Adriana Cruz Afonso Romano

The role of EGCG in breast cancer prevention and therapy

O papel do EGCG na prevenção e terapia do cancro da mama

Abril, 2020

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Bioquímica

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The role of EGCG in breast cancer prevention and therapy.

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Faculdade de Medicina da Universidade do Porto, 07/04/2020

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REVIEW

The role of EGCG in breast cancer prevention and therapy.

Adriana Romano^a, Fátima Martel^{b,*}^a Faculty of Medicine of University of Porto, Porto, Portugal.^b Department of Biomedicine-Unit of Biochemistry, Faculty of Medicine of University of Porto, Porto, Portugal and Instituto de Investigação e Inovação em Saúde(i3S), University of Porto, Porto, Portugal.**Abstract:****Background:** Breast cancer is the most frequent cancer in women. Green tea has been studied for breast cancer chemopreventive and possibly chemotherapeutic effects due to its high content in polyphenolic compounds, including epigallocatechin-3-gallate (EGCG).**Method:** This review is based on a literature research that included papers registered on the Medline® database. The research was conducted through PubMed, with the application of the following query: "EGCG"AND "breast cancer". The result was a total of 88 articles in which this review stands on.**Results:** *In vitro*, EGCG shows antioxidant or pro-oxidant properties, depending on the concentration and exposure time. EGCG blocks cell cycle progression and modulates signaling pathways that affects cell proliferation and differentiation. EGCG also induces apoptosis, negatively modulates different steps involved in metastasis and targets angiogenesis by inhibiting VEGF transcription. *In vivo*, investigations have shown that oral administration of EGCG results in reduction of tumor growth and in antimetastatic and antiangiogenic effects in animal xenograft and allograft models.**Discussion:** Much remains unknown about the molecular mechanisms involved in the protective effects of EGCG on mammary carcinogenesis. In addition, more studies *in vivo* are necessary to determine the potential toxicity of EGCG at higher doses and to elucidate its interactions with other drugs.**Conclusion:** A protective effect of EGCG has been shown in different experimental models and under different experimental conditions, suggesting clinical implications of EGCG for breast cancer prevention and therapy. The data presented in this review support the importance of further investigations.**Keywords:** Epigallocatechin gallate (EGCG), breast cancer, anticarcinogenic effects, cancer prevention, cancer therapy

ARTICLE HISTORY

1. INTRODUCTION

Breast cancer is the most frequent cancer in women. Worldwide, 2.1 million diagnoses were estimated in 2018, contributing to 11.6% of the total cancer incidence burden. Despite the great advances in the diagnosis and treatment of this disease, the incidence of breast cancer is increasing in the developing world as a result of an increase in life expectancy and urbanization and the adoption of western lifestyles [1].

Human breast cancer is a heterogeneous disease and can be classified depending on the presence or absence of expression of progesterone receptors (PR), estrogen receptors (ER), and human epidermal growth factor receptors (HER2). The expression of these receptors determines breast cancer behavior and treatment options [2].

In this context, green tea, one of the most popular beverages worldwide, has been studied for chemopreventive and possibly cancer chemotherapeutic effects due to its high content in polyphenolic compounds [3]. In fact, epidemiological studies and meta-analysis have suggested that green tea consumption is associated with a decrease in incidence and recurrence of breast cancer [4-6].

Green tea extracts contain primarily catechins (monomeric flavonoids) (70%), minor flavanols (10%), and polymeric flavonoids (20%) [7]. The four major catechins (flavan-3-ols) present in green tea are epigallocatechin-3-gallate (EGCG), corresponding to 59% of all catechins, epigallocatechin (EGC), epicatechin-3-gallate (ECG) and epicatechin (EC) [8]. The anticancer effects of green tea catechins on breast cell carcinogenesis have been investigated under different experimental conditions, in different experimental models and results have been obtained both *in vitro* and *in vivo* [9].

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EGCG is one of the most studied catechins due to its extensive pharmacological effects and has been reported to be the most effective green tea catechin against the initiation, progression, and invasion stages of multistage carcinogenesis [10]. This monomeric flavonoid is an ester of epigallocatechin and gallic acid and has a polyphenolic structure with heterocyclic rings with eight free hydroxyl groups [5]. This catechin is featured by di-hydroxyl or tri-hydroxyl substitutions on the B ring and m-5,7-dihydroxyl substitutions on the A ring [7].

(-)-Epigallocatechin-3-gallate

Figure 1. Chemical structure of EGCG

In this review, we will focus on the role of EGCG in breast cancer prevention and therapy, with an emphasis on the molecular mechanisms implicated in its anticancer activity demonstrated both in *in vitro* and *in vivo* studies.

According to its influence on enzyme activities, oxidative stress levels, specific molecular targets and signaling pathways, we classify the mechanisms involved in the breast anticancer effects of EGCG into six categories: antioxidant/pro-oxidant effect, induction of cell cycle arrest, inhibition of proliferation, induction of apoptosis, inhibition of metastasis and inhibition of angiogenesis.

2. METHODS

This review is based on a literature research that included papers registered on the Medline® database. The research was conducted through the official search engine (PubMed), with the application of the following query: EGCG AND "breast cancer". All articles published from 1996 to 2019 were considered. The result was a total of 187 papers, which were further assessed based on title, abstract and full. From these, 26 articles were excluded based on the title, 56 after reading of the abstract, 23 after analysis of the full text and 5 articles were excluded because of the unavailability of the full text. In general, only articles that specifically mentioned anticancer effects of EGCG on breast cell carcinogenesis were included, amongst others with information considered relevant. The reference list of all identified trials was checked for additional relevant articles. The result was a total of 88 articles of several types such *in vitro* studies, *in vivo* studies and reviews in which this review stands on.

3. RESULTS

3.1. In Vitro Effects of EGCG on Breast Cancer

3.1.1. Interference with cellular oxidative stress levels (Table 1)

EGCG reduces oxidative stress levels and levels of lipid peroxidation, protein carbonylation, plasma low-density lipoproteins, C-reactive protein, plasma hydrogen peroxide, oxidative DNA damage and several pro-inflammatory cytokines [11, 12]. It also increases levels of some detoxification enzymes such as xanthine oxidase, glutathione-S-transferase and lipoxygenase [11]. Studies proved that exposure to EGCG abolished chronically-induced cellular carcinogenesis by blocking carcinogen-induced reactive oxygen species (ROS) elevation, contributing to lowering the risk of cancer induction [13]. The polyphenolic structure of EGCG may be responsible for activation of detoxification systems and antioxidant activity as proven in animal studies [14].

Na et al. showed that treatment of human breast epithelial (MCF10A) cells with EGCG activates the ERK1/2 and Akt signaling pathways, facilitating the release of nuclear factor erythroid 2-related factor (Nrf2) for nuclear translocation. Moreover, Nrf2 binding to antioxidant response elements (ARE) was concluded to mediate EGCG-induced expression of antioxidant enzymes such as heme oxygenase-1 (HO-1), glutamate-cysteine ligase and manganese superoxide dismutase (MnSOD) in these cells [12]. So, these results strongly suggest that the chemopreventive effect of EGCG in human breast epithelial cells involves increased antioxidant defense capacities [12]. Additionally, also through activation of Nrf2-ARE signaling, EGCG treatment induces an antioxidant-mediated chemosensitization of the triple-negative breast cancer cell line MDA-MB231 to chemotherapeutics such as cisplatin, by inducing the expression of phase-II enzymes [15].

However, it is now realized that EGCG shows antioxidant or pro-oxidant properties depending on the exposure time and concentration. As a matter of fact, Braicu et al. demonstrated that, in the triple-negative breast cancer epithelial cell line Hs578T, EGCG reduced the levels of ROS at 10 μ M, but increased the levels of ROS at 100 μ M [16]. In a subsequent study, these authors proved that EGCG treatment inhibited the proliferation of Hs578T cells, through activation of apoptotic processes mediated by its capacity to scavenge ROS [17]. Moreover, data from other *in vitro* studies have demonstrated that EGCG can induce programmed cell death in breast cancer cells by a cancer-specific induction of ROS, which are crucially involved in multiple stages of carcinogenesis as it was reviewed by Mokbel [3]. Besides, it was shown that EGCG, in the triple-negative breast cancer cell line MDA-MB-231, may cause cellular DNA breakage by generation of ROS and, in the presence of metals such as cooper, can lead to a pro-oxidant action which contributes to breast cancer cell death [18]. In this manner, EGCG might prevent breast carcinogenesis [11].

To determine whether EGCG acts through antioxidant or pro-oxidant effects, future studies in cells and animals should

employ specific biomarkers of oxidative damage to lipids, proteins and DNA [14].

On the other hand, Ranzato et al. revealed that EGCG affects $[Ca^{2+}]_i$ via ion channel modulation, involving both Ca^{2+} and K^+ currents and a redox mechanism. Indeed, EGCG induces a Ca^{2+} spike which depends on a redox mechanism and involves enhancement of T-type Ca^{2+} channels (Cav 3.2 channels) activity. Besides, EGCG also inhibits K^+ currents. This evidence seems relevant for the possible use of EGCG in breast cancer treatment [19].

Finally, Tran et al. verified that treatment of MCF-7 breast cancer cell line with EGCG caused an increase in oxidative stress-sensitivity, mediated by inhibition of heat shock- or oxidative-stress-induced promoter activity of HSP70 and HSP90 [20].

Table 1: Effect of EGCG on oxidative stress-related mechanisms.

Cellular Line	Main mechanism of action and key signaling pathways involved	Main Findings	References
Breast cancer cell line (MCF-7)	EGCG inhibits heat shock- or oxidative-stress-induced promoter activity of HSP70 and HSP90	EGCG ↑ oxidative stress-sensitivity	20
Breast cancer cell line (MDA-MB-231)	EGCG activates Nrf2-Keap1 pathway and induces phase-II enzymes expression through ARE (Antioxidant Response Element)-signaling	EGCG ↑ chemosensitization mediated by Nrf2-ARE (Antioxidant Response Element) signaling	15
Breast cancer cell line (Hs578T)	EGCG (10 μ M) reduces ROS levels EGCG (100 μ M) increases ROS levels	EGCG shows antioxidant and pro-oxidant properties	16
Breast cancer cell line (Hs578T)	EGCG scavenges ROS	EGCG ↑ apoptotic processes due to its capacity to scavenge ROS	17
Mammary epithelial cell line (MCF10A)	EGCG induces Nrf2-mediated antioxidant enzyme expression, through activation of PI3K and ERK	EGCG ↑ expression of some antioxidant enzymes leading to resistance to oxidative stress	12
Breast cancer cell line (MDA-MB-231)	EGCG generates ROS, causing cellular DNA breakage	EGCG ↓ cell proliferation due to a pro-oxidant effect	18
Breast cancer cell line (MCF-7)	EGCG induces a redox mechanism-dependent $[Ca^{2+}]$ spike	EGCG ↓ cell proliferation	19

3.1.2. Induction of Cell Cycle Arrest (Table 2)

Cell cycle progression consists of four phases (G1, S, G2 and M) and it is timely managed by cyclin-dependent kinases (CDK's) and their cyclin subunits at the G1/S and G2/M

checkpoints. EGCG can suppress tumor growth by blocking certain steps in cell cycle progression, through modification of the expression levels of cell cycle-regulatory proteins, as shown next.

Liang. et al, through a DNA flow cytometric analysis, concluded that 30 μ M EGCG blocks cell cycle progression at G1 phase in asynchronous MCF-7 cells [21]. Moreover, EGCG induces downregulation of cyclin D and Cyclin E, induces the expression of CDK inhibitors such as p21 and p27 and inhibits the activities of the cyclin-dependent kinase 2 (CDK2) and 4 (CDK4) in breast cancer cell lines [7, 21, 22]. Another study demonstrated that inhibition of human breast cancer cell proliferation by EGCG is mediated in part via induction of p27Kip1 CK, which is now known to bind to and to inhibit complexes formed by cyclin E-CDK2, cyclin A-CDK2, and cyclin D-CDK4, thus playing essential roles in transition through the G1 phase [23].

Additionally, treatment with EGCG was found to inhibit telomerase activity by decreasing human telomerase reverse transcriptase (hTERT) activity [74]. hTERT activity is regulated by various post-transcriptional mechanisms including binding of the heat shock proteins, HSP90 and HSP70, and the PI3/Akt pathway, and EGCG inhibits both the expression of HSP70 and HSP90 and the PI3K/AKT pathway [20,24]. Moreover, Meeran et al. showed that, in MDA-MB-231 breast cancer cell line, EGCG treatment led to inhibition of telomerase through epigenetic mechanisms involving DNA methyltransferases (DNMTs) and histone acetyltransferases (HATs) inhibition with subsequent DNA hypomethylation and histone deacetylation (central pathways for hTERT-targeted breast cancer prevention) [25].

Table 2: Effect of EGCG on cell cycle.

Cellular Line	Main mechanism of action and key signaling pathways involved	Main Findings	References
Breast cancer cell line (MDA-MB-231)	EGCG downregulates cyclin D and Cyclin E, involved in G1/S progression	EGCG ↓ culture growth	22
Breast cancer cell line (MCF-7)	EGCG inhibits the activities of cyclin-dependent kinase 2 (Cdk2) and 4 (Cdk4). EGCG induces the expression of the Cdk inhibitor, p21 protein and p27 protein	EGCG ↓ cell growth	21
Breast cancer cell lines (MCF-7 and MDA-MB-231)	EGCG causes DNMTs and HATs inhibition which leads to DNA hypomethylation and histone deacetylation, inhibiting the transcription of hTERT	EGCG ↓ telomerase and ↑ apoptosis	25
Breast cancer cell line (MCF-7)	EGCG downregulates telomerase	EGCG ↓ cell viability and ↑ apoptosis	24
Breast cancer cell line (MDA-MB-231)	EGCG induces the p27Kip1 protein, which causes an arrest of cells at the G1/S phase transition	EGCG ↓ proliferation and/or viability	23

3.1.3. Inhibition of Cell Proliferation and Viability (Table 3)

Similarly to other polyphenolic compounds, EGCG has pleiotropic effects in breast cancer cells, activating and inhibiting multiple signaling pathways and thus affecting many aspects of cellular function, including cell proliferation and differentiation. Importantly, EGCG is capable of selectively inhibiting cell proliferation without affecting healthy cells [26].

As shown next, EGCG inhibits the activities of PTKs (EGFR, FGFR, PDGFR, HER2/neu tyrosine kinases) and Akt kinase via STAT3, PI3K, mTOR, and NF- κ B signaling pathways [27, 28].

Firstly, Pianetti et al. concluded that the reduced signaling to NF- κ B via PI3K and Akt kinase caused by EGCG, occurs through inhibition of basal Her-2/neu receptor tyrosine phosphorylation [29].

In addition, it was shown that EGCG selectively blocks STAT3 phosphorylation, inhibiting STAT3 translocation into the nucleus. Consequently, the expression levels of STAT3-NF- κ B target genes were decreased, which downregulate the CD44 expression of MCF-7 and MDA-MB-231 cell lines [28]. Thus, it has been proven that through these mechanisms, the proliferation capacity of breast cancer cells is reduced.

Additionally, EGCG can inhibit mitogen activated protein kinases (MAPK's) and the transcription factor AP-1. MAPK's modulated by EGCG include ERK, JNK, and p38, which constitute a group of important targets of EGCG for breast cancer therapy and prevention. As proved by Ediriweera et al., EGCG has the potential to inhibit the phosphorylation of MAPK and ERK1/ERK2 in MCF-7 cells [30].

EGCG is able to stop growth of MCF-7Tam cells, a breast carcinoma cell line resistant to tamoxifen, through downregulation of EGFR, EMMPRIM and other molecules implicated in an aggressive biological behavior [31]. This catechin inhibits EGFR signaling pathway, mainly by direct inhibition of ERK1/2 and Akt kinases, which supports the role of EGCG as an inhibitor of breast cancer cell proliferation since this pathway is intrinsically related to the control of cell proliferation [32].

Moreover, EGCG appears to inhibit the chymotryptic activity of the 20S proteasome, thereby inhibiting cell proliferation. This action is recognized as a novel and promising target for treatment of breast cancer [33].

Among the several mechanisms proposed to explain the antiproliferative effect of EGCG on breast cancer cells, interference with energetic metabolism has emerged as an additional mechanism. This is because EGCG has been recently shown to affect the pathways related to glucose metabolism. More specifically, EGCG inhibits glucose uptake and lactate production by breast cancer cells, thus impairing glucose metabolism and decreasing cell viability and proliferation. Interestingly, this effect was found to be ER-independent, and, accordingly, it was observed in both the ER-positive MCF-7 and the triple-negative MDA-MB-231 cell lines [34].

Moreover, EGCG inhibits the expression and activities of the glycolytic enzymes hexokinase, phosphofructokinase, and lactic dehydrogenase, and to a lesser extent the activity of

pyruvate kinase. In addition, EGCG decreases the expression of hypoxia-inducible factor 1 α (HIF1 α), an essential player in regulating glycolysis [35].

Another mechanism involved in the antiproliferative effect of EGCG in breast cancer cells is an epigenetic effect. Indeed, EGCG has been shown to reverse DNA methylation-mediated silencing of tumor suppressor genes, causing inhibition of breast cancer cell growth. The presence of a catechol group in the structure of EGCG plays a key role in inhibiting DNA methyltransferases (DNMT1, DNMT 3a and DNMT3b) activity [36, 37]. Thus, epigenetic modifications by EGCG might be an important event in restriction of breast cancer carcinogenesis.

Additionally, it has been suggested that the increased incidence of breast cancer in women results from bioaccumulation of environmental pollutants such as polycyclic aromatic hydrocarbons (PAHs). In this context, Guo et al. proved that in DMBA (dimethylbenz[a]anthracene, a specific environmental carcinogen) - transformed breast cancer cell line D3-1, treatment with EGCG modulates genes involved in nuclear and cytoplasmic transport, transformation, redox signaling and response to hypoxia and to PAHs [38].

β -catenin is a transcriptional activator that is overexpressed in breast cancer cells. It is known that free cytoplasmic β -catenin may translocate to the nucleus and activate the Wnt signaling pathway, which contributes to tumorigenesis and progression. In this context, Hong et al. proved that EGCG inactivates β -catenin signaling in MDA-MB 231 human breast cancer cells [39].

Moreover, EGCG can downregulate ER- α , thus causing an inhibition of ER-positive breast cancer cell growth [40]. ER- α 36, a variant of ER α , is highly expressed in ER α breast cancer cells, and is critical to keep stemness of both ER β + and ER α + breast cancer cells. So, targeting ER- α 36 is a potential way to eradicate breast cancer stem cells. Nonetheless, studies revealed that ER- α 36 expression was also identified in patients diagnosed as ER-negative [41]. Interestingly, EGCG was found to decrease the expression of ER- α 36, thereby inhibiting tumor sphere growth and reducing CD44 β /CD24-cell population of ER-negative breast cancer cells. These results, showing that EGCG potently inhibits the growth of ER-negative stem cells, through down-regulation of the ER- α 36, supports the development of ER- α 36 as a therapeutic target [42].

Van Aller et al. determined the effect of EGCG on cell proliferation using a CellTiter-Glo luminescence cell viability assay and proved that EGCG potently inhibits PI3K, mTOR and phosphorylated Akt (p-Akt) and decreases MDA-MB-231 cell viability [43]. Similarly, Hong et al. proved that EGCG decreases MDA-MB-231 cell viability through downregulation of the expression of β -catenin, cyclin D1, and p-AKT [39].

Moreover, Filippi et al. demonstrated that EGCG was able to reduce the viability of SK-BR-3 cells, a human breast cancer cell line that overexpresses the Her2 (Neu/ErbB-2) gene product [32]. Finally, EGCG was found to be the most effective inhibitor of MCF-7 cell proliferation and viability, as compared to other epicatechin derivatives such as (-)-epicatechin (EC), (-)-epicatechin gallate (ECG), (-)-epigallocatechin (EGC) [24].

Table 3: Effect of EGCG on cell proliferation and viability.

Celular Line	Main mechanism of action and key signaling pathways involved	Main Findings	References
Breast cancer cell lines (MDA-MB-231 and MDA-MB-436)	EGCG decreases the expression of ER- α 36, inhibiting tumor sphere growth and reducing CD44p/CD24- cells population	EGCG $\downarrow$ cell growth	42
Breast cancer cell lines (MCF-7, MDA-MB-231, MDA-MB-157 and HCC1806)	EGCG decreases DNMT activity, down-regulates miR-221/222, and changes histone acetylation	EGCG $\downarrow$ cell growth and $\downarrow$ cell proliferation	36
Breast cancer cell lines (MCF-7 and MDA-MB-231)	EGCG inhibits DNMT	EGCG $\uparrow$ cancer cell death and reactivate DNA methylation-silenced tumor suppressor genes	37
Breast cancer cell lines (MCF-7 and MDA-MB-231)	EGCG inhibits the PI3K/Akt/mTOR signaling pathway	EGCG $\downarrow$ cell viability and proliferation	43
Breast cancer cell line (4T1)	EGCG inhibits the expression and activity of the glycolytic enzymes hexokinase, phosphofructokinase, and lactic dehydrogenase and pyruvate kinase. EGCG decreases the expression of hypoxia-inducible factor 1 α (HIF1 α) and glucose transporter 1 (GLUT1)	EGCG exerts an anti-tumor effect through a $\downarrow$ of key enzymes that participate in the glycolytic pathway and the suppression of glucose metabolism	35
Breast cancer cell line (MDA-MB-231)	EGCG inactivates the β -catenin signaling pathway	EGCG $\downarrow$ cell proliferation	39
Breast cancer cell line (SK-BR-3)	EGCG reduces Akt and ERK phosphorylation levels	EGCG $\downarrow$ cell viability	32
Breast cancer cell line (MCF-7)	EGCG reduces the increase of ERK1/2 phosphorylation in response to leptin	EGCG shows a protective effect against leptin-induced proliferation	30
Breast cancer cell line (MDA-MB-231)	EGCG inhibits the constitutive activation of EGFR, Stat3 and Akt	EGCG $\downarrow$ cell proliferation	27
Breast cancer cell line (MCF-7)	EGCG downregulates EGFR and EMMPRIN	EGCG $\downarrow$ cell growth	31

Breast cancer cell lines (MCF-7 and MDA-MB-231)	EGCG inhibits STAT3 phosphorylation	EGCG $\downarrow$ cancer stem cell population	28
Breast cancer cell lines (MCF-7 and MDA-MB-231)	EGCG inhibits glucose uptake and reduces lactate production	EGCG $\downarrow$ glucose uptake, impairing glucose metabolism and $\downarrow$ cell viability and proliferation	34
Breast cancer cell lines (MCF-7 and T47D)	EGCG downregulates ER α protein, mRNA and gene promoter activity	EGCG $\downarrow$ cell proliferation	40
Breast cancer cell line (MCF-7)	EGCG inhibits the expression of HSP70 and HSP90 by inhibiting their promoter activity	EGCG $\downarrow$ cell proliferation and colony formation	20
DMBA-transformed breast cancer cell line (D3-1)	EGCG decreases the expression of: Chromosome segregation 1-like (CSE-1), Karyopherina6, Aryl hydrocarbon receptor (AhR), Connective Tissue Growth factor (CTGF), Epithelial cell transforming sequence (ECTS), Hypoxia upregulated 1, SREBP2, Mcl-1, LIM and SH3 protein1, Thrombospondin 1, Tight junction protein 1, H1 histone family member, nucleosome assembly protein 1-like 4, HSP-10. EGCG increases the expression of; AKR C3, C2 and C1, PPAR γ , Carbonic anhydrase	EGCG $\downarrow$ cell proliferation EGCG changes expression of genes involved in nuclear and cytoplasmic transport, transformation, redox signaling, and response to hypoxia	38
Breast cancer cell line (MMTV-Her-2/neu)	EGCG inhibits signaling via PI3K, Akt kinase to NF κ B pathway mediated by inhibition of basal Her-2/neu receptor tyrosine phosphorylation	EGCG $\downarrow$ proliferation and transformed phenotype	29

3.1.4. Stimulation of Apoptosis (Table 4)

In cancer cells, the factors affecting the extrinsic and intrinsic pathways of apoptosis are downregulated, causing the cells to proliferate uncontrollably [44]. Treatment of the 4T1 breast cancer cell line with EGCG resulted in a concentration- and time-dependent induction of apoptosis [45]. Interestingly, EGCG-induced apoptosis in breast cancer cells may be orchestrated by the cooperative effects on both extrinsic and intrinsic apoptosis pathways [46]. Treatment with EGCG led to upregulation of pro-apoptotic genes such as p53, p21, caspase-3, caspase-8, caspase-9, Apaf-1, Bax and PTEN. Activation of caspases increases the Bax/Bcl-2 ratio and stimulates the cleavage of poly (ADP-ribose) polymerase

(PRAP) assuming that both mechanisms play a fundamental role during apoptosis [7]. Zan et al. reported that EGCG might increase PARP, pro-caspase-3 and pro-caspase-9 at protein levels. Furthermore, the same study showed that miR-25 suppression induced by EGCG resulted in inhibition of proliferation and induction of apoptosis of breast cancer cells MCF-7 [47]. EGCG also activates the intrinsic apoptosis pathway by stabilizing and activating p53 and increasing Bax on a p53-dependent manner [7].

Additionally, EGCG induces cell apoptosis by suppressing the expression of anti-apoptotic factors such as Bcl-2, Bcl-xL and survivin, accompanied by the inhibition of NF- κ B, JAK/STAT and PI3K/Akt pathways, in the breast cancer cell lines 4T1, T47D, MCF-7 and MDA-MB-231 [45, 48-51]. The inhibition of activation of PI3K/Akt results in the suppression of survivin, a member of the inhibitor of apoptosis (IAP) family of proteins, followed by increased caspase-9 activity [52].

In another report, EGCG decreased Bcl-2 expression and caused a time- and concentration-dependent loss of mitochondrial membrane potential that led to release of cytochrome c into the cytosol and was directly associated with the activation of pro-caspase enzymes in the breast cancer cell line MCF-7 [53].

Other studies have indicated that EGCG can be a potent fatty acid synthase (FAS) inhibitor, by suppressing HER2/HER3 signaling and by inhibiting basal activation of PI3K/Akt [54, 55].

Additionally, based on bioinformatics analysis, EGCG was described to have a possible influence on breast cancer progression through induction of apoptosis mediated by inhibition of focal adhesion kinase (FAK) signaling pathway [49].

In another study, it was described that EGCG promoted MCF-7 cell apoptosis via signaling pathways associated with the cell membrane, through modification of EGFR signaling, anti-laminin receptor (LR), intracellular Ca^{2+} , osteopontin (OPN) secretion, gelsolin, tropomyosin-4 (Tm-4), tropomyosin-1 (Tm-1) and adherents junction proteins (E-cadherin and β -catenin) [56]. Finally, EGCG induces an increase in endoplasmic reticulum Ca^{2+} and a decrease in cytosolic Ca^{2+} by inhibiting Bcl-2 mediated Ca^{2+} leakage from the endoplasmic reticulum in MCF-7 breast cancer cell line [57].

Table 4: Effect of EGCG on apoptosis.

Celular Line	Main mechanism of action and key signaling pathways involved	Main Findings	References
Breast cancer cell line (MCF-7)	EGCG interferes with the FAK-mediated pathway	EGCG $\uparrow$ apoptosis	49
Breast cancer cell line (MDA-MB-231)	EGCG inhibits the lipogenic enzyme fatty acid synthase (FAS)	EGCG $\uparrow$ apoptosis	55

Breast cancer cell line (MCF-7)	EGCG causes loss of mitochondrial membrane potential as well as release of cytochrome c into the cytosol directly associated with the activation of pro-caspase enzymes	EGCG $\uparrow$ apoptosis	53
Breast cancer cell line (MCF-7)	EGCG induces the expression of p53 but inhibits the expression of Bcl-2	EGCG $\downarrow$ proliferation and $\uparrow$ apoptosis	58
Breast cancer cell line (T47D)	EGCG upregulates pro-apoptotic genes such as p53, p21, caspase-3, caspase-9, Bax and PTEN. EGCG downregulates survival genes including PI3K, Akt and Bcl-2	EGCG $\uparrow$ apoptosis	48
Breast cancer cell line (Hs578T)	EGCG activates extrinsic and intrinsic pathways	EGCG $\uparrow$ apoptosis	17
Breast cancer cell line (MCF-7)	EGCG inhibits miR-25 expression and increases PARP, pro-caspase-3 and pro-caspase-9	EGCG $\uparrow$ apoptosis and disrupts cell cycle progression at G2/M phase	47
Breast cancer cell line (MCF-7)	EGCG inhibits EGFR, LR, FAS, intracellular Ca^{2+} secretion, OPN secretion, caspase-3, gelsolin, Tm-4, Tm-1, and adherents junction proteins, E-cadherin and β -catenin	EGCG $\downarrow$ cell proliferation, $\uparrow$ apoptosis and disrupt adherents junction formation via modification of the signaling pathways in cell membranes	56
Breast cancer cell line (MDA-MB-231)	EGCG downregulates Akt	EGCG $\uparrow$ apoptosis	50
Breast cancer cell line (MCF-7)	EGCG inhibits the basal activation of p-Akt, which leads to the suppression of survivin, followed by increasing caspase-9 activity	EGCG $\uparrow$ apoptosis	52
Breast cancer cell line (MCF-7)	EGCG downregulates FAS by suppressing HER2/HER3 signaling and downstream PI3K/Akt activation	EGCG $\uparrow$ apoptosis	54
Breast cancer cell line (4T1)	EGCG decreases the expression of Bcl-2 and increases expression of Bax, cytochrome c release, Apaf-1, and cleavage of caspase 3 and PARP proteins	EGCG $\uparrow$ apoptosis	45

3.1.5. Inhibition of Metastasis (Table 5)

In cancer patients, metastasis constitutes the leading cause of mortality, contributing to 90% of deaths from solid tumors [59]. The metastatic process is characterized by different steps which can be negatively modulated by EGCG resulting in reduction of metastasis: loss of cellular adhesion, increase of cellular motility and invasiveness, access to the blood circulation, spread into distant tissues and colonization [60]. EGCG suppress metastasis by regulating signal pathways and growth factors/receptors, by affecting the activity of proteolytic enzymes and by repressing the epithelial-to-mesenchymal transition (EMT) process, as shown next [61].

Breast cancer cells undergoing metastasis are characterized by expanded expression of matrix metalloproteinases (MMPs), which disrupt the protective basal membrane [62]. *In vitro* studies using human MDA-MB-231 and MCF-7 breast cancer cells showed that treatment with EGCG downregulates MMP-2 and MMP-9 [31, 63-66]. In addition, EGCG caused downregulation of EGFR and upregulation of TIMP-1 and TIMP-2, with restriction of cellular invasion [31, 63]. On the other hand, EGCG modulates cell matrix adhesion molecules and growth factor receptors through a FAK/Erk/NF- κ B signaling pathway-mediated mechanism [67]. Moreover, in MCF-7 breast carcinoma cell line resistant to tamoxifen, Farabegoli et al proved that EGCG could attenuate the tamoxifen-resistant phenotype, by stopping *in vitro* invasion through downregulation of EMMPRIN (extracellular matrix metalloproteinase inducer), a glycoprotein able to activate MMP-2 and MMP-9 [31]. In addition, Sen et al. proved that this green tea catechin downregulated MT1-MMP transcription. This metalloproteinase is responsible for cleaving pro-MMP-2, which generates the active form of MMP-2. Consequently, this downregulation results in inhibition of the MT1-MMP-driven migration of breast cancer cells [66].

On the other hand, considering that endothelial cell proliferation involves the Wnt pathway, Kim et al. demonstrated that EGCG decreases cellular invasion by suppression of Wnt signaling [68].

In addition to suppression of MMPS, EGCG was found to interfere with the hepatocyte growth factor/scatter factor (HGF/SF) signaling pathway and with its receptor Met. Through this effect, EGCG may also inhibit cancer cells invasion and metastasis in the invasive breast carcinoma cell line MDA-MB-231 [69].

The anti-invasive effects of EGCG in breast cancer cells might also be related to control of cell adhesion and migration. Urokinase plasminogen activator (uPA) and its inhibitor plasminogen activator inhibitor (PAI-1) are biomarkers for metastatic risk in breast cancer. EGCG, through inhibition of AP-1 (activator protein-1) and NF- κ B, suppresses uPA secretion and reduces the metastatic behavior of breast cancer cells [70]. The EGCG derivative 3e could repress metastasis in MDA-MB-231 cells by the same mechanism [71].

Activation of ErbB3/ErbB2 heterodimers by HRG (heregulin β 1) promotes metastasis of breast cancer cells by enhancing tumor cell invasion. Kushima et al. has demonstrated that EGCG inhibited migration and invasion of the breast cancer cell line MCF-7 by suppressing HRG-stimulated activation of

epidermal growth factor receptor-related protein B2 (ErbB2)/ErbB3/protein kinase B (Akt) signaling [72].

Moreover, EGCG also plays a crucial role in inhibiting tumor metastasis by virtue of modulation of signal pathways, including modulation of β 1 integrin-mediated signaling, down-regulation of VASP (vasodilator-stimulated phosphoprotein) via Rac1 pathway [73] and down-regulation of the EGFR signaling pathway [31, 67].

Table 5: Effect of EGCG on metastasis.

Cellular line	Main mechanism of action and key signaling pathways involved	Main Findings	References
Breast cancer cell lines (MCF-7 and MDA-MB-231)	EGCG downregulates MMP-2, MMP-9, EGFR and upregulates TIMP-1 and -2	EGCG $\downarrow$ tumor invasion	63
Breast cancer cell line (MCF-7)	EGCG downregulates EGFR, MMP-2, MMP-9 and EMMPRIN	EGCG $\downarrow$ cellular invasion	31
Breast cancer cell lines (MCF7/DOX and MCF7/DOX500)	EGCG inhibits MMP-2 and MMP-9 activity	EGCG $\downarrow$ cellular invasion	65
Breast cancer cell line (MDA-MB-231)	EGCG derivative 3e reduces the expression of uPA and PAI-1	EGCG derivative 3e $\downarrow$ metastatic behavior (adhesion, invasion and migration)	71
Breast cancer cell line (MCF-7)	EGCG inhibits migration/invasion through downregulation of ErbB2/ErbB3/PI3K/Akt signaling	EGCG $\downarrow$ metastatic process	72
Breast cancer cell line (MCF-7)	EGCG downregulates gelatinase-A (MMP-2) by downregulation of MT1-MMP, VEGF, NF- κ B, FAK	EGCG $\downarrow$ cellular invasion	66
Breast cancer cell line (MDA-MB-231)	EGCG blocks HGF signaling which induces a sustained activation of the MAPK and PI3-kinase pathways	EGCG $\downarrow$ cellular motility	69
Breast cancer cell line (MDA-MB-231)	EGCG represses Wnt signaling by targeting HMG-box protein 1	EGCG $\downarrow$ cellular invasion	68

3.1.6. Inhibition of tumor angiogenesis (Table 6)

Angiogenesis, the induction of new blood vessels, is a processed required for tumor growth and metastasis and has a central role in both mechanisms. Breast cancer cells can regulate angiogenic switch to produce angiogenic factors such as the vascular endothelial growth factor (VEGF). VEGF is the most critical regulator of the development of the vascular system and is overexpressed in a variety of solid tumors including breast cancer [74].

Interestingly, EGCG targets angiogenesis by inhibiting VEGF transcription [75]. One study demonstrated that EGCG

inhibited the activation of Erk-1 and Erk-2, and suppressed the DNA-binding activity of AP-1 to the VEGF promoter [2]. In another study, EGCG demonstrated an anti-angiogenic activity via modulation of VEGF signaling, by inhibition of the phosphorylation of VEGF receptor (VEGFR) [7].

The VEGF-induced angiogenesis signal-pathway is initiated through a multi-component receptor complex composed of VEGF-2, β -catenin, VE cadherin and PI3-kinase. Rodriguez et al. proved that EGCG, in human umbilical vein endothelial cells (HUVECs), inhibited the formation of this receptor complex, resulting in interference with VEGF signaling and inhibition of endothelial cell formation [76]. Furthermore, EGCG itself can also block the binding of VEGF to its receptor, which provides another way to suppress angiogenesis [27].

Angiogenesis is promoted by hypoxia, which causes activation of HIF-1 α and production of oxygen radicals, responsible for activation of NF- κ B pathways. Both pathways increase VEGF expression. Gu et al. concluded that EGCG is capable of inhibiting HIF-1 α and NF- κ B activation in breast tumors, thereby inhibiting tumor proliferation, migration and angiogenesis [74].

One investigation has shown that inhibition of angiogenic fibroblast growth factors is also an important contributor to the antiangiogenic effects of EGCG. More specifically, EGCG was found to decrease the levels of the angiogenic factor bFGF (basic fibroblast growth factor) and aFGF (acidic fibroblast growth factor) in MDA-MB231 cells [77].

Table 6. Effect of EGCG on tumor angiogenesis.

Cellular line	Main mechanism of action and key signaling pathways involved	Main Findings	References
Breast cancer cell line (MDA-MB-231)	EGCG inhibits the constitutive activation of the EGFR, Stat3 and Akt which leads to inhibition of VEGF promoter activity and cellular production of VEGF	EGCG $\downarrow$ cell proliferation and exerts anti-angiogenic activities	27
Human breast cancer cell line (Hs578T)	EGCG inhibits VEGF expression	EGCG $\downarrow$ cellular invasion by $\downarrow$ VEGF expression	75
Breast cancer cell lines (MCF-7 and MDA-MB-231)	EGCG inhibits the activation of HIF-1 α and NF κ B, and VEGF expression	EGCG $\downarrow$ breast tumor angiogenesis and growth	74
Breast cancer cell line (MDA-MB-231)	EGCG inhibits VEGF protein secretion in conditioned media	EGCG $\downarrow$ VEGF transcription	2
Breast cancer cell line (MDA-MB-231)	EGCG decreases the transcription levels of bFGF and aFGF	EGCG $\downarrow$ angiogenic fibroblast growth factors	77

3.2. In Vivo Effects of EGCG on Breast Cancer (Table 7)

In vivo animal experiments are considered as gold standard in chemopreventive studies for EGCG effects on breast cancer prevention and therapy. They give indication on pharmacologic and therapeutic effect of EGCG, which can later be extrapolated to humans [45].

Kaur et al. verified that treatment of C3(1) SV40 T,t antigen transgenic multiple mammary adenocarcinoma (TAg) mice with EGCG delayed carcinogenesis as demonstrated by a significant decrease in the volume and size of tumors [78]. Moreover, they suggested that EGCG affected carcinogenesis through interference with oxidative stress, which plays a key role in carcinogenesis, and through induction of apoptosis. In fact, EGCG decreased the levels of malondialdehyde-DNA, an endogenous product of lipid peroxidation that can induce carcinogenesis through the formation of DNA adduct, in breast tumors, and caused an increase in tumor levels of cleaved caspase-3 protein [78].

In addition, Baliga et al. proved that oral administration of EGCG to 4T1 tumor cells-bearing BALB/c mice resulted in reduction of tumor growth accompanied with an increase in Bax/Bcl-2 ratio, a reduction in proliferating cell nuclear antigen and activation of caspase-3 [45]. Moreover, they observed that oral administration of EGCG to BALB/c mice inhibited metastasis to lungs [45].

Thangapazham et al. revealed that, in nude mice inoculated with MDA-MB-231 breast cancer cells, treatment with EGCG was effective in delaying the tumor incidence as well as reducing the tumor burden. This investigation also described that treatment with EGCG caused induction of apoptosis, by decreasing Bcl-2 and increasing Bax levels, and inhibition of cell proliferation by downregulation of cyclin D and cyclin E, key cell cycle proteins involved in G1/S progression [22].

Gu et al observed that the oral administration of EGCG in the immunocompetent female mice (C57BL/6) allografted with mouse breast cancer (E0771) cells resulted in a VEGF-mediated inhibition of progression of breast cancer growth. A reduction in tumor cross section area and in tumor weight, compared with the control group, was observed [74].

Wei et al. reported that, in Balb/c mice with 4T1 cells subcutaneously injected, EGCG exerts an anti-tumor effect through the inhibition of key enzymes that participate in the glycolytic pathway (hexokinase, phosphofructokinase, lactic dehydrogenase and pyruvate kinase). Furthermore, EGCG decreased the expression of HIF1 α and GLUT1 [35].

In another study, female Sprague–Dawley CD rats exposed to DMBA on day 50 postpartum, and to EGCG throughout life in the drinking water, showed a latency to first tumor development [79]. However, the number of tumors per rat in EGCG-exposed rats, at the dose used, was not significantly different from the controls [79].

Zan et al., using female CB-17 severe combined immunodeficient (SCID) mice inoculated with MCF-7 breast cancer cells, also demonstrated a lower rate of proliferation after treatment with an oral dose of EGCG, through modulation of miR-25 pathway [47].

Tran et al. demonstrated that treatment with EGCG resulted in delayed tumor incidence and reduced tumor size in BALB/c

mice, and that the anti-tumor activity of EGCG is mediated by decreased levels of HSP90 and HSP70 [20]. High levels of HSP's are related with poor therapeutic outcome, suggesting that this inhibition can be therapeutic [20]. In addition, Radhakrishnan et al. showed that treatment with EGCG nanoparticles accomplished improved tumor growth inhibition in C57/BL6 mice [44].

Investigations have also shown that EGCG, like others catechins from green tea, have an antiangiogenic effect in animal xenograft and allograft models, suggesting a possible therapeutic potential. In fact, Sartippour et al. showed that, in female SCID mice, treatment with EGCG suppresses xenograft size and decreases tumor vessel density [80]. Besides, EGCG inhibits breast cancer growth via an antiproliferative effect on endothelial and tumor cells in SCID mice. In this mouse model, EGCG proved to decrease the vessel density in the tumors and researchers observed smaller tumors with fewer blood vessels in the group treated with this catechin [80]. Finally, Mineva et al. established that, in an orthotopic mouse model, EGCG treatment decreases growth of pre-existing tumors derived from ALDH-positive stem-like SUM149 cells and their expression of VEGF-D. This fact is correlated with a decrease in peritumoral lymphatic vessel density [81].

Table 7. In vivo effects of EGCG on breast cancer

Experimental Model	Main mechanism of action and key signaling pathways involved	Main Findings	References
(Tag)mice	EGCG ↓ M1dG levels, which reflect oxidative DNA changes. EGCG induces apoptosis	EGCG ↓ tumor growth	78
Female BALB/c mice	EGCG downregulates HSP70 and HSP90 expression	EGCG delays tumor incidence and ↓ tumor size	20
Female C57BL/6 mice	EGCG downregulates miR-25 expression	EGCG ↓ tumor growth	47
Female BALB/c mice	EGCG inhibits the activity and mRNA levels of the glycolytic enzymes hexokinase, phosphofructokinase, and lactic dehydrogenase and pyruvate kinase. EGCG decreases the expression of HIF-1α and GLUT1	EGCG ↓ tumor growth	35
Female Sprague–Dawley CD rats	Not described	EGCG ↓ latency to first tumor development, although the number of tumors per rat was not significantly different from the controls	79
Female athymic nude mice (NCR-nu/nu)	EGCG downregulates cyclin D and cyclin E	EGCG ↓ cellular growth and ↑ apoptosis EGCG ↓ tumor burden and	22

		delays tumor incidence	
Female BALB/c mice	EGCG increases the Bax/Bcl-2 ratio, reduces proliferating cell nuclear antigen and activates caspase 3	EGCG ↓ tumor growth EGCG ↓ metastasis to lungs	45
Female SCID mice	EGCG has an antiproliferative effect on the tumor cells as well as the endothelial cells	EGCG ↓ tumor volume	80
Female NOD/SCID mice	EGCG downregulates expression of VEGF-D	EGCG ↓ tumor growth	81

DISCUSSION

Breast cancer is a significant health problem, so it is of great importance that new preventive and therapeutic strategies continue to be studied. EGCG has proved, both *in vitro* and *in vivo*, to possess multiple anticarcinogenic activities in various human cancers, including breast cancer [49, 55].

In vivo and *in vitro* studies propose various molecular mechanisms involved in the anticarcinogenic effect of EGCG in relation to breast cancer, which were summarized in this review. Namely, EGCG can inhibit stem cell population, such as CD44⁺ population, and their renewal through Wnt/β-catenin pathway. Moreover, this catechin can modulate various key regulatory genes participating in signaling events involved in self-renewal pathways like angiogenesis (VEGF), cell cycle (cMyc, cyclin D1, p21), signaling (EGFR, Ras, ERK1/ERK2, Nf-kB2, Nrf2), epithelial to mesenchymal transition (E-cadherin) and apoptosis (p53, Bax, Bcl-2, caspase-3 and caspase-9) [11]. Through these mechanisms, EGCG can restrict carcinogenesis progression at early pre-malignant stages from breast cancer initiation and promotion. In addition, EGCG can also target breast cancer resistance to therapy. Firstly, EGCG can modulate P-glycoprotein and breast cancer resistant proteins which are proteins involved in drug efflux, thereby increasing the efficacy of drugs used in chemotherapy. Secondly, this catechin shows the capacity to kill resistant cancer cells through influence in different molecular signaling pathways [64] (Figure 2).

However, much still remains unknown about the molecular mechanisms that play a role in the effect of EGCG effects on mammary carcinogenesis. We next point out some areas for future research.

-Its effects on receptor pathways, growth factor pathways, enzymes and signaling pathways must be better elucidated so that EGCG can be recommended for clinical trials [79].

- EGCG is a very sensitive molecule and it is known that this molecule changes its structure under cell culture conditions by auto-oxidation and dimerization. The factors that influence its molecular stability are pH, temperature and the concentration of the catechin [43, 82]. As a matter of fact, at high concentrations, EGCG change its stereoconformation and its half-life is prolonged. In contrast, at lower concentrations, there is formation of dimers, resulting in molecular instability [83]. The poor stability of EGCG represents a relevant limitation for EGCG's use in humans and to work around this issue new strategies should be investigated.

- A problem in the therapeutic setting of EGCG is its low oral bioavailability. Recent approaches based on the use of micro-particles or nanoparticles (NPs) in which EGCG is encapsulated, have been built to enhance EGCG's bioavailability and stability [84]. The bioactivity, stability and potential side effects of EGCG have been discussed; however, further investigations will be fundamental to improve EGCG oral bioavailability. Moreover, the optimal concentration and administration route of EGCG when functioning in the human body as an anticancer drug should be also investigated [85-87].

- *In vitro* studies that aim to mimic *in vivo* effects in most of cases used EGCG at concentrations higher than 10 μM and even as high as 200 μM , which are unachievable for humans. Indeed, a serum concentration of EGCG <10 μM can be achieved by drinking a couple of cups of green tea or taking a tablet supplement, and the effects of these lower doses have not been well investigated [88]. To investigate how EGCG, at a tolerable and achievable concentration, can be used in treatment of breast cancer patients, studies used a range of 0.1–1 μM . Zenge et al. showed that a EGCG concentration of 0.1–1 μM exerted cancer-selective growth inhibitory and pro-apoptotic effects. Besides, EGCG altered the expression of many key proteins involved in cancer growth and survival [88].

- More studies are necessary to determine the potential toxicity, such as hepatotoxic effects, of EGCG at higher doses [16]. In addition, additional investigations about drug interactions and the influence of EGCG on enzymes such as cytochrome P450 system are crucial [83].

Other catechins present in green tea, such as CG, GC, EC, EGC, ECG, and GCG also suppress the growth of the breast cancer cell line MDA-MB-231[80]. In fact, many of these catechin components appear to be as potent as EGCG. This fact explains why green tea was reported to have the same potency as EGCG (dose per dose), although it contains only half as much EGCG. This conclusion thus supports the theory that natural products exert their positive effects based on their multiple mixed components

In vivo studies demonstrated that treatment with EGCG does not significantly affect angiogenesis, VEGF expression and cell growth in the normal tissues [74]. Breast cancer cells are under greater hypoxia and oxidative stress which, among other factors, justifies the distinct behavior observed. Investigators also proved that there was no significant difference in the body weight and kidney weight between EGCG-treated mice and the control mice.

So, based on *in vivo* evidence, we can conclude that EGCG protects breast cells against carcinogenesis through modulation of multiple targets, suggesting some implications of EGCG in chemoprevention and therapy of breast cancer. In addition, it is important to highlight that EGCG can be a good chemopreventive agent since it can selectively target breast cancer cells with very low toxicity to normal cells. Supplemental investigation to settle EGCG as a powerful inhibitor of cancer progression is thus important.

CONCLUSION

Breast cancer is recognized as a heterogeneous disease and it is known that EGCG, through multi-targeted effects, has the potential to be chemopreventive due to its influence in multiple molecular events and signaling pathways involved in breast carcinogenesis. In this context, the protective action of EGCG against breast cancer via inhibition of proliferation, invasion, angiogenesis, and metastasis is well documented (Figure 2). These findings help validate the value of EGCG as an anti-cancer factor in breast cancer. This protective effect has been shown in different experimental models and under different experimental conditions, suggesting clinical implications of EGCG in the chemoprevention of breast cancer in high-risk women [79]. Studies suggest that the usual consumption of green tea might improve body's cancer preventive machinery. Besides, pure EGCG may be used as therapeutic agent along with conventional therapy for better breast cancer treatment management. The factors which influence the therapeutic benefit are complex in *vivo* and involve steps related to the absorption, digestion, distribution, and metabolism of this drug. In conclusion, more *in vivo* and clinical studies should be executed to confirm the role of EGCG as therapeutic against breast cancer.

Figure 2. Effects of EGCG on breast cancer cells.

AKT: protein kinase B; a FGF and bFGF :acid fibroblast growth factor and basic fibroblast growth factor CDK2 and CDK4: cyclin-dependent kinase 2 and cyclin-dependent kinase 4; DNMT:DNA methyltransferase; EGFR: epidermal growth factor receptor; EMMPRIN: extracellular matrix metalloproteinase inducer; ERK: extracellular signal-regulated kinase; FAS: fatty acid synthase; GLUT1: glucose transporter 1 ; HIF1 α : hypoxia-inducible factor 1 α ; HK: hexokinase; HSP 70 and HSP90: heat shock protein 70 and heat shock protein 90; LDH: lactic dehydrogenase; MMP-2 and MMP-9: membrane matrix metalloproteinase 2 and 9; MT1-MMP:membrane type-1 matrix metalloproteinase; mTOR: mammalian target of rapamycin; PARP: Poly (ADP-ribose) polymerase; PI3K:phosphoinositide-3-kinase; PFK : phosphofructokinase; PKN: pyruvate kinase; PTEN: Phosphatase and tensin homolog; ROS: reactive oxygen species; SAH: S-adenosyl-L-homocysteine; PAI-1: plasminogen activator inhibitor type-1; p21, p27, p53:protein21, protein27 and protein 53; TIMP-1 and TIMP-2: Metalloproteinase Inhibitor 1 and 2; uPA: Urokinase plasminogen activator VEGF: vascular endothelial growth factor;

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Anexos

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Title: (The Title of the Article should be Precise and Brief and Must Not be More Than 120 Characters. Authors should avoid the Use of Non-Standard Abbreviations. The Title Must be Written in Title Case Except for articles, conjunctions and prepositions.)

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Abstract: The abstract of an article should be clear, concise and accurate summary, having no more than 250 words, and including the explicit sub-headings (as in-line or run-in headings in bold). Use of abbreviations should be avoided and the references should not be cited in the abstract. Ideally, each abstract should include the following sub-headings, but these may vary according to requirements of the article.

ARTICLE HISTORY

Received:
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- Background
- Objective
- Method
- Results
- Conclusion

Keywords: Provide 6 to 8 keywords.

1. INTRODUCTION

The Introduction section should include the background and aims of the research in a comprehensive manner.

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Section headings should be numbered sequentially, left aligned and have the first letter capitalized, starting with the introduction. Sub-section headings however, should be in lower-case and italicized with their initials capitalized. They should be numbered as 1.1, 1.2, etc.

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Please provide soft copies of all the materials (main text in MS Word or Tex/LaTeX), figures/illustrations in TIFF, PDF or JPEG, and chemical structures drawn in ChemDraw (CDX)/ISISDraw (TGF) as separate files, while a PDF version of the entire manuscript must also be included, embedded with all the figures/illustrations/tables/chemical structures etc. It is advisable that the document files related to a manuscript submission should always have the name of

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Illustrations may be submitted in the following file formats:

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- Microsoft Word (version 5 and above; figures must be a single page)
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There are a number of software packages available, many of them freeware or shareware, capable of converting to and from different graphics formats, including PNG.

General tools for image conversion include Graphic Converter on the Macintosh, Paint Shop Pro, for Windows, and ImageMagick, available on Macintosh, Windows and UNIX platforms.

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Chemical structures MUST be prepared in ChemDraw/CDX and provided as a separate file.

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Greek symbols and special characters often undergo formatting changes and get corrupted or lost during preparation of a manuscript for publication. To ensure that all special characters used are embedded in the text, these special characters should be inserted as a symbol but should not be a result of any format styling (Symbol font face) otherwise they will be lost during the conversion to PDF/XML.

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All references should be numbered sequentially [in square brackets] in the text and listed in the same numerical order in the reference section. The reference numbers must be finalized and the bibliography must be fully formatted before submission.

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- Data Tables should be submitted in Microsoft Word table format.
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CONCLUSION

A small paragraph summarizing the contents of the article, presenting the final outcome of the research or proposing further study on the subject, may be given at the end of the article under the Conclusion section.

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If the manuscript has an individuals' data, such as personal detail, audio-video material etc., consent should be obtained from that individual. In case of children, consent should be obtained from the parent or the legal guardian.

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Financial contributions and any potential conflict of interest must be clearly acknowledged under the heading 'Conflict of Interest'. Authors must list the source(s) of funding for the study. This should be done for each author.

ACKNOWLEDGEMENTS

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E-citations

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