Original Article

An Overview Of EGCG And Its Potential Effects On Breast Cancer Cells

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Abstract

Emperor Shennong a well-known character in Chinese brewed the first green tea. Green tea has the highest concentration of antioxidants of any tea and, is made from the processed leaf of Camellia Sinensis. This review has two parts, the first part explains an overview of green tea and its major polyphenol Epigallocatechin (EGCG), and the second part explains its therapeutic roles of it in the treatment of breast cancer cells. Green tea is processed and further EGCG is extracted depending on the technique and region. Among other phytochemicals, epigallocatechin is the richest phytochemical present in green tea, and also possesses therapeutic potential in the treatment of various disorders, among which treatment for cancer has been extensively researched. EGCG has various potential molecular targets involved in the processes like apoptosis, metastasis, growth factor, transcription, and cells cycle EGCGhave been postulated for its anticancer effects on breast cell carcinogenesis by inducing cell death via apoptosis, inducing auto phagy, interacting with cellular oxidative stress levels, inhib ting metastasis, and inhibiting tumor angiogenesis.

Keywords: Polyphenol, Metastasis, Tumor, Apoptosis, Autophagy

1. INTRODUCTION

Tea is processed from the leaf of Camellia Sinensis, which is consumed by two-thirds of the world's population. Black (fermented), Green (non-fermented), and oolong tea are the three types of tea (semi-fermented) [1]. In the sovereignty of Monarch Shennong, a legendary sage and well-known character in Chinese agricultural and medical mythology, green tea was first brewed around 2737 BC. Green tea is the most consumed beverage in the world, second only to water is green tea (Camellia sinensis), and regular consumption has long been associated with positive health effects [2]. Green tea is grown in at least 30 different countries worldwide and it contains around 200 components, which include alkaloids like caffeine, minerals, analogs like theanine, polyphenols like catechin and flavonols, vitamins, etc. Polyphenols are the most abundant and active category of chemical components in green tea, accounting for around 40% of the leaf's dry weight. 30–40% of the catechins in an average cup of brewed green tea come from the extract [3]. Green tea contains polyphenols such as 48.6% of epigallocatechin-3-gallate (EGCG), 12.3% of epicatechin gallate (ECG), 4.1% of epigallocatechin (EGC), 4.1% of epigallocatechin (EGC), 4.1% epicatechin (EC), 1.8% of gallocatechin gallate (GCG), 1.8% of gallocatechin (GC), 1.2% catechin, 0.2% gallic acid [4]**.** EGCG is the most prevalent polyphenol in green tea and is an ester of epigallocatechin and gallic acid. In addition to green tea, EGCG may be found in trace levels in plums, pecans, apples, hazelnuts, onions, etc. [5]**.**

Green tea polyphenols have been shown to suppress tumor development and invasion in malignancies such as breast, lung, prostate, liver, and leukemia [6],[7]. In an in vitro study, which used the human MCF-7 and MDA- MB- 231 breast cancer cells, downregulated EGFR and MMP-2 and -9 and upregulated TIMP-1 and -2 and they were involved in signaling pathways like NF-kB/FAK/ERK [8],[9]. It also showed potential effects in an in-vivo study where it suppressed the growth and progress of stomach, lung, esophageal, intestinal, skin, and prostate cancers [10],[11]. Epidemiological studies, albeit inconclusive, show that green tea polyphenols may have cancer-preventive properties. However, the positive effect of tea drinking on cancer prevention or advancement is unclear. High polyphenol intake is required to get adequate serum concentrations. Nonetheless, consistent use of green tea has been linked to a better prognosis in breast cancer patients [12] as well as a possible lower chance of relapse [13].

1.1 Structure of green tea catechins

Flavones, flavanols, and flavonols are the three main families of polyphenols, which are found in significant concentrations in green tea. With the exception of the heterocyclic C-ring, these molecules have the same chemical composition. The C-ring structure of flavones and flavonols is comparable, with double bonds present at one or two sites. The absence of a hydroxyl group at the 3-position in flavones distinguishes the two polyphenols. Flavanols are distinguished from flavonols by the absence of an oxygen group at the C-4 ring's position and the double bond at the 2-3 position. **Figure 1** illustrates the chemical structures of these molecules [14]**.**

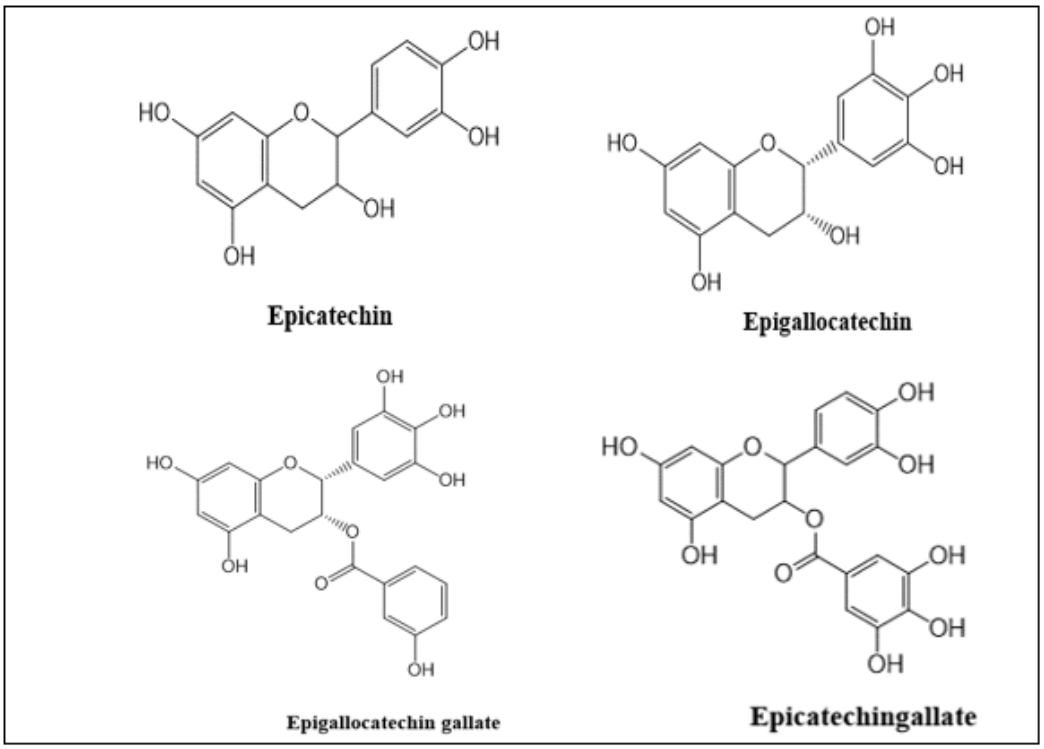

Figure 1: Chemical structures of green tea catechins

1.2 Extraction of EGCG from green tea leaves

1g of green tea leaves dissolved in 10ml of methanol

↓

Vortexed for 30mins and after a while centrifuged at 2000rpm for 15min

↓

Supernatant solution is collected

↓

Supernatant solution is added to 15ml Agilent Dispersive SPE tubes (Pigments Association of Analytical Communities (AOAC) + fat)

↓

20 minutes of vortexing

↓

The tube was centrifuged again (2000rpm, 10min) to get the supernatant - Final solution

↓

A 0.2-micron syringe filter was used to filter the solutions

↓

Identification of EGCG using a UHPLC apparatus

1.3 Molecular targets of EGCG

EGCG and green tea extract demonstrate anti-cancer effects by mediating various mechanisms which include anti-oxidant activity enhancement and detoxification system activation [15], receptor protein kinase (RTKs) and mitogen-activated protein kinase (MAPK) are suppressed [16], epigenetic modifications in gene expression are produced [17], the cell cycle is altered [18] and clonal extension of the tumor-initiating stem cell population is inhibited [19]. In **Figure 2**, the principle molecular targets of EGCG are listed.

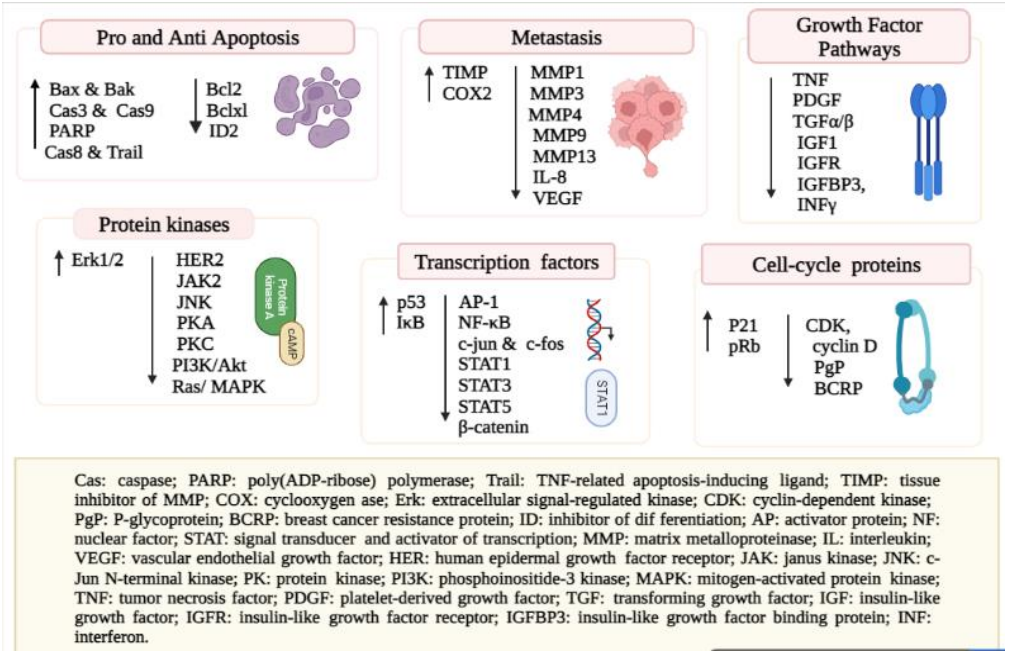

Figure 2: Molecular targets of EGCG

2. BREAST CANCER

The most common malignancy in women is breast cancer. Breast cancer has had the greatest prevalence of all malignancies in women globally for many years. Patients have a greater chance of survival when compared to more lethal malignancies, probably because breast tissue is not a required organ for human existence. Breast cancer is a set of physiologically and molecularly diverse illnesses that originate in the breast. It is a group of distinct cancers that appear in the mammary glands [20]**.** The fifth greatest reason for cancer-related mortality and also one of the maximum diagnosed malignancies nowadays is breast cancer [21]**.** WHO estimates that 685 000 deaths worldwide and 2.3 million new breast cancer diagnoses in females have occurred in the year 2020. By the verge of 2020, seventy-eight lakhs (7.8 million) women had been detected with breast tumor, making it the most prevalent illness in the world. Breast cancer is the type of cancer that results in the most lost disability-adjusted life years (DALYs) for women globally. According to the absence or presence of the molecular markers for progesterone or oestrogen receptors and human EGRF-2, breast cancer is classified into three main subtypes: hormone receptor-positive/ERBB2 negative, triple-negative, and ERBB2 positive [22]**.**

2.1 Metastasis in breast cancer

Around 90% of cancer mortality is caused by metastasis, one of the characteristics of the disease. The migration of cancer cells from their primary tumor to surrounding tissues or other organs is referred to as metastasis [23].

The common metastasis process is as follows:

i. **Detachment of breast cancer cells from the extracellular matrix (ECM) and the onset of local migration and invasion:** The loss of the cell's link to the ECM via cellular adhesion proteins such as integrins causes cancer cells to dissociate from neighbouring cells and the basement membrane. Cells with this improved invasive potential begin to penetrate surrounding tissue by secreting proteolytic enzymes that break down the ECM and offer a channel of invasion.
ii. **Intravasation into the circulatory system:** Cancer cells adhere to the endothelium wall, then invasion and migrate through lymphatic or blood vessel walls.
iii. **Circulation:** Tumour cells migrate to other organs via blood or lymphatic circulation. To survive in an anchorage-independent way, the cells must develop anoikis resistance.
iv. **Arrest, adhesion, and efflux at metastatic sites:** Tumour cells stop their cell cycle and attach to capillary walls inside target organs before extravasating into the location of metastasis.
v. **Metastatic tumor formation:** cancer cells with tumorigenic potential will multiply and form tiny tumors. Because the uncontrolled division of cells is a complicated, multi-step process, metastatic cells require a wide variety of traits to overcome obstacles, including the potential to survive in unattached circumstances, infiltrate, and produce new tumors. Any of these stages that are disrupted will halt cancer's spread. Furthermore, in order to survive, cancer cells must resist the immune response that destroys them and avoid apoptotic signals. Secondary metastases will occur if the tumor cells can complete these processes.

2.2 EGCG's effect on breast cancer cells

EGCG, which has been isolated from green tea leaves, has been postulated for its anticancer effects on breast cell carcinogenesis by inducing cell death via apoptosis, inducing autophagy, interacting with cellular oxidative stress levels, inhibiting metastasis, and inhibiting tumor angiogenesis [24] as illustrated in **Figure 3.**

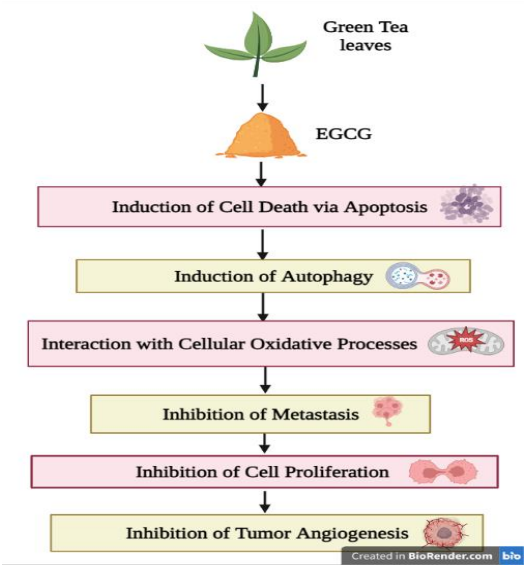

Figure 3: Roles of EGCG in breast cancer cells

2.2.1 Induction of apoptosis

Programmed cell death which means apoptosis, is governed by a collection of genes, in breast cancer cells, EGCG-induced apoptosis might be achieved thru the combined effects on equally intrinsic and extrinsic apoptotic pathways [25],[26]**.** PTEN, caspase-3, Bax, caspase-9, p53, p21, and other pro-apoptotic genes are elevated by EGCG. Furthermore, caspase activation activates the cleavage of poly (ADP-ribose) polymerase (PRAP), suggesting these processes are important during apoptosis. At the protein level, EGCG has been found to enhance CASP3, PARP, and CASP9. By activating p53, and stabilizing and elevating Bax, EGCG also stimulates the intrinsic apoptotic pathway in a p53-dependent manner [27]**.**

Furthermore, EGCG promotes cell death in the breast cancer cell lines 4T1, T47D MDA-MB-231, and Michigan Cancer Foundation-7 by decreasing the expression of anti-apoptotic proteins such as BcL-xL, survivin, and Bcl-2, as well as inhibiting the NF-kB, PI3K/Akt, & JAK/STAT pathways. Survivin, a member of the inhibitor of apoptosis (IAP) family of proteins, is suppressed when PI3K/Akt activation is inhibited, which is followed by increased caspase-9 activity [28],[29]**.** A further study discovered that EGCG promoted MCF7 cell death via altering EGFR signaling, the anti-laminin receptor, intracellular Ca2+, osteopontin secretion, gelsolin, tropomyosin-1, tropomyosin-4, and adherent junction proteins such as catenin and E-cadherin [30]. Inhibiting Bcl-2 results in an increase in Ca2+ leakage from the endoplasmic reticulum. Finally, in the MCF-7 breast cancer cell line, EGCG lowers cytosolic Ca2+ and increases endoplasmic reticulum Ca2+ [31].

2.2.2 Induction of autophagy

The response to anti-cancer treatment is frequently influenced by autophagy, a lysosomal catabolic mechanism of self-degradation and recycling of cellular macromolecules and organelles. The potential of EGCG to promote autophagy has been examined in the research. To assess the impact of EGCG on the autophagy process, researchers have utilized a variety of complementary tests. These included:(1) determining how autophagosomes form using confocal microscopy;(2) identifying the essential proteins for autophagy development; and (3) using transmission electron microscopy to directly assess how autophagosomes form and fuse with lysosomes [27]. In a study, it was observed that EGCG significantly increased the amount of the essential autophagy proteins Beclin1, ATG5, and LC3B in breast cancer cells. Beclin1 is an essential protein for the development of autophagosomes and is crucial for the localization of autophagic proteins to create the pre-autophagosomal structure (PAS) [32]**.** ATG5 binds with ATG12 to target autophagosome vesicles and is crucial for the autophagosome's membrane development [33],[34]**.** Organelles and LC3B-II are broken down by lysosomal hydrolases during the integration of the autophagosome with the lysosome [35].

2.2.3 Interaction with oxidative stress levels in cells

EGCG reduces a variety of pro-inflammatory cytokines, protein carbonylation, lipid peroxidation, C-reactive protein, plasma low-density lipoproteins, plasma hydrogen peroxide, and oxidative DNA damage [24],[36],[37]. Additionally, it raises levels of a few detoxifying enzymes, including glutathione S-transferase (GST), lipoxygenase (LOX), and xanthine oxidase [36]. Studies have demonstrated that EGCG has anticarcinogenic properties that protect DNA from Reactive oxygen species-induced deterioration by reducing Reactive Oxygen Species stress levels [38]**.** Catechins reduce Reactive Oxygen Species stress by increasing the activity of anti-oxidants like catalase, superoxide dismutase (SOD), and glutathione peroxidase (GPx), which straight scavenge Reactive Oxygen Species; and by chelating ferrous iron, they also stop the Fe-induced production of hydroxyl free radicals through the Fenton and Haber-Weiss reactions [39]. Finally, Tran et al. showed that EGCG administration made the MCF-7 breast cancer cell line more vulnerable to oxidative stress. This was achieved by preventing HSP90, and HSP70 which are produced in response to heat shock or oxidative stress, from acting as promoters [40].

2.2.4 Inhibition of metastasis

90% of deaths from solid tumors in cancer patients are due to metastasis, which is the main cause of mortality in this population [41]. EGCG reduces metastasis, by regulating proteolytic enzyme activity, growth factors, receptors, signal pathways, and the epithelial-to-mesenchymal transition procedure [42]. The behavior of tumor invasion and metastasis largely depends on the degradation of proteolytic enzymes and extracellular matrix components by matrix metalloproteinases [43]. A study revealed that when 4T1 cells were treated with EGCG, it led to mitochondrial disruption and a decrease in the expression of Bcl-2 which further resulted in the release of cytochrome C and upregulation of Apaf-1 [44]. The functioning of the anti-metastatic and tumor-suppressive actions of EGCG is due to the involvement of signaling cascade like NO/ PKC δ/Akt / e NOS/ cGMP [45]. EGCG also suppresses the effects of protein tyrosine kinase (PTKs) which includes FGFR, PDGFR, EGFR, HER2 tyrosine kinases, and Protein Kinase B (PKB) also known as Akt through various signaling pathways like NF-kB, STAT3, mTOR, P13K [46],[47]. EGCG also suppresses the activity and expression of MMP- 2 and -9 and this may be the main reason for its anti-metastatic action[48],[49]. The inhibition of Matrix metalloproteinases (MMPs) includes the induction of Tissue inhibitors of metalloproteinases (TIMPs -3) levels by suppressing the enhancing activity of class 1 histone deacetylase (HDACs) and zeste homolog 2 (EZH2) [50]. In addition, by decreasing the TAM infiltration, EGCG can modulate tumor microenvironment [51].

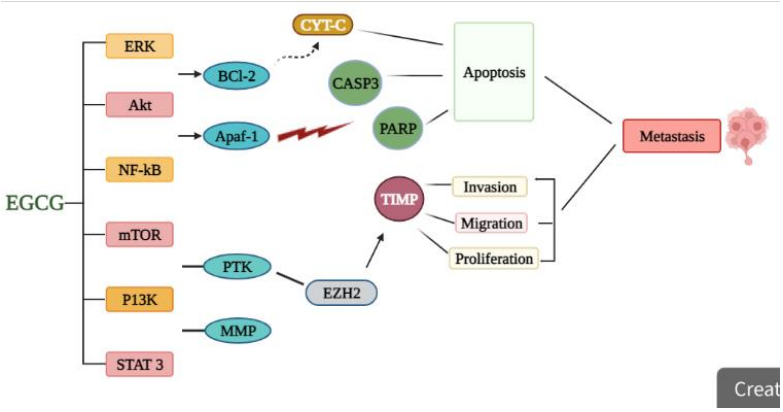

Figure 4: Mechanism of inhibition of metastasis

2.2.5 Inhibition of cell proliferation

In breast cancer cells, EGCG displays pleiotropic effects similar to those of other polyphenolic substances. It activates and inhibits a number of signaling pathways, impacting a variety of cellular processes including cell division and proliferation. It's significant that EGCG may specifically decrease cell growth without harming healthy cells [44]. The effect of EGCG in preventing cell proliferation in breast cancer cells is due to an epigenetic mechanism. In fact, research has shown that EGCG prevents breast cancer cells from growing by undoing DNA methylation-mediated suppression of tumor suppressor genes. The catechol group in EGCG's structure is crucial in preventing the action of the DNA methyltransferases: DNMT-3a, DNMT-1, and DNMT-3b. Therefore, EGCG-induced epigenetic changes may play a significant role in limiting the carcinogenesis of breast cancer [40]. The significant roles of EGCG to inhibit cell proliferation are illustrated in **Figure 5.**

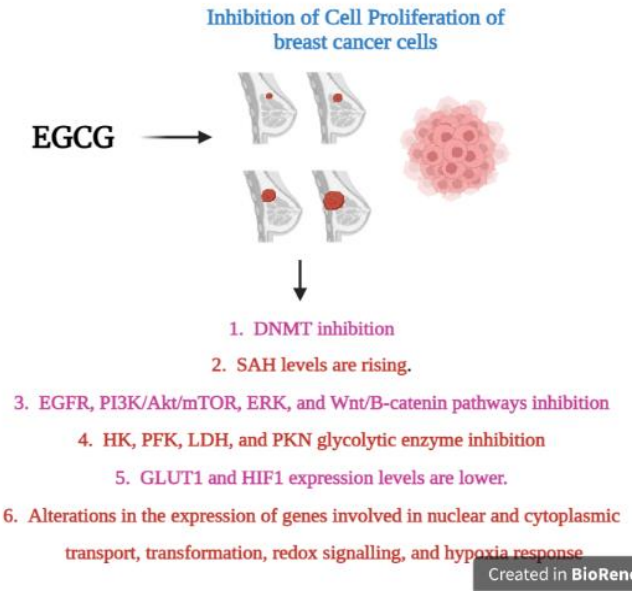

Figure 5: Effect of EGCG in inhibiting cell proliferation

2.2.6 Inhibition of tumor angiogenesis

Angiogenesis is a crucial process for the growth of tumors and can give them oxygen and nutrients [52]. Tea catechins, particularly EGCG, have strong antiangiogenic effects and reduce the incidence of breast cancer [53]. The most important regulator of the vascular system's development is VEGF, which is overexpressed in a number of solid tumors, including breast cancer , and by preventing VEGF transcription, EGCG targets angiogenesis [54]. A multi-component receptor complex consisting of b-catenin, Vascular Endothelial growth factor-2, PI3-kinase, and VE-cadherin initiates the VEGF-induced angiogenesis signal pathway. The development of the multi-component receptor complex was inhibited by EGCG, interfering with VEGF signaling and inhibiting endothelial cell formation [55].

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