

Stem Cells, Cancer Stem-Like Cells, and Natural Products

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Key words

- general methods
- cancer

Abstract

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Somatic stem cells can be found in many rapidly regenerating tissues, e.g., the skin, gastrointestinal mucosa, and hematopoietic system, but are also present at low numbers in non-regenerative organs such as the heart and brain. In these organs, somatic stem cells aid in normal tissue homeostasis and repair after injury as well as self-renewal and the generation of specific progenitor cells during differentiation. Cancer stem-like cells are a small subpopulation of self-renewing cells that are able to proliferate upon appropriate stimulation and differentiate into heterogeneous lineages in tumors. Modulation of the behavior of normal tissue stem cells and cancer stem-like cells is an emerging and thriving new field of research. The present review gives an overview of the state-of-the-art findings and highlights perspectives for future scientific developments and clinical application.

Abbreviations

▼
ABC transporter: ATP-binding cassette transporter

BFU-E: erythroid burst forming units
 BJBTD: Bo-jung-bang-dock-tang
 CD: cluster of differentiation
 CFU-GEMM: granulocyte erythrocyte monocyte macrophage colony-forming units
 CFU-S: spleen colony forming units
 CPA: cyclophosphamide
 EPO: erythropoietin
 ESC: embryonic stem cells
 FAS: fatty acid synthase
 GAS: γ -interferon activated sites
 GM-CSF: granulocyte-macrophage-colony stimulating factor
 HCC: hepatocellular carcinoma
 hMSC: human bone marrow mesenchymal stem cells
 HSC: hematopoietic stem cells
 IL: interleukin
 iPSC: induced pluripotent stem cells
 JAK: Janus activated kinase
 KMKKT: Ka-mi-kae-kyuk-tang
 SCF: stem cell factor
 STAT: signal transducer and activator of transcription
 T-IC: tumor-initiating cells
 TPO: thrombopoietin

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Embryonic Stem Cells and Pluripotent Adult Stem Cells for Tissue Regeneration

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Somatic stem cells can be found in many rapidly regenerating tissues, e.g., the skin, gastrointestinal mucosa, and hematopoietic system, but are also present at low numbers in non-regenerative organs such as the heart and brain. They function in maintaining normal tissue homeostasis and in tissue repair after injury as well as self-renewal and the generation of specific progenitor cells during differentiation. If needed, somatic stem cells can switch from a quiescent state to a highly

proliferative state, differentiate to functionally mature cells and replace other differentiated cells during physiological tissue turnover [1–4]. One of the most promising applications of stem cell biology is tissue regeneration. The concept is to differentiate stem cells into specific tissue and cell types in order to treat neurodegenerative diseases, muscular diseases, diabetes, and others. The challenge in regenerative medicine is the targeted differentiation of stem cells into the desired cell type. Embryonic stem cell-like induced pluripotent stem cells (iPSC) can be generated by inducing expression of defined transcription fac-

tors. The expansion and differentiation of stem cells takes place *in vitro* in cell cultures using cocktails of growth factors and natural or synthetic small molecules, which regulate crucial signaling pathways of stem cell differentiation [5]. Current protocols are based on embryonic stem cells (mainly derived from aborted fetuses) or adult stem cells (i.e., hematopoietic stem cells derived from bone marrow) [6].

Cancer Stem-like Cells and Their Role in Cancer Therapy

▼ Cancer stem cells are small populations of self-renewing cells with the ability to proliferate upon appropriate stimulation and differentiate to heterogeneous lineages in tumors [7]. The term “cancer stem cells” is merely an operational term. Cancer stem cells do not necessarily derive from normal stem cells nor are they multipotent [8,9]. Hence, cancer stem cells may not show lineage-dependent cell differentiation. Therefore, terms such as “cancer-initiating cells” or “cancer stem-like cells” are more appropriate than “cancer stem cells”.

Cancer stem-like cells are characterized by clonal expansion that initiates and sustains tumor growth *in vivo*. Cancer stem-like cells should not be confused with the cell of cancer origin, which is the very first malignantly transformed cell [10]. Cancer stem-like cells can originate from normal stem cells or progenitor cells after acquisition of tumor-initiating mutations [11,12]. It remains a matter of discussion whether cancer stem-like cells can be derived only from normal stem cells [13,14], or if they can also be derived from mature cells that are dedifferentiated or transdifferentiated, which is regulated by genetic and epigenetic factors [9,15].

There is much debate about the identification of cancer stem-like cells, and a variety of different cellular markers have been proposed to differentiate between cancer stem-like cells and the general population of cancer cells within a tumor. Putative markers of high relevance include the cell surface protein CD34 and the glycosylated isoform of CD133/prominin [16]. Another marker, e.g., in breast cancer, is the hyaluronic acid binding receptor CD44 which controls cell-cell interactions and is involved in cross-talk between cancer stem-like cells and their microenvironment [17–19]. CD44+/CD24- cells are associated with breast cancer metastasis to distant sites [20]. CD24 inhibits CXCL12-CXCR4-mediated migration of cells. Loss of CD24 expression, therefore, fosters invasion and metastasis [21]. However, these markers are not exclusively expressed by cancer stem-like cells, but rather are also expressed on normal cells. Furthermore, cancer stem-like cells have been identified that lack these markers [22].

The development of resistance represents a major obstacle to successful cancer therapy. Current protocols in radio- and chemotherapy are able to remove bulk cancer tissue. However, therapy-resistant cancer stem-like cells may survive and resume growth at a later time, leading to disease recurrence and metastasis. This phenomenon, termed minimal residual disease, has been known for many decades. Cancer stem-like cells may be able to survive radio- and chemotherapy by adopting a non-proliferative, dormant state [23,24]. Even after years, cancer stem-like cells can be reactivated and lead to refractory disease [25,26]. Cancer stem-like cells often reside in niches within tissues where they are shielded against the stresses of chemotherapy [27–29].

Hypoxic areas serve as niches, and hypoxic tumors are known to be radio- and drug-resistant [30]. Furthermore, hypoxic areas are less vascularized than oxygenated ones. Therefore, antiangiogenic treatment approaches may affect the niches of cancer stem-like cells [31].

Several mechanisms of drug resistance have been found in cancer stem-like cells:

- ▶ High expression levels of drug transporters of the ATP-binding cassette (ABC) type, particularly ABCB1/MDR1/P-gp and ABCG2/BCRP, especially in side populations [32–34]
- ▶ Highly efficient DNA repair [35]
- ▶ Resistance to commit programmed cell death (apoptosis) [36]
- ▶ Quiescence. Non-proliferating cells are generally more resistant than proliferating ones.
- ▶ Decreased immunogenicity [36].

Activity of Natural Products On Embryonic Stem Cells

▼ Several laboratories have screened small molecule libraries for compounds that promote embryonic stem cell differentiation. Embryonic stem cells (ESCs) are an attractive source of cells for disease modeling *in vitro* and may eventually provide access to cells/tissues for the treatment of degenerative diseases. Staurosporine, a staurosporine derivative, primes ESCs for efficient differentiation through a mechanism that affects expression of the transcription factor and oncogene, c-Myc [37]. Staurosporine is a known kinase inhibitor [38].

Retinoic acid acts by binding to nuclear receptors and inducing transcription of specific target genes during embryonic stem cell and embryonic carcinoma differentiation. The standard murine EC cell line F9 can be induced by retinoic acid to differentiate into primitive, parietal, and visceral endodermal cells. Another EC cell line, P19, differentiated into endodermal and neuronal cells upon retinoic acid treatment [39].

Butyrate, a natural small fatty acid and histone deacetylase inhibitor, significantly increased the efficiency of mouse iPSC generation using the transcription factors Oct4, Sox2, Klf4, and c-Myc. Butyrate not only changed the reprogramming dynamics by reducing the frequency of partial reprogramming and transformation, but also increased the ratio of iPSC colonies to total colonies. This effect was mediated by c-Myc during early stages of reprogramming [40].

Epidemiological evidence indicates that fruits and vegetables possess chemopreventive activity against cancer. Therefore, it is possible that natural products may selectively target cancer stem cells and induce differentiation. Reynertson et al. [41] used an alkaline phosphatase stain to assay plant extracts for the ability to induce differentiation in embryonic stem cells. Alkaline phosphatase is a characteristic marker of undifferentiated embryonic stem cells. The authors investigated approximately 100 fractions obtained from 12 species of ethnopharmacologically used plants and found fractions from 3 species that induced differentiation, decreasing the levels of alkaline phosphatase and pluripotency marker transcripts (Nanog, Oct-4, Rex-1). These fractions affected proliferation of murine embryonic stem cells, human embryonic cells, and prostate and breast carcinoma cells in a concentration-dependent manner. The isolated ellagic acid and gallic acid were cytotoxic towards cultured breast carcinoma cells.

Effects of Natural Products On Pluripotent Adult Stem Cells



Reprogramming of pluripotent adult stem cells

Vitamin C enhanced iPSC generation from both mouse and human somatic cells at least in part by alleviating cell senescence, an important roadblock to reprogramming. In addition, vitamin C accelerated gene expression changes and promoted the transition of pre-iPSC colonies to a fully reprogrammed state [42].

Hematopoietic stem cells in high-dose chemotherapy

Hematopoietic stem cell transplantation is employed in oncology mainly for treatment of lymphoproliferative disorders and leukemia. Multipotent hematopoietic stem cells derived from bone marrow, peripheral blood stem cells, or umbilical cord blood are preserved before the patient is subjected to high doses of ablative chemotherapy in which tumor cells and normal proliferating hematopoietic cells are destroyed. Reinfusion of hematopoietic stem cells induces repopulation of the bone marrow and reconstitution of the blood.

The Kambo (Japanese herbal) extract TJ-48 accelerated recovery from hematopoietic injury induced by radiation and the anticancer drug mitomycin C. The active fraction of TJ-48 is composed of free fatty acids (oleic acid and linolenic acid). Among a panel of 27 different free fatty acids, oleic acid, elaidic acid, and linolenic acid showed potent activity on hematopoietic stem cells. The administration of oleic acid to mitomycin C-treated mice enhanced spleen colony forming units (CFU-S) counts to twice those of the control group when counted on days 8 and 14, suggesting that the fatty acids contained in TJ-48 actively promote the proliferation of hematopoietic stem cells [43].

Bo-jung-bang-dock-tang (BJBDT) is a medicinal herbal cocktail that has been used for cancer prevention and treatment in traditional Korean medicine. Lim et al. [44] suggested that BJBDT can enhance hematopoiesis via the hematopoietic cytokine-mediated JAK2/STAT5 pathway. BJBDT significantly increased expression of the hematopoietic cytokines interleukin (IL-3), stem cell factor (SCF), granulocyte-macrophage-colony stimulating factor (GM-CSF), thrombopoietin (TPO), and erythropoietin (EPO) at the level of mRNA and secretion in hematopoietic stem cells (HSCs). Additionally, BJBDT enhanced the phosphorylation of Janus activated kinase 2 (JAK2) and the signal transducer and activator of transcription 5 (STAT5) as well as STAT binding to γ -interferon activated sites (GAS) in HSCs. Furthermore, BJBDT significantly enhanced the growth rate of granulocyte erythrocyte monocyte macrophage colony-forming units (CFU-GEMM) and erythroid burst forming units (BFU-E) *in vitro*. Moreover, BJBDT increased EPO at the mRNA level in the kidney and plasma. It also increased the number of erythroid-specific antigen Ter-119(+) erythroid cells in mice with aplastic anemia induced by 20% benzene. Consistently, histochemical staining revealed that BJBDT increased the bone marrow and stromal cell populations and decreased macrophage and adipocyte populations in bone marrow tissue of mice with aplastic anemia.

Ka-mi-kae-kyuk-tang (KMKKT) is an Oriental herbal medicinal cocktail that has demonstrated antiangiogenic, anticancer, and antimetastatic activities without considerable side effects in pre-clinical animal models. Seo et al. [45] investigated whether KMKKT alleviated cancer chemotherapy-induced leukopenia or other hematotoxicities *in vivo* using a mouse model. KMKKT was given orally once daily for 10 days to the mice before they were given daily injection of cyclophosphamide (CPA) for 4 days.

KMKKT ameliorated the CPA-induced decrease in red blood cells, hemoglobin content, and total white blood cell/leukocyte counts. Examination of multiple organ sites involved in hematopoiesis and lymphocyte differentiation and maturation revealed that the changes induced by CPA had been attenuated in each and every type of cell examined. Some cell types in the bone marrow were fully restored. In particular, bone marrow stem cells in the Sca-1(+), CD117(+), Sca1(+)/CD117(+), and CD34(+)/CD117(+) mice were overstimulated, supporting a role for KMKKT in stimulating hematopoietic stem cell (HSC) signaling to compensate for CPA-induced destruction of leukocytes and other cell types.

Pluripotent stem cells of normal tissue in cancer therapy

Multiple myeloma is characterized by the accumulation of clonal malignant plasma cells in the bone marrow, which stimulates bone destruction by osteoclasts and reduces bone formation by osteoblasts. In turn, the altered bone microenvironment sustains survival of myeloma cells. A major challenge in treating multiple myeloma is discovering drugs that target not only myeloma cells but also osteoclasts and osteoblasts. Resveratrol promotes the expression of osteoblast markers like osteocalcin and osteopontin in human bone marrow mesenchymal stem cells (hMSC-TERT) in a concentration-dependent manner and stimulates their response to 1,25(OH)₂ vitamin D₃ [1,25(OH)₂D₃] [46]. Moreover, resveratrol upregulates the expression of 1,25(OH)₂D₃ nuclear receptor in a concentration-dependent manner.

PHY906 is a formulation of four herbal compounds traditionally used to treat nausea, vomiting, cramping, and diarrhea. Irinotecan is an established anticancer drug, but like many anticancer drugs, one of its major side effects is diarrhea. The administration of PHY906 with irinotecan in a mouse model of colon cancer resulted in a synergistic reduction in tumor burden, enhanced maintenance of body weight, and stem cell regeneration in the intestinal mucosa [47]. PHY906 did not protect against the initial DNA damage and apoptosis triggered by irinotecan in the intestine, but by 4 days after irinotecan treatment, PHY906 had restored the intestinal epithelium by promoting the regeneration of intestinal progenitor or stem cells via several Wnt signaling components. PHY906 also potentiated Wnt3a activity in human embryonic kidney-293 cells. Furthermore, PHY906 exhibited anti-inflammatory effects in mice by decreasing infiltration by neutrophils and macrophages, upregulating tumor necrosis factor- α expression in the intestine, and increasing proinflammatory cytokine concentrations in plasma. Chemical constituents of PHY906 potentially inhibited nuclear factor κ B (NF κ B), cyclooxygenase-2, and inducible nitric oxide synthase. Our results show that the herbal medicine PHY906 can counteract the toxicity of irinotecan via several mechanisms that act simultaneously.

Effects of Natural Products On Cancer Stem-Like Cells



Cancer stem cells give rise to the tumor bulk through continuous self-renewal and differentiation. Understanding the mechanisms that regulate self-renewal of cancer stem-like cells and the points at which there is potential for intervention by natural products is of greatest importance in the discovery of anticancer drugs targeting cancer stem cells (● Table 1). Relapse in cancer is mostly due to the proliferation of cancer stem cells that could not be eliminated by standard chemotherapy.

Table 1 Effects of natural products towards cancer stem-like cells.

| Natural product | Cell type | Effect | Reference |
|---|--|---|-----------|
| Curcumin, sulforaphane, soy isoflavone, epigallocatechin-3-gallate, resveratrol, lycopene, piperine, vitamin D(3) | breast cancer stem-like cells | signal pathways (Wnt/ β -catenin, Hedgehog, Notch) ↓ | [48] |
| Curcumin | C6 brain tumor cells (side population) | side population ↓ | [58] |
| Resveratrol | mammospheres | apoptosis, DAPK2, BNIP3 and lipid synthesis ↓, fatty acid synthase ↓ | [52] |
| Resveratrol | medulloblastoma stem-like cells | proliferation ↓, radiosensitivity | [57] |
| Z-guggosterole | murine breast cancer metastases | CD44 positive cells ↓, apoptosis markers (caspase-3, ceramide) ↑ | [49] |
| Pomegranate extract | murine WA4 MMTV-Wnt-1 breast tumors | G ₀ /G ₁ cell cycle arrest, caspase-3, apoptosis | [50] |
| Ellagic acid, ursolic acid, luteolin, sulforaphane | breast cancer stem-like cells (xenograft tumors) | proliferation and viability ↓, Wnt/ β -catenin pathway ↓, aldehyde dehydrogenase-positive cells ↓ | [51] |
| All-trans retinoic acid | breast cancer stem-like cells | G ₀ /G ₁ cell cycle arrest, cellular differentiation | [53] |
| <i>Clostridium perfringens</i> enterotoxin | ovarian cancer stem-like cells <i>in vitro</i> and <i>in vivo</i> | proliferation of CD44- and claudin-4 positive cells ↓ | [54] |
| Lupeol | CD133 positive hepatocellular carcinoma cells | CD133 expression ↓, tumorigenicity in nude mice ↓ | [55] |
| Gossypol | prostate cancer <i>in vitro</i> and <i>in vivo</i> | DNA damage, p53 activation, apoptosis | [56] |
| Eckol | glioma stem-like cells <i>in vitro</i> and <i>in vivo</i> | CD133 cell population ↓, PI3K/AKT and Ras/Raf/Erk signaling pathways ↓, anchorage-independent growth ↓, xenograft growth ↓, drug and radio resistance ↓ | [59] |
| Parthenolide | myeloma | preferential toxicity to stem-like cells | [60] |
| Grapholide | stem-like cells | | |
| Cantharidin | primary acute myeloid leukemia stem and progenitor cells <i>in vitro</i> | cytotoxicity | [61] |
| Rakicidin A | hypoxia-adapted chronic myeloid leukemia stem-like cells | apoptosis, synergistic interaction with imatinib | [62] |

Breast cancer

Several dietary compounds including curcumin, sulforaphane, soy isoflavone, epigallocatechin-3-gallate, resveratrol, lycopene, piperine, and vitamin D(3) have been identified as having a direct or indirect effect on self-renewal pathways (Wnt/ β -catenin, Hedgehog and Notch) [48]. Curcumin and piperine have been demonstrated to target breast cancer stem cells. Sulforaphane has been reported to inhibit growth of pancreatic tumor-initiating cells as well as breast cancer stem cells. These studies provide a basis for preclinical and clinical evaluation of dietary compounds for chemoprevention against cancer stem cells.

The cancer stem cells play a critical role in both initiation and relapse, as they are resistant to most cytotoxic agents and are able to proliferate indefinitely. Krishnamurthy et al. [49] found that the bile acid sodium deoxycholate increased the number of intestinal metastases generated from murine mammary carcinoma 4T1 tumors. The metastatic nodes contained slowly dividing cancer cells in the immediate vicinity of newly formed blood vessels. These cells were positive for CD44, a biomarker for breast cancer stem cells. Deoxycholate promoted survival of CD44⁺ cells and concurrently reduced levels of activated caspase-3 as well as ceramide, a sphingolipid that induces apoptosis in 4T1 cells.

Z-guggulsterone, an antagonist of the farnesoid-X-receptor, obliterated this anti-apoptotic effect, indicating that deoxycholate increased cell survival via the farnesoid-X-receptor. Deoxycholate also increased expression of the gene coding for the vascular endothelial growth factor receptor 2 (Flk-1) in tumor cells, suggesting that deoxycholate enhances the initial growth of secondary tumors adjacent to blood vessels. The authors concluded that

treatment with Z-guggulsterone and/or vascular endothelial growth factor receptor 2/Flk-1 antagonists may be a promising strategy for reducing breast cancer metastasis.

Pomegranate (*Punica granatum* L.) is another natural substance known to possess anticancer activities. The effects of a standardized pomegranate extract on a mouse mammary cancer cell line (designated WA4) derived from mouse MMTV-Wnt-1 mammary tumors were examined [50]. The WA4 cell line has been previously characterized, and it has been found that the majority of WA4 cells possess stem cell characteristics. The pomegranate extract inhibited the proliferation of WA4 cells in a time- and concentration-dependent manner due to an arrest of cell cycle progression in the G₀/G₁ phase. Pomegranate extract was also cytotoxic to quiescent WA4 cells and resulted in an increase in caspase-3 enzyme activity by induction of the apoptotic pathway. The phytochemicals ellagic acid, ursolic acid, and luteolin, derived from pomegranate extract, caused a time- and concentration-dependent reduction in cell proliferation and viability, suggesting that they contribute to the inhibitory effect of the extract. The authors conclude that the effects observed in their investigation are due to inhibition of cancer stem-like cells.

Sulforaphane is a natural compound derived from broccoli and broccoli sprouts. Sulforaphane decreased the aldehyde dehydrogenase-positive subpopulation of cells and reduced the size and number of primary mammospheres in tumor xenografts in a nonobese diabetic/severe combined immunodeficient (NOD/SCID) mouse model [51]. Sulforaphane eliminated breast cancer stem-like cells *in vivo*, thereby abrogating tumor growth after re-implantation of primary tumor cells into mice. Western blotting

analysis and a β -catenin reporter assay showed that sulforaphane downregulated the Wnt/ β -catenin self-renewal pathway. These findings support the use of sulforaphane for chemoprevention against breast cancer stem cells.

Resveratrol significantly reduced cell viability and mammosphere formation and subsequently induced apoptosis in cancer stem-like cells [52]. The inhibitory effect of resveratrol was accompanied by a significant reduction in lipid synthesis caused by the downregulation of the fatty acid synthase (*FAS*) gene followed by the upregulation of the pro-apoptotic genes DAPK2 and BNIP3. Resveratrol-induced activation of the apoptotic pathway in cancer stem-like cells was suppressed by TOFA and by fumonisin B1, suggesting that resveratrol induces apoptosis through the modulation of fatty acid acid-mediated cell survival signaling. Resveratrol was able to significantly suppress the growth of cancer stem-like cells in an animal xenograft model with no apparent toxicity.

All-trans retinoic acid (ATRA), a form of vitamin A, has also shown potential as an anticancer agent. The inhibitory effect of ATRA stealth liposomes was more potent in CD44⁺/CD24⁻ cancer stem cells than in cancer cells without this phenotype [53]. Its mechanisms include arrest of breast cancer stem cells at the G0/G1 phase and induction of differentiation. Formation and growth of xenografted tumors were significantly inhibited by ATRA stealth liposomes. Combination therapy of ATRA stealth liposomes with vinorelbine stealth liposomes produced the strongest inhibitory effect on the tumor relapse model compared to free ATRA, and vinorelbine stealth liposomes.

Ovarian cancer

During the characterization of CD44⁺ ovarian cancer stem cells, Casagrande et al. [54] found a high rate of expression of the genes encoding claudin-4. CD44⁺ ovarian cancer stem cells expressed the claudin-4 gene at significantly higher levels than matched autologous CD44⁻ ovarian cancer cells. Because claudin-4, a tight junction protein, is a natural and high-affinity receptor for *Clostridium perfringens* enterotoxin, the authors investigated the sensitivity of ovarian cancer stem cells to *Clostridium perfringens* enterotoxin treatment *in vitro* and *in vivo*. Small-interfering RNA-mediated knockdown of claudin-3/-4 expression in CD44⁺ cancer stem cells significantly protected cancer stem cells from *Clostridium perfringens* enterotoxin-induced cytotoxicity. Multiple intraperitoneal administrations of sublethal concentrations of *Clostridium perfringens* enterotoxin in mice harboring xenografts of chemotherapy-resistant CD44⁺ ovarian cancer stem cells had a significant inhibitory effect on tumor progression. In fact, all treated animals were either cured or achieved long-term survival. Hence, *Clostridium perfringens* enterotoxin may represent an unconventional but potentially highly effective strategy to eradicate chemotherapy-resistant cancer stem cells.

Liver cancer

Lupeol (lup-20(29)-en-3 β -ol) is a triterpene found in fruits and vegetables that inhibited the self-renewal ability of liver tumor-initiating cells in both hepatocellular carcinoma (HCC) cell lines and clinical HCC samples [55]. Furthermore, lupeol inhibited tumorigenicity *in vivo* in nude mice and downregulated CD133 expression. In addition, lupeol sensitized HCC cells to chemotherapeutic agents through the phosphatase and tensin homolog (PTEN)-Akt-ABCG2 pathway. PTEN plays a crucial role in the self-renewal and drug resistance of liver tumor-initiating cells T-ICs; downregulation of PTEN via a lentiviral-based approach

reversed the effect of lupeol on liver T-ICs. Using an *in vivo* drug-resistant HCC tumor model, lupeol dramatically decreased the tumor volumes of MHCC-LM3 HCC cell line-derived xenografts. Lupeol exerted a synergistic effect when combined with established chemotherapeutic drugs (cisplatin, doxorubicin) without any adverse effects on body weight.

Prostate cancer

Gossypol reduced the viability of tumor initiating CD44⁺ prostate cancer cell lines *in vitro*. Additionally, gossypol inhibited prostate tumor-initiating cell growth in a xenograft model. The decrease in viability was associated with increased DNA damage, activation of p53, and induction of apoptosis [56].

Brain cancer

Lu et al. [57] isolated cancer stem-like cells from medulloblastoma patient samples and cultured them as three-dimensional spheroids. They displayed enhanced self-renewal and expressed stem-like genes (Oct-4, Nanog, Nestin, Musashi-1) and anti-apoptotic genes (Bcl-2, Bcl-xL). These spheroids also showed significant resistance to radiotherapy as compared to the parental medulloblastoma cells in 2D culture. Resveratrol effectively inhibited the proliferation of stem-like medulloblastoma cells and significantly enhanced radiosensitivity.

Fong et al. [58] treated rat C6 glioma cells with curcumin and observed a decrease in a specific side population of C6 cells that retained less Hoechst 33342 dye after daily curcumin treatment. Since the side population exhibited stem-like cell markers, curcumin may be a dietary phytochemical with the potential to target cancer stem-like cells.

Treatment of spheroid-forming glioma cells with Eckol, a phlorotannin compound, effectively decreased sphere formation as well as the CD133⁺ cell population, and blocked both phosphoinositide 3-kinase-Akt and Ras-Raf-1-Erk signaling pathways. Eckol treatment suppressed expression of the glioma stem-like cell markers and the self-renewal-related proteins without causing cell death. Moreover, Eckol significantly attenuated anchorage-independent growth on soft agar and tumor formation in xenograft mice. Importantly, Eckol treatment effectively reduced the resistance of glioma stem-like cells to ionizing radiation and temozolomide [59].

Multiple myeloma

Gunn et al. [60] tested two natural product inhibitors of NF κ B, parthenolide and andrographolide, in multiple myeloma stem-like cells. Both compounds demonstrated preferential toxicity toward cancer stem-like cells as compared to non-tumorigenic multiple myeloma cells. Coculture with bone marrow stromal cells in their 3D reconstructed bone marrow model abrogated andrographolide activity while having no effect on parthenolide cytotoxicity.

Leukemia

Cantharidin targeted primary acute myeloid leukemia stem and progenitor cells *in vitro* in contrast to conventional chemotherapeutic agents (e.g., Ara-C and daunorubicin) that mainly targeted more differentiated leukemic cells [61]. Because of concentration-limiting toxicity *in vivo*, neither cantharidin nor norcantharidin proved therapeutically beneficial in acute myeloid leukemia xenograft models as a single agent. However, their potent *in vitro* activity against leukemic stem-like cells and pathway targeting

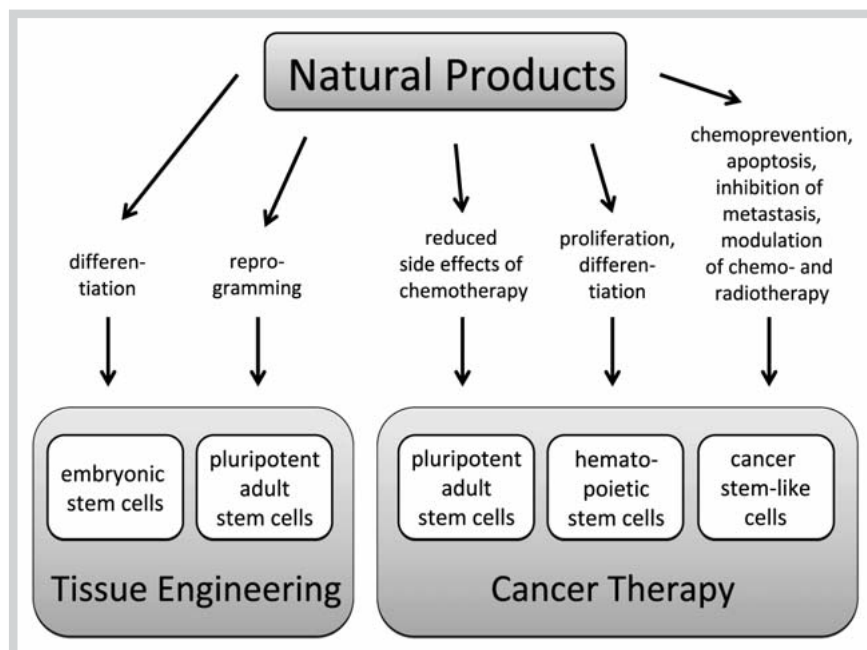


Fig. 1 Therapeutic potential of natural products towards stem cells.

may still be exploited clinically with a new generation of cantharidin derivatives.

Treatment with Abl tyrosine kinase inhibitors drastically improves the prognosis of chronic myeloid leukemia patients. However, quiescent CML cells are insensitive to tyrosine kinase inhibitors and can cause relapse. The hypoxic conditions in the bone marrow allow leukemic cells that reside there to become insensitive to cell death stimuli. Takeuchi et al. [62] described that **rakicidin A**, a natural product produced by a *Micromonospora* strain, induced cell death in hypoxia-adapted chronic myeloid leukemia cells with stem cell-like characteristics. Apoptosis was induced by both caspase-dependent and -independent pathways. Treatment with rakicidin A in combination with the tyrosine kinase inhibitor imatinib resulted in synergistic cytotoxicity.

Conclusion and Perspectives

Many natural products have been described that affect cellular pathways relevant for stem cells and cancer stem-like cells, but have not yet been tested in these cell types specifically. Hence, there are a number of natural compounds to be explored in cancer stem cell biology. Some examples are:

- ▶ Natural small molecules such as vitamin A and retinoic acid inhibit cancer cell growth by induction of differentiation rather than by cytotoxicity [39, 64–67]. The potency of retinoid acids for stem-like cell treatment is promising and needs to be further explored.
- ▶ Numerous phytochemicals that are able to inhibit ATP-binding cassette (ABC)-mediated multidrug resistance (for review see [68]). ABC-transporters such as BCRP are important markers of cancer side-population cells which resemble cancer stem-like cells. ABC transporters contribute to the drug-resistant phenotype of cancer stem-like cells.
- ▶ Phytochemicals that inhibit NF- κ B [69, 70]. NF- κ B is a transcription factor that mediates resistance towards apoptotic stimuli. It promotes tumor progression and drug- and radio-resistance. For example, parthenolide is a well-known NF- κ B

inhibitor that inhibits stem-like breast cancer cells [71]. Another promising drug may be the antimalarial artesunate, which is also active against cancer cells. It has been shown to inhibit growth of rat embryo (stem) cells [72], to induce cellular differentiation [73] and to inhibit NF- κ B [74, 75].

- ▶ Phytochemicals that inhibit signaling pathways of stem cells and cancer stem-like cells. Certain pathways trigger the self-renewal of stem and stem-like cells. Their inhibition suppresses self-renewal and induces cellular differentiation. Interesting examples are:

- ▶ Cyclopamine from *Veratrum californicum*, which inhibited growth of medulloblastoma cells and induced a loss of neuronal stem cell-like character by neuronal differentiation in a mouse model [76].
- ▶ ECGC from green tea (*Camelia sinensis*), which inhibits Wnt/ β -catenin signaling. This pathway is involved in various developmental processes, including growth inhibition in breast cancer cells [77]. Comparable effects have been found for vitamin D on Wnt/ β -catenin signaling in colorectal cancer cells [78]. Artesunate also inhibits the Wnt/ β -catenin signaling [79, 80].
- ▶ Resveratrol, a polyphenol in red grapes and other fruits, that downregulates the expression of Notch [81]. The Notch signaling pathway is also involved in developmental processes and regulates tumor self-renewal in medulloblastoma [82].

Natural products may gain considerable importance in stem cell biology for both tissue regeneration and for protection of normal tissue against the side effects of cancer chemo- and radiotherapy (● **Fig. 1**). Established anticancer drugs kill the bulk of differentiated cancer cells, but leave undifferentiated and quiescent cancer stem-like cells untouched. This population of cancer stem-like cells can give rise to tumor relapse even after chemotherapy has achieved complete clinical remission of tumors [63]. The results obtained with natural products in the past few years are promising, as several compounds have been reported to attack cancer stem-like cells. Furthermore, many of these natural products also improve the efficacy of chemo- and radiotherapy, making them ideal partners for combination therapy regimens. The field of

treating cancer stem-like cells with natural products is still in its infancy, but it can be expected to develop into a rapidly growing area of research.

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Conflict of Interest

There are no conflicts of interest.

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