

Phytochemicals modulate carcinogenic signaling pathways in breast and hormone-related cancers

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Abstract: Over the years, nutrition and environmental factors have been demonstrated to influence human health, specifically cancer. Owing to the fact that cancer is a leading cause of death worldwide, efforts are being made to elucidate molecular mechanisms that trigger or delay carcinogenesis. Phytochemicals, in particular, have been shown to modulate oncogenic processes through their antioxidant and anti-inflammatory activities and their ability to mimic the chemical structure and activity of hormones. These compounds can act not only by influencing oncogenic proteins, but also by modulating noncoding RNAs such as microRNAs and long noncoding RNAs. Although we are only beginning to understand the complete effects of many natural compounds, such as phytochemicals, researchers are motivated to combine these agents with traditional, chemo-based, or hormone-based therapies to fight against cancer. Since ongoing studies continue to prove effective, herein we exalt the importance of improving dietary choices as a chemo-preventive strategy.

Keywords: natural compounds, dietary polyphenols, epigallocatechin gallate, curcumin, genistein, noncoding RNA, cancer

Introduction

Almost two centuries ago, the French lawyer/politician and gastronome Anthelme Brillat-Savarin stated "you are what you eat".¹ Since then, nutrition awareness has become very popular, and over the past years we have witnessed an increase in the number of people who devote time to educate and inform themselves about their selection of food products for consumption. Numerous scientific studies have shown that our eating habits can directly affect weight gain or loss, blood cholesterol levels, blood sugar levels, etc. However, the food we consume has an additional potential to act at a smaller scale, namely at molecular level, thus supporting the fact that foods are nutraceuticals.

Food products are advertised as agents having nutritional value as well as an effect on biologic functions, and they achieve their dynamic properties due to the presence of natural compounds named phytochemicals. These substances can be classified into various categories according to their structure or function (Figure 1, Table 1), but they usually act by mimicking the hormones. Good examples of this are phytoestrogens. In addition, phytochemicals can act by exerting antioxidant and anti-inflammatory effects. Nevertheless, the fact that phytochemicals have the potential of improving the lives of patients who suffer from various malignant diseases, such as cancer, is extremely interesting and exciting. Thus, the purpose of this manuscript is to evaluate the impact of the phytochemicals epigallocatechin gallate (EGCG), curcumin, and genistein on hormone-related cancers, especially breast cancer.

Nowadays, despite the exponential increase in oncological studies, the incidence of cancer continues to increase at an alarming pace. According to the latest GLOBOCAN

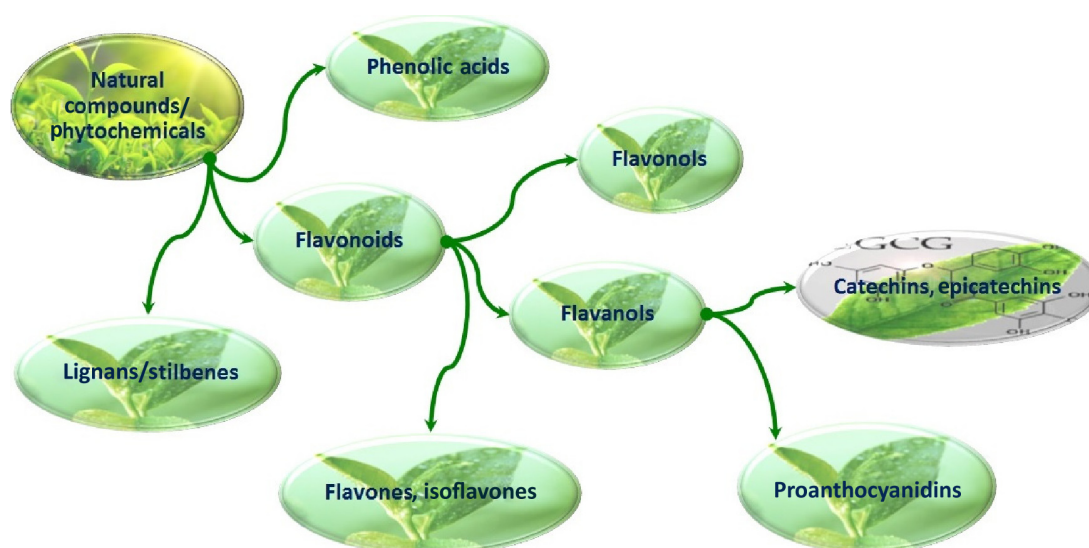


Figure 1 Classification of the principal types of phytochemicals.

statistics, during the year 2012 over 8 million people died of cancer, 32.6 million patients were diagnosed with this malignant disease during the previous 5 years, and a little over 14 million new oncological patients were identified.² These facts are alarming, and most certainly reinforce the importance of making efforts as a scientific community, to elucidate the molecular mechanisms that trigger carcinogenesis, with purpose of improving the lives of cancer patients.

Among the different types of cancer, breast cancer causes the highest number of deaths among women worldwide.² Breast cancer is a highly heterogeneous tumor that is subclassified into several subtypes based on their molecular characteristics. Among the several existing subclassifications of breast cancer, a subtype that generates great concern among caregivers is the one whose cells do not present estrogen receptors (ERs) on their surface, namely, ER-negative

Table 1 Classification of phytochemicals

Classification of phytochemicals, examples				Sources
Phenolics	Phenolic acids	Hydroxybenzoic acids	Gallic, vanillic, syringic	Chestnuts (boiled or roasted), witch hazel, tea leaves, oak bark, rhubarb, etc
		Hydroxycinnamic acids	p-Coumaric, caffeic, ferulic, sinapic	Barley, eucalyptus, coffee, Arabidopsis, Hibiscus, etc
	Flavonoids	Flavonols	Quercetin, kaempferol, myricetin	Aloe vera, European elderberry, soy, St John's wort, tomatoes, red onions, etc
		Flavones	Apigenin, luteolin	Celery, parsley, chamomile tea, green peppers, thyme, oregano, etc
		Flavanols (catechins)	Catechin, epicatechin, epigallocatechin gallate	White tea, green tea, persimmon, pomegranate, cocoa beans, etc
		Flavanones	Eriodictyol, hesperetin	Citrus fruits, rose hip, mountain balm, etc
		Anthocyanidins	Cyanidin, pelargonidin, malvidin	Grapes, berries, red cabbage, red onions, plums, kidney beans, geranium, etc
		Isoflavonoids	Genistein, glycitein	Lupin, fava beans, soy, coffee, etc
		Stilbenes		Grapes, etc
		Coumarins		Tonka bean, vanilla grass, etc
		Tannins		Eucalyptus, geranium, etc
Carotenoids	α -carotene, β -carotene, lutein, zeaxanthin, lycopene			Carrots, broccoli, spinach, zucchini, etc
Alkaloids				Poppy, tomatoes, potatoes, etc
Organosulfur compounds	Isothiocyanates, indoles, allyl sulfur compounds			Cabbage, broccoli, spinach, garlic, onions, etc

tumors. These tumors behave in an aggressive manner, and are resistant/unsusceptible to the effect of hormone therapies, particularly ER modulators.

Genome modulatory role of natural compounds

Both nutritional agents and environmental factors have a direct impact on the epigenetic machinery in human cells. Regarding dietary natural compounds and polyphenols, studies have shown that they are involved in modulating the epigenetic mechanisms and in shaping the epigenome by participating in three crucial processes: DNA methylation, histone modifications, and post-transcriptional regulation of genes via noncoding RNAs (ncRNAs), especially miRNAs.³ As a result of any of these dynamic changes, the fate of each gene is decided in regard to whether it will be translated into a protein or not. The involvement of phytochemicals with regulative behavior such as these could modify the activity of proteins as well as ncRNAs, and for this reason they have been said to have a promising role in pharmacogenomics.

Recent studies are aimed at identifying new, more efficiently targeted anticancer drugs, but more so, they are focused at trying to find innovative means to potentiate and improve existing therapies. This could most likely be achieved by deciphering molecular mechanisms that are or can be made vulnerable to specific therapeutic agents.⁴ For this purpose, phytochemicals have been extensively tested, alone or in combination with common drugs, to observe their modulation capabilities to obtain an additive or synergic therapeutic effect. “Phytochemicals” is a generic term for various compounds naturally occurring in plants, which are mainly responsible for their taste, smell, or color. They have been shown to exhibit different beneficial effects on human health. Most phytochemicals (such as flavonoid compounds) exert their functional roles through their antioxidant, chemopreventive, and anti-inflammatory capabilities,⁵ for example, EGCG is a very important polyphenol compound extracted from green tea. EGCG is mainly known for its role as a cancer modulator because of its antioxidant, proapoptotic, and antiangiogenic effects.^{6–11} It has been extensively studied due to its reduced costs and decreased cellular toxicity. Additionally, it is relatively easy to find in many common foods such as tea, chocolate, grapes, grape seeds, etc.^{12,13}

Alongside the beneficial effects of natural compounds such as EGCG, it is important to consider the fact that these agents could still display cytotoxic effects, which can be sometimes due to inappropriately high doses, unsuitable combinations, or improper use. Indeed, it is like the saying

of the Renaissance scientist Paracelsus (presumed parent of toxicology), “dosis facit venenum – the dose makes the poison”.¹⁴ Some examples of toxicities caused by phytochemicals include the goitrogenic properties of some components of the *Brassica* plants, the hemolytic effects of fava beans on certain genetically susceptible populations, and the agglutination caused by lectins.¹⁵ Nonetheless, if tested thoroughly, phytochemicals can improve the life of patients suffering from various diseases such as cancer, particularly when administered in combination with conventional therapies.

Breast cancers represent a very heterogeneous group of tumors to target, and the application of phytochemicals to treat patients with breast cancer would represent an attractive approach to complement conventional therapies. Regarding the ER-positive tumor type, studies have shown that estrogen generally supports malignant progression by favoring the cancer phenotype, especially in hormone-dependent cancers such as mammary tumors, via mechanisms that alter the routine transcription of estrogen-responsive genes. This occurs when ER dimers, belonging to the nuclear receptor superfamily of ligand-activated transcription factors, bind to the estrogen response elements located on the promoters of the genes that they regulate. Nuclear ERs are not the only structures that are involved in the process of supporting tumor development in breast cancer. Another active player is ER alpha (ER α) located in the cytosol and at mitochondrial level can also contribute to various nongenomic signaling pathways. For instance, mitochondrial ER α signaling takes an active role in the inhibition of intrinsic apoptotic pathways.^{16,17}

For ER-positive tumors, one of the most used conventional therapies is selective estrogen receptor modulator (SERM) such as tamoxifen. This drug acts by specifically binding to ERs on the surface of cancer cells, thereby blocking the hormone-induced signaling pathways that sustain tumor proliferation.^{18–20} While this agent works efficiently and represents a therapeutic alternative for estrogen-positive tumors, the drug is not useful for tumor subtypes that are not driven by ERs. In this sense, phytochemicals such as EGCG, or other natural compounds, could work by modulating the genetic expression of tumors that are not hormone driven, and alter their sensitivity to antihormonal therapies such as SERMs. The molecular mechanisms by which these phytochemicals manifest their effect upon hormone-induced signaling pathways of malignant cells are not completely understood. However, studies have linked their mechanism of action to the expression and function of certain genes or ncRNAs. Similarly, in ER-negative cancers, polyphenols

have been known to regain the ER phenotype,²¹ thereby making tumor cells sensible to the antiestrogenic effects of tamoxifen, and possibly to other anticancer drugs.

Several groups of scientists, including Li et al have worked on the idea of reinstating the sensitivity of tumors that possess a characteristic ER-negative phenotype.²¹ According to them, if reverted, these tumors could regain sensitivity to known chemotherapeutic treatments. Some of their work branches out of the hypothesis that the lack of *ER* gene expression in cancer subtypes, such as triple negative, is not caused exclusively by mutations. More so, it can be the result of other alterations such as epigenetics that modulate what can be referred to as the “hormone resistance machinery”, or genes and proteins that influence hormones. Some mechanisms that have shown to validate the hypothesis of these groups are epigenetic means of gene silencing, which seem to play an important role,^{21,22} especially hypermethylation at the DNA level in the *ER* gene promoter.²³ Additionally, several types of histone modifications,^{24,25} such as acetylations and deacetylations, have been shown to influence reading and interpretation of the genetic code, and this can affect hormone-induced cell signaling pathways.^{23,26}

Since ER-negative tumors behave differently from their receptor-positive counterparts, not only by being more heterogeneous but also by displaying a greater and more aggressive metastatic capacity, more efforts need to be made to re-sensitize these cells to the effects of endocrine therapeutic options, such as SERMs. An option to address this would be to use histone deacetylase inhibitors (HDAC

inhibitors), which have proved their clinical significance in acting against several types of cancer. ER tumors have also shown to increase their sensitivity to known therapies when treated with drugs such as demethylating agents, which are involved in silencing gene expression. An example of a demethylating agent is DNMT, a DNA methyltransferase, which has an effect against the hypermethylation of the promoter of *ER*, leading to a decrease in its mRNA and thereby a depleted protein expression.²⁷

Among the studies of natural compounds focused on discovering and characterizing the molecular mechanisms that underlie the onset, advancement, and development of cancer, many concentrated on discovering new means and novel compounds that can be used to modulate, and eventually control, subcellular events that lead to carcinogenesis. Prevention of cancer through dietary intervention has received an increasing interest, and dietary polyphenols, for example, have become not only important potential chemopreventive but also therapeutic natural agents that have the capability to interfere with oncogenic signaling pathways.²⁸ Nowadays, studies also have a more ambitious aim such as finding and developing targeted drugs that can act at the core of molecular events that trigger or modulate particular steps in the stepwise progress of tumor development and disease progression (Figure 2). In order to achieve this, it is necessary to also address another aspect, namely, the poor bioavailability of phytochemicals, because of which most phytochemical compounds reach the targeted sites at subtherapeutic concentrations.^{29,30} In the case of curcumin,

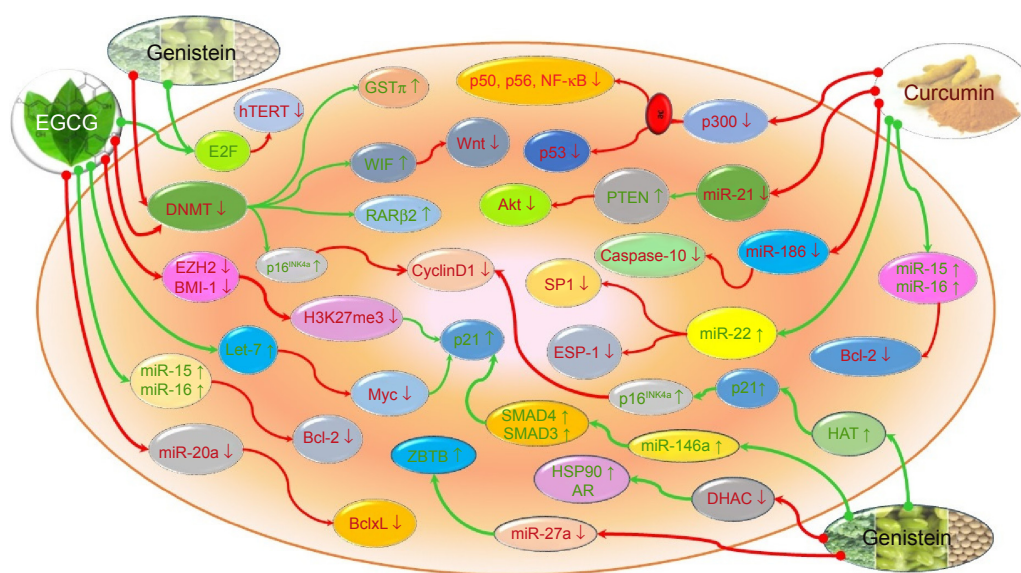


Figure 2 Modulatory effects of EGCG, curcumin, and genistein.
Abbreviation: EGCG, epigallocatechin gallate.

for instance, this limitation has been amended through the synthesis of functionally identical analogs with improved efficiency^{29,31} and by constantly testing novel delivery systems, including liposomes, micelles, and various functionalized nanoparticles.^{32–34} Although these drug carriers still need to undergo further *in vivo* assessments, the promising results support the potential of phytochemicals to be used as chemopreventive and chemotherapeutic agents in the clinic.

Epigallocatechin gallate

Epigallocatechin gallate (EGCG) (PubChem chemical structure identifier CID: 65064, Figure 3) is a polyphenol present in several natural sources, but mainly in green tea, which is among the most common beverages worldwide.³⁵ EGCG has been long studied, and is known to have beneficial effects in various pathologies including neurodegenerative diseases, diabetes, strokes, and obesity. It can also have chemopreventive effects in several types of cancers.³⁶ Its antioxidant effect appears to counteract the inflammatory processes that precede the development of malignancy, such as cellular transformation, sustained proliferation, and other characteristics that lead to tumorigenesis.

One of the ways that EGCG manifests its protective/preventive effect against cancer has been proven *in vitro* in both cervical and breast cancer cells. It does so by is by detaining the cell cycle in the G1 phase^{37,38} and by influencing the synthesis of several proteins, such as CDK1 and cyclins D and E (which tightly regulate cell cycle progression). In addition, this particular polyphenol can induce processes such as apoptosis, positively regulating the function of proteins

such as pRb, p53, p27, etc, especially in breast, prostate, and bladder cancer cells *in vitro*.^{38–40}

One interesting aspect observed in different studies is that malignant cells seem to be more susceptible to the antitumor effect of bioactive polyphenols, when compared with normal, untransformed cells.^{41,42} The ability of phytochemicals to act on cancer cells in a selective manner has great importance in what concerns the concept of targeted therapy. Most conventional chemotherapy is nonspecific for cancer cells and also affects nonmalignant tissues; thus, it has unpleasant and potentially dangerous side effects.⁴³ On the other hand, the lack of toxicity on normal cells displayed by many phytochemicals, together with their ability to act in a synergistic manner with many anticancer drugs,^{44–46} makes phytochemicals interesting and appealing from the therapeutic point of view. Moreover, dietary polyphenols, including EGCG, administered in low doses appear to promote survival genes and protective mechanisms by activating the mitogen-activated protein kinase (MAPK) signaling pathway; however, in higher doses they can induce antitumorigenic processes such as apoptosis.⁴⁷ EGCG can influence programmed cell death by triggering and activating proapoptotic proteins, such as caspases 3, 8, and 9, and by downregulating the natural inhibitors of these cellular process, such as Bcl2, Bid, XIAP, or IAP2.^{48,49} This effect is enhanced alongside by dietary polyphenols upregulating p53, p21, and Bax, as shown in a study conducted by Hastak et al using the RNA interference technique of siRNA-p53 inactivation.³⁹ Additionally, we confirmed in our laboratory the role of EGCG as an activator of apoptosis through thorough studies, some of which showed a synergistic effect on combining the treatment of HeLa cells with EGCG and siRNA-p53, which reversed the oncogenic role of the mutated *p53* gene present in cancer cells. This therapeutic effect was achieved by modulating various genes that belong to families such as caspases, TNF receptor associated factor, Caspase recruitment domain, death domain, or Bcl2. Thus, this suggests that EGCG has a role as gene expression co-activator, and therefore could be used as an anticancer therapy.⁵⁰ Similar results were obtained when treating cervical cancer cells only with EGCG, then performing gene expression analysis using polymerase chain reaction (PCR) array technology. The treatment also contributed to the activation of caspase-mediated apoptosis, via BAK1 upregulation.⁵¹

Previous studies suggest that the hyperactivation of MAPK signaling pathway may constitute one of the mechanisms that lead to the loss of ER α expression in breast cancer, promoting an ER-negative phenotype.¹⁶ Nonetheless, this

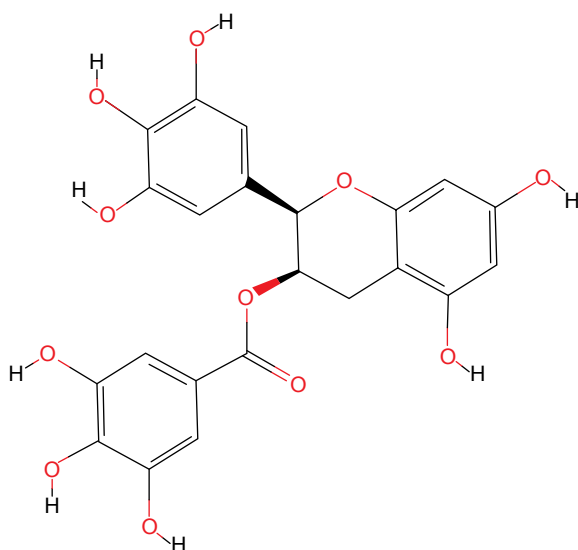


Figure 3 The chemical structure of EGCG.
Abbreviation: EGCG, epigallocatechin gallate.

process is reversible when the MAPK signaling pathway is inhibited, emphasizing the necessity of further studies to elucidate these molecular mechanisms.

Nuclear factor- κ B (NF- κ B) is another signaling pathway that is usually deregulated in cancer. In ER-negative breast cancer cell models, NF- κ B is constitutively active. Nonetheless, it barely shows any type of activity in ER-positive/Her2-negative cell lines. Zhou et al have confirmed this exact prediction by showing that high NF- κ B activity positively correlates with a decreased ER α expression, as obtained in a study conducted on 81 patients.⁵² In this manner, it promotes cell survival mechanisms, while at the same time inhibits apoptosis-mediated cell death.⁵³ Because of this, the role of the dietary polyphenolic compound EGCG has been studied and considered as an approach to help balance the signaling pathway toward the desired direction.

Regarding the effect of certain natural compounds on oncogenic signaling pathways, EGCG was observed to decrease the production of NF- κ B. This diminished the effect that NF- κ B had on the expression levels of ER α .^{48,54} Thus, it may be stated that this phytochemical not only appears to promote the reactivation of the ER expression, but it may be working as a preventive mechanism to lessen the activity of genes that could lead to regaining an ER-positive phenotype. EGCG may be predicted to be achieving this by modulating the levels of significant molecules in key signaling pathways. Alternatively, it is possible that polyphenols, such as EGCG, do not actually revert the hormonal phenotype from ER negative back to ER positive, but just detain a sequence of events responsible for the loss of ER expression. The inhibitory effect that EGCG has on the NF- κ B molecule, and consequently on the signaling pathways it takes part in, appears to achieve a desired effect in breast cancer cells. Regarding patient benefits, this could potentially translate into new therapeutic alternatives for breast cancer tumors.^{55,56}

Curcumin

Curcumin (1,7-bis[4-hydroxy-3-methoxyphenyl]-1E,6E-heptadiene-3,5-dione) (PubChem chemical structure identifier CID: 969516, Figure 4) is a polyphenolic compound derived from turmeric, an aromatic perennial herbaceous plant from the ginger family named *Curcuma longa*.³⁵ This bright yellow powder, also known as “Indian saffron”, is a principal ingredient in curry, and it has also been long used in traditional Asian medicine due to its anti-inflammatory, antimicrobial, and antioxidant properties.^{57,58} Even though curcumin has a reduced bioavailability and is poorly absorbed/rapidly processed, it has promising modulatory

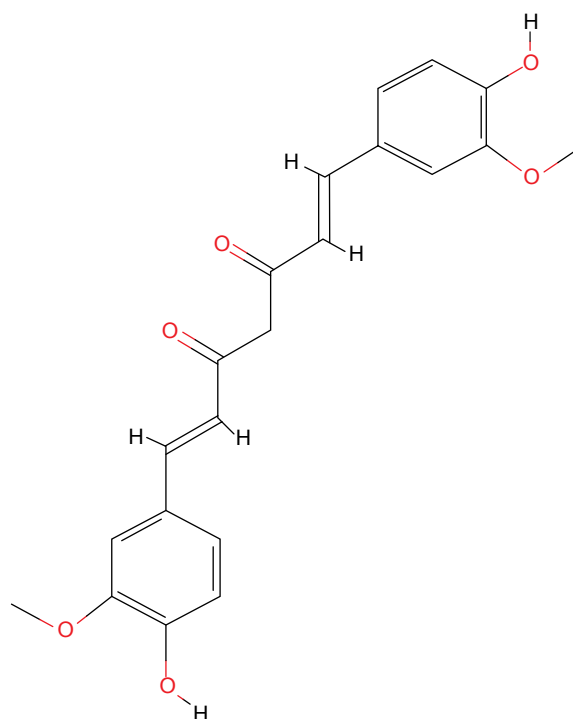


Figure 4 The chemical structure of curcumin.

effects that have led researches to put effort into either enhancing its bioavailability or developing synthetic analogs to further study its antitumorigenic effect.

As mentioned, curcumin is a known anti-inflammatory compound used in traditional Asian medicine. Besides from inhibiting COX2, a known key player in the inflammatory process,⁵⁹ it has been shown to downregulate NF- κ B. Pathways activated by NF- κ B are triggered by inflammation, as shown by cellular studies in vitro and on peripheral blood samples from oncogenic patients.⁶⁰ The inhibitory effect that curcumin has on NF- κ B resembles the effect that EGCG has on it. In this manner, it may also interfere with processes that lead to diminished ER expression.

The influential effect of curcumin on NF- κ B also extends to other molecules that are part of its signaling pathway, such as COX2, MMP9, BclxL, Bcl2.⁶¹ Further studies have shown that curcumin may also have inhibitory effects on other oncogenic signaling pathways, such as Akt. As a result, it can initiate apoptotic processes and inhibit tumor cell proliferations.^{62,63} Thus, curcumin could potentiate the effect of antihormonal therapy in cancer by positively influencing processes that can reinstate ER expression. Furthermore, it has overall beneficial effects for cancer patients by promoting apoptosis in tumor cells. Nonetheless, further studies still need to be conducted in order to better understand the effects of this dietary polyphenol on breast cancer and how it can be combined with the known therapies.

Genistein

Another important dietary natural compound is genistein or 4',5,7-trihydroxyisoflavone (PubChem chemical structure identifier CID: 5280961, Figure 5).³⁵ It is an isoflavone found in large quantities in soybeans and, consequently, in most products that contain soy protein. Its structure resembles very much that of estradiol; thus, genistein functions as a phytoestrogen, binding to the ERs, causing its effects in a dose-dependent manner. At low doses ($<1\text{ }\mu\text{M}$), genistein acts as an estrogen agonist, promoting cell proliferation. On the other hand, when administered in concentrations higher than $5\text{ }\mu\text{M}$, it displays an antagonistic activity by inhibiting the growth of ER-dependent tumor cells.⁶⁴ This fact has been confirmed in recent studies. For example, Lavigne et al worked on a human mammary tumor cell line, MCF-7, and observed that genistein, at low physiological concentrations, sustains the mitogenic activity of cells. However, when administered at pharmacological doses, it promotes programmed cell death, decreasing proliferation.⁶⁵

Genistein is a downregulator of several molecules and signaling pathways known for their involvement in tumorigenesis, cancer development, and progression. For example, it can inhibit protein tyrosine kinases and induce proapoptotic effects in this way.^{66,67} Additionally, isoflavones found in soybeans have antioxidant potential which inhibits the activation of the NF- κ B and the signaling pathways that it takes part in. In both breast and prostate cancers, genistein inhibits tumor cell growth and proliferation in vitro.^{68,69} Interestingly, both of these are cancer types influenced by hormones.⁷⁰ In vivo, the effects produced by both breast and prostate cancers appear to be similar, as displayed by experiments conducted by Davis et al.⁷¹ By studying the lymphocytes harvested from the blood of volunteers that were administered soy supplements, they observed a protective effect of this leguminous plant against the tumor necrosis factor alpha (TNF α)-induced NF- κ B upregulation.

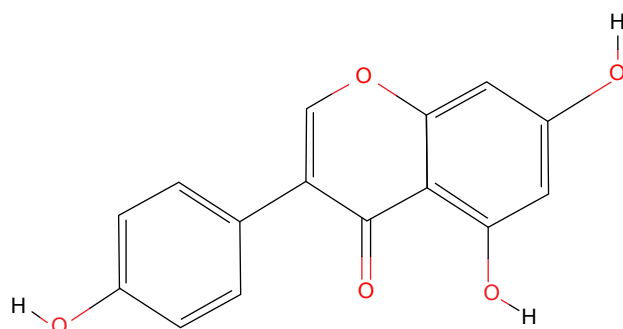


Figure 5 The chemical structure of genistein.

Finally, an additional target inhibited by the isoflavone genistein is the Akt kinase. Genistein can downregulate its activity by preventing its phosphorylation. This fact was observed in several studies conducted on hormone-related cancer cell lines.^{69,72,73}

According to Kikuno et al genistein inhibits both Akt and NF- κ B in prostate cells by reducing multiple epigenetic mechanisms.⁷⁴ In addition, it could contribute to the prevention of prostate cancer onset by modulating an antiestrogenic effect mediated through heat shock proteins HSP90 and HDAC6.⁷⁵ Other signaling pathways that promote tumorigenesis, such as Wnt/ β -catenin and Notch signaling, are also inhibited by genistein, and all these data represent promising indications that this natural compound may also be used in combination with traditional, conventional chemotherapy or with hormone therapy to regain/potentiate their effect.^{76,77}

The Asian paradox

A remarkable observation has been noted when comparing breast cancer incidences in Asian women to those in Caucasian and Asian-American women.⁷⁸ Principally, it was observed that when Asian-American women continued to consume a typical Asian diet, with high quantities of soy, fish, rice, tea, and greens, their risk of developing breast cancer was lower than that of Western and Caucasians, in general. On the other hand, when Asian-Americans switched to the Western diet, their breast cancer risk increased just as that of women of the Caucasian descent.⁷⁹

Genistein in particular and soy, in general, have been extensively studied, specifically in regard to their relation with breast cancer. In Asian countries, soy is a staple food, and it is consumed by virtually everybody throughout their lives. Asian women are exposed to soy isoflavones from the very beginning of their existence, and this might be a possible explanation of the risk differences observed between them and Caucasians. Therefore, the increased intake of soy and soy-derived products in childhood and early adolescence seems to confer a degree of protection against the development of breast cancer.^{79–83} Regarding Asian-American women, a possible explanation for them having a higher risk than Asian women might be that the soy-based foods that are produced in the Western countries vary considerably from the traditional Asian ones due to the higher degree of processing. This most likely alters the bioavailability and accessibility of soy isoflavones.^{84,85} These interesting facts raise questions regarding the preventive potential that genistein can have on breast cancer risk. Thus, more studies need to be conducted on this topic in order to elucidate the mechanisms that appear to improve the lives and overall survival of cancer patients.

ncRNAs, cancer, and natural compounds

Since the central dogma of molecular biology has recently shifted from the clear, straightforward model of “DNA–mRNA–proteins” to the complex labyrinth of ncRNAs, it is not surprising that the effects of natural compounds have also begun to raise interest in the context of miRNA modulation. ncRNAs, and miRNAs in particular, are not only potential biomarkers for the diagnosis and prognosis of cancer, but, as studies have shown, can also be the targets for therapeutic agents, as one miRNA can participate in the modulation of many different molecular pathways involved in malignant initiation, development, and progression.

ncRNAs, best represented by miRNAs, have been known to play an active role in different molecular pathways that are involved in the development and progression of tumors. It has been noticed that changes in miRNA expression levels have effects on cancer phenotypes. In this way, miRNAs can be involved – either as targets or as effectors – in the development of new therapeutic approaches.⁸⁶ On the other hand, dietary phytochemicals have been shown to modulate the expression of miRNAs that are involved in cancer development by acting either as tumor suppressors or as oncogenic miRNAs. Thus, natural compounds such as these have the potential for being used either as direct antitumor agents, or as “helpers” that can assist in reinstating a phenotype that would allow cancer cells to become receptive to chemotherapeutic drugs.^{87–93}

Natural compounds have been shown to influence the expression of several miRNAs, which, in turn, modulate important cellular processes, such as reducing inflammation, cell growth, invasion, and metastasis, and promoting epithelial to mesenchymal transition, while stimulating cell differentiation and cell cycle arrest (Figure 2). For example, in the case of EGCG, the natural compound has been shown to upregulate the expression levels of let-7, miR-15, and miR-16, and to inhibit miR-20a.^{86,94} An increase in miR-16 expression causes a decrease in the expression of Bcl2 protein, and consequently apoptosis is enhanced. A similar mechanism occurs when genistein downregulates the expression of miR-20a; this, in fact, leads to an increased expression of proapoptotic gene *BclxL*.⁹⁵ Furthermore, in vivo studies conducted by Siddiqui et al confirmed findings of a similar kind with an animal model study conducted on mice with prostate cancer, in which reverse transcription PCR (RT-PCR) showed that mice treated with EGCG presented lower levels of the oncomir miR-21 and upregulated levels of the tumor suppressor miR-330.⁹⁶ Once again, these

arguments support our claim that green tea polyphenols have anticancer effects, and that their use should be considered in cancer types such as breast and prostate.

Curcumin, the polyphenolic compound from turmeric, displays similar effects on miRNAs: it inhibits oncogenic ones such as miR-21 and miR-186*, while it upregulates tumor suppressors such as miR-15, miR-16, miR-22, miR-27a, and miR-34.⁹⁴ For example, by upregulating miR-22 in pancreatic cancer, curcumin exerts an inhibitory effect on ER α and transcription factor Sp1.⁹⁷ Similarly, the downstream effect is also the promotion of apoptosis, differentiation, and cell cycle arrest, while inhibiting cell growth, invasion, metastasis, and EMT phenotype.^{83,86} Studies have shown that similar effects were also displayed by the synthetic analog of curcumin, which stimulated the expression of tumor suppressor PTEN through the downregulation of miR-21 and miR-200.⁹⁸

The effects that genistein has on hormone-related cancers are similar. In prostate cancer, it upregulates the expression levels of miR-221 and miR-222, leading to the overexpression of tumor suppressor ARH1, which is usually downregulated in this malignancy.⁹⁹ Furthermore, in ovarian cell lines treated with genistein, 53 miRNAs were differentially expressed compared to the untreated samples. Moreover, genistein induced the expression of ERs alpha and beta, while reducing migratory and invasion capabilities of cells.¹⁰⁰

The results obtained from recent studies support the need to continue shedding light on the way that dietary natural compounds influence tumorigenesis by influencing the levels of miRNAs. Moreover, there is a need to further study other types of ncRNAs that are affected by phytochemical intake. In this regard, Liu et al showed in a microarray study, conducted on hepatocyte cell lines, that EGCG has a modulatory effect on the expression of long ncRNAs potentially involved in cholesterol metabolism.¹⁰¹ In a similar way, more studies like this should be performed to see the effect of phytochemicals on long ncRNAs that are involved in tumorigenesis.

In summary, many studies presented in the literature have shown that phytochemicals either act directly on miRNAs and post-transcriptionally modulate gene expression affecting tumor development and progression, or, alternatively, cause a synergistic effect when administered together with conventional drugs. A slight change in the expression of one miRNA can generate a signaling cascade that has the potential to involve many molecular networks and trigger various responses in the tumor cell, specifically in the cancer types that are hormone related. Further studies on this could lead to a more significant understanding of the beneficial effects

Table 2 List of completed clinical trials, with results, conducted on the effects of diet on cancer

Search words	Number of completed trials	Name of trial	Number/identifier	Enrollment (persons)	Study start date	Study completion date	Results (where available – quoted from the publications)	Articles generated (DOI) (where available)
“Only with “All results” studies”								
“Breast cancer” and “phytochemical”	0	The Effects of Red Wine and White Wine on Blood Estrogen and Progesterone Levels	NCT00572351/IRB 6110	44	January 2006	March 2007	“These data suggest that red wine is a nutritional AI and may explain the observation that red wine does not appear to increase breast cancer risk.”	10.1089/jwh.2011.3001
“Breast cancer” and “diet”	8	Dietary Fat, Eicosanoids, and Breast Cancer Risk*	NCT01824498/UMN914	18	January 2004	November 2010	“These results do not support a role of n-3 FA (Omega-3 fatty acids) in the reduction of sex hormone levels.”	10.1080/01635581.2011.589957, 10.1016/j.nutres.2011.06.003, 10.1016/j.nutres.2011.12.006, 10.1186/1743-7075-10-36
		Coenzyme Q10 in Relieving Treatment-Related Fatigue in Women With Breast Cancer (CoQ10)*	NCT00096356/CCCFWU-97202, U10CA081851	236	August 2004	August 2009	“Supplementation with conventional doses of CoQ10 led to sustained increases in plasma CoQ10 levels but did not result in improved self-reported fatigue or QOL after 24 weeks of treatment.”	10.1016/j.suponc.2012.03.003
		Study of Palatexate in Female Patients With Previously treated Breast Cancer*	NCT01118624/PDX-014, 2008-006425-14	22	March 2010	July 2012	–	–
		The Role of Serotonin in Hot Flashes After Breast Cancer*	NCT00228943/0501-03, DOD-BC043199, 043199	28	July 2005	November 2008	“Physiologically documented and self-reported hot flashes were not exacerbated by tryptophan depletion.”	10.1097/gme.0b013e318199e9f6
		Do Aromatase Inhibitors (AIs) Decrease Intestinal Calcium Absorption?*	NCT00766532/H-2008-0144	12	January 2009	October 2010	“The null effect of AI therapy on TFCA (total fractional calcium absorption) might relate to the brief duration of AI therapy, the minimal effect of AI therapy on estradiol levels, subjects’ high calcium intake or excellent vitamin D status.”	10.1007/s10549-012-1982-z
		Exercise and Phytoestrogens: Effect on Factors Predisposing to Cardiovascular Disease in Postmenopausal Women*	NCT01048606/IDionne_ phyto_2008-2011	45	January 2009	November 2012	“While phytoestrogens combined with mixed exercise were not sufficient to improve menopausal symptoms, it seemed to be a better strategy than exercise alone to improve the general quality of life in postmenopausal women.”	10.1097/gme.0b013e3181da7915, 10.3109/13697137.2011.566652, 10.1016/j.metabol.2011.06.025, 10.3109/13697137.2011.643515, 10.3109/13697137.2012.662251

(Continued)

Table 2 (Continued)

Search words	Number of completed trials	Name of trial	Number/identifier	Enrollment (persons)	Study start date	Study completion date	Results (where available – quoted from the publications)	Articles generated (DOI) (where available)
"Breast cancer" and "EGCG"	0	Pyridoxine in Preventing Hand-Foot Syndrome in Patients Who Are Receiving Liposomal Doxorubicin for Cancer*	NCT00245050/CASE5Y03, P30CA043703	34	April 2004	September 2011	–	–
		Evaluation of SAME for Hot Flashes*	NCT01140646/MC09C4, NCI-2010-01224	45	October 2010	November 2012	–	–
		Defined Green Tea Catechin Extract in Treating Women With Hormone Receptor Negative Stage: I-III Breast Cancer	NCT00516243/IRB-AAAB7638, CDR0000653465, 2006-0521	40	July 2007	March 2010	"In conclusion, using a novel clinical trial design for Phase I testing which evaluates long-term toxicity, we determined the MTD for Polyphenon E to be 600 mg bid (total of 1,200 mg EGCG daily), which will serve as the upper safety limit in future long-term intervention trials."	10.1158/1940-6207.CAPR-12-0117
		Green Tea and Reduction of Breast Cancer Risk	NCT00917735/0806M36121	1,084	July 2009	June 2014	–	–
		Curcumin for the Prevention of Radiation-induced Dermatitis in Breast Cancer Patients	NCT01042938/URCC1106, 5KL2RR024136-03, 05-238-80	35	January 2008	April 2011	–	–
"Breast cancer" and "genistein"	0	Gemcitabine Hydrochloride and Genistein in Treating Women With Stage IV Breast Cancer	NCT00244933/CDR0000446089, P30CA022453, WSU-C-2597	19	February 2004	October 2009	–	–
		Genistein in Preventing Breast or Endometrial Cancer in Healthy Postmenopausal Women	NCT00099008/UNC-GCRC-2107, CDR0000393450	30	March 2004	July 2006	"Unconjugated soy isoflavones appear to be safe and well tolerated in healthy postmenopausal women at doses of 900 mg/day."	10.1097/gme.0b013e318167b8f2

Note: *Indicates the studies obtained when selecting the "studies with results" option while searching.

Abbreviations: EGCG, epigallocatechin gallate; MTD, maximum tolerated dose; QOL, quality of life; bid, twice a day.

of natural compounds in cancer therapy, and this could lead the way to more effective treatment approaches.

Clinical trials

At least for the time being, a vast majority of studies have been conducted in an in vitro setting, and the results generated are not yet reaching therapeutic applications, but can be used for a chemopreventive approach. Nonetheless, the increasing number of studies that report success in modulating or influencing the course of hormone-dependent cancers by using natural bioactive compounds offers valuable data that can be useful in the sense of applying the use of dietary supplements such as polyphenols, for example, luteolin, apigenin, EGCG, quercetin, genistein, etc. The ever increasing interest in these phytochemicals and their potential action as nutraceuticals is proven by the large number of studies conducted by different teams worldwide. On the other hand, when it comes to clinical trials, the situation is different. Looking on the www.clinicaltrials.gov website, which, according to its own description, is a resource that offers information on publicly and privately financed clinical trials, the number of studies on humans is not that large. Although the list of trials that was obtained when searching for “diet” and “cancer” consisted of a total of 1,714 interventional studies, the numbers become drastically lower after filters are applied. The results obtained when searching for specific keywords, such as “only completed trials”, “only trials that have results”, and “exclude unknown status”, are presented in Table 2.^{102–116}

Nonetheless, there are a lot of researchers interested in continuing studies because of the positive effects that these phytochemicals have on various pathologies. Although at the moment most of the studies are focused on in vitro work, the majority of them need to further validate their results in human subjects. This is bound to change in the future, since the scientific and medical communities are continuously searching for ways to improve human life, also by using natural compounds together with the standard treatments of care.

Conclusion

Food components have been shown to influence the microenvironment that dictates the cellular response of an organism with regard to immunity, metabolism, responses to stress stimuli, etc.¹¹⁷ These scientific approaches promise to evolve to the point where dietary phytochemicals will be used, in a multimodal approach, as multifunctional agents that are capable of stopping or reversing transformation of premalignant

cells, before they reach their full tumor phenotype, as well as for the preservation of healthy cells.¹¹⁸

As a result of their structure and function, biologically active natural compounds have long been known for their antioxidant, anti-inflammatory, proapoptotic, and antiangiogenic effects, which have been proved by numerous studies conducted mostly on in vitro models. Recent studies, facilitated by modern research tools and concepts, have shown that phytochemicals have the potential of modulating various molecular processes, including signaling pathways involved in the development and progression of tumors. Although we are only beginning to understand the precise way in which these compounds exert their action, there is a clear motivation driving their application together with traditional, chemotherapy, or antihormonal therapy, especially in the case of hormone-dependent cancers. Finally, these types of studies aim to create a greater conscience in human beings in the sense that they can improve their dietary choices, and in this way prevent the development of malignancies such as cancer.

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Disclosure

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