Dietary Phytochemicals Target Cancer Stem Cells for Cancer Chemoprevention

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Abstract Cancer is a multistep process involving genetic and epigenetic changes in the somatic genome. Genetic mutations as well as environmental factors lead to the initiation, promotion, and progression of cancer. Cancer progression ends in tumor metastasis to distant sites, and metastasis is the major reason for cancer patient deaths. Recent experimental evidence suggests the pivotal role of cancer stem cells. A tumor is heterogeneous and composed of different cell types. The cancer stem cells in the tumor have the capacity both to self-renew and differentiate to sustain the tumor. Features of cancer stem cells are described in this review, with an emphasis on the role that dietary phytochemicals may play in cancer chemoprevention. Ingredients in the diet can inhibit cancer cells and cancer stem cells. These compounds include curcumin from curry, epigallocatechin gallate from green tea, resveratrol from red wine, genistein from soy, sulforaphane from broccoli, and many others. Current research findings advocate the beneficial effects towards cancer chemoprevention via uptake of a combination of different dietary phytochemicals.

Keywords Phytochemicals • Cancer stem cells • Chemoprevention • Curcumin • Epigallocatechin gallate • Resvertrol • Genistein • Sulforaphane • Combination therapy

Cancer and Cancer Stem Cells

Biology of Cancer

A somatic cell undergoes changes in its genome and epigenome during its lifetime. If the accumulated changes involve regulation of cell growth and death, the

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resulting uncharted growth may alter the normal somatic cell to turn into a cancer cell. During the carcinogenesis process, there are malfunctions of genes, including gain of function of proto-oncogenes and loss of function of tumor suppressor genes.

Cancer cells exhibit hallmark alterations in their physiology, namely: (1) self-sufficiency of growth signals, (2) insensitivity to growth inhibitory signals, (3) evasion of programmed cell death, (4) limitless replicative potential, (5) reprogrammed cellular energetics, (6) induction of angiogenesis, (7) presence of tumor-promoting inflammation, (8) avoidance of immune destruction, (9) maintenance of genome instability and mutation, and (10) activation of tissue invasion and metastasis (Hanahan and Weinberg 2000, 2011).

Because cancer is a multistep process, it may take decades to develop. Carcinogenesis can be traced from its initiation, followed by promotion, progression, and metastasis. Accumulation of a handful of mutations (10–20) is adequate for cancer development. The cancer cell will then self-renew, migrate, and invade, as a clonal expansion in its complex tissue ecosystem (Greaves and Maley 2012). The niche or microenvironment surrounding the cancer cell can shape the path of carcinogenesis; as an example, the host immune system will attempt to destroy the developing cancer, although results may vary, as seen in the outcomes of cancer immunoediting (Schreiber et al. 2011). The tumor evolves from being benign to being malignant, and finally leads to the death of its host, the cancer patient. Investigators are interested in details of this process of carcinogenesis, and ways to halt its progression. Dietary phytochemicals have been shown to modulate the carcinogenetic procession.

Cancer Stem Cells

History and Definition

By histological examination, the tumor is heterogeneous, composed of tumor cells at various stages of cell differentiation, plus nontumor cells such as fibroblasts and immune cells. Thus, not all cells within a tumor are equal. Clonal evolution and competition within the tumor result in the dominance of the cell that is resistant to therapy and can both self-renew and differentiate its progeny cells. This observed tumor heterogeneity has been explained by the cancer stem cell (CSC) hypothesis. A workshop of the American Association for Cancer Research in 2006 settled with a consensus definition: CSC "is a cell within a tumor that possesses the capacity to self-renew and to cause the heterogeneous lineages of cancer cells that comprise the tumor" (Clarke et al. 2006). The putative CSC is also known as a tumor-initiating cell, a term preferred by some investigators, as seen in this example: "tumor-initiating cells (popularly known as cancer stem cells)" (Zhou et al. 2009). In this chapter, we use the popular term, CSC.

In a review published in 2001, the abstract starts with "stem cell biology has come of age" but ends with "cancer cells may include 'cancer stem cells'—rare

cells with indefinite potential for self-renewal that drive tumorigenesis" (Reya et al. 2001). Research on various aspects of CSCs has been reported in the intervening years. In addition to research and review publications, CSCs are the topic of edited monographs. The first book dedicated to CSCs came out in 2009 (Bapat 2009); its editor was a scientist from India who first isolated human ovarian CSCs (Bapat et al. 2005). Other volumes subsequently appeared (Farrar 2010; Allan 2011; Scatena et al. 2012). One addressed CSC methods (Yu 2009). There are even Web-published volumes (Shostak 2011a, 2011b) and a series dedicated to cancer cells and CSCs (Hayat 2012). So, has CSC biology come of age?

In reality, the CSC hypothesis and the CSC concept have a long history. The subject has been billed as "old concepts, new insights" (Vermeulen et al. 2008) and "an evolving concept" (Nguyen et al. 2012). Linking stem cells and cancer, histological similarities were noted between embryonic and tumor tissues in the nineteenth century. This became the "embryonic rest" theory: cancers arise from cells with properties similar to those of early embryos. Later investigations concluded that cancer can be viewed as a "caricature" of normal development. The uncharted cell proliferation seen in cancer is the result of distortion of normal development, and a lack of coordination between growth and differentiation (Nguyen et al. 2012). In addition, knowledge on stem cell biology in normal tissues has been translated to the concept of CSCs in cancerous tissues.

Perhaps the best known normal stem cell is the hematopoietic stem cell (HSC). HSC has a hierarchy that permits an organized process of self-renewal and differentiation, giving rise to progeny of differentiated cell types including erythrocytes, macrophages, lymphocytes and many others. Immunologists have identified cluster of differentiation (CD) markers for the different cell types, and these cell surface antigens allow for the isolation of cells by methods such as fluorescence-activated cell sorting (FACS). The rare HSC exhibits the surface marker CD34, the member of a family of single-pass transmembrane sialomucin proteins. It should be noted that although CD34$^+$ is seen in human HSC, the mouse equivalent is CD34$^-$ or CD-34^{low}. Thus, there are species differences in CD distribution.

In studying hematopoietic malignancies, in 1937, Jacob Furth and Morton Kahn provided the first quantitative assay for the assessment of the frequency of cancer cells maintaining the hematopoietic tumor. They showed that a single mouse leukemic cell was capable of transmitting the systemic disease when transplanted into a recipient mouse (as quoted by Clevers 2011). By definition, this has to be the CSC of murine leukemia.

Like its murine counterpart, the human CSC was first demonstrated in leukemia and then extended to solid tumors. Bonnet and Dick (1997) reported the presence of CD34$^+$/CD38$^-$ CSCs in human acute myeloid leukemia. Isolated patient CSCs will reconstitute the leukemia when transplanted in immunodeficient mice. Al-Hajj et al. (2003) reported the presence of CD44$^+$/CD24$^{-/low}$ CSCs in human breast cancer. They wrote: "As few as 100 cells with this phenotype were able to form tumors in mice, whereas tens of thousands of cells with alternate phenotypes failed to form tumors." Singh et al. (2003) reported the presence of CD133$^+$ CSCs in human brain cancer. They commented:

> The increased self-renewal capacity of the brain tumor stem cell (BTSC) was highest from the most aggressive clinical samples of medulloblastoma compared with low-grade gliomas. The BTSC was exclusively isolated with the cell fraction expressing the neural stem cell surface marker CD133. These CD133⁺ cells could differentiate in culture into tumor cells that phenotypically resembled the tumor from the patient.

These initial studies on CSCs were expanded to many cancers in the following decade.

Biomarkers and Experimental Studies

Cellular antigens of CSCs can serve as biomarkers. The more common ones are CD24, CD34, CD44, and CD133 (Woodward and Sulman 2008; Keysar and Jimeno 2010). They are used in the isolation of CSCs from leukemia, breast cancer, and many other cancers. CD24 is a heat-stable antigen, a sialoprotein that acts as a ligand for P-selectin, thus enabling the cell to bind to platelets and protecting tumor cells in the blood stream. Although CD24 is negative in breast CSCs, it is present in ovarian CSCs (Gao et al. 2010). CD44 is a transmembrane glycoprotein which is a hyaluronic acid receptor. Besides breast cancer, CD44 is found in CSCs from pancreatic, gastric, head and neck, ovarian, and colon cancer. CD133, also named prominin-1, is a glycoprotein consisting of five transmembrane domains with a restricted expression within the plasma membrane protrusion sites. In addition to brain cancer, CD133 is found in CSCs from colon, liver, pancreatic, and prostate cancer (Alison et al. 2011; Zobalova et al. 2011; Hu and Fu 2012). It should be noted that these antigens are also present in the respective normal stem cells, although they have been used as biomarkers for their cancerous counterparts. Hence, the significance of CD24 and CD44 as CSC markers has been seen as "an enduring ambiguity" (Jaggupilli and Elkord 2012). Another important point is the lack of a single CD antigen common to all CSCs. However, a universal marker may be close at hand. In a recent drug screening study specifically targeting CSCs, dopamine receptors have been discovered as a biomarker for CSCs but not normal stem cells. Because only leukemic and breast CSCs have been tested so far, the novel biomarker requires confirmation and validation (Sachlos et al. 2012).

In addition to identification through biomarkers, CSCs can also be characterized by functional assays, namely, the detection of side population (SP) activity and the assessment of aldehyde dehydrogenase (ALDH) activity (Keysar and Jimeno 2010; Tirino et al. 2013). The SP assay measures the ability of cells to expel the fluorescent dye, Hoechst 33342, caused by the activity of ATP-dependent drug transporters on the plasma membrane, especially the ABCG2 transporter (Mo and Zhang 2012). As analyzed by flow cytometry, cells with fast drug exit form a side population, as opposed to the main population composed of the majority of cells. Initially identified in HSCs, SP has been found in many CSCs. Similarly, ALDH is a detoxifying enzyme and acts by the oxidation of aldehydes to carboxylic acids for further metabolism or liver exit. A fluorescent substrate assay using ALDEFLUOR, biodipy-aminoacetaldehyde (BAAA), allows for the isolation of ALDH⁺ cells. The ALDH

Table. 1 Markers for Cancer Stem Cells. (Adapted from Woodward and Sulman 2008; Keysar and Jimeno 2010; Clevers 2011; Hu and Fu 2012; Sachlos et al. 2012 and other references cited in text)

Marker	Description	Examples of tumor types
CD24	Heat stable sialoglycoprotein	Ovarian cancer
CD34	Hematopoietic progenitor surface glycoprotein	AML (acute myeloid leukemia)
CD44	Hyaluronic acid receptor	Breast/colorectal/gastric/liver/head and neck/ovarian/pancreatic cancer
CD90	Thymocyte differentiation antigen-1 (Thy-1)	Glioma/liver cancer
CD133	Prominin-1	Brain/colorectal/lung/liver/ovarian/pancreatic/prostate cancer
CD326	Epithelial surface antigen (ESA) or epithelial cell adhesion molecule (EpCAM)	Colorectal/pancreatic cancer
ABCG2	ATP-binding cassette drug transporter	Brain/liver/lung/ovarian/pancreatic cancer
ABCG5	ATP-binding cassette drug transporter	Melanoma
ALDH	Aldehyde dehydrogenase	Breast/colorectal/lung/ovarian/pancreatic/prostate cancer
DR	Dopamine receptor	AML (acute myeloid leukemia)

assay was initially used to isolate HSCs and normal breast stem cells, and then extended to CSCs (Ginestier et al. 2007). Inasmuch as both SP and ALDH assays involve drug extrusion and metabolism, exhibiting these capacities indicates drug resistance of normal and CSCs. For example, SP and ALDH assays, in addition to specific biomarker (such as CD44 and CD133) screening, have been utilized for the identification of human ovarian CSCs (as summarized by Bapat 2010). However, it should be emphasized that these assays only enrich CSCs. The biomarker and functional assays may not characterize all the CSCs in the analyzed samples; therefore, additional CSCs not exhibiting the attribute being tested may still be present. For a list of biomarkers currently being used in the isolation of cancer stem cells, please see Table 1.

In addition to biomarkers and functional assays, another in vitro method to study CSCs is the use of serum-free spheres or spheroid cultures. Tissue culture cells are usually grown as a monolayer in a nutrient-rich medium containing fetal bovine serum as a source of the necessary growth factors and other components. However, in the absence of serum but in the presence of growth factors such as fibroblast growth factor (FGF) and epidermal growth factor (EGF), cells will grow as spheres in suspension in unattached/untreated tissue culture plastic dishes, flasks, or plates. Spheroid cultures mimic the three-dimensional nature of a tissue. Furthermore, it should be noted that oxygen will be less available to the cells located at the interior of a sphere. The hypoxic condition may modulate their differentiated state: towards stemness. Originally developed for neurobiological studies, the spheroid culture is used to "identify stem cells based on their reported capacity to evaluate self-renewal

and differentiation at the single-cell level in vitro" (Pastrana et al. 2011). Spheroid cultures have been developed for normal tissues and tumors (Chen et al. 2012a). When isolated, individual sphere-forming cells from a tumor will form secondary and tertiary spheres upon sequential subcultures; by operational definition these will be CSCs. However, there have been critiques on this spheroid assay. Spheres are prone to aggregate. Hence, cell density will influence clonality. The quiescent stem cell may be missed by this method (Pastrana et al. 2011). In fact, the quiescent nature of putative normal and CSCs has been utilized for their characterization. These cells are known as label-retaining cells, because they can retain labels such as the lipophilic dye PKH26, which is diluted in subsequent cell divisions but not so with slow-dividing cells (Pece et al. 2010; Xin et al. 2012).

From a tumor sample, putative CSCs may be characterized by the in vitro techniques discussed above. In addition to isolating CSCs from tumor samples, it is also possible to isolate them from established cancer cell lines (Drewa et al. 2011; Mather 2012). The advantage of the latter is the absence of nontumor cells as contaminants; the disadvantage is the additional accumulated changes during the long period of in vitro culture. As an example, our interest in CSCs and SP analysis led us to isolate SPs from the rat C6 glioma cell line, deriving putative CSCs from an established cell line (Fong et al. 2010). However, the C6 stemness state is dynamic: although SPs give rise to both SP and non-SP progenies, as expected, non-SPs can do the same (Fong et al. 2010; Fong and Chan 2012).

Complementary to in vitro studies of CSCs, in vivo models are available. The isolated cells are tested by their ability to initiate new tumor growth after xenotransplantation into immunocompromised mice (Cheng et al. 2010). Limiting dilution xenotransplantation yields an estimate of CSC abundance in the tumor sample; sequential transplantation yielding the original tumor will confirm the presence of CSCs. Different murine models with varying degrees of immunodeficiency have been applied to CSC studies. Examples include athymic nude mice, nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mice, and NOD/SCID interleukin-2 receptor gamma chain null ($Il2rg^{-/-}$) mice. For example, using the severely immunodeficient NOD/SCID $Il2rg^{-/-}$ mice in single-cell transplants (in an appropriate extracellular matrix, Matrigel) from human melanoma samples, 27% of unselected melanoma cells develop tumors in mice (Quintana et al. 2008). Does this finding detect the abundance of human skin CSCs, whereas CSCs from other cancers are rare? There have been critiques on this xenotransplantation assay. Placing human cancer cells in immunodeficient mice is artificial because the human cancer cell will not encounter its normal host immune response. Therefore, results from this assay cannot reflect the physiological fate of the CSC in its native environment. Despite these objections, the limiting dilution murine in vivo xenotransplantation assay has been held as the "gold standard" for CSC identification (Ghiaur et al. 2012). However, other in vivo assays have been attempted for identifying CSCs. One example is the zebrafish model (Blackburn et al. 2011). Nonetheless, we found this quote: "despite being considered the gold standard assay for CSCs by many in the field, there is no reason to assume that growth in immunocompromised mice is in fact a relevant assay for CSC activity" (Ghiaur et al. 2012).

Controversy and Evidence

Even now, the CSC hypothesis is controversial. In 2009, one review asked: "Cancer stem cells: mirage or reality?" (Gupta et al. 2009a); another discussed: "Cancer stem cells and cancer nonstem cells: from adult stem cells or from reprogramming of differentiated somatic cells" (Trosko 2009); a third commented: "The investigation and study of cancer stem cells (CSCs) have received enormous attention over the past 5–10 years but remain topics of considerable controversy" (Rosen and Jordan 2009). Similar statements were made in 2013: "The investigation and study of CSCs have received enormous attention only over the past 5–10 years and remain topics of considerable controversy. Opinions about the validity of the CSC hypothesis, the biological properties of CSCs, and the relevance of CSCs to cancer therapy differ widely" (Tirino et al. 2013).

However, there is accumulating evidence favoring the CSC hypothesis. The CSCs may explain concepts of cancer and therefore are relevant to cancer therapy. The major supporting data are: (1) the presence of CSCs in minimal residual disease (MRD) and (2) the demonstration of CSCs in cell lineage tracing studies of murine tumors.

MRD is a term first used in leukemia, to denote the small numbers of leukemic cells that remain in the patient during or after treatment, when the patient is in remission (no symptoms/signs of disease). It is the major cause of relapse in cancer. MRD has been applied to solid tumors. If CSCs are resistant to therapy, they should be enriched after chemo- and radiotherapy and found in MRD. This has been shown to be the case. For example, resident breast CSC populations surviving conventional treatment (such as docetaxel) have been found to be enriched for $CD44^+/CD24^{-/low}$ breast CSCs that express epithelial-mesenchymal-transition (EMT)-associated genes (Creighton et al. 2009). Thus, the presence of CSCs after therapy predicts recurrence (Ghiaur et al. 2012).

Lineage tracing is a common technique for studying cell origins in developmental biology. Tracking cells with fluorescent proteins such as green fluorescent protein (GFP) in three different murine solid tumors, as reported in 2012, has been cheered as "resolving the stem-cell debate" (Gilbertson 2012; Graham 2012). In murine intestinal adenoma, 5–10% of the cells were CSCs, exhibiting the marker leucine-rich repeat-containing heterotrimeric guanine nucleotide-binding protein-coupled receptor 5 (Lgr5). The cell lineage tracing was made possible through the use of Cre-reporter at the *Rosa26-Confetti* locus, showing fluorescence (with four colors: green, red, yellow, blue) and even color conversion during tumor development. Lgr5 cells generated additional Lgr5 cells, as well as other adenoma cell types (Schepers et al. 2012). In murine papilloma, a benign skin tumor, 20% of cells were stem cells (tracked by yellow fluorescent protein) that divided twice a day whereas the others became terminally differentiated tumor cells (Driessens et al. 2012). In murine glioblastoma, a transgene was created to label both the quiescent adult neural stem cells and a subset of the endogenous glioma tumor cells (expressing GFP). The transgene also contained a viral thymidine kinase gene that could be targeted by the drug ganciclovir. Gliomas were treated with the drug temozolomide (TMZ);

but TMZ treatment alone led to the regrowth of a subpopulation of CSCs, that were then controlled by ganciclovir. TMZ-ganciclovir cotreatment impeded tumor development, by destroying both cancer cells and CSCs. Hence, this last study demonstrated the existence of murine glioma CSCs and their selective targeting (Chen et al. 2012b).

Resistance to Therapy and Stem Cell Pathways

With the assumption that findings in mice are extrapolatable to humans, the demonstration of CSCs in murine glioma and TMZ-ganciclovir cotreatment shows clinical relevance of CSCs. CSCs are resistant to therapy; they are or become chemo- and radio-resistant during or after therapeutic treatments (Donnenberg and Donnenberg 2005; Krause et al. 2011). These characteristics are due to the activity of drug transporters and metabolism enzymes, and a DNA repair system activated by genomic instability. CSCs may possess less reactive oxygen species (ROS), and thus are less susceptible to radiation therapy (Diehn et al. 2009). Depending on individual cases of cancer, CSCs may arise from either mutated normal stem cells, or dedifferentiated cancer cells exhibiting stem cell features. They display pathways of gene expression in common with those of normal stem cells. Therefore, thinking along therapeutic approaches, compounds targeting CSCs must be capable of differentiating them from the normal stem cells and sparing the latter, otherwise unforeseen problems with normal tissue homeostasis can occur.

Several signal transduction pathways are active in CSCs and may be amenable for intervention. The self-renewal pathways seen in CSCs relate to the expression of proteins involved in Hedgehog, Wnt, and Notch signaling. Additional pathways include PI3K and NFκB pathways (Garvalov and Acker 2011; Alison et al. 2011, 2012; Hu and Fu 2012).

The Hedgehog (Hh) signaling pathway starts with a secreted morphogenetic factor. The term Hh comes from the fruit fly genetic mutation Hh that leads to spiny-looking larva; the gene is essential for arthropod segmentation and mammalian development. The mammalian Hh morphogen, as a ligand, binds to its receptor, Patched 1. This binding activates another plasma membrane protein, Smoothened, which eventually leads to activation of the transcription factor known as Gli (glioma).

The Wnt signaling pathway also starts with a secreted morphogenetic factor. The term Wnt comes from the fruit fly genetic mutation Wingless (Wg), which is important for arthropod polarity and segmentation, and the murine gene Integration 1 (Int1), a gene activated in breast cancer of mice infected with mouse mammary tumor virus. Wnt morphogen binds to its receptor, and after a series of intermediate steps, results in the mobilization of a cytoskeletal protein, *beta*-catenin, from the cytoplasm to the nucleus to activate its specific transcription factor known as lymphoid enhancer binding factor/T-cell factor (LEF/TCF).

Instead of secreted factors, the Notch signaling pathway starts with the membrane-associated Notch protein. The term Notch also comes from a fruit fly genetic

mutation, which results in a notch in the fly wing. The binding of Notch by its ligand, such as the membrane protein Delta from a neighboring cell, initiates the pathway. Notch protein undergoes limited proteolysis by the proteinase named *gamma*-secretase to yield the Notch intracellular domain (NICD), which mobilizes from the cytoplasm to the nucleus to activate its specific transcription factor named recombination signal binding protein for immunoglobulin kappa J (RBPJ), alias CSL (after the mammalian centromere promoter binding factor 1, CBF-1, the fruit fly suppressor of hairless mutation, and the nematode *lag-2* gene). Notch gene mutations/polymorphisms have been found in cancer patients, and may be involved in CSC chemoresistance (Crea et al. 2011).

The three signaling pathways initiated by Hedgehog, Wnt, and Notch are functional in embryonic stem cell development and may be dysregulated in CSCs. Activation of stem cell signaling pathways results in the expression of stemness genes (pluripotency) in CSCs. Examples are Oct4 (octamer-binding transcription factor 4, a homeodomain transcription factor), Nanog (a homeobox protein, another transcription factor), and Sox2 (sex determining region Y-box 2, a transcription factor with a high mobility group domain) commonly found in aggressive, poorly differentiated tumors (Ben-Porath et al. 2008).

Besides Hedgehog, Wnt, and Notch pathways, additional ones are PI3K and NFκB (Alison et al. 2012). Phosphoinositide 3-kinase (PI3K) is linked to the mammalian target of rapamycin (mTOR) that relates to cellular energetics. The signaling pathway that leads to the activation of nuclear factor kappa B (NFκB) is important for cytokine expression and the inflammatory response. Activation of these pathways results in the expression of stemness phenotype. The CSCs proliferate and differentiate, and may become resistant to treatments by chemicals and radiation. Molecules targeting these pathways, especially dietary phytochemicals, are discussed with respect to the CSCs.

Niche and Metastasis

Normal stem cells/CSCs reside in specific places. All cells are influenced by their surrounding environment, a local ecosystem. This concept is known as the stem cell microenvironment, or the stem cell niche. Thus, for "normal stem cells and cancer stem cells: the niche matters" (Li and Neaves 2006); and "location, location, location: the cancer stem cell niche" (Sneddon and Werb 2007).

Each resident cell is affected by its niche. The best characterized normal stem cell is the HSC. It behaves differently depending on the niche. In its bone niche, HSC is quiescent in the bone marrow. In its vascular niche, HSC undergoes proliferation and differentiation within the blood vessels. Similarly, the niche concept applies to the CSCs. In addition to the primary tumor site, there are secondary tumor sites after tumor metastasis. Each tumor site consists of a variety of cellular and extracellular components. In addition to cancer cells and CSCs, other cell types include blood and lymphatic endothelial cells, fibroblasts, adipocytes, and immune and inflammatory cells. These cells may secrete molecules that can either promote or inhibit the

tumor depending on changes in the microenvironment. The cytokines, chemotactic factors, and extracellular matrix produced by the various cells affect the behavior of cancer cells. Changes always occur within the tumor; the niche is dynamic. For example, there are cancer-associated fibroblasts and tumor-infiltrating macrophages. Separating the macrophage subsets as M1 and M2 with pro- and anti-inflammatory attributes, respectively, M1 protects against and M2 facilitates tumor development. Furthermore, the niche within the tumor is hypoxic. Hypoxia can be a determinant for CSCs, as CSCs may express hypoxia-inducible factors (Fábián et al. 2013).

Biology of a tumor can only be understood by studying tumor cell heterogeneity and the niche that is constructed during the course of carcinogenesis. Hence, the situation becomes "the bed and the bugs: interactions between the tumor microenvironment and cancer stem cells" (Castaño et al. 2012). There is interdependence between the CSC and its niche. For example, paracrine signaling between carcinoma cells and mesenchymal stem cells (MSCs) has been reported (Li et al. 2012; Rasanen and Herlyn 2012). MSCs are recruited to the tumor niche. Carcinoma cells produce the cytokine interleukin-1, which induces the MSCs to produce the lipid molecule prostaglandin E_2 (PGE_2). PGE_2 induces changes in carcinoma cells by the activation of the *beta*-catenin signaling pathway and the formation of CSCs, via a process known as epithelial–mesenchymal transition (EMT). This example suggests that the niche allows CSC development.

Cells communicate within an epithelial cell layer. This comprises gap junctional intercellular communication (GJIC), an essential feature for cellular homeostasis (Trosko 2009). During EMT, epithelial cells undergo a transformation to become more mesenchymal in nature, from the tightly packed, highly differentiated, and immobile cells into the more loosely packed, less differentiated, and mobile cells. A special group of transcription factors is activated, including Snail and Slug (named after fruit fly zinc finger gene regulatory proteins). The adhesive protein E-cadherin (E for epithelial) is downregulated, leading to more motile cells. The absence of E-cadherin also allows for drug resistance, because its elevated expression has been associated with high drug sensitivity. Within the tumor, activation of the EMT program generates a reservoir of CSCs. EMT enhances cell survival by expressing genes that aid cells in avoiding apoptosis (programmed cell death), anoikis (cell death due to loss of cell adhesion), cellular senescence (the process of limited cell replication), and even host immune response (Tiwaria et al. 2012; Scheel and Weinberg 2012).

In addition to the contribution to the expression of stem cell phenotypes, EMT facilitates early stages of cancer cell invasion and metastasis. CSCs within the primary tumor leave this location to colonize distant sites; thus, metastasis is the major cause of human cancer deaths. The CSCs may be genetically unstable; multiple CSC clones may be present within the same tumor. The intrinsic genetic alterations keep the cells in the EMT and in the stemness state (Baccelli and Trumpp 2012). Once they have migrated to secondary sites, the cells may convert back to the epithelial state. Mesenchymal–epithelial transition (MET) can occur, but this reverse process is less well characterized. Nonetheless, there is phenotypic plasticity between the EMT–MET processes (Brabletz 2012).

Cancer, Cancer Stem Cells, and Mitochondria

Are there interactions among cancer, CSCs, and mitochondria? Almost all mammalian cells have mitochondria as their subcellular organelles, except mature erythrocytes. Mitochondria are essential for cell metabolism. In tumor cells, their mitochondria resist apoptosis-associated permeabilization. Tumor cell mitochondria also contribute to altered cell metabolism by stimulating cell growth and anabolic metabolism. Tumor cell metabolism can become a target for cancer treatment (Kroemer and Pouyssegur 2008).

An early discovery on tumor metabolism became known as the *Warburg effect*, after Otto Warburg, who reported the increased glycolysis in leukemic and solid tumor cells. The dominance of this work may have delayed investigations on cancer and mitochondria for some time. However, recent studies on mitochondrial enzymes and metabolism have shown that they play a significant pathogenic role in cancer (Scatena 2012).

When the cell experiences stress, its mitochondria respond. The rapid mitochondrial fission and fusion depend on GTPases, notably dynamin-related protein-1 (Drp-1) promoting fission and mitofusin-2 (Mfn-2) promoting fusion. The processes lead to elimination of damage (via fission) or compensation of damage (via fusion). Defective mitochondria are destroyed via autophagy (known as mitophagy). If all else fails, the stressed cell undergoes apoptosis (Youle and van der Bliek 2012).

That mitochondria fission is important for cancer has been demonstrated in lung cancer. Using human lung adenocarcinoma cell line A549 xenografts in nude mice, Mfn-2 gene therapy (with adenovirus vector) leads to smaller tumors as compared to control cancer cells. Similar tumor size reduction is also seen with the pharmacological approach using Mdiv-1, a selective inhibitor of mitochondrial division in yeasts and mammalian cells. Thus, impaired fusion and enhanced fission contribute to the proliferation/apoptosis imbalance in cancer (Rehman et al. 2012). Targeting cancer cell mitochondria has generated the development of a group of compounds known as *mitocans* (mitochondrially targeted anticancer drugs). One such example is *alpha*-tocopheryl succinate, an analogue of vitamin E (Neuzil et al. 2007).

Thus far, metabolic differences between normal and cancer cell mitochondria have been shown, and the latter may be a therapeutic target of cancer. Is there a relationship to CSCs? Breast CSCs, derived from the human breast cancer cell line MCF7, is susceptible to mitochondrially targeted vitamin E succinate (named MitoVES) in vitro. Additionally, it has been proposed that the CSC marker CD133 may select the stem cells by both evading host immune response and by overcoming stress-induced apoptosis via a mitochondrial connection (Zobalova et al. 2011). In a study using the CD44$^+$/CD24$^{-/low}$ breast CSCs from MCF7 as xenografts to NOD/SCID mice, the mitochondrial targeting liposome carrying daunorubicin and quinacrine inhibits tumor development (Zhang et al. 2012a). In summary, CSCs can be targeted by mitochondrial drugs.

Chemoprevention and Phytochemicals

Occupational cancer was first identified by Percivall Pott in 1775: young chimney sweeps later got scrotal cancer because they worked naked to avoid dirtying their clothes. The remedy was to wear clothes during chimney work and to clean and bathe afterwards. Such measures were followed in Holland but not in England, resulting in individuals with less and more scrotal cancers, respectively. Prevention of cancer became a topic of interest; concepts of chemoprophylaxis and chemoprotection began being proposed. In 1976, Michael Sporn introduced the word "chemoprevention" while investigating vitamin A and its analogues, and this became the commonly accepted term (Sporn and Suh 2000; Lippman and Hawk 2009). Chemoprevention refers to the use of agents to inhibit, reverse, or delay tumorigenesis.

Fruit and vegetable intake has been part of the human diet. Being omnivorous, humans also consume various meats and other foods. Bioactive components from plants are nonnutrient dietary phytochemicals that may modulate gene expression and signal transduction pathways (Manson 2003; Surh 2003; Tan et al. 2011). Phytochemicals have been isolated and characterized from fruits such as grapes and apples, vegetables such as broccoli and onions, spices such as turmeric, and beverages such as green tea and red wine, as well as numerous other sources. Collectively, these compounds became known as chemopreventive agents. As chemopreventive agents, phytochemicals have been shown to interact with multiple cellular targets (Aggarwal and Shishodia 2006a; Lee et al. 2011) and even with the epigenome (Vanden Berghe 2012). With respect to chemoprevention, phytochemicals target inflammation (Murakami and Ohigashi 2007; Kim et al. 2009). This is important because inflammation may initiate cancer, which is the loss of cell cycle control (Meeran and Katiyar 2008). In addition, the chemopreventive phytochemicals are applicable to cancer therapy, because molecular mechanisms may be common to both chemoprevention and cancer therapy. Phytochemicals may modulate cancer development and even metastasis (Pan and Ho 2008; Gupta et al. 2010).

Among the diverse phytochemicals implicated in the inhibition of carcinogenesis, one major group comprises polyphenols. These compounds are virtually ubiquitous in plant materials and may occur at very high levels. Mostly synthesized from phenylalanine, plant phenolics serve as structural polymer components and are also responsible for ultraviolet light screening. They have antioxidant activity and act as signaling molecules. The most important role of plant phenolics may be in plant defense against pathogens and herbivore predators. With the discovery of health benefits of plant polyphenols, it has been proposed to optimize the phenolic content of the diet so as to obtain favorable consequences for the general health of the population (Parr and Bolwell 2000). Within the plant phenolics, one group of importance for chemoprevention is known as the flavonoids (Yao et al. 2011). Another group has been classified as phytoestrogens (Moutsatsou 2007). Phytochemical action on CSCs is discussed in the following sections. CSCs are known to be resistant to chemotherapy. Previously we reviewed "overcoming cancer drug resistance by phytochemicals" (Chan and Fong 2009). Relevant information on phytochemicals discussed earlier has been updated and incorporated in this chapter.

Dietary Phytochemicals Targeting Cancer Stem Cells

Dietary phytochemicals that target CSCs are timely research areas, as seen in the abundance of publications and reviews on the topic. Before covering the major phytochemicals involved, we discuss recent reviews on this subject.

Aptly the very first review we found has the title: "Targeting Cancer Stem Cells with Phytochemicals" (Kawasaki et al. 2008). Stem cell pathways are presented, as well as a list of clinical trials to evaluate various phytochemicals. A review on CSCs concluded that "dietary phytochemicals are natural products found in our diet and can be used to target cancer stem cells" (Subramaniam et al. 2010). Another review suggested the tissue-specific stem cells as a target for chemoprevention and concludes that "known chemoprevention agents including sulforaphane, vitamin D_3, curcumin, quercetin, genistein, vitamin E, and EGCG may attribute their success at least in part to regulating self-renewal and differentiation of tissue-specific stem cells" (Maund and Cramer 2011). In a chapter titled "Towards New Anticancer Strategies by Targeting Cancer Stem Cells with Phytochemical Compounds" (Tanveer et al. 2011), a suggestion was to target Oct4 function in CSCs by phytochemicals. In a review titled "Implications of Cancer Stem Cell Theory for Cancer Chemoprevention by Natural Dietary Compounds" (Li et al. 2011b), stem cell pathways and phytochemicals were discussed; the authors concluded that:

> Naturally-occurring dietary compounds are advantageous in several aspects as chemoprevention agents: (1) they are present in commonly consumed food, which is readily available to most people in daily life; (2) they usually have very low or no toxicity, in contrast to most chemotherapy drugs; (3) many of these compounds have shown potential as an adjunct to chemotherapy drugs in some clinical trials. Although the reports were very limited for dietary compounds to inhibit CSCs, many of them have been shown to be involved in modulation of CSC self-renewal pathways.

The same conclusions were reiterated in their contributed book chapter (Li et al. 2011a).

In addition to dietary phytochemicals, CSCs can be targeted by phytochemical analogues (as reviewed by Dandawate et al. 2013), other natural products (Efferth 2012), and even Chinese herbs (Weber et al. 2012). To end this section, an appropriate quote is from a review titled "Cancer Stem Cells: Potential Target for Bioactive Food Components" (Kim et al. 2012): "Unquestionably, a diet-induced shift from deregulation to regulation in cancer stem cells could have profound influence on cancer relapses and therefore is of immense societal importance."

Curcumin

Health Effects

Curcumin is a diferuloylmethane from the Indian spice turmeric. (See Fig. 1 for the chemical structure of curcumin.) It is responsible for the yellow color of curry
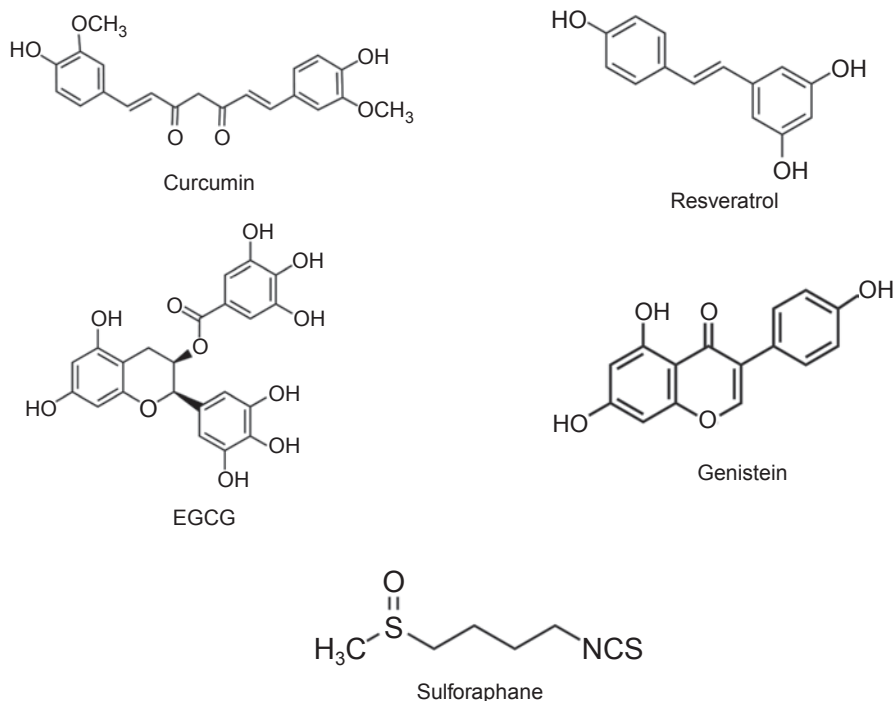
Fig. 1 Chemical structure of phytochemicals

powder. Turmeric is produced from the rhizome of the plant *Curcuma longa*. In addition to its use as a spice, turmeric has multiple applications in traditional Asian Indian medicine, ranging from treating insect bites to wound healing. Multiple health benefits have been associated with curcumin and turmeric, as summarized in a monograph (Aggarwal et al. 2007) and many reviews (Strimpakos and Sharma 2008; Goel et al. 2008). Multiple and diverse bioactivities have been attributed to curcumin, including cancer chemopreventive and anti-inflammatory activities (Bisht et al. 2010). Curcumin acts on many cellular targets (Ravindran et al. 2009). These include enzymes such as cyclooxygenase, lipoxygenase, and inducible nitric oxide synthase (iNOS); transcription factors such as NFκB and activating protein-1 (AP1); cytokines such as tumor necrosis factor (TNF); and many gene products linked with cell survival, proliferation, invasion, and angiogenesis. For example, we found that curcumin inhibits NFκB and TNF in a human monocytic cell line Mono Mac 6. With the endotoxin-induced murine sepsis model, curcumin ingestion by gavage induces bioactivity as shown by downregulating iNOS gene expression in the murine liver (Chan 1995; Chan et al. 1998). Synergistic effects between curcumin and chemotherapeutic drugs have been demonstrated: we have shown that curcumin enhances the cisplatin-mediated killing of the human ovarian cancer cell lines CAOV3 and SKOV3 in vitro (Chan et al. 2003; Chan and Fong 2007).

We end this section with a note on curcumin and patentability. In March 1995 the USPTO awarded the patent "Use of turmeric in wound healing" (US Patent 5,401,504) to Suman Das and Hari Har Cohly, with the University of Mississippi as assignee. The patent was withdrawn in August 1997, after protest from India's Council of Scientific and Industrial Research (CSIR) and demonstration of the lack of novelty criterion required for patenting, inasmuch as turmeric has been used for centuries by the Indian population for wound healing (Jayaraman 1997). Whereas CSCs are drug- and radiation-resistant, curcumin is a chemosensitizer and radiosensitizer for tumors (Goel and Aggarwal 2010). However, we should also point out the "dark side of curcumin" (Burgos-Morón et al. 2010), as we note the potential of turmeric consumption in reducing the risk of cancer (Hutchins-Wolfbrandt and Mistry 2011).

Effects on Cancer Stem Cells

Effects of curcumin on CSCs have been shown. Using SP as selection for CSCs in the rat glioma cell line C6 in vitro, we found that curcumin (5 µM daily for 10 days via medium change) reduced SP, likely via the inhibition of drug transporter (Fong et al. 2010). Curcumin targets human glioblastoma CSCs in vitro (2 µM) and in vivo, the latter via intracranial implantation of human CSCs with intraperitoneal injections of curcumin (300 mg/kg every 3 days for 5 weeks); the investigators proposed the mechanism as induction of autophagy and promotion of differentiation (Zhuang et al. 2012). Curcumin targets CSCs of human esophageal squamous carcinoma cell lines in vitro (40–60 µM), showing a decrease in aldehyde dehydrogenase (ALDH) activity as well as CD44 and NFκB expression (Almanaa et al. 2012).

Curcumin (30–60 µM) inhibits spheroid culture of human esophageal CSCs in vitro via the Notch signaling pathway (Subramaniam et al. 2012). Both human breast CSCs (from cell lines MCF7 and SUM159) and normal breast stem cells are targeted by curcumin (5–10 µM) together with another phytochemical piperine (from black pepper, 5–10 µM). There was a decrease in spheroid culture numbers as well as a decrease in ALDH activity (Kakarala et al. 2010). Both normal and CSCs were affected, therefore as a potential phytochemical for cancer therapy, its effect on normal stem cells must be clearly differentiated from CSCs.

Curcumin can complement other drugs. In vitro, cotreatment of curcumin (20 µM) and FOLFOX/FUOX (50 µM fluorouracil and 1.12 µM oxaliplatin) for 5 days inhibited the spheroid cultures of human colon CSCs from the cell lines HCT116 and HT29 (Yu et al. 2009). Induction of apoptosis in human brain tumor stem cells (commercially available from Celprogen) in vitro has been shown with cotreatment of curcumin (20 µM) and paclitaxel (10 nM) (Hossain et al. 2012). These results show the feasibility of combining conventional drugs with phytochemicals to target CSCs in clinical cancer.

However, the solubility of curcumin is a concern. To overcome this hurdle, various carriers have been developed. A polymeric nanoparticle formulation of curcumin, nanocurcumin trademark as NanoCurc (5–20 µM), induced apoptosis and reduced the CD133^{+} stem cells in malignant brain tumors in vitro

(Lim et al. 2011). A hyaluronic acid (HA)-based nanogel-curcumin conjugate, using cholesteryl-HA, had 8 times higher cytotoxicity than curcumin alone towards human pancreatic CSCs expressing CD44 (Wei et al. 2013). The authors also reported similar effects with nanogel-drug conjugates of two other compounds targeting CSCs, namely etoposide and salinomycin. A novel micelle formulation of curcumin, composed of stearic acid-g-chitosan oligosaccharide (CSO-SA) as a polymeric, biocompatible, and biodegradable drug carrier, inhibited CD44⁺/CD24⁺ colorectal CSCs in vitro and in vivo (nude mice xenografts), the latter at a dose of 2 mg/kg daily for 14 days in a 15-day experiment (Wang et al. 2012a). Another approach for curcumin solubility is to synthesize analogues. Difluorinated-curcumin (CDF) inhibited spheroid cultures of human colon CSCs from the cell line HCT116 (at 2–8 μM) in vitro (Kanwar et al. 2011). CDF (2 μM) plus FOLFOX/FUOX (100 μM fluorouracil and 2.5 μM oxaliplatin) inhibited the growth of the HT-29 colorectal cancer cell line CSCs in vitro via downregulation of microRNA-21 (miR-21) and promotion of differentiation (Yu et al. 2013). GO-Y030, a monoketone analogue of curcumin (Sato et al. 2011), inhibited human colorectal CSCs from the cell lines HCT116 and SW480 in vitro (induced apoptosis at 0.5–2.5 μM) and in vivo (NOD/SCID mice xenografts), the latter at a dose of 50 mg/kg daily for 14 days in a 29-day experiment (Lin et al. 2011). These results show that human CSCs can be targeted by curcumin and analogues in vivo using mouse models.

Resveratrol

Health Effects

Resveratrol is a stilbene (3,4′,5-tri-hydroxy-trans-stilbene to be exact) from red wine, grapes, peanuts, and pine nuts. (See Fig. 1 for the chemical structure of resveratrol.) Red wine is made from whole grapes (including grape skin) from the fruits of the grape vine plant *Vitis venifera* and related species. Resveratrol is present in red wine because the molecule is associated with the skin portions of the fruit to fight against fungal infections of the plant. It is an example of the phytoalexins, compounds produced by plants against pathogens. Similar to plant fungal pathogens, we demonstrated it is active against human dermal fungal pathogens: resveratrol may be useful for treating athlete's foot (Chan 2002). Health benefits associated with resveratrol are summarized in a monograph (Aggarwal and Shishodia 2006b) and many reviews (Athar et al. 2007; Gescher 2008; Harikumar and Aggarwal 2008; Kundu and Surh 2008; Saiko et al. 2008). It is "one molecule, many targets" (Pirola and Fröjdo 2008). Resveratrol's recent claim to fame involved its action as an activator of sirtuin deacetylases, leading to lifespan extension from organisms as diverse as yeasts, nematodes, fruit flies, and fish (Baur and Sinclair 2006). Resveratrol's prior claim to fame involved a phenomenon known as the "French Paradox" (Opie and Lecour 2007), referring to the observation that the French benefit from a relatively low risk of developing cardiovascular disease despite a diet that is high in saturated fat.

Resveratrol improves general health in mice and seems to delay their ageing parameters (Barger et al. 2008; Pearson et al. 2008). In this sense, it mimics a phenomenon known as caloric restriction, a method that retards aspects of the ageing process in mammals (Maxmen 2012). Indeed, it has anti-inflammatory activity (Bisht et al. 2010) and shows health benefits, especially cardioprotection and cancer chemoprevention. With respect to the French Paradox, it is believed resveratrol and alcohol in red wine may be part of the reason. We have shown a synergistic effect between resveratrol and alcohol in the inhibition of iNOS (Chan et al. 2000). Moderate consumption of red wine may be beneficial to human health.

We end this section with a commercial note. In April 2008, the FDA granted Orphan Drug Status to resveratrol, for the treatment of MELAS syndrome: mitochondrial encephalomyopathy with lactic acidosis and strokelike episodes, caused by mitochondrial DNA mutations. The sponsor was Sirtris Pharmaceuticals, a company in Massachusetts with expertise in resveratrol and its analogues (SRT501 was their resveratrol formulation). Orphan drug designation provides a company with seven years of marketing exclusivity. In late April 2008, Sirtris was purchased by GlaxoSmithKline for $ 720 million. In December 2010, Sirtris announced that the clinical trial of SRT501 was halted but studies with other synthetic resveratrol-mimicking drugs would continue. Currently, the role of resveratrol as an antiageing molecule seems to be in doubt (Wade 2011). However, we must emphasize that resveratrol possesses cancer chemopreventive properties. The difficulty seems to be deciding on dietary-relevant doses (Scott et al. 2012).

Effects on Cancer Stem Cells

Effects of resveratrol on CSCs have been shown. These include CSCs from acute myeloid leukemia (AML), brain, breast, head and neck, and pancreatic cancer. Inasmuch as resveratrol has multiple cellular targets, several of the cellular targets in CSCs have been revealed. In AML patients there is an increase in the cytokine interleukin 6 (IL-6) in their plasma. IL-6 induced activation of the Sonic Hedgehog signaling pathway has been shown in the human HL-60 cell line, and resveratrol (25 μM) inhibits this activation and decreases cell viability (Su et al. 2013). In malignant brain tumor medulloblastoma CSCs that were grown in spheroid cultures, resveratrol (100 μM) inhibited proliferation in vitro and enhanced radiosensitivity (Lu et al. 2009). In primary central nervous system atypical teratoid/rhabdoid tumors, resveratrol (150–200 μM) induced apoptosis of the isolated CD133^{+}CSCs in vitro and also enhanced radiosensitivity. The in vitro resveratrol-treated and irradiated CD133^{+}CSCs resulted in better survival of SCID mice in a 14-week xenograft experiment (Kao et al. 2009). In glioblastoma multiforma, comparison of CD133^{+} and CD133− cells has shown the involvement of signal transducer and activator of transcription 3 (STAT3). Resveratrol acts via inhibiting the STAT3 cytokine signaling pathway. CSCs treated in vitro with resveratrol (200 μM) and irradiation resulted in better survival of SCID mice; this effect of resveratrol can be mimicked with small hairpin (sh) RNA of STAT3 (Yang et al. 2012a).

Moving from the central nervous system to other organs, breast CSCs have been targeted by resveratrol. Using $CD44^+/CD24^-$ CSCs isolated from cell lines MCF7, MDA-MB231, and 231LM, resveratrol (50–100 µM) inhibited spheroid cultures by inducing apoptosis. The mechanism involved the action of resveratrol on lipid synthesis, by its downregulation of fatty acid synthase (FAS). Luciferase-expressing CSCs from MDA-MB231 showed smaller-sized tumors in nude mice xenografts with resveratrol administration (22.4 mg/kg via oral gavage or intraperitoneal injection) every 2 days in a 28-day experiment (Pandey et al. 2011). Similar results have been reported by the same investigators using CSCs isolated from another human breast cancer cell line, MCF10DCIS.com (Pandey et al. 2012). In human head and neck cancer, resveratrol (25–100 µM) eliminates aldehyde dehydrogenase (ALDH) activity, spheroid formation, and epithelial mesenchymal transition (EMT) in vitro, as well as reduces tumor xenograft volume and weight in vivo in immunodeficient mice (40 mg/kg daily for 20 days) (Hu et al. 2012). Human pancreatic $CD133^+/CD44^+/CD24^+$CSCs formed spheroid cultures and expressed stemness genes such as Oct4, Nanog, and Sox2. These activities were inhibited by resveratrol (10–30 µM) in vitro. In a special transgenic mouse model, $KRAS^{G12D,}$ the mice were designed to develop pancreatic ductal adenocarcinoma. The increase in size of the pancreas was inhibited by resveratrol (40 mg/kg) administered 5 days a week for 10 months. In addition to inhibiting the stemness genes, alias pluripotency maintaining factors, resveratrol inhibited EMT (Shankar et al. 2011). In summary, resveratrol aims at different cellular targets of CSCs, depending on the cancer type.

Epigallocatechin Gallate

Health Effects

EGCG is a polyphenol from green tea. (See Fig. 1 for the chemical structure of EGCG.) Tea is made from leaves of the plant *Camellia sinensis*, originally from China. EGCG is the major green tea polyphenol, accounting for 30–40% dry weight of the water-extractable material of green tea. It is converted to dimers and polymers during black tea production. Tea is one of the most popular beverages consumed in the world. Multiple health benefits have been associated with EGCG and tea consumption (Zaveri 2006; Yang et al. 2007; Khan and Mukhtar 2008). These include cancer chemoprevention, as well as the reduction of atherosclerosis, hypercholesterolemia, Alzheimer's and other ageing-related diseases. EGCG targets multiple signaling pathways. For example, we reported EGCG inhibition of iNOS both at the gene expression level and at the enzyme activity level (Chan et al. 1997). EGCG acts synergistically with cisplatin to kill ovarian cancer cells. Even in the cisplatin-resistant cell line C200, enhanced cell death by cisplatin was seen in the presence of EGCG in cell culture (Chan et al. 2006).

We end this section with a commercial note. In October 2006, the FDA approved an extract of green tea as a prescription drug for topical treatment of genital warts (Gross et al. 2008). The extract is Polyphenon E (Veregen) ointment (from a German biotech company MediGene and its marketing partner Bradley Pharmaceuticals,

Inc.). This was the first prescription botanical (herbal) drug approved by the FDA both under its current regulatory policy for botanical products as well as the FDA's new Drug Application Process (Blumenthal 2007).

Effects on Cancer Stem Cells

Effects of EGCG on CSCs have been shown. Actually, early reports used normal stem cells. Spheroid cultures for CSCs followed the initial work with normal neurospheres. In rat neurospheres, EGCG (10–50 µg/ml) and related compounds inhibited neurosphere adhesion and migration (Chen et al. 2003). Extending to CSC spheroid cultures, epigallocatechin (EGCG without the gallate moiety) and polyphenon 60 (characterized green tea extract, 10 µM) prevented cell shedding from mouse mammary cancer mammospheres in vitro. The mechanisms proposed are decreased ROS production and downregulation of matrix metalloproteinase-9 (Günther et al. 2007). EGCG (50 µM) inhibited spheroid cultures of human neuroblastoma BE(2)-C cells in vitro. These CSCs exhibit ALDH activity as well as the expression of stemness genes such as Oct4 and Nanog (Nishimura et al. 2012).

Both EGCG and its analogues act on CSCs. Several chemically synthesized analogues (5–40 µM) target human breast CSCs cultured as mammospheres from the cell line MDA-MB231. The mechanism has been attributed to activation of the AMP-activated protein kinase (AMPK) pathway, because a known activator of this pathway, metformin (5–10 mM), behaves in the same way (Chen et al. 2012c).

Synergistic effects of EGCG and another phytochemical, quercetin, on CSCs have been investigated. In human pancreatic CSCs, EGCG (20–60 µM) inhibited spheroid cultures, EMT, and the Hedgehog pathway in vitro. This activity was synergistic with quercetin (20 µM), as shown by increased apoptosis (Tang et al. 2012). In another publication, the same investigators also reported similar effects on human prostate CSCs (Tang et al. 2010). In summary, EGCG can act on CSCs either alone or in concert with another phytochemical.

Genistein

Health Effects

Genistein is an isoflavone (4,5,7-trihydroxyisoflavone to be exact) from soybeans. (See Fig. 1 for the chemical structure of genistein.) Soybeans are seeds from the legume plant *Glycine max*, originally from China, although China currently imports soybeans from the United States. It is believed that the isoflavone in soy-rich foods contributes to lower rates of prostate and breast cancers observed in China and Japan as compared to Western countries. Genistein is a molecule with many cellular targets, including inhibition of the NFκB pathway, modulation of the cell cycle, and induction of apoptosis (Banerjee et al. 2008). In addition, genistein has been classified as a phytoestrogen. However, it is lipid soluble and exhibits low oral

bioavailability in pharmacokinetics studies (Yang et al. 2012b). Furthermore, there is a "dark side" to "the whole soy story" (Daniel 2005). Soybeans contain protein-ase inhibitors that can be "toxic" to digestion. Also, people may develop allergic reactions to soy.

We end this section with an interesting demonstration of the transgenerational effect of genistein. The agouti mouse model, as noted by color change in the fur, is useful as an epigenetic biosensor for nutritional and environmental alterations on the fetal genome (Dolinoy 2008). Maternal dietary supplementation with genistein (250 mg/kg in the diet) led to DNA hypermethylation in the embryo and the meth-ylation state was maintained until adulthood. Thus, phytochemicals such as genis-tein modify the epigenome, and the effect starts early in embryogenesis (Dolinoy et al. 2006).

Effects on Cancer Stem Cells

Effects of genistein on CSCs have been shown. Genistein was reported as a protein tyrosine kinase inhibitor. In an in vitro study on chronic myelogenic leukemia in 1996, the stem and progenitor cells were more sensitive to genistein (200 µM) than the nor-mal progenitor cells (Carlo-Stella et al. 1966a, 1996b). In human prostate cancer, the CSCs from the cell line PC3 had been targeted by genistein (15 µM) in vitro, which aided in the death of these CSCs by docetaxel. In this work, the CSCs have been named "the Achilles' heel of cancer" (McCubrey et al. 2011). Another study on prostate cancer extended its focus to in vivo xenografts, and discovered the cellular target as inhibiting the Hedgehog pathway (Zhang et al. 2012b). Genistein (30 µM) inhibited spheroid cultures of prostate CSCs from the cell lines 22RV1 and DU145, and inhibited tu-mor development in vivo in nude mice (twice weekly of genistein and/or docetaxel at 10 mg/kg) in a 28-day experiment. Although the CSCs were resistant to docetaxel, genistein plus docetaxel decreased tumor volume more than genistein alone. Another interesting report involved the feeding of genistein to mice, "at concentrations present in soy protein isolate (250 mg/kg food;" Montales et al. 2012). Sera from the genistein-fed animals were used to treat human breast CSCs from the cell lines MCF7 and MDA-MB231. When 1–5% of these sera was added to the medium, spheroid formation was inhibited, suggesting "factors found in sera of mice consuming" genistein were active against CSCs. Molecular details of the factors were not characterized. However, this last investigation is interesting with respect to chemoprevention: genistein in the diet leads to the animals having factors in the circulation that inhibit CSCs.

Sulforaphane

Health Effects

Sulforaphane, alias 1-isothiocyanato-4-methylsulfinylbutane, is an isothiocya-nate found in brassicaceous vegetables. (See Fig. 1 for the chemical structure of

sulforaphane.) Broccoli, cabbage, cauliflower, Brussel sprouts, and kale are cultivars of the plant known as *Brassica oleracea*. These common vegetables belong to the plant order Brassicales. An older name is Cruciferae; they are also named crucifers or the cabbage family. The wild cabbage originally came from Europe. Broccoli and related vegetables contain many phytochemicals (Latte et al. 2011). In addition to sulforaphane, there are other compounds, such as indole-3-carbinol (I3C) and quercetin. Sulforaphane is released from the plant cell wall by activity of the enzyme myrosinase via cooking or chewing. The compound has multiple cellular targets: as an antioxidant, an activator of phase II detoxification enzymes, an anti-inflammatory agent, an inducer of apoptosis, and other roles (James et al. 2012). Broccoli has been used, as an example, for a "nutritional genomics" approach "in conveying to the consumer" that "the dietary regime being marketed will positively affect their health" (Ferguson and Schlothauer 2012).

We end this section with a quote on broccoli from a former US president (Dowd 1990). In March 1990, President George H. W. Bush, in response to queries about a broccoli ban he had imposed upon Air Force One, said: "I do not like broccoli. And I haven't liked it since I was a little kid and my mother made me eat it. And I'm President of the United States, and I'm not going to eat any more broccoli!" In fact, the president might be a "supertaster." People who find Brussel sprouts unbearably bitter carry a variant of the bitter taste receptor (T2R38) which results in their enhanced innate immunity (Lee et al. 2012a).

Effects on Cancer Stem Cells

Effects of sulforaphane on CSCs have been shown. Sulforaphane has been shown to inhibit human breast CSCs from the cell lines MCF7 and SUM159 in vitro and in vivo (Li et al. 2010). Spheroid cultures were inhibited (10–20 µM), as well as ALDH expression. In NOD/SCID mouse xenografts, two successive generations elegantly showed a decrease in tumor volume and an increase in survival (50 mg/kg daily for 14 days) in a 35-day experiment. Sulforaphane inhibited spheroid cultures in human pancreatic CSCs from the cell line MIA-PaCa2 in vitro (20 µM) and in vivo (Rausch et al. 2010). Sulforaphane inhibits renewal of pancreatic cancer stem cells in vitro (20 µM) by inhibiting the Sonic Hedgehog signaling pathway (Rodova et al. 2012). In nude mice xenograft experiments, sulforaphane (60 mg/kg) or co-treatment with the multikinase inhibitor sorafenib (3 mg/kg), daily for 3 days in a 5-day experiment, reduced tumor size via inhibition of the NFκB pathway (Rausch et al. 2010). The same research group also extended their report on pancreatic CSCs to include results on prostate CSCs derived from the cell line DU145 (Kallifatidis et al. 2011). Sulforaphane was added in vitro to taxol, cisplatin, gemcitabine, fluro-uracil, or doxorubicin. Targeting was specific for CSCs, because normal cells (primary human fibroblasts and umbilical vein endothelial cells) were not affected. In vivo nude mice xenografts for pancreatic CSCs used sulforaphane in combination with gemcitabine (3 mg/kg consequently for 3 days; treatment started after tumor development was observed, in a 40-day experiment). In a different experiment, the

pancreatic CSCs had been pretreated with the sulforaphane–gemcitabine combination before xenograft. Both protocols resulted in decreased tumor volume (Kallifatidis et al. 2011). In fact, sulforaphane alone could reduce human pancreatic CSC growth in immunodeficient mice, through inhibiton of the Sonic Hedgehog signaling pathway (20 mg/kg once a day for 5 days in a 6-week experiment; Li et al. 2013). In addition to solid tumors, sulforaphane enhancement of drug efficacy has been reported for leukemia. The CD34^{+} chronic myeloid leukemia CSCs previously resistant to imatinib were killed by a combination of imatinib (0.1–1 µM) and sulforaphane (20–30 µM) in vitro, via activation of the Wnt pathway and ROS induction (Lin et al. 2012).

Other Dietary Phytochemicals

Many phytochemicals may serve as chemopreventive agents. In some cases, their activities on CSCs have been investigated. In addition to sulforaphane, another important phytochemical found in the brassicaceous vegetables is indole-3-carbinol (I3C). I3C is a derivative of glucobrassicin, a secondary plant metabolite. I3C can be further metabolized into a dimer (3,3"-diindolmethane, DIM). I3C, DIM, and related synthetic analogues are potential agents of chemoprevention and cancer therapy (Weng et al. 2008; Safe et al. 2008). DIM inhibited spheroid culture formation, for CSCs from human and mouse cancer cell lines (the human non-small–cell lung carcinoma H460 and the murine melanoma B16/F10) in vitro. DIM-treated melanoma CSCs showed smaller tumor volume in vivo (Semov et al. 2012).

Quercetin is a flavonol found in common fruits and vegetables such as apples, broccoli, and onions. It has antioxidant activity and has been reviewed as safe in vivo, although mutagenic in vitro (Harwood et al. 2007; Boots et al. 2008; Gibellini et al. 2011). We reported that quercetin sensitized the killing by cisplatin of the human ovarian cancer cell line SKOV3 (Chan et al. 2003). Quercetin–EGCG's synergistic activity on human pancreatic CSCs has been discussed (Tang et al. 2011). In fact, quercetin by itself inhibited spheroid cultures of pancreatic CSCs, and it exhibited a synergistic effect with sulforaphane. In nude mice xenografts, quercetin and sulforaphane decreased tumor size (Zhou et al. 2010). Quercetin also targets the heat shock protein Hsp27. In human breast CSCs, inhibition of spheroid cultures by the Hsp90 inhibitor geldanamycin was enhanced by quercetin (Lee et al. 2012b). In human lung CSCs from the cell line A549 as xenografts to NOD/SCID mice, tumor volume was decreased by treatment with quercetin, cisplatin, and gemcitabine (Hsu et al. 2011a).

Cucurbitacin I, a cell-permeable triterpenoid compound, is found in members of the Cucurbitacea family (cucumber, squash, melons, pumpkins) as well as brassicaceous vegetables. Investigators from Taiwan showed its effect on CD44^{+}/ALDH^{+} CSCs from head and neck squamous cell carcinoma (Chen et al. 2010), as well as CD133^{+} CSCs from non-small–cell lung cancer (Hsu et al. 2011b), thyroid cancer (Tseng et al. 2012), and pediatric brain cancer medulloblastoma (Chang et al. 2012). Its cellular target is the STAT3 cytokine pathway.

Lupeol is another triterpene found in fruits and vegetables. A research group in Hong Kong demonstrated its effect on CSCs from liver cancer (Lee et al. 2011). Its

cellular target is the phosphatase and tensin homologue (PTEN)-protein kinase B (Akt)-ABCG2 pathway.

Extending from purified phytochemicals to plant extracts, a blueberry preparation fed to mice has been shown to be effective towards CSCs. Sera from blueberry-fed animals inhibited spheroid cultures of human breast CSCs (Montales et al. 2012). A pomegranate extract also inhibited mouse mammary CSCs in vitro (Dai et al. 2010)

In summary, individual phytochemicals and plant extracts from fruits, vegetables, spices, and beverages may be harnessed to inhibit CSCs, but their cellular targets may vary depending on the cancer type. A list of probable molecular targets for phytochemicals in CSCs can be found in Table. 2.

Other Molecules Targeting Cancer Stem Cells

In addition to dietary phytochemicals, many other molecules target CSCs. We start with compounds isolated from plant species. Parthenolide (PTL), from the feverfew plant (*Tanacetum parthenium*), is commonly used in herbal medicine. PTL targets human acute myelogenous leukemia stem cells (Guzman et al. 2005). As an inhibitor of NFκB, it inhibited human breast CSCs from the cell line MCF7 (Zhou et al. 2008). Screening in silico for compounds with similar effects on gene expression as PTL has revealed others, including celastrol, found in thunder god vine (*Tripterygium wilfordii*) and used in Chinese herbal medicine (Hassane et al. 2008). Another compound, berberine, from plants such as goldenseal (*Hydrastis canadensis*) and the Chinese goldthread (*Coptis chinensis*), also used in herbal medicine, targets MCF7 SP cells (Kim et al. 2008). MCF7 SP cells were also inhibited by the alkaloid oxymatrine, from the Chinese herbal medical plant *Sophora flavescens* (Zhang et al. 2011). Eriocalyxin, an analogue of the natural diterpene from the Chinese herbal medical plant *Isodon eriocalyx*, inhibited human ovarian CSCs via NFκB inhibition (Leizer et al. 2011). Cyclopamine from corn lily (*Veratrum californicum*) targets glioblastoma CSCs by inhibiting the Hedgehog pathway (Bar et al. 2007). However, it should be noted that cyclopamine is a teratogen that causes holoprosencephaly (cyclopia). Silybin, alias silibinin, is a flavonoid compound from the herbal medical plant milk thistle (*Silybum marianum*). The phytochemical targets colorectal CSCs (Wang et al. 2012b). Butein is a chalconoid compound from the Chinese lacquer tree (*Toxicodendron vernicifluum*) with aromatase-inhibiting effects. It targets human breast CSCs (Cioce et al. 2010). Gossypol is a polyphenol from the cotton plant (genus *Gossypium*) and at one time had been tested as a male contraceptive. It targets prostate CSCs via activation of the p53 gene regulatory protein (Volate et al. 2010). From the above studies, it is obvious that many phytochemicals can target CSCs of different cancers.

In addition to plants, molecules targeting CSCs been found in other sources. 3-O-methylfunicone (OMF), a secondary metabolite of the soil fungus *Penicillium pinophilum*, inhibited spheroid cultures of human breast CSCs from the cell line MCF7 (Buommino et al. 2011). Thus, fungal metabolites may act on CSCs.

Vitamins target CSCs. Retinoic acid, a metabolite of vitamin A, is a molecule essential for growth and development; it also induces cell differentiation. All-*trans*

Table. 2 Probable Molecular Targets for Phytochemicals in Cancer Stem Cells (CSCs). (Adapted from Kawasaki et al. 2008; Subramaniam et al. 2010; Li et al. 2011, and the references cited in text)

Targeted process	Molecular targets	Phytochemicals (Examples Of Sources)
Signaling Pathways		
Hedgehog	Gli	Cyclopamine [Corn lily]
	Smo	EGCG [Green tea]
		Genistein [Soy]
		Quercetin [Apples, onions]
		Sulforaphane [Broccoli, cabbages]
Wnt	β-catenin	EGCG [Green tea]
		Sulforaphane [Broccoli, cabbages]
Notch	Notch	Curcumin [Turmeric]
		Resveratrol [Grapes, peanuts]
Kinases	Protein tyr kinase	Genistein [Soy]
Inflammation	NFκB	Curcumin [Turmeric]
		Parthenolide [Feverfew]
	IL-6	Resveratrol [Grapes, peanuts]
	STAT-3	Cucurbitacin [Cucumber, pumpkins]
		Resveratrol [Grapes, peanuts]
Drug Resistance		
Efflux	ABCG2	Curcumin [Turmeric]
		Piperine [Black pepper]
Metabolism	ALDH	Curcumin [Turmeric]
		Sulforaphane [Broccoli, cabbages]
	AMPK	EGCG [Green tea]
	Hsp 27	Quercetin [Apples, onions]
	ROS	EGCG [Green tea]
		Sulforaphane [Broccoli, cabbages]
EMT	Slug	Resveratrol [Grapes, peanuts]
Metastasis	MMP-9	EGCG [Green tea]
Apoptosis	Bax	I3C [Broccoli, cabbages]
	Caspase	Resveratrol [Grapes, peanuts]
Autophagy	LC3	Curcumin [Turmeric]
Differentiation	RAR	Retinoic acid [Spinach, carrots]

ABCG-2 ATP-binding cassette transporter 2, *ALDH* aldehyde dehydrogenase, *AMPK* AMP-activated protein kinase, *Bax* B cell lymphoma 2 (Bcl-2) associated X protein is a proapoptotic protein, *β-catenin* beta catenin, a protein in the Wnt signaling pathway, *Caspase* cysteine-dependent aspartate-directed protease are proteinases functional in apoptosis, *EGCG* epigallocatechin gallate, *EMT* Epithelial mesenchymal transition, *Gli* zinc finger transcription factor in Hedgehog signaling pathway, *Hsp 27* heat shock protein 27 is a chaperone protein, *I3C* indole-3-carbinol, *IL-6* interleukin 6, *LC3* microtubule associated protein light chain 3 is a marker for autophagy, *MMP-9* matrix metalloproteinase 9, NFκB, transcription factor nuclear factor kappa B, *Notch* a membrane protein in the notch signaling pathway, *RAR* retinoic acid receptor, *ROS* reactive oxygen species, *STAT 3* signal transducer and activator of transcription 3, *Slug* transcription factor Slug is important for EMT, *Smo* plasma membrane Smoothed in Hedgehog signaling pathway, *Tyr* tyrosine

retinoic acid (ATRA) inhibited human breast CSCs (Ginestier et al. 2009); ATRA stealth liposomes prevented relapse of breast cancer arising from breast CSCs (Li et al. 2011). Gemini vitamin D, a vitamin D analogue, targets human breast CSCs (So et al. 2011). *Gamma*-tocotrienol, a vitamin E constituent, targets human prostate CSCs (Luk et al. 2011).

Some drugs may also target CSCs. The telomerase inhibitor imetelstat inhibited both breast and pancreatic CSCs (Joseph et al. 2010). Statins, including simvastatin and lovastatin, suppressed abnormal human embryonic stem cells and thus may play a role in CSC inhibition (Gauthaman et al. 2009). Probably the most significant drug reported thus far for targeting CSCs is metformin (Hirsch et al. 2009; Vazquez-Martin et al. 2011; Iliopoulos et al. 2011). Metformin is currently being used to treat diabetes. There is a definite future in its application to CSCs (Dowling et al. 2012).

High-throughput screening assays on currently available compounds have been established for embryonic and CSCs. In one human embryonic stem cell screen, retinoic acid and others have been found to modulate Oct4 expression (Desbordes et al. 2008). In a breast CSC screen, salinomycin has been found to target CSCs. This compound, originally isolated from *Streptomyces albus*, is an ionophore and has been added to chicken feed as a coccidiostat to control protozoan parasites in poultry (Gupta et al. 2009b). In another ovarian CSC screen from 1,200 clinically approved drugs, the compound niclosamide has been found to target CSCs (Yo et al. 2012). Originally developed as a molluscicide (for snails that are intermediate hosts of blood flukes, parasites causing schistosomiasis) and anthelminthic (for cestodes or tapeworms), niclosamide has a proton carrier mode of action and is also active against viruses (such as the one that causes severe acute respiratory syndrome, SARS; Jurgeit et al. 2012). In a combined normal and CSC screen, the antipsychotic drug thioridazine has been shown to target only CSCs, whereas salinomycin targets both normal and CSCs (Sachlos et al. 2012). It is expected that more compounds targeting CSCs will be uncovered in future screenings.

A complementary approach is to look for novel sources that target CSCs. Along this line of research, an extract from the marine sponge *Crambe crambe* has been shown to target pancreatic and prostate CSCs (Ottinger et al. 2012). Thus, unusual animal and plant species may yield novel molecules targeting CSCs.

Desirable Properties of Compounds Targeting Cancer Stem Cells

Phytochemicals and other compounds that target CSCs have just been reviewed. Here we discuss desirable properties needed for application to cancer therapy. Topics of concern are antagonistic effects, combination therapy, multiple molecular targets, safety, and bioavailability.

Whereas synergistic effects among phytochemicals are welcome in cancer prevention and therapy (Liu 2004; HemaIswarya and Doble 2006), antagonistic effects are not. We can use curcumin, resveratrol, and EGCG as examples. Curcumin is

synergistic with resveratrol in colon cancer inhibition (Majumdar et al. 2009), but antagonistic with EGCG towards keratinocytes. EGCG enhances keratinocyte differentiation whereas curcumin inhibits its differentiation; curcumin activates apoptosis whereas EGCG does not (Eckert et al. 2006). Potential antagonistic effects among compounds must be analyzed before combination use.

Despite the potential opposing effects between individual phytochemicals, their combinatorial applications have been promoted. It has been suggested that "Specific combinations of phytochemicals may be far more effective in protecting against cancer than isolated compounds" because "combinations of dietary chemopreventive agents can sometimes result in significant activities at concentrations where any single agent is inactive" (De Kok et al. 2008). Thus, combinatorial use of phytochemicals is an attractive approach in targeting CSCs.

Each CSC has multiple molecular targets. Dietary phytochemicals such as curcumin, resveratrol, and EGCG tend to aim at a multitude of cellular targets. Perhaps it is because of these characteristics that definitive mechanisms of action are not available despite decades of research (Francy-Guilford and Pezzuto 2008). This multitarget nature of phytochemicals may be advantageous in targeting CSCs because the multifaceted mode of action may hinder the cell's ability to develop resistance to the phytochemicals.

How safe are the dietary phytochemicals? Because they can interact with drug transporters and drug metabolizing enzymes (known as phase I and phase II enzymes), the potential toxicity and safety of phytochemicals are major concerns. As an example, they have antioxidant capacities against ROS. However, under special conditions they also exhibit pro-oxidant capacity. Whereas ROS is harmful in general, the removal of too much ROS interferes with body functions, as seen in the warning presented by scientists to the food industry (Finley et al. 2011). With an emphasis on their putative cancer chemopreventive uses, the safety of a group of phytochemicals has been reviewed (Verschoyle et al. 2007). For curcumin, given to cancer patients at 3,600 mg/day for 4 months or 800 mg/day for 3 months, only minor adverse effects were seen. For EGCG, daily oral doses for 4 weeks at 800 mg/day in 40 volunteers caused only minor adverse effects. For resveratrol, a single oral dose at 5 g in 10 volunteers caused only minor adverse effects (Boocock et al. 2007). However, "High consumption of dietary phytochemicals should be considered with caution taking into account their dosage regimes, toxicity, metabolic conversion, transport mechanisms, tissue availability, synergistic interaction with drugs, and interferences with key enzymes, receptors, metabolic pathways, and normal GI microflora" (Priyadarsini and Nagini 2012). More extensive toxicological testing is recommended, if phytochemicals are to be taken as cancer chemopreventive agents on a long-term basis by healthy individuals.

How bioavailable are the dietary phytochemicals? Bioavailability may differ, depending on conditions varying from pure compounds and plant extracts, to mixtures with other food components (EGCG vs. green tea extract, genistein vs. soymilk or tofu, quercetin vs. apple cider or apples; Manach et al. 2005). For phytochemicals such as plant polyphenols, the effects in vivo, although significant, are more limited than those observed in vitro (Williamson and Manach 2005). However, it is possible to design appropriate interactions that affect the bioavailability of phytochemicals in vivo (Scholz

and Williamson 2007), such as enhancement of curcumin efficacy with piperine, as previously mentioned. We have used oral gavage plus starvation (an empty stomach) to enhance curcumin uptake in mice (Chan et al. 1998; Adapala and Chan 2008) so as to demonstrate its in vivo inhibition of the inflammatory iNOS in the murine liver (beneficial outcome) and the exacerbation of murine visceral leishmaniasis, a protozoan parasitic disease (detrimental outcome). The last report recommends caution with regard to the potential clinical use of curcumin: it may re-activate latent infections.

A second look at bioavailability relates to the concept of hormesis. The effect of curcumin has been described as an example of xeno-hormesis (Salvioli et al. 2007). The concept of hormesis suggests that the fundamental nature of the dose–response curve to be neither linear nor threshold, but rather U-shaped or J-shaped. This shows a low dose stimulatory response known as the hormetic effect, representing over-compensation in response to disruptions in homeostasis (Calabrese and Baldwin 2001). For phytochemicals among an estimated 10,000 secondary products (natural pesticides), it has been proposed that human ancestors evolved a generalized defense mechanism against low levels of phytochemicals to enable their consumption of many different plant species containing variable levels of natural pesticides (carcinogens) without subsequent ill health. Traces of phytochemicals found in fruits and vegetables may potentiate the immune system and help to protect against cancer (Trewavas and Stewart 2003). A biphasic dose response is observed. At high concentrations, phyto-chemicals can be toxic, whereas subtoxic doses may induce adaptive stress responses. There is the activation of signaling pathways that results in increased expression of genes encoding cytoprotective proteins. It is suggested that hormetic mechanisms of action may underlie many of the health benefits of phytochemicals (Mattson 2008). These benefits would include their action against CSCs.

Implementation of the Phytochemical Approach Towards Chemoprevention

Among all the compounds undergoing clinical trials, the success rate of anticancer drugs is less than 5%, the lowest as compared to other drugs for cardiovascular, central nervous system, and infectious diseases; and it takes $ 2 billion to bring a drug to market (Bhattacharjee 2012). Examples of anticancer drugs from plants are taxol and vinblastine. Plants are a rich source of drugs. "Only a small fraction of the immense diversity of plant metabolism has been explored for the production of new medicines and other products important to human well-being" (De Luca et al. 2012). Therefore, new phytochemicals targeting CSCs are expected in the future.

In the meantime, currently available phytochemicals need more definitive studies, because an evidence-based approach is favored (Higdon 2007). "Facts and fiction" of each phytochemical must be differentiated (Espín et al. 2007). A phyto-chemical may act in concert with conventional drugs, as visualized by the following metaphor. When used in low concentrations, the phytochemical causes multiple wounds without killing the CSC. Nevertheless, the treatment increases the CSC's

vulnerability to the magic bullet represented by the chemotherapeutic drug (Russo 2007). In this sense, dietary phytochemicals may serve as adjuvant anticancer agents with practically minimal or no side effects.

There is an urgent need to bridge the gap between apparent in vitro efficacy and clinical use of dietary phytochemicals (Manson et al. 2007). Biomarkers and decisions on dosages for clinical trials still have to be worked out (Scott et al. 2009). As an example, transcription analyses (microarrays of gene expression) of peripheral blood mononuclear cells (PMBCs) from dietary intervention studies "have not resulted yet in clear confirmation of candidate genes related to disease risk" (De Mello et al. 2012).

What is the current status of dietary phytochemicals for cancer chemoprevention? The focus lies in the identification of molecular mechanisms and cellular targets. The "concept of combination chemoprevention by multiple agents or by the consumption of 'whole foods' has become an increasingly attractive area of study" (Mehta et al. 2010).

Concerning "whole foods" consumption, the current US dietary guidelines have been illustrated as MyPlate, with advice to cover half the plate with fruits and vegetables (Willett and Ludwig 2011). From Europe, there is Mediterranean Diet Pyramid Today, with a plant-based core of the dietary pattern for consuming fruits and vegetables everyday (Bach-Faig et al. 2011).

How effective are fruits and vegetables for cancer chemoprevention? The early case-control studies tout the reduction in cancer risk, but the more recent prospective cohort studies show only a weak association. For example, a pooled analysis of 14 cohort studies of pancreatic cancer concludes that "fruit and vegetable intake during adulthood is not associated with a reduced pancreatic cancer risk" (Koushik et al. 2012). Therefore, this is "turmoil in the produce section" but: "A very weak or undetectable association between fruits and vegetables and risk of cancer does not exclude the possibility that one or a small group of fruits or vegetables, or a specific substance in some of these foods, has an important protective effect" (Willett 2010). This latter point has been well proven by the catalogue of dietary phytochemicals that can target CSCs covered in this review. Furthermore, "although the evidence for benefits of fruits and vegetables against cancer was waning, data supporting benefits for cardiovascular disease were accumulating" (Willett 2010).

Perspectives

In September 2011, the United Nations General Assembly convened a High Level Meeting on Non-communicable Diseases (NCDs) because the four main NCDs—cardiovascular disease, cancer, chronic lung diseases, and diabetes—kill three in five people worldwide, and cause great socioeconomic harm within all countries, particularly developing nations. In September 2012, *Science* magazine published a special section including a cover photo on the topic of disease prevention (Ash et al. 2012). One contributed article titled: "Can Noncommunicable Diseases Be

Prevented?" suggested "effective approaches for large-scale NCD prevention include," among these, "increasing the consumption of fresh fruits and vegetables" (Ezzati and Riboli 2012).

When is the best time to start eating fruits and vegetables? First Lady Michelle Obama started a campaign against childhood obesity (Willett and Ludwig 2011). It has been suggested that "research should focus more sharply on specific fruits and vegetables and their constituents and on earlier periods of life" (Willett 2010). We have already mentioned genistein's effect on fetal DNA methylation. Thus, here comes "the maternal womb: a novel target for cancer prevention" (Simmen and Simmen 2011). Dietary interventions prior to and during pregnancy may confer cancer chemoprevention. The first sentence in a discussion on the "myth or reality" aspects of dietary molecules begins with the 400 BC statement of Hippocrates: "Let thy food be thy medicine and thy medicine be thy food" (Neergheen-Bhujun et al. 2012).

We end this chapter with a comment on cell differentiation and the CSC. In October 2012 the Nobel Prize in Physiology or Medicine was announced for the discovery of cell reprogramming (Abbott 2012). In 1962 John Gurdon published his work on the plasticity of a differentiated nucleus from an amphibian: the nucleus from a tadpole's intestinal cell replaced the egg nucleus and generated a frog. In 2006 Shinya Yamanaka published his work on the reprogramming of a differentiated cell type from a mouse: the mouse fibroblast became an embryonic stem cell by adding just four genes. The latter work is transferable to humans and the induced pluripotent stem (iPS) cells will be important for future clinical applications. The differentiated state of a cell is dynamic. A CSC plays its essential role in maintaining the particular tumor. If dietary phytochemicals, or specific fruits and vegetables, are available to destroy this CSC, or reverse its stemness characteristics, or induce its cell differentiation, there will be chemoprevention of cancer.

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