DIETARY PHYTOCHEMICALS ON ELIMINATION OF CANCER STEM CELLS THROUGH TARGETING ITS QUIESCENCE AND SURVIVAL MECHANISMS

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Abstract

According to tradition, plants and herbs are potential cures for numerous illnesses. In recent decades, they have gained worldwide recognition as critical sources of new drugs, especially for cancer, and the focus on medicinal plant research has increased tremendously. Currently, research scientists attribute the occurrence of post-chemotherapy tumour exacerbation to the presence of cancer stem cells hidden in the bulk of the malignant tumour, forming a rare subpopulation that plays a major role in tumour re-initiation, progression and recurrence. There is increasing evidence of cancer stem cells in a wide array of tumours, and these cells could be the crucial target for future chemotherapy approaches. This review of the major plant-based phytochemicals with documented antagonistic activities against cancer stem cells, their molecular targets and the mechanisms involved, may serve as a guide for developing from the existing natural agents, more effective, and broader spectrum anti-cancer stem cell compounds with the potential for clinical application.

Keywords: *Phytochemical, Chemotherapy, Cancer Stem Cell, Plant-Derived Product, Natural Product*

Introduction

Cancer is a primary cause of morbidity and mortality worldwide, with an estimated 27 million new cases and 17 million deaths by the year 2030. It takes about 20 to 30 years for cancer to form and grow, as a result of long term inappropriate diet and lifestyle, and various hereditary and environmental factors (1, 2). In recent decades, scientists have come to recognise that a tumour is made up of different types of cells, among which cancer stem cells (CSCs) form a minor population within the tumour mass, differentiating into heterogeneous cells to add to the existing tumour mass or establish a secondary tumour (3). CSC is an operational term for the subpopulations commonly referred to as cancer-initiating cells, cancer stem-like cells or side population cells with the ability to self-renew and differentiate, induce angiogenesis, resist apoptosis, and with a longer lifespan than other cancer cells (3).

There are two primary speculations on the origin of CSCs. In the first theory, CSCs are thought to arise from the mutated normal stem cells with multiple shared characteristics, including the ability to activate a self-renewal program, with differentiation, and telomerase induction. However, these CSCs lack the tight regulation of proliferation and the genomic integrity that the normal stem cells possess. In the second theory, CSCs are derived from the matured and differentiated cancer cells through de-differentiation or trans-differentiation, regulated by epigenetic or genetic factors. Regardless of their origin, these cells exhibit unique properties of self-renewing and differentiation with heightened invasion and migration capacity, enhanced telomere-lengthening activity, resistance to chemotherapeutic drugs, and stimulation of anti-apoptotic activity (4-6). The initial hypothesis of CSC marked a significant milestone in cancer research as it provided a convincing explanation for the tumour relapse phenomenon frequently observed in the patient who has undergone multiple cancer therapies.

The subpopulation of CSCs was first reported in patients with acute myeloid leukaemia (AML) where two distinct leukaemic cell populations were identified from their surface markers CD34 and CD38. These cells were injected

separately into immunocompromised mice, and it was found that only the cell population marked by CD34+CD38- successfully mimicked the AML disease. The relatively more mature cell population designated by CD34+CD38+ were not able to. Characteristically, the recapitulated leukaemic cells in the mice displayed similar morphology and bone marrow localisation as in the original AML patient (7). Since then, tumorigenic CSCs have been reported in numerous other solid tumours. For instance, the presence of the hyaluronic acid-binding receptor (CD44), and the loss of the CD24 expression marked the CSC population correlated with higher invasive and metastatic capacity. Other surface markers significantly associated with the CSC population included CD34, ALDH1 and glycosylated CD133 surface proteins (8).

For a relapse to occur, CSCs must survive the harsh tumour microenvironment and cancer therapies, and drive its self-renewing and uncontrolled proliferation (9). As a part of survival, mechanism CSCs are relatively quiescent with a lower cycling rate in the dormant stage of the cell cycle. As a result, the cells become insensitive to many of the chemotherapeutic drugs which target the highly proliferative cancer cells. Additionally, they also express a higher number of drug efflux ABC transporter proteins or detoxifying enzymes that preserve the genome from the chemical attacks of the chemotherapeutic drug that have successfully penetrated into the CSCs (10). Like most cancer cells, CSCs also display an ability to evade cell death by expressing the FLICE-inhibitory protein, which renders them resistant to TRAIL-induced apoptosis. Overexpression of the anti-apoptotic proteins such as IAP, CXCR1 and the BCL2 family of proteins also contributes to the enhanced survival of CSCs. Furthermore, CSCs usually reside in a hypoxic tumour microenvironment or niche that offers another protection from the external assaults (4, 10).

The ability of CSCs to initiate tumour growth and metastasis are attributed to a self-renewing capacity characterised by symmetric and asymmetric cell division. Symmetric division occurs when two daughter cells produced, have equivalent cell fates, and asymmetric division refers to the production of two daughter cells with distinct cell fates. True self-renewing CSCs can undergo serial transplantation that is defined by the capability to regenerate secondary and tertiary tumours upon serial grafting, for example, from the parental mouse tumour cells into subsequent mouse recipients. The steps are often governed by stem cell regulatory pathways, which include Wnt/β-catenin, Notch, Hedgehog, PI3K/AKT/mTOR, NF-κB, JAK/STAT3 and BMI-1, collectively termed the stemness genes that function to maintain the CSC population (4, 11). The CSCs may also play a role in promoting metastasis by acquiring mesenchymal properties, releasing angiogenic and lymphangiogenic factors that stimulate the growth of new blood and lymph vessels. A study by Hong et al. demonstrated that breast cancer cell lines that possess greater lymphatic metastatic activity contain a higher percentage of CSCs and that cancer cells derived from lymph node metastasis also present higher similarity to the CSC phenotype (10).

Dietary Phytochemicals Targeting CSCs

To find a potential cure for cancer, CSC eradication is exceptionally crucial with the targeting of the numerous CSC regulatory signalling pathways for an effective anti-cancer treatment. Some dietary phytochemicals have been reported to inhibit self-renewal, tumour regrowth and/or CSC maintenance through their anti-proliferative, anti-oxidative, apoptosis-inducing ability, as well as other anti-CSC activities.

Dietary phytochemicals are beneficial as they are well tolerated, are found in various food products and are easily added to the daily diet or taken as a supplement. Due to their minimal toxicity, the phytochemicals could also be consumed on a long term basis as a chemopreventive agent (13). A summary of natural products documented with promising anti-CSCs efficacy is presented in Table 1. The molecular structures for most of the phytochemicals that will be discussed in the following sections are illustrated in Figure 1.

Table 1: Dietary phytochemicals, plant source and their anti-CSCs functions

Phytochemical	Plant source	Functions
Curcumin and derivatives	*Curcuma longa*	Induce quiescent CSCs to proliferate Inhibit CSCs drug resistance ability Inhibit CSCs self-renewal activity Induce CSCs apoptosis Induce CSCs differentiation Induce oxidative stress in CSCs Induce methylation of tumorigenic genes such as EGFR
Baicalein and Scutellaria extract	*Scutellaria Baicalensis*	Inhibit CSCs drug resistance ability Inhibit CSCs self-renewal activity Induce CSCs apoptosis
Berberine	*Berberis Hydrastis canadensis Tinospora cordifolia*	Inhibit CSCs drug resistance ability Inhibit CSCs self-renewal activity Induce CSCs apoptosis
Epigallocatechin-3-gallate and derivatives	Tea	Inhibit CSCs drug resistance ability Inhibit CSCs self-renewal activity Induce CSCs apoptosis Regulate CSCs microenvironment Reverse methylation of tumor-suppressive genes such as RARβ Induce mesenchymal-to-epithelial transition in CSCs Inhibit CSCs migration and invasion

Phytochemical	Plant source	Functions
Genistein	Soybean	Inhibit CSCs drug resistance ability Inhibit CSCs self-renewal activity Induce mesenchymal-to-epithelial transition in CSCs Inhibit CSCs migration and invasion
Berbamine	*Berberis amurensis*	Inhibit CSCs self-renewal activity
Honokiol	*Magnolia spp*	Inhibit CSCs self-renewal activity Induce CSCs apoptosis Induce mesenchymal-to-epithelial transition in CSCs Inhibit CSCs migration and invasion
Psoralidin	*Psoralea corylifolia*	Inhibit CSCs self-renewal activity Induce CSCs apoptosis
Sulforaphane	Broccoli/ broccoli sprouts	Inhibit CSCs self-renewal activity Induce chemo-sensitisation of CSCs to anti-cancer drugs Induce CSCs apoptosis Induce oxidative stress in CSCs Induce mesenchymal-to-epithelial transition in CSCs
Aronia melanocarpa juice	*Aronia melanocarpa*	Inhibit CSCs self-renewal activity Induce CSCs apoptosis Induce methylation of tumorigenic genes such as UHRF1
AR seed extract	*Alcea rosea*	Inhibit CSCs self-renewal activity Induce CSCs apoptosis
YMGKI-1	*Antrodia cinnamomea*	Inhibit CSCs self-renewal activity Induce CSCs autophagy Induce CSCs differentiation
Parthenolide and derivatives	*Tanacetum parthenium*	Induce oxidative stress in CSCs Inhibit CSCs self-renewal activity Induce CSCs apoptosis
Vitamin A compounds	Fruits and vegetables that are dark green or orange in color	Inhibit CSCs self-renewal activity Induce CSCs differentiation Induce chemo-sensitisation of CSCs to anti-cancer drugs
Oxymatrine	*Sophorae flavescens*	Inhibit CSCs self-renewal activity
Cyclopamine	*Veratrum californicum*	Inhibit CSCs self-renewal activity
Isoliquiritigenin	Licorice root	Inhibit CSCs self-renewal activity Reverse methylation of tumor-suppressive genes such as WIF1
Quercetin	Commonly found in apples, cranberries, blueberries and onions	Inhibit CSCs self-renewal activity Induce CSCs apoptosis
Eckol	*Ecklonia cava*	Inhibit CSCs self-renewal activity Induce chemosensitisation of CSCs to anti-cancer drugs
Vitamin E and derivatives	Plant foods such as oils, nuts, grains, fruits and wheat germ oil, sunflower, and safflower oils	Inhibit CSCs self-renewal activity Alter oxidative state in CSCs Alter CSCs metabolic activity
Indole-3-carbinol	*Brassica* genus such as broccoli and cabbage	Inhibit CSCs self-renewal activity Induce CSCs apoptosis
Resveratrol	Grapes, peanuts, berries	Inhibit CSCs self-renewal activity Induce CSCs autophagy Induce mesenchymal-to-epithelial transition in CSCs Inhibit CSCs migration and invasion
Ginsenoside F2	Ginseng	Induce CSCs autophagy Induce CSCs apoptosis
Gossypol	*Gossypium* species	Inhibit CSCs self-renewal activity Induce CSCs apoptosis
Koenimbin	*Murraya koenigii*	Inhibit CSCs self-renewal activity Induce CSCs apoptosis
Lupeol	Vegetables (white cabbage, pepper, cucumber, tomato) and fruits (olive, fig, mango, strawberry, red grapes)	Inhibit CSCs self-renewal activity Induce chemosensitisation of CSCs to anti-cancer drugs
Metformin	*Galega officinalis*	Inhibit CSCs self-renewal activity Induce mesenchymal-to-epithelial transition in CSCs Inhibit CSCs migration and invasion
Mulberry leaf extract	*Morus* genus	Induce CSCs differentiation

Phytochemical	Plant source	Functions
Pomegranate extract (PE)	*Punica granatum*	Induce quiescent CSCs to proliferate Inhibit CSCs self-renewal activity Induce CSCs apoptosis
Pomiferin	*Maclura pomifera*	Inhibit CSCs self-renewal activity
Triptolide	*Tripterygium wilfordii*	Inhibit CSCs self-renewal activity Induce CSCs apoptosis
3, 3-diindolylmethane	Cruciferous vegetables such as broccoli, brussels sprouts, cabbage and kale	Inhibit CSCs self-renewal activity Induce CSCs apoptosis
6-shogaol	*Zingiber officinale*	Inhibit CSCs self-renewal activity Induce CSCs autophagy
Rooperol	*Hypoxis hemerocallidea*	Inhibit CSCs self-renewal activity Induce CSCs apoptosis Induce oxidative stress in CSCs
Glabridin	*Glycyrrhiza glabra*	Inhibit CSCs self-renewal activity Induce mesenchymal-to-epithelial transition in CSCs

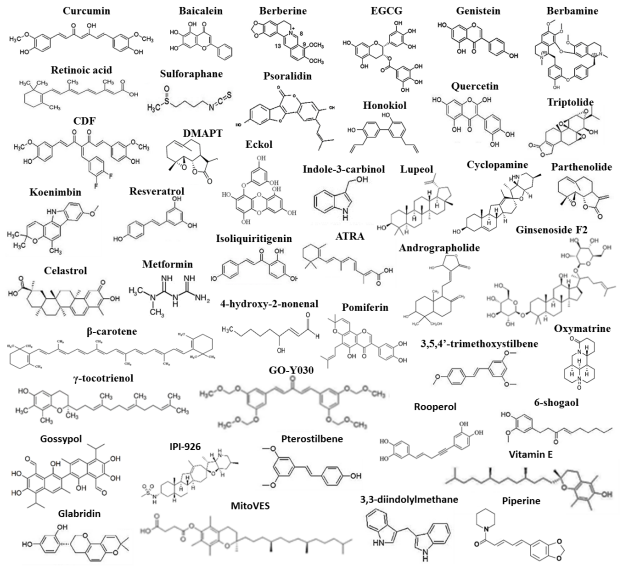

Figure 1: Plant phytochemical molecular structures

Dietary phytochemicals targeting CSCs quiescence and drug resistance

A relatively slower proliferating or quiescent CSC population is a contributing factor to drug resistance as these cells do not undergo a complete cell cycle arrest and can, therefore, be reactivated to enter an active cell cycle. Many *in vivo* and *in vitro* studies have reported that this quiescent cell population can survive through conventional chemotherapy, which mainly kills the highly proliferating mature cancer cells. This explains why a tumour could regrow in a patient whose primary tumour seemed to have fully regressed. The quiescence state in CSCs is usually achieved by regulating the quiescence signature that includes increased expression of cell cycle regulators such as p53, cyclin D2 and MXI1; upregulation of the key stem cell-associated pathways such as Notch signalling, which control the reversibility of cell quiescence by prevention of cell entry into an irreversible cell cycle of arrest and differentiation; and expressing cyclin-dependent kinase inhibitors such as p21 that govern the maintenance and cell entry into the quiescence state (14-16). In order to allow these quiescent CSCs to be targeted by the anti-cancer drugs, the cells must first be triggered to enter an active proliferative state. This was demonstrated by Gardana et al. using low concentrations of curcumin isolated from *Curcuma longa* to treat the quiescent leukaemia-like stem cells (QLC). The induced proliferation of QLC was observed with an increase in cell yield due to a lower doubling time of the cells. These proliferating leukaemia cells that had been pushed into the S phase of the cell cycle exhibited greater sensitivity to the toxicity effects of 5-fluorouracil (17).

Enhanced expression of drug efflux transporters remains the primary cause of drug resistance in CSCs. There is accumulating evidence that CSCs overexpress chemoresistance-conferring adenosine triphosphate–binding cassette (ABC) transporters that facilitate the translocation of compounds against a concentration gradient across the biological membrane by ATP hydrolysis (18, 19). For instance, the CD133+ CSCs population isolated from a glioblastoma demonstrated greater resistance to the chemotherapeutic drugs such as carboplatin, etoposide, paclitaxel and temozolomide, with a higher cancer recurrence rate and the formation of a secondary tumour most probably from the overexpression of drug efflux transporters. Upregulation of ABC transporters has also been correlated with drug resistance of CSCs in other cancers in the liver, colon and rectum, breast and pancreas, and in the melanoma (19). The selection of clones of CSCs with enhanced drug resistance phenotype after each round of the treatment cycle will eventually lead to an emergence of a more aggressive tumour recurrence. Formulation of more effective therapy is, therefore, necessary to solve this issue of drug resistance in CSCs. With curcumin, Zhang et al. were able to reduce the CD133+ population by sensitising the CSCs to cisplatin as a result of decreased ABCG2 drug resistance gene expression, thus reducing the CSCs proliferation and clonogenicity in laryngeal carcinoma Hep-2 cells (20). Zhou et al. showed that the anti-cancer activity of mitomycin C was improved by reducing the resistance of $CD44^{+}CD24^{-/low}$ breast cancer stem-like cells via inhibition of the expression of *ABCG2* and *ABCC1* genes. These sensitised CSCs were unable to propagate to the fifth generation due to the lowered self-renewing ability (21). In addition to curcumin, other studies have also confirmed the therapeutic efficacy of the various phytochemicals to eliminate chemoresistant CSCs. Baicalein (*Scutellaria baicalensis Georgi*) (22, 23), berberine (*Berberis, Hydrastis*

canadensis and *Tinospora cordifolia*) (24), epigallocatechin-3-gallate or EGCG (green tea) (25) and genistein (soybean) (26) have all demonstrated inhibition of *ABCG2* expression in myeloma, breast cancer, head and neck cancer as well as gastric cancer, respectively.

Dietary phytochemicals targeting CSCs self-renewal, differentiation and survival regulatory pathways

Normal stem cells maintain their stemness phenotype via multiple regulatory pathways, and the primary players include Wnt/β-catenin, Notch, Hedgehog, STAT3, PI3K/AKT/mTOR and apoptotic signalling. These key signalling pathways could transform the cells into CSCs if they are abnormally activated or mutated in the cancer cells, thus making them potential therapeutic targets (27).

First and foremost, Wnt/β-catenin signalling is essential for the regulation of CSCs. This signalling is activated upon binding of the Wnt ligand to both its secreted and membrane-associated proteins, such as the transmembrane receptors frizzled (Frz) protein, that results in the stimulation and recruitment of the Dishevelled (Dsh) protein. This Wnt/Frz/Dsh protein complex will subsequently bind to Axin and repress Gsk3β activity, thus preventing the phosphorylation and degradation of β-catenin. The accumulated β-catenin will then be translocated into the nucleus to activate the transcription of the downstream target genes responsible for proliferation, self-renewal and survival. Through controlling β-catenin delocalisation, this pathway is also crucial for the maintenance of a balance between cell proliferation and differentiation. Wnt/β-catenin is one of the evolutionarily conserved signalling pathways that are critical during embryogenesis and the maintenance of adult organs, including the gastrointestinal tract. These signalling pathways could also be expressed aberrantly in the cancer cells to give rise to the CSC population as a result of genetic and/or epigenetic modifications. Human colorectal tissues that exhibit nuclear delocalisation of β-catenin express higher levels of CSCs markers including CD133, CD44 and CD166. Studies have demonstrated that the knocking down of β-catenin or deletion of the Wnt target gene in the intestinal CD44+ population could eliminate the CSCs and inhibit tumorigenesis (4, 10, 28).

Notch signalling is also implicated during embryogenesis to regulate the differentiation process to ensure cellular homeostasis. It functions through four non-covalent heterodimeric Notch receptors (Notch 1-4) in conjunction with five ligands (DLL1, DLL3, DLL4, JAG 1 and JAG2) to activate the downstream target genes such as *cyclin D1*, *NF-κB*, *Akt*, *mTOR*, *Mcl1, cMyc* and vascular endothelial growth factor (*VEGF*). Aberrant Notch signalling has been shown to confer proliferative and survival advantages in both haematopoietic and solid tumours. More recently, Notch receptors 1 and 2 were reported to be upregulated in pancreatic CSCs with an enhanced CD44 and EpCAM expression. The overexpression of the Notch intracellular domain in ductal carcinoma in situ has been correlated with a higher chance of relapse after cancer therapy. The addition of a Notch pathway inhibitor to the medulloblastoma cells led to a significant reduction in its anchorage-independent growth and xenograft formation, attributed to the elimination of its CD133+ CSCs population. The knockdown of Notch 1 or 2 also suppressed the expression of Akt and Mcl-1 proteins that led to an improved radio-sensitivity of the glioma CSCs (4, 28).

Being a part of the stemness regulatory signalling pathways, Hedgehog signalling has been implicated in the embryonic development, normal tissue repair and the EMT process through the control of proliferation, survival and angiogenesis. It is frequently deregulated in CSCs. Hedgehog signalling is triggered when one of its three ligands (Shh, Dhh or Ihh), is bound to the transmembrane receptor Patched1 (PTCH1). In the unbound state, the function of PTCH1 is to attach to the Smo receptor and repress its activity. However, when the Hedgehog ligand binds and internalises the PTCH1 receptor, Smo inhibition will be lifted, and subsequently, the GLI family zinc transcription factors could be expressed and translocated into the nucleus. GLI1 is primarily a transcriptional activator while GLI2 could be a suppressive or promoting factor and their target genes include HES family of proteins, SNAIL1, Cyclin D1, c-MYC, BCL2 and VEGF. In colorectal, liver, breast and pancreatic cancers, upregulation of Hedgehog signalling components (PTCH1, GLI1 and GLI2) and its target genes such as *Snail1* which is correlated with EMT, have been observed in the CSCs population with an enhanced metastatic and tumour sphere-forming capacity (4, 28).

Inflammation could be another promoting factor for CSCs formation through the actions of several inflammatory cytokines, including IL-6, which had been shown to activate STAT3 signalling. Activated STAT3 signalling is commonly involved in the governing of biological functions mainly related to cancer development such as cell proliferation, survival, angiogenesis and immune evasion. Aberrant regulation of STAT3 signalling has been reported not only in solid tumours such as hepatocellular and colorectal cancers but more importantly, in their CSCs compartment. More recently, activation of IL-6/STAT3 signalling has been associated with the anti-cancer drug resistance phenomenon (10, 28).

PI3K, AKT and mTOR are distinct pathways which are highly interconnected to one another that they are often thought of as a single unique signalling pathway. These pathways also play critical roles in cell growth and survival during cellular stress. Upregulation of mutated PI3K, AKT (AKT1 and AKT2) and mTOR (mTORC1 and mTORC2) were reported in numerous cancers such as colorectal, hepatic, gastric and pancreatic. Activated PI3K/AKT/mTOR signalling often leads to inactivation of pro-apoptotic signals (procaspase-9, Bad and the Forkhead family of transcription factors that trigger FasL expression) while stimulating the proliferative factors (IRS-1, Raf-1 and Cyclin D1), as well as suppressing the anti-proliferative factors (GSK3, p21CIP1/WAF1 and p27KIP1). Radiotherapy resistance in intestinal cancers is frequently

associated with increased CSCs features due to an active PI3K/Akt/mTOR signalling. Inhibiting the PI3K/AKT/mTOR pathway in the colon or hepatic cancer cells could repress CSCs proliferation by reducing their stemness markers such as CD133, LGR5, ALDH1 and EpCAM. Suppression of this pathway could also promote the conversion of CSCs into mature cancer cells (28, 29). Alternatively, PTEN, which is a downstream target of this signalling, could also be exploited as it has a tumour suppressor function by limiting cell growth and sensitising cancer cells to apoptosis. In many cancer types such as breast, endometrial and thyroid, PTEN had mutated or had lost its native function, therefore, allowing a constitutive activation of PI3K/AKT/mTOR signalling in the cancer cells (29).

Mitogen-activated protein kinase (MAPK) pathways, especially ERK signalling, are among the regulatory pathways that function in the regulation of cellular proliferation and differentiation. It is of particular interest because the activation status of ERK signalling will determine the early cell differentiation commitment during embryonic development. For instance, deletion of the upstream ERK pathway activator gene, *Grb2*, could lead to the blockage of primitive endoderm and trophectoderm development. In a separate study, an active ERK signalling had also been shown to be essential for the pre-adipocyte differentiation into a mature adipocyte. ERK is mainly stimulated by a cascade of upstream activators beginning with Ras molecule, followed by several tyrosine kinases (MAPK and MAPKK) and typically involves a series of threonine and tyrosine residues phosphorylation processes. The activated ERK will then be translocated into the nucleus to activate its downstream target genes such as Pdx1, *Elk1* and *neuroD1*, which are all differentiation-inducing genes (30-32).

Survival is another important characteristic of CSCs, primarily by evading apoptosis, the active and energy-dependent programmed cell death process. There are two major apoptotic pathways: the intrinsic or mitochondrial signalling and the extrinsic or death receptor signalling. As expected in several tumours and CSCs, dysregulated apoptosis or survival mechanisms are usually active and so cell death evasion becomes a significant hallmark for both cancer and CSCs. Apoptotic pathway dysregulation is usually attributed to the overexpression of the anti-apoptotic BCL-2 family of proteins such as BCL-2, BCL-xL and MCL-1, with down-regulation of the pro-apoptotic BCL-2 family of proteins, including BAK, BID, BAX and PUMA. For example, glioma and breast CSCs usually have a higher expression of anti-apoptotic BCL-2, BCL-xL and FLIP proteins. In some cancer types such as the colon cancer cell line (SW1222), the isolated CSCs expressed not only an increase in BCL-2 expression but also showed paclitaxel resistance via an active autophagy signalling. Besides the BCL-2 family of proteins, the upregulated expression of anti-apoptotic proteins such as survivin is also common in AML and glioma CSCs (4).

Seeing the importance of the above signalling pathways in maintaining stemness and survival characteristics of CSCs, they, therefore, present an important avenue for targeting by the dietary phytochemicals in our combat against CSCs, as is discussed below.

Curcumin

Curcumin is a diferuloylmethane compound found in *C. longa*, and its anti-CSCs properties have been well studied. Treatment with curcumin could reduce the ability of primary and secondary spheres formation among the oesophageal cancer cells. The glioma side population cells were also decreased at low concentrations of curcumin (33). In addition, it can inhibit the migratory and EMT activity of breast CSCs by blocking the nuclear β-catenin translocation, therefore repressing Slug activation and restoration of the E-cadherin expression (34). Furthermore, curcumin downregulates the IGF1, STAT3 and Hedgehog signalling pathways to inhibit brain tumour cell proliferation through G2/M phase cell cycle arrest, reducing the clonogenicity of the CSCs (35). Curcumin also possesses the ability to induce cellular differentiation in the glioma cells as demonstrated in both *in vivo* and *in vitro* studies. The results showed an increased differentiation of cancer markers which include GFAP, bIII-tubulin TUJ1 and OLIG2 as well as decreased stemness markers (SOX2 and Nestin). Concomitantly, autophagy which is a cell self-degradative process is induced in the glioma CSCs as shown by an elevated amount of the autophagy marker, LC3-II (36).

The presence of microtentacles on the cell surface is a prominent feature of breast CSCs. These microtentacles are supported by an enhanced vimentin expression and α-tubulin detyrosination activity that allows quicker reattachment of suspended CSCs. Following treatment with curcumin, however, these microtentacles residing on breast CSCs surface were attenuated, and they slowed down CSCs reattachment efficiency (37). Upon observing the potential of curcumin against their formation by suppressing *Stat3* and its downstream target genes activation, as well as CSCs, Lin et al. generated a novel curcumin analogue (GO-Y030) which inhibited tumour spheres, inducing apoptosis in the colon CSCs (38). Drug resistance is one of the CSC hallmarks. However, curcumin could also act as a chemo-sensitisation agent in a combination treatment with cisplatin to synergistically increase the percentage of apoptotic and migratory non-small cell lung CSCs with higher *p21* and *Apaf1* gene expression and lower *cyclin D1* expression (39). Similarly, when curcumin or difluorinated-curcumin (CDF) treatment is combined with other drugs and phytochemicals such as FOLFOX, EGCG and piperine, isolated from *Piper nigrum*, the anti-CSCs activity is more potent than the curcumin treatment alone. This occurs primarily through blocking of STAT3 phosphorylation, suppression of Wnt/β-catenin signalling and apoptosis induction (40-43).

Epigallocatechin gallate (EGCG)

EGCG is a major polyphenolic compound in tea products that are effective against cancers including prostate,

nasopharyngeal, head and neck, and the breast. With EGCG treatment, the CSCs in these solid tumours decreased from the loss of self-renewal with the inhibition of tumour spheres formation. EGCG plays its role through several mechanisms; inhibition of NANOG signalling; induction of transcriptional repressor, HBP1 to inhibit Wnt/β-catenin signalling and its targets which include MYC, SNAIL, SLUG, vimentin, nuclear β-catenin and LEF-1/TCF activity; repression of STAT3 phosphorylation and its target genes including *Bcl-2*, *survivin*, and *cMyc*; inhibition of Notch activation and its targets, HEY1 and HES1; induction of cellular apoptosis by stimulating expression of caspases 3,6, 7; and suppression of chemoresistant pump expression, sensitising CSCs to chemotherapeutic drugs such as cisplatin and 5-fluorouracil. Manipulation of these signalling pathways by EGCG will, therefore, reduce the stemness markers (SOX2, OCT4, CD44 and NANOG), and the survival, migration and invasion of the CSCs (25, 44-46). The action of EGCG could be synergised with other phytochemicals. When glioma CSCs were treated with a combination of EGCG and temozolomide, (a DNA methylating agent), the CSC population showed a dramatic decrease in their stemness properties with lowered self-renewal and differentiation abilities, heightened cellular apoptosis with the downregulation of BCL-2, PARP cleavage and p-AKT expression, and an arrested cell cycle at G0/G1 phase due to the elevated expression of P-glycoprotein or MDR1 gene expression (47). Chen et al. synthesised EGCG analogues that displayed heightened CSC inhibitory activities, compared to its parental compound, by activating the AMP-activated protein kinase pathway and downregulating the mTOR pathway (48). Peracetylated EGCG that can be applied topically also showed greater effectiveness than EGCG by restoring ERK signalling while inhibiting the protein kinase D1 (*PKD1*) gene expression and PI3K/AKT signalling to prevent tumour proliferation (49).

Sulforaphane

Sulforaphane is another widely studied anti-CSCs phytochemical that is abundant in broccoli or broccoli sprouts and other cruciferous vegetables. It is effective, especially against the CSCs population at low concentrations (0.5-5.0 µmol/L) which usually have no effects on the mature cancer cells. Sulforaphane acts by suppressing the key CSCs signalling including the Wnt/β-catenin pathway by blocking GSK-3β phosphorylation, thereby preventing β-catenin accumulation, suppressing TCF/LEF transcription and reducing cyclin D1 expression to cause breast CSC proliferation arrest (50). Sulforaphane also inhibits the Hedgehog pathway which is highly expressed in pancreatic CSCs, leading to the downregulation of its downstream components such as GLI1, GLI2 and SMO, leading to repression of the pluripotency-maintaining factors (OCT4, NANOG, PDGFRα, VEGF, cyclin D1, TWIST1, Vimentin and ZEB1). Furthermore, apoptosis is induced in CSCs with the downregulation of the anti-apoptotic BCL-2 family members, resulting in reduced primary and secondary sphere generation (51-53).

Moreover, sulforaphane treatment can increase CSC sensitivity of the leukaemic, prostate and pancreatic cancers to the common chemotherapeutic drugs such as doxorubicin, 5-fluorouracil, gemcitabine and imatinib. This chemosensitisation effect is achieved through inhibition of the Notch and β-catenin signalling that resulted in the suppression of MDR-1 gene expression thus inducing CSCs apoptosis with the increased expression of the pro-apoptotic BCL-2 family (54, 55). When sulforaphane is combined with a TRAIL agent, an additive effect is also observed, showing a stronger antagonistic activity against the prostate and pancreatic CSCs. This effect is correlated with the suppression of TRAIL-induced NFκB binding that leads to reduced ALDH1 activity and CSCs self-renewal potential (56, 57).

Parthenolide

Parthenolide, a sesquiterpene lactone isolated from *Tanacetum parthenium*, is effective in eliminating several cancer CSCs, including prostate and breast. CSCs treated with parthenolide showed a reduction in the CSC population with a decrease in CD44 surface marker expression and a drastic drop in the number of colonies formed. This phenomenon is correlated to the capability of parthenolide to inhibit NFκB signalling, non-receptor tyrosine kinase (SRC) signalling and its components, such as FAK, PKC, FGFR2, ELK-1 and CaMKo, as well as repression of numerous transcription factors, including C/EBP-α, c-MYB, SNAIL, SP, HOXA-4, FRA-1, STAT3, XBP-1, SRF and p53 (58, 59). In combination with andrographolide, a phytochemical extracted from the plant *Andrographis paniculata*, both phytochemicals act in synergy to become a potent killer of multiple myeloma CSCs by activating caspase-mediated apoptosis signalling (60). Dimethylamino-parthenolide (DMAPT), which is an improved version of parthenolide, showed an even greater oral bioavailability of up to 70%. It could specifically eliminate leukaemic CSCs via the production of a stress response to suppress NFκB while inducing p53 signalling (61). Similarly, parthenolide-like agents such as celastrol and 4-hydroxy-2-nonenal have also been shown to display selective toxicity against the CD34+/CD38- leukaemic CSCs by simultaneously causing a stress environment and the suppression of NFκB signalling, with decreased NFκB/p65 nuclear localisation and Nrf2 activation, to impair the engraftment of AML CSCs in the sublethally irradiated mice (62).

Vitamin A and metabolites

Vitamin A is a fat-soluble dietary phytochemical that has been associated with carcinogenesis ever since its discovery. It is found in fruits and vegetables that are dark green or orange in colour. In the liver, this vitamin is processed into several important metabolites and circulated throughout the body(63). Among these metabolites, retinoic acid caused breast CSC proliferation arrest at G0/G1 phase by inducing cellular differentiation with a decreased expression of side population cells marked by CD44+ and CD24- surface markers (64). Another

vitamin A metabolite, β-carotene, reduced neuroblastoma CSCs in two studies. The β-carotene-treated cells exhibited lower stemness genes expression (*Oct4*, *Sox2*, *Notch1* and *DLK1),* but an increase in the expression of differentiation markers (vimentin, neurofilament and peripherin). These changes are most probably initiated by the activation of ERK signalling with an inhibition of the hypoxia pathway and their downstream genes. The β-carotene metabolite also demonstrated chemosensitisation by potentiating the effect of cisplatin to suppress the self-renewal activity of neuroblastoma CSCs (65, 66). All-trans retinoic acid (ATRA), another common metabolite of vitamin A, also inhibited the breast CSCs population through induction of cellular differentiation via several mechanisms: downregulating miR-20a, a tumour suppressing microRNA, thereby resensitising the breast CSCs to cell killing mediated by natural killer cells; increasing expression of differentiation markers, estrogen receptor alpha and keratin 18; suppressing stemness regulatory genes, *Sox2*, *Notch3*, *Slug* and *Jag1*; repressing pro-inflammatory NFκB/IL-6 signalling; and inducing apoptotic signalling (67-69).

Resveratrol

Various berries, grapes, peanuts and red wine are dietary foods rich in the resveratrol phytochemical that is effective against the CSC population. Lu et al. reported that resveratrol treatment at 100μM successfully stopped medulloblastoma CSC proliferation (70) by blocking the G1/S stage cell transition, increasing the expression of CDK inhibitors, such as p21 and p27, while downregulating the expressions of cyclin D2, CDK2, cyclin E, CDK 6 and c-MYC (71, 72). Treatment of glioblastoma, CSCs with resveratrol for 48 hours caused an S phase arrest followed by induction of Bax-mediated apoptosis at 96 hours post-treatment (73). In resveratrol-treated CSCs, common observations included a reduction in the CSCs population due to repressed tumour sphere formation capacity (with reduced pluripotency markers such as SOX2, OCT4, NANOG and c-MYC), increased apoptotic CSCs (activated executioner caspases and XIAP), induced autophagy (upregulation of LC3-II, ATG7 and Beclin-1), suppressed CSCs invasion and migration (decreased SLUG, ZEB-1 and SNAIL), an elevated proportion of senescence CSCs (enhanced β-galactosidase activity), as well as inhibited Wnt/β-catenin signalling (73-75). Hagiwara et al. showed that resveratrol treatment in breast CSCs could upregulate the activity of a central RNA interference molecule, Argonaute 2 (*Ago2*). This molecule promotes the expression of multiple tumour-suppressing microRNAs including miR-141, miR-16, miR-200c and miR-16 that exerts long term silencing effect on the CSCs regulatory genes. A natural dimethylated resveratrol analogue, pterostilbene, has a similar anti-CSCs function by stimulating the expression of tumour-suppressive microRNAs via *Ago2*-dependent actions (76). MR-3 (3,5,4'-trimethoxystilbene), methoxylated resveratrol, also eradicates CSCs population by increasing epithelial marker (E-cadherin) while decreasing mesenchymal markers (Snail, Slug and Vimentin), to inhibit EMT process; and repressing Wnt/β-catenin signalling and its downstream target genes while restoring GSK3β activity to reduce nuclear β-catenin accumulation; and by inactivating PI3K/AKT signalling (77).

Genistein

Genistein is an isoflavone phytoestrogen that is found plentifully in soybean related products, and it has a strong antagonistic activity against CSCs of various cancer types. CSC populations treated by Ggenistein often present with diminished colony and sphere formation ability, decreased level of pluripotency markers such as ALDH, CD44, SOX2, OCT4, CD90, EpCAM and NANOG, reduced cell migration, invasion and EMT phenotype (26, 78, 79). These anti-CSCs characteristics effects are mainly achieved via several mechanisms: inactivation of Hedgehog signalling resulting in decreased SMO and GLI1 expression (80); inhibition of Notch signalling that modulated expression of cyclin D1, NOTCH-1, NFκB p65 subunit, Vimentin, SNAIL, ZEB2 and E-cadherin(79); upregulating PTEN expression to repress PI3K/AKT signalling and its downstream components while activating E-cadherin and estrogen receptor β genes expression n (81, 82); and stimulating re-expression of tumour-suppressive microRNAs such as miR-200b/c and let-7a/b/c to suppress the expression of CSCs regulatory genes which included *FoxM1*, *Zeb1/2*, *Snail2* and V*imentin* while upregulating *E-cadherin* expression (78).

Cyclopamine

Cyclopamine is an alkaloid found in *Veratrum californicum*. It is a well-known phytochemical that depletes CSCs population in a variety of cancers such as glioblastoma (83), breast (84), multiple myeloma (85) and acute lymphocytic leukaemia (86), primarily by inhibiting the Hedgehog signalling pathway. This pathway is usually active in the CSCs to regulate self-renewing and multilineage differentiation activities. Upon treatment with cyclopamine or its derivatives such as IPI-926, the clonogenicity of CSCs was reduced as a result of depletion of the CSCs population, and this effect could likely be caused by Bmi-1-associated Hedgehog signalling inhibition.

Metformin

Metformin is a clinically approved medication for the treatment of diabetes, and it is a compound that is extracted from the plant *Galega officinalis*. Metformin, in clinically acceptable non-cytotoxic dosage, obstructed the generation of breast CSC population and sensitised the resistant CSCs to radiation therapy. These effects were achieved by blocking the mTOR pathway and its downstream targets such as 4EBP1 and S6K1 thus preventing oestrogen receptor-mediated OCT4 activation by repressing the transcription of *EMT* genes which included *Twist1*, *Slug*, *Snail2*, *Zeb2* and the *TGFβ* cytokines (87-89). Insulin itself has a growth-stimulatory function for the thyroid cancer cells and CSCs, but the addition of metformin antagonised this effect by stopping cell proliferation at the G1 phase. This triggered caspases activation, reducing clones and spheres formation together with the potential to

accentuate the cytotoxicity of chemotherapeutic drugs. This was mostly attributed to the inhibition of the insulin-mediated ERK phosphorylation while activating the AMPK/mTOR signalling to exert an anti-proliferative activity (48). Re-expression of the tumour-suppressive microRNAs was another mechanism of metformin to repress the CSCs. For instance, ectopic expression of miR-26a decreased the pancreatic CSCs regulatory markers, EpCAM and EZH2 (90).

Berberine

Berberine is an isoquinoline alkaloid that can be found in various plants such as *Berberidaceae* genus, *Hydrastis canadensis* and *Coptis chinensis*. Treatment with berberine reduced the pancreatic side population cells as indicated by the decreased expression of the stemness genes, *Nanog, Sox2 and POU5F1* (91). Berberine can be packaged in the form of liposomes to gain entry into the breast CSCs and selectively accumulate in the mitochondria, thereby modifying the mitochondrial permeability transition pores to release cytochrome C and trigger the apoptotic cascade. Berberine also inhibited the drug reflux ABC transporters, ABCC1-3 and ABCG2, stimulating the pro-apoptotic BCL-2 members while inactivating anti-apoptotic BCL-2 members (92).

Honokiol

Honokiol phytochemical can be isolated from the Magnolia plants *Magnolia officinalis*. It exhibits greater antagonistic activity towards the CSCs population as demonstrated by Yao et al., who showed that the sorted side population cells were more sensitive to the honokiol compared to the parental renal cancer cells. The treated side population cells showed a decrease in their stemness features as indicated by the reduced CD44 surface marker expression and repressed Wnt/β-catenin pathway components with a lower expression of TCF4, β-catenin, c-MYC, cyclin D1, SNAIL and SLUG and with a higher expression of GSK-3α/β that degrades β-catenin. Honokiol also induces programmed cell death in the side population cells by upregulating the activities of executioner caspases and downregulating the anti-apoptotic BCL-2 proteins (93). Honokiol by activating miR-141 to target *Zeb2* gene expression to suppress renal carcinoma cells proliferation reverses the EMT process of the cells and eradicates the CSCs. *Zeb2* gene is the master regulator of EMT and the maintenance of CSCs characteristics (94). A later study demonstrated the ability of honokiol in reducing cancer stemness through downregulation of ALDH1 and CD44 in oral carcinoma stem cells (OCSC) and suppressing its migration and invasion ability. The OCSC self-renewal, invasion and colony formation ability was inhibited by honokiol through suppressing IL-6 production and phosphorylation of STAT3 (95).

Oxymatrine

Oxymatrine is an alkaloid commonly found in the plant *Sophorae flavescentis*. Zhang et al. discovered that this phytochemical was capable of reducing the proliferation of MCF-7 breast cancer cells and its side population through inhibition of the Wnt/β-catenin pathway (96). This finding was in agreement with a separate study by Xu et al. who investigated compound Kushen injection (CKI) which primarily contained Θoxymatrine, where the molecular components and downstream targets of the canonical Wnt/β-catenin pathway were downregulated in the treated side population cells including WNT1, β-catenin, TCF4, LEF1, c-MYC and cyclin D1 (96, 97).

Pomegranate extract (PE)

Pomegranate extract (PE) is obtained from the plant *Punica granatum* and has been demonstrated to possess anti-CSC ability in two separate studies. The first study is reported by Dai et al., who showed that PE could inhibit the proliferation of mouse mammary CSCs via the induction of cell cycle arrest at G0/G1 phase and lead to cell apoptosis. This finding is further supported by Nair et al. who confirmed that PE treatment caused a G1 phase arrest of the cell cycle to inhibit pancreatic CSCs cell proliferation thereby increasing the cell population that lacks CD44 and CD24 surface markers expression. In PE, ursolic acid, ellagic acid and luteolin are most probably the phytochemicals that elicit the anti-CSCs activities (98, 99). Intriguingly, Nallanthighal et al. discovered the unique properties of PE in reducing CSCs self-renewal properties through minimising mammosphere formation and inhibiting cell migration through downregulating *EMT* genes such as TWIST1 (100).

Other phytochemicals or phytoextracts

Berbamine found in *Berberis amurensis* overrides the tyrosine kinase (TKI)-resistance of chronic myeloid leukaemia CSCs by specifically binding to the ATP binding pocket of the protein kinase II (CaMKIIγ) which is a crucial regulatory switch for NFκB, STAT3 and Wnt/β-catenin signalling pathways to maintain the self-renewing and survival activities in CSCs. CaMKIIγ binding by berbamine would cause growth inhibition of the leukaemic CSCs (101). On the other hand, psoralidin from the plant *Psoralea corylifolia* inhibited the breast CSCs mammosphere proliferation and formation by diminishing the *Notch* and *Hes1* genes expression, inhibiting NFκB signalling, inducing apoptosis (low BCL-2 but high BAX, caspases and PARP expression) and decreasing *EMT* genes (low *Vimentin*, β-*catenin*, *Twist* and *Slug* but high *E-cadherin* expression) (102). Sharif et al. discovered that *Aronia melanocarpa* juice could be used to selectively reduce the stemness with decreased OCT4 expression of the teratocarcinoma CSCs by causing an S phase cell cycle arrest, attributed to the reduced expression of cyclin B1 as well as activating the p53 and p73 apoptotic cascade (103). Similarly, *Alcea rosea* seed extract can prohibit CSCs proliferation at the G0/G1 phase by reducing cyclins B and D1 genes expression. The numbers of colonospheres were also diminished as a result of induced apoptosis and decreased CSCs markers due to the suppression of Notch and Wnt/β-catenin pathways

(104). The maleic and succinic acid derivative (YMGKI-1) isolated from *Antrodia cinnamomea,* inhibits head and neck CSCs by inducing cellular differentiation as shown by the decreased stemness markers (ALDH, GRP78, CD133, OCT4, NANOG and NOTCH2), with increased expression of differentiation markers (CK-18 and E-cadherin). CSCs treated with YMGK-1 also exerted an induced autophagy activity that led to cellular apoptosis due to repression of various transcriptional proteins, including mTOR, AMPK, HER2, MAPK, EGFR and PI3K (23). Isoliquiritigenin is a chalcone-type compound found in the liquorice root can increase Wnt inhibitory factor 1 (WIF1), resulting in the inhibition of Wnt/β-catenin signalling and its downstream target genes to cause a G0/G1 cell cycle arrest in breast CSCs (105). Apart from this, quercetin derived from apples, onions, cranberries and blueberries targets the NFκB pathway to inhibit EMT process and pancreatic CSCs proliferation while inducing the programmed cell death machinery (106). Eckol, a phlorotannin from *Ecklonia cava*, suppresses glioma CSCs stemness and sensitises them to anti-cancer therapy by inhibiting PI3K/Akt and ERK signalling pathways (107). Gamma-tocotrienol, a type of vitamin E present in various plant foods such as nuts, grains, fruits, sunflower oil, safflower oil and wheat germ oil, represses mevalonate-mediated Stat3 signalling and its subsequent target genes to diminish the drug-resistant ALDH+ population in breast cancer (108). *Brassica* genus such as broccoli and cabbage are abundant in indole-3-carbinol. This compound activates the p53 signalling by disrupting the MDM2-p53 interaction to trigger the apoptotic pathway and anti-proliferative response in breast CSCs (109). Other anti-CSCs agents that also stimulate apoptosis or autophagy to eradicate the CSCs population include ginsenoside F2 (a dammarane-type triterpene saponin from ginseng) (110), gossypol from *Gossypium* species (111), triptolide (from *Triptergyium wilfordii)* (112), 3,3-diindolylmethane or DIM (from cruciferous vegetables such as broccoli and) (113), koenimbin(from *Murraya koenigii)* (114), and 6-shogaol (from *Zingiber officinale*)115). Besides, koenimbin also induces CSCs differentiation and suppresses the Wnt/β-catenin pathway to decrease the serial passaging ability of the breast CSCs (114). At the same time, 6-shogaol reduces the *Notch1* and the target genes, *Hes1* and *Cyclin D1* transcriptional level to cause a drastic drop in the number of the secondary sphere (115). Lupeol, a triterpene from various fruits and vegetables, blocks the self-renewal capability of liver CSCs by downregulating the stemness markers (OCT4, NANOG, SOX2, Nestin and β-catenin), and sensitising the CSCs to chemotherapeutic drugs via repression of the PI3K/AKT/ABCG2 axis (116). Similarly, pomiferin extracted from *Maclura pomifera* could eliminate glioma CSCs by decreasing their stemness-related genes (117). Mulberry leaf extracts from the genus *Morus* typically activate the ERK signalling in neuroblastoma CSCs that results in promotion of cellular differentiation activity to inhibit its clone and sphere formation abilities (87).

Dietary phytochemicals targeting CSCs redox regulation

Reactive oxygen species (ROS) are the oxidative byproducts of normal cell metabolism upon stimulation by various stimuli such as ultraviolet (UV) light, gamma and x-ray irradiations, lipid peroxidation, mitochondria-catalysed electron transport reactions, chronic inflammation and environmental pollutants. These highly reactive molecules include hydroxyl peroxide, and superoxide and hydroxyl radicals. A balanced level of ROS is critical to maintaining normal cellular processes, most importantly involving programmed cell death and cellular senescence. High ROS levels in healthy cells create a condition known as oxidative stress. This often leads to genomic instability and DNA repair deficiency that promotes cellular transformation into malignant cells with higher proliferative and survival capacity, hence increasing chances of cancer formation (118). Therefore, antioxidant, natural products such as green tea, which contains high concentrations of polyphenols, could act as ROS scavengers to control or prevent cancer development (119). However, this therapy becomes particularly ineffective in CSCs, which usually exhibit low levels of ROS as a result of the high amounts of ROS scavengers expressed. This is usually the causal factor for the high CSC tumorigenicity and their radiation therapy resistance. For example, gastrointestinal cancer stem cells, identified by CD44+, possessed an enhanced defence mechanism against ROS with an increased expression of cysteine-glutamate exchange transporters. Levels of ROS were much lower in these malignant stem cells than the non-malignant cells. This was attributed to an upregulation of the ROS scavenging that helped to maintain the low ROS levels, thus contributing to the resistance to radiation (120). To target this CSCs population, parthenolide could potentially be used as a pro-oxidant agent. When human AML stem cells were treated with parthenolide, the cells showed an increased ROS level that resulted in NFκB pathway suppression and p53 activation to stimulate cellular apoptosis. Moreover, parthenolide displayed good specificity as it only targeted the AML stem cells without affecting the normal haematopoietic stem or progenitor cells (121). Curcumin can induce nitric oxide (NO) production in the leukaemic stem cells to cause oxidative cellular injury.

Curcumin also inhibits the mitochondrial respiration, which produces high levels of ROS which potentially damage the biomacromolecules due to lipid peroxidation, amino acid residues oxidation as well as DNA oxidative damage. This would eventually drive the cell to undergo apoptosis (17). Rooperol extracted from *Hypoxis hemerocallidea* exhibits no side effect on the normal embryonic fibroblast cells as it only targets the human teratocarcinoma CSCs via modification of its membrane potential that leads to ROS production, hence activating the apoptotic p53 mechanism with an up-regulation of the cleaved executioner caspases and PARP molecules. Concurrently, rooperol also causes

down-regulation of the stemness factors such as OCT4, SOX2 and NANOG that are essential for the maintenance of cell pluripotency (122). Tocopherol or vitamin E is a well-known fat-soluble antioxidant commonly found in plant foods such as nuts, grains, sunflower oils, safflower oils, fruits and wheat germ oil. In tumours with high levels of angiogenesis and metastatic activity, it was determined that the ROS level was also elevated and treatment with vitamin E could abolish those abnormal blood vessel formation and metastases. Another common food-rich pro-oxidant agent is resveratrol that has been found to protect the normal cells while causing oxidative stress in the cancer cells under low pH environment. A low pH environment is a common condition in the core of the tumour niche due to its glycolytic-dependent metabolism. This specific metabolic activity accumulates high amounts of lactate which lead to nuclear DNA base rotation that results in an exposure of its copper metal ion. The exposed copper ion would be mobilised by resveratrol and high levels of ROS via the Fenton reactions would be generated, thus leading to oxidative DNA damage and cell death (71, 72). In a study by Lin et al., sulforaphane was also shown to eliminate leukaemic CSCs by inducing elevated amounts of intracellular ROS that re-sensitised the CSCs to the actions of imatinib (54).

Dietary phytochemicals targeting CSCs metabolism

Like the normal stem cells, CSCs tend to survive on a hyper-glycolytic metabolism, more commonly known as aerobic glycolysis. This is due to the altered function of several key glycolytic metabolic enzymes in CSCs such as lactate dehydrogenase, hexokinase, pyruvate dehydrogenase kinase, glucose transporters, glutaminase and fatty acid synthase (124). Glycolysis is favoured in CSCs as it is thought to be an adaptive response during proliferation to take up and incorporate nutrients into the biomass necessary for new cell production (125). Glycolytic metabolism creates an acidic microenvironment that is crucial for the selection of an acid-induced cell toxicity-resistant phenotype. This phenotype confers an advantage to the cancer cells for an unrestricted proliferation and invasion capability (126, 127). Therefore, inhibition of glycolytic enzymes is crucial to eradicating the CSC population. Among the metabolic enzymes that are important in CSCs, pyruvate kinase isoenzyme 2 serves to convert glucose into lactate for energy production or to manufacture cell building units. Treatment with resveratrol has been shown to inhibit the expression of this enzyme through mTOR signalling suppression that leads to a decrease in lactate production and glycolytic metabolism in the CSCs(71, 72). CSCs are also characterised by a reduced mitochondrial function as compared to the mature tumour cells due to an alteration of the mitochondrial enzymes in function and biogenesis. This includes succinate dehydrogenase, pyruvate kinase M2, fumarate hydratase, as well as isocitrate dehydrogenase 1 and 2 (124). For this, vitamin E succinate or MitoVES that targets succinate dehydrogenase could be used to treat the CSCs. MitoVES is a mitocan from the vitamin E group. Mitocan is a small molecule that targets mitochondria to induce cancer cell apoptosis. Yan et al. discovered that in the presence of MitoVES, the mammospheres derived from the breast cancer cells were suppressed and their stemness markers, including CD44, ALDH, EpCAM, CD133, Oct4 and ABCG2 were also reduced (128). Lipid metabolism is another important factor in CSCs as lipogenesis has been demonstrated to be capable of tumorigenesis, as indicated by the higher expression of lipogenic genes in the ductal carcinoma in situ as well as isolated CSCs from breast cancers. These cells frequently exhibit greater tumour-initiating capacity. This is evident from work by Pandey et al., who ectopically expressed the lipogenic gene master regulator, SREBP1 into breast cancer cells. Such transformation increased their lipogenic metabolism, mammosphere formation and survival abilities. Upon treatment with resveratrol, the expression of the lipogenic genes in the breast CSCs were reduced together with the reduction in the number of ductal carcinoma in situ formed in the xenografts as well as in the formation of their mammospheres (129).

Dietary phytochemicals targeting CSCs epigenetic profile

Epigenetic modification of the genome is one of the main contributing factors for cancer development. There are a few different types of extra-genetic processes which change gene expression without altering the coding DNA sequence. Among these, DNA methylation is one of the key mechanisms that regulate the genome epigenetically to manipulate gene expression by the coupling of a methyl group to the CpG dinucleotides at the C-5 position of the cytosine ring. This process is generally mediated by DNA methyltransferases (DNMTs) that will lead to gene silencing because hypermethylation of the CpG-rich promoter region would prohibit binding by the transcription factor. The major types of DNMTs are DNMT1 (maintenance methyltransferase) and DNMT3A/B (*de novo* methyltransferases). DNMT1 plays an important role to maintain the methylation pattern of DNA during genome replication while the cells are dividing.

Meanwhile, DNMT3A/B is involved in the differentiation process during human development. The actions of DNMTs are usually coordinated by the intracellular level of metabolites such as S-adenosyl-methionine, ketoglutarate and acetyl-CoA, which are associated with the metabolic status of the cells. Therefore, cellular metabolism is also a determinant for the epigenetic regulation of the cells (130, 131). In cancer cells, hypermethylation of tumour suppressor genes is a common phenomenon and accumulation of these aberrant epigenetic alterations predisposes these cells to induce genomic instability that subsequently leads to cells with improved stemness phenotype and self-renewal capacity to form the CSCs (132). Accordingly, a possible approach to target the CSCs could be through the demethylation process to activate tumour-suppressing genes and stemness-inhibiting factors or through methylation of stemness and tumorigenic genes.

For example, isoliquiritigenin was found to demethylate WIF1 (WNT inhibitory factor 1) gene by deactivating Sp1/DNMT1-dependent signalling. Hence, the mammary carcinogenesis that is induced by the breast CSCs could be blocked, attributed to the reduction of the *Wnt* stemness gene expression (105). Retinoic acid receptor beta (RARβ) is another gene that helps to control cell proliferation via regulation of cell growth and differentiation, but it is commonly hypermethylated in cancer cells that allow their uncontrolled proliferation. By using a combination of EGCG and catechins, the unmethylated *RARβ* gene expression in breast cancer cells such as MCF7 and MDA-MB-231 was found to increase with a parallel decrease in its methylated counterpart. This demethylation activity by EGCG and catechins could be achieved via direct repression of the DNMTs or indirect inhibition of the DNMTs facilitated by the catechol-O-methyltransferase (133). Glabridin is a phytochemical present in the *Glycyrrhiza glabra* plant. This compound also can demethylate and activate miR-148a that prevented the activation of SMAD2 expression. SMAD2 deactivation would lead to the restoration of epithelial cell features (increased E-cadherin and ZO-1 and decreased *vimentin* genes expression), enhanced cell adhesiveness, reduced xenografted tumour growth, and attenuated CSCs-like characteristics (decreased expression of DNMTs)(134). Alternatively, methylation of the tumorigenic gene might be another potential method to eliminate the CSC population. It is well known that *EGFR* gene is frequently overexpressed in various cancer types, and *EGFR* overexpression is frequently associated with poor prognosis. As confirmed by Yu et al., cells surviving the treatment with a combination of 5-fluorouracil and oxaliplatin displayed an increased EGFR expression due to hypomethylation of this gene. Treatment with curcumin successfully eradicated these surviving cells as demonstrated by the hypermethylation of *EGFR* gene accompanied by an upregulation of the DNMT1 gene expression (43). *UHRF1* (Ubiquitin-like, containing PHD and RING Finger domains 1) gene is an epigenetic integrator that is related to the proliferation and survival abilities in cancer cells. Treatment with *Aronia melanocarpa* juice could reduce *UHRF1* gene expression and result in the killing of teratocarcinoma CSCs (103).

Conclusion and Future Perspectives

The CSC subpopulation presents a great challenge to the effective treatment of cancer by conventional chemotherapy as the drugs are unable to kill the undifferentiated and quiescent CSCs. In many cases, the tumour relapses and becomes more aggressive than the parental phenotype. To date, plants and herbs remain an important source of medicinal drugs for both traditional and modern medicine. Plant-derived phytochemicals are well known for their antioxidant and immunomodulatory potentials. These compounds have been described as effective candidates against the CSC population. CSC-targeting phytochemicals are beneficial, as they could either act alone or as an adjunct to the current chemotherapy regimens, to potentiate their efficacy. Moreover, these phytochemicals could be used for a prolonged time in the patients whose tumour had regressed after the chemotherapy, to limit the chances of CSC regrowth.

Nevertheless, there are still several fundamental questions that need to be answered before phytochemicals possessing anti-CSCs could be approved for clinical application. First, CSC itself is a heterogeneous population that may not respond homogeneously to all anti-CSC phytochemicals as seen in the simplified *in vitro* study models, compared to the highly complicated host tumour microenvironment. Comparative studies of phytochemicals against different CSC cell lines, model and signalling pathway associated with the anti-CSCs activities should be encouraged with the objective to search for the potential candidate ~~in~~ targeting the heterogeneous CSC population. Second, efforts on phylogenetically mapping plant species with their anti-CSCs mechanisms of action should be made to determine if closely related species exhibit the same or stronger activities with the evolutionarily conserved phytochemistry. Additive and synergistic interactions of phytochemicals in combination with existing chemotherapeutics are worth investigating to enhance therapeutic efficiency through regulating multiple signalling pathways while reducing the therapeutic doses and toxicity concerns. Suitable drug formulations for the phytochemicals are essential for effective clinical therapy. Detailed studies on the pharmacokinetic and pharmacodynamic aspects of the phytochemicals are also crucial for any potential clinical drug, to derive the therapeutic dosage and drug clearance rate so that the drug could be safe in man. Despite the encouraging anti-cancer properties of phytochemicals, the bioavailability and stability limitations of phytochemicals remain of concern. Efforts in developing delivery systems such as polymeric nanoparticles, liposomes, micelles and immunoconjugates can be devoted to circumventing the drawbacks. The studies on plant phytochemicals against CSCs have been encouraging in recent years and CSCs-targeting with phytochemicals though still in its early stage, is now rapidly moving into the spotlight of anti-cancer research.

Abbreviations

ABC	Adenosine-triphosphate-binding cassette
ALDH1	Aldehyde dehydrogenase 1
AML	Acute myeloid leukaemia
CSC	Cancer stem cell
DNA	Deoxyribonucleic acid
DNMT1	DNA methyltransferase
EGCG	Epigallocatechin-3-gallate
EGFR	Epidermal growth factor receptor
EMT	Epithelial-mesenchymal transition
MXI1	MAX-interacting protein 1
QLC	Quiescent leukaemia-like stem cells
PE	Pomegranate extract
ROS	Reactive oxygen species

UHRF1	Ubiquitin-like, containing PHD and Ring Finger domains 1
UV	Ultra-violet
VEGF	Vascular endothelial growth factor
WIF1	WNT inhibitory factor 1

Ethics Statement

This manuscript reviews dietary phytochemicals in eliminating cancer stem cells. No animal experiments and human subjects were involved in this paper.

Competing Interests

The authors declared that there is no conflict of interest.

Acknowledgements

We would like to thank Dr Le Cheng Foh and Miss Christen Mayer for editing this manuscript.

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