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Role of oxidative stress in angiogenesis and the therapeutic potential of antioxidants in breast cancer

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Abstract. – The escalation of cancer cases globally, especially breast cancer, is of concern. Angiogenesis is hallmark of cancer pathogenesis and plays an important role in cancer progression and metastasis. Pro-angiogenic agents, secreted by tumor cells, form new blood vessels, and produce reactive oxygen species (ROS). ROS promote angiogenesis via two major pathways: namely Vascular Endothelial Growth Factor (VEGF) dependent and non-VEGF dependent pathways. As a consequence of unbalanced ROS overproduction and low antioxidants levels, oxidative stress occurs and promotes angiogenesis in breast cancer tissues. Thus, the potential use of antioxidants as a preventive therapy in breast cancer. Preclinical studies depict that vitamins A and E may counter oxidative stress resulting in reduction of metastasis and viability of breast cancer. Furthermore, clinical studies demonstrate a decline in breast cancer risk in postmenopausal women upon the consumption of antioxidants. Herein, we discuss various pro-angiogenic agents that may play an important role in breast cancer angiogenesis. Moreover, the contribution of oxidative stress in inducing the angiogenic process is extensively reviewed here. Furthermore, the findings of pre-clinical and clinical studies on the use of antioxidants, namely vitamins A and E, in breast cancer are deliberated upon, along with the role of angiogenesis in cancer therapy.

Key Words:
Breast cancer, Angiogenesis, Oxidative stress, ROS, Antioxidants, Vitamins.

Abbreviations
ROS, Reactive Oxygen Species; VEGF, Vascular endothelial growth factor; VEGFR, Vascular endothelial growth factor receptor; NF-κB, Nuclear factor kappa B; NICD, Notch intracellular domain; Dll, Delta like ligand; NRP, Neuropilin; PI-3K, phosphatidylinositol 3′ kinase; PLC-γ, phospholipase C gamma; FAK, focal adhesion kinase; Tsad, T Cell-Specific Adapter; PTP, protein tyrosine phosphatase; Nox, NADPH oxidase; HIF, Hypoxia-inducible factors; VHL, von Hippel-Lindau protein; HRE, hypoxia response elements; PHDs, prolyl hydroxylase enzymes; PDGF, platelet derived growth factor; PAI, plasminogen activator inhibitor; MMP, Matrix metalloproteinases; Ang, Angiopoietin; DOKR, docking protein; PAK, p21-activated protein; NOS, nitric oxide synthase.

Introduction

The escalation in cancer cases is of significant concern worldwide, despite the presence of various therapeutic interventions and supportive care. Breast cancer is one of the most diagnosed cancers in women globally, and the first leading cause of cancer-related deaths among women[1]. A significant increase in comprehension of the breast cancer pathogenesis has led to its 'molecular' classification. Based on receptors and tumor grades, breast cancers are typically sub-grouped into; luminal/estrogen and progesterone receptors- positive (ER/PR+), human epidermal growth factor receptor 2- positive (HER2+) and basal like/triple negative breast cancer (ER-/PR-/HER2-)[2,3].

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Angiogenesis is hallmark of cancer that leads to the formation of new blood vessels. This hallmark is well-known to play an essential part in the progression and metastasis of cancer[4]. Oxidative stress is caused by the disturbance of redox homeostasis, which is an important factor that contributes to angiogenesis process[5]. Under physiological conditions, redox homeostasis helps in tissue growth and repair. However, under pathological conditions like cancer, oxidative stress plays a vital role in the vascularization of cancerous tissue growth and metastasis[5]. Poor nutritional intake, smoking exposure, and other environmental toxins may increase reactive oxygen species (ROS) levels, which in turn resulted in oxidative stress- DNA damage[6,7]. The accumulation of DNA damage over time can ultimately lead to cancer[6]. In addition, breast cancer is an example where the gene-environment interactions have a major role in cancer development[6,7]. On the other hand, antioxidant usage scavenges free radicals decreasing oxidative stress and preventing oxidative DNA damage[8].

In this review, we discuss the various pro-angiogenic agents that may play a role in breast cancer angiogenesis. Moreover, we deliberate upon the contribution of oxidative stress in inducing the angiogenic process. In addition, we discuss the findings of pre-clinical and clinical studies on the use of antioxidants, namely vitamins A and E, in breast cancer.

Search Strategy

A comprehensive literature search was conducted in this narrative review, following Gasparyan recommendations[9]. Using the following keywords, with Boolean combinations; "breast cancer", "angiogenesis", "oxidative stress", "ROS", "antioxidant", and "vitamin", PubMed (MEDLINE), EMBASE, Scopus and Web of Science were searched without data or time restriction till June 2020.

Angiogenesis in Breast Cancer

Angiogenesis is the formation of new blood vessel from existing vessels[4]. In breast cancer status, the new formed vessels become primarily noticeable at the pre-invasive stage of high-grade ductal carcinoma *in situ* in breast cancer[10]. Under normal physiological conditions, angiogenesis is tightly regulated by a balance between pro- and anti-angiogenic agents. However, the genetic and epigenetic triggers within the microenvironment of tumor cells; as hypoxia, and metabolites, disrupted this balance and tends to support pro-angiogenic agents[11]. The released angiogenic agents by tumor cells cause vasodilation and increase the vascular permeability of blood vessels in the vicinity of the tumor[12]. This results in the degradation of the basement membrane, loosening of the pericyte covering and, plasma protein diffusion, which helps in matrix formation for migration of endothelial cells[13]. Continuous exposure to angiogenic agents contributes to the extensive degradation of the basement membrane and inhibition of endothelial-pericyte interaction[13]. Therefore, vessels formed are irregularly shaped, unstable, dilated, tortuous, immature, and dysfunctional. However, they are in full capacity to nourish the increasing requirements of cancer cell[14].

Prevalent Pro-Angiogenic Agents and Their Role

Vascular Endothelial Growth Factor (VEGF)

Vascular endothelial growth factor (VEGF) is one of the major proangiogenic agents known to be involved in breast cancer. It is mainly produced by tumor rim cells, which surrounded the tumor mass[15]. Besides being a potent angiogenic factor, it is also a vasodilator, that increase the vascular permeability[16].

VEGF ligands are described as homodimer glycoproteins of 40-45kDa, which have five isoforms: VEGF-A/B/C/D and placental growth factor (PIGF)[17]. VEGF-A binds to both VEGF receptor (VEGFR) isoforms 1 and 2 to promote new blood vessel formation. Studies have suggested that VEGF-A is estrogen responsive in breast cancer[18]. VEGF-B and PIGF mainly bind to VEGFR-1. Whereas VEGF C and D are produced in their immature form and then activated after cleavage by proteases[19,20].

VEGF receptors (VEGFR) are tyrosine kinase receptors with seven immunoglobulin-like structures with 3 domains: ectodomain, transmembrane domain and intracytoplasmic domain containing tyrosine residues[21]. These residues undergo autophosphorylation when bound to VEGF ligands and then activates the downstream signaling for angiogenesis[21]. These receptors have primarily three isoforms: VEGFR-1/2/3. VEGFR-1 has weaker angiogenic and tyrosine kinase properties.

This isoform is crucial in embryogenesis, acts as decoy receptors and negatively regulates VEGFR-2 activation[22]. VEGFR-2 is a major angiogenic receptor; and presents in endothelial cells and lymphatic vessels[22]. Similarly, VEGFR-3 presents in lymphatic vessels and plays an important role in lymph angiogenesis[22,23].

Fibroblast Growth Factors (FGF)

Fibroblast growth factors (FGF) are important angiogenic factors and positively associated with breast cancer[24]. They are ubiquitously expressed as small polypeptides, which stimulate the proliferation of pericytes and fibroblasts. Basic fibroblast growth factor (bFGF/ FGF-2) is the most common FGF; which binds to all isoform of FGF receptors[24]. FGF bound to heparin sulphate in extracellular matrix (EMC) and activated upon degradation[25].

Angiopoietins and Tie Receptors

Angiopoietins (Ang) are approximately 70-kDa and have 3 isoforms: Ang 1, Ang 2, and Ang 4[26]. Tie receptors are type 1 tyrosine kinase receptors and primarily have 2 isoforms: Tie 1 and Tie 2. Ang 1 mainly bind to Tie 2 and its potent stabilizer of the vasculature. Ang/Tie system is crucial for blood vessel remodeling[26]. Under physiological conditions, Ang 1 is low in breast tissues. However, studies show there is an increase level of Ang 1/ Ang 2 ratio in breast cancer, which associated with poor survival[27,28].

Interleukin (IL)-8

IL-8 produced by endothelial and tumor cells and activates cell migration and angiogenesis when binds to CXCR1 and CXCR2[29]. IL-8 also stimulates VEGF expression in endothelial cells by CXCR2 in an autocrine fashion[29]. Breast cancers have an elevated level of IL-8 which associated with invasive and aggressive tumors[30,31].

Matrix Metalloproteinases (MMPs)

Matrix metalloproteinases (MMPs) are class of zinc dependent proteolytic enzymes that degrade the extracellular matrix (ECM) adjacent to the blood vessel wall, leading to migration of endothelial cells[32]. A meta-analysis study by Song et al[33], suggested that the overexpression of MMPs is associated with poor survival in breast cancer patients. However, larger studies are required to completely understand the association of MMPs with breast cancer patients' prognosis.

Methods to Detect Angiogenesis in Breast Cancer

Microvessel Density (MVD)

Microvessel density (MVD) was first described by Weidner et al[34] in 1991. It used to detect intertumoral angiogenesis through the immunohistochemical staining of pan endothelial proteins with staining factor VIII in breast cancer[34]. However, Hayes et al[35] did not recommend its use as prognostic marker as the generated data are so confounded. Furthermore, the growing complexity and understanding of cancer types and pathologies requires the standardization to utilize this technique[36].

Raman-AFM (Atomic Force Microscopy)-Imaging

Raman atomic force microscopy is utilized to detect the biochemical and architectural alteration of tissues around the blood vessels in breast cancer[37]. Type 3 collagen, fibroblast and glycocalyx stiffness around the newly formed vessels can be detected, as well as increased lactic acid and glycogen levels can be monitored. This imaging technique helps visualized angiogenesis development in breast cancer[37].

Oxidative Stress and Angiogenesis

Estrogen compounds have been shown to produce ROS[38]. The high ROS level can lead to misbalance in redox hemostasis, which results in cell proliferation, activation of redox sensitive proteins and pro-survival mechanisms in cancer cells[38]. ROS are an oxygen derived group containing both free radicals: superoxide ($O_2^{\bullet-}$), hydroxyl ($HO^{\bullet}$) and peroxyl ($ROO^{\bullet}$), and non-radical reactive molecules: hypochlorous acid (HOCl), ozone (O_3), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2). Specifically, superoxide ($O_2^{\bullet-}$) radicals have an unpaired electron which makes them extremely reactive and biologically relevant species along with hydrogen peroxide (H_2O_2)[39]. The hallmark of oxidative stress in breast cancers is characterized by the overproduction of ROS[38].

Mechanism of Production of ROS

All the cells in the vasculature are capable of producing ROS, specifically endothelial cells and pericytes[40]. There are many enzymes responsible for ROS production such as lipoxygenase, cytochrome P450 enzymes, cyclooxygenase[40]. The most relevant cascades of ROS production are briefly described as follows:

NADPH Oxidase Signaling Cascade

Nicotinamide adenine dinucleotide phosphate (NADPH) oxidases produce ROS through converting oxygen burst to superoxide radicals ($O_2^{\bullet-}$) by donating one electron[40,41]. The NADPH oxidase family consists of seven members, Nox1-5, and dual oxidases 1 and 2. These enzymes are present in the plasma membrane, ER and endosomes. Their structure consists of a catalytic subunit, transmembrane-spanning subunit, and various regulatory subunits in cytoplasm which depend on the type of NOX isoforms, p22phox, p40phox, p47phox, p67phox. These enzymes are activated by various stimuli including growth factors[40,41].

Mitochondrial Electron Transfer Chain (ETC)

Mitochondria is the main organelle in the cell which produces ATP[42]. It contains enzyme complexes I, II, III and IV which reduce oxygen by four electrons to form two molecules of water and small quantity of superoxide ($O_2^{\bullet-}$). Further, under physiological conditions very small quantities of $O_2^{\bullet-}$ escape, which is scavenged by superoxide dismutase (SOD) and glutathione peroxidase. While in dysfunctional status of mitochondria, the extra production of $O_2^{\bullet-}$ creates redox imbalance and consequent oxidative stress[42].

Method of Oxidative Stress Detection

Direct Measurement

Fluorogenic probes, dichlorodihydrofluorescein diacetate (DCFDA) and dihydroethidium (DHE) have been used to detect hydrogen peroxide (H_2O_2) and Superoxide ($O_2^{\bullet-}$), respectively[43,44]. Another assay named reactive oxygen metabolites (D-Roms) test can also be used for quantification of ROS molecules[43,44].

Indirect Measurements

Oxidative stress could also be assessed alternatively by extent of damage done to proteins, lipids, and DNA. Proteins damage can be measured by advanced oxidation protein products (AOPP)[45]. Detection of Malondialdehyde (MDA) by using thiobarbituric acid reactive substances is to estimate lipid oxidation[46]. More recently, oxidized levels of low-density lipoproteins (LDL) could also be measured using enzyme-linked immunosorbent assay (ELISA)[46,47]. Further, DNA damage measured by detecting 8-Hydroxy-2-deoxyguanosine (8-OHdG) by ELISA or by other immunohistochemical analysis techniques[48].

ROS and Angiogenesis

ROS plays a very crucial role in angiogenesis. Under physiological conditions, small amounts of ROS acts as a signaling molecule which has an important role in wound healing, reproduction, and tissue repair. However, in pathological conditions, the overproduction of ROS activates growth factors and has various effects on cell signaling resulting in angiogenesis[49]. Furthermore, inhibiting NADPH oxidase reduce endothelial cell migration, VEGF-induced angiogenesis, and tubule formation[50,51].

Oxidative Stress VEGF Dependent-Angiogenesis Pathways in Breast Cancer

VEGF Signaling Pathway

VEGFR-2 has a vital role in angiogenesis in breast cancer. Its expression has been shown to be elevated in invasive breast carcinomas[52]. The binding of VEGF causes the dimerization of VEGFR-2 and then autophosphorylation of tyrosine residues in the intracytoplasmic tail, triggering various downstream signaling pathways, that will be discussed thoroughly[23,53]. Activation of mitogen-activated protein-kinase pathway (MAPKs), MEK, p38, p42/p44 MAPK and ERK, resulted in the proliferation of endothelial cells. Phospholipase C gamma (PLC-γ) binds to phosphorylated tyrosine residue 1175 in the VEGFR-2 cytoplasmic tail and activates protein kinase C which further activates RAF/MEK/ERK pathway, leading to vascular proliferation. The activated Akt by phosphatidylinositol 3′ kinase (PI-3K) induces vasodilation and favors cell survival via nuclear factor kappa B (NF-). Furthermore, additional networks are activated including focal adhesion kinase (FAK) and T Cell–Specific Adapter (Tsad); which promote cellular permeability and migration[23,53] (Figure 1).

ROS are potent signaling molecules that affect the VEGF signaling pathway extensively. Exogenous ROS can enter endothelial cells and increase the expression of VEGF, which activates EGFR in an autocrine or paracrine manner[54]. Further, VEGF also induces endogenous ROS production mainly by NADPD oxidase (Nox) (55). Nox 4

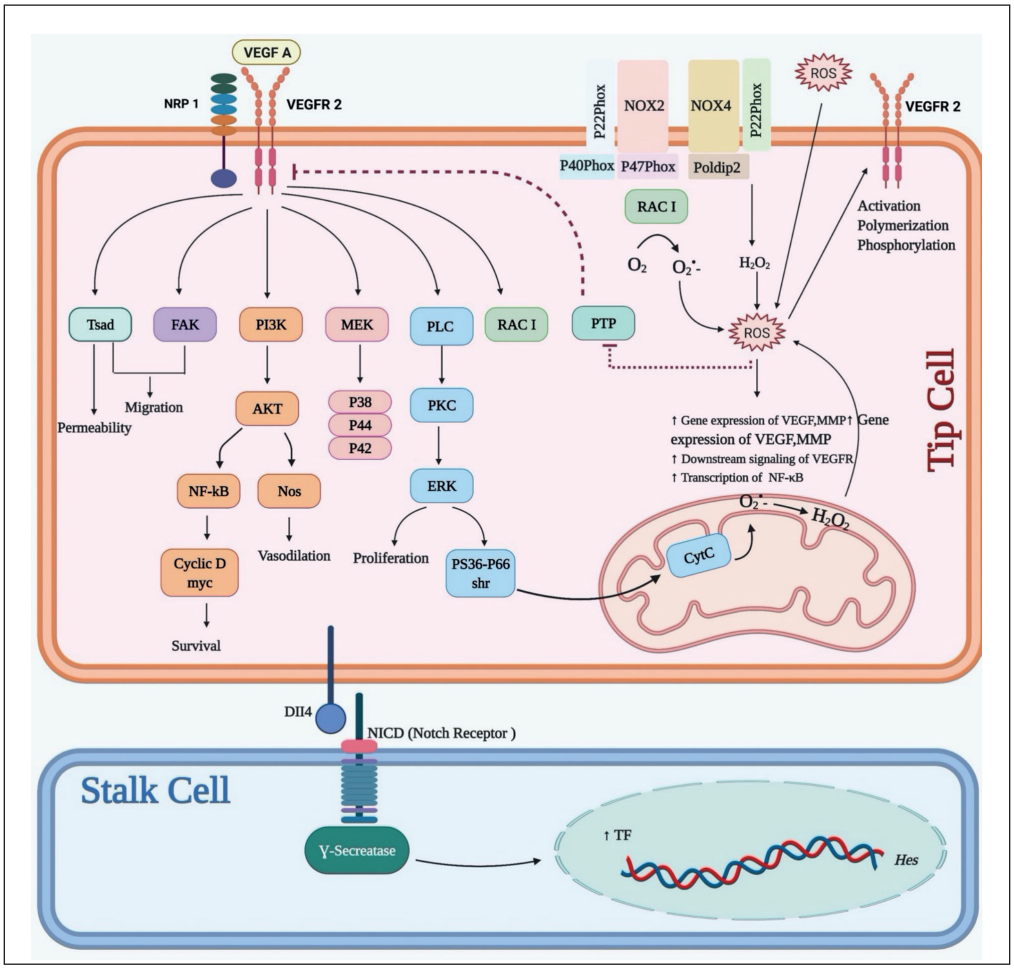

Figure 1. VEGFR, Notch and Neuropilin (NRP) pathway in relation to oxidative stress in angiogenesis. Schematic representation of oxidative stress VEGF dependent-angiogenesis pathways described in the text. Abbreviations: VEGF: Vascular endothelial growth factor; VEGFR: Vascular endothelial growth factor receptor; NF-: Nuclear factor kappa B NICD: Notch intracellular domain; Dll: Delta like ligand; NRP: Neuropilin; PI-3K: phosphatidylinositol 3′ kinase; PLC- : phospholipase C gamma; FAK: focal adhesion kinase; TSAd: T Cell-Specific Adapter; PTP: protein tyrosine phosphatase; Nox: NADPH oxidase; ROS: reactive oxygen species.

and Nox 2 are prominent sources of ROS in endothelial cells[55]. Nox 2 contains p22phox and two cytoplasmic regulatory subunits GTPase Rac1and p47phox. VEGF/VEGFR binding activates Rac 1 production which attached to Nox 2 and produce superoxide radicals[56]. The exact mechanism of activation of Nox 2 and Nox 4 is not yet completely understood. $O_2^{\bullet-}$ produced by Nox 2 is converted to hydrogen peroxide by superoxide dismutase (SOD)[57]. This can be the reason for initial increase in cytosolic ROS increase after VEGF/VEGFR binding. Nox 4 does not require subunits like Nox 2 except poldip2 and p22phox[58,59]. Its activation produces hydrogen peroxide, which are sensed by Nox 2 resulting in its activation and more $O_2^{\bullet-}$ production. Furthermore, activation of PKC, ERK/JNK phosphorylate p66Shc independently also produces hydrogen peroxide. p66Shc is an adaptor protein with unique collagen homology domain (CH2)[58,59]. Phosphorylation of Ser 36 enable p66Shc translocation to mitochondria; where acts as a catalyst, enhancing electron transfer from cytochrome c to oxygen molecule resulting in $O_2^{\bullet-}$ and H_2O_2 production[60]. Thus, increased oxidative stress inhibits protein tyrosine phosphatase (PTP) which have a negative regulatory effect on VEGFR[61]. In addition, ROS activates the redox sensitive transcription factor and downstream signaling protein, phosphorylation, and dimerization of VEGFR enhancing angiogenesis[61] (Figure 1).

Neuropilin (NRP)

Neuropilins (NRPs) are evolutionarily conserved plasma membrane spanning proteins that have two isoforms NRP 1 and NRP 2[62]. Its structure consists of 5 subunits of ectodomain transmembrane domain and PDZ binding motif in its short cytoplasmic domain. NRP-1 acts as co-receptor VEGF/VEGFR signaling cascade[62]. The increased expression of NRP-1 is associated with invasive and metastatic behavior of the breast cancer[63]. During angiogenesis, initially the vessels start sprouting, then the endothelial cells undergo tip and stalk cell selection[64]. The tip morphology of cell is responsible for navigation of the endothelial cells, while the stalk morphology is responsible for proliferation, finally the formation of new blood vessels. Tip/stalk morphology is regulated by VEGF and notch signaling. NRP 1 is known to promote tip cell morphology which inhibited by notch receptors[64].

Notch Signaling Pathway

Notch receptors are well-conserved single pass plasma membrane receptors with isoforms Notch 1-4[65]. The ligands, Delta like ligand (Dll) and Jagged ligands are present on the cell surface of neighboring cells. Endothelial cells depicting tip cell morphology have strong VEGFR-2 expression and overexpress Dll-4[65]. Breast cancer reveals high expression of Notch receptors, and the isoforms differ according to the type of breast cancer[66,67]. Binding of Dll-4 and notch receptors induces cleavage of receptor fragment by γ-secretase complex (S3)[68]. This resulted in the release of Notch intracellular domain (NICD) and translocation to the nucleus[69]. NICD activates transcriptional factors like *Hes-1*, *Hes-5*, and cyclin D which regulates proliferation and migration of endothelial cells. Notch signaling lead to the suppression of Nox4, thereby negatively modulating ROS generation[69].

Oxidative Stress VEGF Independent Angiogenesis Pathways in Breast Cancer

Hypoxia Signaling Pathway

Oxygen utilization is escalated during uncontrolled cell division giving rise to hypoxia in breast cancer. Hypoxia-inducible factors (HIF 1, and 2) activated upon hypoxia and targets various genes which regulate angiogenesis, cell survival, epithelial–mesenchymal transition (EMT), and mobility[70]. Elevated expression of HIF-1α is linked with adverse outcome in breast cancer[71, 72]. Hydroxylation of proline residues by oxygen dependent prolyl hydroxylase enzymes (PHDs) result in binding of von Hippel–Lindau protein (VHL) on HIF-1α[73]. VHL is an E3-ubiquitin ligase with renders HIF-1α degradation by proteasomes. However, in hypoxia, hydroxylation of HIF-1α is decreased leading to its dimerization with HIF-1β[73]. The heterodimeric protein translocates to the nucleus and binds to hypoxia response elements (HRE) activating transcription of various genes[70,74,75]. HIF-1α stimulates angiogenesis by increasing the transcription of VEGF/VEGFR, platelet derived growth factor (PDGF), plasminogen activator inhibitor (PAI-1), Tie 2 receptors, angiopoietin, ephrin A1 ligands and glycolysis enzymes. In oxidative stress, elevated ROS inactivates PHD2 and activates NF-kB, which increases HIF-1α transcription[70,74,75] (Figure 2).

Angiopoietin-Tie2 System

Angiopoietin (Ang) expression is increased by VEGF, PDGF and HIF-1α in the endothelial cells. As described earlier, Ang binds to Tie receptor leading to its dimerization and phosphorylation in tyrosine residues at the cytoplasmic tail[26]. The downstream signaling pathways includes PI3K/Akt, which increases the survival of endothelial cells, and MAPK pathway inducing the proliferation[77,78]. Ang1/Tie2 complex also activates docking protein, Dok-R, which further stimulates the p21-activated protein (PAK-1) promoting migration and proliferation of endothelial cells[76]. ROS is generated by activation of Rac-1 which simulates Nox. However, activation of p38, p44, p42 by phosphorylation is still controversial since some studies have reported opposite results[77,78] (Figure 3).

TLR2/MyD88

Toll Like receptor (TLR) activation in tumor cell results in increased proliferation, metastasis, and pro-survival phenotype[79]. Phospholipid membranes undergo oxidation in the presence of ROS. 2-(ωcarboxyethyl) pyrrole (CEP) is one of the carboxyalkyl pyrrole (CAP) protein adducts generated due to ROS reactions. CEP binds to TLR-2 and activates MyD88 which in turn activates Rac-1. As previously discussed, Rac-1 is potent angiogenic and NADPH oxidase activating protein[80].

Cell Metabolism-Glycolysis

Angiogenesis is a high-energy consumption process. Endothelial cells increase their glycolytic flux in order to meet their increasing metabolic needs in

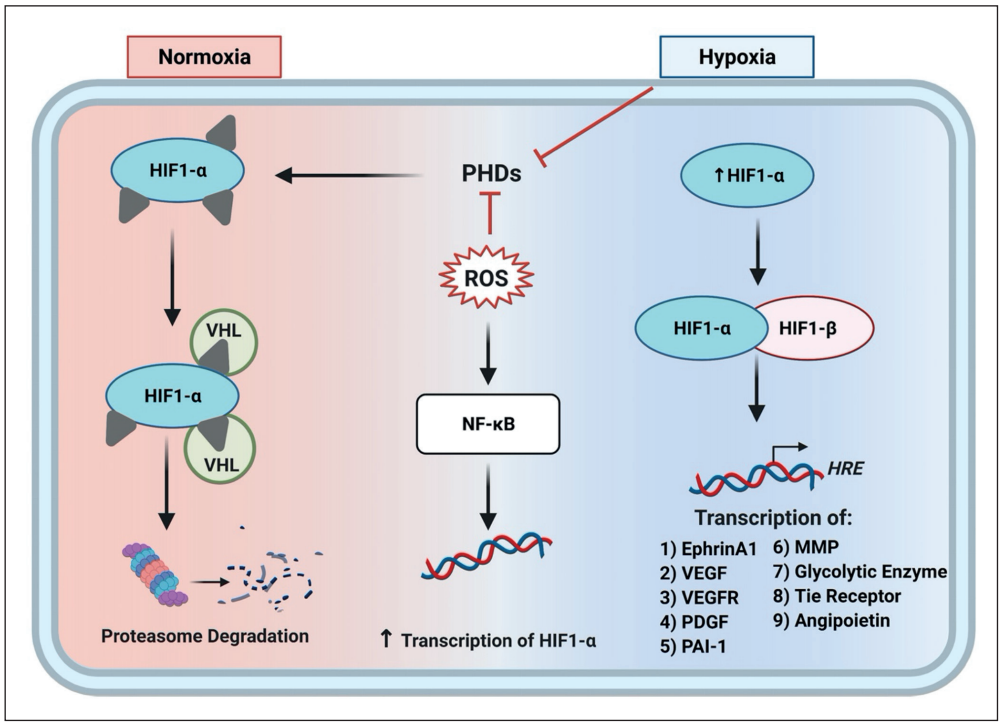

Figure 2. Hypoxia signaling pathways in association with ROS in angiogenesis. Schematic representation of hypoxia signaling pathways illustrated in the text. Abbreviations: HIF 1, and 2: Hypoxia-inducible factors; VHL: von Hippel-Lindau protein; HRE: hypoxia response elements; PHDs: prolyl hydroxylase enzymes; PDGF: platelet derived growth factor; PAI 1: plasminogen activator inhibitor; MMP: Matrix metalloproteinases; VEGF: Vascular endothelial growth factor; VEGFR: Vascular endothelial growth factor receptor.

cancer status. VEGF/VEGFR signaling increases the expression of glucose transporter 1 (GLUT-1), lactate dehydrogenase (LDH), and 6-phosphofructo2-kinase/fructose-2,6-bisphophatase (PFKFB3)[81]. In addition, glutamine and asparagine are utilized for energy production and protein synthesis. Thus, the enzymes necessary for this function are also increased, glutaminase 1 (GLS-1) and asparagine synthetase (ASNS)[81]. In endothelial cells, ROS are involved in the oxidation and reduction of reactive cysteines to undergo further glutathionylation, which is crucial to maintain nitric oxide synthase (NOS) levels and function[82]. ROS also reduces the activity of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a key enzyme in glycolysis pathway[82]. However, more studies are required to understand the metabolomics which affect angiogenesis especially in various types of breast cancer.

Preclinical Studies of Antioxidants on Breast Cancer

The imbalance between a high level of free radicals and a low level of antioxidants is considered to be a causative factor in cancer development[83]. Thus, the use of antioxidant supplements to prevent breast cancer is a noteworthy area of research. The most commonly used antioxidant supplements are vitamins. Vitamin A and vitamin E are recognized to modulate several signaling pathways which are associated with different stages of breast cancer. These vitamins have antitumoral effects representing potential breast cancer chemo-preventive strategies, sparing normal cells[8,84]. For the purposes of this review, the antioxidant effects of vitamin A and E along with their association with breast cancer in premenopausal and postmenopausal status are discussed thoroughly.

Vitamin A

The vitamin A forms in the human diet are retinol, retinoid, retinoic acid, and provitamin A carotenoids[85,86]. The main functions of vitamin A are aiding in growth and development processes, maintaining the immune system and good vision. Vitamin A is found in yellow food sources, such as carrot and mango[85,86].

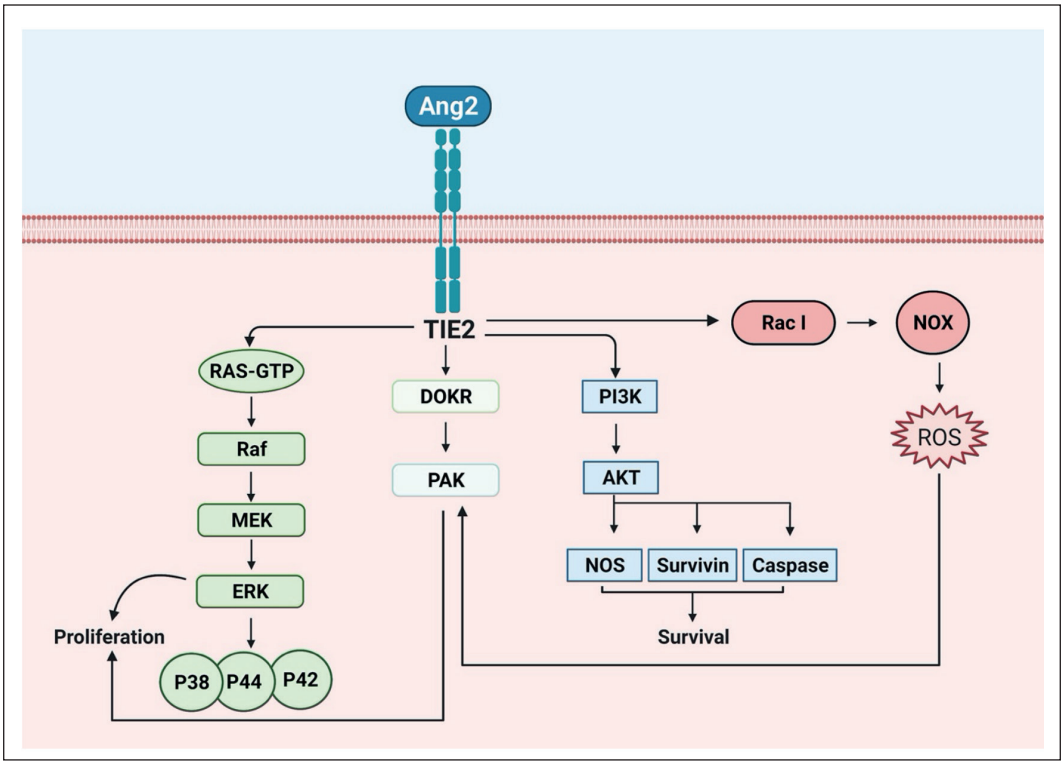

Figure 3. Ang/Tie signaling pathways with ROS production in angiogenesis. Schematic representation of Ang/Tie signaling pathways defined in the text. Abbreviations: Ang: Angiopoietin; DOKR: docking protein; PAK: p21-activated protein; PI-3K: phosphatidylinositol 3′ kinase; Nox: NADPH oxidase; ROS: reactive oxygen species; NOS: nitric oxide synthase.

Growing evidence demonstrate that vitamin A in its different forms has antitumor effects on breast cancer *in vitro* and *in vivo*[87-92]. This is mediated by retinoic acid receptors (RARs) and the retinoid X receptor which target tumor suppressor gene, RARbeta2 gene[87]. The synergistic cross talk between retinoic acid and PI-3K signaling pathways, has shown to regulate the proliferation and migration in MCF-7 breast cancer cell line[88]. Retinoic acid can inhibit the growth of both breast cancer cells and breast cancer stem cells. Interestingly, the stem cells are more sensitive to retinoic acid as it impairs the self-renewing ability of stem cells and promotes their differentiation[89]. HER2-positive breast cancer cell lines exhibit RARα overexpression which in turn reduces the cell viability and increases the sensitivity to vitamin A treatment[90]. Furthermore, carotenoids have an antiproliferative effect on MCF-7 breast cancer cell line through the downregulation of bcl-2 gene and induction of cell cycle arrest[91]. Kawata et al[92] reported that β-carotene mediated the anti-inflammatory effects, inhibition of Cox2, Nos2 and TNF-α gene expression, through ROS scavenging activity. Nonetheless, further studies are needed to verify the beneficial effects of vitamin A on preventing and alleviating breast cancer.

Vitamin E

Vitamin E are fat soluble compounds and mainly grouped in two categories, tocopherol and tocotrienol. The main food source of vitamin E is vegetable oils. The antioxidant function of vitamin E protects the cell membrane from ROS[86].

Several preclinical studies show that vitamin E has anti-cancerous effects on breast cancer cell lines[93-98]. Tocotrienol possessed the antiproliferative activity *via* the suppression of the Ras signaling pathway by inhibiting both MAPK and PI3K/Akt signaling pathways[93]. Also, it arrested the cell cycle in MCF-7 cancer cell line by downregulation of the cell cycle regulator proteins, Rb/E2F complex, cyclin D1/cdk4 and cyclin B1/cdk1[94]. Vitamin E suppressed the cell viability of breast cancer cell lines, MDA-MB-231 and MCF-7. Interestingly, it selectively induced apoptosis and inhibited VEGF gene expression in MDA-MB-231 triple negative breast cancer cell line[95]. Tocotrie-

nol stimulated the cell apoptosis by inducing endoplasmic reticulum stress which characterized by excessive unfolded proteins and upregulation of the death receptor 5 (DR5)[96,97]. Specifically in triple negative breast cancer MDA-MDB-231, the β-tocotrienol has been shown to promote apoptosis by inducing mitochondrial stress and suppressing PI3K/Akt signaling pathway[98]. Also, β-tocotrienol possessed antiproliferative effects regardless of the hormonal status of breast cancer by inducing mild G1 arrest[98].

Clinical Studies of Antioxidants on Breast Cancer

There are several clinical studies that have been conducted on the usage of antioxidants supplementation in breast cancer. Antioxidants have been used by healthy individual to decrease the risk of breast cancer and by breast cancer patients to reduce treatment toxicity, tumor recurrence and enhance tumor response to treatment. However, a recent case-control study reported that there was no significant association between dietary antioxidants and breast cancer risk among 150 breast cancer cases regardless of their menopausal status[99]. Interestingly, a comprehensive review on 22 different studies concluded that antioxidant supplements, such as vitamin C, vitamin E, glutamine, glutathione, melatonin, or soy isoflavones, during conventional breast cancer treatments have no effect on toxicity, tumor response, recurrence, or survival of the patient[100]. Another study revealed that vitamin E decreases hot flashes among 120 breast cancer patients caused by tamoxifen therapy in randomized, placebo-controlled trial[101].

Nonetheless, the inadequate number of clinical studies as well as experimental limitations, for example: the route of administration; achievement of desired therapeutic dosage; low numbers of patients; has made it difficult to determine the precise therapeutic potential of antioxidants in breast cancer patients. Thus, further studies into the role of supplementation and their effects on breast cancer patients are warranted.

Premenopausal Breast Cancer Women

In 1991, 90,655 premenopausal women were enrolled to investigate the effects of vitamins A, C, and E, folate, and carotenoids intake in reducing the risk of breast cancer. Following these women for 8 years; no effects were observed[102]. European Prospective Investigation into Cancer and Nutrition (EPIC) recruited 116,957 premenopausal women and followed them from 8 years[103]. The risk of breast cancer among premenopausal women does not decrease by dietary intake of β-carotene, vitamin C and vitamin E[103]. Another case-control pairs study in 2015 showed that there is no inverse association between plasma levels of carotenoids and the breast cancer risk in 1,179 premenopausal women who were followed for almost 9 years[104].

Thus, it seems that consumption of vitamin A and E do not have any correlation in the prevention of breast cancer in premenopausal women, perhaps due to their hormonal protective mechanisms.

Postmenopausal Breast Cancer Women

A recent study was conducted on 200 postmenopausal breast cancer survivors who were previously enrolled in the Women's Healthy Eating and Living (WHEL) study and were successfully treated form early-stage breast cancer[105]. A significant reduction in oxidative stress biomarker, 8-hydroxy-2′-deoxyguanosine (8-OHdG) was observed in urine samples, in relation to high level of plasma carotenoid concentrations. However, the dietary carotenoid levels were not significantly associated with reduction of oxidative stress indicators[105]. A nested case control study was conducted in 2002 on two cohorts of 1974 and 1989[106]. These two cohorts enrolled women who donated blood from Maryland and in 1994 they developed breast cancer; and a majority of them at the time of their diagnosis were postmenopausal. There was a significant inverse association of high carotenoid concentration with low risk of breast cancer in both cohorts, but retinol and tocopherol did not show any associations[106]. Another nested case-control study in 2005 used plasma from a newly diagnosed 969 women with breast cancer prior to end of Nurses' Health study (NHS); around 89.5% of newly diagnosed women were in the postmenopausal[107]. This study reported that high levels of carotenoids lead to decrease the risk of breast cancer, specifically α-carotene has significant inverse association with invasive breast cancer with nodal metastasis. However, retinol and tocopherols revealed no significant associations[107]. A case-control study in 2003 enrolled 385 postmenopausal women who were diagnosed with breast cancer between 1986 and 1988[108]. This case-control study concluded that the usage of vitamin E supplements for more than 3 years reduces both the risks of recurrence and mortal-

ity related to breast cancer[108]. Further, a population-based case-control study in Canada, in 2011 was conducted on 2,362 breast cancer cases, and 63.3% of the cases were postmenopausal[109]. A significant reduction in breast cancer cases after the menopause was observed when intake of multiple vitamins, beta-carotene, vitamin C, vitamin E and zinc, was longer than 10 years either from diet or supplements, but this effect was abolished if these antioxidants have been taken less than 10 years[109]. This suggests that indeed, the intake of carotenoids, and vitamin E depict breast cancer prevention in postmenopausal women, even for the invasive types. However, vitamin E should be administered for long durations to exert its effects.

Free Radicals as Risk Factor in Postmenopausal Breast Cancer

As previously discussed, antioxidant intake seems to be the most beneficial in postmenopausal status. Various studies have been conducted to determine the potential reasons behind this observation. It has been reported that estrogen hormone has a beneficial antioxidant effect via the prevention of guanine DNA bases oxidation at the 8th position[110]. This tends to happen in premenopausal women patients who display high estrogen concentrations. As estrogen levels drop, oxidative stress increases in the body. Additionally, low levels of estrogen have detrimental pro-oxidative effect; estrogen contains catechol structure in its chemical arrangement which induces oxidative DNA damage[110].

Almost 200 women were recruited to evaluate the redox status in healthy and breast cancer in response to the hormonal alterations of menopause[111]. Interestingly, lipid peroxidation and protein oxidation were significantly higher in post-menopausal women, compared to premenopausal women in both the healthy and breast cancer women. Furthermore, antioxidant defense systems, glutathione (GSH) levels and enzymatic superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), were significantly lower in both the healthy and breast cancer menopausal women. In this study, they concluded that normal redox status is disturbed by breast cancer and by the changes of hormonal status via menopause[111]. Another reason for high oxidative stress in postmenopausal breast cancer is due to the high levels of hydrogen peroxide (H_2O_2)[112]. Further, the single nucleotide polymorphisms (SNPs) in thioredoxin system, CYBA (NADPH oxidase: rs3794624) and MT2A (metallothionein 2A: rs1580833), are associated with postmenopausal breast cancer risk[113]. Another polymorphism in the promoter region of the CAT gene (catalase: rs1001179) are correlated with increased risk of estrogen receptor–positive (ER+) breast cancer in postmenopausal women who are on hormone replacement therapy[114]. In 2014, a prospective study on 216 consecutive postmenopausal breast cancer patients found that ROS are highly correlated with ER+ than ER- patients[115]. The breast tumor with low ERα/ERβ ratio has poor prognosis in postmenopausal breast cancer patients and correlates with greater oxidative damage[116]. In 2013, a nested case-control study was conducted using a population-based cohort among 24,697 postmenopausal women[117]. The positive association between 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxo-dG) excretion in the urine, a biomarker of oxidative stress, and risk of especially ER-positive breast cancer[117]. Collectively, postmenopausal status characterized by high free radicals' level, mediated by low antioxidant defense systems which could be the cause of breast cancer.

Adverse Effects of Vitamins A and E

The other side of the antioxidant story is vitamins A and E toxicity. Several randomized, double-blind clinical studies have used α-tocopherol and β-carotene on cohort of smoker elder men for primary prevention of cancer. Unexpectedly, those studies have reported that receiving β-carotene increased the incidence of lung cancer and death from cardiovascular diseases[118-120], while α-tocopherol increased the incidence of prostate cancer and the risk for hemorrhagic stroke[119]. The meta-analysis of the dose-response relationship between α-tocopherol supplementation and total mortality by Miller et al[121], has concluded that high dosage of vitamin E supplements > 400 IU/d for 1 year increased all-cause mortality[122]. From five vitamin E trials, the risks for all-cause mortality, published since the meta-analysis of Miller et al[123], are cardiovascular diseases, head and neck cancer, diabetes, and peripheral arterial diseases. Nonetheless, avoiding β-carotene supplementation in all smokers specifically the elder men regardless of the type of cigarette smoked is highly recommended[124]. However, future studies are warranted to clarify this issue and perhaps focus on the dual role of these vitamins.

Conclusions

Angiogenesis is crucial for progression and metastasis of breast cancer. Various pro angiogenic agents escalate the proliferation of endothelial cells and vascular remodeling. Oxidative stress generated due to angiogenesis further complicates the web of signaling pathways involved. ROS affects VEGF/VEGFR signaling, a major pathway for angiogenesis by increasing its expression and generation of more ROS leading to endothelial cell proliferation. Similarly, ROS increases transcription of HIF 1 alpha *via* NF kappa B and inhibits PHD. HIF 1 alpha increases the transcription of various proangiogenic, glycolytic, and pro survival genes. Angiopoietin-Tie system is one of the angiogenic receptors which increase ROS and in return it activates its downstream signaling molecules resulting in proliferation and survival of endothelial cells. Metabolomics of the blood vessel is also affected by ROS. Endothelial cells produce lactate by glycolytic efflux which is also a pro-angiogenic agent and ROS modification of proteins and lipids helps in sustaining NOS levels and increases the formation of blood vessels.

Furthermore, oxidative stress is a risk factor of breast cancer because of the irreversible formation of oxidative DNA damage. Vitamins A and E have been shown to have antioxidative effects by scavenging free radicals and reducing oxidative stress through altering different cell signaling pathways in different breast cancer cell lines. Studies on postmenopausal breast cancer patients reveal high levels of free radicals and low antioxidant defense systems profile. Moreover, antioxidants have been shown to exert preventive effects in postmenopausal women against breast cancer in different clinical studies. However, more studies are still required to understand the precise role of ROS in angiogenesis and in breast cancer.

As described in this review, antioxidants may present minimum effects on breast cancer, whereas some antioxidants may exhibit favorable effects on cancer treatment. Thus, to comprehend the potential role of antioxidants in breast cancer prevention or therapy, it will be necessary to characterize the redox status in each subtype, and cell types, for patients[118]. Moreover, identification of tumor-type specific oxidative stress genetic profiles and how this might aid in the prognosis is still in preliminary stages, albeit the increasing availability of cancer genetic expression profiles, mutations, survival, and epigenetic data, it will be to utilize bioinformatic techniques to understand the role of oxidative stress genes[119]. For example, mitochondria can result in enhanced ROS levels reported in triple-negative breast cancer (TNBC), thus targeting ROS and/or mitochondria is suggested could as a therapeutic option in this situation. Future studies should focus on clarification of whether antioxidants have a dual role depending on their dosage and bioavailability, and their interaction with different therapies. Personalized therapies based on genetic studies may improve clinical outcomes with supplementation. Moreover, antioxidants could be a promising preventive therapy in postmenopausal women with breast cancer, and this needs to be explored thoroughly.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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