Chapter 10

Role of Endothelial Nitric Oxide Synthase in Breast Cancer

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Additional information is available at the end of the chapter

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Abstract

Breast cancer (BC) is the most common form of carcinoma and a primary cause of morbidity and mortality globally. Oxidative stress represents as an important factor in carcinogenesis and may play a role in initiation and progression of tumors. Oxidative stress–induced NO• damage to DNA includes a multitude of lesions, many of which are mutagenic and have multiple roles in cancer and aging. It is caused by an unfavorable balance between reactive oxygen species/reactive nitrogen species (ROS/RNS) and antioxidant defenses. ROS/RNS are generated during normal cellular metabolism, as a result of the influence of various environmental factors, as well as during pathological processes. Nitric oxide (NO•) is a ubiquitous, short-lived free radical produced from L-arginine by nitric oxide synthases (NOSs), and isoforms of NOS exist, depending on the site of origin: endothelial (eNOS), neuronal (nNOS), mitochondrial (mtNOS), and inducible (iNOS). eNOS is responsible for the endothelial synthesis of NO• and has shown to modulate cancer-related events such as inflammation, angiogenesis, apoptosis, cell cycle, invasion, and metastasis. Genetic studies also showed that eNOS gene polymorphisms are associated with the development of breast cancer. Therefore, selective targeting of eNOS may prove a potential strategy for prevention and treatment of breast cancer.

Keywords: breast cancer, oxidative stress, nitric oxide, endothelial nitric oxide synthase, therapeutics

1. Introduction

Cancer, a multifaceted disorder, represents one of the most important health problems worldwide, with approximately 14 million new cases and 8.2 million cancer-related deaths every

year [1]. The transformation from a normal cell into a tumor cell is a multistage process, typically a progression from a precancerous lesion to malignant tumors.

Cancers originate from a single abnormal cell (clonal origin) with an altered DNA sequence (mutation). Successive rounds of mutation and selective expansion of these cells result in the formation of a tumor mass and leads to tumor growth and progression, which eventually breaks through the basal membrane barrier of surrounding tissues and spreads to other parts of the body (metastasis) (**Figure 1**).

Cancers may be classified by their primary site of origin such as brain cancer, oral cancer, lung cancer, prostate cancer, liver cancer, renal cell carcinoma (kidney cancer), breast cancer, etc.

Breast cancer is defined as a malignant growth that begins in the epithelium of the breast. It is estimated as one of the most commonly diagnosed cancers worldwide (11.9%) [2]. In India, breast cancer has overtaken cervical cancer and has become the most prevailing cancer among women [3]. The most frequent type of breast cancer is ductal carcinoma *in situ* (DCIS), which affects the cells of the milk ducts. Cancer that starts in lobes or lobules is called lobular carcinoma *in situ* (LCIS); it is the second most common type of breast cancer. While rare breast cancer types include tubular, medullary, metaplastic, mucinous carcinoma, and Paget's disease.

Breast cancer is a clinically heterogeneous disease. Breast cancer cells generally overexpress estrogen receptor (ER)/progesterone receptor (PR), and/or human epidermal growth factor-2 (HER-2) receptor and lead to the tumor formation and progression. Thus, breast cancer can be classified into two subgroups on the basis of receptor status namely: (i) ER/PR positive (luminal A/B) and (ii) triple negative (ER, PR, and HER-2 negative) (basal-like) to know the prognosis and clinical outcome of breast cancer (**Figure 2**). Early breast cancer usually does not cause symptoms. As the cancer grows, symptoms may include: lump in the armpit, change in the size, shape, or feel of the breast or nipples, nipple discharge, etc. Symptoms of advanced breast cancer may include: breast pain or discomfort, bone pain, skin ulcers, and weight loss.

Breast cancer etiology is complex and multifactorial where there is a strong interplay between genetic and environmental factors. The strongest nonmodifiable determinants of breast cancer risk are female gender and age. Other risk factors associated with breast cancer can be grouped into three broad determinants: family history (hereditary) factors, hormonal and reproductive factors, and environmental (including lifestyle) factors (**Figure 3**).

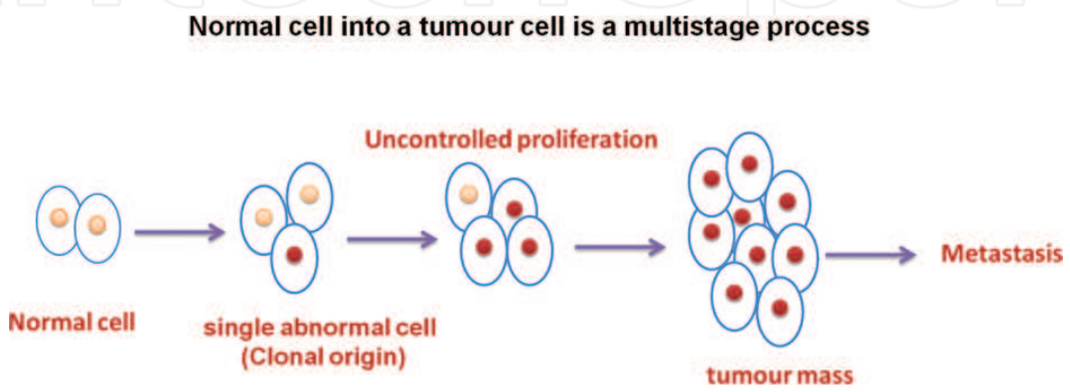

Figure 1. Carcinogenesis, a multistep process.

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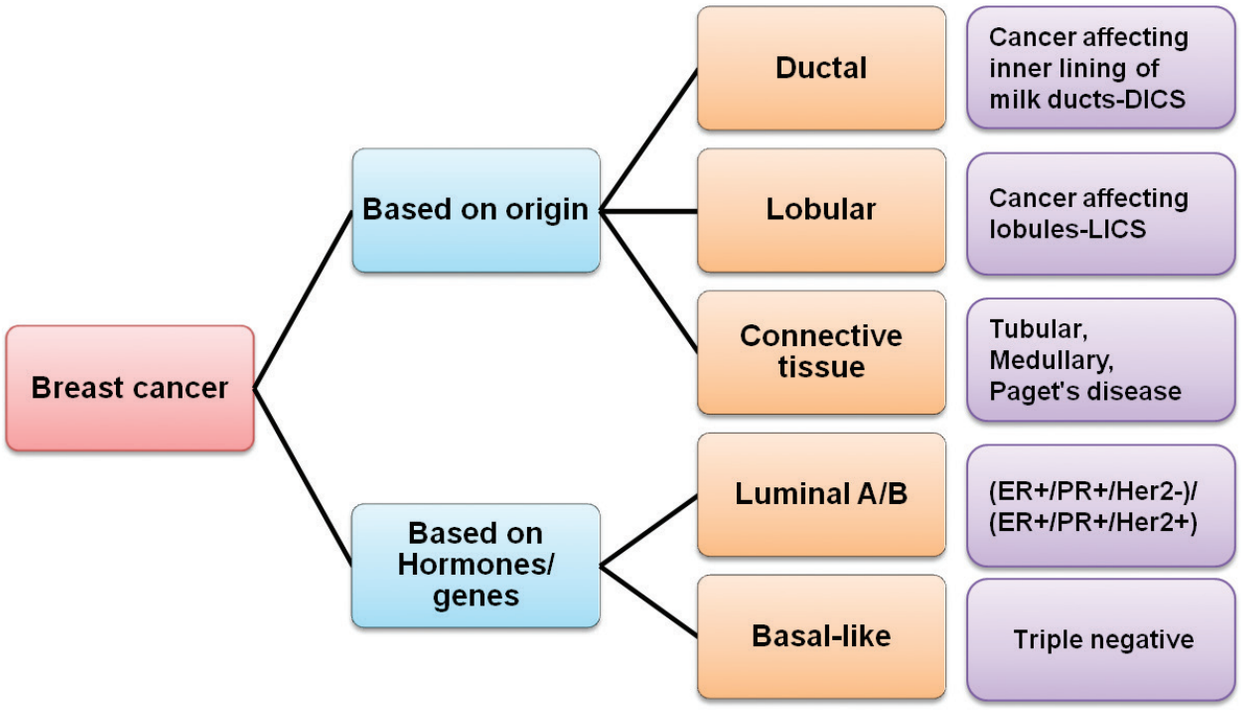

Figure 2. Classification of breast cancer.

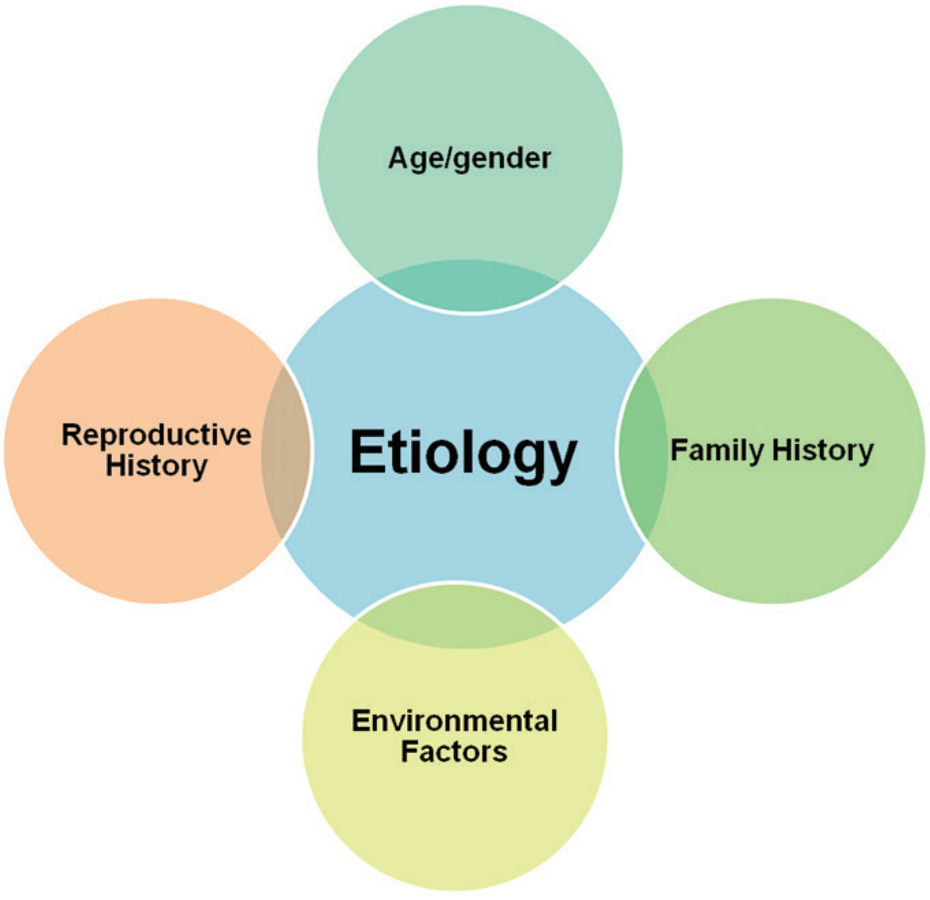

Figure 3. Breast cancer etiology.

Family history (hereditary) factors are associated primarily with early-onset of breast cancer. Previous genetic analyses of breast cancer–prone families have identified the BRCA1 and BRCA2 genes (breast cancer type 1 and 2 susceptibility protein). Women with mutations in either BRCA1 or BRCA2 (over 250 mutations have been identified) are at a significantly elevated lifetime risk (55–85% compared with 12% for the general population) for developing breast or ovarian cancer [4].

Reproductive and hormonal factors may increase the time and/level of steroid hormone exposure, consequently stimulating cell growth, and have been associated with breast cancer susceptibility. Major reproductive factors such as early menarche, late menopause, later age at first full-term pregnancy, and nulliparity are known to be associated with a higher risk of breast cancer [5].

Environmental (including lifestyle) factors such as the use of exogenous estrogens, radiation exposure, alcohol consumption, and socioeconomic status are also well-known risk factors for the disease [6]. Physical inactivity is a major risk factor for several types of cancer [7–9]. Exposure to ionizing radiation such as X-rays at a young age, alcohol consumption, and smoking habits have consistently been shown to increase breast cancer risk.

Radiations are classified into two major types namely: ionizing radiation and nonionizing radiation. The ionizing radiation transmits energy via X-rays and alpha particles disrupt chemical bonds, which results in chemically reactive free radical formation, a phenomenon known as ionization. These ionizing radiations may directly pass through the cell and may cause DNA damage. Such damage if unrepaired can result in nonlethal DNA modifications or cell death. The nonlethal DNA modifications thus eventually may cause malignant transformations. Ionizing radiation is, therefore, a known mutagen and an established breast carcinogen. Nonionizing radiation (e.g., microwaves and extremely low-frequency electric and magnetic fields (ELF-EMF)) does not have enough energy to break chemical bonds and produce ionization [10].

Several epidemiological studies have shown that alcohol consumption has been associated with breast cancer susceptibility. The alcohol metabolism occurs via multiple stages which may increase the risk of carcinogenesis. Alcohol metabolized into an acetaldehyde and other products subsequently damages DNA by inducing DNA modifications. Apart from this, acetaldehyde alone may also cause breast tumorigenesis by interfering with DNA repair mechanisms. Free radicals generated in the second stage of alcohol metabolism are thought to cause DNA damage, strand breakage, and base alterations and have been implicated for their role in alcohol-associated carcinogenesis [11].

There are over 60 carcinogens in cigarette smoke, which have been evaluated by the International Agency for Research on Cancer [12]. Cigarette smoke is rich in carcinogens such as nitrosamines and polycyclic aromatic hydrocarbons and induces oxidative damage. The gas phase of freshly generated cigarette smoke has large amounts of nitric oxide and other unstable oxidants. The presence of such free radicals and oxidants can lead to oxidative DNA damage [13]. The environmental risk factors that alter the levels of free radicals (reactive species) generated in the body are known to react with DNA to cause mutations in critical genes and consequently promote carcinogenesis.

2. Free radicals

Free radicals are molecules with high instability and reactivity due to the presence of an odd number of electrons in the outermost orbit of their atoms; their action derives from their attempts to attain "balance" by binding with electrons of neighboring atoms, giving rise to chain reactions (**Figure 4**) [14].

Free radical can be classified as reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS include the superoxide anion (O_2^-), hydroxyl radical (OH^-), hydrogen peroxide (H_2O_2), while RNS include nitric oxide ($NO\bullet^-$) and peroxynitrite ($ONOO^-$) the radical nitrogen dioxide (NO_2^-), and nitrite (NO_2^-) [15].

Free radicals are key players in the initiation and progression of tumor cells and enhance their metastatic potential. They are now considered a hallmark of cancer.

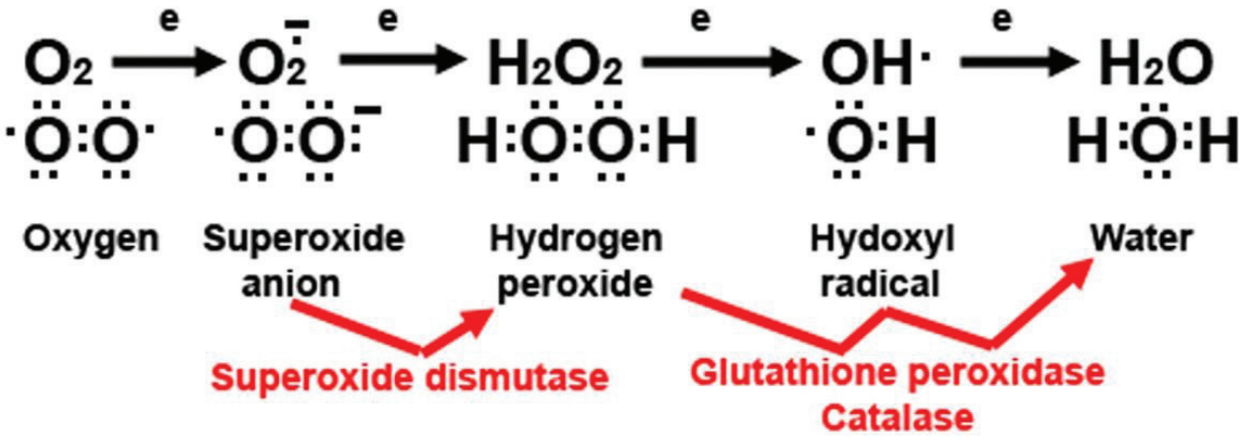

Figure 4. Formation and elimination of ROS.

3. Oxidative stress

The cell generates free radicals and also degrades, which is strictly necessary to avoid the damage derived from free radicals. However, various intrinsic and extrinsic circumstances and the biochemical activity of the cell can make it lose control over the formation and management of free radicals and results in "oxidative stress." It results from a disturbance in balance between the formation of ROS/RNS and the defense provided by cell antioxidants. This imbalance may cause damage related to various human diseases (**Figure 5**) [16].

During endogenous metabolic reactions, aerobic cells produce ROS such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH\bullet$), and organic peroxides as normal products of the biological reduction of molecular oxygen [17]. Under hypoxic conditions, the mitochondrial respiratory chain also produces nitric oxide ($NO\bullet^-$), which can generate other reactive nitrogen species (RNS) [18].

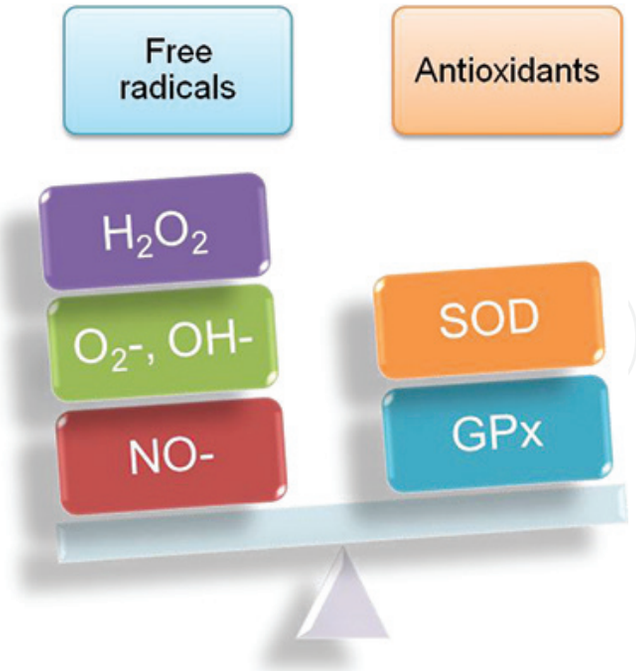

Figure 5. Oxidative stress.

4. Antioxidants

Cells have natural defense systems against ROS that consist of antioxidant enzymes, vitamins, etc., some of these antioxidants are produced inside the human body, mostly falling into the enzymatic category, as they are predominantly protein in nature. These proteins include the superoxide dismutase (SOD) enzymes (which have differential subcellular localization and superoxide dismutation to H_2O_2), glutathione peroxidase (GPx), and catalase (both of which clear peroxide), thioredoxins (Trxs; reduce oxidized proteins), and glutathione synthetase (GSS; synthesizes glutathione [GSH], an important antioxidant), among others. Vitamins are mostly obtained from nutritional sources and include ascorbic acid (vitamin A), tocopherol. Therefore, a fine balance exists between the levels of ROS and antioxidants within the cell [19]. Increased ROS/RNS can result in a greater number of mutations, oxidation of critical proteins, and other alterations, finally culminating in cell death. Identification of potentially modifiable factors that affect oxidative stress in breast cancer patients is an increasingly important task.

5. Nitric oxide

Nitric oxide (NO•) is a ubiquitous, short-lived, water soluble and endogenously produced gas that exerts a wide range of biological effects. It is a pleiotropic regulator, critical to numerous biological processes, including vasodilation, neurotransmission, and macrophage-mediated immunity.

The general function of NO• protects against the effects of free radicals but at excessive concentrations, NO• or its derivatives may lead to DNA damage and impair the tumor suppressor function of p53, which may cause cancer development [20].

5.1. Intracellular mechanisms of $NO^{\bullet -}$

When NO• is synthesized, it has a half-life of only a few seconds. Its bioavailability is reduced due to the high affinity binding of superoxide anion (high reactivity in both molecules is due to the unpaired electrons). NO• has an ability to tightly bind to the heme moiety of both hemoglobin (Hb) and guanylyl cyclase (GC) and found mostly in the vascular smooth muscle cells and other cells. Thus, NO• when produced in endothelium is quickly diffused into the blood circulation, binds to hemoglobin in blood and forms nitrates. NO• may also activate guanylyl cyclase (GC), an enzyme that catalyzes the dephosphorylation of guanosine triphosphate (GTP) to 3′,5′-cyclic guanosine monophosphate (cGMP) and serves as a second messenger for many important cellular functions, particularly for signaling smooth muscle relaxation [21].

5.2. $NO^{\bullet -}$ biological functions

Nitric oxide ($NO^{\bullet -}$) is known to play important functional roles in a variety of physiological systems [22].

1. $NO^{\bullet -}$ induces vasodilation—NO initiates and maintains vasodilation through a cascade of biological events that culminate in the relaxation of smooth muscle cells that line arteries, veins, lymphatics. NO also inhibits the aggregation of platelets and thus keeps inappropriate clotting from interfering with blood flow.
2. $NO^{\bullet -}$ regulates programmed cell death (apoptosis)—NO has been shown to both induce and inhibit apoptosis. The activity of the caspases can be directly affected by antiapoptotic effects of NO through nitrosylation of the active site, leading to inhibition of protein function. NO can also affect the expression of many members of the Bcl-2 protein family, including both proapoptotic proteins.
3. $NO^{\bullet -}$ alters kidney function—The release of NO around the glomeruli of the kidneys increases blood flow through them thus increasing the rate of filtration and urine formation.
4. $NO^{\bullet -}$ induces smooth muscle cell contractility—The relaxing effect of NO on the smooth muscle allows wavelike motions in the gastrointestinal tract.

While, NO also inhibits the contractility of the smooth muscle wall of the uterus. During child birth, as the moment of birth approaches the production of NO decreases.

5. $NO^{\bullet -}$ affects secretion from several endocrine glands—NO regulates the release of Gonadotropin-releasing hormone (GnRH) from the hypothalamus and adrenaline from the adrenal medulla.

In mammals, under normoxic conditions, NO• is generated endogenously "on demand" when the guanidino nitrogen of L-arginine undergoes a five-electron oxidation to yield the gaseous free radical, nitric oxide, and citrulline in equimolar concentrations (**Figure 6**) [23].

The constitutive forms of NOS are endothelial NOS (eNOS; type III) and inducible NOS (iNOS; type II). Cofactors for NOS include oxygen, NADPH, tetrahydrobiopterin, and flavin adenine nucleotides.

The inducible (calcium-independent) isoform (iNOS) produces much larger amounts of NO and is only expressed during inflammation. Whereas iNOS can produce injurious amounts of RNS (check), eNOS and nNOS produce beneficial amounts under physiological conditions. The constitutive (calcium-dependent) isoform and endothelial NOS (eNOS) produce small amounts of NO, which act as a vasodilator. The third form, neural NOS (nNOS; type I) serves as a transmitter in the brain and in different nerves of the peripheral nervous system to produce vasodilation. mtNOS constitutively expressed and membrane-bound nNOS isoform alpha, precluding a novel alternative spliced product. The mitochondrial production of nitric oxide is catalyzed nitric-oxide synthase (mtNOS). This enzyme has the same cofactor and substrate requirements as other constitutive nitric-oxide synthases (**Figure 6**).

NOS1 is the neural (or brain) isoform, also known as *nNOS*. It plays an important role in neural communication via synaptic transmission from nerve to nerve across synapses, and from peripheral nerves to the brain.

NOS2 is also known as *iNOS*. It generates extremely elevated concentrations of NO, to participate in a host defense mechanism. It also takes several hours to be activated in response to an injury or infection. Unlike nNOS, which takes part in normal neural communication, an abnormal stimulus (a wound, tissue damage, hypoxia, bacterial infection, etc.) may induce iNOS.

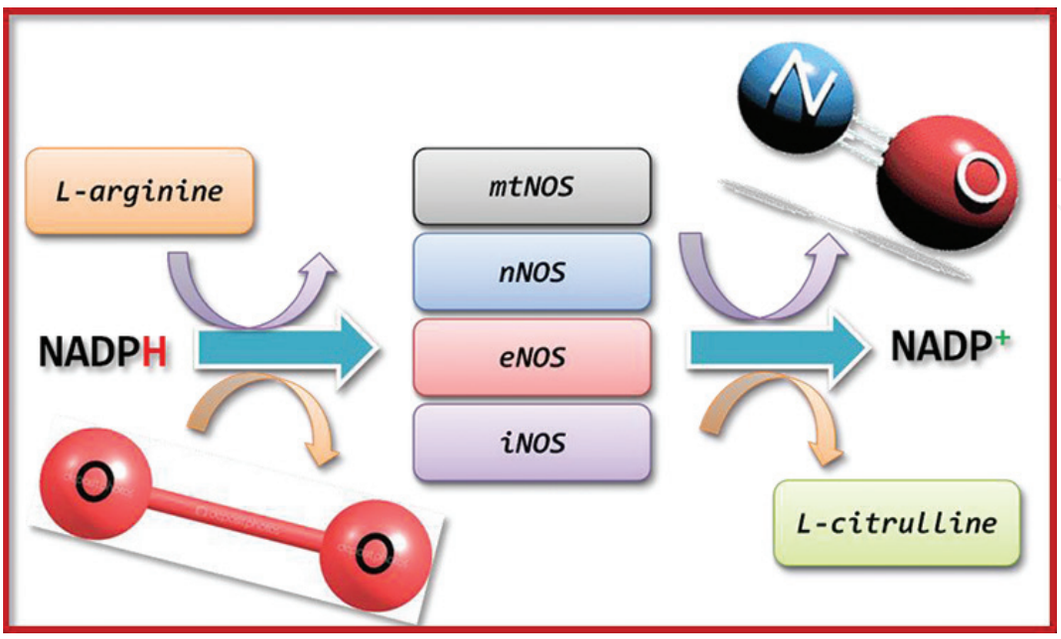

Figure 6. Mechanism of NO production from NOS.

The third isoform is *eNOS* (or NOS3) which stands for "endothelial cell" NOS. This isoform is active at all times and is found in endothelial cells which are the cells that line the inner surface of all blood vessels and lymph ducts. The eNOS is activated by the pulsatile flow of blood through vessels and maintains the diameter of the blood vessels at an optimal level. In addition, it also promotes angiogenesis, a process of new blood vessels formation.

A fourth type, mitochondrial NOS (*mtNOS*), differentiated by its subcellular localization in the mitochondrial inner membrane, regulates various functions of mitochondria in liver, heart, kidney, breast, etc. The mitochondrial utilization of NO involves superoxide anion and H_2O_2, a species freely diffusible outside the mitochondria that participate in the modulation of cell proliferation and apoptosis and in cell transformation leading to cancer [24, 25].

6. Endothelial nitric oxide synthase (eNOS)

Endothelial nitric oxide synthase enzyme, also known as nitric oxide synthase-3 (NOS-3) or constitutive NOS (cNOS), has been shown to be a critical regulator of carcinogenesis. It is a dimeric structure in their active form containing two identical monomers of 134 kD represented by *a reductase domain* containing the binding sites for nicotinamide adenine dinucleotide phosphate (NADPH), flavin mononucleotide (FMN), and flavin adenine dinucleotide (FAD). While *an oxidase domain* comprises the binding sites for the heme group, zinc, the cofactor tetrahydrobiopterin (BH4), and the substrate L-arginine [26]. The reductase domain is attached to the oxidase domain via calmodulin-binding sequence [27].

The eNOS expression is regulated by a range of transcriptional and posttranscriptional mechanisms, generates nitric oxide (NO•) in response to a number of stimuli. Constitutively expressed eNOS oxidizes L-arginine to generate L-citrulline and NO•. The essential cofactors for catalysis of this reaction include as calmodulin (CaM), flavin mononucleotide, flavin adenine dinucleotide, tetrahydrobiopterin (H4B) and NADPH.

Typically, the eNOS isoforms can be activated as a result of calmodulin (CaM) binding following a rise in intracellular calcium. They may also be activated and/or inhibited by phosphorylation via various protein kinases. Oxygen levels also regulate NOS levels in a cell type and isoform-specific manner by altering enzyme expression and by limiting the availability of oxygen, a key substrate for NO• synthesis.

Experimental and epidemiological evidence has shown the contributory role of eNOS induced NO• in tumor progression, suggesting a possible implication of endothelium expressed eNOS in tumors. The role of NO• in cancer biology is widespread, including its involvement in cellular transformation, malignant lesions, initiation, progression of the metastatic process, and induction of genotoxicity [28]. NO•-mediated DNA damage may be due to direct modification of DNA, or inhibition of DNA repair mechanisms [29]. Similarly, most of the RNS can cause DNA strand breaks and result in multiple mutations in DNA [30].

Breast carcinogenesis involves the transformation of a normal cell into a tumor cell mediated by a sequence of cellular and molecular events. These events consist of the attainment of

precise characteristics, such as uncontrolled proliferation, avoidance of mitotic control, resistance to apoptosis, replicative immortality, escape from immune surveillance, progression by stimulating invasion and metastasis, angiogenesis, genomic instability, and deregulated metabolism [31].

Tumor-derived eNOS has shown to modulate cancer-related events (inflammation, apoptosis, cell cycle, angiogenesis, invasion, and metastasis) (**Figure 7**) and genetic studies showed that eNOS gene polymorphisms are associated with the development of multiple cancers [32].

Figure 7. Multiple roles of eNOS in tumor development.

6.1. Inflammation

Inflammation a localized protective response elicited by injury is known to cause DNA damage [33]. Chronic inflammation due to infection or injury is estimated to contribute to 25% of all cancers in the world [34]. A growing body of laboratory research has shown that inflammation is a key mediator in the promotion of malignant transformation, where pro-inflammatory cytokines can facilitate tumor growth and metastasis by altering tumor cell biology and activating stromal cells in the tumor microenvironment, such as vascular endothelial cells, tumor-associated macrophages, and fibroblasts.

NO• is closely related to inflammatory status and regarded as a critical inflammation mediator. Pro-inflammatory cytokines can modulate the expression of eNOS and can accelerate the growth and development of cancer. eNOS, for example, can regulate the expres-

sion of the pro-inflammatory molecules nuclear factor-κB (NF-κB) and cyclooxygenase-2 [32, 35, 36].

6.2. Apoptosis

Apoptosis is an ordered and orchestrated cellular process that occurs in physiological and pathological conditions. Impaired apoptosis has been associated with initiation and development of cancer. The mechanism of apoptosis is regulated by an array of factors triggering and activating signaling cascade, subsequently leading to cellular death [33]. eNOS may be a molecular node in growth factor–mediated inhibition of apoptosis [37]. The antiapoptotic mechanism is understood on the basis of gene transcription of protective proteins and direct inhibition of the apoptotic executive effectors (caspase family protease).

The mechanisms of action that lead to the proneoplastic activity of NO• are via apoptosis inhibition by S-nitrosylation-inactivation of caspases- 1, 2, 4, 8 and 3, 6, 7 and disruption of the apoptotic protease activating factor 1/caspase-9 complex (Apaf-1/caspase-9 apoptosome is an essential initiator of caspase activation that initiates an apoptotic protease cascade) [38]. Other antiapoptotic effects of NO• depends on the interaction of NO•/cGMP, which inhibits the release of cytochrome C, stimulation of heat-shock protein (Hsp) 70 and Hsp 32, elevated Bcl-2 expression, repression of ceramide generation [39], and induces cyclooxygenase-2 activity [40]. In the animal model study, the lipopolysaccharide (LPS)-induced hepatic apoptosis was increased by the administration of NOS inhibitors [41]. Thus at high concentration, NO• has a potential role in cancer, i.e., inhibition of eNOS activity specific to tumor cells may be a viable option for the stimulation of apoptosis and treatment of cancer alone or in combination with chemotherapeutic agents.

6.3. Angiogenesis

Angiogenesis in mammary tumors can be stimulated by inflammation, which induces proliferation and morphogenesis of vascular endothelial cells in response to a large number of cytokines or angiogenic molecules produced by tumor and host cells [42]. Angiogenesis is essential for tumor growth and metastasis and has been considered the most important prognostic indicator for predicting overall survival [43]. eNOS strongly affects tumor growth by promoting angiogenesis [44]. Tumor growth–enhancing effects of vascular endothelial growth factor (VEGF) are associated with increased NOS activity and inhibition of apoptosis in human breast carcinoma xenografts [45].

Endogenous NO• promotes tumor blood flow via dilatation of arteriolar vessels. It decreases leukocyte-endothelial adhesive interactions and increases vascular permeability [46]. Several cancer treatment methods influence eNOS expression and activity. Low-dose irradiation-induced angiogenesis is believed to be mediated by NO• from eNOS [47]. Studies have shown that VEGF released as a purified protein or produced by tumor cells requires a functional NO•/cGMP pathway within the end compartment to promote neovascular growth. NO• also has an invasion-stimulating effect, which is mediated by upregulation of MMP-2 and MMP-9 (matrix metalloproteinases) and downregulation of TIMP-2 and possibly TIMP-3 (tissue inhibitors of MMP) [48].

6.4. Tumor progression/invasion

NO• has been investigated regarding its possible involvement in the promotion of breast carcinoma. Increased amounts of NO• have been observed in the blood circulation of advanced grade breast cancer patients [49] where the increased levels of NO• have shown to promote tumor angiogenesis. Nitrotyrosine, a marker derived from NO•, was correlated with expression of VEGF-C and has been associated with lymph node metastasis in breast carcinoma patients, implicating the role of NO• in the development and progression of breast cancer [50]. In relation to the link of eNOS with cell proliferation, the eNOS expression has been detected in tumor cells specifically in breast cancer [51].

Several studies have observed NO• released by eNOS, can stimulate cancer cell cycle progression and proliferation. More specific to eNOS, studies have shown that the eNOS/NO• pathway plays a role in cancer cell DNA/RNA synthesis and proliferation apart from promoting angiogenesis [52]. The eNOS gene plays an essential role in endothelial cell proliferation in cell culture models and is a central mediator of several endothelium growth stimulators, such as vascular endothelial growth factor (VEGF) and prostaglandin E2. In human breast cancer, eNOS appears to be expressed in tumor epithelial cells, and its presence is correlated with histological grade and lymph node status. Higher NOS activity has been found in invasive breast tumors when compared with benign or normal breast tissue carcinoma [53].

6.5. Metastasis

Metastasis is a complex process by which the malignant cancer cells from the breast expand into other regions of the body. Lymphatic metastasis is a critical determinant of cancer prognosis. Recent findings indicate that eNOS mediates VEGF-C-induced lymph-angiogenesis and, consequently, plays a critical role in lymphatic metastasis [54]. Investigational studies on tumors have provided substantial evidence of the contributory role of NO• in tumor development, where series of tests were performed on tumor-bearing mice using NOS inhibitors showed a delayed tumor growth through eNOS inhibition and barred metastasis, signifying the potential role of endothelium-derived eNOS in metastasis [55].

7. Regulation OF *NOS3* gene expression

The most fundamental level of NOS regulation is reflected in the tissue-specific expression of the different isoforms. The amount of NO produced results from the expression level and activity of eNOS. It is regulated by several interlinking mechanism such as transcriptional, post-transcriptional, and posttranslational modifications. The activity of NOS3 is also controlled by avid binding transcription factors namely Ets-1, Elf-1, Sp1, Sp3, and YY1 to the NOS3 promoter region. Posttranscriptionally, NOS3 activity is controlled by modifications of the primary transcript, stability of mRNA, its subcellular localization, and nucleocytoplasmic transport. Posttranslational modifications of NOS3 consist of fatty acid acylation, substrate, and cofactor

availability, protein-protein interactions, and amount of phosphorylation. Another significant epigenetic mechanism for NOS3 gene expression is differential promoter methylation [47].

The gene-encoding eNOS located on chromosome 7q35-36 and is composed of 26 exons (coding sequences) and introns (sequences between exons) with an entire length of 21 kb and has more than 168 polymorphisms [56]. Over the last few years, polymorphisms of the gene have been identified, and their association with various diseases has been explored. Genetic comparison studies on healthy people and cancer patients have shown that polymorphisms in eNOS are associated with the development of cancers.

A single nucleotide polymorphism (SNP), T-786C, was identified in the 5′ flanking region involving a substitution of thymine (T) to cytosine (C) at a locus 786 base pairs upstream [57]. Another common variant of eNOS with a G to T transversion at nucleotide position 894 (G894T) leading to a change in amino acid at 298 (Glu298Asp) has been reported [58], and a 27-bp variable number of tandem repeats (VNTR) polymorphism in intron 4 (intron 4b/4a) [59] and high numbers of CA, which have been repeated in intron 13 of eNOS gene, are also known to be associated with complex disorders. These polymorphisms seem to be functional and have been widely investigated for their associations with cancer risk [60, 61]. Molecular studies of eNOS −786T > C, intron 4b/4a, and 894G > T polymorphisms (**Figure 8**) if performed in large and unbiased can provide valuable insights into the association between the eNOS gene and breast cancer risk.

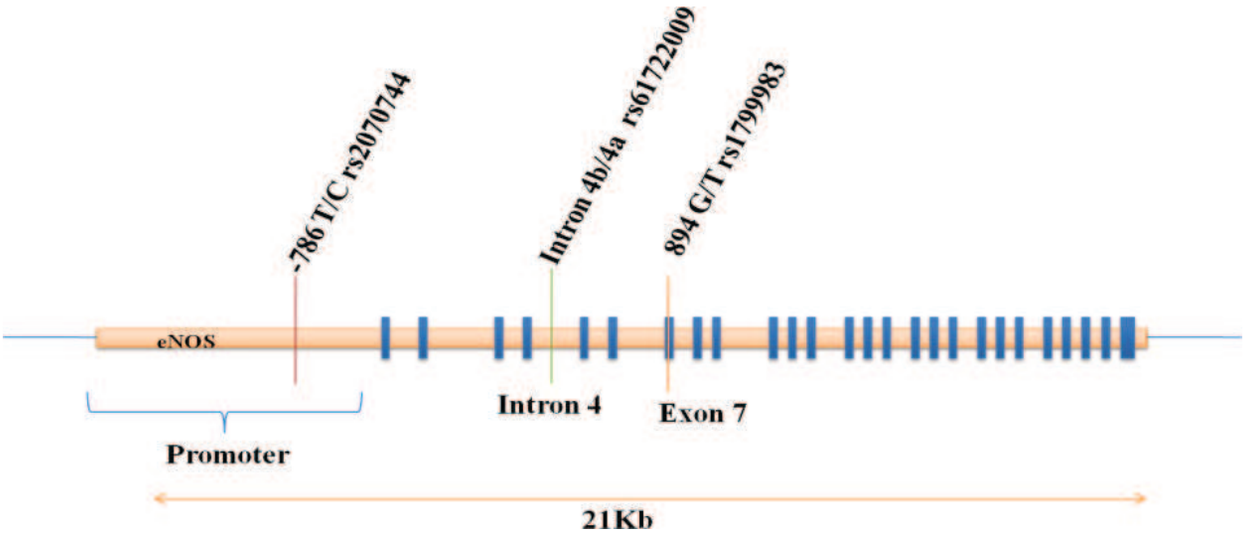

Figure 8. Organization of eNOS gene.

8. Conclusion

All these findings suggest that the expression of eNOS in breast cancer may be a critical event in carcinogenesis. Understanding different actions of NO• induced by eNOS in breast cancer

at the molecular level can help in providing diagnostic or prognostic markers and also in devising potential strategies for prevention of breast cancer. The ability of many tumors to exploit eNOS/NO for a survival, proliferative, and metastatic advantage suggests that pharmacological use of eNOS inhibitors might attenuate these effects. Therefore, selective targeting of eNOS may prove a useful therapeutic or chemopreventive measure. However, further careful studies are needed to confirm the potential therapeutic role of eNOS as a novel target for breast cancer therapy.

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