EGCG may combat Breast Cancer Through Oxidative Stress Inhibition, Tumor Suppressor Gene Reactivation, and Cancer Pathway Regulation

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Received September 02, 2024
Accepted October 15, 2024
Electronic access October 31, 2024

Breast cancer is one of the leading cancers affecting women around the globe. EGCG, the primary catechin in green tea, may serve as a promising candidate for breast cancer treatment due to its antioxidant and anti-inflammatory properties. The objective of this review paper is to present EGCG's beneficial properties and determine if it could be leveraged towards breast cancer treatment. To this end, scholarly articles from 2010 to the present were gathered from databases Google Scholar and PubMed. Each article was analyzed and key information such as experimental findings and current research gaps were extracted. This paper uncovered that through inhibiting reactive oxygen species generation, suppressing angiogenesis, reactivating tumor suppressor genes, and modulating oncogenic pathways, EGCG successfully targets and addresses the core mechanisms of breast cancer. However, further research is required to fully understand the therapeutic potential of EGCG, including how other foods can affect its bioavailability, molecular mechanisms behind its interactions with other biological molecules, and its toxicity and efficacy in clinical settings. In addition to summarizing the most recent information on EGCG, this review contributes to existing literature by exploring the compound's role in overcoming resistance to breast cancer therapies, highlighting gaps in current studies, and providing future directions to enhance EGCG's efficacy. The paper provides a comprehensive review of the various mechanisms through which EGCG can exhibit its anticancer effects.

Introduction

Among the various types of cancers, breast cancer has the highest incidence in women, and it is estimated that 1 in 8 women in the U.S. will develop invasive breast cancer (a type of breast cancer in which cancer cells have spread beyond the breast to other organs)[1,2]. Breast cancer is characterized into several main subtypes, including estrogen receptor (ER) positive, ER-negative, human epidermal growth factor receptor 2(HER2) positive, and triple-negative breast cancer (TNBC). Key risk factors include mutations in essential apoptotic pathways, genetic mutations, malfunctions of the immune system, and high levels of cholesterol and estrogen exposure[1]. Current treatment options including conventional surgery, radiotherapy, chemotherapy, endocrine therapy, immunotherapy, cancer stem cell therapy, and targeted drug delivery have exhibited severe side effects, which highlights the need to explore less invasive therapeutic options[3].

One such potential treatment to consider is epigallocatechin gallate (EGCG), the most abundant catechin found in green tea. It is known for its anti-cancer, anti-inflammatory, antioxidant, and other biological properties, with fewer side effects compared to other breast cancer therapies[4]. EGCG's relevance to breast cancer lies in its ability to regulate oncogenic pathways and its potential to target aggressive breast cancer subtypes including ER-negative and TNBC, which lack effective therapeutic options compared to other subtypes.

This review introduces EGCG's potential therapeutic effects by focusing on its ability to inhibit oxidative stress, reactivating tumor suppressor genes, and regulating crucial signaling pathways involved in breast cancer.

Methods

This systematic review process adheres to PRISMA guidelines. Scholarly articles from 2010 to the present retrieved from databases including Google Scholar and PubMed were used. Eight key phrases were utilized in searching the literature: (a) Properties of EGCG, (b) Breast cancer, (c) EGCG and cancer, (d) Bioavailability of EGCG, (e) EGCG and breast cancer, (f) Breast cancer treatment, (g) EGCG, and (h) Cancer review. Specific information including what essential breast cancer pathways EGCG is involved in, how EGCG interacts with the molecules involved in these pathways, and what properties EGCG exhibits were searched for to analyze EGCG's role as a potential breast cancer therapeutic. To make connections in the literature reviewed, information from the various sources as indicated above were collected. Identified gaps in the research were filled in by combining information from multiple articles.

Subsequently, common themes, as well as the established mechanisms of action of EGCG were used as a foundation to generate inferences to answer the question of its potential as a breast cancer therapeutic.

Most cited articles are systematic reviews and only a small portion of primary research articles are included. Thus, the review process did not use any effect measures, and this might be a potential bias of the paper. Of the primary research articles, most of the research conducted used double blind, placebo, controlled, and randomized trials, which are the most effective research methods that prevents underlying biases. However, selection bias and attrition bias might still exist. For instance, the participant recruitment process could include inherent biases such as the exclusion or the inclusion of individuals with specific health conditions. Participants dropping out of the study can potentially affect the results and the outcomes as well.

Cancer Overview

Cancer Stages

As one of the most lethal diseases worldwide, cancer is caused by the abnormal growth of cells. This eventually results in the formation of tumors that can invade and destroy other body tissues. Cancerous cells arise from mutations in the DNA, malfunctions of mitotic control regulators and pathways, and failures of apoptosis. Many different stages exist in cancer with increasing malignancy. At stage 0, also known as in situ and localized, abnormal cells are present, but they have not spread to nearby tissues. At this point, it is unsure if these abnormal cells will develop into cancerous cells. The following stages include stages I, II, and III, also known as regional. In these stages, cancerous cells already exist, have grown in number, and large tumors have already formed and started to invade surrounding tissues. The last cancer stage is stage IV, also known as distant, in which tumors have already spread to distant parts of the body[5].

Conventional Cancer Treatments

A few different treatments for cancer exist, and they can be categorized as conventional treatment and emerging, modern treatment groups. The most utilized conventional cancer treatment is surgery along with radiotherapy or chemotherapy. Surgery treatment directly removes cancer tumors, but this treatment is the most effective only at early stages of cancer before the tumor spreads to distant parts of the body. Radiotherapy utilizes high energy radiation to kill cancer tumors. However, due to the large surface area that the radiation covers, radiotherapy has severe side effects including damaging healthy cells, tissues, and organs. Like radiotherapy, utilizing chemotherapy contains the risk of damaging growing healthy cells and tissues. The major limitation of chemotherapy, however, is drug resistance. Debela et al. found that cancer cells originally controlled by anti-cancer drugs can develop an immunity to the drugs[6].

Modern/Emerging Cancer Treatments

Emerging therapies, on the other hand, include stem cell therapy, targeted drug delivery, ablation cancer therapy, gene therapy, and natural antioxidants. Stem cell therapy utilizes undifferentiated cells in the bone marrow (BM) that can differentiate into various cell types. By utilizing the undifferentiated BM cells, stem cell therapy can replace the damaged healthy cells that resulted from chemotherapy. Mesenchymal stem cells (MSCs) are currently being tested in clinical trials. Even though there is success in stem cell therapy, challenges such as therapeutic dose control, low cell targeting, and retention in tumor sites exist. Limitations of stem cell therapy lie in its side effects, which include tumorigenesis, drug toxicity and resistance, and viral infection. Targeted drug delivery utilizes drugs and substances that interfere with growth molecules. This leads to blocking the growth and spread of cancer. One such drug is apoptosis-inducing drugs. They target parts of the cell that control whether cells live or die, or in other words, prevent the tumors from making new blood vessels. Consequently, when this occurs, it helps to cut off the tumor's blood supply. Compared to chemotherapy, the targeted drug delivery method is more specific and effective because it offers treatment in cell cycle arrest, apoptosis induction, and proliferation prevention. However, small nanoparticles and drugs may not reach target tumors successfully due to the complications of the blood vessels. At the same time, side effects of targeted drug delivery exist[6].

Ablation cancer therapy utilizes thermal ablation, including hyperthermia and hypothermia to destroy tumor tissue. Compared to surgery, ablation cancer therapy is an improvement because it is done through injections and a non-invasive approach. Radiofrequency, microwave, high intensity focused ultrasound, ablation, and cryoablation are currently being used in clinical settings. All of these treatments use hyperthermia except for cryoablation. However, the limitation of this treatment is that it is difficult to destroy tumors near major blood vessels or organs because normal, healthy tissues can be damaged. Gene therapy is the insertion of a normal copy of a defective gene, which can replace oncogenes (a mutated gene that fails to regulate mitosis and thus promotes tumor growth) and mutated tumor suppressor genes. There are approximately 2900 gene therapies under clinical trials and two thirds of the therapies are related to cancer. The challenge of gene therapy consists of unsuccessful genome integration, limited efficacy, and high chances of being neutralized by the immune system. Natural antioxidants have been utilized to treat cancer because of the molecules' ability to prevent oxidative stress by inhibiting radical oxygen species (ROS). ROS are molecules that are constantly produced by metabolizing organelles including the mitochondria, peroxisomes,

and the endoplasmic reticulum. ROS can change the regulation of transcription factors by damaging the DNA and other macromolecules such as antioxidant enzymes and lipids. Damaged antioxidant enzymes may disrupt anti-inflammatory pathways and lead to inflammation, and the degradation of lipids will lead to the breakdown of cellular membranes and lipid chains. Compounds such as vitamins have antioxidant effects and this treatment method is currently under clinical trials. The major limitation of utilizing natural antioxidants is their limited bioavailability[6].

Breast Cancer Overview

Breast cancer is one of the primary causes of mortality in women around the globe[1]. It is characterized by the formation and growth of cancerous cells around the tissues of the mammary glands. Breast cancer is characterized into several subtypes, including hormone receptor (ER) positive, ER-negative, human epidermal growth factor receptor 2(HER2) positive, and triple-negative breast cancer (TNBC). It has the highest incidence of all cancers in women, and it is estimated that 1 in 8 women in the U.S. will develop invasive breast cancer. In 2018, over 260,000 new cases of invasive breast cancer were diagnosed, with more than 3.1 million women in the U.S. having a history of breast cancer[7]. The primary risk factors of breast cancer include mutations in essential apoptotic pathways, genetic mutations, malfunctions of the immune system, and high levels of cholesterol and estrogen and carcinogen exposure[1]. Of the genetic mutations, mutations in the BRCA1 or BRCA2 genes carry the firmest relationship with breast cancer. BRCA1 is considered to be the cause of 5-10% of breast cancer cases[2].

Breast Cancer Stages

There are different stages of breast cancer as is true for cancer in general. These include stage 0, 1, 2, 3, and 4. At stage 0, the tumor is growing, but it remains non-invasive. It is at stage 1 where the tumor starts its invasive process. There are two subsets of stage 1, stage 1A and 1B. Stage 1A includes tumors that measure up to approximately 2 centimeters wide with no lymph nodes involved. Stage 1B describes smaller tumors larger than 0.2 millimeters that are found in the lymph node. Stage 2 also contains substages 2A and 2B. At stage 2A, the tumor is found in the axillary lymph node but not in the breast. At this stage, cells are no larger than 5cm. At stage 2B, tumor cells are larger than 5cm, but they fail to reach axillary lymph nodes. Stage 3 contains substages 3A, 3B, and 3C. At stage 3A, tumors can be found in 4-9 axillary nodes. At stage 3B, tumors of any size have caused swelling or ulcers on the skin of the breast, which is the condition in inflammatory breast cancer. At stage 3C, tumors have spread to up to 10 or more auxiliary nodes and nodes below and above the clavicle. The last stage of breast cancer is stage 4. At this stage, tumors have spread to other locations of the body[2].

Breast Cancer Treatments

Similar to cancer in general, the most utilized treatments for breast cancer include surgery, radiotherapy, chemotherapy, and endocrine therapy[2,3]. Surgery approaches include mastectomy (total excision of the breast), lumpectomy (excision of the breast tumor with a margin of surrounding normal tissue), and axillary lymph node dissection (ALND). Studies have shown that mastectomy combined with lumpectomy can improve overall survival. However, the limitations of the three surgery types include the presence of diffuse microcalcifications (calcium deposits within the breast tissue that can re-develop into tumors, if malignant)[3]. Radiotherapy utilizes high energy radiations that are applied to the whole chest, breast, and regional lymph nodes. A meta-analysis demonstrated that radiotherapy following surgery offered more benefits to patients with higher-risk breast cancer[8]. The major side effect of radiotherapy is cardiotoxicity, which is difficult to avoid because radiation can cover heart and lung locations while treating the chest. Moreover, advanced invasive breast cancer can develop radiotherapy resistance due to oxygen deprivation of tumor cells. The lack of oxygen can lead to cell proliferation and radiotherapy and apoptosis resistance. A major protein involved in this resistance is hypoxia-inducible factor 1 alpha (HIF-1α). Chemotherapy, on the other hand, utilizes taxanes, anthracyclines and cyclophosphamide (chemotherapeutic agents) to target and kill tumor cells. Similar to radiotherapy, chemotherapy results in severe side effects. Early side effects include fatigue, alopecia (hair loss), cytopenia (low amounts of red blood cells), muscle pain, neurocognitive dysfunctions, and chemo-induced peripheral neuropathy (damaged peripheral nerves due to chemotherapy). Late side effects include cardiomyopathy (disorders that affect the heart muscle), secondary cancers (such as myelodysplastic syndrome), early menopause, sterility, and psychosocial impacts[9].

Endocrine therapy is the most effective in treating ER-positive invasive breast cancer. The purpose of this therapy is to target estrogen receptor modulators and regulators that lead to estrogen stimulation by either inhibiting or degrading them. Examples of competitive inhibitors that compete with estrogen to bind to estrogen receptors include tamoxifen, toremifene, bazedoxifene, and raloxifene[3]. The major side effect of endocrine therapy is resistance. Micro-RNAs (miRNAs) such as miR-155 and miR-221/222 have been found to be involved in resistance mechanisms. miR-155 targets SOC6 (inhibitor cytokine signaling) and stimulates the activation of STAT3 signaling pathway. STAT3 is involved in cell survival and resistance to the competitive inhibitor tamoxifen. miR-221/222 regulate various oncogenic pathways, and their high expressions were found to be related to tamoxifen resistance as well[9]. Even though breast cancer

treatments exist, they are lacking in efficacy mainly due to their severe side effects. Herein, this current paper proposes EGCG (a natural product (NP)) as a potential therapeutic for breast cancer as it is less toxic compared to traditional treatment methods and harbors a variety of properties that can support anticancer activity and overcome therapy resistance [10].

EGCG as A Potential Therapeutic

Metabolization Process and Molecular Overview of EGCG

EGCG is the most prevalent catechin and is mainly responsible for the bioactive properties of green tea. EGCG has attracted attention due to its antioxidant, anti-inflammatory, anti-cancer, and other biological properties. One cup of green tea contains approximately 177 mg of EGCG, but this amount varies depending on tea brewing preparation methods including brewing time and the type of green tea [10]. After oral consumption, EGCG is metabolized into biologically active substances via methylation (catalyzed by catechol-O-methyltransferase (COMT) and methylated to form 4-O-methyl-EGCG, 4-Omethyl-EGCG and 4-4-di-O-methyl-EGCG metabolites), glucuronidation (conjugation reaction where glucuronic acid is covalently linked to a substrate containing a nucleophilic functional group), and microbial metabolization [11]. Studies assessing absorption, distribution, metabolism, and excretion in rats and dogs have shown that EGCG is rapidly absorbed by the intestinal system, distributed to various tissues (including the liver, kidneys, brain, and lungs), metabolized in the liver, and excreted through biliary and urinary pathways [4]. EGCG can be found in the form of an odorless white powder or crystal. EGCG's health-promoting abilities are attributed to its structure [4]. It is composed of three aromatic rings: A, B, and D linked together by a pyran ring (C). EGCG's ability to interact with other biological molecules is due to the hydroxyl groups on its rings, and its antioxidant property is achieved as the hydroxyl groups form hydrogen bonds or donate electrons or hydrogen atoms to other molecules. EGCG can enter cells including cancer cells because it has a strong affinity with the lipid bilayer [12].

Antioxidant and Anti-inflammatory Properties of EGCG: Molecular Mechanisms

EGCG's antioxidant property can be attributed to its ability to scavenge ROS and to inhibit superoxide-forming enzymes based on its role as a flavanol. As previously mentioned, ROS can activate signaling pathways that inflict DNA damage. Thus, ROS can lead to the emergence of malignant cells and the growth of cancer cells. EGCG reduces ROS by donating a hydrogen atom and an electron from its hydroxyl group on ring B and the 2,3 double bonds conjugated with the 4-oxo group. This results in a relatively stable flavonoid radical (a class of polyphenolic compounds found in plants that have an essential role in preventing oxidative stress) that can inhibit superoxide-forming enzymes such as xanthine oxidase (XO), cyclooxygenase 2 (COX2), and NADPH oxidase (NOX). The overexpression of these enzymes can lead to cancer progression. EGCG exhibits its anticancer property by inhibiting the expression and activity of these enzymes. For instance, it acts as both a competitive and a non-competitive inhibitor of XO. Its hydroxyl groups in position 5 and 7 on ring A plays the most important role in its inhibition of XO by interacting with amino acids near the active site of the enzyme via hydrogen bonding. As for its inhibition of COX2, EGCG acts by suppressing its transcription and modulating its signal transduction. EGCG exhibits its anti-inflammatory property by blocking the activation of nuclear factor kappa B (NF-B) (an inflammatory protein) to suppress COX2 expression. This results in the inhibition of the activity of the COX2 promoter, which leads to decreased COX2 mRNA and protein expression. EGCG can also inhibit COX2 transcription by suppressing transforming growth factor alpha (TGFα), which drives the COX2 promoter. Finally, EGCG inhibits NOX activity by disrupting the assembly of the multiprotein complex that is essential for NOX's full enzymatic activity through its 4' hydroxyl group on ring B [13]. During the pathophysiology of breast cancer, there is an upregulation in the production of ROS species, which will increase the secretion of inflammatory markers. EGCG may exert therapeutic effects for breast cancer by reducing these key markers mentioned above.

Interactions of Superoxide Enzymes in Breast Cancer

The superoxide enzymes previously mentioned are heavily involved in breast cancer progression. In a study that examined low-density lipoprotein (LDL) induced breast cancer, XO inhibitor febuxostat (FBX) was found to significantly decrease LDL induced breast cancer cell migration *in vitro*, due to the fact that ROS production was inhibited [14]. Because EGCG is also an inhibitor of XO, it could demonstrate a similar inhibitory mechanism for LDL induced breast cancer cells such as that of FBX. EGCG's ability to inhibit COX2 expression can be leveraged towards breast cancer treatment. Recently, it has been discovered that elevated COX2 expression levels strongly correlate with reduced survival ER-negative breast cancer. Compared to ER-positive breast cancer, ER-negative breast cancer is one of the most challenging malignancies due to restricted treatment options [15]. Since EGCG is capable of inhibiting COX2 by blocking NF-B, this mechanism should be further explored in additional *in vitro* studies and animal models to solidify its potential as a treatment option for ER-negative breast cancer. NOX enzymes generate ROS as their main function, and they are involved in virtually all steps of carcinogenesis, including initiation, promotion, and progression19. High expression of NOX2 mRNA levels were found in claudin low (breast can-

cer subtype with poor prognosis and therapy resistance) and ER-negative breast cancer subtypes. In general breast cancer cells, NOX2 was found in lipid rafts (LR); when the LR were destroyed, the activity of NOX2 decreased, which indicates that NOX2 performs its superoxide activity in LR[16]. EGCG can serve as a potential treatment for claudin low breast cancer since it can decrease NOX2 mRNA levels by suppressing TGFα. It can also disrupt the multiprotein complex that is essential for NOX's full enzymatic activity. Due to EGCG's ability to inhibit superoxide enzymes, it can be a potential therapeutic for breast cancer including claudin low and ER-negative breast cancer subtypes.

Current Use of EGCG

The habit of drinking green tea dates back several thousands of years, and green tea is one of the popular ancient medicines that can improve diets[17,18]. Dried green tea leaves serve as medicine used to relieve fatigue, stress, and weakness[19]. In addition, green tea extract is used to treat genital and anal warts in Europe and the U.S.[19]. The practice of consuming green tea is still widely popular today, especially in countries such as China and Japan. The medical benefits of tea catechins including EGCG have influenced the argument that green tea may harbor therapeutic potential in obesity and cancer due to its anti-inflammatory and antioxidant properties[20]. Green tea is currently being used as food and dietary supplements and drink beverages. In Japan, green tea has already been recognized as a cancer treatment option. However, its role as a cancer therapeutic is still in the developing process in the United States and its anticancer roles are still widely unexposed to the public[21]. It has been found that when green tea is combined with other anticancer drugs, it results in stronger antitumor activity[21]. When combined with curcumin (a molecule in turmeric), EGCG inhibited the growth of oral epithelial cells and estrogen receptor alpha (ERα) breast cancer cells. EGCG was also found to induce apoptosis in PC-9 cells (lung cancer cell line) and MCF-7 cells (breast cancer cell line) when combined with tamoxifen (anticancer drug)[21]. This demonstrates EGCG's ability to treat both oral, lung, and breast cancer; it also interacts well with other anticancer drugs, which can effectively reverse anticarcinogenic activity of existing anticancer drugs. These current uses of green tea have demonstrated EGCG's potential for treating cancer.

EGCG and Breast Cancer

Epigenetic Mechanisms of EGCG

EGCG's anticancer properties in breast cancer are not limited to its ability to inhibit superoxide enzymes, as previously mentioned. EGCG can also decrease breast cancer progression through epigenetic mechanisms. Its main function in altering epigenetic mechanisms involves inhibiting DNA methyltransferase (DNMT), an enzyme that adds a methyl group to the DNA and thus leads to decreased transcription[22]. DNA methylation can lead to the progression of breast cancer due to the fact that this process can inhibit essential tumor suppressor genes. Major genes involved in breast cancer that EGCG regulates through inhibiting gene expression include human telomerase reverse transcriptase (hTERT), signal peptide-CUB-EGF domain-containing protein 2 (SCUBE2), miR221/222, miR25, and estrogen receptor 1 (ESR1).

hTERT is expressed in a majority of breast cancer cells, and EGCG is found to reduce MCF-7 breast cancer cells by inhibiting the transcription of hTERT. The mechanism behind how EGCG inhibits hTERT transcription involves primary inhibition of DNMT and histone acetyltransferases (HATs)[23]. This inhibition leads to E2F-1 (hTERT repressor) binding to the promoter region of the hTERT gene, which suppresses hTERT transcription[23]. EGCG's ability to inhibit DNMT also explains its interaction with SCUBE2, a tumor suppressor gene. Because DNA methylation inhibits the transcription of SCUBE2, this results in an inability of the gene to successfully suppress tumor growth. EGCG can reactivate SCUBE2 expression by inhibiting DNMT and thus preventing its methylation, which then leads to breast cancer tumor suppression.

In triple negative breast cancer, high expressions of miR221/222 were found[24]. These are microRNAs that can act as oncomiR (microRNAs that act as oncogenes) that promote breast cancer cell proliferation. In a study that examined EGCG's effect on various breast cancer cell lines, EGCG was found to decrease proliferation in the following cell lines: MCF-7, MDA-MB-157, MDA-MB-231, and HCC1806[24]. EGCG significantly decreased miR221/222 expressions in MDA-MB-157 and HCC1806 cell lines and it also enhanced p27 protein levels. p27 is a kinase inhibitory protein that arrests the G1 phase of the cell cycle, and it is also one of the targets of miR221/222[25]. Once miR221/222 is activated, they target the 3' untranslated region (UTR) of p27 mRNA and as a result, p27 expression is inhibited[26]. Inhibition of p27 can lead to uncontrolled regulation of the cell cycle and thus contributing to breast tumor proliferation. EGCG can reactivate p27 through stimulating the activity of its transcription factors (FoxO) and thus allow p27 to exhibit its function as a breast cancer tumor suppressor[24,25]. Another microRNA that has been implicated in breast cancer progression is miR25. miR25, similar to miRNA221/222, is an oncomiR that exhibits increased expression in MCF-7 human breast cancer cells[27,28]. EGCG is capable of inhibiting miR25 expression through potentially epigenetic mechanisms. The specific underlying mechanism of how EGCG can reduce miR25 expression remains unclear. The downregulation of miR25 can promote MCF-7 breast cancer cell apoptosis because BTG2 (tumor suppressor gene) is no longer suppressed by miR25[27]. The apoptosis rate increased up to 43% when MCF-7 cells were

treated with EGCG at 20 g/ml[28]. Because EGCG treatment of breast cancer cells lead to apoptosis, the breast tumors will be killed and their proliferation will be inhibited, leading to decreased breast cancer symptoms.

EGCG can also inhibit ESR1, which is a gene that codes for ERα[29]. ERα is a subtype of estrogen receptor, and it is largely expressed in ER positive breast cancer. Because ERα is present, estrogen can bind to ERα and promote breast cancer cell proliferation[30]. EGCG is capable of down regulating the ESR1 promoter region between -2769bp to -1000 bp[30]. On the other hand, ER negative breast cancers lack ERα because the promoter region of ESR1 is methylated. This makes hormonal therapy ineffective for ER negative breast cancer because there are no available receptors that the treatment hormones can bind to. However, EGCG can reactivate the methylated ESR1. By inhibiting DNMT and promoting acetyl-H3, acetyl-H3K9, and acetyl-H4 (histone acetylation activators), EGCG can reactivate the expression of ESR1[29]. This can increase ERα levels, which allows hormonal therapy to be more effective in treating ER- breast cancers. Through these epigenetic mechanisms, EGCG can serve as a strong candidate for breast cancer treatment options.

EGCG's Involvement in Cancer Pathway Regulation

Other than its involvement in epigenetic mechanisms, EGCG also participates in many cancer related pathways including phosphoinositide 3-kinase/protein kinase B (PI3k/AKT), vascular endothelial growth factor (VEGF), MAPK, and EGFR. In the context of breast cancer, these pathways can contribute to its pathophysiology when they become dysregulated through the mutation of key genes. Abnormal regulation such as hyperactivation of the PI3k/AKT pathway is induced by mutations and increase resistance to apoptosis and over-multiplication of cells, which contributes to tumor formation and is the hallmark of breast cancer progression[31,32]. In the PI3K pathway, the frequently mutated gene is PIK3CA, which codes for the p110α (a subunit of the PI3k enzyme)[32].VEGF is a signaling molecule that controls angiogenesis (growth of new blood vessels), which can aid cancer cells in growth since these blood vessels serve as a means of transporting nutrients and oxygen[33]. High expression of mutated EGFR and MAPK have been found in breast cancer which results in oncogene transformation, promoting cancer cell growth[34,35]. EGCG can potentially mitigate breast cancer progression by suppressing the activation of oncogenic forms of MAPK and EGFR, inhibiting VEGF mRNAs, and impeding the expression of PI3k/AKT pathways[36,37]. This inhibition would prove significant since inhibition of PI3k/AKT can potentially lead to apoptosis and the death of breast cancer cells. Low levels of VEGF will lead to the decrease of blood vessel growth, which means that there will be less nutrient being transported to the tumor, leading to its death.

Limitations of EGCG

This paper is mainly theoretical based and presents the biochemical functions of EGCG in the context of breast cancer. This section of the review demonstrates that EGCG has not been widely explored in the field of cancer therapy through presenting that several mechanisms and aspects of EGCG still remain unclear. This calls for the need of more exploration and specific investigation of EGCG in the context of breast cancer through *in vitro* and animal studies so that eventually it can be tested in clinical trials.

Green tea catechins exhibit many anticarcinogenic functions that justify EGCG's potential as a potential breast cancer treatment. However, the bioavailability of EGCG is relatively low, meaning that the amount of EGCG released successfully in the blood stream is little. If EGCG concentrations are low, its effectiveness in reaching its destination to treat breast cancer would decrease. EGCG is relatively unstable and can be easily degraded before reaching the targeted destination[38]. Compared to other green tea catechins, EGCG has a longer half-life (5.0-5.5 hours), but in order for it to exhibit clinical effects, its low bioavailability renders it ineffective[38].

Factors Influencing EGCG's Bioavailability

Factors that affect the bioavailability of EGCG include EGCG's surrounding environment, such as pH levels and other molecules. The digestion process of EGCG exhibits large impacts on EGCG's effectiveness as well. When EGCG is ingested orally, esterases in the saliva first break down EGCG by hydrolyzing EGCG. Moving into the gut, EGCG can be degraded and deconjugated by gut microbiota, which lowers its bioavailability. The molecular mechanisms behind how exactly EGCG gets degraded by the gut microbiota remains unclear and requires further exploration. Then, EGCG is absorbed by enterocytes (absorptive cells located in small and large intestines) and modified by the enzymes in enterocytes mainly in the small intestine. EGCG is then, as previously mentioned, methylated by catechol-O-methyltransferase (COMT), glucuronidated by UDP-glucuronosyltransferase (UGT), and sulfated by sulfotransferase (SULT) in the liver[39]. Finally, EGCG is released into the bloodstream and dispersed to other tissues[40].

Catechins, including EGCG, were found to be the most stable at pH 4 and 5.4[41]. Higher pH results in the epimerization of EGCG[19]. Yet the human gastrointestinal tract has an acidic condition of pH 1.5, which is not an ideal condition for the catechins nor EGCG to fully function because this pH is out of the optimal range[42]. In addition, EGCG has a low intestinal permeability because of there is lack of receptors that can transfer EGCG into intestinal epithelial cells, which explains EGCG's low bioavailability[43]. EGCG transmission thus relies heavily

on passive diffusion. EGCG was also found to be substrates for protein efflux pumps including multidrug resistance-associated protein (MRP) efflux pumps and P-glycoprotein (P-gp)[44]. These efflux pumps send the absorbed EGCG molecules back to the extracellular spaces, which further contributes to EGCG's low bioavailability. A potential solution that can be investigated for the protein efflux pumps is to apply a compound similar in structure to EGCG. This compound can act as an inhibitor of EGCG and prevent it to bind to protein efflux pumps, which then will improve EGCG's bioavailability. In addition, EGCG can be degraded through auto-oxidation and epimerization, potentially due to its reactive hydroxyl groups[45]. EGCG is auto oxidized in alkaline solutions, where oxygen takes electrons and results in the formation of hydrogen peroxide, superoxides, and ROS[46]. The formation of these products can pose toxic effects. EGCG can also be epimerized, where the bond between B and C rings changes in formation and arrangement. Epimerization of EGCG converts it to gallocatein gallate (GCG), and this degradation occurs during tea brewing[47]. Therefore, if EGCG is consumed via green tea drinking, it has already been degraded even before it is ingested orally. However, EGCG's maximum concentration can be preserved if the tea is brewed between 75 to 85 degrees Celsius under 5 minutes[45]. Temperature beyond 85 degrees Celsius decreases EGCG concentration due to epimerization of the molecule[48]. Thus, when ingesting green tea, it is crucial to maintain proper temperature and brewing duration to preserve the maximum concentration of EGCG. It has also been found that when EGCG is ingested with other liquids such as water or milk with high Ca^{2+} and Mg^{2+} concentrations, these liquids, because of the existing metals, will lead to the inactivation and low absorption of EGCG[49]. Mechanisms behind how these metals can impede EGCG functions remain unclear. According to Naumovski et al., EGCG was the most effective when ingested without food on an empty stomach54. Thus, it is also crucial to consider food pairings while ingesting EGCG since other molecules in foods can prevent EGCG from exhibiting its functions.

Toxicity

EGCG can be toxic because of its susceptibility to auto-oxidation. The formation of hydrogen peroxide, superoxides, and ROS can increase carcinogenicity if EGCG is applied in an excessive dosage. According to Kucera et al., EGCG at high concentrations (greater than or equal to 20 uM) will result in an increase in ROS and thus induce toxicity[50]. High dosage of EGCG (1500 mg/kg) has also shown hepatotoxicity (liver toxicity) due to the increase in oxidative stress in the liver[51]. In a study examining oral administration of EGCG in CF-1 mice, a single dose of EGCG (1500 mg/kg) one time during the day resulted in a reduced survival rate of 85%[51]. However, the absence of hepatotoxicity in mice was also reported in studies, and this varying result needs to be investigated through more animal studies of EGCG[52]. It seems that EGCG exhibits toxic properties only at high dosages and according to the EFSA panel, intake of EGCG less than 800 mg/day does not show signs of hepatotoxicity[19]. In addition, most studies mentioned above which supported EGCG's anticancer properties utilized EGCG way below concentrations of 800 mg (most studies used 20ug) which demonstrates EGCG's potential as a therapeutic agent. Nevertheless, since its toxicity and especially the safe dosage for human consumption remains unclear, EGCG requires further investigation[19].

Methods to Enhance EGCG's Effectiveness

Nanoparticle-based Delivery System

Various methods and delivery systems have been developed to improve green tea catechins' bioavailability once ingested. One such delivery system is nanoparticle-based delivery, specifically food nanoparticles (particles between 1-100 nm)[53], which was found to enhance catechin stability under harsh conditions such as pH through catechin encapsulation[54]. This delivery system releases drugs at a slow rate, which enhances drug solubility and reduces toxicity. Macro-and-micro-food molecules can serve as carriers of the catechins by loading the catechins inside of them. Through this mechanism, the carriers serve as a shielding material that protects the catechins from rapid degradation and instability. The size of carriers can be modified, and encapsulation complexes can be created through techniques such as electrospraying, emulsion, and self assembly[54]. Current carriers found to be effective in delivering EGCG include lipids, proteins, and carbohydrates[54].

Lipids

Lipids found in fats, oils, soy, and milk can be artificially modified to form liposomes, which are vesicles with multiple membranes that exhibit both hydrophobic and hydrophilic behaviors[55]. Lipids are firstly dried with organic solvent, dispersed in aqueous solution, and purified[55]. The multiple bilayers of liposomes are effective in entrapment of catechins, and the addition of cholesterol further strengthens the rigidity of liposomes. Liposomes are found to be effective drug delivery carriers that have low toxicity and the ability to target specific tumor sites as well as prevent their contents from being degraded by enzymes and extreme pH conditions[55]. EGCG was found to be encapsulated at a 99.6% effective rate by liposomes containing egg phosphatidylcholine (PC), a phospholipid found in chicken eggs59.

Proteins

Another effective carrier is proteins. Although many of them exist, gelatin and milk proteins seem to be the most effective carriers of EGCG. Gelatin is a protein derived from collagen, and its thermal flexible property contributes to its high efficacy as a carrier. Gelatin-A was found to successfully encapsulate EGCG with 100% efficiency when 10% of EGCG was loaded. It preserved EGCG's antioxidant properties compared to when EGCG was ingested alone61. Another effective protein for carrying EGCG is milk protein, which includes caseins and -lactoglobulin (main protein in whey). The interaction between EGCG and milk proteins alters the protein structure through EGCG's hydroxyl groups, which enhances EGCG's affinity with milk proteins and allows the proteins to protect EGCG's antioxidant properties[38,56].

Carbohydrates

Carbohydrate delivery systems are also being used for EGCG delivery, especially chitosan. Chitosan is found in exoskeletons of crustaceans, and it is a linear polysaccharide that is deacylated by chitin. Chitosan's nontoxic, biodegradable, and cationic properties facilitate its interactions with negatively charged portions of membranes, proteins, and molecules such as EGCG, which explain its wide usage in nanoparticle delivery[54,57,58]. Chitosan encapsulates EGCG through the automatic formation of hydrogen and weak ionic bonds between the atoms65. This encapsulation prevents EGCG from degradation and oxidation in the gastrointestinal tract and improves EGCG's effectiveness[59]. Even though nanoparticle-based delivery methods are proven to be effective in delivering and further enhancing EGCG's biological properties, its limitation lies in the fact that the encapsulation complex is prone to change and may be affected by surrounding molecules and environments and fails to successfully protect EGCG[60]. The mechanisms by which the encapsulation complex is affected remain unclear and require further investigation, including studies on the gut microbiota and their impact on this delivery system.

Co-administration of Food Molecules

Other than nanoparticle-based delivery systems, molecular modifications and co-administration with other food or drug molecules of EGCG can also improve its effectiveness. Ingesting tea alongside milk or black pepper has enhanced EGCG's bioavailability[39]. When tea was served with milk, the catechins' recovered from 20% to 52%[39]. This is potentially due to the milk proteins that protects catechins from degradation in the intestinal environment; as previously mentioned, milk proteins are efficient carriers of EGCG in the nanoparticle delivery system. However, Mereles Hunstein found that when ingested with milk, as previously mentioned, the cations in milk decreased EGCG's bioavailability[49]. Thus, it is currently unsure if milk can serve as an enhancement or a limitation for EGCG since research has found contradictory results. Black pepper, on the other hand, can also improve EGCG's bioavailability due to its component piperine. Piperine was found to prevent EGCG degradation by inhibiting the glucuronidation of EGCG[39].

Molecular Modifications of EGCG

Currently, the primary chemical and enzymatic modification method utilized for EGCG is esterification. Through this method, non-polar aliphatic hydrocarbon chains can be added to EGCG's reactive hydroxyl groups, forming esters and water, and therefore improve EGCG's stability[61]. This stabilization decreases EGCG's reactivity since it prevents its hydroxyl groups from forming ROS. In chemical esterification, EGCG and acyl group donors such as anhydride, acyl chloride, and carboxylic acid are heated with catalysts in solvent. Adding the acyl groups preserves the phenolic hydroxyl structure of EGCG and also enhances its stability and lipophilicity (the ability for a molecule to dissolve in lipids). However, this method's safety is in question if EGCG is going to be utilized in food because of the carcinogenic nature of some catalysts, such as pyridine[61]. Oxyacylation, on the other hand, is a form of esterification that utilizes anhydride and chlorination to attach fatty chain groups to the phenolic hydroxyl group of EGCG. This procedure can be conducted at moderate temperature with less toxic solvents, and therefore it is easy to manipulate for reaction conditions in food application[61]. Enzymatic esterification entails EGCG being transesterified with other esters through enzymatic reactions involving lipases and proteases. Compared to chemical esterification, enzymatic esterification is less toxic and contains more specificity (in terms of binding to the EGCG molecule), which facilitates enzyme separation at the end of the reaction[61]. Because esterification changes EGCG's hydroxyl groups, this method enhances EGCG's antioxidant activity, which also strengths EGCG's role in breast cancer treatment, and therefore allows EGCG to be more effective when ingested.

Discussion

The objective of this systematic review paper was to elucidate the potential of green tea catechin EGCG as a therapeutic treatment for breast cancer. The paper is theoretical based, and it mainly provides biochemical properties of the compound EGCG in relation to breast cancer. This review seeks to provide researchers potential areas to explore in the future, including the need to conduct *in vitro* and clinical studies of EGCG.

EGCG is the most abundant catechin in green tea and exhibits significant antioxidant, anti-inflammatory, and anti-cancer

properties. This is largely due to its chemical structure, which allows it to interact effectively with various biological molecules, such as superoxide enzymes. The compound's comprehensive properties position it as a promising candidate for breast cancer therapy as it is less invasive, more targeted to the cancer cells, and exhibits less side effects compared to current treatments such as chemotherapy, radiotherapy, endocrine therapy, and surgery. Moreover, its natural existence provides a safer option compared to current treatments in which radiation or large area applications are involved.

EGCG's Antioxidant Property: Superoxide Enzyme Inhibition

EGCG's ability to inhibit enzymes such as XO, COX2, and NOX, which are involved in the progression of breast cancer, supports its potential role as a breast cancer therapeutic. The inhibition of XO by EGCG, as discussed, highlights its antioxidant property and its potential in reducing oxidative stress and mitigating the migration of cancer cells, similar to the effects observed with the XO inhibitor FBX. This suggests that EGCG could serve as an additional treatment option for the management of breast cancer, particularly in scenarios where oxidative stress plays a critical role in cancer progression. Moreover, EGCG's ability to inhibit COX2 through multiple mechanisms, including the suppression of NF-B and TGFα, offers a targeted approach to managing ER- breast cancer, which is known for its aggressive nature and limited treatment options. Compared to chemotherapy and radiotherapy, EGCG can interact with specific pathways involved in breast cancer progression which makes it a more targeted therapeutic option. Thus, it would be less likely to exhibit side effects after treatment. The role of NOX enzymes in carcinogenesis, particularly in claudin-low and ER- breast cancer subtypes, further emphasizes the potential efficacy of EGCG in cancer therapy. By disrupting the assembly of the NOX enzyme complex (which increases superoxide) and suppressing TGFα (Drives COX2's promoter), EGCG may inhibit the superoxide activity crucial for cancer cell survival and proliferation. This further strengthens the hypothesis that EGCG is a strong therapeutic candidate not only in common breast cancer subtypes but also in those with poor prognosis and therapy resistance.

Epigenetic Modulation

In addition to its enzymatic inhibition, EGCG's epigenetic modulation capabilities present a compelling argument for its use as a breast cancer therapy. The compound's ability to inhibit DNMT and HATs highlights its potential to inhibit cancer cell proliferation by reactivating tumor suppressor genes such as SCUBE2 and hTERT. This epigenetic reprogramming is particularly relevant in triple-negative breast cancer, where EGCG has been shown to decrease the expression of oncogenic microRNAs such as miR221/222 and miR25 by inhibiting DNMT. It is this reprogramming that results in enhanced tumor suppressor activity and increased apoptosis, which can successfully halt uncontrolled cell division and breast cancer tumor proliferation.

Breast Cancer Signaling Pathways Regulation

Furthermore, EGCG's potential to modulate key signaling pathways involved in breast cancer progression, such as PI3K/AKT, VEGF, MAPK, and EGFR, underscores its versatility as a therapeutic agent. Its inhibition of these pathways has implications in mitigating breast cancer progression through a variety of mechanisms. These implications include decreasing tumor growth through reduced angiogenesis, which limits blood supply and nutrients to the tumor. This reduction in angiogenesis restricts tumor cell survival and ultimately leads to the overall suppression of breast cancer progression.

Remaining Gaps in EGCG Research

In light of all of the beneficial properties of EGCG that make it a strong therapeutic candidate, gaps still remain in the research regarding several aspects of EGCG, which includes its efficacy and application. Firstly, the optimal dosage of EGCG for human and animal consumption is still an enigma, and since the compound can be toxic at very high concentrations and breast cancer treatment is long-term, it is therefore crucial to conduct more *in vivo* studies and clinical trials in determining the safety and long-term effect of EGCG. Secondly, molecular mechanisms, such as the mechanisms behind how EGCG can decrease miR25 expression and how the gut microbiota degrades EGCG, remain unclear, which require more molecular investigation and research on EGCG. Additionally, the impacts of other foods such as milk on EGCG's bioavailability is contradictory, requiring more clinical trials in investigating how milk affects EGCG. The mechanisms by which the encapsulation complex is affected also remain unclear and require further investigation, including studies on the gut microbiota and their impact on this delivery system. This current paper brings attention to EGCG's anticancer properties and its ability to effectively treat breast cancer by providing conceptual and foundational knowledges of EGCG. This compound has not been widely explored in the field of cancer therapy, and its potential to treat breast cancer remains theoretical, which is a limitation that calls for the need of more exploration and specific investigation of the compound through *in vitro* studies and clinical trials. Several classical hallmarks, including uncontrolled cell growth, increased angiogenesis, evasion of apoptosis, and impaired expression of tumor suppressor genes characterize breast cancer. EGCG, with its comprehensive biological activities, addresses each of these hallmarks through various mechanisms. EGCG halts uncontrolled cell growth by inhibiting key enzymes XO, COX2, and NOX that reduce the

production of ROS, which are critical drivers of uncontrolled cell growth. In addition, EGCG's ability to reduce VEGF mRNA levels disrupts angiogenesis, successfully depriving tumors of the necessary blood supply for their growth. Moreover, EGCG's inhibition of DNMT and HATs reactivates silenced tumor suppressor genes, such as p27 and SCUBE2, restoring their ability to regulate the cell cycle and promote apoptosis. Furthermore, EGCG modulates key cancer-related signaling pathways, including PI3K/AKT, MAPK, and EGFR, which further promotes breast cancer cell death. Through these mechanisms, EGCG effectively targets the fundamental hallmarks of breast cancer, making it a well-rounded and promising therapeutic candidate.

Conclusion

This current paper presents EGCG as a potential therapeutic for breast cancer, emphasizing its various properties that reduce breast cancer risk including its ability to target key breast cancer hallmarks. The antioxidant, anti-inflammatory, and anticancer properties of EGCG promote the benefits of green tea consumption and enable it to interact and inhibit critical breast cancer pathways. By reducing ROS, suppressing angiogenesis, reactivating tumor suppressor genes, and modulating oncogenic pathways, EGCG successfully targets and addresses the core mechanisms of breast cancer, which supports its candidacy as a breast cancer treatment option. Despite these promising findings, however, further research is required to fully understand the therapeutic potential of EGCG, including how other foods can affect its bioavailability, molecular mechanisms behind its interactions with other biological molecules, and its efficacy in clinical settings. Future research in these areas could strengthen EGCG as a valuable component of breast cancer treatment strategies.

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