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Chemopreventive and Therapeutic Potential of Phytochemicals Targeting Cancer Stem Cells

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Abstract Cancer stem cells (CSCs) constitute a subpopulation of transformed cells that possess intrinsic ability to undergo self-renewal and differentiation, which confers resistance to conventional anti-cancer therapy and cancer recurrence. The development of more effective cancer therapies hence requires identification of target subpopulations with distinct CSC phenotypes. Therefore, targeting CSCs is now considered as a rational and fundamental approach in the management of cancer. Components of signaling network involved in maintaining stemness of some CSCs and their self-renewal capability have been recently discovered. Some edible phytochemicals modulate signal transduction involved in self-renewal and survival of CSCs, thereby improving the efficacy of the current anti-cancer strategies. This article deals with chemopreventive/chemotherapeutic potential of selected phytochemicals, such as genistein, sulforaphane, curcumin, and epigallocatechin gallate in the context of their modulation of signal transduction networking among components of CSCs.

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Introduction

Despite an enormous progress in the development of anti-cancer therapy, cancer is still a major cause of death. Although anti-cancer drugs can considerably shrink the tumor size, they often fail to eradicate residual cancer cells. The cancer often recurs even after surgical removal of tumor, which is the major stumbling block in the cancer treatment [1, 2]. The tumor recurrence has been associated with innate ability of cancer cells to grow and propagate at a specific site of its origin. Recent studies have suggested the existence of stem cells in several human malignancies, which have the intrinsic capability of self-renewal and differentiation [3, 4]. The self-renewal potential of a tumor is dependent on a small subpopulation of cells within the tumor microenvironment, termed "tumor-initiating cells" and/or "cancer stem cells (CSCs)."

CSCs share several properties with normal stem cells. These include the ability to undergo self-renewal, differentiation and migration, active telomerase expression, increased membrane transporter activity, and activation of anti-apoptotic signaling [5]. Many tumor cells are maintained by a subpopulation of cells that exhibit tumor-initiating stem cell-like properties with variable morphology, differentiation status, and molecular features within a tumor mass [6, 7]. Tumor-initiating cells have intrinsic capacity for uncontrolled proliferation, survival, invasion, and metastasis [8, 9]. Stem cell regulatory pathways include those mediated by Notch, Hedgehog, Wnt, NF-κB, and JAK/STAT3 [10–14]. These signaling molecules involved in the maintenance of CSCs

are commonly considered as products of "stemness" genes [15]. Some pharmaceuticals and dietary phytochemicals have the ability to suppress self-renewal, growth, or maintenance of CSCs [16, 17]. In the present review, we focus on some chemopreventive phytochemicals that target one or more of the molecular signaling pathways exploited by CSCs.

Key Molecules of the Signaling Pathways that Regulate CSCs

Identification of Cell Surface Markers in CSCs

It is now possible to isolate CSCs from tissue specimens of patients with malignant tumor and well-established cancer cell lines. The different populations within a tumor can be identified according to the signature of proteins expressed on the surface of a particular cell. Fluorescence-activated cell sorting (FACS) using the specific antibodies directed against stem cell-like surface markers, such as CD44, CD24, CD34, CD138, CD20, CD90, and CD133, is the universal method generally applied for the isolation of CSCs (Table 1). For example, it has been reported that CD133 is a marker expressed in various types of stem cells, including those of brain and hematopoietic origin [18, 19]. In addition, CD44, a cell surface receptor for hyaluronic acid, is a multifunctional class I transmembrane glycoprotein. It was found to be associated with cell migration in normal cells and highly expressed in cancer cells [20]. CD44-positive cells exhibit capability to promote tumorigenesis in breast and colorectal cancer models displaying stem cell properties [21••, 87]. Table 1 summarizes the list of generally accepted CSC markers.

EMT in CSCs

Epithelial-to-mesenchymal transition (EMT) is a crucial event in initiation of the tumor metastasis [22]. During EMT, cancer cells lose epithelial cell–cell junctions and acquire migratory characteristics to become motile fibroblastic cells with metastatic capacity [23]. Some pathophysiological conditions allow EMT phenotypic cells to attain a multipotent stem cell-like property [24]. Thus, it has been reported that metastatic cancer cells undergoing EMT exhibit CSC-related traits. For instance, the induction of EMT in immortalized human mammary epithelial cells resulted in enrichment for a $CD44^+/CD24^-$ CSC-like subpopulation and increased formation of mammospheres. In addition, stem-like cells isolated from mammary carcinomas regulate the expression of EMT markers [24]. These cells displayed a strong reduction in the E-cadherin protein and increased expression of EMT-inducing transcription factors, such as forkhead box protein C2 (FOXC2), Smad-interacting protein 1 (SIP1), and Snail and Twist [24]. CD44 has been known to be a β-catenin/TCF-4 target gene, supporting a role for the EMT-associated Wnt pathway in maintenance and growth of CSCs [25]. Moreover, in an in vivo model of orthotopic pancreatic cancer, implantation of distinct subpopulation of $CD133^+/CXCR4^+$ CSCs induced the metastatic activity of the developing tumors [6]. Interestingly, poorly differentiated aggressive breast cancers exhibited an embryonic stem cell-like gene expression, such as Nanog, Oct4, and SRY-box 2 (Sox2) [26].

Table 1 Cell surface markers associated with cancer stem cells

Tumor type	Cell surface markers	References
Breast	$ESA^+/CD44^+/CD24^-/Lineage^-$	[86–88]
Colorectal	$CD133^+/CD166^+/EpCAM^+/CD44^+$	[21••] [89]
Gastric	$CD24^+/CD44^+$	[90]
Brain (glioma)	$CD133^+/CD15^+$	[39] [91]
Melanoma	$CD20^+/CD133^+$	[92, 93]
Liver	$CD90^+/CD133^+/EpCAM^+/CD45^-$	[94, 95]
Prostate	$CD44^+/\alpha2\beta1^+/CD133^+/ALDH^+$	[80] [96]
Lung	$CD133^+/CD44^+/CD90^+$	[97, 98]
Pancreatic	$CD44^+/CD24^+/EpCAM^+/CD133^+$	[99, 100]

Self-renewal Signaling in CSCs

Self-renewal capability of (cancer) stem cells has been known to be regulated by the Hedgehog, Notch, and Wnt/β-catenin pathways and the transcription factor B lymphoma Mo-MLV insertion region 1 (Bmi-1).

Activation of the Hedgehog signaling pathway is initiated by ligand binding to one of the three secreted glycoproteins found in mammals: Sonic (Shh), Desert (Dhh), and Indian (Ihh) Hedgehog. After secretion, these ligands bind to Patched (Ptch1), which is a 12-pass transmembrane spanning receptor. In the absence of ligands, Ptch1 constitutively represses the activity of Smoothened (Smo), a 7-pass transmembrane spanning protein, by directly binding to Smo receptors. Following Hh ligand binding to Ptch, the repression of Smo is relieved, and the expression of zinc transcription factors of Gli family is upregulated, leading to their translocation into the nucleus [14, 27]. Gli1 acts as a transcriptional activator, whereas Gli2 can either activate or repress gene expression depending on post-transcriptional and post-translational modifications [27]. Hedgehog signaling components including Ptch1, Gli1, and Gli2 are highly expressed in normal human mammary stem/progenitor cells cultured as mammospheres, and activation of these signaling increases the number of mammosphere-initiating cells and the mammosphere size [14]. In contrast, cyclopamine, the Hedgehog signaling inhibitor, decreased mammosphere formation of primary human mammary stem cells isolated from patients undergoing breast reduction surgery [14]. Silencing of Shh and Gli1 decreased

the proportion of aldehyde dehydrogenase (ALDH)-positive cell population and the thyrospheres size in anaplastic thyroid carcinoma (KAT-18) cells [28•].

Notch receptors 1–4 are non-covalent heterodimers consisting of an extracellular subunit and a transmembrane subunit. Notch signaling has been reported to be implicated in growth of hematopoietic and solid tumors [29], and inappropriate Notch activation stimulates cancer cell proliferation and prevents apoptosis [29]. High expression of Notch intracellular domain (NCID) in ductal carcinoma in situ (DCIS) was associated with the increased recurrence rate after surgery. A γ-secretase inhibitor, DAPT, which blocks Notch signaling, was shown to suppress the mammosphere-forming capacity of DCIS [30]. In addition, blockade of the Notch pathway by GSI-18, another γ-secretase inhibitor, abrogated the growth of DAOY medulloblastoma cells in culture [31]. Likewise, the anchorage-independent growth of DAOY cells was also markedly reduced when these cells were seeded in soft agar. Intraperitoneal administration of GSI-18 also blocked xenograft formation with no apparent side effects. These effects were attributed to depletion of cancer stem cells, as subpopulations expressing the stem cell marker CD133 as well as the stem-like side population were profoundly reduced following Notch blockade by GSI-18 treatment [31]. Moreover, inhibition of the Notch pathway rendered the glioblastoma stem cells more sensitive to radiotherapy. Thus, GSI treatment enhanced radiation-induced cell death and impaired clonogenic survival of glioma CSCs, but not bulk non-stem glioma cells. Knockdown of Notch1 or Notch2 increased the radiosensitivity of glioma stem cells through downregulation of Akt and Mcl-1 expression [32].

Wnt ligands interact with both secreted and membrane-associated proteins including, at least, ten 7-pass transmembrane Frizzled (Fzd) receptors, two low-density lipoprotein receptor-related proteins (LRP), and a number of extracellular Wnt-modulating proteins. When Wnt binds to Fzd/LRP, Dsh is recruited to the membrane and binds to the receptor complex. This, in turn, leads to recruitment of Axin and GSK3β, which facilitates phosphorylation of LRP by glycogen synthese kinase (GSK)-3β. When Axin and GSK3β are recruited to FZD/LRP, phosphorylation of β-catenin is prevented. β-Catenin accumulated in cytoplasm diffuses to the nucleus and binds to and activates the lymphoid-enhancer binding factor (LEF)/T cell-specific transcription factors (TCFs) [33, 34]. A role of Wnt/β-catenin signaling in maintenance of various CSCs has been reported [35]. Vermeulen et al. have reported that myofibroblast-secreted factors, such as hepatocyte growth factor, activate β-catenin-dependent transcription and subsequently enhance clonogenicity in myofibroblast (MFCM) and primary colon cell lines (CRC-MF49 and CRC-MF66). In MFCM transfected with TCF/LEF TOP-GFP lentiviral reporter gene, cells with a high Wnt signal activity exhibited elevated expression of colon cancer stem markers. In addition, TOP-GFP^{high} cells induced tumor growth in a xenograft model [35]. Expression of CD44, CD133, and CD166 CSC markers and nuclear localization of β-catenin were found to be higher in human colorectal tissues carrying *K-Ras* mutation [21••]. Likewise, spheres derived from *APC* mutant isogenic *K-Ras*-mutant cells showed elevated mRNA levels of Oct4, Nanog, and Sox2. In this study, injection of *K-Ras*-mutant cells into the spleen of mice caused liver metastasis. These findings suggest that oncogenic *K-Ras* induces distal metastasis of colorectal cancer cells harboring *APC* mutation through activation of CSCs [21••].

Apoptotic Signaling in CSCs

Apoptosis is an active and energy-dependent cell death process, which is mediated by two different pathways: the extrinsic (death receptor) and intrinsic (mitochondrial) pathways. Disordered apoptosis is frequently observed in various cancer cells, and apoptosis evasion is one of the hallmarks of cancer [36]. Apoptotic signaling pathways are also deregulated in CSCs. For example, survivin, known as an anti-apoptotic protein, was overexpressed in CD34$^+$/CD38$^-$ acute myeloid leukemia (AML) and glioma CSCs [37, 38]. Dysregulation of the intrinsic pathway in CSCs is accompanied by altered expression of Bcl-2 family proteins, which are composed of anti-apoptotic (i.e., Bcl-2, Bcl-xL, and Mcl-1) and pro-apoptotic proteins (i.e., Bax, Bak, Bid, and Puma). For instance, CD133$^+$ glioma CSCs express a high level of FLIP, Bcl-2, and Bcl-xL [39]. Similarly, CD44$^+$ CSCs from breast cancer patients also overexpress the anti-apoptotic protein Bcl-2 [40]. Compared to parent cells, cancer stem-like CD44$^+$/CD24$^+$ cells isolated from colon cancer (SW1222) cells exhibit not only increased Bcl-2 expression but also resistance to paclitaxel-induced cytotoxicity, which is mediated through activation of autophagy signaling [41]. Therefore, activation of pro-apoptotic pathways and inactivation of anti-apoptotic signaling molecules in CSCs may improve anti-tumor efficacy.

Dietary Cancer Preventive Phytochemicals Targeting CSCs

The discovery of CSCs and elucidation of their role in manifestation and recurrence of malignancy have helped us better understand tumor development, metastasis, and drug resistance. It has been suggested that some cancer chemopreventive phytochemicals target multiple pathways involved in stem cell maintenance and proliferation (Fig. 1). This results in sensitization of CSCs to chemotherapeutic agents, induction of their differentiation, and inhibition of self-renewal signaling. The following section summarizes how selected phytochemicals interfere with the signal transduction involved in self-renewal and survival of CSCs (also summarized in Table 2).

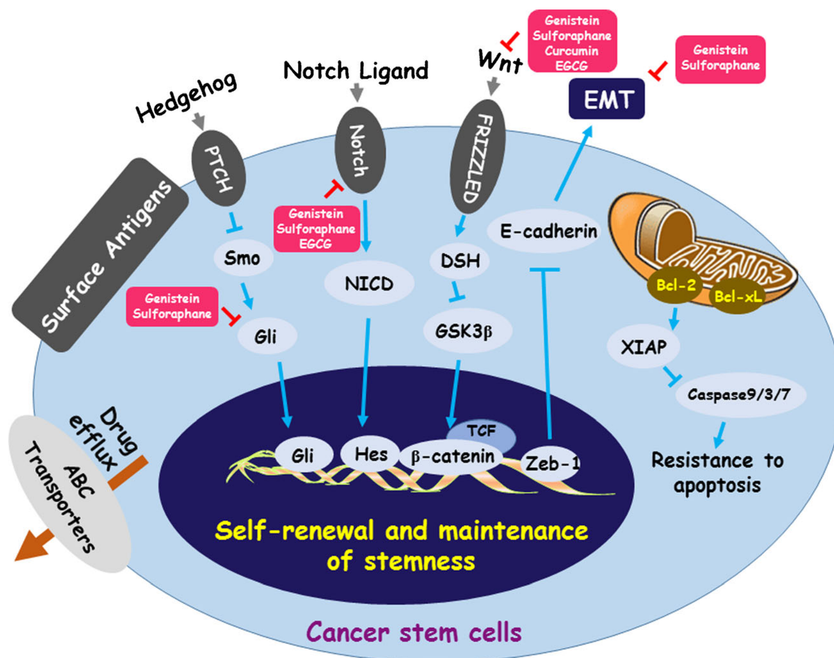

Fig. 1 Modulation of signaling pathways that regulate CSC self-renewal activity and stemness maintenance by chemopreventive/therapeutic phytochemicals

Genistein

Genistein (4,5,7-trihydroxyisoflavone), a major isoflavone constituent of soybeans and soy products, has been shown to exert an inhibitory effect on proliferation of various cancer cells. Epidemiological studies have revealed that consumption of genistein as part of soybean-based diet contributes to the reduced incidence of breast cancer [42]. In addition, genistein can overcome cancer drug resistance and attenuate the metastatic activity of tumor cells [43, 44]. Genistein has cancer cell growth inhibitory effects over a physiologically achievable concentration ranging from 10 nM to 20 μM [45]. Though physiologic concentration of genistein are considered to rarely exceed the nanomolar or low micromolar range, some Japanese individuals who intake relatively large amounts of soya products on regular basis exhibit submicromolar concentrations of this isoflavone [46].

Genistein (15–30 μM) was found to inhibit the expression of Shh, Gli1, and CD44 in tumorsphere cells of prostate origin

Table 2 Natural dietary compounds that regulate signal transduction involved in self-renewal maintenance and growth of CSCs

Tumor type	Phytochemicals	Effects
Breast cancer	Genistein	↓ ALDH expression; ↓ Smo and Gli expression ↓ $CD44^+/CD24^-/ESA^+$ subpopulation through inactivation of PI3K/Akt pathway
	Sulforaphane	↓ ALDH expression; ↓ β-Catenin and cyclin D1 expression
	Curcumin	↓ Wnt signaling; ↓ Microtentacles; ↓ $CD44^+/CD24^-$ subpopulation
	EGCG	↓ ALDH expression; ↓ VEGF-D expression ↓ Cyclin D1, RhoC, Bcl-xL and fibronectin expression
Prostate cancer	Genistein	↓ EMT phenotype; ↓ Shh, Gli1 and CD44 expression
	EGCG	↓ $CD44^+/\alpha2\beta1^+/CD133^+$ subpopulation; ↓ Vimentin, slug and snail expression ↓ β-Catenin nuclear accumulation; ↑ Apoptosis
Pancreatic cancer	Genistein Sulforaphane	↓ CD44 and EpCAM expression ↓ CD133, CD44, CD24 and ESA; ↓ Smo, Gli1 and Gli2 expression ↓ Oct4 and Nanog expression; ↓ Notch1 expression
Gastric cancer	Genistein	↓ Oct4, Sox2, Nanog, CD44 and CD90 expression; ↓ ABCG2 expression
Leukemia	Genistein	↓ $CD34^+/CD38^-$ subpopulation; ↑ Apoptosis
Colon cancer	Curcumin	↓ ALDH, CD44, CD133 and CD166 expression
HNSC	EGCG	↓ Oct4, Sox2, Nanog and CD44 expression ↓ Notch transcriptional activity
Skin cancer	EGCG	↓ CD34 expression
Glioma	EGCG	↑ Apoptosis, ↓ P-glycoprotein expression

[47]. As a consequence, tumorsphere formation of prostate cancer cells was suppressed. Moreover, intraperitoneal (i.p.) administration of genistein (10 mg/kg, twice a week for 3 months) inhibited prostate tumor growth in a subcutaneous xenograft model of tumorsphere cells derived from prostate cancer (22RV1 and DU145) cells [47]. It has been reported that low concentrations of genistein (0.2–15 μmol/L) inhibit the invasion by reversing the EMT phenotype in LNCaP human prostate cancer cells stably overexpressing HIF-1α and 1A8-ARCaP [48]. Similarly, genistein at a low concentration (5 or 10 μM) was shown to reduce the size and the number of mammospheres formed by breast cancer (MCF-7) cells, which was mediated by blocking the Hedgehog-Gli1 signaling pathway [49•]. According to a study by Gabriela Dontu and colleagues, 50,000 ALDH-negative cells failed to form tumors, while <500 ALDH-positive cells were able to generate a breast tumor in 40 days [50]. By utilizing a xenograft model of MCF-7 cells in nude mice, daily i.p. injection of 20 and 50 mg/kg genistein for 2 weeks suppressed the expression of ALDH and its mRNA transcript in the grafted tumors. In addition, expression of Smo and Gli was reduced by genistein treatment in MCF-7 cells and in xenograft model of MCF-7 cells [49•]. These findings suggest that downregulation of the Hedgehog pathway may contribute to the loss of stemness of CSCs in the presence of genistein.

Pretreatment of Notch-1- and FoxM1-overexpressing human pancreatic cancer (AsPC-1) cells with genistein (10–60 μM) reduced expression of some cell surface proteins including CD44 and EpCAM, which are putative markers of CSCs [51•, 52]. In AsPC-1 cells, overexpression of Notch-1 and FoxM1 is responsible for the acquisition of EMT and the CSCs phenotype, and this was attenuated by genistein treatment [51•, 52]. Bao et al. have reported that genistein inhibits growth, clonogenicity, migration and invasion, EMT, and sphere formation in pancreatic cancer cells, which were associated with reduced expression of CD44 and EpCAM [51•, 52]. In mammary glands of rats fed genistein diet, Wnt activation was decreased [53]. In another study, mammosphere formation in MCF-7 and MDA-MB-231 cells was inhibited by sera collected from adult female mice subjected to dietary intake of genistein [54]. In addition, treatment of sera to epithelial cells isolated from MMTV-Wnt-1 transgenic mouse mammary tumors reduced the size and the number of tumorspheres [54]. Under the same treatment condition, the proportion of $CD44^+/CD24^-/ESA^+$ subpopulation was decreased which appeared to be mediated through attenuation of PI3K/AKT signaling and upregulation of PTEN expression. When the Akt inhibitor perifosine was treated to MCF-7 cells, primary mammosphere formation was dramatically suppressed [54]. It has been reported that a low concentration of genistein (15 μM) inhibits self-renewal properties of human gastric cancer (MGC-803 and SGC-7901) cells by blocking expression of gastric CSC markers, such as Oct-4, Sox2, Nanog, CD44, and CD90 [55]. Moreover, in gastric cancer cells, resistance to 5-fluorouracil was attenuated by genistein (15 μM), which was associated with suppression of ABCG2 expression [55].

Sulforaphane

Sulforaphane, an isothiocyanate present in cruciferous vegetables, has been reported to have multiple mechanisms underlying its chemopreventive and chemotherapeutic effects. These include the induction of cytoprotective proteins and carcinogen-detoxifying enzymes [56], inhibition of inflammatory responses [57], induction of tumor cell apoptosis [58], and the blockade of angiogenesis and metastasis [59]. In recent studies, sulforaphane was found to be effective in targeting breast [60], prostate [61, 62], pancreatic [61], and leukemia [63] CSCs. The plasma levels of sulforaphane in rats reached 46 ng/ml after administration of 0.5 mg/kg, and human studies consistently indicated similar levels of 12 and 18.2 ng/ml in plasma after broccoli consumption [64–66].

In breast CSCs, sulforaphane (1–5 μmol/L) decreased the proportion of ALDH-positive cell population from 65 to 80 % and reduced the size and the number of primary mammospheres by 8- to 125-fold and 45 to 75 %, respectively. In a xenograft model of human breast cancer (SUM159) cells inoculated into NOD/SCID mice, daily injection of sulforaphane (50 mg/kg) for 2 weeks inhibited tumor growth as well as expression of β-catenin and cyclin D1 by as much as 77 % [60]. In pancreatic CSCs, activation of the sonic Hedgehog pathway has been known to drive the self-renewal of these cells [67]. Oral administration of sulforaphane (20 mg/kg) by gavage, 5 days a week for 6 weeks, inhibited the self-renewal activity of pancreatic CSCs ($CD133^+/CD44^+/CD24^+/ESA^+$) isolated from human pancreatic tumors and resulted in a 45 % reduction in growth of tumors derived from primary pancreatic CSCs orthotopically implanted into the pancreas of humanized NOD/SCID/IL2Rγ mice [67]. Sulforaphane reduced expression of Smo, Gli1, and Gli2- components of the Shh signaling pathway in mouse tumor tissue. In addition, sulforaphane inhibited the expression of Oct4 and Nanog as well as platelet-derived growth factor receptor (PDGFRα) and vascular endothelial growth factor (VEGF) which are downstream targets of Hedgehog signaling [68]. Moreover, sulforaphane treatment resulted in a significant reduction of the EMT marker Zeb-1 transcription factor and an increase of E-cadherin expression [68].

Combination of an anti-cancer drug (one of cisplatin, gemcitabine, doxorubicin, and 5-fluorouracil) with sulforaphane synergistically inhibited clonogenicity, spheroid formation, and ALDH activity in CSC-enriched prostate cancer (DU145) cells [61]. In another study, combination of sulforaphane and recombinant soluble TRAIL was found to be

superior to single treatment in reducing tumor growth and CSCs marker expression in the xenotransplantation model of androgen-independent prostate cancer (PC3) cells into chorioallantoic membrane of fertilized chicken eggs. This was associated with the inhibition of TRAIL-induced NF-κB DNA binding activity and expression of CXCR4, Jagged1, Notch1, Sox2, and Nanog [62]. Notably, pancreatic CSCs treated with sulforaphane (5 μM) downregulated Notch1 expression, whereas gemcitabine (25 nM) treatment led to induction of Notch1. However, co-treatment of sulforaphane and gemcitabine inhibited spheroid formation of pancreatic cancer cells, which was associated with downregulation of Notch1 mediated by blocking c-Rel expression [61]. Isolated imatinib-resistant leukemia stem cells ($CD34^+/CD38^-$) showed higher expression of Oct4, CD133, β-catenin, and Sox2 than did $CD34^+/CD38^+$ counterpart cells. Co-treatment sulforaphane (30 μM) sensitized the $CD34^+/CD38^-$ leukemia stem cells to imatinib-induced apoptosis. This increased sensitivity to the anti-cancer drug was attributable to activation of caspase-3, PARP, and Bax as well as decreased Bcl-2 expression [63].

Curcumin

Curcumin, a principal yellow coloring ingredient found in turmeric, has been used for centuries in traditional oriental medicine to treat inflammatory disorders. The anti-inflammatory properties of curcumin account for its anti-carcinogenic and anti-mutagenic effects in many experimental studies [69] and some clinical studies [70]. Howells et al. reported that in vitro studies with curcumin in the 10 μM or lower micromolar range have human physiological relevance [71]. Daily treatment of 5 μM curcumin gradually decreased the proportion of the stem-like cancer cell subpopulation in rat glioma (C6) cells from day 3 to day 10 [72]. Overexpression of Wnt-1 in the mammary gland of MMTV-Wnt-1 transgenic mice resulted in formation of mammary adenocarcinomas [73]. The mammosphere formation decreased by curcumin (50 μM) treatment was found to be associated with the inhibition of the Wnt signaling pathway in normal human breast tissue cells isolated from women undergoing elective reduction mammoplasty and also in breast cancer (MCF-7) cells. More recently, Charpentier et al. have reported that curcumin treatment results in a significant reduction of reattachment efficiency in stem-like cancer cells [74••]. It has been recognized that breast cancer cell lines with more stem-like properties display higher levels of microtentacles (McTN), a type of tubulin-based protrusion of the plasma cell membrane that forms in detached or suspended cells and stimulate cell reattachment. In addition, McTNs are more abundant in metastatic breast carcinoma cell lines [75]. Curcumin, when treated at a non-apoptotic concentration, significantly reduced not only the proportion of cells expressing the $CD44^+/CD24^-$ stem cell marker phenotype but also the McTN frequencies in triple-negative mammary gland carcinoma (BT-549) cells which exhibit a high degree of stem cell characteristics [74••].

Treatment of 10 μM curcumin decreased the sphere formation in the ALDH-positive population cells isolated from primary human breast cells [76]. Likewise, combined treatment of curcumin and dasatinib by gavage inhibited expression of several CSC markers including ALDH, CD44, CD133, and CD166 in remnants of spontaneous adenomas from *APC*$^{Min+/-}$ mice [77]. Several in vitro studies have demonstrated that co-treatment of an anti-cancer drug and curcumin suppresses the development of stemness characteristics [77, 78]. In chemo-resistant colon cancer cells treated with dasatinib and curcumin, decreased colonosphere formation was found to be associated with the 25–30 % inhibition of mRNA expression of CD133, CD44, CD166, and ALDH [77]. In addition, in the laryngeal carcinoma cell line HEp-2, about 1.5–3.5 % of cells showed the characteristics of CD133-positive CSCs, which are responsible for resistance to cisplatin [78]. When cisplatin was treated together with curcumin, the CD133-positive population was markedly reduced in HEp-2 cells. The reduced expression of ABCG2 by curcumin in CD133-positive sorting cells has been shown to account for the induced sensitivity of CD133-positive cells to cisplatin [78].

EGCG

The green tea polyphenol epigallocatechin gallate (EGCG) possesses antioxidant, antitumorigenic, anti-inflammatory, and antiangiogenic activities [79]. It has been reported that the plasma bioavailability of EGCG is in the range of 0.1–7 μM in humans and concentrations over 100 μM EGCG is observed in saliva [71]. Most in vitro studies were conducted at concentrations ranging from 10 to 100 μM that exceed physiological serum levels of EGCG in humans. However, consumption of green tea has potential health benefits in human epidemiological studies. EGCG (30–60 μM) inhibited self-renewal capacity of prostate CSCs expressing $CD44^+/\alpha2\beta1^+/CD133^+$ and human prostate cancer (PC-3 and LNCaP) cells [80]. As an underlying mechanism of its self-renewal inhibitory activity, EGCG inhibited EMT in prostate CSCs by blocking expression of Vimentin, Slug, Snail, and nuclear β-catenin as well as LEF-1/TCF reporter gene activity. EGCG induced apoptosis through activation of caspase-3/7 and inhibition of Bcl-2, survivin, and XIAP protein expression. EGCG retarded migration and invasion in prostate CSCs [80]. Suppression of self-renewal capacity of head and neck squamous carcinoma (HNSC) CSCs by 5 μM EGCG was found to be associated with decreased expression of stem cell markers, such as Oct4, Sox2, Nanog, and CD44 [81]. When these cells treated with cisplatin (10 μM) and EGCG (5 μM) were subcutaneously injected into female BALB/c nude mice, combination treatment inhibited tumor formation and induced

apoptosis. In addition, EGCG decreased the transcriptional activity of the Notch in HNSC CSCs [81]. Moreover, i.p. injection of 16.5 mg/kg EGCG for 5 days a week suppressed the growth and lymphangiogenic capacity of breast tumors derived from ALDH-positive stem-like breast cancer (SUM-149) cells in a murine xenograft model [82]. Tumorsphere formation by SUM-149 cells was also significantly inhibited by EGCG (40 μg/ml) treatment, suggesting its effects on self-renewal ability, which was associated with downregulation of VEGF-D. In addition, EGCG substantially decreased mRNA levels of cyclin D1, RhoC, Bcl-xL, and fibronectin in SUM-149 cells [82]. Topical application of peracetylated EGCG (1–5 μmol) markedly inhibits the number of tumors in 7,12-dimethylbenzo[*a*]anthracene-initiated and 12-*O*-tetradecanoylphorbol-13-acetate-promoted mouse skin tumorigenesis [83••]. Expression of the cutaneous CSCs marker $CD34^+$ and activation of protein kinase D1 (PKD1) is known to be involved in the process of skin promotion. In this study, PKD1 was found to be strongly expressed in $CD34^+$ cells, which was diminished by treatment of peracetylated EGCG. The compound also remarkably suppressed activation of nuclear factor-κB, CREB, and C/EBPs by inhibiting the phosphorylation of JNK1/2, p38, and PI3K/Akt and by attenuating protein expression of downstream target molecules, such as iNOS, COX-2, ODC, and VEGF [83••].

More recently, it has been reported that EGCG can induce apoptosis in some CSCs. Treatment of glioma (U87) stem-like cells (GSLCs) with EGCG induced apoptotic cell death, which was mediated via inactivation of Akt, leading to downregulation of Bcl-2 and induction of PARP cleavage. In this study, EGCG (50–200 μM) also inhibited neurosphere formation and migration of U87 GSLCs. In addition, EGCG enhanced the sensitivity of U87 GSLCs to temozolomide, which was mediated by blocking expression of drug resistant genes, such as P-glycoprotein, but not that of ATP-binding cassette transporter subfamily G member 2 and O^6-methylguanine-DNA methyltransferase [84]. Likewise, inhibition of sphere growth by EGCG (20–40 μM) was associated with suppression of STAT3 phosphorylation and expression of genes related to cell growth and survival, including Bcl-2, c-Myc, and survivin in nasopharyngeal carcinoma (NPC; TW01 and TW06) cells [85].

Conclusion

A wide variety of phytochemicals modulate CSC markers and related signaling pathways that are involved in the self-renewal capacity. CSCs are responsible not only for tumor initiation, development, and metastasis but also for resistance to anticancer therapy. Thus, attempts have been made in recent years to explore therapeutic/preventive potential of phytochemicals targeting CSCs, thereby overcoming the limitations of conventional anticancer treatment. Up to date, some dietary components have been shown to inhibit self-renewal and EMT signaling at relatively low concentrations. Further studies are needed to determine their efficacy and effective concentrations against CSCs and to elucidate their mechanism of action. It is expected that edible phytochemicals, by targeting CSCs, can reduce cancer therapeutic resistance and tumor recurrence and improve patient survival.

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Compliance with Ethics Guidelines

Conflict of Interest Do-Hee Kim and Young-Joon Surh declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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