Journal of Bioanalysis & Biomedicine

Hamilton and Olszewski, J Bioanal Biomed 2013, S9
DOI: 10.4172/1948-593X.S9-003

Review Article Open Access

Chemotherapy-induced Enrichment of Cancer Stem Cells in Lung Cancer

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Abstract

Small cell lung cancer (SCLC) is a neuroendocrine tumor of the lung that is clinically characterized by early recurrence after initial complete response to combination chemotherapy, resulting in dismal prognosis. Cancer stem cells (CSCs) are suspected to survive the initial cycles of treatment and trigger relapse in form of a chemoresistant tumor. Whereas a profound amount of results reporting on CSCs exists for non-small cell lung cancers (NSCLC), far less data which are restricted to a few cancer cell lines and CD133-positive subpopulations of fresh tumor tissues are available for SCLC. Enrichment of CSCs in response to chemoradiotherapy and in recurrent tumors would prove their clinical relevance. The GLC14, GLC16 and GLC19 series of SCLC cell lines established from serial biopsies of one patient before, during and after therapy, respectively, provides an opportunity to investigate the phenotypical changes during chemoradiotherapy. We demonstrated by whole genome expression analysis that CSC markers such as CD44, CD133, CD47, ALDH1A1, AKR1C and members of the WNT and Notch pathways are increasingly expressed after chemoradiotherapy in GLC16 and GLC19, compared to the treatment-naïve GLC14 SCLC cells. These findings in addition to the available literature suggest a role for CSCs in SCLC in tumor recurrence and in the resistant phenotype. Thus, targeting of CSCs may constitute a promising approach to delay or prevent recurrences that would eventually result in treatment failure and poor survival rates in SCLC patients.

Keywords: Small-cell lung cancer; Chemotherapy; Cancer stem cells; Microarray; Chemoresistance

Small Cell Lung Cancer

Lung cancer is the leading cause of cancer death due to inadequate therapeutic options, thus equaling morbidity with mortality rates and causing an unsolved health problem [1,2]. Among the neuroendocrine tumors that account for approximately 20% of lung cancers, SCLC comprises the majority of cases [3]. This highly malignant tumor originates from neuroendocrine cells (Amine Precursor Uptake and Decarboxylation/APUD cells) in the bronchus called Feyrter cells and in consequence of its rapid growth, early dissemination and progression to drug resistance after successful first-line therapy treatment of SCLC has limited efficacy [4-7]. Lung cancer is the most preventable cancer worldwide owing to the fact that the predominant risk factor is tobacco consumption and women are increasingly affected [8]. Non-neuroendocrine lung tumors which are subsumed as non-SCLC (NSCLC), including adenocarcinoma, squamous cell carcinoma and large cell carcinoma, differ by a lower growth fraction and aggressiveness from SCLC. Without treatment, SCLC has an aggressive clinical course, with median survival times from diagnosis of only several months [7]. Because the majority of patients with SCLC present with disseminated disease, localized therapy is not possible in most cases. The current chemotherapy regimens prolong survival, nevertheless, the overall survival at 5 years is only 5 to 10% [5-7]. Patients with SCLC confined to the hemithorax of origin, the mediastinum, or the supraclavicular lymph nodes are classified as having limited-stage disease with an expected median survival of 16 to 24 months and the other patients with extensive-stage disease have a median survival of only 6 to 12 months [5-7].

In patients with limited-stage SCLC, combination chemotherapy produces results that are clearly superior to single-agent treatment with overall objective response rates of 65 to 90% and complete response rates ranging from 45 to 75%. The combination of etoposide and cisplatin/carboplatin chemotherapy with concurrent chest irradiation is standard and achieves median survivals of 18 to 24 months and 40% to 50% 2-year survival with less than a 3% treatment-related mortality [5-7]. The prognosis for patients with relapsing SCLC is exceedingly poor, with expected median survival between 2 to 3 months. Topotecan is the single approved chemotherapeutic drug for second line treatment of SCLC prolonging live for several months [9]. In summary, the treatment of SCLC is characterized by a lack of progress and extremely short survival rates in advanced disease, that have been not seen much improvement in the past decades. There are many proposals as to why this may be, including the presence of resistant CSCs, accelerated development of acquired resistance, redundancy in cell survival/signaling pathways, etc., but the underlying cause remains unclear so far.

Cancer Stem Cells Concept

According to a popular newer concept, CSCs are a rare population of undifferentiated cells driving tumor initiation, maintenance and spreading [10,11]. This contrasts or expands the former model of cancer development as a stochastic process with outgrowth of well-adapted tumor cell clones. CSCs display unlimited proliferation potential, ability to self-renew and capacity to generate a progeny of differentiated cells, recapitulating the major tumor populations. CSC-like cells have been characterized from leukemia, and a range of solid tumors, including melanoma, glioblastoma as well as breast, prostate, pancreatic, and colon carcinomas [10-12]. These cells can be expanded *in vitro* as tumor spheres, i.e. mamma- and neurospheres, and produce

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Received March 30, 2013; **Accepted** June 04, 2013; **Published** June 08, 2013

Citation: Hamilton G, Olszewski U (2013) Chemotherapy-induced Enrichment of Cancer Stem Cells in Lung Cancer. J Bioanal Biomed S9: 003. doi:10.4172/1948-593X.S9-003

original tumors in immunodeficient mice at lower cell numbers than the unfractionated tumor cell population. Brain, hematopoietic, prostate and colon cancer CSCs feature the membrane antigen CD133, whose expression is shared by normal stem cells of different lineages [13]. In a combined model of complex tumor development integrating the stochastic and CSC model, genetically distinct CSCs exist on top of each heterogenous tumor subclones [11]. Most therapies are directed at the bulk of rapidly dividing tumor cells but not the slow dividing CSCs. CSCs are resistant to both radiation and chemotherapy, and, therefore, this subpopulation persists following therapy and seems to be responsible for relapses which occur in some cases after an extended period of dormancy of cells residing in a CSC niche [14,15]. Resistance to therapy is believed to be a complex, multistep process that begins with the short-term, inherent plasticity of subpopulations of cancer cells. The cytotoxic stimuli resulting from inhibiting a target can result in enhanced expression/activity of stemness factors in persisting subpopulations of cells [16]. Expression of these stemness factors results in genetic plasticity that allows these cells to remain in a dormant, drug-tolerant state. Since the role of CSCs is still not firmly established, these cells are also alluded to as tumor initiating cells [TICs], cancer initiating cells [CICs] and tumor propagating cells [TPCs]. Recently, independent studies using lineage-tracing technique in mouse models have corroborated the existence of CSCs in brain, skin and intestinal tumors [17,18]. Eradicating CSCs, in addition or instead of the fast growing tumor mass seems to constitute a promising approach to achieve a long-lasting response and thereby to improve cancer therapy.

Although work and publications on CSCs mounted in recent years, a stringent definition of CSCs in solid tumors and specific markers for CSCs in distinct types of tumors are still lacking that some phenotypic and functional features of CSCs can reversibly turn on and off constituting a transient state rather than a hierarchically determined characteristic of rare CSCs [20]. CSCs may be defined by their high tumorigenicity *in vivo*, but in practice they are usually detected by the presence of combinations of various antigens such as CD24, CD44, CD133, EpCAM, CD166, Lgr5, CD47, and ALDH activity in various malignant tumors [10,20]. In the CSC model, targeting embryonic pathways like Wnt, Hedgehog and Notch by smoothened inhibitors, gamma-secretase inhibitors, anti-DLL4 antagonists, Wnt antagonists, and CBP/β-catenin inhibitors have shown promising anticancer effects in early studies [21].

Although a large body of evidence is in favor of the CSC concept, several aspects of its foundations were questioned. For example, the proof and enumeration of CSCs in xenograft transplantation experiments is subject to the degree of immune system incompetence of the host and appropriate microenvironmental conditions [19,22]. While distinct surface antigens selectively expressed on CSCs are used to isolate these cells, no marker or pattern of makers are known to prospectively identify CSCs in many tumor types. One common characteristic of CSCs from different tumors is the capacity to extrude dyes in anABCG2 /BCRP1-dependent manner, defining a small so-called side population (SP) of cells [23]. Although these SP cells share characteristics of CSCs, pure populations were difficult to obtain and non-SP cells were found to comprise CSCs.

Neuroendocrine tumors and CSCs

CSCs were described for a wide range of tumor entities and neuroendocrine tumors being no exception. For example, cells with CSC features were detected in carcinoids, neuroendocrine tumors of the pancreas and medullary thyroid carcinoma [MTC]. Metastatic gastrointestinal neuroendocrine tumors (NETs) are frequently are refractory to chemotherapy [24]. By using the Aldefluor assay (Stem Cell Technologies, Grenoble, France), $ALDH^+$ cells were found to comprise approximately 6% of cells in samples of 19 patients. The $ALDH^+$ cells formed spheres in anchorage-independent conditions and initiated tumors in *in vivo* assays, whereas $ALDH^-$ cells did not. Moreover, $ALDH^+$ cells demonstrated increased expression of activated Src, Erk, Akt, and mammalian target of rapamycin (mTOR) compared with non-CSCs. Selective expression of the CSC marker CD44 in neuroendocrine tumor cells of prostate cancer, in combination with their other known features, further supports the significance of such cells in therapy resistance and tumor recurrence [25].

MTC is a cancer of the parafollicular C cells of the thyroid commonly caused by an inherited or acquired mutation of the RET proto-oncogene. Therapeutic resistance and frequent recurrence of the disease imply the presence of CSCs in MTC and actual CD133 positivity was identified by immunostaining patient MTCs and in two MTC cell lines [26]. The $CD133^+$cells could be expanded by sphere formation assay, passaged multiple times, and expressed neural progenitor markers β-tubulin-3 and glial fibrillary acidic protein. These data support the existence of CSC-like cells in MTC, which exhibit the features of self-renewal and of multiple lineage differentiation that is dependent on ret proto-oncogene receptor activity.

Accumulating evidence point to the occurrence of CSCs in neuroendocrine lung tumors and this data on SP cells, CD133-, CD166-, CD44- and ALDH1-expressing cell subpopulations, as discussed in the following sections, are beginning to clarify the true phenotype of the lung CSCs [21].

Lung Cancer and CSCs

In comparison to the rapidly advancing research on breast and colon cancer CSCs, investigations on this cell subpopulation in the area of lung cancer is lagging behind.Identifying these stem cells in lung cancer and finding ways for their elimination may lead to new clinical approaches to delay or prevent disease recurrence [27-30].

The tumorigenic cells in SCLC and NSCLC were first characterized as a rare population of undifferentiated cells expressing CD133, a marker of normal and CSCs of the hematopoietic, neural, endothelial and epithelial lineages [31]. Lung cancer $CD133^+$ cells were able to grow indefinitely as tumor spheres in serum-free medium containing epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF). The injection of a low number of lung cancer $CD133^+$ cells in immune-compromised mice generated xenografts matching the original tumor. Upon differentiation, lung cancer $CD133^+$ cells acquired the specific lineage markers and lost CD133 expression as well as tumorigenicity. Furthermore, a panel of 15 primary lung cancer cell lines representing SCLC, large cell, squamous cell carcinoma and adenocarcinoma, comprised a small population of cells strongly positive for CD44 with coexpression of CD90 in the spheroid-forming cell fraction [32]. These $CD44^+CD90^+$ cells revealed increased expression of mesenchymalembryonic stem cell-related markers and resistance to irradiation, indicative of CSCs, in case of SCLC and large cell carcinoma. The description of different stem cell markers for the same tumor entities demonstrate that this subject is not settled so far for lung cancer.

NSCLC and CSCs

CD44, CD133 and CD90 have been widely accepted for isolation of CSCs in human hematological malignancies as well as in solid tumors [32]. The most prominent CSC marker found in NSCLC lung cancer tissues is CD133, commonly associated with the maintenance, metastasis and drug-resistance of tumor cells [33,34]. The different approaches to identify CSCs in NSCLC are discussed below.

Sphere formation of NSCLC CSCs: Sphere formation in tissue culture is particularly useful to enrich the potential CSC subpopulations when specific CSC makers have not been defined [35,36]. To obtain tumor cell spheres, culture conditions have to be adjusted to serum-free media, supplemented with several growth factors, such as EGF, bFGF, leukemia inhibitory factor (LIF) and various other additives, upon which the spheroid growth of subpopulations of the tumor cell lines which display gene expression profiles consistent with CSCs is favored. Furthermore, sphere cells exhibit increased expression of CSC surface markers and oncogenes compared to adherent cultures and are associated with enhanced tumorigenicity and chemoresistance.

In a study by Eramo et al. 0.6 to 22.0% of SCLC and NSCLC tumor cells were capable of forming spheres with varying frequency of $CD133^+$expression [32]. Lung cancer $CD133^+$ cells were able to grow indefinitely as tumor spheres in serum-free stem cell selection media. These $CD133^+$ lung cancer sphere showed expression of stemness genes such as Oct-4 and NANOG, self-renewal potential, chemotherapy resistance, and ability to recapitulate tumor heterogeneity *in vivo*. Sphere growth from 10 NSCLC patient samples and five NSCLC lung cancer cell lines sorted for $CD133^+$ expression displayed higher Oct-4 expression and the multiple drug-resistant marker ABCG2 in conjunction with significant resistance to chemotherapy agents (i.e., cisplatin, etoposide, doxorubicin, and paclitaxel) and radiotherapy [37]. The treatment of Oct-4 siRNAcould specifically block the capability of $CD133^+$ to form spheres and enhanced response to chemoradiotherapy. Sphere growth was obtained for 11 out of 15 lung adenocarcinoma malignant pleural effusion patient samples in another study [38]. Compared to adherent cells, these sphere cells were associated with enhanced ALDH1 activity and Oct-4, Nanog, Notch3, and Stat3 mRNA expression. In summary, according to their sphere-forming capability and specific markers, NSCLC specimens contain a subpopulation of cells with CSC characteristics. The NCI-H1915 large cell NSCLC line forms typical CSC tumor spheres and recapitulates original tumor histologies in brain xenotransplantation models [39]. Furthermore, brain metastases of patients also expressed CD15 and CD133, markers suggestive of a stem-like population.

Chemotherapeutic selection of NSCLC CSCs: According to the CSC hypothesis, chemotherapy preferentially targets the rapidly dividing mass of differentiated cells in tumors and spares the slowly growing, chemoresistent CSC cells. Vice versa it should be possible to enrich CSC cells *in vitro* by cultivation of cell lines in the presence of chemotherapeutics. This was demonstrated in drug-selected cells (doxorubicin, cisplatin, or etoposide) of the human lung cancer cell line H460, which displayed spheroid formation, stem cell characteristics, self-renewal capacity and ability to differentiate, as well as high tumorigenic and metastatic potential [40]. Inherent and acquired cisplatin resistance reduces the effectiveness of this agent in the treatment of NSCLC. An isogenic model of cisplatin resistance was generated in a panel of NSCLC cell lines (A549, SKMES-1, MOR, H460) by selecting cisplatin resistant sublines over a period of twelve months [41]. Resistant cells accumulated in the G_0/G_1 phase of the cell cycle and showed enhanced clonogenic survival. Moreover, these cells displayed a CSC-like signature with increased expression of $CD133^+$/$CD44^+$ Nanog, Oct-4 and SOX-2 markers, increased ALDH activity and upregulation of the epithelial-mesenchymal transition (EMT) markers, namely c-Met and β-catenin. Concordantly, these resistant sublines demonstrated decreased uptake of cisplatin and reduced cisplatin-GpG DNA adduct formation compared to the parental cell lines. In a similar experimental approach, cisplatin treatment of lung cancer cells *in vitro* resulted in enrichment of the $CD133^+$cell fraction, both after acute cytotoxic exposure and in cells with a stable cisplatin-resistant phenotype [42]. Subpopulations of $CD133^+ABCG2^+$ and $CD133^+CXCR4^+$ cells were spared by *in vivo* cisplatin treatment of lung tumor xenografts established from primary tumors.

Cell surface markers of NSCLC CSCs: $CD133^+$ cells sorted from patient lung cancer tissue samples,displayed enhanced expression of Oct-4, enhanced self-renewal ability, increased expression of ABCG2, enhanced resistance to chemoradiotherapy, increased invasiveness and tumorigenicity, as well as increased spheroid formation compared to $CD133^-$ cells [37]. In a similar study, Bertolini et al. reported enhanced tumorigenic potential *in vivo*, elevated expression of ABCG2, CXCR4, ITG6A, Oct-4, and NANOG as well as cisplatin resistance *in vitro* and *in vivo* of $CD133^+$ NSCLC cells [42]. The $CD133^+$and epithelial-specific antigen-positive ($CD133^+ESA^+$) subpopulation is increased in primary NSCLC compared with normal lung tissue. It is dinstinguished by expression of stemness genes, adhesion, higher motility and drug efflux than the $CD133^-$ counterpart and has higher tumorigenic potential in immune-compromised mice [42]. Accordingly, a tendency toward shorter progression-free survival was observed in $CD133^+$ NSCLC patients treated with platinum-containing regimens. In tumor specimen of 45 patients with respectable NSCLC, expression of CD133 showed a higher correlation with deceased patients and a strong significant association with patients exhibiting progressive disease [43]. As further CSC marker, expression of CD44 on 62-96% of tumor cells was described for 6 out of 10 human NSCLC lines. Compared to $CD44^-$ cells, $CD44^+$ cells displayed spheroid formation, resistance to cisplatin treatment *in vitro*, enhanced tumorigenicity *in vivo*, and elevated expression of stemness genes Oct-4, NANOG, and SOX2 [44].

Cellular activities of NSCLC CSCs: CSC cells may be detected as side cell (SP) subpopulation by assessment of the Hoechst 33342 efflux which is mainly effected by the ATP-cassette-binding ABCG2 [23]. Dye efflux assays were employed to isolate SP cells were from six human NSCLC cell lines (H460, H23, HTB-58, A549, H441, and H2170) [45]. Xenograft experiments in immune-compromised mice showed that these SP cells were enriched in tumor-initiating and disseminating capability, displayed elevated expression of ATP-binding cassette transporters (ABCG2) and chemoresistance. In this case, unlimited proliferative potential was conferred by high expression of human telomerase reverse transcriptase (hTERT) and the G_0 quiescent state indicated by a lower expression of minichromosome maintenance (MCM)-7 protein. In another study, sixteen clinical lung cancer samples also displayed a smaller but persistent SP population [46]. However, here expression of CD133 was restricted to the SCLC line H446 in contrast to the NSCLC lines A549, H157, H226, Calu-1 and H292, as identified by real-time PCR and fluorescence-activated

cell sorting after chemotherapeutic drug selection. The sorted $CD133^+$ H446 SCLC subset exhibited self-renewal, differentiation, proliferation and tumorigenic capacity in subsequent assays.

The measurement of ALDH1 activity and expression represents a universal marker for the identification and isolation of CSCs from multiple sources [47]. Functional tests for this marker CSCs rely on the fluorescent ALDH1 substrate Aldefluor followed by fluorescence-activated cell sorter analysis [48]. In human lung cancer cell lines ALDH1 activity was associated with self-renewal and differentiation, resistance to chemotherapy, expression of CD133 and enhanced tumorigenicity, as well as ability to recapitulate the original tumor heterogeneity *in vivo* [49]. From 303 clinical patient specimens and controls, overexpression of ALDH1 was positively correlated with stage and grade of the tumor and associated with poorer prognosis for patients with early-stage lung cancer.

SCLC and CSCs

For the characterization of putative CSCs in SCLC only a limited number of studies have become available so far [50].

Sphere formation of SCLC CSCs

Stem-like cells were enriched from the SCLC cell line H446 by growing them as spheres in a defined serum-free medium [51]. These cells showed increased *in vitro* clonogenic and *in vivo* tumorigenic potential as well as drug resistance and contained a higher proportion of cells expressing the stem cell surface markers CD133 and urokinase-type plasminogen activator receptor (uPAR), when compared with unfractionated cells. However, both $CD133^+$ and $CD133^-$ cell fractions were capable of forming spheres, whereas only cells derived from the $uPAR^+$ fraction exhibit this capability. Moreover, formation of transplantable tumors was restricted to $uPAR^+$ cells which could also differentiate into $CD56^+$cells, CK^+(cytokeratin-positive) cells, and $uPAR^-$ cells. These data indicated the existence of a population of tumor sphere-forming cells with CSC properties in the H446 SCLC cell line. A similar enrichment of SCLC stem-like cells by isolation of sphere-forming cells from SCLC cell lines was applied for screening differentially expressed miRNAs [52]. Of the five downregulated miRNAs (let-7, miR-20, 21, 27a and 30b), miR-27a was found to enhance the stem-like properties of SCLC cells *in vitro* and to constitute critical factor in maintaining a stem cell function in SCLC.

Chemoresistance of selected SCLC cell populations

The relationship between expression of the CSC markers CD133 and uPAR/CD87 was investigated in six SCLC cell lines [53]. The SBC-7 cell line showed the highest expression levels of both CD133 and CD87 and was used to isolate distinct cellular subsets. Both $CD133^+$/$CD87^-$ and $CD133^-$/$CD87^+$ subpopulations showed a higher resistance to etoposide and paclitaxel as well as greater repopulating ability than the $CD133^-$/$CD87^-$ subpopulation. $CD133^+$/$CD87^-$ cells contained more G_0 quiescent cells than $CD133^-$/$CD87^-$ cells, although $CD133^-$/$CD87^-$ cells showed the highest tumorigenic potential.

Cell surface markers of SCLC CSCs

SCLC and NSCLC with neuroendocrine features express achaete-scute complex homologue 1 (ASCL1), where the factor may play a role in the primitive neuroendocrine phenotype of these tumors. Knockdown of ASCL1 in cultured SCLC resulted in inhibition of soft agar clonogenic capacity and induction of apoptosis [54]. Two stem cell markers, namely CD133 and ALDH1A1, are directly regulated by ASCL1 in SCLC. In SCLC xenografts, a relatively abundant CD133 (high)-ASCL1 (high)-ALDH1(high) subpopulation is associated with markedly enhanced tumorigenicity compared with cells with weak CD133 expression. This finding suggests that a broad range of SCLC cells has tumorigenic capacity rather than a small discrete population. The urokinase plasminogen activator (uPA) and its receptor uPAR/CD87 are major regulators of extracellular matrix degradation and are involved in cell migration and invasion. The uPAR-positive cells in six SCLC lines demonstrated multidrug resistance, high clonogenic activity and coexpression of CD44 and MDR1, putative cancer stem cell markers [55].

Cellular activities of SCLC CSCs

SP cells in SCLC cell line H446 account for approximately 5% of the whole cell population, have a stronger capability of forming tumor spheres and exhibit increased mRNA expression levels of ABCG2, CD133, and nucleostemin [56]. *In vivo*, SP cells showed better proliferative ability, tumorigenesis and higher drug resistance as well as differentiation into non-SP cells. Rare SP cells (< 1%) with high proliferative capacity *in vitro*, efficient self-renewal and reduced cell surface expression of the neuronal differentiation markers, CD56 and CD90, were described for several SCLC cell lines [50]. As few as 50 SP cells from H146 and H526 SCLC cell lines rapidly reconstituted tumors in mice and these cells overexpressed many genes associated with CSCs, such as ABCG2, FGF1, IGF1, MYC, SOX1/2, WNT1, as well as genes involved in drug resistance, angiogenesis, Notch and Hedgehog pathways.

The expression of ALDH1A1 and ALDH3A1 was investigated in patients with lung cancers: squamous cell cancer, adenocarcinoma, and SCLC [57]. These aldehyde dehydrogenases were significantly expressed in NSCLC and at lower levels in SCLC. Atypical pneumocytes demonstrated significantly higher levels of expression than normal pneumocytes, pointing to an upregulation during malignant transformation. Further characterization of the $ALDH^+$cells is not available so far.

GLC14/16/19 series of SCLC cell lines and CSC markers

Longitudinal biopsies, viable cells or cell lines collected before and during chemotherapy are rarely available for SCLC patients. Due to the aggressive clinical course and disseminated disease at presentation, these patients rarely undergo tumor resection and the diagnosis is performed thin needle biopsy specimen, leaving hardly tissue samples for further investigations [58]. However, one series of three biopsies that were used to constitute the corresponding cell lines GLC14, GLC16 and GLC19, were described for a single SCLC patient by Berendsen et al. in 1988 [59]. In detail, the GLC14 cell line was established using a biopsy from a right supraclavicular node of this 55-yr-old woman revealing SCLC extended disease. Treatment was started with cyclophosphamide, doxorubicin and etoposide (CDE), resulting in complete response after five cycles. Prophylactic cranial irradiation (10 times, 30 Gy) was given. After three months the chest X-ray showed SCLC recurrence which was treated by reinduction chemotherapy with CDE. After four cycles of chemotherapy a partial response was observed and the GLC16 cell line established from a biopsy specimen of the remaining tumor. After further radiotherapy the chest X-ray normalized. However, the tumor

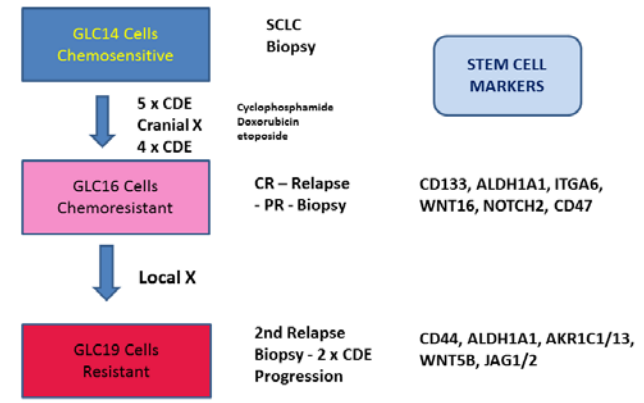

Figure 1: Schematic representation of the isolation of the GLC14/16/19 series of SCLC cell lines and CSC marker expression. GLC14 cell line was established from the first biopsy upon diagnosis, GLC16 line following radiochemotherapy (CDE – cyclophosphamide/doxorubicin/ etoposide chemotherapy; X – radiation therapy; CR – complete response; PR – partial response) and GLC19 line after local radiation therapy and before progression and termination of therapy. Stem cell markers exhibiting increased expression at the transcript level relatively to GLC14 cells are shown at the right side of the figure for GLC16 and GLC19 cells, respectively.

recurred short-term and while the GLC19 cell line was established from a biopsy at this time the patient died with progressing tumor 17 month after first presentation. These GLC cell lines were reported to have retained the morphological, biochemical, and immunohistological characteristics of the biopsy specimens. The parent tumor progressed from an initially chemosensitive state (GLC14) to one exhibiting increased drug resistance for doxorubicin, etoposide, melphalan, and vinblastine *in vitro* (GLC16 and GLC19) which allows for a gene expression analysis of the molecular changes acquired during relapse and eventual treatment failure [59].

A comparative analysis of the overexpressed gene transcripts using GLC14 versus GLC16 and GLC19 cells was performed employing the Applied Biosystems Human Genome Survey Microarray V2.0 (Figure 1). Upregulated molecular pathways in the GLC16/19 cells compared to the GLC14 cells are expected to be associated with tumor progression and chemoresistance in this case of SCLC. In GLC16 cells, transcripts for the stem cell markers CD133, ALDH1A1, WNT16, NOTCH2 and CD47 were significantly upregulated (> 2.5fold) compared to chemonaĭve GLC16 cells. Furthermore, increased expression of c-KIT (CD117), fibroblast growth factor receptor 1 (FGFR1) and integrin alpha-6(ITGA6) seems to supplement this cancer stem cell phenotype. CD133 and ALDH1A1 constitute classical cancer stem cell markers found expressed in a wide range of critical subpopulations of solid tumors [27]. The other markers are characteristics of CSC-related, tumor-initiating (TIC) or chemotherapy-induced cell populations. A chromosomal aberration frequently found in lung cancer affects the 1p region and comprises alterations in genes belonging to the Wnt or the Notch developmental pathways, particularly NOTCH2 [60]. Furthermore, during the search for CSC-related gene expression in residual tumor cells of neoadjuvant-treated gastric cancer patients immunohistochemical analysis demonstrated a chemotherapy-associated increase in the intensity of NOTCH2 staining [61]. Engagement of CD47 is associated with an induction of downstream signaling via G-proteins, leading to the activation of the PI3K/Akt survival pathway [20]. For the case of invasive bladder cancer, a subpopulation of $CD44^+CK5^+CK20^-$ cells was identified as the TIC subpopulation, distinguished by expression of CD47. Moreover, high tumorigenic and metastatic properties of lung NSCLC CSCs are associated with expression of the stem cell factor (SCF) and its receptor c-kit (CD117) play likewise an important role in survival and proliferation of lung CSCs [28]. A highly tumorigenic $ITGA6^+$ subpopulation of cancer cells was recently been identified in primary and metastatic breast cancer samples [62]. This subpopulation is capable of growth as spherical organoids, displays resistance to proapoptotic agents and high tumorigenicity in immunodeficient mice depending on the expression of this antigen. In summary, the GLC16 cell line obtained after CDE chemotherapy exhibits expression of classical CSC markers and of a number of additional antigens which are involved in tumor initiation and dissemination. Consistent with the exposure to cyclophosphamide GLC16 cells express high levels of DNA repair protein O^6-methylguanine-DNA-methyltransferase (MGMT) transcript, known to be involved in chemotherapy resistance, particularly resistance of in $CD133^+$ glioblastomamultiforme stem cells to the alkylating agent temozolomide [63].

Similarly, genome-wide gene expression of GLC19 cells was compared to GLC14 cells [64]. Putative classical markers of cancer stem cells overexpressed in GLC19 cells included CD44, ALDH1A1 and the aldoketoreductase family 1, members C1/13 (AKR1C1 and AKR1C13). Again, several mediators upregulated in GLC19 cells belong to the Wnt and Notch signaling pathways. They comprise secreted frizzled-related protein 1 (SFRP1), WNT5B, coactivator mastermind-like 2 (MAML2), transducin-like enhancer of split 2 (E(sp1)) homolog (TLE2) and Notch receptor ligands jagged 1/2 (JAG1 and JAG2). GLC19 cells are distinguished from GLC14 by a reduced growth rate, expression of CSC markers, increased expression of components of the Wnt and Notch signalling pathway, as well as receptors for the growth factors FGFs and EGF. Thus, GLC19 reveals overexpression of CSC markers which are only partially overlapping with those found for GLC16 cells, most likely due to the different therapy regimen used in this later phase of treatment.

Conclusion

The aggressive course of SCLC and the fast development of recurrent and resistant disease may be associated with the presence of tumor cells with CSC-like characteristics. Lung CSCs could derive from bronchioalveolar stem cells (BASCs) and alveolar epithelial type II cells (AEC2), as well as from cells which express the CSC marker glycoprotein CD133 or markers for SPs [29]. Normal stem cells that have undergone mutational events or transit-amplifying or differentiated cells subjected to dedifferentiation could be the source of CSCs [10]. Activation of pathways that normally regulate embryonic lung development and injury repair, including the Wnt, Hedgehog and Notch pathways, has also been identified in lung tumor cells [11,30]. Among the most significantly mutated genes in SCLC, SOX2 amplification was detectable in 27% of the samples [65]. Expression of SOX2 is linked to stem cells, self-renewal, and embryonic development and together with Oct-4, Klf4 and c-Myc, is sufficient to artificially reprogram normal cells into stem-like cells [66].

The cell surface marker CD133 has previously been identified as a reliable marker for CSCs in some of tumor entities [34,37]. However,

no mechanism has so far been proposed to link CD133 expression with the CSC phenotype [13]. The reliability of this marker as a CSC marker has recently been disputed [67]. In case of colon cancer CSCs, CD133 expression was found not to be restricted to intestinal stem or cancer-initiating cells, and that during the metastatic transition $CD133^+$ tumor cells might give rise to a more aggressive $CD133^-$ subset [68]. In NSCLC, both $CD133^+$ and $CD133^-$ subpopulations were described to comprise CSCs [69]. It has been reported that CD133 may play a role in response to hypoxia, cell cycle regulation and proliferation of cells but not necessarily tumor initiation [33,67]. Independently of its role as marker of CSCs, immunohistochemical assessment of 161 NSCLCs surgically resected revealed CD133 expression was correlated with tumor stage and predictive of unfavorable prognosis in patients with stages II-IV NSCLC [70]. In contrast, characterization of the chemoresistant residual NSCLC tumor cells which cause tumor regrowth, so-called tumor reinitiating cells (TRICs), were studied in xenograft and genetically engineered mouse models, revealing no consistent enrichment of CSC marker-positive cells, but expression of EMT characteristics [34,71]. Furthermore, the NCI-H446 cell line used in characterization of CSCs in an SCLC cell line actually represents biochemical and morphological variant of SCLC with an atypical morphology.

Genome-wide expression analysis of the GLC14/16/19 series of SCLC revealed the increased expression of CSC features after chemoradiotherapy. Although at this time chemotherapy consisted of cyclophosphamide, doxorubicin and etoposide instead of the current standard therapy combining cisplatin/carboplatin and etoposide, these findings are expected to apply for both protocols. Upregulation of the transcription for CD133, ALDH1A1, WNT16, NOTCH2 and CD47 in the GLC16 cells are consistent with a regrowth of CSC-like populations in this early relapsing SCLC. Interestingly, after partial response to chemotherapy, the remaining tumor cells (GLC19) exhibited a different CSC-like gene expression pattern including CD44, ALDH1A1 and AKR1C1/13 in combination with several mediators belonging to the WNT and Notch signaling pathways. These results demonstrate a chemoradiotherapy-triggered increase in CSC markers in tumor cell populations of this SCLC patient, suggesting a role for this kind of tumor cells in resistance and recurrence *in vivo*. An increase in the CSCs population after primary systemic therapy was found to present a poor prognostic factor in breast cancer [72]. Thus, recurrent SCLC seems to express the same markers, namely CD133, CD44, and ALDH1 that were published to predictrelapse of lung adenocarcinoma (Hazard Ratio> 3.6) [73].

The CSC model also would offer an explanation for relapses of SCLC after a complete response to initial therapeutic interventions, and cancer therapy failures with a wide range of new therapeutics tested clinically [74-76]. CSC markers in SCLC seem to match those observed in other solid tumors and, therefore, the drugs showing preferential activity against CSCs of other tumors should be tested preclinically for the elimination of these resistant cells in SCLC to open the way to tackle the refractoriness of this tumor entity by radically different approaches [77,78]. In terms of the rapid recurrence of SCLC after primary therapy, this tumor entity would be ideally suited to test clinically the efficacy of CSC-directed therapies in combination with the elimination of the tumor bulk using conventional chemotherapy.

Conflict of Interest Disclosure and Acknowledgment

The author has no conflict of interest. This work was supported in part by a grant from the “Medical Scientific Fund of the Mayor of the City of Vienna“, project number 11016. We wish to thank the team of the “Oncology Lab” (Prof. Dr. R. Zeillinger) of the University Clinic of Gynecology, Medical University of Vienna, Austria, for help with the microarray experiments.

References

1. Siegel R, Naishadham D, Jemal A (2012) Cancer statistics, 2012. CA Cancer J Clin 62: 10-29.
2. Kalemkerian GP, Akerley W, Bogner P, Borghaei H, Chow LQ, et al. (2013) Small cell lung cancer. J Natl Compr Canc Netw 11: 78-98.
3. Travis WD (2010) Advances in neuroendocrine lung tumors. Ann Oncol 21 Suppl 7: vii65-71.
4. Modlin IM, Champaneria MC, Bornschein J, Kidd M (2006) Evolution of the diffuse neuroendocrine system--clear cells and cloudy origins. Neuroendocrinology 84: 69-82.
5. Murray N, Turrisi AT 3rd (2006) A review of first-line treatment for small-cell lung cancer. J ThoracOncol 1: 270-278.
6. Hann CL, Rudin CM (2008) Management of small-cell lung cancer: incremental changes but hope for the future. Oncology (Williston Park) 22: 1486-1492.
7. Califano R, Abidin AZ, Peck R, Faivre-Finn C, Lorigan P (2012) Management of small cell lung cancer: recent developments for optimal care. Drugs 72: 471-490.
8. Thun MJ, Carter BD, Feskanich D, Freedman ND, Prentice R, et al. (2013) 50-year trends in smoking-related mortality in the United States. N Engl J Med 368: 351-364.
9. Spigel DR (2012) Treatment update in small-cell lung cancer: from limited to extensive disease. Curr Treat Options Oncol 13: 505-515.
10. Bohl SR, Pircher A, Hilbe W (2011) Cancer stem cells: characteristics and their potential role for new therapeutic strategies. Onkologie 34: 269-274.
11. Baccelli I, Trumpp A (2012) The evolving concept of cancer and metastasis stem cells. J Cell Biol 198: 281-293.
12. Hu Y, Fu L (2012) Targeting cancer stem cells: a new therapy to cure cancer patients. Am J Cancer Res 2: 340-356.
13. Grosse-Gehling P, Fargeas CA, Dittfeld C, Garbe Y, Alison MR, et al. (2013) CD133 as a biomarker for putative cancer stem cells in solid tumours: limitations, problems and challenges. J Pathol 229: 355-378.
14. Dean M, Fojo T, Bates S (2005) Tumour stem cells and drug resistance. Nat Rev Cancer 5: 275-284.
15. Malik B, Nie D (2012) Cancer stem cells and resistance to chemo and radio therapy. Front Biosci (Elite Ed) 4: 2142-2149.
16. Basile KJ, Aplin AE (2012) Resistance to chemotherapy: short-term drug tolerance and stem cell-like subpopulations. Adv Pharmacol 65: 315-334.
17. Driessens G, Beck B, Caauwe A, Simons BD, Blanpain C (2012) Defining the mode of tumour growth by clonal analysis. Nature 488: 527-530.
18. Schepers AG, Snippert HJ, Stange DE, van den Born M, van Es JH, et al. (2012) Lineage tracing reveals Lgr5+ stem cell activity in mouse intestinal adenomas. Science 337: 730-735.
19. Welte Y, Adjaye J, Lehrach HR, Regenbrecht CR (2010) Cancer stem cells in solid tumors: elusive or illusive? Cell Commun Signal 8: 6.
20. Gires O (2011) Lessons from common markers of tumor-initiating cells in solid cancers. Cell Mol Life Sci 68: 4009-4022.
21. Quintana E, Shackleton M, Foster HR, Fullen DR, Sabel MS, et al. (2010) Phenotypic heterogeneity among tumorigenic melanoma cells from patients that is reversible and not hierarchically organized. Cancer Cell 18: 510-523.
22. O'Flaherty JD, Barr M, Fennell D, Richard D, Reynolds J, et al. (2012) The cancer stem-cell hypothesis: its emerging role in lung cancer biology and its relevance for future therapy. J ThoracOncol 7: 1880-1890.
23. Chang JT, Mani SA (2013) Sheep, wolf, or werewolf: Cancer stem cells and the epithelial-to-mesenchymal transition. Cancer Lett.

24. Wu C, Alman BA (2008) Side population cells in human cancers. Cancer Lett 268: 1-9.

25. Gaur P, Sceusi EL, Samuel S, Xia L, Fan F, et al. (2011) Identification of cancer stem cells in human gastrointestinal carcinoid and neuroendocrine tumors. Gastroenterology 141: 1728-1737.

26. Palapattu GS, Wu C, Silvers CR, Martin HB, Williams K, et al. (2009) Selective expression of CD44, a putative prostate cancer stem cell marker, in neuroendocrine tumor cells of human prostate cancer. Prostate 69: 787-798.

27. Zhu W, Hai T, Ye L, Cote GJ (2010) Medullary thyroid carcinoma cell lines contain a self-renewing CD133+ population that is dependent on ret proto-oncogene activity. J Clin Endocrinol Metab 95: 439-444.

28. Peacock CD, Watkins DN (2008) Cancer stem cells and the ontogeny of lung cancer. J Clin Oncol 26: 2883-2889.

29. Gorelik E, Lokshin A, Levina V (2010) Lung cancer stem cells as a target for therapy. Anticancer Agents Med Chem 10: 164-171.

30. Lundin A, Driscoll B (2012) Lung cancer stem cells: Progress and prospects. Cancer Lett S0304-3835: 00485-5.

31. Morrison BJ, Morris JC, Steel JC (2013) Lung cancer-initiating cells: a novel target for cancer therapy. Target Oncol .

32. Eramo A, Lotti F, Sette G, Pilozzi E, Biffoni M, et al. (2008) Identification and expansion of the tumorigenic lung cancer stem cell population. Cell Death Differ 15: 504-514.

33. Wang P, Gao Q, Suo Z, Munthe E, Solberg S, et al. (2013) Identification and characterization of cells with cancer stem cell properties in human primary lung cancer cell lines. PLoS One 8: e57020.

34. Wu Y, Wu PY (2009) CD133 as a marker for cancer stem cells: progresses and concerns. Stem Cells Dev 18: 1127-1134.

35. Wang S, Xu ZY, Wang LF, Su W (2013) CD133+ cancer stem cells in lung cancer. Front Biosci 18: 447-453.

36. Rappa G, Mercapide J, Anzanello F, Prasmickaite L, Xi Y, et al. (2008) Growth of cancer cell lines under stem cell-like conditions has the potential to unveil therapeutic targets. Exp Cell Res 314: 2110-2122.

37. Morrison BJ, Steel JC, Morris JC (2012) Sphere culture of murine lung cancer cell lines are enriched with cancer initiating cells. PLoS One 7: e49752.

38. Chen YC, Hsu HS, Chen YW, Tsai TH, How CK, et al. (2008) Oct-4 expression maintained cancer stem-like properties in lung cancer-derived CD133-positive cells. PLoS One 3: e2637.

39. Mancini R, Giarnieri E, De Vitis C, Malanga D, Roscilli G, et al. (2011) Spheres derived from lung adenocarcinoma pleural effusions: molecular characterization and tumor engraftment. PLoS One 6: e21320.

40. Nolte SM, Venugopal C, McFarlane N, Morozova O, Hallett RM, et al. (2013) A cancer stem cell model for studying brain metastases from primary lung cancer. J Natl Cancer Inst 105: 551-562.

41. Levina V, Marrangoni AM, DeMarco R, Gorelik E, Lokshin AE (2008) Drug-selected human lung cancer stem cells: cytokine network, tumorigenic and metastatic properties. PLoS One 3: e3077.

42. Barr MP, Gray SG, Hoffmann AC, Hilger RA, Thomale J, et al. (2013) Generation and characterisation of cisplatin-resistant non-small cell lung cancer cell lines displaying a stem-like signature. PLoS One 8: e54193.

43. Bertolini G, Roz L, Perego P, Tortoreto M, Fontanella E, et al. (2009) Highly tumorigenic lung cancer CD133+ cells display stem-like features and are spared by cisplatin treatment. ProcNatlAcadSci U S A 106: 16281-16286.

44. Pirozzi G, Tirino V, Camerlingo R, La Rocca A, Martucci N, et al. (2013) Prognostic value of cancer stem cells, epithelial-mesenchymal transition and circulating tumor cells in lung cancer. Oncol Rep 29: 1763-1768.

45. Leung EL, Fiscus RR, Tung JW, Tin VP, Cheng LC, et al. (2010) Non-small cell lung cancer cells expressing CD44 are enriched for stem cell-like properties. PLoS One 5: e14062.

46. Ho MM, Ng AV, Lam S, Hung JY (2007) Side population in human lung cancer cell lines and tumors is enriched with stem-like cancer cells. Cancer Res 67: 4827-4833.

47. Cui F, Wang J, Chen D, Chen YJ (2011) CD133 is a temporary marker of cancer stem cells in small cell lung cancer, but not in non-small cell lung cancer. Oncol Rep 25: 701-708.

48. Douville J, Beaulieu R, Balicki D (2009) ALDH1 as a functional marker of cancer stem and progenitor cells. Stem Cells Dev 18: 17-25.

49. Alison MR, Guppy NJ, Lim SM, Nicholson LJ (2010) Finding cancer stem cells: are aldehyde dehydrogenases fit for purpose? J Pathol 222: 335-344.

50. Jiang F, Qiu Q, Khanna A, Todd NW, Deepak J, et al. (2009) Aldehyde dehydrogenase 1 is a tumor stem cell-associated marker in lung cancer. Mol Cancer Res 7: 330-338.

51. Salcido CD, Larochelle A, Taylor BJ, Dunbar CE, Varticovski L (2010) Molecular characterisation of side population cells with cancer stem cell-like characteristics in small-cell lung cancer. Br J Cancer 102: 1636-1644.

52. Qiu X, Wang Z, Li Y, Miao Y, Ren Y, et al. (2012) Characterization of sphere-forming cells with stem-like properties from the small cell lung cancer cell line H446. Cancer Lett 323: 161-170.

53. Miao Y, Li J, Qiu X, Li Y, Wang Z, et al. (2013) miR-27a regulates the self renewal of the H446 small cell lung cancer cell line in vitro. Oncol Rep 29: 161-168.

54. Kubo T, Takigawa N, Osawa M, Harada D, Ninomiya T, et al. (2013) Subpopulation of small-cell lung cancer cells expressing CD133 and CD87 show resistance to chemotherapy. Cancer Sci 104: 78-84.

55. Jiang T, Collins BJ, Jin N, Watkins DN, Brock MV, et al. (2009) Achaete-scute complex homologue 1 regulates tumor-initiating capacity in human small cell lung cancer. Cancer Res 69: 845-854.

56. Gutova M, Najbauer J, Gevorgyan A, Metz MZ, Weng Y, et al. (2007) Identification of uPAR-positive chemoresistant cells in small cell lung cancer. PLoS One 2: e243.

57. Wang B, Yang H, Huang YZ, Yan RH, Liu FJ, et al. (2010) Biologic characteristics of the side population of human small cell lung cancer cell line H446. Chin J Cancer 29: 254-260.

58. Patel M, Lu L, Zander DS, Sreerama L, Coco D, et al. (2008) ALDH1A1 and ALDH3A1 expression in lung cancers: correlation with histologic type and potential precursors. Lung Cancer 59: 340-349.

59. Berendsen HH, de Leij L, de Vries EG, Mesander G, Mulder NH, et al. (1988) Characterization of three small cell lung cancer cell lines established from one patient during longitudinal follow-up. Cancer Res 48: 6891-6899.

60. de Vries EG, Meijer C, Timmer-Bosscha H, Berendsen HH, de Leij L, et al. (1989) Resistance mechanisms in three human small cell lung cancer cell lines established from one patient during clinical follow-up. Cancer Res 49: 4175-4178.

61. Garnis C, Campbell J, Davies JJ, Macaulay C, Lam S, et al. (2005) Involvement of multiple developmental genes on chromosome 1p in lung tumorigenesis. Hum Mol Genet 14: 475-482.

62. Bauer L, Langer R, Becker K, Hapfelmeier A, Ott K, et al. (2012) Expression profiling of stem cell-related genes in neoadjuvant-treated gastric cancer: a NOTCH2, GSK3B and Î²-catenin gene signature predicts survival. PLoS One 7: e44566.

63. Cariati M, Naderi A, Brown JP, Smalley MJ, Pinder SE, et al. (2008) Alpha-6 integrin is necessary for the tumourigenicity of a stem cell-like subpopulation within the MCF7 breast cancer cell line. Int J Cancer 122: 298-304.

64. Pistollato F, Abbadi S, Rampazzo E, Persano L, Della Puppa A, et al. (2010) Intratumoral hypoxic gradient drives stem cells distribution and MGMT expression in glioblastoma. Stem Cells 28: 851-862.

65. Hamilton G, Ulsperger E, Geissler K, Olszewski U (2012) Therapy-Induced changes of gene expression in a matched pair of small cell lung cancer (SCLC) cell lines. J Cancer Ther 3: 442-451.

66. Rudin CM, Durinck S, Stawiski EW, Poirier JT, Modrusan Z, et al. (2012)

Comprehensive genomic analysis identifies SOX2 as a frequently amplified gene in small-cell lung cancer. Nat Genet 44: 1111-1116.

67. Bernhardt M, Galach M, Novak D, Utikal J (2012) Mediators of induced pluripotency and their role in cancer cells - current scientific knowledge and future perspectives. Biotechnol J 7: 810-821.

68. Donovan LK, Pilkington GJ (2012) CD133: holy of grail of neuro-oncology or promiscuous red-herring? Cell Prolif 45: 527-537.

69. Shmelkov SV, Butler JM, Hooper AT, Hormigo A, Kushner J, et al. (2008) CD133 expression is not restricted to stem cells, and both CD133+ and CD133- metastatic colon cancer cells initiate tumors. J Clin Invest 118: 2111-2120.

70. Meng X, Li M, Wang X, Wang Y, Ma D (2009) Both CD133+ and CD133- subpopulations of A549 and H446 cells contain cancer-initiating cells. Cancer Sci 100: 1040-1046.

71. Mizugaki H, Sakakibara-Konishi J, Kikuchi J, Moriya J, Hatanaka KC, et al. (2013) CD133 expression: a potential prognostic marker for non-small cell lung cancers. Int J Clin Oncol.

72. Hegde GV, de la Cruz C, Eastham-Anderson J, Zheng Y, Sweet-Cordero EA, et al. (2012) Residual tumor cells that drive disease relapse after chemotherapy do not have enhanced tumor initiating capacity. PLoS One 7: e45647.

73. Lee HE, Kim JH, Kim YJ, Choi SY, Kim SW, et al. (2011) An increase in cancer stem cell population after primary systemic therapy is a poor prognostic factor in breast cancer. Br J Cancer 104: 1730-1738.

74. Okudela K, Woo T, Mitsui H, Tajiri M, Masuda M, et al. (2012) Expression of the potential cancer stem cell markers, CD133, CD44, ALDH1, and Î²-catenin, in primary lung adenocarcinoma--their prognostic significance. Pathol Int 62: 792-801.

75. Tirino V, Desiderio V, Paino F, De Rosa A, Papaccio F, et al. (2013) Cancer stem cells in solid tumors: an overview and new approaches for their isolation and characterization. FASEB J 27: 13-24.

76. Demedts IK, Vermaelen KY, van Meerbeeck JP (2010) Treatment of extensive-stage small cell lung carcinoma: current status and future prospects. Eur Respir J 35: 202-215.

77. William WN Jr, Glisson BS (2011) Novel strategies for the treatment of small-cell lung carcinoma. Nat Rev Clin Oncol 8: 611-619.

78. Salama R, Tang J, Gadgeel SM, Ahmad A (2012) Lung cancer stem cells: current progress and future perspectives. J Stem Cell Res Ther S7: 007.

This article was originally published in a special issue, **Cancer Stem Cells** handled by Editor(s). Dr. Daotai Nie, Southern Illinois University, USA