C, D, E (viral VEGF) and F (snake venom VEGF) also contributes to the variety of effects that are generated by VEGF. PlGF is also responsible for the formation of placental tissue. EG-VEGF, which stands for “endocrine gland-derived vascular endothelial growth factor”, was very recently added to this category (13).

The first identified subtype, VEGF-A, has been shown to play a significant role in both normal and abnormal angiogenic processes, including tumor angiogenesis, where it is overexpressed. There is strong evidence linking this to tumor development. VEGF-A and its receptor VEGFR-2 have been investigated as potential therapeutic targets for a wide range of diseases and conditions, including cancer, peripheral artery disease (PAD), diabetic macular edema, and diabetic retinopathy (15). Expression of VEGFA in healthy conditions as well as diseased states, Endothelial cells aren't the only cells that produce VEGF-A in response to hypoxia; other cells, such as tumor cells, platelets, kidney mesangial cells, pericytes and vascular smooth muscle cells (VSMCs), macrophages, keratinocytes, activated T-cells, astrocytes, leukocytes, retinal pigmentary epithelial cells, dendritic cells, osteoblasts, Muller cells in the (14, 16).

Myocardial fibroblasts (myofibroblasts) have been demonstrated to express VEGF-A, suggesting a potential function in tissue healing and remodeling following infarction. Alternative mRNA splicing results in the production of a variety of VEGF-A isoforms. The human VEGF-A gene has eight exons that are separated by seven introns. During the process of vascular system development and differentiation, each isoform contributes to the process in a unique way. Because blood vessels are necessary for the growth of tumors, the tumors themselves produce pro-angiogenic factors like VEGF-A. This allows the tumors to continue their growth. This causes what's known as a "angiogenic switch," which leads in the formation of new capillaries within and around the tumor and enables the tumor to grow at an exponential rate. There is a possibility that these blood arteries are physically aberrant, bleeding, and leaking, all of which contribute to a high interstitial pressure. As a consequence of this, the blood supply through the tumor is inadequate, which results in hypoxia and additional generation of VEGF-A. Because of this, abnormally high levels of its expression are found in a wide variety of cancers, including breast cancer. The significance of VEGF-A in breast cancer and its relevance In mice models of breast cancer, VEGF-A has been shown in a number of studies to have the ability to stimulate the growth of tumor cells. Increased VEGF-A, VEGFR-1, and VEGFR-2 levels have been identified specifically in greater malignancy mammary carcinomas, such as HER2-positive and TN normal-like carcinomas, according to the findings of researchers who have studied breast cancer in cats. These findings have been shown to produce similar results (17).

According to the findings of Obermair and colleagues, the quantities of VEGF found within breast cancer tissues are much higher than those found within fibromas or the normal epithelial tissues of the breast. In addition to this, it has been discovered that VEGF-A functions in breast cancer cells as an autocrine survival factor (18).

Under both normal and hypoxic conditions, blocking VEGF-A with VEGF-neutralizing antibodies or siRNAs led to direct death of tumor cells. Through either VEGFR-1 or VEGFR-2, VEGF signaling has been found in certain research to be responsible for inducing the survival of tumor

cells. In the cells of malignant breast tumor, the inhibition of protein kinase B (AKT) phosphorylation caused by targeted reduction of VEGFR-1 expression had a major impact on the cells' ability to survive. On the other hand, decreasing the expression of NRP1 or VEGFR-2 particularly had minimal effect on the cancer cells' ability to survive. In addition to this, it has been demonstrated that breast cancer cells can be driven to migrate and/or invade their surroundings by VEGF via an autocrine loop. It was found that there existed a loop here (19).

Therefore, VEGF-A functions via selective autocrine effects to enhance tumor cell survival, adhesion, chemotaxis, and proliferation. It also stimulates neovascularization, which involves both blood vessels and tumor cells, and vasodilation, which refers to the maturation of blood vessels. Breast cancer cells produce VEGF-A and activate VEGF receptors on their surface, suggesting the presence of a unique autocrine signaling loop. By phosphorylating and activating VEGFR-1/2 or VEGF-induced NRP signaling, breast cancer cells are able to boost their own growth, migration, and survival (20).

VEGF over-expression was validated by other investigations, both at the protein and the mRNA level. It was found to be significantly elevated in human breast carcinomas, but it was found to be low in non-neoplastic tissues. It has been discovered that breast cancer tissues that include malignant tumors express VEGF mRNA at a higher frequency than breast tissues that do not contain tumors. As a consequence of this, a discernible relationship between the presence of VEGF mRNA positivity and high vascular counts as well as positive axillary lymph node counts has been found (14).

Genetic regulation of VEGF

The expression of VEGF is significantly influenced by the post-transcriptional regulation of this protein (21). Most mRNA transcripts require ribosome interaction with a molecular "cap" at the 5' end of the untranslated region (UTR) before they can be translated into proteins (22). This cap-dependent translation can be suppressed under situations of cellular stress such as hypoxia. (21). Two internal ribosome entry sites (IRES) are located in the guanine-cytosine-rich 5'-UTR of VEGF mRNA, which initiate cap-independent protein synthesis. MicroRNAs (miRNAs) are short noncoding RNA sequences that regulate gene expression post-transcriptionally. They are typically around 22 nucleotides in length (23).

They also play a role in the regulation of cell proliferation, apoptosis, the advancement of the cell cycle, migration, and angiogenesis (24), which they do by targeting the 3'UTRs of the mRNAs that they are intended to control. The inhibition of translation or the breakdown of mRNA is the result of its binding to the RNA. It has been demonstrated that a number of miRNAs have the ability to control the development of blood vessels, which is an essential process for the growth and spread of tumors (25). For instance, it has been discovered that miR-15b, miR-16, miR-20a and miR-20b can function as effective anti-angiogenic miRNAs by targeting VEGF. On the other hand, miR-874 and miR-379 both have different effects on the survival and proliferation of tumor cells (26).

VEGF-A overexpression in tumors

Cancer's angiogenesis is a pivotal step because it's required for initial tumor growth, in addition to invasiveness and metastasis. Multiple tumor tissues were found to have an excessive amount of VEGF expression. Lymph angiogenesis, also known as the recruitment of blood vessels and lymphatic vessels to a developing tumor, is a process that is involved in breast cancer. An elevated level of VEGF expression has been linked to both the formation of tumors and their spread to other parts of the body in a large number of studies that were conducted in vitro and in vivo. In addition, the suppression of tumor development and tumor-induced angiogenesis that arises from the inhibition of VEGF signaling (27).

Overexpression of VEGF mRNA can be found in the vast majority of human malignancies and has been shown to correlate with vascular density, invasiveness, prognosis, metastasis, and recurrence of the disease. Micro-vessel density has been observed to have an effect on the disease-free and overall survival of patients with breast cancer in a number of studies that have been conducted on the prognosis of the disease. As can be shown, the expression of low levels of VEGF-A mRNA serves as an effective predictive biomarker across the board for breast cancer subtypes. It is interesting to note that triple-negative breast cancers (TNBC) and HER2 enriched breast tumors benefit more from low VEGF-A expression. Their hazard ratios (HR) are 2.44 and 2.22, respectively, and their p-values are significant ($p = 0.007$ and $p = 0.0013$, respectively). These findings lend credence to a previous investigation carried out by Howard and colleagues, in which the researchers observed no statistically significant connection between elevated levels of HER2 expression and elevated levels of VEGF activity (28).

It was hypothesized that HER2 is not responsible for regulating VEGF expression in aggressive breast carcinomas, but that other mechanisms, such as hypoxia-inducible factor 1 (HIF-1) expression, do play a role. They continued to believe in the validity of their idea despite evidence against it (HER2 overexpression boosts VEGF via HIF-1). Furthermore, it was shown that hypoxia or activation by tumor-associated angiogenic factors and growth factors like VEGF activates the gamma subunit of heterodimeric HIF-1 (14).

Laughner and colleagues also demonstrated that HER2 signaling led to an increase in HIF-1 expression and, as a consequence, VEGF mRNA expression when it was generated by heregulin-1 stimulation of human MCF-7 breast cancer cells or by overexpression of HER2 in mice 3T3 cells. In contrast, luminal tumors were classified according to their HR values, which ranged from 1.57 (luminal A) to 1.45 (luminal B). To this day, practically all research on cancer

have found that genes that repress tumors are silenced, while genes that stimulate tumor growth, known as oncogenes, are turned up. Therefore, having a low level of VEGF-A mRNA expression can have a protective effect on the development of tumors. Increased EGFR expression in triple-negative breast tumors has been shown by researchers, suggesting that in the absence of expressed hormone receptors, tumor growth is more reliant on growth factors. This was shown by the increased EGFR expression found in triple-negative breast tumors (29).

In comparison, the expansion of breast cancers that fall into the luminal A and luminal B categories is more strictly controlled by hormone receptors. These hormone receptors also play key roles as therapeutic targets. Luminal breast cancers are more likely to be found in women with a family history of the disease. This helps to explain why breast cancers that are HER2 rich or triple negative benefit more from having lower levels of VEGF mRNA expression, as seen in the following diagram. In conclusion, inhibiting the activity of VEGF-A could be an effective therapeutic method for treating breast tumors that have HER2 and triple-negative subtypes (30).

VEGF levels and breast cancer

The studies emphasize the importance of using new biochemical markers as a non-invasive liquid biopsy that give full information's about the site, type and metastatic state of tumors. Studies reported that vascular endothelial growth factor (VEGF) assumed to have this role to predict and investigate new early tumors. Combined analysis with classical breast tumor markers such as CA 15-3 and CEA can provide a novel diagnostic panel that reveals a promising diagnostic value of VEGF in the early stages of breast cancer, for assessing the efficacy of surgical treatment for breast cancer, and in detecting breast cancer recurrence (31).

Previous studies reported that the levels of VEGF were increased in cancerous patients due to its role in angiogenesis (32) which is considered as a one of the hallmark features of cancer (33). Vascular endothelial growth factors (VEGF) play a critical part in this intricate and well-coordinated process, together with other growth factors such as angiopoietins, transforming growth factor and platelet-derived growth factor (1). VEGF is a potent stimulator for endothelial cells and plays a crucial role in both healthy and malignant angiogenesis. It has pro-inflammatory effects and increases tumor vascular permeability as well as endothelial cell proliferation, migration, differentiation, and capillary formation. (34). Previous researches also demonstrated that the VEGF levels showed a significant increase in patient with malignant tumor in comparison with both controls and also patients with benign breast tumor which may provide a powerful tool for early diagnosis of breast cancer beside the other classical tumor markers such as CA 15-3 (31). Recently, several studies focused the levels of VEGF and its expression in cancer in an attempt to discover a powerful treatment for cancers in that it halts the expression of VEGF which may cause prevention and hindered cancer progression (35-37).

Association Between Vascular Endothelial Growth Factor Gene Polymorphisms with Breast Cancer Risk

As was noted before, vascular endothelial growth factor, often known as VEGF, is a powerful angiogenic factor that plays an essential role in the formation, progression, and metastasis of tumors (1). Tumor cells can better avoid being killed by apoptotic stimuli when VEGF signaling is activated, which is also helpful for tumor migration and invasion. A number of solid tumors, including breast cancer, have been shown to have an elevated biomarker called VEGF. When compared to normal or benign breast tissue, malignant breast tumors demonstrated much greater amounts of the growth factor VEGF. The expression of VEGF in tumor tissue has been found to have a substantial link with the density of micro vessels and is associated with a bad prognosis, according to the findings of several investigations (38).

The human VEGF gene is found on the short arm of chromosome 6 (6p12–p21), and it is made up of a total of eight exons that are separated by a total of seven introns. This gene is capable of alternative splicing, which results in the formation of a family of proteins. During embryogenesis, skeletal growth, and reproduction activities, VEGF, which is also known as VEGFA, plays a crucial role as a key regulator of physiological angiogenesis. Additionally, it plays a role in the malignant process of angiogenesis that is connected with malignancies. Due to its importance in driving initial tumor growth, invasiveness, and metastasis, angiogenesis is a hallmark of the cancer progression. It was shown that VEGF expression is abnormally high in some tumor tissues (39). Lymphangiogenesis, or the development of new lymphatic and blood vessels to supply a tumor, is linked to several different types of cancer, including breast cancer. Research in both in vivo and in vitro settings has linked increased VEGF expression to tumor growth and metastasis. Tumor growth isn't the only thing that can be stopped by blocking VEGF signaling; tumor-induced angiogenesis can be stopped as well (40, 41).

SNPs, or single nucleotide polymorphisms, can have an effect on the production of the corresponding protein as well as its function. SNPs can occur in the promoter, the intron, the exon, or the untranslated regions (3′ and 5′-UTR). A number of single nucleotide polymorphisms (SNPs) have been discovered within the VEGF gene, and each of these SNPs has been reported as being associated with different amounts of VEGF expression. One of these SNPs is located in exon 1 of the VEGF gene16, another one of these SNPs (+936) is located in exon 8, which corresponds to the 3′UTR area of the gene, and two of these SNPs are located in the VEGF promoter region, which is located at 2578 and 1154, respectively (42). In addition, other SNPs have been defined, but it has not been determined whether or not they are associated with the expression of VEGF. Several

investigations were conducted to study the VEGF genetic polymorphisms in BC in various ethnic groups, and the results of these studies resulted to contradictory findings(43).

In patients with metastatic breast cancer, a good response to bevacizumab was linked in studies conducted utilizing pharmacogenomics to single nucleotide polymorphisms (SNPs) in VEGF and VEGFR-2. Therefore, investigating the genetic variability of VEGF and VEGFR-2 as a possible biomarker for bevacizumab, which makes perfect sense, is a rational course of action. (44). Previous research has revealed that SNPs within the VEGF gene have a significant biological impact on the ability to forecast the risk of developing cancer, especially breast cancer, as well as the likelihood of a favorable outcome.Gene polymorphism in the promoter region, intron region, exon region, or untranslated regions (3′ and 5′-UTR) has been shown to have the potential to influence the synthesis of the corresponding protein as well as its function (45).

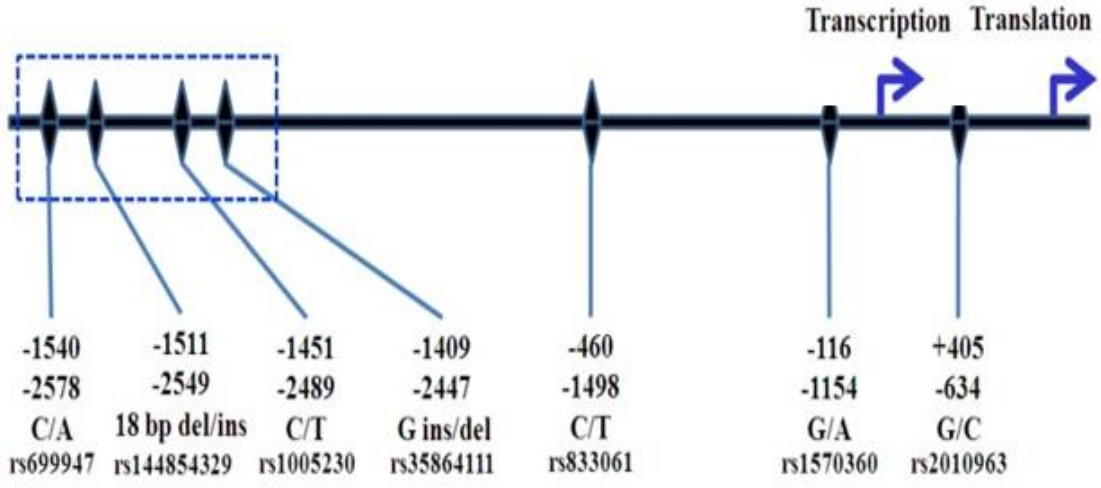
Up to this time, there have been reports of a large number of different VEGF gene polymorphisms, some of which are represented in Figure (3). Both the +405G/C (rs2010963) SNP that is located in the 5'-untranslated region of the VEGF gene as well as the -2578C/A (rs699947) SNP that is positioned in the promoter region of the VEGF gene have been associated to altered VEGF secretion (46). Both of these SNPs may be found in the VEGF gene. As a direct result of this, it has been theorized that these polymorphisms have an association with the progression of cancer and the prognosis of the disease. Inflammation and angiogenesis are both thought to play a role in the prognosis of breast cancer; however, the specific impact that individual germline mutations play in associated genes is not known. It is believed that each of these aspects have some sort of bearing on the development of the disease. Studies that evaluated the effects of the antiangiogenesis medication bevacizumab (BEV) on the outcome of breast cancer (BC) found that the treatment had variable implications on progression-free survival and overall survival, which indicates that only a subgroup of patients may benefit from this treatment (47).

Individuals suffering from a variety of cancers, including those of the breast, uterus, gastrointestinal tract, lung, and prostate, have been found to have elevated levels of circulating VEGF. However, the results have been inconsistent, showing that the link between the VEGF gene polymorphism and cancer requires further investigation. Several studies explored the VEGF genetic polymorphisms in Breast cancer in different ethnic groups and led to varied conclusions however, the results have been inconsistent, suggesting that further inquiry is required (27).

Several studies reported that the polymorphism In SNPs of VEGF gene showed to be correlated with an overall increased risk of breast cancer as it links to the rate of the expression of VEGF that influenced by the polymorphism of these SNPs that located in the promoter region of the VEGF gene (27, 43).

CONCLUSION

According to the studies presented in this review, VEGF showed to have a crucial role in the progression and development of breast cancer and its serum level can be considered as a tumor marker that can be used in future for diagnosis and prognosis which can provide a promising marker with a higher sensitivity and specificity than the classical markers. Additionally, Several SNPs in the gene of VEGF can be considered as a susceptibility genetic marker for the breast carcinoma as it affect the rate of VEGF expression which need a more profound study to elucidate the exact role of VEGF levels in addition to the role of these SNPs.



Nomenclature of *VEGFA* SNPs. The positions in the upper row correspond to the transcription start site, while the positions in the lower row correspond to the translation start site. The polymorphisms in the box are in complete LD. - 2578C is linked with - 2549 18 base pairs deletion, - 2489C. and - 2447G insertion. - 2578A is linked with - 2549 18 base pairs insertion, - 2489T and - 2447G deletion.

Figure (3) Nomenclature of *VEGFA* SNPs (27)

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