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Contents

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Clinical relevance of stem cells in lung cancer

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Abstract

Lung cancer is the major cause of cancer-related deaths worldwide, it has one of the lowest 5-year survival rate, mainly because it is diagnosed in the late stage of the disease. Lung cancer is classified into two groups, small cell lung cancer (SCLC) and non-SCLC (NSCLC). In turn, NSCLC is categorized into three distinct cell subtypes: Adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. NSCLC is the most common lung cancer, accounting for 85% of all lung cancers. Treatment for lung cancer is linked to the cell type and stage of the disease, involving chemotherapy, radiation therapy, and surgery. Despite improvements in therapeutic treatments, lung cancer patients show high rates of recurrence, metastasis, and resistance to chemotherapy. Lung stem cells (SCs) are undifferentiated cells capable of self-renewal and proliferation, are resistant to chemotherapy and radiotherapy and, due to their properties, could be involved in the development and progression of lung cancer. The presence of SCs in the lung tissue could be the reason why lung cancer is difficult to treat. The identification of lung cancer stem cells biomarkers is of interest for precision medicine using new therapeutic agents directed against these cell populations. In this review, we present the current knowledge on lung SCs and discuss their functional role in the initiation and progression of lung cancer, as well as their role in tumor resistance to chemotherapy.

Key Words: Lung cancer; Cancer stem cells; Biomarkers; Chemoresistance

Core Tip: Lung cancer stem cells (CSCs) could have a functional role in primary tumor initiation, invasion and metastasis, resistance to chemotherapeutic drugs, and recurrence in lung cancer. To improve lung cancer treatments, it is necessary to identify and characterize CSCs populations in lung tissue and develop targeted therapies against these cell types. This review discusses the current knowledge on CSCs in lung tissue and future perspectives in lung cancer treatment.

INTRODUCTION

Lung cancer is the leading cause of cancer death worldwide, in both men and women. Lung cancer is classified into two histological types, small cell lung cancer (SCLC) derived from cells of the neuroendocrine lineage and non-SCLC (NSCLC) derived from epithelial cells. The latter is the most frequent type of lung cancer in the population, since represents approximately 85% of all lung tumors, while SCLC represents the remaining 15%[1].

Based on the morphology of the transformed cells, NSCLC is classified into three subtypes: Squamous cell carcinoma (SCC), adenocarcinoma (AD), and large cell carcinoma (LCC). AD arises in peripheral lung tissue from type II alveolar epithelial cells that line the small alveoli, while SCC arises in the central lung region from cells of the stratified squamous epithelium that line the airways from the trachea to the main bronchi. LCC is a heterogeneous group of tumors that lack the morphological characteristics of AD and SCC sub-types or SCLC and arise from epithelial cells that line the lungs[1,2].

SCLC arises from lung epithelial cells from the main bronchi to the terminal alveoli. Their histologic features correlate with the site of origin and reflect epithelial variations of the airways[2]. The classification and main characteristics of lung cancer are shown in Figure 1.

All forms of lung cancer have a poor prognosis, mainly SCLC and SCC, which are typically seen in smokers. SCLC is the most aggressive lung cancer because it grows quickly and spreads to other tissues, generating metastases in the liver, brain, bones, and adrenal glands[3]. LCC has a better prognosis, which depends on the cell subtype. The poor prognosis is generally associated with the ability of the cells to metastasize and the late diagnosis of the disease, since the symptoms and signs do not usually appear in the early stages[4]. People with lung cancer may have chest pain, frequent coughing, blood in the mucus, trouble breathing, swallowing or speaking, loss of appetite and weight, and tiredness[5].

The prevalence of lung cancer worldwide is 13%, with an incidence of 1.8 million new cases per year. Lung cancer is responsible for about 20% of all cancer-related deaths worldwide and has one of the lowest 5-year survival rates (7% in SCLC and 11-17% in NSCLC, depending on the subtype and stage of the cancer[6].

The main cause of lung cancer is long-term smoking, with a 25-fold increase in the risk of developing the disease. However, there are other risk factors such as genetic factors, long-term menopausal hormone replacement therapy, exposure to radon, asbestos, metals (arsenic, cadmium, and chromium), organic chemicals found in smoke coal, and a history of lung disease such as tuberculosis, emphysema, or chronic bronchitis[7]. Smoking can cause all types of lung cancer, but is most strongly linked to SCLC and SCC, while AD is the most common type of lung cancer in patients who have never smoked. In the first two, cancer development frequently occurs in people 60 to 70 years of age with a long-term history of tobacco smoking, while AD occurs in younger people[7].

In this review, we update knowledge about the properties of SCs and their participation in the initiation and progression of lung cancer, pointing out the biomarkers that may be useful in therapeutic strategies or as a prognosis of the disease. In addition, we discuss the role of SCs in the resistance to therapeutic treatments.

SOMATIC MUTATIONS IN LUNG CANCER

Lung carcinogenesis is a chronic process that involves multiple genetic, cellular, and tissue alterations as a consequence of mutations in genes that regulate growth, differentiation, and apoptosis. Cancers are caused by the accumulation of mutations in critical genes, specifically those that control cell growth and division or the repair of damaged DNA. These changes allow cells to grow and divide uncontrollably to form a tumor. In almost all cases of lung cancer, these genetic changes, which are present only in certain lung cells, are acquired during life as a result of exposure to carcinogens, long-term hormone therapy, or chronic lung disease[8].
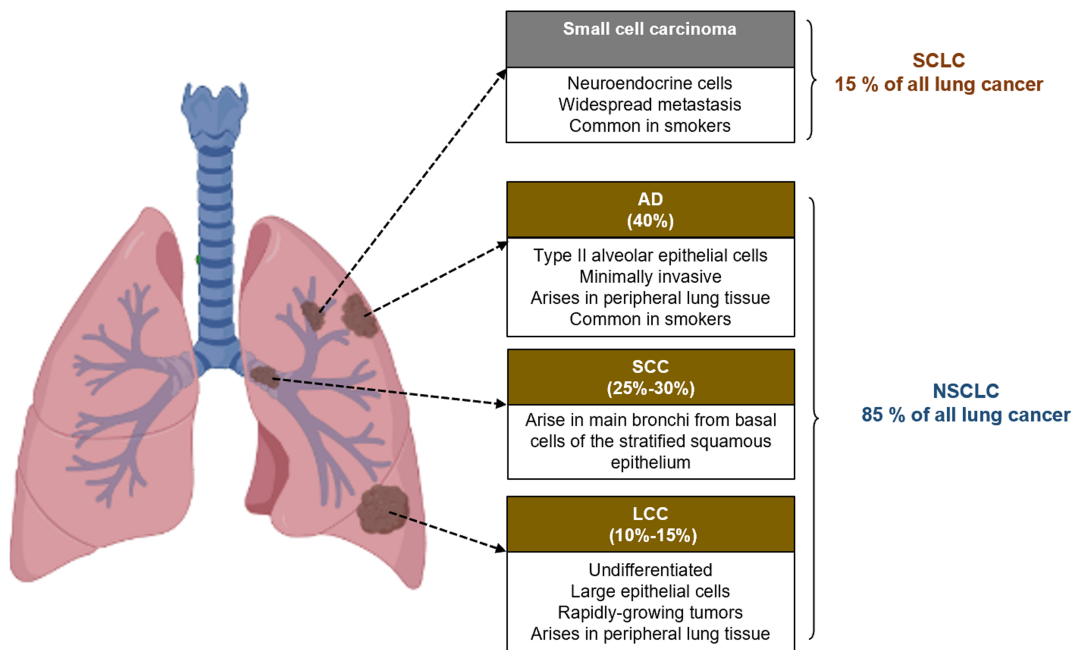
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Figure 1 Classification and main characteristics of lung cancer. Lung cancer is classified into two histological types, small cell lung cancer (SCLC) derived from cells of the neuroendocrine lineage and non-SCLC (NSCLC) derived from epithelial cells. NSCLC is classified into three subtypes: Adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. SCLC: Small cell lung cancer; NSCLC: Non-SCLC; AD: Adenocarcinoma; SCC: Squamous cell carcinoma; LCC: Large cell carcinoma.

The most frequently mutated genes in lung cancer are *epidermal growth factor receptor* (*EGFR*), *Kirsten rat sarcoma virus* (*KRAS*) and *tumor protein 53* (*TP53*)[9,10]. The proteins encoded by these genes participate in signaling pathways that contribute to lung tumorigenesis. Mutations in the *EGFR* gene cause the expression of receptors on the cell membrane that are constitutively activated, triggering signaling pathways within cells involved in lung tumorigenesis. Binding of epidermal growth factor (EGF) to EGFR induces receptor phosphorylation at tyrosine residues and activates multiple downstream signaling pathways, such as the Ras-Raf-MAPK, PI3K, and STAT pathways, which induce proliferation and cell invasion, angiogenesis, inhibition of apoptosis and metastasis. The constitutive activity of EGFR has been observed in more than 60% of patients with NSCLC and is due to different mutations present in the receptor[11]. *KRAS* was first identified as a viral oncogene in the Kirsten RAt Sarcoma virus. The *KRAS* gene encodes the Ras proteins, low molecular weight enzymes with guanosine triphosphate hydrolase (GTPase) activity located on the inside of the plasma membrane. Ras GTPases are activated in response to the binding of ligands to several receptors such as EGF, platelet-derived growth factor, integrin, and cytokine receptors. Mutations in the *KRAS* gene cause loss of the ability of Ras to hydrolyze GTP to guanosine diphosphate leading to constant activation of Ras[12]. Similar to constitutive activation of EGFR, mutated Ras stimulates multiple signaling pathways relevant to tumor development. Mutations in the *KRAS* gene are of high frequency in NSCLC but are also present in other types of cancer such as colorectal and pancreatic cancer[13]. Another protein associated with the onset of lung cancer is the p53 tumor suppressor protein encoded by the *TP53* gene. This tumor suppressor responds to several cellular stresses to regulate expression of target genes, thereby inducing DNA repair or cell cycle arrest and apoptosis[14]. Mutations in the *TP53* gene synthesize a non-functional p53 protein, causing accumulation of damaged DNA in cells and dysregulation of the cell cycle. Mutations in the *KRAS* and *TP53* genes have been found in up to 30% of patients with lung cancer and have been considered as predictors of poor prognosis[9]. Mutations in other genes such as *CDKN2A*, *STK11*, *BRAF*, *PIK3CA*, *RB1*, *PTEN* or *MET* have been associated with the onset of lung cancer, although they showed a lower mutation rate than the *EGFR*, *KRAS* or *TP53* genes. Most of them are involved in the regulation of cell proliferation, differentiation, or apoptosis[9].

Lung tumor tissue is characterized by a heterogeneity of cell types with different phenotypic characteristics and properties as a result of the accumulation of gene mutations and differential cell signaling in the tumor microenvironment. However, it has been shown that only a small fraction of cells in tumor tissue have the ability to form tumor spheroids *in vitro* and develop tumors when they are transplanted into immunodeficient animals. These observations raise the need to study which are the cell populations in the tumor with tumorigenic potential.

ROLE OF STEM CELLS IN TUMORIGENESIS

Cancer stem cells (CSCs) are defined as a subpopulation of tumor cells residing in the tumor with SCs characteristics. CSCs have the ability to self-renew, generate identical daughter cells with stem cell properties, differentiate into multiple cancer cell lineages, facilitate tumor growth and survival, and metastasize to distal sites. In addition, they could be the cause of evasion of the immune system, resistance to chemotherapy and radiation therapy, and cancer relapse[15]. CSCs differ from normal tissue-specific SCs only in their uncontrolled growth and altered genotypes[16].

The seminal proposition that CSCs could be responsible for tumor initiation was introduced by the end of XIX century[17], however the first conclusive evidence for CSCs in acute myeloid leukemia was demonstrated in 1997 by Bonnet and Dick[18]. These authors isolated a subpopulation of CD34+/CD38-leukemic cells capable of initiating tumors in non-obese diabetic/severe combined immunodeficiency disease (NOD/SCID) mice histologically similar to the donor. In recent years, CSCs have been identified in several tumors, including breast, brain, colon, and lung cancer[19].

CSCs are a small proportion of the cell population of a tumor (less than 1%) that exhibit high tumorigenic potential. This is supported by experiments where thousands of tumor cells need to be inoculated into syngeneic or immunodeficient animals for a solid tumor to develop. Some years ago, this requirement was thought to be due to the loss of viability of tumor cells during transfer or the absence of a suitable niche for tumor growth. The new paradigm indicates that only CSCs (a small fraction of the transplanted cells) has the potential to generate a tumor[20]. This hypothesis can be reinforced by the fact that metastases are histologically heterogeneous and similar to the primary tumor, which implies that the cell that gave rise to it has the potential to differentiate into multiple cell types, such as a SCs[19].

Several questions have been raised about the origin of CSCs in tumor tissue. The first hypothesis is that CSCs could originate from tissue-specific normal SCs that have dysregulated cell growth. In this sense, it has been shown that all tissues harbor SCs that play an essential role in tissue repair[16]. These normal SCs could be transformed into CSCs due to the accumulation of gene mutations or the action of chemical mediators released under conditions of tissue damage[21]. This hypothesis is accepted for tissues with a high degree of cell renewal, such as intestinal epithelium or skin, but it is controversial for tissues that are quiescent or that renew slowly, such as lung epithelium. In this sense, some authors have identified a fraction of cells in active division (less than 1.3%) located in specific niches of the tracheal and bronchiolar epithelium with characteristics of SCs that could become CSCs[22,23]. The second hypothesis is that CSCs could originate from more differentiated progenitor cells that have acquired the capacity for self-renewal or differentiated tumor cells (called CSCs-like cells) due to mutations in genes that regulate the cell cycle, hypoxia, or chemical mediators of the tumor microenvironment such as nitric oxide or certain interleukin[24].

STEM CELL MARKERS IN LUNG CANCER

Tumor tissue is heterogeneous, showing different cell populations that have their own signaling pathways, leading to more complex therapeutic strategies. Factors contributing to intratumoral heterogeneity include genetic mutations, epigenetic changes, interactions with the microenvironment and CSCs[25]. CSCs represent only a small group of cells within the heterogeneity of tumor tissue. SCs trigger specific signaling pathways, such as Notch, Hedgehog, and Wnt, that allow them to self-renew and proliferate in tissue repair processes. These pathways are tightly controlled, but their aberrant activation in CSCs can induce the expression of tumor molecular markers, leading to tumorigenicity and chemoresistance[26].

CSCs have been identified in several types of cancer through proteins that are differentially expressed in these cells. These proteins include cell membrane receptors, cell adhesion molecules, cell membrane transporters, enzymes involved in metabolism, and transcription factors. Its are used as biomarkers to predict the prognosis of the disease, evaluate the most appropriate chemotherapy treatments and the efficacy of the therapies in tumor remission[15].

Biomarkers that have been identified in lung cancer include cluster of differentiation-133 (CD133), cluster of differentiation-44 (CD44), epithelial cell adhesion molecule (EpCAM), aldehyde dehydrogenase 1A1 (ALDH1A1), ATP-binding cassette sub-family G member 2 (ABCG2) and the transcription factors octamer-binding transcription factor 4 (Oct-4), sex-determining region Y-box 2 (Sox2), and Nanog[15,26]. Although these biomarkers have different structures and functional roles, its are linked to the SCs properties and uncontrolled proliferation of tumor cells. The biomarkers identified in lung CSCs are shown in Figure 2.

Biomarkers are not specific for lung CSCs and may be expressed in other types of cancer, such as breast, brain, colon or liver cancer. Since a single marker is not sufficient to accurately identify CSCs, combinations of several markers are used to identify and isolate CSCs in tumor tissue[27].
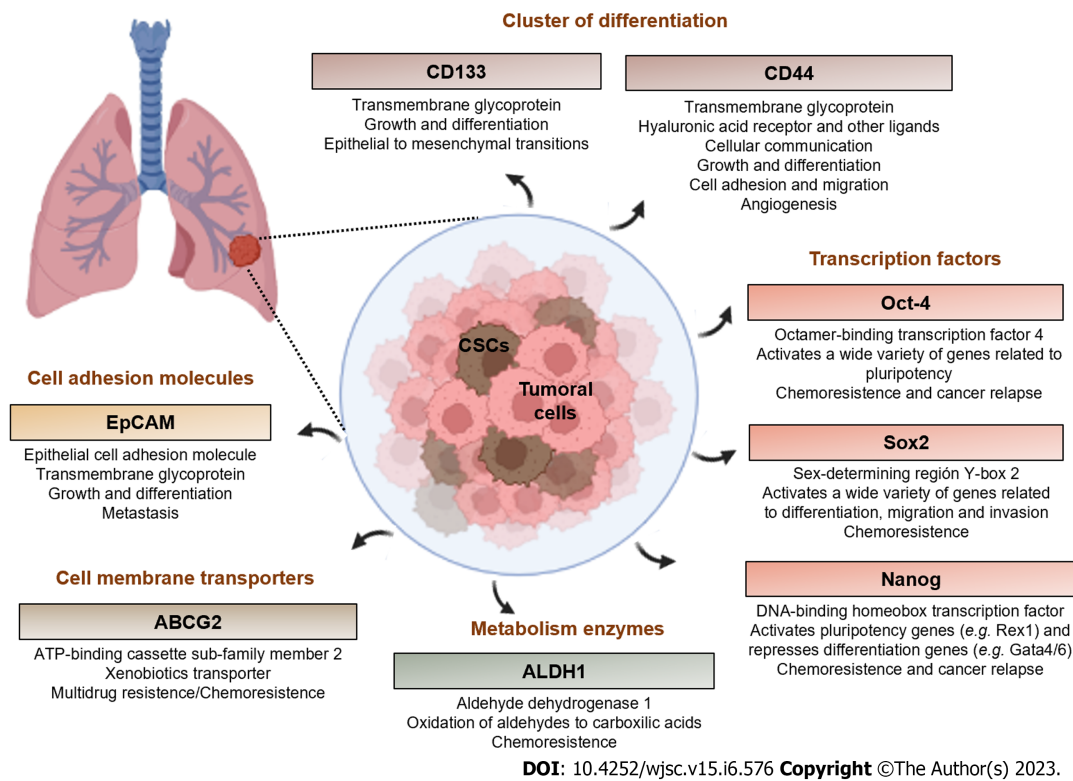
Figure 2 Stem cell biomarkers in lung cancer. Biomarkers identified in cancer stem cells in lung tissue include clusters of differentiation, cell adhesion molecules, cell membrane transporters, enzymes of cell metabolism, and transcription factors. CSCs: Cancer stem cells; ABCG2: ATP-binding cassette subfamily G member 2; ALDH: Aldehyde dehydrogenase.

Cluster of differentiation

CD133: CD133, also known as prominin-1, is a transmembrane glycoprotein involved in cell growth and differentiation through its involvement in multiple signaling pathways[27,28]. It has been identified as the main biomarker of SCs in normal tissue and of stemness in tumor tissue from patients with NSCLC or SCLC.CD133 expression is essential to maintain CSCs characteristics such as tumor cell proliferation, migration, and invasion, and the ability to resist chemotherapy[27].

In lung cancer, the high level of expression of CD133 has been correlated with epithelial to mesenchymal transitions and the formation of metastases in lymph nodes and other tissues, which reveals that CD133 is a biomarker of tumor aggressiveness and poor prognosis of the pathology[29-31]. The molecular mechanisms involved in CD133-mediated cell growth and resistance to chemotherapy are still under study, but appear to be associated with the Wnt/catenin, PI3K-AKT and SRC-FAK signaling pathways[32,33]. Furthermore, CD133 has been shown to directly interact with vascular endothelial growth factor (VEGF), stimulating angiogenesis and leading to tumor growth[34]. CD133 is also used as diagnostic, predictive, or therapeutic biomarkers in other types of cancer including breast, stomach, liver, prostate, colorectal, pancreatic, and renal[28].

CD44: CD44 is a transmembrane glycoprotein of high structural and functional diversity due to alternative splicing processes and post-translational modifications. It can bind to a wide variety of ligands on the cell surface, including hyaluronic acid (HA), and trigger multiple cellular processes such as cell-cell signaling, cell growth and differentiation, cell adhesion and migration, angiogenesis, or cell survival[26].

CD44 regulates several signaling pathways to promote cancer progression, including Notch, Hedgehog, and Wnt pathways[26]. In addition, it can act as a co-receptor and heterodimerizes with growth factor receptors [EGFR, fibroblast growth factor receptor (FGFR), hepatocyte growth factor receptor, VEGF receptor, transforming growth factorβ receptor] and leads to activation of the PI3K-AKT and MAPK pathways[15]. It is hypothesized that CD44 could play an important role in tumorigenesis mediated by the constitutive activation of EGFR, whose expression is frequently mutated in patients with NSCLC. In this last sense, CD44 could be an important marker to predict the efficacy of chemotherapy using EGFR-specific tyrosine kinase inhibitors in patients with lung cancer[15].

CD44 is expressed in almost all tumor types, but it has been identified as the main CSCs biomarker in lung ADs and in SCC. Its expression is associated with a more aggressive tumor phenotype, with the ability to metastasize and resist chemotherapy[35].

Cell adhesion molecules

EpCAM: EpCAM is a transmembrane protein and acts as an adhesion molecule on the lung epithelium. It is a potential biomarker for lung tumors of epithelial origin[36]. EpCAM expression in lung tumor epithelium is upregulated by metastasis-associated protein 1 and leads to increased metastatic capacity of tumor cells[36]. The co-expression of EpCAM with CD44 and CD166 in NSCLC indicates a greater self-renewal capacity, clonal heterogeneity and stemness. These biomarkers in lung tumor tissue indicate a poor prognosis for the disease[37].

Cell membrane transporters

ABCG2: ABCG2 is a transporter of xenobiotic compounds into the extracellular space, which has been implicated in the development of multidrug resistance (MDR) in cancer. The wide variety of ABCG2 substrates includes several antitumoral drugs such as paclitaxel, doxorubicin, cisplatin, topotecan, mitoxantrone and irinotecan. ABCG2 also expels the fluorescent dye Hoechst 33342 out of the cell, a property that is unique to SCs and is used for identification of these cells[38]. The expression of ABCG2 in SCs has been conserved in many tissues, such as the pancreas, lung, heart, testis, liver, brain, prostate, and embryonic tissue, demonstrating the importance of this transporter for cell survival[39]. In lung cancer, increased expression of ABCG2 has been found in SCs, associated with the upregulation and activity of the transcription factors Sp1, Sp3, YAP1 and Nrf2[40-42]. Its expression in lung CSCs is associated with cancer relapse and poor prognosis[42].

Enzymes involved in metabolic pathways

ALDH1: ALDH1 is an enzyme that participates in cellular metabolism through the oxidation of aldehydes to carboxylic acids and is a marker of SCs of normal and tumor tissue[43]. It has three main isotypes, ALDH1A1, ALDH1A2 and ALDH1A3, but its activity is mainly attributed to the ALDH1A1 isotype[43].

Increased expression of ALDH has been found in NSCLC overexpressing a subpopulation of CD44+/EpCAM+ cells[44]. Overexpression of the ALDH1 and CD133 markers was found exclusively in SCC and AD[45]. ALDH1A1 overexpression is associated with an aggressive chemotherapy-resistant tumor phenotype. Despite this, ALDH1 could be a useful therapeutic target for tumors growing in tissues that do not normally express high levels of ALDH1A1, such as the lung, breast, colon, and stomach[43].

Transcription factors

Otherwise, the transcription factors involved in the normal activity of SCs could be deregulated in cancer, and activate cellular pluripotency genes and suppress differentiation genes, triggering signaling pathways responsible for the characteristics of CSCs. Transcription factors that regulate the functions of lung CSCs, such as Oct-4, Sox2, and Nanog, have been identified.

Oct-4: Oct-4 also known as POU5F1, is a member of the POU transcription factor family that contains a binding domain to the ATGCAAAT sequence in target genes[15]. The *Oct-4* gene encodes four protein isoforms called Oct4A, Oct4B-190, Oct4B-265, and Oct4B-164. Of these, Oct4A is known to transcribe a wide variety of genes that regulate SCs stemming. Several Oct-4A target genes have been identified in CSCs of lung cancer, including Fgf4, Utf1, Opn, Rex1/Zfp42, and Fbx15[15].

Oct-4A transcriptional activity is upregulated by post-translational modifications, through phosphorylation at residues Ser229, Ser236 and tyr327 or sumoylation at residue Lys118. Its expression is high in SCs, however Oct-4 is ubiquitinated and degraded by the proteasome in the cell differentiation process[15].

In lung cancer, Oct4 activity is associated with chemotherapy resistance, cancer relapse, and worse outcomes[15].

Sox2: Sox2 is a member of the high mobility group (HMG) box gene family encoded by the sex-determining region Y-box gene. HMG is a sequence of 80 amino acids that acts as a DNA-binding domain in several target genes related to the maintenance of pluripotency in embryonic SCs and CSCs [46]. Sox2 is closely associated with early embryonic development, neuronal differentiation, bronchial morphogenesis, and airway epithelial maturation[46].

In lung cancer, Sox2 can regulate the transcription of the c-MYC, Wnt and Notch oncogenes and increase metastatic capacity through the FGFR-ERK1/2-SOX2 signaling pathway[47]. In SCC, Sox2 can induce expression of tumor-related factors p63 and keratin 6 and lead to cancer differentiation, migration, and invasion. In SCLC, Sox2 is crucial in the PI3K-Akt-Sox2 signaling pathway and may mediate chemoresistance[47]. In addition, Sox2 together with Oct-4 increase its expression under hypoxia in tumor tissue, which induces the expression of the CD133 marker and the self-renewal and maintenance of lung CSCs[48].

Sox2 transcriptional activity can be increased by phosphorylation at amino acid residue Thr118 or methylation at Arg113, which inhibits its proteasome degradation or promotes its homodimerization, respectively[49].

Sox2 and Oct4 have been located in the cell nucleus of SCLC and lung AD, but not in their paracancerous tissues or benign tumor tissues, pointing to the importance of Sox2 and Oct4 as potential markers for cancer therapies[50].

Nanog: Nanog, a DNA-binding homeobox transcription factor, may promote cell proliferation, renewal, and stem properties. Nanog can regulate cell pluripotency through two mechanisms of action. Thus, Nanog can repress the transcription of genes essential for cell differentiation, such as Gata4 and Gata6, or it can activate the transcription of genes necessary for self-renewal, such as Rex1[15].

Nanog expression is upregulated by Nr5a2 promoting CSCs properties and tumorigenesis in NSCLC [51]. Nanog is highly expressed in pluripotent cells and its expression is downregulated during differentiation. Its transcriptional activity is increased by phosphorylation at amino acid residues Ser52, 65 and 71 and Thr287, which abolishes its ubiquitination and degradation by the proteasome[52].

Nanog is increased in many types of carcinomas, including lung cancers, and is associated with chemoresistance, cancer relapse, and poor prognosis[15,52].

STEM CELL MARKERS AS THERAPEUTIC TARGETS FOR THE TREATMENT OF LUNG CANCER

Most clinical trials involve the use of drugs whose targets of action are protein tyrosine kinases, regulators of the cell cycle or cell signaling pathways. Several chemotherapy drugs are not effective in controlling tumor growth and metastases. Thus, novel therapeutic agents directed against CSCs are a hope for patients who do not respond to conventional therapies or who relapse after cancer treatment. These therapies include the use of synthetic or natural inhibitors, monoclonal or bispecific antibodies (BsAb), antibodies-drug conjugates, aptamer-drug conjugates, or chimeric antigen receptor T (CAR-T) therapies. Novel therapies using stem cell biomarkers as pharmacological targets are summarized in Table 1.

Inhibitory drugs

Salazosulfapyridine in combination with cisplatin and pemetrexed is under phase I study for advanced NSCLC (Trial registration-UMIN 000017854). This drug inhibits the intracellular uptake of cysteine by the cysteine-glutamate antiporter in CSCs that overexpress CD44 marker, preventing the synthesis of the antioxidant GSH, essential for cellular redox homeostasis, and leading to the inhibition of tumor growth[53].

Phase II trial using disulfiram in combination with cisplatin plus vinorelbine have shown to be effective for the treatment of metastatic NSCLC (Trial registration-NCT 00312819)[54]. *In vitro* studies in NSCLC cell lines treated with cisplatin have shown the growth of chemoresistant ALDH1+ cell subpopulations. These cells were sensitive to the cytotoxic effects of cisplatin after treatment with diethylaminobenzaldehyde and disulfiram, inhibitors of ALDH1 activity[55]. In xenograft models in NOD/SCID mice, it has been shown that disulfaran inhibits the activity of ALDH1A1 and the expression of Sox2, Oct-4 and Nanog, reducing the size of tumors derived from ALDH+ CSCs and cancer recurrence [56].

ABCG2 transporter is one of the main CSCs markers under study due to its role in MDR. It has been found that the secalonic acid D, a metabolite of marine-derived mangrove endophytic fungus, can down-regulate the expression of ABCG2 by activation of Calpaina 1, a protease that shortens the half-life of the transporter[57]. In addition, Verteporfin, an inhibitor of the YAP1 transcription factor, was found to down-regulate the expression of the ABCG2 transporter in lung CSCs[41].

A-803467, a tetrodotoxin-resistant sodium channel blocker, inhibits ABCG2 transporter activity, increasing the sensitivity to chemotherapeutic drugs such as topotecan, doxorubicin and mitoxantrone in multidrug resistant cells. A combination of A-803467 and ABCG2 substrates may potentially be a novel therapeutic treatment in tumors overexpressing the ABCG2 protein[58].

FL118, a topoisomerase 1 inhibitor similar to camptothecin analogues (such as irinotecan or topotecan) used in the clinic, selectively inhibits the expression of several members of the proapoptotic protein family such as Survivin, Xiap, CIAP2 and BCL-2. It has also been found that FL118 down-regulate the expression of ABCG2, ALDH1A1 and Oct-4. FL118 improves sensitivity to chemotherapy and inhibits the growth of CSCs and metastases. Moreover, FL118 is effective for human tumors that acquire irinotecan, topotecan and cisplatino resistance due to its ability to bypass the drug resistance induced by ABC transporters[59].

Monospecific and Biespecific antibodies

mAbMEM-85 is a monoclonal antibody of therapeutic interest since it inhibits the growth of lung cancer cells in murine models. It has been shown that mAbMEM-85 recognizes the hyaluronate binding site in the C-terminal region of CD44 in lung cancer SCs[60].

Axitinib is a monoclonal antibody that inhibits the activity of the ABCG2 transporter in CSCs, and reverses MDR. Studies in A549 human lung cancer cells and nude mice bearing S1-M1-80 xenografts

Table 1 Potential therapeutic strategies targeting lung cancer stem cell markers

Stem cell marker-target	Therapeutic strategies in clinical trials and preclinical studies
Differentiation cluster	
CD133	Docetaxel-loaded liposomes conjugated to anti-CD133 aptamers
	Gefitinib-loaded nanomicelles conjugated to anti-CD133 aptamers
	Salinomycin sodium-loaded nanoparticles conjugated to anti-CD133 antibody
	CD133-specific CAR-T cells plus anti-PD-1 antibody and a CD73 inhibitor
CD44	Salazosulfapyridine plus cisplatin and pemetrexed (Phase I clinical trial for the treatment of advanced non-squamous NSCLC)
	mAb MEM-85 (monoclonal antibody)
	HA-Cisplatin conjugated
	HA-Irinotecan conjugated (Phase II study for treatment of SCLC)
	HA-Apoferritin conjugated
	HA-conjugated cisplatin-loaded nanoparticles
Cell adhesion molecules	
EpCAM	Doxorubicin-loaded nanoparticles conjugated to EpCAM aptamer
	Catumaxomab BsAb anti-human EpCAM/CD3 T-cell antigen (Phase I study for treatment of NSCLC)
	MT110 BsAb anti-EpCAM/CD3 T-cell antigen (Phase I clinical trial for treatment of lung adenocarcinoma)
	MuS110 BsAb anti-human EpCAM/CD3 T-cell antigen
	2C11x4-7 BsAb anti-murine EpCAM/CD3 T-cell antigen
Cell membrane transporters	
ABCG2	Secalonic acid D
	Axitinib in combination with topotecan or mitoxantrone
	FL118 (topoisomerase 1 inhibitor) in combination with irinotecan, topotecan or cisplatino
	A-803467 (tetrodotoxin-resistant sodium channel blocker) in combination with topotecan, doxorubicin or mitoxantrone
	Verteporfin (YAP1 inhibitor)
Metabolic enzymes	
ALDH	Disulfiram in combination with cisplatin plus vinorelbine (Phase II clinical trial for the treatment of NSCLC)
	Disulfiram alone or in combination with diethylaminobenzaldehyde and cisplatin
	FL118 in combination withirinotecan, topotecan or cisplatino
Transcription factors and signaling pathway	
Oct-4	FL118 in combination with irinotecan, topotecan or cisplatino
Notch-signaling pathway	BsAb-5 directed against c-MET and CTLA-4 in CD166+ NSCLC

mAb: Monoclonal antibody; HA: Hyaluronic acid; BsAb: Bispecific antibody; CAR-T: Chimeric antigen receptor T; c-MET: Cellular mesenchymal-to-epithelial transition factor; CTLA-4: Cytotoxic T-lymphocyte-associated protein 4; NSCLC: Non-small cell lung cancer; ABCG2: ATP-binding cassette subfamily G member 2; CD133: Cluster of differentiation-133; CD44: Cluster of differentiation-44; EpCAM: Epithelial cell adhesion molecule; ALDH: Aldehyde dehydrogenase; Oct-4: Octamer-binding transcription factor 4.

that overexpress ABCG2 have shown that axitinib increases tumor cell apoptosis induced by chemotherapeutic drugs such as topotecan and mitoxantrone without causing additional toxicity[61].

BsAb target two different epitopes or antigens simultaneously. One of the Fab fragments recognizes epitopes on cytotoxic drugs or CD8+ T or NK immune cells, while the other Fab fragment can bind epitopes on CSCs. Then, BsAbs can selectively direct effector cells or chemotherapeutic drugs towards CSCs, promoting their destruction. BsAb have been extensively explored in translational and clinical studies in lung cancer.

Catumaxomab is a BsAb with binding sites directed to human EpCAM and CD3 T-cell antigen. A phase I study has shown that catumaxomab is safe and tolerable when administered intravenously in patients with NSCLC[62]. Other anti-CD3-EpCAM BsAbs have been studied in immunocompetent mice bearing lung tumors, showing potent inhibition of local and disseminated tumor growth[63]. MT110 BsAb targeting EpCAM/CD3 T-cell antigen has been tested in patients with colorectal, gastric, and lung cancer[64]. MuS110 BsAb was found to have similar *in vitro* characteristics and *in vivo* antitumor activity as MT110[65].

BsAb-5 target cellular mesenchymal-to-epithelial transition factor (c-MET) and cytotoxic T-lymphocyte-associated protein 4 in CD166+ lung CSCs with high affinity and specificity. BsAb-5 has been shown to reduce tumor size in mouse models by a mechanism that involves inhibition of the c-MET-Notch pathway in CSCs and the up-regulating effector T cells. BsAb could be a possible drug for the treatment of human NSCLC[66].

Drugs conjugated to specific ligands of CSCs

The extracellular domain of CD44 contains a HA binding site, a property that is used for the development of HA-conjugated antitumor drugs. Preclinical studies have shown that HA can be effectively used to deliver chemotherapy and selectively decrease CD44+ lung CSCs. Thus, a phase II clinical trial (ACTRN 12611000520932) using HA-irinotecan has shown to be effective in the treatment of SCLC[67]. Other studies show that cisplatin or apoferritin conjugated to HA are effective in eliminating lung CSCs[68,69].

Drugs-loaded nanoparticles

In recent years, nanoparticulate systems have been developed to encapsulate antitumor drugs. The efficacy of these systems in the treatment of cancer has been improved by the conjugation of the nanoparticles to specific ligands, antibodies or aptamers directed against SC markers. In this sense, it has been shown that the intratracheal administration of HA-conjugated cisplatin-loaded nanoparticles attenuated lung cancer growth in mice[70]. Other systems such as salinomycin sodium-loaded nanoparticles conjugated to anti-CD133 antibody, doxorubicin-loaded nanoparticles conjugated to EpCAM aptamer and gefitinib or docetaxel-loaded liposomes conjugated to anti-CD133 aptamers have been shown to be effective in inhibiting tumor growth[71-74].

CAR-T therapies

CAR-T cell is a new therapeutic approach that involves the development of a receptor expressed in T cells that recognizes certain specific antigens on the membrane of cancer cells, triggering an antitumor immune response. The efficacy of specific CAR-T therapy against the AC133 epitope of CD133 has been studied in a mouse model with orthotopic xenograft. AC133-specific CAR-T cells reduced tumor size and prolonged survival in the humanized orthotopic SCLC model but were unable to eliminate tumors completely. However, the combination of AC133-specific CAR-T cells with an anti-PD-1 antibody and a CD73 inhibitor was able to eliminate chemoresistant CSCs[75].

CONCLUSION

Current chemotherapies for lung cancer involve the use of drugs whose targets of action are protein tyrosine kinases, regulators of the cell cycle or cell signaling pathways. Despite improvements in treatments, some patients do not respond to therapies or have cancer relapses months or years after treatment. It is hypothesized that one of the main causes of cancer relapse is the ineffectiveness of anticancer drugs to eliminate CSCs in tumor tissue. Chemotherapy and radiotherapy induce senescence of tumor cells. Factors released by senescent cells into the tumor microenvironment could activate signaling pathways that induce phenotypic and functional changes in SCs, increasing their plasticity and uncontrolled growth. In addition, CSCs could be chemoresistant due to the expression of transporters in cell membranes that expel xenobiotic compounds into the extracellular space.

In lung cancer, several biomarkers have been identified in CSCs associated with the maintenance of tumorigenicity, metastasis and chemoresistance. These biomarkers could be useful as targets for the effective treatment of lung cancer. Thus, new drugs directed against CSCs include the use of inhibitors, monoclonal and BsAb, antibody-drug conjugates or aptamer-drug conjugates, and CAR-T therapies. These therapies are a hope for patients who do not respond to conventional treatments or relapse in lung cancer.

FOOTNOTES

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

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