Flavonoids and Cancer Stem Cells Maintenance and Growth

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Abstract

Normal stem cells are known to possess three important characteristics of self-renewal, restriction on stem cell numbers and ability to divide and differentiate. Compared to normal stem cells, the cancer stem cells (CSCs) have no control on the stem cell numbers. CSCs constitute a miniscule number of cells in the tumour and are responsible for tumour growth, recurrence and progression. CSCs play a vital role in drug resistance, EMT and metastasis, which are responsible for approximately 90% of cancer-related deaths. Thus, targeting CSCs has now gained significant importance in the control and treatment of various cancers. Traditional cancer therapy regimens have not been successful against cancer drug resistance and metastasis. In the recent past, numerous dietary compounds derived from natural sources have been found effective in chemoprevention and treatment of various cancers. Flavonoids are one of such naturally occurring polyphenolic compounds that are found abundantly in fruits, vegetables, tea, seeds, grains, nuts and some traditional medicinal herbs. Various flavonoids have also been shown to have an inhibitory effect on the self-renewal potential and survival of cancer stem cells of different origins. The aim of this chapter is to focus on cancer stem cells and their role in tumour progression and drug resistance and how chemoprevention using flavonoids can become an effective tool to control cancer growth.

Keywords

Cancer stem cells · EMT · Metastasis · Drug resistance · Chemoprevention

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26.1 Introduction

Cancer is defined as the group of diseases in which cells divide uncontrollably by breaking normal rules of cell division. Normal cells follow a set of instructions that dictate whether and when cells should divide or differentiate, but in cancer cells these instructions are not followed. Cancer cells grow uncontrollably and invade nearby tissues and spread through the body. In the year 2012, International Agency for Research on Cancer (IARC) estimated 14.1 million new cancer cases and 8.2 million cancer deaths worldwide. Cancer is caused by various factors that include environmental factors and genetic and/or epigenetic factors within a single cell. Cancer cells alter important mechanisms of cells such as rate of proliferation, invasion and metastasis ability, replicative immortality and angiogenesis [1]. The cancer cells may comprise CSCs which are a small population of cancer cells having indefinite potential for self-renewal and frequently develop drug resistance [2]. Ever since the identification of CSCs in 1994, they have been a subject of intense study. Their properties, such as the capability to initiate and propagate the tumour growth and develop resistance to the conventional therapies, have garnered focus of the cancer researchers [3]. The fundamental difference between CSCs and normal stem cells is that in the case of the latter, the number of cells generated through cell division is similar to cells that terminally differentiate, thus keeping the number of normal stem cells constant. In contrast, cancer stem cells keep on proliferating and do not differentiate, while the mature cells do not die. Although, in both the cases, some cells do not actively proliferate and function as reserve cell population [4]. Stem cells have been implicated in various important cellular processes, and they are identified and isolated based on various cell surface markers.

CSCs are speculated to have an imperative role in tumour cell proliferation, invasion and metastasis. One of the most important cellular events that are related to heterogeneity and stemness of tumour is epithelial-mesenchymal transition (EMT). In EMT, epithelial cells lose their cell adhesion property and get converted into motile cells having a distinctive mesenchymal morphology, thus allowing the cells to migrate to a different location within or outside the tissue. Apart from these pro-invasive and metastasis-inducing functions, EMT has been shown to promote stemness in tumours in both in vitro and in vivo models [1]. EMT confers metastatic potential to the cancer cells with the help of EMT transcription factors such as Twist and Zeb1 [5]. A few recent studies have shown that CSCs express various EMT markers which promote the generation of the cancer stem cell-like population leading to chemotherapeutic resistance [6, 7]. Thus, CSCs play a pivotal role in EMT and metastasis of cancer cells and vice versa. Indeed, approximately 90% of cancer mortality has been attributed to metastasis and not primary tumours [8]. Therefore, it is pertinent that CSCs are targeted along with the usage of conventional therapies for successful cancer therapy to minimize or negate the chances of relapse (Fig. 26.1).

Prevalent therapies such as chemotherapy, radiation therapy and surgery have not proved significantly successful in reducing the burden of cancer. In addition, chemotherapy and other traditional therapies are confined by toxicity and development

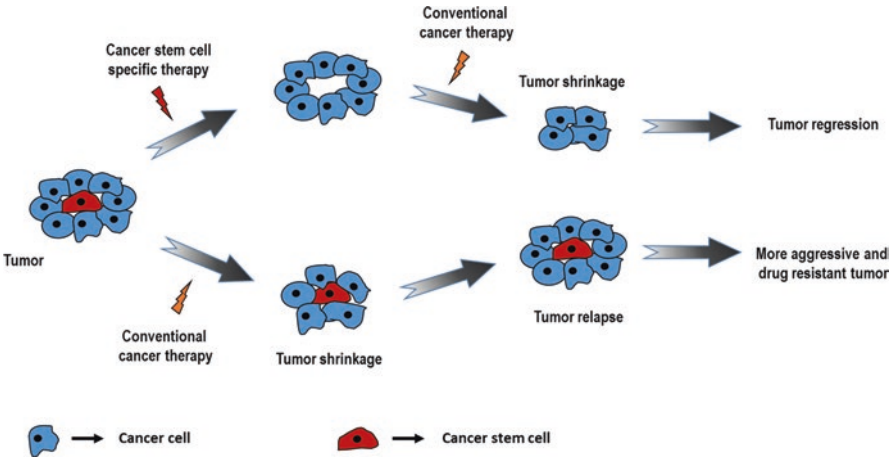

Fig. 26.1 Cancer stem cell therapy versus conventional cancer therapy

of resistance that eventually results in relapse of tumours [9]. Studies have shown that chemoprevention is a much better approach for controlling cancers [10]. Usage of dietary compounds in cancer chemoprevention has been a topic of interest among researchers for the past few years as it has been shown in various studies that there is a clear link between dietary habits and cancer prevention [11]. Flavonoids, available in various food items such as citrus fruits, vegetables, tea and cocoa, are labelled as one of the important components of functional foods, and they exhibit several beneficial properties for human health [12]. These properties include anti-allergic, anti-inflammatory, antioxidant, antiviral and anticancer effects [13]. Most flavonoids arise from natural sources and are found to be safe for human consumption [14]. Numerous epidemiological studies support the use of flavonoids for their chemopreventive abilities as well as for their anticancer potential [15]. Several flavonoids have been shown to inhibit CSCs in vitro and in vivo [9, 16]. Flavonoids have been shown to target or intervene signalling pathways that are critical for CSC maintenance and growth such as Hedgehog, Wnt/β-catenin and Notch pathways. Flavonoids have been used in combination with several chemotherapeutic drugs and found to exert synergistic effect to control growth, maintenance, cell survival and stemness of cancer cells and CSCs [14]. Thus, flavonoids can prove to be promising molecules that can target CSCs.

26.2 The Biology of Cancer Stem Cells

26.2.1 Cancer Stem Cells: Origin

It is a well-established fact that cancer arises from a mutation in a single cell, yet tumours eventually become heterogeneous in nature containing undifferentiated and proliferative cells expressing different markers [17]. Heterogeneous nature of

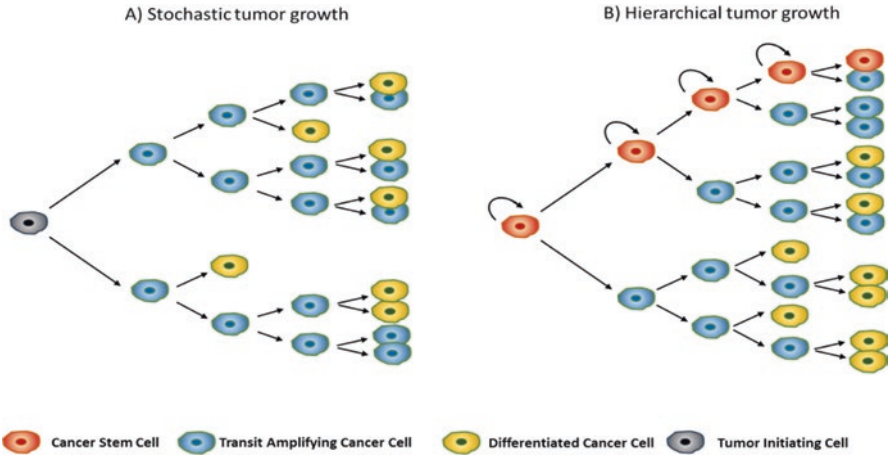

Fig. 26.2 Two models proposed for tumour growth

tumours is believed to be the reason for tumour progression, metastasis, relapse and resistance to therapy. Heterogeneity in tumours is of two types, intra-tumoural and inter-tumoural, and they arise due to mechanisms such as clonal evolution, tumour microenvironment and presence of CSCs [18]. Two models have been proposed for the tumour growth (Fig. 26.2). A stochastic model hypothesizes that tumours arise as a homogenous group of cells and the heterogeneity is the outcome of arbitrary stochastic events. However, hierarchical model propounds that a stem-like precursor cell results in the generation of a heterogeneous group of cells that differentiate with distinct phenotypical as well as biological attributes [1].

Stem cells (SCs) are responsible for various functions such as tissue homoeostasis and regeneration. After division SCs generate transit-amplifying cells, which after some rounds of divisions eventually differentiate terminally and develop the specific tissue. Apart from this, stem cells can also be activated when injuries occur and participate in tissue repair [19]. Another noteworthy feature of stem cells is that they do not express a specific marker for different tissues and are mostly determined by their functional properties such as their scope for long duration self-renewal and their efficiency to differentiate into one or multiple cell lineages [1].

The concept of adult haematopoietic stem cells motivated Dick and colleagues to carry out experiments and demonstrate that in human acute leukaemia, all the leukaemia cells were not able to initiate leukaemia when introduced into immune-deficient mice. Terminally differentiated leukaemia cells were not able to propagate the disease; however, those leukaemia cells which had similar markers as of adult HSCs had more efficacy for initiating leukaemia and thus were termed as leukaemic stem cells (LSCs) [20].

Inspired by the work of Dick and colleagues, many researchers diverted their focus on CSCs and their presence in solid tumours. CSCs in solid tumours were first derived from breast cancer cells when a group of researchers injected the $CD44^{+}CD24^{-/low}$ population of cells into immune-deficient mice [21]. Subsequent studies using the same approach by various research groups led to the discovery of

CSCs in various cancers such as pancreatic cancer, squamous cell carcinomas, lung cancer and melanoma [1].

26.2.2 Cancer Stem Cell Biomarkers in Various Human Malignancies

The first evidence of CSCs in cancer was reported by John Dick in 1994 in AML [20]. Subsequently, CSCs were reported in numerous human malignancies such as brain, colon, ovary, breast, pancreas, prostate and lung [22]. Some of the biomarkers prevalent in CSCs of various malignancies are discussed below.

Specific biomarkers can be used to differentiate CSCs from normal cells and other tumour cells [23]. CSCs express several markers simultaneously, and it is very difficult to define or describe CSCs using one biomarker. Understanding the role of CSC biomarkers may help us to uncover unprecedented facts that will in turn improve current cancer therapy and prognosis [24]. CD133 is the highly investigated biomarker for CSCs. It is a 120 kDa cell surface glycoprotein comprised of two glycosylated extracellular loops and transmembrane domains which are five in number [25]. CD133 and its epitope AC133 have been frequently demonstrated as cell surface marker for CSCs in multiple cancers including central nervous system tumours as well as colon, breast, prostate, ovarian and lung cancers [25]. $CD133^{+}$ cells were more competent in tumour sphere formation and differentiation than $CD133^{-}$ cells in lung cancer cell lines like NSCLC and SCLC [26]. Oct-4 and NANOG, which are well-known embryonic stem cell markers, were expressed by $CD133^{+}$ cells. Upon knockdown of Oct-4 in $CD133^{+}$ cells, clonogenicity and chemosensitization of cells reduced significantly [27]. Further, $CD44^{+}/CD49f^{hi}/CD133/2^{hi}$ phenotype cells have been shown to have increased tumorigenicity and self-renewal capacities in vivo and give rise to molecular and functional heterogeneity [28].

CD44 is a multifunctional membrane-bound glycoprotein which binds to hyaluronic acid that is abundant in stem cell niches and performs various functions such as differentiation, migration, cell adhesion, homing and interaction with extracellular matrix [25, 29]. A recent study by Wang et al. suggested an important role of CD44 in identification of lung CSCs. They showed that the expression of CD44 variant exon 6 (CD44v6) in 79 lung cancers was 67.6% (48/71) in NSCLC and only 0% (0/8) in SCLC. This data demonstrates that the expression of CD44v6 is associated with histologic type of tumour [25, 30]. $CD44^{+}/CD24^{-/low}$ cell surface marker has been most commonly used to characterize breast CSCs. The stemness-inducing properties of $CD44^{+}/CD24^{-/low}$ cells have been further implicated in colony formation, migration and invasion assays [31].

Aldehyde dehydrogenases (ALDH) are a family of $NAD(P)^{+}$-dependent enzymes which are involved in differentiation, cellular detoxification and drug resistance by exploiting cellular aldehyde oxidation. Increased activity of ALDH was observed in stem cell populations of different cancers which suggests its role as a common biomarker for both normal and cancer stem cell populations [25, 29]. Overexpression of ALDH1 is observed in lung tumours compared to the normal lung. A study has demonstrated the expression level of ALDH in 12 different human lung cancer cell

lines using flow cytometry. A higher expression level of cytosolic forms, ALDH1A1 and ALDH3A1, in some NSCLC cell lines and patients with lung cancer was observed [29]. NSCLC (non-small-cell lung cancer) patients having tumour cells with elevated expression of ALDH1A1 show significant resistance to EGFR tyrosine kinase inhibitors and chemotherapy drugs which is associated with poor clinical outcome [32, 33].

CD338 or ABCG2 (BCRP) is a member of the ATP-binding cassette transporters. The main function of ABCG2 is to pump out wide range of molecules out of cells providing multidrug resistance [34, 35]. ABCG2 could impart subpopulation (SP) phenotype of CSCs and serve as a promising CSC biomarker. Increased expression of ABCG2 has been observed in mammary gland, liver cancer SP cells and lung CSCs. Elevated expression of ABCG2 was also reported in SP cells isolated from different lung cancer cell lines (H460, H23, HTB-58, A549, H441 and H2170). The SP cells isolated from A549 cells were completely vanished after treatment with selective ABCG2 inhibitor suggesting it as an important biomarker in lung cancer [29]. $CD338^{+}$ cells displayed stemness and tumorigenic potential when compared to $CD338^{-}$ cells in BRCA1-mutated breast cancer cell line HCC1937 [36].

Alpha-6 integrin has been showed as essential for tumorigenicity of a CSC-like subpopulation within the breast cancer cell line MCF-7 [37]. Mammary progenitor marker $CD61^{+}$, also known as beta-3 integrin, has been demonstrated to have a CSC-like population having highly enriched tumorigenic capability when compared to $CD61^{-}$ subset of cells in the mouse model [38]. Expression of Ca^{2+}-dependent cell-cell adhesion glycoprotein P-cadherin has been associated with the expression of breast CSC markers and an onset of transient EMT required for the metastasis [39].

Other prominent stem cell marker is nestin, an intermediate filament protein which is marked with cell signalling, cytoskeletal organization, proliferation and attributing stemness properties to the cells [40]. Musashi-1 is RNA-binding protein which is additional protein focused towards the presence of stem cells in neurosphere culture. Its involvement in tumorigenesis is well accepted [41]. Also, the level is associated with the mitotic activity, determining the grade and aggressiveness of the brain tumour [42]. CD15 is a novel putative marker present on astrocytes; adult neurogenic zones had been shown in the glioma-derived neural sphere [43]. The presence of this trisaccharide 3-fucosyl-N-acteyllactosamine (FAC) group is attributed with the tumour-propagating properties in medulloblastoma [44].

All these above-mentioned markers are useful in cell signalling mechanisms employed between the cells and their microenvironments. Over the period of time, several other cell surface markers were identified in various CSCs; some of them have been enlisted in the table below (Table 26.1).

26.2.3 Cancer Stem Cells: EMT and Metastasis

EMT in cancer cells is orchestrated by the involvement of various environmental factors; signalling pathways; group of pleiotropic transcription factors (TFs) such as Snail1, Twist1 and Zeb1; microRNAs (miRNAs); and other numerous mechanisms

Table 26.1 Cancer stem cell markers in different cancers

Tumour type	Cancer stem cell marker	References
Acute myeloid leukaemia	$CD38^-$, $CD34^+$	[45]
Breast cancer	$EPCAM\ (ESA)^+$, $CD44^+$, $CD24^-$, ALDH, CD29, CD133	[21]
Colorectal cancer	$CD133^+$, $CD44^+$, $CD26^+$, ALDH	[46]
Liver cancer	$CD133^+/CD44^+$, $EpCAM^+$, $CD90^+$	[47]
Glioblastoma	$CD133^+$, $CD15^+$	[48]
Head and neck cancer	$CD44^+$, ALDH1	[49]
Hepatocellular carcinoma	$CD45^-$, $CD90^+$	[50]
Lung cancer	$CD133^+$, ABCG2, CD90, CD117, ALDH1	[51, 52]
Medulloblastoma	$CD133^+$, $CD15^+$	[53]
Melanoma	$CD20^+$, $CD271^+$	[54]
Multiple myeloma	$CD138^+$	[55]
Osteosarcoma	$CD117^+$ (c-Kit), CD133+, Stro-1+	[56]
Ovarian cancer	$CD133^+$, $CD44^+$, $CD117^+$, $CD24^+$	[57]
Pancreatic cancer	$CD44^+$, $CD24^+$	[58]
Prostate cancer	Integrin α2/β1, BMI-1, CD49f (integrin α6), $CD133^+$, $CD44^+$, ABCG2/Hoechst 33342, SCA-1, $CD166^+$, $CD151^+$, $p63^+$	[59, 60]
Renal carcinoma	$CD133^+$	[58]
Bladder cancer	$CD44^+$, $CD47^+$, $CK5^+$	[61]

that foster the loss of epithelial and adhesive attributes and gain of mesenchymal and migratory characteristics. Aberrant expression level of EMT TFs in primary tumours has been linked with poor prognosis, tumour invasion and metastasis. [62]. Activation of EMT through the repression of E-cadherin is carried out by several signalling pathways such as transforming growth factor beta (TGF-β), Notch, Wnt and integrin pathway [63, 64]. E-cadherin is known to maintain epithelial state, and decrease in its expression has been acknowledged to be a hallmark of EMT [65]. Recent study has indicated that EMT cascade helps in the genesis of many traits of CSCs in numerous cancers such as colorectal, breast, hepatocellular and pancreatic carcinoma [66]. The work of various researchers suggests that EMT induction and various EMT markers expressed by CSCs are linked with genesis of CSC-like population that in turn impart resistance to chemotherapy [6, 7, 67, 68].

Overexpression of various EMT TFs is downregulated at the time of metastatic colonization. In a study, it has been shown that transient overexpression of Twist1 facilitated the lung metastasis in squamous cell carcinoma [69]. The change in mesenchymal state to epithelial is imminent in formation of secondary cancer and is called as MET (mesenchymal-epithelial transition). In MET, expression of EMT TFs gets switched off to facilitate the metastatic colonization of cancer cells. Similarly, another EMT TF Prrx1, a member of the homoeobox proteins, is an established inducer of EMT in cancer cells. Nonetheless, Prrx1 downregulation has been implicated in metastasis and induction of tumour stemness in various cancer

cell lines [70]. Further, increased expression of a well-known EMT inducer Snail in prostate cancer cells was surprisingly shown to inhibit metastasis and colonization [71]. Collectively, these studies suggest that tumour stemness, EMT and metastasis are regulated by diverse mechanisms and point out that for the completion of metastatic colonization, MET is as important as EMT.

Acquisition of EMT and CSC-like phenotype cannot be achieved only by overexpression and downregulation of EMT TFs but also requires the regulation by tumour microenvironment or niche. Circulating tumour-associated fibroblasts are extensively endowed with stem cell markers and augment the metastatic capability of tumours by boosting their migration and extravasation [72, 73]. CSCs are directly involved in establishing distant metastasis, for example, in breast cancer metastasis to the bone, it was shown that the cells present in the bone metastatic site contained cells with characteristic breast cancer stem cell markers [74]. Another study showed that breast CSCs having putative markers such as $CD44^{+}$ and $CD24^{-/low}$ were able to initiate tumours in distant sites and could induce lung metastasis [75]. In pancreatic cancer it was demonstrated that subpopulation of cells with $CD133^{+}/CXCR4^{+}$ phenotype were localized in the invasive front of the tumour and had more migratory potential than $CD133^{+}/CXCR4^{-}$ cells. Only $CD133^{+}/CXCR4^{+}$ cells were able to metastasize to the liver [76].

Metastasis, in most cases, occurs several years after the successful treatment of the initial tumour, thus displaying the unique features of latency in metastatic cells [1]. Cancer stem cells with metastatic characteristics have unique features such as dormancy and plasticity. For example, the cancer cells present in the bone marrow are dormant in nature and thus are protected from cytotoxic agents. To come out of the dormant phase to proliferate and induce metastasis, they require the activation of several signalling pathways such as BMP and MAPK pathways [71]. Some unique markers such as thrombospondin-1 induce the sustained tumour quiescence during the dormant phase of the tumour, but when the dormant phase is over, these quiescent cells enter the cell cycle with the help of active TGF-β1 and periostin secretion from endothelial tip cells that are present in the tumour niche. These evidences suggest that tumour microenvironment and niche play a very important role to sustain tumour dormancy and late metastasis [77].

26.2.4 Cancer Stem Cells: Drug Resistance

Chemotherapy is one of the traditionally used methods for the treatment of many cancers. However, tumour cells frequently acquire the ability to resist the effects of chemotherapeutic drugs [78, 79]. Recent studies suggest that inhabitancy of cancer cells provides them varying capabilities to both initiate and metastasize tumours [20, 21]. CSCs are believed to be predominantly responsible for the genetic heterogeneity present in tumours. When success of treatment in cancer patients is considered, the main concern is about the relative resistance of CSCs to many standard chemotherapeutic drugs [80]. The cancer cells develop capability of synchronous resistance to several drugs that are not structurally related and have varied mechanism of actions,

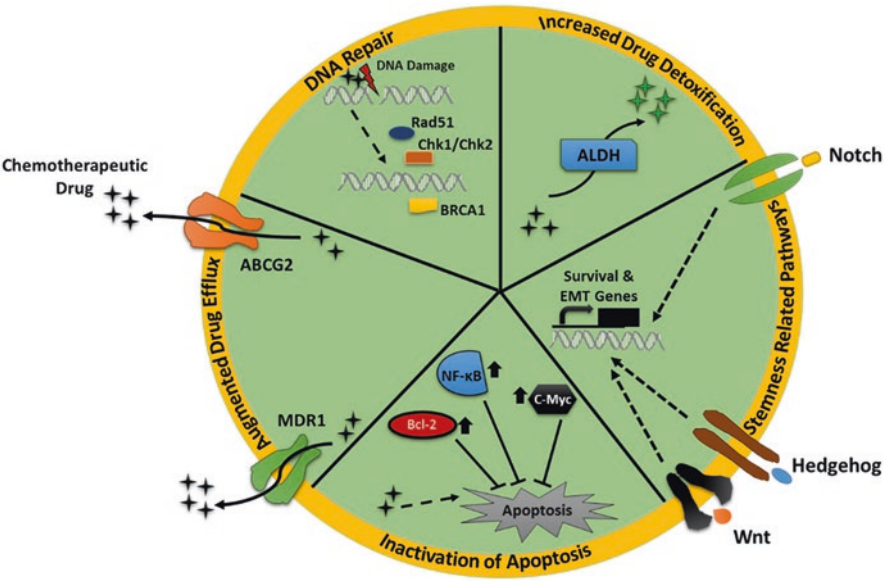

Fig. 26.3 Molecular mechanisms involved in CSC-associated chemoresistance

known as multidrug resistance (MDR). Various strategies used by CSCs for establishing and maintaining drug resistance include the efflux of drugs by ABC transporters such as ABCC1, ABCG2 and ABCB1/P-glycoprotein, secretion of drugs into vesicles and consequent exclusion by exocytosis, reduced uptake of drugs, glutathione system, detoxifying pathways such as cytochrome P-450 pathway and modulations in the apoptotic signalling (Fig. 26.3) [78]. Thus, CSCs are hard to treat because of their abilities to efflux the drugs and their metabolites easily. Therefore, focus should be on tackling the mechanisms involved in induction of MDR.

26.2.4.1 CSCs Chemoresistance and Enhanced Drug Efflux Mechanisms

ABC transporters have been shown to have a vital role in efflux of most chemotherapeutic drugs, and elevated expression of ABC transporters is the chief mechanism of chemotherapeutic resistance in CSCs [81–83]. ABC transporters efflux a wide variety of chemotherapeutic drugs that include vinblastine, doxorubicin, colchicine, etoposide and paclitaxel and are frequently correlated with MDR in cancer [80]. The side population of CSCs is a subgroup of stem cells that have a high capacity for efflux of mitosis-inhibiting drugs. The drug-transporting ability of these SP cells is due to the presence of certain ABC transporters [78]. ABCB1/P--glycoprotein/MDR1 is one of the well-studied ABC transporters and is usually involved in chemotherapeutic resistance in various cancers, including AML and gastrointestinal cancer [84]. Further, meta-analysis studies showed that ABCB1 may be expressed in 40% or as high as 66% of breast cancers [85]. Some recent evidence showed that chemotherapy may elevate expression of ABCB1. This may

suggest why at least some acquired resistance in breast cancer is associated with elevated expression of ABCB1 after neoadjuvant chemotherapy treatment [86, 87]. ABCG2, another ABC transporter known as breast cancer resistance protein (BCRP) because it was first identified in resistant variety of MCF-7 breast cancer cells [88], is also linked to chemotherapeutic resistance in AML patients [80, 89].

Increasing evidences show the correlation between the chemotherapeutic resistances of CSCs to MDR1 expression. $CD133^+$ prostate CSCs [80, 90] and $CD117^+$, $CD44^+$ and $CD133^+$ ovarian CSCs have higher expression of MDR1 [91]. Glioblastoma CSCs also showed elevated expression of MDR1 and resistance to carboplatin, etoposide and doxorubicin [92]. Increased levels of MDR1 and resistance to daunorubicin were observed in $CD34^+/CD38^-$ AML CSCs [93]. CSCs or cells with CSC-like characteristics express other ABC transporters. For example, ABCG2/BCRP is overexpressed in $CD34^+$ and $CD38^-$ AML CSCs which show resistance to mitoxantrone [94]. Thus, these studies indicate a clear link between the expression of MDR proteins in CSCs and chemoresistance in these cells.

26.2.4.2 Aldehyde Dehydrogenase and CSCs Chemoresistance

Increased ALDH activity is another mechanism through which CSCs attain the ability for chemoresistance. ALDH1 is an enzyme that is localized in cytosol which catalyses the oxidation of aldehyde into carboxylic acids [95]. The main function of ALDH enzymes is to remove toxic aldehydes produced during metabolic processes [96]. This detoxification capability of ALDH enzymes implies that it has a role in imparting resistance to cells in the case of certain chemotherapeutic drugs [80]. A study by ALDEFLUOR-positive staining showed that on an average, ALDH activity was present in 8% of normal mammary epithelial cells [97]. Breast cancer cells having high ALDH activity compared to normal epithelial cells were able to develop xenograft tumours with as low as 500 cells [98]. The ability of ALDH expression to impart resistance against cyclophosphamide has been showed in tumours of different origin, and its inhibition can sensitize CSCs to cyclophosphamide [99]. Knockdown of ALDH1A1 in pancreatic cancer suggested that ALDH may also be resistant towards gemcitabine [100]. It suggests that ALDH expression imparts resistance in CSCs against chemotherapeutic drugs and its inhibition can sensitize CSCs to these drugs [99].

26.2.4.3 Chemoresistance of CSCs by Apoptosis Inactivation

B-cell lymphoma-2 (BCL-2) family proteins, traditionally identified as the potential oncogene in acute B-cell leukaemia, are involved in chemotherapeutic resistance of CSCs [98]. BCL-2 expression has also been seen in various cancers as well as in haematopoietic lineage cells [101, 102]. Increased BCL-2 expression in myeloid progenitor cells aggravated leukaemia development in transgenic mice. This suggests an overlay between BCL-2 expression and the capacity of cancer-initiating cells to induce tumour formation [80]. In glioblastoma cells, $CD133^+$ CSCs showed higher BCL-2 expression and increased chemoresistance [103, 104]. A study explained that BCL-2 expression was elevated in $CD44^+/CD24^{-/low}$ breast cancer stem cells; however, the mechanism involving the expression of these proteins is

still not clear [105]. Similarly, $CD133^+$ hepatocellular CSCs had higher expression of BCL-2 and also showed resistance to doxorubicin and 5-fluorouracil [106]. Further, in $CD133^+$ colon CSCs where IL-4 utilization was found via autocrine system, IL-4 neutralizing antibodies reduced BCL-XL expression and increased sensitivity towards 5-fluorouracil and oxaliplatin [107]. Aurora-A is an oncogenic serine/threonine kinase that regulates the cell cycle, and BCL-2 family members may be induced in CSCs via this protein. Recent studies in $CD133^+CD29^+CD20^-$ colorectal CSCs showed that these cells have an elevated expression of Aurora-A as well as BCL-2, MCL-1 and BCL-XL. Aurora-A knockdown with shRNA showed a strong decrease in MCL-1 and BCL-2 expression along with a moderate reduction in BCL-XL expression [108, 109, 98].

26.2.4.4 CSCs and Dysregulated DNA Damage Response

Another important mechanism that regulates tumour chemoresistance and progression is elevated DNA damage response. Under hypoxia, cancer cells can stimulate a strong DNA damage response via proteins such as HIF transcription factors [110]. Subsequently, ataxia telangiectasia-mutated (ATM) and ataxia telangiectasia and Rad3-related (ATR) protein are activated after the induction of hypoxia and in response to DNA damage [111]. CSCs are protected from DNA-damaging radiation and chemotherapeutic drugs via these signalling pathways [98]. A study showed that $CD133^+$ glioma CSCs were resistant to ionizing radiation than $CD133^-$ cells [112]. Activated DNA damage response factors ATM, CHK1 and CHK2 were highly expressed in $CD133^+$ cells as compared to $CD133^-$ cells after treating with radiation. Further, treatment of debromohymenialdisine, which is a CHK1/CHK2 inhibitor, to $CD133^+$ glioma stem cells reversed radio resistance. This suggests that knockdown of CHK1 and the reduction in DNA damage response may be an effective approach to target chemoresistant CSCs [98].

26.2.4.5 Chemoresistance and CSC-Related Signalling Pathways

Several pathways participate in imparting chemoresistance to CSCs. Wnt/β-catenin pathway is often required for self-renewal of both normal stem cells and CSCs in different cell types [113, 114]. It has been shown that Wnt signalling pathway activation increased renewal of $OV6^+$ HCC hepatic CSCs, whereas knockdown of β-catenin using lentiviral microRNA showed a decrease in self-renewal capacity [115]. In neuroblastoma cells, Wnt pathway activation by FZD1 induced resistance against Dox [116]. Resistance to paclitaxel and cisplatin in C-kit^+ ovarian cells was mediated by ABCG2, which was downregulated by knockdown of β-catenin and reversed chemoresistance to cisplatin and paclitaxel [117]. Further, oxaliplatin treatment induced Notch pathway activation in colon cancer cells, which, when knocked down or inhibited, could prevent chemoresistance of colon CSCs towards oxaliplatin [118]. Furthermore, Notch proteins were found to be upregulated in ovarian CSCs. GIS treatment sensitized ovarian CSCs to cisplatin via inhibition of Notch-mediated maintenance of MDR1 expressing CSCs [119, 120]. The redox transcription factor NF-κB, a key inflammatory mediator, is also associated with chemoresistance of different CSCs. In $CD44^+$ ovarian CSCs, constitutive activation

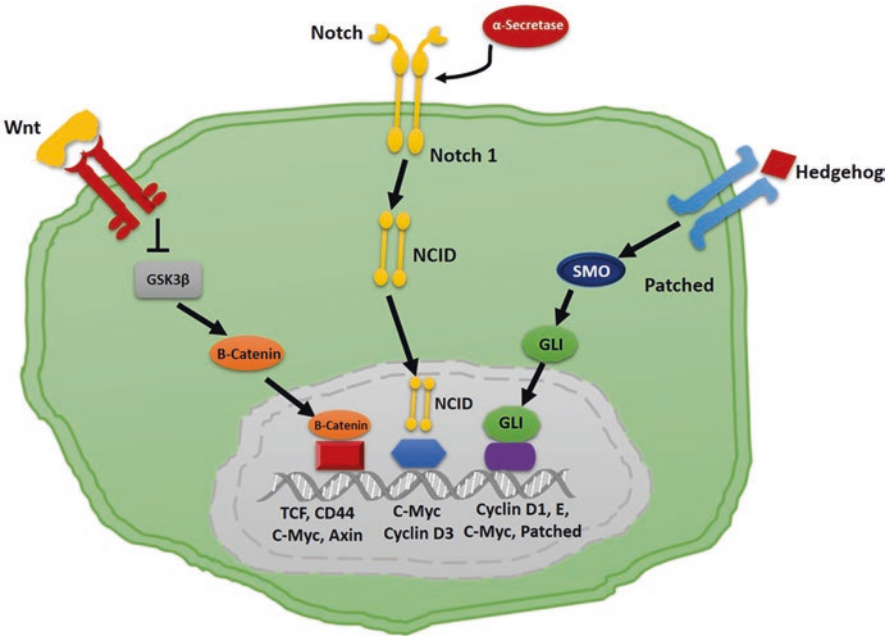

Fig. 26.4 Molecular pathways regulating CSC growth and maintenance

of NF-κB and other pro-inflammatory signals was correlated with resistance towards carboplatin and paclitaxel. Inhibition of NF-κB by eriocalyxin B increased cell death in resistant $CD44^{+}$ ovarian CSCs [121] (Fig. 26.4).

26.3 Flavonoids in Cancer Chemoprevention and Their Implications in Cancer Stem Cell Biology

26.3.1 Flavonoids: Occurrence and Characteristics

Flavonoids are naturally occurring plant phytochemicals, which are widely distributed and are important constituents of the diet working as functional foods. Flavonoids have been known to mankind from the last 60 years when for the first time they were identified by Szent-Gyorgyi and colleagues. These are the largest group of the phenolic phytonutrients, which have shown beneficial health-promoting effects and pharmacological properties due to which they have inspired various researchers [122]. There are six major important flavonoid classes as summarized in Table 26.2, which shows different classes of the flavonoids, sources and some of their properties.

Table 26.2 Different classes of the flavonoids

Class of the flavonoids	Rich food sources	Examples of the class	Remarks	Colour	References
Flavanones	Chick peas, cumin, berries, peppermint, citrus fruits	Hesperidin Narirutin Naringenin glycosides Liquiritigenin	Flavour of the citrus fruits like bitter taste	Colourless to pale yellow	[124]
Flavones	Grains, herbs like parsley and rosemary	Apigenin Luteolin Nobiletin Sinensetin Tangeretin	Gives plant tissue colour, gives bitter taste to fruits	Pale yellow	[125]
Flavans (monoflavans, biflavans, triflavans) or flavan-3-ol or flavanol	Fruits and teas (green and black). Biflavans: fruits, hops, nuts, beverages (cocoa and tea), sorghum, barley grains	Catechin Epicatechin Luteoforol Procyanidin Theaflavin	Complicated flavonoids and are not glycosylated but esterified with gallic acid. Contribute to the astringent taste of beer and wines	Colourless	[126]
Flavonols	Fruits, vegetables, berries, herbs, legumes, maize and tea	Quercetin Kaempferol Isorhamnetin Myricetin	Found predominantly in the skin of fruits	Pale yellow	[125]
Isoflavones	Legumes, black beans, soya beans, green split peas, clover sprouts	Daidzein Genistein Biochanin A Formononetin	Best known for their oestrogenic activity	Colourless	[127]
Anthocyanins	Cherries, berry fruits, plums, eggplant and radishes	Delphinidin Cyanidin Petunidin Peonidin Malvidin	Red at 3.5 pH becoming colourless and then shifting to blue as the pH increases	Blue and red coloration	[125]

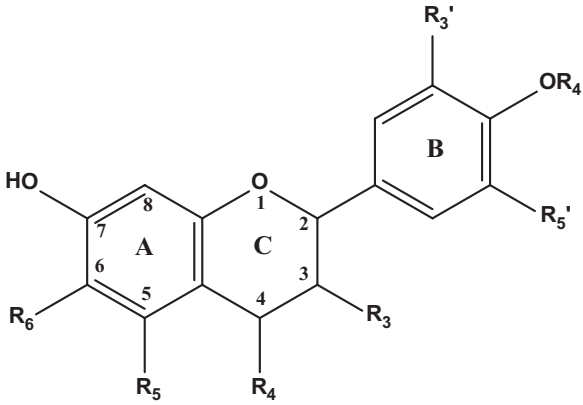

Fig. 26.5 General structure and numbering pattern for common food flavonoids

26.3.1.1 Chemical Structure of Flavonoids

Flavonoids consist of two-benzene ring system (aromatic ring structure) containing aromatic hydroxyl group and are connected by a carbon-carbon bridge. Structurally characterized as C_6-C_3-C_6 carbon skeleton, they occur as both aglycones (without sugar moiety) and glycosides (with sugar moiety). In flavonoids, A ring is joined with a six-member C ring through three-carbon bridge (Fig. 26.5). The different classes of flavonoids formed differ in the substitution at the C ring and level of oxidation. In addition, within a class the variation among the flavonoids is due to different substitution at A and B rings [123].

26.3.2 Flavonoids in Health and Disease

Researchers have shifted their focus on dietary source-derived flavonoids because of the accumulating versatile evidences that implicate their beneficial effects in human health and disease. Fruits and vegetables are the main sources of flavonoids and phenolic compounds that show antioxidant properties both in vitro and in vivo models [128–130]. Numerous epidemiological studies showed the protective roles of flavonoids in cardiovascular diseases, cancer and other age-related diseases [128]. Further, some flavonoids have shown anti-inflammatory, free radical scavenging, antibacterial, antiviral, hepato-protective, anti-allergic and antidiabetic activities [131, 132].

26.3.2.1 Antioxidant Activity

The most widely described property of flavonoids is their potential to act as antioxidants. Due to the potential to scavenge free radicals and ROS, flavonoids have been known as “high-level” natural antioxidants [128, 133]. Antioxidant activity of flavonoids depends on how the functional groups are arranged on the nuclear structure. The number of hydroxyl groups present and their configuration and substitution influence several mechanisms of antioxidant activity including metal ion chelation

and radical scavenging ability [134]. Flavonoids exert their antioxidant activity via different mechanisms such as chelation of trace elements that contribute in free radical generation, ROS scavenging and by upregulation of antioxidant enzymes [135, 136]. Flavonoids also inhibit several enzymes that are involved in ROS generation such as glutathione S-transferase, microsomal monooxygenase and NADH oxidase [137]. For example, quercetin exhibits its anti-oxidative property by iron chelation and stabilization [138]; Rutin and epicatechin have demonstrated strong radical scavengers and lipid peroxidation inhibiting ability in vitro [139].

26.3.2.2 Anti-inflammatory Activity

Cyclooxygenase and lipoxygenase are two major enzymes responsible for inflammation and provoking the release of cytokines. Some phenolic compounds are known to inhibit both these enzymes and suppress inflammation [140]. Flavonoids have been shown to inhibit the synthesis of eicosanoids including prostaglandins and leukotrienes [141]. Quercetin inhibits both these pathways [142]. Other anti-inflammatory property of flavonoids includes the capacity to inhibit neutrophil degranulation as it directly hampers with the release of arachidonic acid by neutrophils and other immune cells [143].

26.3.2.3 Protective Effects on Cardiovascular System

Flavonoid consumption can prevent various cardiovascular diseases such as hypertension and atherosclerosis [144]. Endothelial dysfunction is a crucial event for the development of cardiovascular disease as it leads to arterial thrombus formation and eventually atherosclerosis. Consumption of flavonoids has been beneficial in preventing endothelial dysfunction [145]. Quercetin and its glycosides have been shown to protect LDL against oxidative modification [146]. A study has reported an inverse correlation between flavonoid consumption and total plasma cholesterol concentrations [147]. Along with decreasing cholesterol levels, flavonoids are also known to lower blood pressure levels that may be beneficial for heart patients [148]. Aggregation of platelet also plays a pivotal role in cardiovascular diseases as it has been shown that platelets contribute in generation of ROS and inhibit endothelial formation. Tea pigment phenolics have been reported to decrease blood coagulation and prevention of platelet aggregation [149, 150].

26.3.3 Targeting Cancer Stem Cells Using Dietary Flavonoids

26.3.3.1 Flavonoids and Cancer Chemoprevention

Various studies have pointed out the fact that long-term consumption of flavonoid-rich diet can prove to be beneficial and can help in reducing the risk of various chronic diseases including cancers [151]. Epidemiological studies have displayed that flavonoid-rich diet has an inverse relationship with cancers. Some examples include the less incidence of colorectal cancer in Asian population when compared to western population (31 colorectal cancer cases per 100,000 people in the UK and 4 colorectal cancer cases per 100,000 people in India). Apart from this, almost 70%

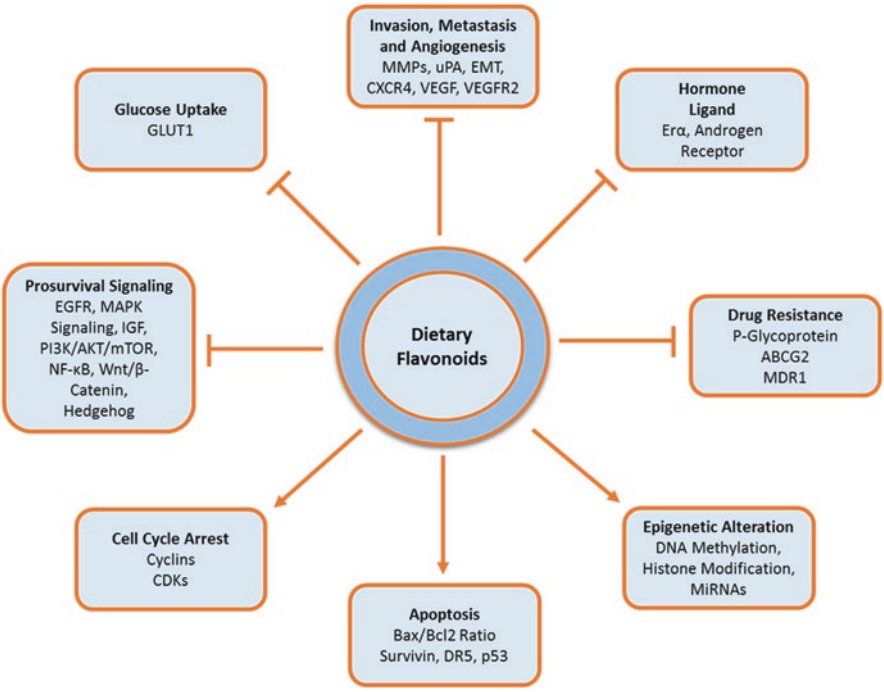

Fig. 26.6 Anticancer properties of dietary flavonoids

of cancers are now linked to the dietary intake [9]. Flavonoids are known as chemopreventers because they impart a cancer-preventive effect, for example, flavonoids such as curcumin, genistein, quercetin, EGCG and luteolin and several others [152]. Cancer chemopreventive efficiency of flavonoids may be because of several factors such as free radical scavenging; induction of apoptosis; downregulation of cell proliferative, adhesion and invasion markers; supressing inflammation; increased cell differentiation; induction of cell cycle arrest; regulation of steroid hormone and oestrogen metabolism; regulating the expressions of oncogenes; tumour suppressor genes; and various growth factor receptors [153]. Flavonoids are indeed shown to modulate the expression level of several proteins that play a role in carcinogenesis. Examples of such proteins affected by flavonoids include p53, Bcl-2, p21, Bax, NF-κB, COX-2, p73, GSH and catalase. EGCG is shown to inhibit cell proliferation and exert apoptosis in several tumour cell lines such as HeLa, CaSki, Hep-2 and SW780, melanoma cells, adrenal NCI-H295 cancer cells and A549 cells [152]. Genistein is shown to scavenge free radicals and exert its chemopreventive effect [154]. Flavonoids such as EGCG and quercetin are also known to exert their anticancer efficacy by modulating epigenetic proteins such as HDAC and DNMT-1 [155]. Several other mechanisms are exploited by flavonoids to exert their chemopreventive effect on cancer as shown in Fig. 26.6.

26.3.3.2 Flavonoids Targeting CSCs

Several recent studies have suggested that flavonoids can also target CSCs and may sensitize them towards anticancer drugs. Although much precise mechanisms are not known, it is hypothesized that flavonoids can target CSCs by either inhibiting their growth, self-renewal and metabolism or by targeting their niche and microenvironment [156].

Flavonoids Targeting CSC Signalling Pathways

Flavonoids can target CSCs by various mechanisms that may include modulating the self-renewal signalling pathways involved in CSC maintenance such as Wnt/β--catenin, Hedgehog and Notch signalling. Flavonoids such as curcumin, genistein and EGCG have been shown to directly or indirectly modulate these signalling pathways and contribute in the reduction of CSC growth and maintenance [157]. EGCG showed alteration in Wnt/β-catenin signalling in breast cancer cells [158]. EGCG also inhibited the downstream molecules of Wnt/β-catenin signalling and inhibited TCF/LEF binding and c-Myc expression [159]. Genistein decreased mammosphere formation and inhibited CSC growth in ER^{+ve} MCF7 and ER^{-ve} MDA-MB-231 breast cancer cell lines [160]. Another well-known flavonoid quercetin was shown to induce apoptosis; downregulate EMT, angiogenesis and stemness proteins; and inhibit CSC-derived xenografts in pancreatic cancer [161]. Curcumin, a flavonoid derived from *Curcuma longa*, has displayed its chemopreventive effect by downregulating CSCs' self-renewal pathways via inhibiting notch signalling in oesophageal cancer [162]. Chemosensitization to 5-FU by curcumin is attributed to its ability to decrease the number of CSC marker positive cells [163]. Further, curcumin treatment induced apoptosis and reduced the TGF-β1-induced cell invasion and proliferation in pancreatic cancer cell line (Panc-1) and also reduced the expression of Shh, GLI1 and vimentin. Further it was also showed that curcumin treatment resulted in the increased E-cadherin expression [164]. Curcumin is also shown to reduce cell growth and downregulate Notch 1, Hes-1 and Bcl-XL expression and induce apoptosis in pancreatic cancer cells. Curcumin pretreatment in combination with Notch-1 downregulation by siRNA synergistically increased growth inhibition and apoptosis [165]. Treatment of curcumin and resveratrol in combination was shown to exert synergistic antitumour activity in vitro in MCF-10A-Tr (cigarette smoke-mediated transformed cells) breast epithelial cells and in tumour xenograft in vivo models. In MCF-10A-Tr cells, Bax to Bcl-xL ratio increased along with the increased curcumin uptake in cells when resveratrol was treated in combination, and this leads to increased apoptosis along with PARP cleavage, release of cytochrome C and cleavage of caspase-3. This combination also modulated Hedgehog pathway and downregulated the expression of Shh, Smo, Gli and c-myc and enhanced the level of p21 in in vitro and in vivo models. Curcumin also inhibited the transactivation of Slug by accumulation of β-catenin and expression of c-Myc and cyclin D1 in azoxymethane-induced colon cancer in rat model [166]. Further, genistein was also shown to decrease cell viability and increase apoptosis in colon cancer cells, through reduction in nuclear β-catenin and increase in mRNA expression of Sfrp2, which is an antagonist of Wnt signalling pathway

[167]. Effect of genistein was also evaluated on breast cancer (MCF-7) cells where it reduced the cell growth and induced apoptosis along with the downregulation of Hedgehog signalling cascade [168]. EGCG inhibited the expression of GSK3-α and GSK3-β along with a reduction in β-catenin phosphorylation in HT29 cells [169]. In pancreatic cancer cells, EGCG downregulated the expression of self-renewal pathway proteins such as Nanog, c-Myc and Oct-4 and also decreased the number of CSCs along with sonic hedgehog pathway proteins such as smoothened, patched, Gli1 and Gli2. EGCG was also reduced EMT by downregulating transcriptional activity of various EMT TFs such as snail, slug and ZEB1. Further, EGCG act synergistically with quercetin and inhibited CSCs' self-renewal potential by inhibiting TCF/LEF and Gli activities [170].

Flavonoids Targeting CSC Markers and Niche

Combination of Src inhibitor dasatinib and curcumin was used against chemoresistant colon cancer cells that were enriched with CSC subpopulations. The combination treatment exhibited an elevated response by reducing cell growth, invasion and colony-forming ability of cancer cells and was also shown to reduce the expression of various CSC markers such as ALDH, CD133, CD166 and CD44 [171]. $ALDH^{+}$/$CD133^{+}$ colon cancer cells were shown to have increased expression of phosphorylated STAT3 in comparison to $ALDH^{-}$/$CD133^{-}$ cells, thus implying that cancer stem cells have more pSTAT3. Curcumin and its analogue GO-Y030 were shown to reduce STAT3 phosphorylation along with a reduction in cell viability, increased apoptosis and decreased sphere formation [172]. Further, curcumin-treated colon cancer cells displayed reduction in levels of CD44, CD166 and EGFR expression suggesting that curcumin can be used as an effective anticancer agent [173]. Difluorinated curcumin (CDF) is a novel curcumin analogue that was shown to have inhibitory effects on colon cancer stem-like cells. Combination of CDF with 5-FU+Ox exerted better anticancer activity than curcumin alone. CDF in combination with 5-FU decreased CD44, CD166 and ABCG2 expression along with apoptosis induction and growth inhibition in colon cancer cells [174].

In GEM-resistant MIAPaCa-2 pancreatic cancer cells, which contain high number of CSC, CDF decreased sphere forming along with reduction in CSC markers such as EpCAM and CD44 [175]. CDF also showed effectiveness in orthotopic xenograft models and decreased the expression of CD44, EpCAM and EZH2 by increasing the expression of let-7, miR-101 and miR-26a [176]. Genistein also displayed anti-CSC properties in pancreatic cancer cells by decreasing the expression of CD44 and EpCAM [177]. Kaempferol, a flavonoid isolated from *Delphinium*, witch hazel, grapefruit and other plant sources, was shown to downregulate ABCG2 expression, thus downregulating CSCs in oesophageal cancer [178]. Luteolin is also investigated for its anticancer properties in various studies, and one such study has shown that it is indeed successful in inhibiting various stem cell markers such as CD44, ALDH1 and many others in breast cancer cells [179]. Many other flavonoids have been investigated for their anti-CSC properties, and most of these flavonoids have shown potential to be very potent anticancer and anti-CSC agents. These flavonoids are summarized in Table 26.3.

Table 26.3 Flavonoids targeting molecular markers of different cancers

Flavonoid	Cancer type	Stem cell target	References
Epigallocatechin gallate (EGCG) [green tea]	Breast cancer	ALDH, c-myc, miR-21, miR-27, Wnt/β-catenin, BCRP	[180–182]
	Head and neck carcinoma	BMI-1, Twist1 and NF-kBp65	[183]
	Prostate cancer	Detoxification enzymes, androgen receptor, Wnt/β-catenin, CD133, CD44, NF-κB	[184, 185]
	Lung cancer	PI3K/AKT, Wnt/β-catenin	[181]
	Pancreatic cancer	Nanog, c-Myc, Oct-4, Hedgehog	[170]
	Liver cancer	Phase II detoxifying enzymes	[186]
	Colon cancer	Wnt/β-catenin	[187]
Curcumin	Breast cancer	ALDH1A1 expression, $CD44^{high}$/$CD24^{low}$ phenotype, Wnt/β-catenin, Hedgehog, STAT-3, NF-κB, Notch-1	[21, 157, 165, 181, 188, 189]
	Lung cancer	miRNA-186	[190]
	Colorectal cancer	DCKL-1, Nanog, ALDH1A, Lgr5, ABCG2, ALDH, CD44, CD133 and CD166 Hedgehog, Wnt/β-catenin	[165, 191, 192, 181]
	Gastric cancer	Wnt/β-catenin	[193]
	Intestinal cancer	Wnt/β-catenin	[194]
	Liver cancer	Side population	[195]
	Pancreatic cancer	NF-κB, CD44, EpCAM, Notch1, Nanog, Hedgehog, miR-21, miR-200, Wnt/β-catenin, let-7, miR-26a, miR-101, Hes-1 and Bcl-XL	[165, 181, 196, 197]
	Prostate cancer	Wnt/β-catenin, androgen receptor, let-7, miR-26a, miR-101, miR-21, miR-200	[181, 197]
	Melanoma	NF-κB	[198]
	Glioma (rat)	SP phenotype	[199]
	Oesophageal	Notch-1	[165]
Quercetin	Pancreatic cancer	CSC population identified using fluorescent tag (Gdeg), $CD44^{high}CD24^{low}$ NF-κB, Wnt/β-catenin	[161, 200, 201]
	Head and neck cancer	ALDH1, Oct-4, Nanog and Nestin	[202]
	Colorectal cancer	CD44,CD133, Wnt/β-catenin	[185, 187, 201]
	Prostate cancer	Wnt/β-catenin	[185]

(continued)

Table 26.3 (continued)

Flavonoid	Cancer type	Stem cell target	References
Genistein	Breast cancer	ALDH, BCRP/K562, NF-κB, Notch-1, Hedgehog, Bcl-2	[168, 203, 204]
	Prostate cancer	miR-221, miR-222, Hedgehog, CD44	[205, 206]
	Pancreatic cancer	CD44, EpCAM, Notch-1, let-7b, c, d, e, miR-26a, miR-146a, miR-200, miR-21	[181, 197, 207]
	Colon cancer	Wnt/β-catenin	[166]
	Renal cell carcinoma	Wnt/β-catenin	[208]
	Gastric cancer	CD44	[209]
	Ovarian cancer	CD133, CD44 and ALDH1	[210]
Silibinin	Breast cancer	Notch-1, NF-κB, Bcl-2, Wnt/β-catenin	[211, 212]
	Colon cancer	NANOG, SOX2, CD44	[213]
	Prostate cancer	ZEB1, NF-κB, Bcl-2, Wnt/β-catenin	[214]
Apigenin	Glioblastoma	CD133, Nanog and Sox2	[215]
	Head and neck cancer	CD44, NANOG, and CD105	[216]
	Prostate cancer stem cells	NF-κB, Oct3/4	[217]
	Breast cancer	Wnt/β-catenin	[187]
Chrysin	Liver cancer	Wnt/β-catenin, CD133 and CD44	[218, 219]
Kaempferol	Breast cancer	BCRP/K562	[203]
	Oesophageal cancer	ABCG2	[178]
Fisetin	Melanoma	Wnt/β-catenin	[220]
Naringenin	Breast cancer	BCRP/K562	[203]
Acacetin	Breast cancer	BCRP/K562	[203]
Luteolin	Breast cancer	ALDH, $CD44^{+}$, Notch-1	[179, 221]
	Colon cancer	Wnt/β-catenin	[222]
	Oral cancer	ALDH1, CD44	[223]

26.3.4 Role of Flavonoids in Cancer Stem Cell Biology and Drug Resistance

The major drawback of using chemotherapy is the presence of the MDR which makes the treatment challenging. There are list of proteins like P-gp, lung resistance protein (LRP), BCRP and ATP-binding cassettes which diminish the bioavailability of the drug to the target causing these drugs to efflux out from the cells [224, 225]. If higher concentration of the drugs is used to combat these problems, it may lead to cytotoxicity and other redundant cellular resistances; hence, there is the need for the development of potential chemosensitizers to overcome the problems of chemoresistance and hazardous toxicity leading to side effects [226, 224].

In this direction, flavonoids containing compounds are useful in targeting the cancer cells. The flavonoids present in honey such as chrysin, genistein, biochanin,

quercetin, kaempferol and naringenin have shown induction of apoptosis by generating ROS in both colon cancer and bladder cancer cell lines. These flavonoids have been shown to be effective on various other cancer cell models including CaCo-2 cells and MCF-7 cells where they inhibit P-gp transporters, MRP-2 and BCRP and interact with ATPase's binding proteins reversing MDRs [227–229]. Many of the flavonoids also target drug substrates by increasing their accumulation, hence increasing their efficacy. For example, EGCG present in green tea reduces drug effluxes by targeting P-gp transporters leading to accumulation of rhodamine 123 that reverses MDR [230–232]. A study in MRP1-expressing HEK293 cells showed the inhibitory potential of flavonoids such as quercetin, naringenin, hesperetin, silibinin and daidzein on ABC transporters and MRP1, 4 and 5 and inhibited efflux. Further in L1210/Adr cells (a multidrug-resistant mouse leukaemia cell line), curcumin treatment resulted in decreased expression of *mdr1b* through regulating PI3K, Akt and NF-κB pathways. In addition, curcumin treatment also reversed MDR in ABCG2-expressing HEK cells, which facilitated accumulation of mitoxantrone and doxorubicin and sensitized the cells towards chemotherapeutic drugs [182]. Another flavonoid fisetin decreased the elevated expression of plasma membrane drug transporter P-glycoprotein in various human tumours through increasing cellular glutathione (GSH) content.

Biochanin, a substrate of both P-gp and CYP3A, has been shown to help in paclitaxel accumulation, which alters the bioavailability of the drug in the intestine and helps in raising the efficacy of anticancer drugs [233, 234]. The inhibition of MRP-1-mediated transport by various flavonoids including biochanin A, morin, apigenin, diosmetin, luteolin, baicalein, genistein, robinetin, chrysin, kaempferol, myricetin, naringenin and silymarin has been demonstrated [235].

The major studied mechanisms by which flavonoids augment the multidrug resistance reversal properties include:

1. Inhibiting the overexpression of multidrug resistance gene-1 (MDR1) [236]
2. Binding to the nuclear binding domains (NBD) of P-gp, which is involved in ATP hydrolysis to provide energy for efflux of the drugs out of the cells, thereby enhancing accumulation, bioavailability and efficacy of the drug to efficiently target the cancer cells [237]
3. Suppressing ATPase activity [238]
4. Hindering the ABC transporter functioning by competitively binding to substrate-binding sites [238, 239]

Many evidences have been documented about flavonoid's unique properties of reversing the multidrug resistances in various cancers. Still mechanistic studies are required for a clear understanding in different cross-signalling molecular mechanisms that target the multimodal different transporters, modulate signalling pathways which contributes to drug effluxing and lower the efficacy and concentration of anticancer drugs (Fig. 26.7).

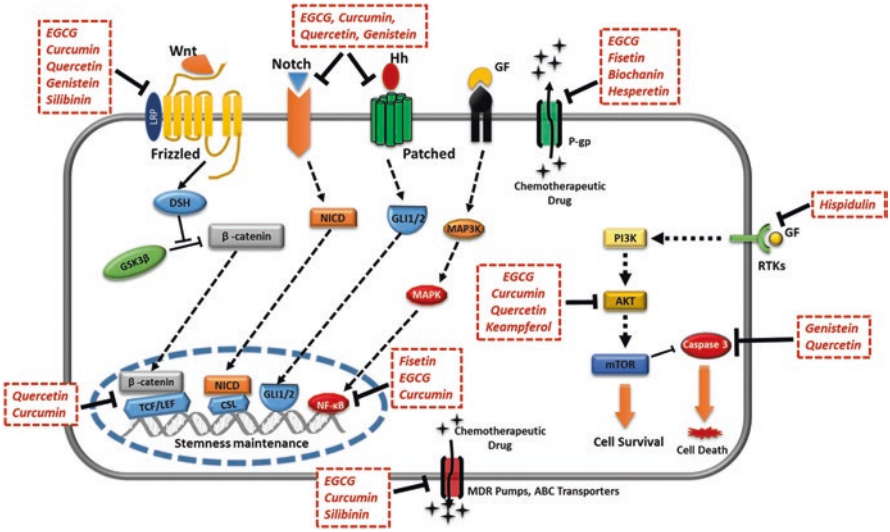

Fig. 26.7 Flavonoids targeting different CSC biomarkers and pathways

26.4 Summary and Future Directions

Cancer remains a major problem due to treatment failure, metastasis and recurrence after surgery, chemotherapy and radiotherapy. Recent reports showed that CSCs comprise a small cell population exhibiting resistance to anticancer drugs and also have been implicated in cancer relapse. A number of studies have identified signalling pathways that are responsible for acquiring and maintaining CSC in tumour mass. Current anticancer chemotherapy has failed to target CSCs. Hence, there is a need of novel compounds, which can target CSCs to achieve complete elimination of cancer. Functional foods and their active constituents, which are consumed regularly as dietary components, have been implicated in inhibiting these signalling pathways and target CSCs in many preclinical and clinical studies. Therefore, a regimen involving functional foods and their components may be helpful in the treatment of cancer in combination with current anticancer therapy to achieve complete remission of cancer. These active constituents will also serve as lead compounds for future anticancer drug discovery targeting CSCs as well as other types of cancers.

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