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Review

Lung Cancer Stem Cells and Cancer Stem Cell-targeting Natural Compounds

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Abstract. *The novel information regarding molecular and translational research have created a paradigm shift in the understanding of lung cancer biology, revealing the more precise target for anti-cancer drug discovery. Lung cancer is a leading cause of cancer death worldwide accounting for approximately 1 in 5 of all cancer-related deaths. The most important causes of death in such a cancer involves the treatment failure as well as the spreading of cancer cells to distant sites which the cancer stem cell (CSC) within the tumor is accepted as a key driver. CSC is a rare special population of cancer cells exhibiting high tumorigenic properties together with self-renewal and differentiation capability. CSC is not only linked with high tumor-initiating activity, but is also implicated in chemotherapeutic resistance, metastasis, epithelial to mesenchymal transition, and recurrence. Thereafter, novel therapeutic strategies targeting these CSCs are considered in order to improve long-term clinical outcome. Here, we provide sufficient data regarding the biology of CSC in lung cancer, known CSC markers and cellular signals, and promising compounds targeting the stem cell signals in lung cancer that may benefit the development of novel anti-cancer treatment.*

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Lung cancer has been long recognized as a life-threatening cancer for several years. The death rate of such a cancer is very high in comparison to that of other cancers comprising approximately 20% of the world's cancer related deaths (1). A total of approximately 1.8 million new cases of lung cancer were estimated in 2012 and this number accounted for 13% of all new cancer cases (1). Generally, more than three-fourths (nearly 80%) of all lung cancers are non-small cell lung cancers (NSCLC) and the rest are small cell lung cancers (SCLC) (2). NSCLC can be further classified to adenocarcinoma, squamous cell carcinoma, and large cell carcinoma (2). Worldwide, a very high number of NSCLC new cases are diagnosed annually and the five-year survival is only about 17.8%. Therefore, the NSCLC is considered very lethal with both high incidence and low survival. NSCLC is frequently found in the elderly population and smoking is accounted as the most important risk factor in most patients (3). Other contributory factors are exposure to environmental toxicants including asbestos, radon, and certain metals such as arsenic, cadmium, and chromium (4). In addition, the exposure to organic chemicals found in coal smoke and fuel burning have been reported to be the risk factors of lung cancer (5).

The concept of cancer stem cells (CSC) was introduced in 1977 (6) and has now become a very interested topic in cancer research. CSCs are a small rare fraction (in most solid tumor <1%) of the whole cancer cell population that exhibit high tumorigenic potential (7). CSCs were first recognized as cancer initiating cells as they are believed to be the root cause or seed of cancer (8, 9). The characteristics of CSCs which play pivotal role in driving aggressiveness of cancer include their self-renewal capacity, differentiation (asymmetric cell division), high invasion and migration characteristics, high tumorigenicity, and resistance to chemotherapy (10). Based on such information, CSCs are thought to be the main mediators of all cancer hallmarks including high tumorigenic, high metastasis potential, evading from immune system, resistance to chemotherapy, and cancer relapse (8, 9).

The knowledge at the present day has pointed that the currently used therapeutic approaches comprising surgery, radio-, chemo-, and targeted therapy, in particular for lung cancer management have failed to eradicate the CSC population, a main cause of disease relapse. Natural product-derived compounds that specifically target these CSCs could unravel a more precise and efficient way to eradicate this disease.

In addition, the better understanding of CSC biology in relevant to the basic mechanisms enhancing CSC properties and aggressive behaviors of lung and other high mortality cancers may influence the development of cancer management strategies including anti-metastatic, control of CSC activities, and inhibition of cancer relapse.

Cancer Stem Cell in Lung Cancer

It is now well accepted that lung cancer is characterized by a heterogeneity of cancer cells which exhibit different cell phenotypes as resulted from their distinct cellular signaling.

Among the different lineage of cancer cells residing in the tumor, there is a specific small population of cancer cells that has stem cell characteristics including the self-renewal capacity and multi-lineage differentiation (11, 12). Nevertheless, the cancer cells containing the stem cell capacities exhibit highly aggressive phenotypes such as tumorigenic potency, migration and invasion, evading from anoikis, and chemotherapeutic resistance (9, 12-14). These specific subpopulation of cancer cells were first named "cancer initiating cells", as they were believed to be the beginning seed of the whole cancer and have now become the main focus of cancer cell biology as well as anti-cancer drug discovery researches.

CSCs were first identified in acute myeloid leukemia (AML) by their cell surface marker CD34+Cd38–. These cells were shown to have a high capacity to self-renew in bone marrow and differentiate to leukemic cells when transplanted into severe combined immunodeficient (SCID) mice (15). After these observations were made, CSCs of various cancer types including lung cancer were discovered through specific cell-surface proteins by numerous research groups (16). However, recent studies suggested that not only CSCs but also certain populations of cancer cells within tumors can have stem cell properties (stemness) (17). Moreover, fully differentiated cancer cells can be transformed to be cancer stem-like cells (CSC-like cells) by the activity of certain cancer microenvironment substances such as nitric oxide, hypoxia condition, and interleukin (18-20) or by the mutation of specific gene including *TP53* (21, 22). These indicated that "stemness" is a phenotype which can be acquired *via* proper extracellular stimulation signals or accumulation of specific gene mutations within the cells. Nevertheless, both CSC and CSC-like cells show the same characteristics as normal stem cells.

The key CSC properties compose of:

- Self-renewal capacity; an unique ability of the CSC (just like normal stem cells) to generate the identical daughter cells with identical stem cell characteristics.
- Ability to drive tumor heterogeneity and survival of tumor; an ability to differentiate into different cancer cell linages, facilitate cell growth, and survival of whole tumor.
- High tumorigenic potential; an ability of CSC to proliferate and create non-CSC lineages and form new tumors.

Based on the discovery of CSC in lung cancer together with the knowledge from clinical pathology, lung cancer is well recognized as a disease of heterogeneity. Researches have shown that the cancer cells derived form same tumor have a distinct ability to form tumor spheroid *in vitro* as well as *in vivo* (23, 24). Cancer cells within the same tumor have diverse cellular properties and signaling resulting from the accumulation of genetic mutations and epigenetics alterations. These differences in cellular properties and signaling are associated with plasticity and heterogeneity of cancer stem cells which could be identified by certain biomarkers.

Cancer Stem Cell Markers and Regulatory Proteins

As CSCs maintain high stem cell signaling that is similar to that in normal tissues, certain cellular markers used for the identification of normal stem cells are also utilized for CSC identification. For lung cancer, the well-recognized CSC markers are summarized in Table I and described as followed.

Cluster of differentiation-133 (CD133, prominin-1, PROM-1). CD133 is an 865 amino acids penta-span transmembrane protein which has been accepted as a principle marker of stemness in several solid tumors (25). In human, this protein is a 120 kDa protein product of a single-copy gene on chromosome 4 (4p15.32) (25). In general, CD133 is an important marker used for the isolation and identification of the stem cells from normal tissue like human hematopoietic stem cells (26, 27). The function of CD133 in the cells is not fully known yet but several studies have shown that CD133 expression is linked with stem and progenitor cell characteristics as well as the stage of cell regeneration and differentiation (27). Emerging evidence has also shown that CD133 is involved in cell growth and development (28).

In cancers, evidence has shown that the expression of CD133 is associated with tumor aggressiveness through up-regulation of certain proteins (29-32).

Later on, evidence has shown that CD133 is not only a biomarker and is functioning in normal stem cells, but also in cancer cells. Expression of CD133 has been used for the identification of CSC in several cancers including lung (33), pancreatic adenocarcinoma (34), hepatocellular carcinoma (35), prostate (36), neural (37), colorectal (38), and renal

Table I. *Cancer stem cell marker for lung cancer.*

Cancer stem cell marker	Description and Function
Aldehyde dehydrogenase isoform 1 (ALDH1)	• Cytosol protein involving in aldehyde detoxification. • Protection of stem cells against the against oxidative aldehydes. • Marker for poor prognosis in lung cancer.
CD133 (prominin-1)	• A cell-surface glycoprotein. • Role in lung cancer is still unclear. • May acts as an organizer of cell membrane topology. • Marker for poor prognosis in lung cancer.
CD44	• A cell-surface glycoprotein involves in cell to cell interactions, adhesion and motility of cells. • A receptor for several extracellular matrix component including hyaluronic acid, collagens, osteopontin, and matrix metalloproteinases.
CD166 (activated leukocyte cell adhesion molecule (ALCAM))	• A transmembrane glycoprotein member of the immunoglobulin superfamily of proteins. • Control cell clustering and cell migration
Wnt/β-catenin (canonical Wnt pathway)	• Wnt pathway that stabilizes and causes a cellular β-catenin accumulation. The β-catenin then translocates into the nucleus and acts as a transcriptional coactivator of transcription factors that belong to the TCF/LEF family. • Controls embryonic development and adult homeostasis. • Essential for stem cell properties. • Participates in CSC phenotypes in lung cancer. • Marker for poor prognosis in lung cancer.
Octamer-binding transcription factor 4 (Oct4, POU5F)	• A homeodomain transcription factor of the POU family. • Function in the self-renewal of undifferentiated embryonic stem cells. • Absolutely required for the stemness properties of murine and primate embryonic stem cells. • Essential for somatic cell reprogrammation. • Participate in the tumorigenicity and malignancy of non-small cell lung cancer (NSCLC). • Associated with gefitinib resistance in NSCLC.
Nanog	• A transcription factor involves with self-renewal of undifferentiated embryonic stem cells. • Important for inner cell mass and embryonic stem (ES) cells proliferation and self-renewal. • Marker for poor prognosis in lung cancer.
Sex determining region Y-box 2 (**Sox2**)	• A member of the SoxB1 transcription factor family • Important transcription factor in pluripotent stem cells (PSCs). • Function with Oct4 and Nanog to control gene expression in PSCs and maintain stem cell properties.
ATP-binding cassette sub-family G member 2 (ABCG2, BRCP, ABCP, MXR)	• A constitutively expressed ATP-binding cassette (ABC) transporter that protects many tissues against xenobiotic molecules. • Controls porphyrin homeostasis. • Involves in drug resistance mechanism of cancer cells.

cancers (39). In particular, CD133 is frequently used for indicating stemness in lung cancer following immune-histochemical analysis of non-small cell lung cancer (NSCLC) (29) or small cell lung cancer (SCLC) (40) patient samples. Subsequent transplantation of CD133+cells cultured from these tumors into severe combined immune-deficient (SCID) mice generated tumor xenografts phenotypically identical to the original tumor.

Cluster of differentiation-44 (CD44). CD44 is 742 amino acids cell surface glycoprotein member of the tumor necrosis factor receptor superfamily. In human, this protein is approximately 80-95 kDa and is the product of the gene in the short arm of chromosome 11 (11p13). This protein is implicated in the activities of several types of cells including hematopoietic, epithelial, endothelial, and tumor cells (41). The function of CD44 is involved in multiple cellular processes such as cell growth and differentiation, cellular movement, angiogenesis and release of protease enzyme from cell membrane (41). The activation of CD44 by heterodimerization with growth factor receptors (EGFR, FGFR, HGFR, VEGFR, TGF-βR) leads to the activation of PI3K-AKT and MAPK pathways (42). CD44 has been recognized as a marker for lung cancer stem cells (43) by analyzing the CSC marker expression in 10 NSCLC related to CSCs properties (43). Moreover, CD44 positive cell has high tumorigenicity in transplantation, increased capacity to resist to chemotherapy, and higher expression of stem cell transcription factor Oct-4 and Nanog. However, analysis of lung tumor samples by immunohistochemical revealed that CD44 expression was a prognostic marker only in adenocarcinomas but not in squamous cell carcinomas.

Aldehyde dehydrogenases 1A1 (ALDH1A1). Aldehyde dehydrogenase (ALDH) is a member of a group of enzymes that catalyze the oxidation of aldehydes to carboxylic acids. ALDH1A1 is 501 amino acid protein member of the ALDH1 family and is also known as retino aldyhyde dehydrogenase1 (RALDH1). In human, this protein is an approximately 55 kDa protein, product of the *ALDH1A1* gene located in chromosome 9 (9q21.13). ALDH1A1 is a putative hematopoietic stem cell marker associated with increased drug resistance in many cancers (44). ALDH1A1 has been used to develop the assay named "Aldefluor assay" which is used to distinguish stem and progenitor cells from normal cells. This assay also has been used in the identification of potential CSCs in leukemia, breast, neural, head and neck, colon, liver and lung cancers (45). More recently, lung CSCs have been identified using this method (46). NSCLC cells with relatively high ALDH1 activity, displayed *in vitro* features of CSCs, including an increased capacity for proliferation, self-renewal, differentiation and expression of the CSC surface marker, CD133 (47, 48). Xenograft transplantation of these cells in NOD/SCID mice demonstrated increased tumorigenicity, in addition to increased ALDH1 protein expression which correlated with poor clinical outcome and advanced stage of disease in NSCLC.

ATP-binding cassette sub-family G member 2 (ABCG2). ABCG2 (BRCP, ABCP, MXR) is the member of ATP binding cassette (ABC) superfamily that consists of transmembrane proteins. ABCG2 is 655 amino acids with approximately 72kDa protein product of a gene in chromosome 4 (4q22). ABCG2 is a half-transporter predominantly localized at plasma membranes, while dimerization is required for active function (49). The side population phenotype (SP) is one well-known characterization method of CSC. This method measures the ability of stem cells to efflux the fluorescent dye Hoechst 33342 from the cells. ABCG2 has been proved to be a molecular determinant responsible for SP phenotype (50). ABCG2 expression is a conserved feature of stem cells from a wide variety of tissues, including pancreas, lung, limbal epithelium, heart, testis, muscle, cornea and conjunctiva, brain, prostate, and embryo (51). Moreover, ABCG2 was high frequently identified in various types of cancer including carcinomas of the digestive tract, lung, breast, ovarian, and melanoma (49). The expression level of ABCG2 is also associated with high pathological grade of tumor and poor prognosis outcome of patients (52, 53).

Transcription factors regulating cancer stem cells. CSCs characteristics are thought to be regulated by the numerous set of molecular signals that are tightly controlled by stem cell transcription factors which enrolled the activity to maintain normal stem cell functions. There are several well-characterized stem cell transcription factors that are used for CSC identification, and among them Octamer-binding transcription factor 4 (Oct-4), Sex determining region Y-box 2 (Sox2), and Nanog are intensively used for lung CSC identification.

Octamer-binding transcription factor4 (Oct-4). Oct-4 is a POU domain-containing transcription factor that binds to the octamer sequence, ATGCAAAT, of the target genes. Oct-4 is encoded by *POU5F1* gene located at chromosome 6p21.31. The human Oct-4 gene consists of five exons which can be spliced into three main isoforms *OCT4A, OCT4B* and *OCT4B1*. These gene isoforms provide four isoform proteins Oct4A, Oct4B-190, Oct4B-265, and Oct4B-164. All forms of Oct-4 are functionally and structurally divided into three domains including an N-terminal transcriptional activation domain, a central POU domain, and a C-terminus containing a cell type-specific transactivation domain. Oct4A, is generally referred as Oct-4, and regulates the stemness of embryonic stem cells. Oct-4 is highly expressed in embryonic stem (ES) cells and low expressed when ES cell differentiates and there is consequently loss of pluripotency. Oct-4 and its activities were shown to be required for maintaining the ES cell capacity (54). Several target genes of Oct-4 in ES cells have been identified, including Fgf4, Utf1, Opn, Rex1/Zfp42, and Fbx15 (55). Additionally, high levels of Oct-4 have been identified in highly aggressive tumors, poor prognosis patients, and relapse cancer. It has been documented that overexpression of Oct-4 is associated with tumorigenicity,

metastasis, and cancer relapse in certain cancers (56). High expression of Oct-4 was detected in prostate and breast cancer stem cells and in the tumor initiating cells in a p53–/– tumor mice model (57). Oct-4 level and activity are known to be regulated by several steps, such as transcription, translation, and post-translational modifications. The key regulatory control of Oct-4 is found to be the post-translational modifications *via* phosphorylation. Oct-4 can be phosphorylated by various types of protein kinases at Ser229, Ser236 and tyr327. Phosphorylation at these residues regulates Oct-4 stability and transcriptional activity (58, 59). Ubiquitination is the main pathway responsible for eliminating short-lived proteins. Oct-4 can be ubiquitinated by an HECT-type E3 ubiquitin ligase, Wwp2. Wwp2 regulates Oct-4 level by mediating its ubiquitination and degradation during ESC differentiation. Both the Wwp2 and Oct4 levels decrease when ESCs are induced to differentiate (60). The small ubiquitin-related modifier (SUMO), which is functionally divergent from ubiquitin, can modify many nuclear proteins to affect their subcellular localization, thus altering their interaction with cooperative molecules. Studies show that Oct-4 can be sumoylated at a single lysine, lysine 118, which is located at the end of the N-terminal transactivation domain and next to the POU DNA-binding domain. Sumoylation of Oct-4 significantly increases its stability, DNA binding, and thus the transcriptional activity (59).

Sex determining region Y-box 2 (Sox2). SRY (sex determining region Y)-box2, Sox2, is a member of the Sox transcription factor of HMG-family that occupies many gene targets including self-renewal maintaining genes (61). Sox2 shares an approximately 80 amino acids region with its family known as high-mobility group (HMG) box domains which are DNA binding domains (62). Sox2 and Oct-4 cooperative to activate gene transcription by binding at non-palindromic sequences. In normal tissue, Sox2 regulates the morphogenesis of various tissue types including control branching morphogenesis of the bronchial tree and maturation of the epithelium of airways, development of gastric and intestinal basal cells, and growth and differentiation of neuronal cells. The amplification of DNA at 3q26.3 region which encodes the *Sox2* gene is frequently observed in NCLCs squamous cell carcinoma. Sox2 expression is also observed in high grade prostate cancer, colorectal cancer and breast cancer (63). Additionally, overexpression of Sox2 is associated with chemo-resistance, cancer migration and anchorage-independent growth (63). The regulation of Sox2 level and transcriptional activity are found to be mainly by post-translational modifications. Sox2 can decrease its DNA binding capacity by sumoylation at lysine 247 which is triggered by phosphorylation at triplet serine (ser249, 250, and 251). Therefore, phosphorylation at Thr118 promotes Sox2 stability by blocking its ubiquitination. In addition, Sox2 is directly methylated at Arg113 by protein arginine methyltransferase 4 (PRMT4, known as CARM1). This methylation promotes Sox2 self-association *via* HMG-box domain. The acetylation by p300/cAMP-response element-binding protein (CBP) at Lys75 of Sox2 promotes its proteasomal degradation (59).

Nanog. Nanog is a 350 amino acid protein with a homeobox-containing motif which facilitates binding with DNA. Nanog is encoded by the *NANOG1* gene, located on chromosome 12 (12p13.31), which is activated to maintain the pluripotent state of the cell. Based on the differences in gene expression between wild-type and Nanog null cells, it has been proposed that Nanog regulates pluripotency mainly as a transcription repressor for downstream genes that are important for cell differentiation such as Gata4 and Gata6 (64). However, Nanog can also activate the genes necessary for self-renewal such as *Rex1*. Nanog is highly expressed in pluripotent cells and its expression is down-regulated during differentiation. High expression of Nanog has been documented in many types of carcinomas such as tumor of brain, colon, breast, gastric, liver, kidney, and lung. Importantly, the expression of Nanog in certain tumors have provided positive correlation with treatment failure and poor prognosis of patients. Moreover, up-regulation of Nanog expression promotes tumorigenicity. These demonstrated that Nanog is associated with tumor progression, resistance to chemo/radiotherapy, and disease relapse (65). Nanog can be phosphorylated at four Ser/Thr-Pro motifs. These phosphorylation sites suppress the ubiquitination of Nanog and enhance its stability by promoting the interaction between Nanog and the prolyl isomerase Pin1 (66).

CSC and Lung Cancer Metastasis

Metastasis is a process comprising several steps where cancer cells disseminate form their original tumor to generate new tumors at distant parts (67). Like in other types of malignant human cancers, metastasis in lung cancer is considered as a key determining factor of prognosis of patients (68). Lung cancers are frequently diagnosed in metastatic stages (stage IV or more advance stages) at the time of first diagnosis (69). To successfully metastasize, the cancer cells encompass many biological processes including increased motility, induction of epithelial-mesenchymal transition (EMT), intravasation, survival in the blood or lymphatic circulations, extravasation, mesenchymal-epithelial transition (MET), and ability to form new tumor (tumorigenesis) (70). A rare population (less than 1%) of cancer cells could succeed in metastasizing. Several natural compounds have been tested for their possible role in preventing cancer cell dissemination as explained in our previous work (71).

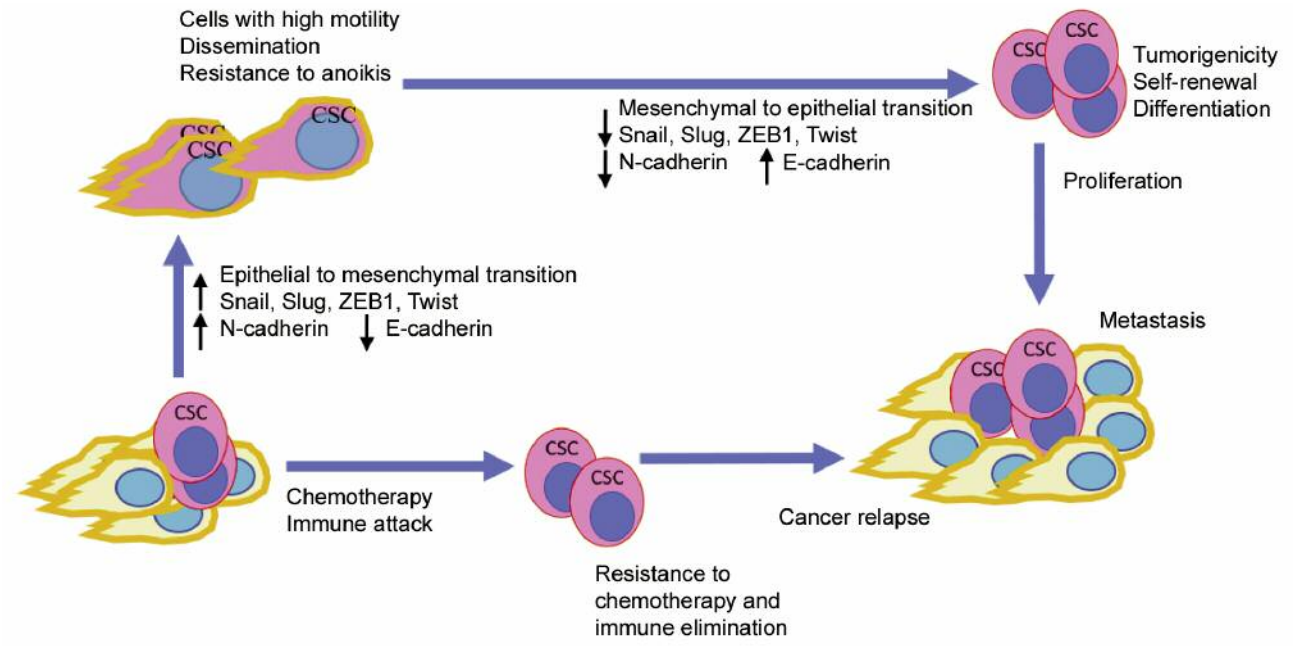


Figure 1. *Schematic figure of cancer stem cell in implication to metastasis, chemotherapeutic resistance, epithelial to mesenchymal transition, and cancer relapse.*

The process of cancer cell transition from epithelium-like cells to mesenchymal-like cells has been shown to be implicated in several behaviors of normal as well as cancerous cells (72). Epithelial to mesenchymal transition (EMT) is an essential process of the cancer cell to metastasize as it provides many important capabilities including ability to survive in detached conditions (anoikis resistance), increased invasion, and enhanced cell motility (73). It has been identified that the EMT in cancer cells involves several pleiotropic transcriptional factors, such as Snail (74), Slug (75), Zeb1 (76), Bmi-1 (77), and deltaEF1 (78). Such transcription factors enable EMT cells to disrupt epithelial adhesion, and cell to cell interaction (79). The EMT cells will then detach, leave the primary tumor mass, migrate, and invade into the circulation (80). It is known that EMT in cancer cells is relatively transient. Importantly, at the final step of cell dissemination when the cancer cells have reached the site of new establishment, the conversion of EMT named mesenchymal to epithelial transition (MET) is required to ensure stable cell interaction and adhesion to the surrounding new microenvironment (81). Therefore, the dynamic drive of EMT-MET processes is considered as a critical capability of cancer cells to metastasize (82).

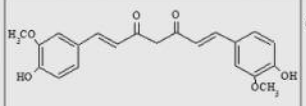
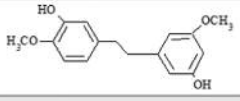
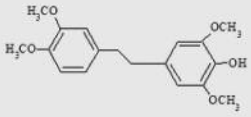
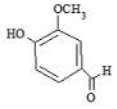
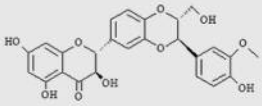
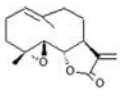
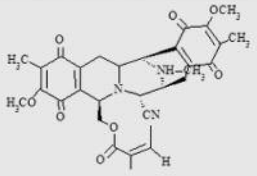
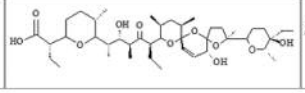
After cancer cells have reached the site of metastasis, the tumorigenic potential, the unique property found in CSCs, of the cells is required for the establishment of new tumor (83, 84). These contexts have suggested the involvement of EMT and CSC in the process of metastasis. Even though the clear picture of how EMT relates to CSC is not yet verified, recent evidence has linked EMT to CSCs (85). EMT related transcription factors or inducers such as Epidermal Growth Factor (EGF), Transforming Growth Factor β (TGF-β), Hepatocyte Growth Factor (HGF), Wnt/β-catenin, Hedgehog, Notch pathways, are essential for CSCs (86, 87). Certain studies have pointed out that induction of EMT mediated by EMT inducers in fully differentiated epithelial cells can trigger CSC-like phenotypes including increased CD44, decreased CD24, and increased stem cell phenotypic markers (85, 88, 89). Furthermore, the decrease in E-cadherin and increase in MMP-2, an indicator of EMT, were found to be critical for CSCs to metastasize (90).

In conclusion, evidence has pointed out that the key players of metastasis are the cell possessing ability of CSC to generate new tumor, increased motility, and EMT-MET transition. It has been assumed that metastasis-initiating cells are overlapping with CSCs to some degree. In addition, genetic evaluation of CSCs revealed their relevance in tumor recurrence and metastases, supporting the conclusion that CSCs may be metastatic precursors. The involvement of CSC in lung cancer metastasis, EMT-MET, and cancer relapse is presented in Figure 1.

Natural Product Targeting Lung Cancer Stem Cells

Natural products have been a rich source of novel lead compounds which may be useful for anti-cancer approaches. Here we summarized the discovered natural product-derived compounds (Table II) that have been demonstrated to influence

Table II. *Natural products targeting lung cancer stem cells and related mechanism*.

Compounds	Structure	Source	Proteins/signals	References
Curcumin		*Curcuma longa*	DNA damage, DNA repair, JAK2/STAT3/Wnt/β-catenin, Sonic Hedgehog CD133, CD44, ALDHA1, Nanog, Oct4	(105, 106)
Gigantol		*Dendrobium draconis*	AKT, CD133, ALDH1A1	(114)
Chrysotoxine		*Dendrobium pulchellum*	Src-AKT-Sox2, CD133, CD44, ABCG2, ALDH1A1	(116)
Vanillin		*Vanilla planifolia*	AKT-proteasomal degradation, CD133, ALDH1A1,Oct4, Nanog	(121)
Silibinin		*Silybum marianum*	ALDH activity	(123)
Parthenolide		*Tanacetum parthenium*	ER stress, Apoptosis, ATF4, DDIT3, PMAIP	(129)
Renieramycin M		*Xestospongia sp.*	CD133, CD44, ALDH1A1	(131)
Salinomycin		*Streptomyces albus*	OCT-4, Nanog, Sox2	(134)

key CSC signaling pathways such as WNT/β-catenin, Hedgehog, Notch and PI3K/AKT/mTOR pathways (91-93).

Curcumin, a yellow polyphenol, is a key compound derived from the rhizome of Turmeric (*Curcuma longa*) belonging to the ginger family (Zingiberaceae) (94). Previous studies have revealed the promising activities of curcumin against various kinds of cancer. Curcumin is able to suppress cancer signaling pathways, inhibit metastasis and angiogenesis, induce apoptosis, and sensitize tumor cells to cancer therapies (95-98). Several studies have suggested that curcumin can target CSCs in a variety of human cancer types (99-102) including lung cancer (103). Curcumin can diminish the self-renewal capacity of lung CSCs by inducing DNA damage or suppressing DNA repair mechanisms. Sheefa *et al*., studied the effect of curcumin on circulating CSCs isolated by sphere formation assay and observed a significant inhibition of sphere formation (104). Moreover, the result from single cell gel electrophoresis assay showed that 95.47±0.72% of DNA material was present in comet tail (104). Wu *et al*., reported that curcumin disturbed JAK2/STAT3 signaling pathway resulting in the reduction of tumor sphere growth of H460 lung cancer cells *in vitro* and *in vivo* (105). Curcumin has also been reported to reduce CSC marker (CD133, CD44, ALDHA1, Nanog and Oct4) expression, inhibit proliferation and induce apoptosis *via* down-regulation of WNT/β-catenin and Sonic Hedgehog pathways in A549 and H1299 cells (106). Furthermore, curcumin is an attractive candidate for combination therapy because curcumin can target CSCs which are responsible for cancer recurrence and therapy resistance. Baharuddin *et al*., combined curcumin with low dose cisplatin (3 μM) and found that curcumin increased the sensitivity of the highly migratory CD166+/EpCAM+ CSC subpopulation in the A549 and H2170 cells to cisplatin-induced apoptosis and inhibited migration (107).

Many studies have revealed that natural products isolated from *Dendrobium* species possess anticancer properties including anti-proliferation, anti-migration, anti-metastasis, and apoptotic induction in lung cancer (108-110). Gigantol, a bibenzyl phenolic compound derived from several medicinal orchids, has been shown to inhibit proliferation, migration, EMT and CSC phenotype in lung cancer cells (111-114). At non-toxic doses (below 20 μM), gigantol isolated from *Dendrobium draconis* could suppress tumor spheroid formation and decrease lung CSC marker proteins, including CD133 and ALDH1A1, in non-small-cell lung cancer NCI-H460 cells (112). Additionally, gigantol inhibited cancer stem cell-like phenotypes through down-regulation of AKT signaling pathway which lead to reduced levels of Oct4 and Nanog (114). Chrysotoxine, a bibenzyl compound isolated from stems of *Dendrobium pulchellum*, has been reported to sensitize anoikis and inhibit metastasis of lung cancer cells in an anchorage-independent fashion (115). Bhummaphan *et al*. investigated the suppressive effects of chrysotoxine on CSC-rich populations of H460 and H23 cells and primary CSCs in three-dimensional (3D) culture and showed that non-toxic doses (≤20 μM) of chrysotoxine inhibited CSC-like phenotypes and decreased CSC markers CD133, CD44, ABCG2 and ALDH1A1 which were mediated through a Src-AKT-Sox2-dependent mechanism (116).

Vanillin, a 4-hydroxy-3-methoxybenzaldehyde isolated from the seed of *Vanilla planifolia*, is widely used as a flavoring agent in food and cosmetics (117). Vanillin inhibited cell migration, lamellipodia formation and angiogenesis and induced apoptosis in many cancer types including lung cancer (117-120). Non-toxic doses (below 100 μM) of vanillin could inhibit spheroid and colony formation, major hallmarks of the cancer stemness, and reduce the CSC markers CD133 and ALDH1A1 and the related transcription factors, Oct4 and Nanog in H460 cells through the reduction of AKT and downstream CSC transcription factors (121).

Silibinin is a natural polyphenolic flavonoid derived from milk thistle seed (*Silybum marianum*) which has the ability to diminish many cancer types including lung cancer (122). To determine the suppressive effect of silibinin on lung cancer stem cells, Corominas-Faja *et al*. developed erlotinib-refractory cells (PC-9/Erl-R cells) by growing NSCLC PC-9 cells expressing the *EGFR* exon 19 deletion in routine culture medium containing a high dose of erlotinib (1 μM) (123). The results from flow cytometry and the ALDEFLUOR® reagent showed that silibinin reduced aldehyde dehydrogenase (ALDH)-expressing CSC-like cells in erlotinib-refractory cell populations and inhibited lung cancer spheres formation in a dose-dependent manner.

Isoflavone VF166, a derivative of soy isoflavone daidzein, can inhibit growth of various cancers including lung cancer. VF166 could suppress cell adhesion, migration and invasion of NSCLC *in vitro*. Moreover, the results from real time RT-PCR revealed that treatment with VF166 up- and down-regulated various genes including *DKK1, KLF4, MUC1, ErbB2, PTCH1* and *SMO* in NSCLC cells involved in the regulation of invasion associated signaling pathways such as WNT/β-catenin, Hedgehog, STAT3, and SPARC (124).

Parthenolide, a natural sesquiterpene lactone isolated from the shoots of feverfew (*Tanacetum parthenium*), has anticancer effects on cancer cells and cancer stem cells from various types of cancer including lung cancer (125-128). Parthenolide has been shown to selectively kill cancer stem-like cells *via* ER stress and apoptosis signaling pathway in A549/shCDH1 cells in which CDH1/E-cadherin was knocked down with shRNA. Its underlying mechanisms are up-regulation of activating transcription factor 4 (ATF4) and DNA damage-inducible transcript 3 (DDIT3) expression which lead to up-regulation of Poly (ADP-ribose) polymerase-1 (PMAIP1) expression (129).

Renieramycin M (RM) isolated from the blue sponge *Xestospongia* sp. has been reported to have anti-invasion, anti-migration, and apoptosis-inducing activities in lung cancer cells (130). RM treatment at non-toxic concentrations reduced significantly colony and spheroid formation of H460 cells. Furthermore, RM can also down-regulate the CSC markers CD133, CD44 and ALDH1A1 of CSC-enriched H460 cells (131).

Salinomycin is polyether ionophore antibiotic derived from *Streptomyces albus* (132). Salinomycin showed time- and dose-dependent cytotoxic activity evaluated by sulforhodamine B and colony formation assay in LNM35 and A549 lung cancer cells (133). Treatment with salinomycin for 24 h has been shown to significantly inhibit the tumor sphere formation using flow cytometry and reduce stem cell markers OCT-4, NANOG and SOX2 expression by real-time RT-PCR analysis in ALDH A549 lung cells (134). Zhang *et al*. developed salinomycin-nanoparticles (salinomycin-NPs) and gefitinib-nanoparticles (gefitinib-NPs) by the emulsion/solvent evaporation approach to kill both lung CSCs and lung cancer A549 and A431 cells (135). Both salinomycin and salinomycin-NPs could selectively target CD133 positive CSCs and reduce tumor sphere formation in lung cells, while gefitinib and gefitinib-NPs preferably target lung cancer cells. Consistent with their *in vitro* results, salinomycin or salinomycin-NPs decreased CSC population in the tumors from nude mice bearing A431 xenografts. Furthermore, combination of Salinomycin-NPs and gefitinib-NPs has a more efficient suppressive effect on tumor growth than the combination of salinomycin and gefitinib or single salinomycin-NPs or gefitinib-NPs.

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