Chapter 8
Lung Cancer Stem Cells

Gavitt A. Woodard and David M. Jablons

Abstract Lung cancer remains the leading cause of cancer mortality and novel therapies are desperately needed to treat metastatic and recurrent disease. The cancer stem cell hypothesis is based on data that within each tumor there is a small sub-population of cancer stem cells that display the stem cell properties of self-renewal, pluripotency, a high proliferative capacity, and the ability to resist chemotherapy and radiation. These cancer stem cells are a likely cause of tumor resurgence after initial response to treatment and are an important therapeutic target. Distinct populations of epithelial cells in the airway and lung have been identified as the cells of origin for the major types of lung cancer: adenocarcinoma, squamous cell cancer, and small cell lung cancer. As we develop new therapies that target these cancer stem cell populations important work is underway to identify reliable cancer stem cell markers and to better understand the major pathways that fuel cancer stem cells. There is great interest in developing antibodies and small molecule inhibitors to the Wnt, Sonic Hedgehog, and Notch pathways to target cancer stem cells in lung and other malignancies, and multiple new drugs are in various stages of clinical trials. Lung cancer stem cells are a promising therapeutic target and important work remains to be done to better understand the role that lung cancer stem cells play in tumor development and recurrence.

Keywords Lung cancer • Lung cancer stem cells • Surface markers • Wnt • Sonic Hedgehog • Notch

1 Introduction: The Cancer Stem Cell Model in Lung Cancer

Lung cancer remains a highly aggressive cancer and is the leading cause of cancer-related mortality in the United States and worldwide with an overall 5 year survival rate of 19.3 % (Howlader et al. 2013). Only 20 % of lung cancer patients are

G.A. Woodard • D.M. Jablons (✉)
Department of Surgery, University of California at San Francisco, San Francisco, CA, USA
e-mail: david.jablons@ucsfmedctr.org

S. Babashah (ed.), *Cancer Stem Cells: Emerging Concepts and Future Perspectives in Translational Oncology*, DOI 10.1007/978-3-319-21030-8_8

surgical candidates at the time of presentation and 30–50 % of these early-stage tumors will recur following a complete surgical resection (Kelsey et al. 2009). Once patients have developed metastatic disease, only 15 % will be alive after 1 year and there are virtually no long term survivors (Groome et al. 2007). This highlights the importance of developing treatment strategies that target the mechanisms leading to tumor invasion and metastasis. For patients with more advanced disease, platinum based chemotherapy, targeted kinase inhibitors, and radiation can result in dramatic responses; however, almost all of these tumors recur within 2–3 years (Lin et al. 2014).

A stem cell is defined by high proliferative capacity, ability for self-renewal, and multipotency in producing daughter cells of varying types. The cancer stem cell (CSC) model is based on clinical and experimental data that a subpopulation of tumor cells displays stem cell like properties including the capacity for self-renewal, differentiation, and the ability to resist cell death from chemotherapy and radiation. Not all cancer cells posses these traits, nor do all cancer cells have the ability to generate a metastasis or a new tumor as cancer cells may be found circulating in the blood of patients who do not always develop metastases (Reya et al. 2001). CSCs have the unique ability to support new growth in xenograft models, whereas other cell populations from the same tumor are unable to repopulate a tumor in the same growth environment. The CSC model has mounting evidence in hematologic malignancies and solid tumors. CSC were first demonstrated in lung cancer in 1982 by Carney et al. who showed that a subpopulation of cells from adenocarcinoma and small cell lung cancer (SCLC) had stem cell-like properties, were able to form colonies in agar, and grow new tumors in athymic nude mice (Carney et al. 1982).

The CSCs give rise to highly proliferative progenitor cells which produce the differentiated cells that define the histologic type of lung cancer. Standard chemotherapy and radiation target the more rapidly dividing differentiated cells and can melt away the bulk of a tumor. However the CSCs divide at a lower rate and have additional mechanisms to resist chemotherapy. Treatment may result in a significant reduction in tumor bulk, but the remaining small number of CSCs has the capacity to eventually repopulate the tumor. Developing therapies that target these CSCs is crucial in preventing cancer recurrence.

2 Pulmonary Histology

In the tracheal and bronchial epithelia, endogenous stem cells in make up just 0.06–1.3 % of all proliferating cells, a smaller number than in the epithelia of the gut or skin where there is a much higher rate of cell turnover and epithelial repopulation (Snyder et al. 2009). The relative quiescence of pulmonary stem cells has made them more challenging to identify and isolate than stem cells in other tissues. Research using xenograft models of airway injury has identified cells throughout the airway that are responsible for repairing epithelial damage. By further investigating these cells, distinct cell populations in the lung have been identified that

display the properties of self-renewal, proliferation, and multipotency that define a stem cell.

2.1 Lung Development

Lung development in humans begins during the fourth week of embryogenesis with structures arising from the laryngotracheal groove. The trachea splits ventrally from the foregut by forming tracheoseophageal ridges which then fuse to create the tracheoseophageal septum. Caudal to this process the lung bud appears and divides into a right and left bronchial bud. In the fifth week the right and left primary bronchial buds divide into secondary buds, which will ultimately form the five lobes of the lungs, and then into tertiary buds which are the basis for the 19 lung segments in the fully developed lung. From this point through the sixteenth week, the lung will continue to grow and develop its major anatomic structures. Alveoli do not begin to form until after the 16th week when the bronchi become well vascularized and enlarged. The terminal airway structures continue to grow and mature until the 26th week of embryogenesis when the blood-air barrier is created. The alveoli saccules develop at the end of each terminal bronchiole and specialized alveolar cells for gas exchange and surfactant production develop (Schoenwolf et al. 2009).

The stem cell pathways Wingless type (Wnt), Sonic Hedgehog (Shh), and Notch, which will be discussed later, play important highly conserved roles in embryogenesis and in maintaining endogenous lung stem cells. During gestation Wnt regulates lung epithelial and mesenchymal development (Morrisey 2003). Mice knockouts have shown that specifically *Wnt-2*, *Wnt-5a*, and *Wnt-7b* are crucial for proper lung maturation (Yamaguchi et al. 1999; Shu et al. 2002). The critical role of the Shh pathway in lung development in mice has been extensively studied, but comparatively less is known about its role in human lung development. Zhang et al. (2012) demonstrated Shh in human lung development has many similarities with murine lung development. Shh is expressed in the developing lung epithelium, as are the Shh receptors Ptch1 and Smo and the Shh signaling effectors Gli1, Gli2, and Gli3. Notch signaling, which plays a role in cell fate decisions, is present early in development at the time of the lung epithelial buds (Tsao et al. 2008). These pathways are integral to normal lung development, cell maintenance, and injury repair, and represent important potential lung CSC targets.

2.2 Cell Diversity

The lung epithelium has many important functions including warming inspired air, performing gas exchange, and defending against pathogens. The epithelium is exposed to a number of insults during normal respiration. The crucial role of maintaining the integrity of the epithelium is performed by airway stem or progenitor

cells. The epithelium in the proximal portion of the upper airways and trachea is a pseudostratified epithelium consisting of ciliated, Clara, and goblet cells. More distally in the smaller airways and bronchioles the epithelial cells become more columnar. There is an increased number of Clara cells, with basal cells and rare neuroendocrine cells found at intervals between the columnar cells.

Within the terminal alveoli, the pneumocytes that comprise the alveolar wall are the alveolar type 1 (AT1) cells, alveolar type 2 (AT2) cells, and macrophages. The squamous AT1 cells are responsible for the structure of the alveolar wall and provide the surface for gas exchange. Cuboidal AT2 cells produce surfactant, a phospholipid and protein mixture which lowers the surface tension and facilitates gas exchange. These AT2 cells are responsible for repairing and repopulating the AT1 cells after injury (Desai et al. 2014).

2.3 Injury Response

During lung development, epithelial branching leads to distinct functional zones along the airway. Within each zone there is a unique cellular composition and set of local progenitor stem cells responsible for repopulating each area. These regional stem cells reside in discrete areas known as the stem cell niche within each portion of the airway. Recognizing these populations is important for CSC research as these endogenous stem cells may be the cells of origin in many cancers and there is insight to be gained from the pathways and mechanisms that confer stem cell properties.

Identifying the cells and stem cell niche in the lung has posed a challenge as the respiratory tract undergoes relatively slower cell turnover rates compared with other systems like the gastrointestinal tract and skin. Therefore the endogenous stem cells populations in the adult lung have been identified via a series of mouse injury models. In the trachea and upper airways the submucosal glands harbor the basal cells which repair and repopulate damaged tissue. A subpopulation of basal cells has been shown to behave like stem cells in response to injury. Borthwick et al. (2001) used the cell surface marker keratin-5 to label for a pluripotent population of tracheal basal cells. Later, Rock et al. (2009) confirmed this finding by labeling keratin-5 and using lineage tracing to demonstrate subsequently labeled Clara and ciliated cell populations in a steady state of airway maintenance, demonstrating that the basilar cells have an important role in tracheal and upper airway maintenance and repair. In addition there was an increase in the number of labeled cells following airway damage, suggesting that the basal cells were the progenitors of cells used for airway repair. In contrast, ciliated cells have been shown to be terminally differentiated and unable to self-renew (Rawlins et al. 2007). In addition to keratin-5, human lung basal cells can be purified using the surface markers ITGA6 and NGFR, and those purified cells are capable of self-renewal and generating luminal daughter cells in vitro (Rock et al. 2009). Collectively, these data suggest that basal cells act as the stem cell of the proximal upper airway.

More distally, the stem cell niches are the branch points of the smaller airways and the bronchoalvoelar duct junctions (BADJs) where the bronchi become alveoli. To identify the stem cells of the BADJ, many experiments have been performed to elucidate which cells display stem cell-like properties. Marked neuroepithelial bodies (NEBs) are found in the BADJ. The NEB consists of two cell types: "variant" Clara cells which express Clara cell secretory protein (CCSP) and pulmonary neuroendocrine cells which are marked by calcitonin gene-related peptide (CGRP) (Giangreco et al. 2007). Cells that express both surface markers CCSP and CGRP proliferate within the NEB during embryogenesis, airway maintenance, and repair. The "variant" Clara cells, defined by the surface protein CCSP, were identified by Reynolds et al. (2000) by exposing mice to naphthalene, a chemical that causes selective Clara cell death. In the presence of naphthalene Clara cells were destroyed, however "variant" Clara cells showed up-regulated activity and were able to subsequently repair airway damage (Giangreco et al. 2002).

AT2 cells produce surfactant and can therefore be recognized by secretory vesicles that containing surfactant protein C (SP-C) (Desai et al. 2014). At the BADJ, Giangreco et al. (2002) identified a population of bronchioalveolar stem cells which mark positive for CCSP and SP-C. These CCSP+ SP-C+ cells were shown to be the predominant proliferative cell population following bronchiolar damage and had the ability to differentiate into Clara, AT1, and AT2 cells. In addition, the CCSP+ cells at the BADJ retained their function even outside of the NEB microenvironment. There are likely multiple possible progenitor cell populations at the BADJ. Other interesting data has shown that SP-C negative cells are capable of regenerating AT2 cells following injury, indicating another potential alveolar progenitor stem cell population (Chapman et al. 2011).

Unfortunately, not all evidence on CCSP+ cells has been consistent. CCSP+ labeled populations have been shown to repopulate damaged AT2 cells following influenza or bleomycin-induced alveolar damage (Zheng et al. 2013) but not after naphthalene or oxygen exposure (Rawlins et al. 2009). Other cell populations may be active under these circumstances and more investigation is needed to identify additional repair mechanisms.

In mouse models, there are data that the important role of maintaining epithelial integrity is performed by committed Clara and AT2 progenitors with Clara cells repopulating the ciliated cell populations, and AT2 cells giving rise to lost AT1 cells in the alveoli (Rawlins et al. 2009; Evans et al. 1976). However, in humans data shows that the airway epithelium is maintained not by these specific subpopulations, but by a large number of progenitor basal cells which divide as necessary to maintain and repair the airway without pre-programmed stem cells (Teixeira et al. 2013).

These endogenous stem cell populations play a crucial role in repairing and maintaining airway epithelial integrity. In chronic lung disease compromise of the airway stem and progenitor cell populations is seen in chronic obstructive pulmonary disease and asthma (Staudt et al. 2014). Conversely, inappropriate up-regulation of these stem cells is implicated not only in lung cancer but in other diseases like

idiopathic pulmonary fibrosis where there are increases in the Wnt/β-catenin stem cell pathway (Chilosi et al. 2003).

3 Models of Cancer Development from Progenitor Stem Cells

There are two theories of tumorigenesis linking stem cells to cancer. In one theory endogenous stem cells are present long enough over a lifetime to accumulate a series of genetic mutations which ultimately become oncogenic and lead to development of a cancer. The other theory is based on the idea that cancers develop from a differentiated, restricted progenitor cell which through oncogenic mutations, acquires more mutations and ultimately these mutations confer the stem cell properties of self-renewal, pluripotency and immortality. There is evidence to support both theories and most likely tumors develop as a combination of these two mechanisms and continue to evolve with natural selection favoring the survival of cells with the most oncogenic, proliferative mutations.

In lung cancer this has been explored by attempting to link different histologic types of lung cancers with specific endogenous stem cell populations. Using mouse models of lung cancer, researchers have identified likely cells of origin and potential stem cells targets for the major histologic types of lung cancer. Different histologic types of lung cancer generally develop centrally or peripherally along the airway and each type of cancer shares characteristics with the lung stem cell population found within each of these anatomic areas (Giangreco et al. 2007).

3.1 Adenocarcinoma

Non-small cell lung cancer (NSCLC) is a broad grouping of primary lung tumors including adenocarcinoma, squamous cell carcinoma, and large cell neuroendocrine carcinoma which combined comprise 80 % of all lung and bronchus tumors (Howlader et al. 2013). Adenocarcinoma is the most common form of NSCLC and lung cancer overall, accounting for 38 % of all newly diagnosed lung cancers (Travis 2011). Recent advancements in targeted therapies for adenocarcinoma with specific tyrosine kinase inhibitors and targeted antibodies have led to modest improvements in survival times for certain subgroups of patients; however, additional therapeutic strategies are desperately needed (Yang et al. 2014; Rossi et al. 2014). In particular, therapies which target the quiescent and chemotherapy resistant stem cell population within each tumor would be a useful adjunct to current therapies that target more rapidly dividing cells.

In identifying the cells of origin in lung adenocarcinoma, much research has utilized *K-ras* mutated xenograft models. Approximately 25 % of human lung

adenocarcinomas have an activating *K-ras* mutation. These mutations are seen more commonly in smokers and are predictive of chemotherapy resistance and poor prognosis (Riely et al. 2008). Transgenic mouse models with activating *K-ras* mutations have been used to induce lung adenocarcinoma development, and used to identify the adenocarcinoma stem cells of origin. Xu et al. (2012) have demonstrated that lung hyperplasia, a precursor to cancer, originates from AT2 cells, terminal bronchial Clara cells, and putative bronchoalvoelar stem cells. However, only hyperplastic AT2 cells in the distal lung actually progress into lung adenocarcinoma. There is other evidence that Clara cells or AT2 cells are the originators of adenocarcinoma since lung adenocarcinomas frequently co-express the markers CCSP and SP-C which are co-expressed by Clara or AT2 cells (Giangreco et al. 2002). Based on current information, the stem cell origin of adenocarcinomas is mostly the Clara or AT2 cells found at the BADJ.

3.2 Squamous Cell Lung Cancer

The second most common type of lung cancer is squamous cell carcinoma (SCC) which accounts for 30 % of all NSCLC. SCC is recognized by the histologic characteristics of keratin pearls and intercellular bridges (Linnoila 1990). SCC in the lung is thought to develop through a process of dysplastic changes over several years similar to the way that squamous cell cervical cancer develops. The lung stem cell most likely linked to SCC is the basal cell since SCCs tend to develop in areas with the highest basal cell concentration at the submucosal gland duct junctions and at intracartilaginous borders. SCC likely occur as basal cell hyperplasia develops into metaplasia, to dysplasia, and ultimately to carcinoma in situ and invasive SCC disease (Jeremy George et al. 2007). Throughout this progression the squamous cells have been shown to maintain a basal cell phenotype and have persistent keratin-5 expression (Barth et al. 2000).

3.3 Small Cell Lung Cancer

Small cell lung cancer (SCLC) is distinct from NSCLC and is characterized by rapidly dividing cells, early development of widespread metastasis, and markedly worse survival outcomes (Elias 1997). Based on the most recent National Cancer Institute's data, SCLC comprises only 11 % of all new lung cancer diagnoses (Howlader et al. 2013). Outcomes in SCLC are so poor the disease is not staged by standard TNM criteria but by grouping patients into limited-stage and extensive-stage categories. Outcomes have remained poor over the past several decades with only 4.6 % of all patients remain alive 2 years following diagnosis. In the 40 % of patients that present with more favorable limited-stage disease there is still only a

10 % 5-year survival rate (Govindan et al. 2006). While most patients with SCLC will initially respond to chemotherapy and radiation, disease recurrence remains a major problem (Stupp et al. 2004).

SCLC has long been considered to arise from neuroendocrine cells as a more aggressive form of a carcinoid tumor of the lung. Phenotypically, SCLC cells have neuroendocrine characteristics and exhibit dense neurosecretory granules. They express the neural cell adhesion molecule synaptophysin and calcitonin gene-related peptide (CGRP). CGRP is also expressed by neuroendocrine cells in the NEB, implicating those neuroendocrine cells as the stem cells of origin for SCLC. Genetically, 70 % of SCLCs have a mutation or loss of heterozogosity in *Rb1* and *p53* (Meuwissen and Berns 2005). Sutherland et al. (2011) demonstrated that SCLC arises most frequently from the NEB when *Rb1* and *p53* are knocked out in Clara, AT2, and neuroendocrine cells of transgenic mice. Of those three cell types, neuroendocrine cells were the most easily transformed into SCLC and Clara cells were the most resistant. These data suggest that the SCLC cells of origin may be not only neuroendocrine cells, but a small number of AT2 and an even rarer population of Clara cells that also have the potential to develop into SCLC.

4 Identification of Lung Cancer Stem Cells

The ability to correctly identify and isolate CSCs is crucial for ongoing research and development of potential therapeutics. In order to classify the cell as a CSC, the isolated cells must display the properties of extensive proliferation and self-renewal in in vitro experiments. Thus far, most CSC research has used cells identified by surface proteins detected using flowcytometry, magnetic bead isolation, fluorescent protein tagging, and immunostaining.

4.1 Surface Markers

The best studied surface marker in lung cancer stem cells is CD133 (prominin-1 or AC133), which was first described in human hematopoietic stem cells (Miraglia et al. 1997). In lung cancer $CD133^+$ cells have been identified in both SCLC and NSCLC tumors. $CD133^+$ cells are rarely found in normal lung tissue but are seen more commonly in lung tissue in the process of regeneration. In both SCLC and NSCLC, $CD133^+$ cells have been shown to possess stem cell properties of pluripotency, self-renewal, and immortality. Eramo et al. (2008) showed that both SCLC and NSCLC $CD133^+$ cells were able to grow indefinitely in media in vitro. They also demonstrated that SCLC and NSCLC $CD133^+$ cells were able to generate phenotypically identical tumors in immunocompromised mice and were able to self-renew and generate unlimited progeny of non-tumorigenic cells in the same xenograft model. Bertolini et al. (2009) showed that injection of a purified pool of

$CD133^+$ cells, but not $CD133^-$ cells, into immunodeficient mice led to tumor development. And compared with $CD133^-$ cells, $CD133^+$ cells had higher expression of the genes involved in stemness, adhesion, motility, and drug efflux.

There is interesting data to suggest that $CD133^+$ cells are more resistant to chemotherapy and therefore can evade standard treatments and later repopulate tumor bulk as a mechanism for tumor recurrence. Bertolini et al. (2009) have also shown that cisplatin treatment can reduce the size of lung tumors in mice xenotransplanted with lung cancer stem cells. However the cells that remain following treatment are universally $CD133^+$ suggesting that the population of cells resistant to chemotherapy are the CSC. This may explain why following a complete radiographic remission patients will later present with tumor regrowth and widespread metastasis, produced by a handful of remaining CSC. In vitro treatment of lung cancer cells with cisplatin enriches for $CD133^+$ cells, and cisplatin treatment of lung cancer xenografts spares subpopulations of $CD133^+$ $ABCG^+$ cells and $CD133^+$ $CXCR4^+$ cells. $CD133^+$ cells express high levels of ABCG2, implying possible overlap with side population cells, as well as the embryonic stem cell markers Oct-4 and Nanog (Eramo et al. 2008). NSCLC patients whose cancers are $CD133^+$ have a tendency towards shorter progression-free survival after treatment with a platinum-containing chemotherapy regimen (Bertolini et al. 2009).

Similar evidence was provided by Levina et al. (2008) who showed that treatment of lung cancer cell lines with cisplatin, etoposide and doxorubicin enriched for $CD133^+$ expression and the side population phenotype. Following treatment the resulting cells were more tumorigenic when xenotransplanted into immunodeficient mice. Also of note, the cells that were able to survive chemotherapy contained two to threefold higher levels of human angiogenic and growth factors cytokines. These data indicate that traditional chemotherapy kills many cancer cells and shrinks tumor bulk but leaves behind a chemotherapy-resistant, aggressive CSC population. This suggests that chemotherapy in face selects for the survival of the most oncogenic cells with a higher expression of genes that promote tumor growth and metastasis.

While CD133 is a promising marker, not all human lung cancers have a population of $CD133^+$ cells (Bertolini et al. 2009) and there is data that $CD133^-$ cells from human lung cancer cell lines are also capable of producing tumors in a xenograft model (Meng et al. 2009). In addition, there has not yet been a consistently proven prognostic correlation between $CD133^+$ cells and survival (Salnikov et al. 2010). A recent meta-analysis of 13 different studies found that CD133 expression was not associated with disease free survival but was associated with shorter overall survival (Wang et al. 2014).

Another well studied stem cell surface marker is CD44, a transmembrane glycoprotein found in about half of NSCLC tumors and in particular in squamous cell NSCLC (Leung et al. 2010). Of note and unlike CD133, $CD44^+$ cells have not been observed in SCLC cell lines (Qiu et al. 2012). Like $CD133^+$ cells, $CD44^+$ cells are also enriched for stem-cell like properties. $CD44^+$ cells from lung cancer cell lines are able to initiate tumor growth in nude mice and perform in vivo differentiation by growing tumors containing both $CD44^+$ and $CD44^-$ cells. Isolated $CD44^+$ cells and

not CD44⁻ cells express the pluripotency genes *OCT-4/POU5F1*, *NANOG*, and *SOX2*. CD44⁺ cells also display resistance to cisplatin treatment in vitro with less apoptosis than CD44⁻ cells (Leung et al. 2010).

As a prognostic marker, high CD44 expression in human NSCLC has been correlated with more advanced regional lymph node metastasis (Ko et al. 2011). However, there is mixed data that CD44⁺ expression has prognostic significance. In squamous cell cancer, some studies have shown that CD44 expression is an independent marker for better overall survival in squamous cell lung cancer (Sterlacci et al. 2014) but others have shown that there is no correlation between CD44⁺ cells and survival (Ko et al. 2011). There is also conflicting data regarding CD44 expressing adenocarcinomas, with some data that high CD44 expression is an independent negative prognostic marker (Ko et al. 2011) and another study which showed that patients with CD44⁺ adenocarcinomas have longer overall survival (Leung et al. 2010).

CD166 is another marker that enriches for cells with stem-cell properties. It has been shown that in immunocompromised mice, transplantation of CD166⁺ cells can create tumors with heterogeneous cell compositions that mirror that of the primary tumor. CD166 expression may be a poor prognostic indicator as CD166⁺ cells overexpress glycine decarboxylase, which has been shown to correlate with worse survival prognosis in NSCLC (Zhang et al. 2012).

Urokinase-type plasminogen activator (uPA) and its receptor uPAR/CD87 are regulators of extracellular matrix degradation and are important for cell migration and invasion. In cancer uPA it is a strong predictor of poor outcomes. In SCLC cell lines, uPAR⁺ cells have been shown to have enhanced clonogenic activity and multidrug resistance in vitro (Gutova et al. 2007) and in vivo are tumorigenic in athymic nude mice (Qiu et al. 2012). Cancer cells that are uPAR⁺ also co-express other CSC surface markers including CD133, CD44, and MDR1 (Qiu et al. 2012; Gutova et al. 2007).

4.2 Side Population Cells

In addition to surface markers, CSC can be identified by functional attributes. In the course of studying lung cancer cells with flowcytometry, an incidentally found side population (SP) phenotype of cancer cells was repeatedly seen on flowcytometry (Fig. 8.1). This SP cell population has an ATP-binding cassette (ABC) family transporter cell membrane protein which pumps out the fluorescent nuclear dye Hoechst 33342. By pumping out the nuclear dye these SP cells appear as a side population to the reminder of the tumor on cell sorting and can be isolated from the rest of the tumor. SP has been detected in studies of SCLC and NSCLC cell lines. In squamous cell cancer SP cells have higher rates of proliferation and greater clonogenic ability in vitro, and a significantly lower concentration of isolated SP cells compared with non-SP cells are capable of producing tumors when xenotransplanted into immunocompromised mice (Loebinger et al. 2008). Isolated SP cells are multipotent and

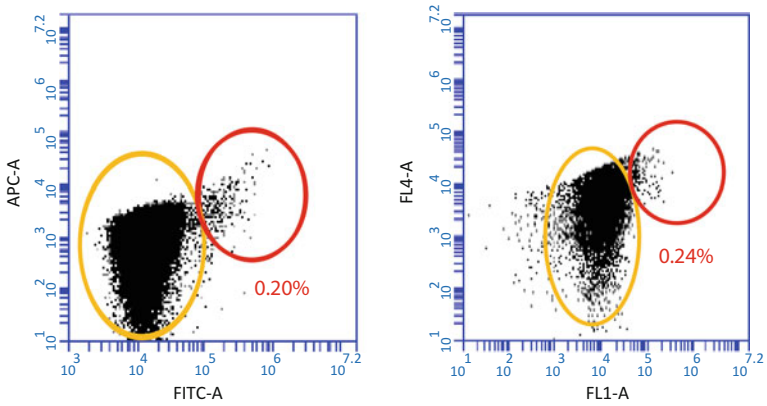

Fig. 8.1 *Side population cells*. The cancer stem cell side population cells have an ABC transporter cell membrane protein which pumps out the fluorescent nuclear dye Hoechst 33342 and separates these rare cancer stem cells (*red circle*) from the majority of the tumor cell population (*yellow circle*) on flow cytometry. These cells make up less than 1 % of all tumor cells. Side population cells have been identified from both lung cancer cell lines (*left*) and from fresh lung cancer surgical specimens (*right*).

produce both SP and non-SP cells in culture. It has been shown in vitro invasion assays that SP cells also have a higher potential for invasiveness than non-SP cells (Ho et al. 2007).

SP cells are significantly more resistant to chemotherapy in assays in vitro and are more capable of maintaining a large colon formation than non-SP cells in the setting of chemotherapy (Ho et al. 2007). The increased ABC transporters that remove Hoechst dye from SP cells and provide a mechanism for the SP cell's relative chemoresistance, and this is further proven by data that SP cells can be sensitized to chemotherapy by blocking the ABC transporter with verapamil (Loebinger et al. 2008). Studies of SP cells suggest that they are more quiescent than other tumor cells as they have higher levels of telomerase and have less mini-chromosome maintenance 7, a marker of proliferation (Ho et al. 2007).

4.3 Aldehyde Dehydrogenase

Aldehyde dehydrogenase (ALDH) is involved in early stem cell development where it oxidizes retinol to retinoic acid (Chute et al. 2006). ALDH has been recognized as a stem cell marker in multiple studies. Jiang et al. (2009) showed that ALDH1$^+$ cells were able to self-renew, had proliferative tumorigenic potential in in vivo studies, and showed resistance to chemotherapy. ALDH1$^+$ cells have been shown to overlap with cells that express the stem cell marker CD133 but there is limited other data to suggest that these stem cells markers occur within the same cell population. ALDH activity is associated in particular with squamous histology (Moreb et al. 2007).

High ALDH1 protein expression has been associated with poor prognosis in early NSCLC (Jiang et al. 2009; Sullivan et al. 2010). This may be partially explained by work by Moreb et al. (2008) who showed that if ALDH protein was inhibited by siRNA, tumor cells had a reduced ability to proliferate and migrate in vitro.

A single, universal marker for lung cancer stem cells has yet to be identified. The current surface makers, SP cell type, and ALDH lack sensitivity and specificity for consistently selecting cells with stem cell properties. Akunuru et al. (2012) have demonstrated that non-SP, $CD133^-$, $ALDH^-$ cells can produce SP, $CD133^+$, and $ALDH^+$ cells in culture, exhibiting the phenotypic switching from a non-cancer stem cell type to a cancer stem cell phenotype. This study and the relative paucity of data showing overlap between the currently identified stem cell surface markers cast some doubt over the accuracy of our current methods to identify stem cell populations and better markers for lung cancer stem cells are needed.

5 Stem Cell Pathways and Molecular Targets

The properties which enable endogenous stem cells to perform their crucial role in normal tissue repair also serve as protective mechanisms against cell death. Stem cells have ABC transporter proteins which lead to rapid toxin and drug efflux as well as high levels of anti-apoptotic proteins. CSCs share these properties which confer an increased resistance to traditional chemotherapy and radiation. In addition, most chemotherapy agents work by targeting rapidly proliferating cells and for this reason may be less effective against the CSCs which are more quiescent in nature. Without eliminating the CSCs, these cells remain in the body as a small but potent tumor reservoir that can ultimately repopulate a cancer recurrence.

For this reason CSCs are important, if not the most important therapeutic target and research into treatments which target the CSC specific signaling pathways such as Wingless type (Wnt), Sonic Hedgehog (Shh), and Notch is of interest in lung and other cancers (Takebe and Ivy 2010). Targeted therapies to block these pathways poses a challenge as these are the same signaling pathways used by normal proliferating cells and there are protective cross-talk mechanisms between pathways that preserve their important role.

5.1 Wnt Pathway

Wingless type (Wnt) glycoproteins are a highly conserved family of 19 secreted signaling molecules that bind to cell surface receptors and regulate downstream gene expression (Angers and Moon 2009). The Wnt pathway plays a crucial role in embryogenesis, lung development, and endogenous stem cell regulation. During embryogenesis and development Wnt proteins control cell fate determination and

direct the development of the cardiovascular, pulmonary, renal, and central nervous systems (Grigoryan et al. 2008). Later in life the Wnt pathway regulates tissue self-renewal, including the renewal of hair follicles, intestinal crypts, and bone growth plates (Clevers 2006; Andrade et al. 2007). Deregulated Wnt signaling has been shown in a large variety of cancers including hepatocellular carcinoma, hepatoblastoma, colorectal cancer, acute and chronic myelogenous leukemia, multiple myeloma, gastric cancer, Wilms' tumor, and NSCLC (He et al. 2005a). Wnt signaling has been shown to promote stem cell self-renewal in hematopoietic stem cells (Reya et al. 2003). Wnt pathway activation, specifically β-catenin signaling, has been shown to be required for cancer stem cells to maintain their tumorigenic potential (Malanchi et al. 2008). The Wnt pathway is therefore an important potential target to eliminate or inhibit cancer stem cell growth.

In the canonical pathway, a Wnt ligand binds to a Frizzled (Fz) receptor or an LDL-receptor related protein (LRP) on the cell surface. This activates one of three intracellular Dishevelled (Dvl) proteins, Dvl-1, Dvl-2, or Dvl-3, which then inhibit glycogen synthase kinase-3β (GSK-3β). When Dvl inhibits GSK-3β, it prevents GSK-3β from phosphorylating β-catenin. In the presence of Wnt signaling free β-catenin stabilizes, accumulates in the cytosol, and ultimately translocates to the nucleus. There β-catenin interacts with either p300 or cyclic AMP response element-binding protein (CBP) and with members of the T-cell factor-lymphocyte enhancer factor (TCF/LEF) family of transcriptional factors which activate target genes including *Myc, Cyclin D1, TCF-1, PPAR-δ, MMP-7, Axin-2, CD44, Cox2* (Takebe et al. 2011; Mazieres et al. 2005). On the cell's surface 10 different Fz receptors have been identified providing multiple Wnt-Fz receptor combinations that can subtlety modify the downstream effects of Wnt (Schulte and Bryja 2007). Wnt has also been shown to be active in at least two noncanonical pathways by activating calmodulin kinase II and protein kinase C in the Wnt/Ca^{++} pathway and by activating Jun N-terminal kinase in the planar cell polarity pathway (Veeman et al. 2003).

In lung cancer multiple mechanisms of increased Wnt activation have been identified. Uematsu et al. (2003a) demonstrated Dvl overexpression as one mechanism by which the Wnt pathway can be activated in NSCLC. Another studies showed that Wnt-1 and Wnt-2 are overexpressed in both NSCLC cell lines and in primary tumor tissue (He et al. 2004; You et al. 2004b). Other Wnt proteins such as Wnt-7a and Wnt-5a appear to behave as tumor suppressors. Wnt-7a is down-regulated in most lung cancer cell lines and primary tumor samples (Calvo et al. 2000). During development Wnt-7a functions via a non-canonical β-catenin independent pathway in developing human limbs (Kengaku et al. 1998). In lung cancer Wnt-7a appears to activate the canonical Wnt pathway, but does not directly target TCF-LEF transcriptional activity. It has been shown to positively regulate the epithelial-mesenchymal transition (EMT) marker E-cadherin expression in lung cancer cells (Ohira et al. 2003). Like Wnt-7a, Wnt-5a activates a non-canonical pathway in development, the Wnt/Ca^{++} pathway. In some cancers Wnt-5a is up-regulated and associated with increased tumor invasion, including the development of lung metastasis in sarcoma (Saitoh et al. 2002; Nakano et al. 2003). However, in hematopoietic malignancies Wnt-5a acts as a tumor suppressor and its role in primary NSCLC has not been studied.

The expression of downstream proteins in the canonical Wnt pathway is an area of ongoing research in NSCLC. Dvl-3 is overexpressed in 75 % of NSCLC tumor samples compared with autologous matched normal tissues from the same patient. Deletion of the PDZ protein binging domain of Dvl blocks Dvl activity and suppresses tumorigenesis in pleural malignant mesothelioma (Uematsu et al. 2003b). Data regarding β-catenin are more controversial. Mutations in the β-catenin gene are rare in lung cancer cell lines and in primary lung tumor tissue (Sunaga et al. 2001; Shigemitsu et al. 2001; Ueda et al. 2001). In NSCLC increased expression of β-catenin is associated with a high proliferative index but is unexpectedly associated with a better prognosis (Hommura et al. 2002). Other independent studies have corroborated this finding by showing that reduced β-catenin expression is associated with a better lung adenocarcinoma prognosis (Retera et al. 1998; Kase et al. 2000). These data may reflect β-catenin's involvement in the Wnt pathway and as a cadherin-mediated cell adhesion component implying a complex, multifacited role in NSCLC which is not yet fully understood (Barker et al. 2000).

Given the importance of the Wnt pathway in maintaining cancer stem cells there are a number of experimental agents in development to inhibit Wnt signaling with promising results. In vitro apoptosis can be induced if either Wnt-1 or Wnt-2 is inhibited by siRNA or a monoclonal antibody. Extracellular Wnt inhibition with monoclonal antibodies to Wnt and the Fz receptor has shown antitumor activity in vitro (He et al. 2005b; You et al. 2004a). And in vivo anti-Wnt-1 and anti-Wnt-2 monoclonal antibodies are also able to suppress tumor grown in a mouse model (He et al. 2004; You et al. 2004b). These results are promising but these monoclonal antibodies have not yet been tested in humans.

The highly conserved gene *Wnt inhibitory factor-1 (WIF-1)* has been shown to be down regulated in several cancers including prostate, breast, bladder, and lung (Wissmann et al. 2003). Mazieres et al. demonstrated that WIF-1 expression is down-regulated in 83 % of human NSCLC tumor specimens and proposed a mechanism of hypermethylation of CpG islands in the functional *WIF-1* promoter region (Mazieres et al. 2004).

Endogenous secreted frizzled-related proteins (sFRP) modulate *Wnt* signaling by competing with Wnt ligand binding to the Fz receptors and have been shown to be down-regulated in colon, gastric, and breast cancer. In lung cancer, Lee et al. (2004) demonstrated that sFRP are down-regulated in NSCLC and mesothelioma cell lines and that 80 % of mesothelioma tissue specimens have hypermethylation of the sFRP gene promoter. Dvl is another important therapeutic target for Wnt pathway inhibition. In lung cancer cell lines, targeted inhibition of Dvl-1, Dvl-2, or Dvl-3 decreases β-catenin expression, decreases TCF-dependent gene transcription, and inhibits tumor cell growth (Uematsu et al. 2003a).

IGC-001 (Institute for Chemical Genomics) is a small molecule that interrupts β-catenin binding to transcriptional cofactor CBP. In colon cancer cell lines ICG-001 results in apoptosis in cancer cells but spares the normal colon epithelial cells (Emami et al. 2004). Intracellular inhibitors NSC668036 (Sigma-Aldrich) and FJ9 are two other compounds in development which target the PDZ domain of Dvl and

inhibit both the canonical and non-canonical Wnt pathways (Fujii et al. 2007; Shan et al. 2005). Chen et al. have identified two additional small molecule inhibitors that block the Wnt pathway in vivo via different mechanisms. One small molecular is a membrane-bound acyltransferse small molecule Porcupine inhibitor, which is essential for Wnt synthesis, and the other small molecule inhibits the destruction of Axin, which suppresses Wnt activity (Chen et al. 2009).

5.2 Sonic Hedgehog Pathway

The Sonic Hedgehog (Shh) pathway is best known for its role in embryogenesis where it controls the migration, polarity, differentiation, proliferation, and transformation of progenitor cells (Varjosalo and Taipale 2008). If unregulated, thoses cellular processes also give the Shh pathway a significant role in carcinogenesis and the transformation of adult stem cells into CSCs. Activated Shh has been implicated in tumorigenesis and metastasis in multiple types of cancers including lung, brain, breast, prostate, and skin. In the canonical Shh pathway, the absence of the Shh ligand leads the transmembrane receptor Patched (Ptch) to inhibit the transmembrane receptor Smoothened (Smo). Inhibited Smo causes cleavage of Gli to the N-terminal repressor form. Therefore when Shh binds to Ptch, the inhibitory effect on Smo is released and active full length Gli is transported into the nucleus and Gli1, Gli2, and Gli3 transcription factors activates transcription of Gli-dependent target genes such as *Gli1*, *Ptch1*, *cyclinD1* and *Wnt* (Hooper and Scott 2005; Huangfu and Anderson 2006; Altaba et al. 2007; Mullor et al. 2001). Gli1 activates Shh target genes, Gli2 has a role in both gene activation and repression and Gli3 represses target gene transcription. The balance between activation and repression by the three forms of Gli appears to control Shh downstream signaling (Altaba et al. 2007).

In addition to the Shh pathway, non-canonical Gli activation independent of Shh, has been shown in many cancer cells types, (Lauth and Toftgard 2007; Mimeault and Batra 2010) and there is evidence for Gli activation independent of Shh, stimulated by other oncogenic signaling pathways such as transforming growth factor β (TGF-β), epidermal growth factor receptor (EGFR), RAS and AKT/PI3K pathways (Guo and Wang 2009; Schnidar et al. 2009; Pasca di Magliano et al. 2006; Stecca et al. 2007). As Gli transcription factors constitute the final effectors of the Shh pathway and are implicated in multiple other oncogenic signaling pathways, they represent an important downstream target for potential cancer therapeutics (Lauth and Toftgard 2007).

The Shh pathway contains many potential CSC therapeutic targets and drug development is an area of active research. The first Shh pathway inhibitor identified was cyclopamine (11-deoxojervine), a plant-derived steroidal alkaloid that binds to and deactivates Smo (Taipale et al. 2000). Park et al. (2011) have shown that Shh signaling is involved in SCLC development in genetically engineered mice and that

Shh inhibition can help prevent tumor recurrence. It has also been shown that inhibition of Shh signaling in SCLC with the Smo antagonist cyclopamine leads to loss of tumorigenicity (Watkins et al. 2003). Cyclopamine remains the only naturally derived compound but there are a growing number of synthetic small molecules designed to inhibit the Shh pathway at different points.

Vismodegib (GDC-0449, Genentech) is a Smo inhibitor approved by the U.S. Food and Drug Administration to treat adult patients with basal cell carcinoma (Ng and Curran 2011; LoRusso et al. 2011; Sekulic et al. 2012; Dlugosz et al. 2012). Response rates in the phase I clinical trial in metastatic or locally advanced basal cell cancer were encouraging, with over half of patients having at least a partial response. Common side effects of vismodegib include dysgeusia, hair loss, nausea, vomiting, anorexia, dyspepsia, weight loss, hyponatremia, and fatigue. A quarter of patients experienced adverse events including fatigue, hyponatremia, muscle spasm and atrial fibrillation and only one of 33 paitents developed a major dose limiting toxicity of grade 3 lymphopenia (Von Hoff et al. 2009). Vismodegib is currently being investigated in clinical trials to treat other types of cancer including ovarian, pancreatic, colorectal, and lung cancer due to its ability to selectively target Shh signaling (Ng and Curran 2011; Agarwal et al. 2011). In SCLC vismodegib is in clinical trials in combination with cisplatin and etoposide (ClinicalTrials.gov 2014).

Another small molecule Smo inhibitor is BMS-833923, XL139 (Bristol-Myers Squibb). BMS-833923 has completed phase I clinical trials in combination with carboplatin and etoposide as a treatment for SCLC. It is also being tested as part of a multidrug regimen in multiple myeloma and metastatic gastric and esophageal cancers (ClinicalTrials.gov 2014). Infinity Pharmaceuticals has developed a cyclopamine-derived inhibitor Shh inhibitor IPI-926 (Infinity Pharmaceuticals) which is in clinical trials for advanced-stage solid tumors and metastatic pancreatic cancer. Early clinical trial data in patients with basal cell carcinoma showed that IPI-926 is well tolerated and nearly a third of patients experienced a partial or complete clinical response (Jimeno et al. 2013). Multiple phase 2 trials are currently underway testing IPI-926 alone or in combination with other agents in for a myriad of advanced-stage malignancies (ClinicalTrials.gov 2014). In addition to the direct effect on Gli transcription factors there is emerging evidence that Shh signals have some control over the architecture of the stromal microenvironment. In a mouse model of pancreatic cancer IPI-926 improved access of chemotherapy agents potentially via this mechanism (Olive et al. 2009).

Other potential emerging therapies which have shown effects in in vitro testing include Robotnikinin, a small molecule that binds to extracellular Shh, and small synthetic molecules called Hedgehog Protein Inhibitors (HPI) 1–4 which inhibit downstream Gli activation through different mechanisms (Stanton et al. 2009). HPI-1 has been shown to inhibit activation of Gli1 and Gli2, HPI-2 and HPI-3 both inhibit Gli2, and HPI-4 inhibits formation of cilia when Smo is active and therefore prevents activation of Gli transcription factors (Hyman et al. 2009). Ongoing investigation into Shh is likely to elucidate other mechanisms by which this pathway drives cancer cell growth and uncover additional therapeutic targets.

5.3 Notch Pathway

The Notch pathway regulates cellular proliferation and differentiation via cell-to-cell communication and has a highly conserved role in determining cell fate during embroygenesis. It also plays a critical role in cellular proliferation, differentiation, apoptosis, hematopoiesis, breast development, colorectal epithelial maturation, immune regulation, and neural stem cell survival (Artavanis-Tsakonas et al. 1999).

When a Notch ligand pairs with a receptor on an adjacent cell it results in a coordinated cell-to-cell communication. In mammals, the membrane-bound Notch ligands are either Delta-like ligands 1, 3, and 4, or Jagged ligands 1 and 2 which are structurally distinct. These membrane ligands interact with four transmembrane, heterodimer Notch receptors 1, 2, 3, and 4 which contain multiple epidermal growth factor-like domains (EGF). The affinity of the ligand for the receptor depends on the EGF domain fucosylation by Fringe proteins Lunatic, Radical, and Maniac (Takebe et al. 2011). After Notch ligand-receptor binding the receptor undergoes a conformational change that exposes a site to proteolytic cleavage by metalloprotease which releases an extracellular fragment and cleavage by **γ**–secretase which releases an active Notch intracellular domain (NICD) fragment into the cytoplasm (Gordon et al. 2007). NICD then modulates Notch-specific gene expression by undergoing nuclear translocation and binding to the translocation initiation complex.

In endogenous stem cells, activated Notch signaling guides asymmetric cell division and retains stem cell viability (Artavanis-Tsakonas et al. 1999). In the healthy mouse lung, suppression of Notch signaling by knocking out *hairy and enhancer of split 1 (Hes1)*, increases the number of cells which differentiate into neuroendocrine cells and decreases the number of cells which become Clara cells (Ito et al. 2000). Constitutive activation of Notch in mice leads to delayed differentiation and accumulation of distal airway stem cells (Dang et al. 2003).

In human lung cancer cell lines, Chen et al. (1997) have shown elevated levels of Notch transcripts and Westhoff et al. (2009) reported possible oncogenic mutations in Notch1 receptor in NSCLC. However, there is other data to show that Notch may have a tumor suppressor effect in squamous epithelial in mice and in human myeloid cancers (Nicolas et al. 2003; Klinakis et al. 2011). These data that the Notch pathway can play both an oncogenic and tumor suppressor role suggest that the Notch pathway is complex. While further investigation is needed to better understand its role, Notch signaling remains an attractive potential therapeutic target.

Research by Moreb et al. (2008) demonstrated that $ALDH1^+$ cells express Notch pathway transcripts and that inhibition of the Notch pathway with γ–secretase inhibitors led to a reduction in $ALDH1^+$ cells. Osanyingbemi-Obidi et al. (2011) showed inhibiting Notch3 with γ–secretase suppressed clonogenic survival in cell lines and that this clonogenic survival could be restored by reintroducing the Notch3 receptor domain. γ–secretase inhibitors decrease tumor growth and the number of $CD133^+$ glioma stem cells in human glioma xenografts (Fan et al. 2006). Early studies of γ–secretase inhibitors in rodent models showed excessive toxicity and

therefore researchers are currently working to develop more specific inhibitors of the Notch transcriptional complex (Imbimbo 2008).

There are clinical trials underway to test novel Notch inhibitors in many malignancies including NSCLC (ClinicalTrials.gov 2014). The primary focus thus far has been on inhibiting γ–secretase mediated Notch cleavage. MK0752 (Merck) is a γ–secretase inhibitor which has been tested in the treatment of T-cell acute lymphoblastic leukemia. It was found to have dose limiting toxicity of gastrointestinal globet cell hyperplasia and secretory diarrhea, however these toxic effects have been shown to be reduced in a mouse model with co-administration of glucocorticoids (Real and Ferrando 2009).

5.4 Transcription Factors

In addition to the central role that the Wnt, Shh, and Notch pathways play in lung cancer stem cells, many niche factors and interactions with other signaling pathways have been shown to play an important part in maintaining a cancer stem cell phenotype. The transforming growth factor-β (TGF-β) family of cytokines has been shown along with Wnt, Shh, and Notch to induce EMT, the complex process by which cells down-regulate E-cadherin, lose their adhesive properties and cell polarity, and gain invasive and migratory properties (Massague 2008). The EMT process and loss of E-cadherin allows some cancer stem cells to become metastatic and has been associated with tumor metastasis and poor prognosis (Kim et al. 2009; Mareel et al. 1997). TGF-β interaction maintains stem cell characteristics in cells which have undergone EMT and may be a therapeutic target in eliminating metastatic cancer stem cells (Bailey et al. 2007).

Another transcription factor which has been shown to play a role in cancer stem cells is Octamer-binding transcription factor 4 (Oct-4). Oct-4 is a homebox transcription factor that is crucial for embryonic stem cell self-renewal along with Nanog and Sox2. There is also mounting evidence for its use as a stem cell marker. In NSCLC there is data to shows that Oct-4 regulates stem cell activity in $CD133^+$ cells (Chen et al. 2008). Ectopic expression of Oct-4 and Nanog, another homebox transcription factor, increases the percentage of lung adenocarcinoma cells that are $CD133^+$, enhances drug resistance, and promotes epithelial-mesenchymal transformation. Oct-4 is present in high grade tumors and is a negative prognostic marker of lung adenocarcinoma survival (Chiou et al. 2010). Chen et al. (2008) demonstrated that siRNA knockdown of Oct-4 reduces clonogenicity and increases sensitivity to chemotherapy in $CD133^+$ cells. CSCs maintain their stem cell properties by up-regulating certain highly conserved cell development and fate determination pathways. Inhibiting these pathways with novel therapies is a highly promising area of research. Chemotherapy that targets the resistant CSC population at the core of a tumor would be incredibly useful in the treatment of lung cancer and other malignancies.

6 Conclusion

The treatment of lung cancer remains challenging with dismal survival outcomes over the past several decades despite advances in chemotherapy and medical care. Lung cancer stem cells are the crucial target in developing new therapeutic strategies. The combined stochastic model of cancer stem cells explains mechanisms of tumor development from local endogenous stem cell populations within the airway, and provides mechanisms for tumor metastasis and recurrence. Within each lung cancer resides a small population of cancer stem cells that maintain the properties of self-renewal, pluripotency, immortality, and chemotherapy resistance. These lung cancer stem cells produce large numbers of progeny cells that comprise the bulk of a tumor. Current lung cancer chemotherapy eliminates the non-stem tumor cells and generates radiographic responses. However, CSCs possess mechanisms that prevent apoptosis and have been shown to survive these standard treatments. The remaining small number of potent lung CSCs over time repopulates a tumor leading to cancer recurrence with cells that have been selected for a phenotype that is even more oncogenic, treatment resistant, and aggressive.

There are significant challenges in developing treatments that target cancer stem cells. The first barrier is accurately identifying and successfully isolating lung cancer stem cells from human cancers into an in vitro research environment. Great progress has been made in identifying surface markers and sorting methods to isolate subpopulations of cells such as $CD133^+$, $CD44^+$ and the SP cells that exhibit stem-cell properties. However no universal stem cell marker has been identified and the variety of stem cell markers that have been discovered are not consistently co-expressed on the same cell.

As we continue to work towards improved methods of cancer stem cell identification there has been promising early work in exploring stem cell signaling pathways and discovering possible therapeutic targets. Wnt, Shh, and Notch signaling pathways all play important roles in embryogenesis and have been shown to be crucial regulators of cancer stem cell activities. Developing therapies that inhibit these cancer stem cells pathways remains tricky as they are highly conserved and used by endogenous stem cells in their normal function of tissue maintenance and repair. Multiple Wnt, Shh, and Notch inhibitors are in various stages of development. The Shh pathway Smo inhibitor vismodegib (Genentech) is already approved for metastatic or recurrent locally advanced basal cell carcinoma with further studies currently underway to expand its application to multiple other solid tumors, including a trial as a combined therapy in lung cancer with cisplatin and etoposide. As research into cancer stem cell pathways and potential drug targets continues to expand we can expect to see multiple new agents designed to target lung cancer stem cells. Understanding and eliminating cancer stem cells remains a promising and exciting area of research and a crucial component in the future treatment of lung cancer.

References

Agarwal V, Lind MJ, Cawkwell L (2011) Targeted epidermal growth factor receptor therapy in malignant pleural mesothelioma: where do we stand? Cancer Treat Rev 37(7):533–542

Akunuru S, James Zhai Q, Zheng Y (2012) Non-small cell lung cancer stem/progenitor cells are enriched in multiple distinct phenotypic subpopulations and exhibit plasticity. Cell Death Dis 3, e352

Altaba R, Mas C, Stecca B (2007) The Gli code: an information nexus regulating cell fate, stemness and cancer. Trends Cell Biol 17(9):438–447

Andrade AC, Nilsson O, Barnes KM, Baron J (2007) Wnt gene expression in the post-natal growth plate: regulation with chondrocyte differentiation. Bone 40(5):1361–1369

Angers S, Moon RT (2009) Proximal events in Wnt signal transduction. Nat Rev Mol Cell Biol 10(7):468–477

Artavanis-Tsakonas S, Rand MD, Lake RJ (1999) Notch signaling: cell fate control and signal integration in development. Science 284(5415):770–776

Bailey JM, Singh PK, Hollingsworth MA (2007) Cancer metastasis facilitated by developmental pathways: Sonic hedgehog, Notch, and bone morphogenic proteins. J Cell Biochem 102(4):829–839

Barker N, Morin PJ, Clevers H (2000) The Yin-Yang of TCF/beta-catenin signaling. Adv Cancer Res 77:1–24

Barth PJ, Koch S, Muller B, Unterstab F, von Wichert P, Moll R (2000) Proliferation and number of Clara cell 10-kDa protein (CC10)-reactive epithelial cells and basal cells in normal, hyperplastic and metaplastic bronchial mucosa. Virchows Arch 437(6):648–655

Bertolini G, Roz L, Perego P, Tortoreto M, Fontanella E, Gatti L, Pratesi G, Fabbri A, Andriani F, Tinelli S, Roz E, Caserini R, Lo Vullo S, Camerini T, Mariani L, Delia D, Calabro E, Pastorino U, Sozzi G (2009) Highly tumorigenic lung cancer CD133+ cells display stem-like features and are spared by cisplatin treatment. Proc Natl Acad Sci USA 106(38):16281–16286

Borthwick DW, Shahbazian M, Krantz QT, Dorin JR, Randell SH (2001) Evidence for stem-cell niches in the tracheal epithelium. Am J Respir Cell Mol Biol 24(6):662–670

Calvo R, West J, Franklin W, Erickson P, Bemis L, Li E, Helfrich B, Bunn P, Roche J, Brambilla E, Rosell R, Gemmill RM, Drabkin HA (2000) Altered HOX and WNT7A expression in human lung cancer. Proc Natl Acad Sci USA 97(23):12776–12781

Carney DN, Gazdar AF, Bunn PA Jr, Guccion JG (1982) Demonstration of the stem cell nature of clonogenic tumor cells from lung cancer patients. Stem Cells 1(3):149–164

Chapman HA, Li X, Alexander JP, Brumwell A, Lorizio W, Tan K, Sonnenberg A, Wei Y, Vu TH (2011) Integrin alpha6beta4 identifies an adult distal lung epithelial population with regenerative potential in mice. J Clin Invest 121(7):2855–2862

Chen H, Thiagalingam A, Chopra H, Borges MW, Feder JN, Nelkin BD, Baylin SB, Ball DW (1997) Conservation of the Drosophila lateral inhibition pathway in human lung cancer: a hairy-related protein (HES-1) directly represses achaete-scute homolog-1 expression. Proc Natl Acad Sci USA 94(10):5355–5360

Chen YC, Hsu HS, Chen YW, Tsai TH, How CK, Wang CY, Hung SC, Chang YL, Tsai ML, Lee YY, Ku HH, Chiou SH (2008) Oct-4 expression maintained cancer stem-like properties in lung cancer-derived CD133-positive cells. PLoS One 3(7), e2637

Chen B, Dodge ME, Tang W, Lu J, Ma Z, Fan CW, Wei S, Hao W, Kilgore J, Williams NS, Roth MG, Amatruda JF, Chen C, Lum L (2009) Small molecule-mediated disruption of Wnt-dependent signaling in tissue regeneration and cancer. Nat Chem Biol 5(2):100–107

Chilosi M, Poletti V, Zamo A, Lestani M, Montagna L, Piccoli P, Pedron S, Bertaso M, Scarpa A, Murer B, Cancellieri A, Maestro R, Semenzato G, Doglioni C (2003) Aberrant Wnt/beta-catenin pathway activation in idiopathic pulmonary fibrosis. Am J Pathol 162(5):1495–1502

Chiou SH, Wang ML, Chou YT, Chen CJ, Hong CF, Hsieh WJ, Chang HT, Chen YS, Lin TW, Hsu HS, Wu CW (2010) Coexpression of Oct4 and Nanog enhances malignancy in lung

adenocarcinoma by inducing cancer stem cell-like properties and epithelial-mesenchymal transdifferentiation. Cancer Res 70(24):10433–10444
Chute JP, Muramoto GG, Whitesides J, Colvin M, Safi R, Chao NJ, McDonnell DP (2006) Inhibition of aldehyde dehydrogenase and retinoid signaling induces the expansion of human hematopoietic stem cells. Proc Natl Acad Sci USA 103(31):11707–11712
Clevers H (2006) Wnt/beta-catenin signaling in development and disease. Cell 127(3):469–480
ClinicalTrials.gov (2014) ClinicalTrails.gov, U.S. National Institutes of Health. http://clinicaltrials.gov. Accessed 20 Oct 2014
Dang TP, Eichenberger S, Gonzalez A, Olson S, Carbone DP (2003) Constitutive activation of Notch3 inhibits terminal epithelial differentiation in lungs of transgenic mice. Oncogene 22(13):1988–1997
Desai TJ, Brownfield DG, Krasnow MA (2014) Alveolar progenitor and stem cells in lung development, renewal and cancer. Nature 507(7491):190–194
Dlugosz A, Agrawal S, Kirkpatrick P (2012) Vismodegib. Nat Rev Drug Discov 11(6):437–438
Elias AD (1997) Small cell lung cancer: state-of-the-art therapy in 1996. Chest 112(4 Suppl):251s–258s
Emami KH, Nguyen C, Ma H, Kim DH, Jeong KW, Eguchi M, Moon RT, Teo JL, Kim HY, Moon SH, Ha JR, Kahn M (2004) A small molecule inhibitor of beta-catenin/CREB-binding protein transcription [corrected]. Proc Natl Acad Sci USA 101(34):12682–12687
Eramo A, Lotti F, Sette G, Pilozzi E, Biffoni M, Di Virgilio A, Conticello C, Ruco L, Peschle C, De Maria R (2008) Identification and expansion of the tumorigenic lung cancer stem cell population. Cell Death Differ 15(3):504–514
Evans MJ, Johnson LV, Stephens RJ, Freeman G (1976) Renewal of the terminal bronchiolar epithelium in the rat following exposure to NO2 or O3. Lab Invest 35(3):246–257
Fan X, Matsui W, Khaki L, Stearns D, Chun J, Li YM, Eberhart CG (2006) Notch pathway inhibition depletes stem-like cells and blocks engraftment in embryonal brain tumors. Cancer Res 66(15):7445–7452
Fujii N, You L, Xu Z, Uematsu K, Shan J, He B, Mikami I, Edmondson LR, Neale G, Zheng J, Guy RK, Jablons DM (2007) An antagonist of dishevelled protein-protein interaction suppresses beta-catenin-dependent tumor cell growth. Cancer Res 67(2):573–579
Giangreco A, Reynolds SD, Stripp BR (2002) Terminal bronchioles harbor a unique airway stem cell population that localizes to the bronchoalveolar duct junction. Am J Pathol 161(1):173–182
Giangreco A, Groot KR, Janes SM (2007) Lung cancer and lung stem cells: strange bedfellows? Am J Respir Crit Care Med 175(6):547–553
Gordon WR, Vardar-Ulu D, Histen G, Sanchez-Irizarry C, Aster JC, Blacklow SC (2007) Structural basis for autoinhibition of Notch. Nat Struct Mol Biol 14(4):295–300
Govindan R, Page N, Morgensztern D, Read W, Tierney R, Vlahiotis A, Spitznagel EL, Piccirillo J (2006) Changing epidemiology of small-cell lung cancer in the United States over the last 30 years: analysis of the surveillance, epidemiologic, and end results database. J Clin Oncol 24(28):4539–4544
Grigoryan T, Wend P, Klaus A, Birchmeier W (2008) Deciphering the function of canonical Wnt signals in development and disease: conditional loss- and gain-of-function mutations of beta-catenin in mice. Genes Dev 22(17):2308–2341
Groome PA, Bolejack V, Crowley JJ, Kennedy C, Krasnik M, Sobin LH, Goldstraw P (2007) The IASLC Lung Cancer Staging Project: validation of the proposals for revision of the T, N, and M descriptors and consequent stage groupings in the forthcoming (seventh) edition of the TNM classification of malignant tumours. J Thorac Oncol 2(8):694–705
Guo X, Wang XF (2009) Signaling cross-talk between TGF-beta/BMP and other pathways. Cell Res 19(1):71–88
Gutova M, Najbauer J, Gevorgyan A, Metz MZ, Weng Y, Shih CC, Aboody KS (2007) Identification of uPAR-positive chemoresistant cells in small cell lung cancer. PLoS One 2(2), e243

He B, You L, Uematsu K, Xu Z, Lee AY, Matsangou M, McCormick F, Jablons DM (2004) A monoclonal antibody against Wnt-1 induces apoptosis in human cancer cells. Neoplasia 6(1):7–14

He B, Barg RN, You L, Xu Z, Reguart N, Mikami I, Batra S, Rosell R, Jablons DM (2005a) Wnt signaling in stem cells and non-small-cell lung cancer. Clin Lung Cancer 7(1):54–60

He B, Reguart N, You L, Mazieres J, Xu Z, Lee AY, Mikami I, McCormick F, Jablons DM (2005b) Blockade of Wnt-1 signaling induces apoptosis in human colorectal cancer cells containing downstream mutations. Oncogene 24(18):3054–3058

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(10):4827–4833

Hommura F, Furuuchi K, Yamazaki K, Ogura S, Kinoshita I, Shimizu M, Moriuchi T, Katoh H, Nishimura M, Dosaka-Akita H (2002) Increased expression of beta-catenin predicts better prognosis in nonsmall cell lung carcinomas. Cancer 94(3):752–758

Hooper JE, Scott MP (2005) Communicating with Hedgehogs. Nat Rev Mol Cell Biol 6(4):306–317

Howlader N, Noone AM, Krapcho M, Garshell J, Neyman N, Altekruse SF, Kosary CL, Yu M, Ruhl J, Tatalovich Z, Cho H, Mariotto A, Lewis DR, Chen HS, Feuer EJ, Cronin KA (eds) (2013) SEER cancer statistics review, 1975–2010. National Cancer Institute, Bethesda. http://seer.cancer.gov/csr/1975_2010/. Based on Nov 2012 SEER data submission, posted to the SEER web site, Apr 2013

Huangfu D, Anderson KV (2006) Signaling from Smo to Ci/Gli: conservation and divergence of Hedgehog pathways from Drosophila to vertebrates. Development 133(1):3–14

Hyman JM, Firestone AJ, Heine VM, Zhao Y, Ocasio CA, Han K, Sun M, Rack PG, Sinha S, Wu JJ, Solow-Cordero DE, Jiang J, Rowitch DH, Chen JK (2009) Small-molecule inhibitors reveal multiple strategies for Hedgehog pathway blockade. Proc Natl Acad Sci USA 106(33):14132–14137

Imbimbo BP (2008) Therapeutic potential of gamma-secretase inhibitors and modulators. Curr Top Med Chem 8(1):54–61

Ito T, Udaka N, Yazawa T, Okudela K, Hayashi H, Sudo T, Guillemot F, Kageyama R, Kitamura H (2000) Basic helix-loop-helix transcription factors regulate the neuroendocrine differentiation of fetal mouse pulmonary epithelium. Development 127(18):3913–3921

Jeremy George P, Banerjee AK, Read CA, O'Sullivan C, Falzon M, Pezzella F, Nicholson AG, Shaw P, Laurent G, Rabbitts PH (2007) Surveillance for the detection of early lung cancer in patients with bronchial dysplasia. Thorax 62(1):43–50

Jiang F, Qiu Q, Khanna A, Todd NW, Deepak J, Xing L, Wang H, Liu Z, Su Y, Stass SA, Katz RL (2009) Aldehyde dehydrogenase 1 is a tumor stem cell-associated marker in lung cancer. Mol Cancer Res 7(3):330–338

Jimeno A, Weiss GJ, Miller WH Jr, Gettinger S, Eigl BJ, Chang AL, Dunbar J, Devens S, Faia K, Skliris G, Kutok J, Lewis KD, Tibes R, Sharfman WH, Ross RW, Rudin CM (2013) Phase I study of the Hedgehog pathway inhibitor IPI-926 in adult patients with solid tumors. Clin Cancer Res 19(10):2766–2774

Kase S, Sugio K, Yamazaki K, Okamoto T, Yano T, Sugimachi K (2000) Expression of E-cadherin and beta-catenin in human non-small cell lung cancer and the clinical significance. Clin Cancer Res 6(12):4789–4796

Kelsey CR, Marks LB, Hollis D, Hubbs JL, Ready NE, D'Amico TA, Boyd JA (2009) Local recurrence after surgery for early stage lung cancer: an 11-year experience with 975 patients. Cancer 115(22):5218–5227

Kengaku M, Capdevila J, Rodriguez-Esteban C, De La Pena J, Johnson RL, Izpisua Belmonte JC, Tabin CJ (1998) Distinct WNT pathways regulating AER formation and dorsoventral polarity in the chick limb bud. Science 280(5367):1274–1277

Kim MA, Lee HS, Lee HE, Kim JH, Yang HK, Kim WH (2009) Prognostic importance of epithelial-mesenchymal transition-related protein expression in gastric carcinoma. Histopathology 54(4):442–451

Klinakis A, Lobry C, Abdel-Wahab O, Oh P, Haeno H, Buonamici S, van De Walle I, Cathelin S, Trimarchi T, Araldi E, Liu C, Ibrahim S, Beran M, Zavadil J, Efstratiadis A, Taghon T, Michor F, Levine RL, Aifantis I (2011) A novel tumour-suppressor function for the Notch pathway in myeloid leukaemia. Nature 473(7346):230–233

Ko YH, Won HS, Jeon EK, Hong SH, Roh SY, Hong YS, Byun JH, Jung CK, Kang JH (2011) Prognostic significance of CD44s expression in resected non-small cell lung cancer. BMC Cancer 11:340

Lauth M, Toftgard R (2007) Non-canonical activation of GLI transcription factors: implications for targeted anti-cancer therapy. Cell Cycle 6(20):2458–2463

Lee AY, He B, You L, Dadfarmay S, Xu Z, Mazieres J, Mikami I, McCormick F, Jablons DM (2004) Expression of the secreted frizzled-related protein gene family is downregulated in human mesothelioma. Oncogene 23(39):6672–6676

Leung EL, Fiscus RR, Tung JW, Tin VP, Cheng LC, Sihoe AD, Fink LM, Ma Y, Wong MP (2010) Non-small cell lung cancer cells expressing CD44 are enriched for stem cell-like properties. PLoS One 5(11), e14062

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(8), e3077

Lin Y, Wang X, Jin H (2014) EGFR-TKI resistance in NSCLC patients: mechanisms and strategies. Am J Cancer Res 4(5):411–435

Linnoila I (1990) Pathology of non-small cell lung cancer. New diagnostic approaches. Hematol Oncol Clin North Am 4(6):1027–1051

Loebinger MR, Giangreco A, Groot KR, Prichard L, Allen K, Simpson C, Bazley L, Navani N, Tibrewal S, Davies D, Janes SM (2008) Squamous cell cancers contain a side population of stem-like cells that are made chemosensitive by ABC transporter blockade. Br J Cancer 98(2):380–387

LoRusso PM, Rudin CM, Reddy JC, Tibes R, Weiss GJ, Borad MJ, Hann CL, Brahmer JR, Chang I, Darbonne WC, Graham RA, Zerivitz KL, Low JA, Von Hoff DD (2011) Phase I trial of hedgehog pathway inhibitor vismodegib (GDC-0449) in patients with refractory, locally advanced or metastatic solid tumors. Clin Cancer Res 17(8):2502–2511

Malanchi I, Peinado H, Kassen D, Hussenet T, Metzger D, Chambon P, Huber M, Hohl D, Cano A, Birchmeier W, Huelsken J (2008) Cutaneous cancer stem cell maintenance is dependent on beta-catenin signalling. Nature 452(7187):650–653

Mareel M, Boterberg T, Noe V, Van Hoorde L, Vermeulen S, Bruyneel E, Bracke M (1997) E-cadherin/catenin/cytoskeleton complex: a regulator of cancer invasion. J Cell Physiol 173(2):271–274

Massague J (2008) TGFbeta in cancer. Cell 134(2):215–230

Mazieres J, He B, You L, Xu Z, Lee AY, Mikami I, Reguart N, Rosell R, McCormick F, Jablons DM (2004) Wnt inhibitory factor-1 is silenced by promoter hypermethylation in human lung cancer. Cancer Res 64(14):4717–4720

Mazieres J, He B, You L, Xu Z, Jablons DM (2005) Wnt signaling in lung cancer. Cancer Lett 222(1):1–10

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(6):1040–1046

Meuwissen R, Berns A (2005) Mouse models for human lung cancer. Genes Dev 19(6):643–664

Mimeault M, Batra SK (2010) Frequent deregulations in the hedgehog signaling network and cross-talks with the epidermal growth factor receptor pathway involved in cancer progression and targeted therapies. Pharmacol Rev 62(3):497–524

Miraglia S, Godfrey W, Yin AH, Atkins K, Warnke R, Holden JT, Bray RA, Waller EK, Buck DW (1997) A novel five-transmembrane hematopoietic stem cell antigen: isolation, characterization, and molecular cloning. Blood 90(12):5013–5021

Moreb JS, Zucali JR, Ostmark B, Benson NA (2007) Heterogeneity of aldehyde dehydrogenase expression in lung cancer cell lines is revealed by Aldefluor flow cytometry-based assay. Cytometry B Clin Cytom 72(4):281–289

Moreb JS, Baker HV, Chang LJ, Amaya M, Lopez MC, Ostmark B, Chou W (2008) ALDH isozymes downregulation affects cell growth, cell motility and gene expression in lung cancer cells. Mol Cancer 7:87

Morrisey EE (2003) Wnt signaling and pulmonary fibrosis. Am J Pathol 162(5):1393–1397

Mullor JL, Dahmane N, Sun T, Ruiz i Altaba A (2001) Wnt signals are targets and mediators of Gli function. Curr Biol 11(10):769–773

Nakano T, Tani M, Ishibashi Y, Kimura K, Park YB, Imaizumi N, Tsuda H, Aoyagi K, Sasaki H, Ohwada S, Yokota J (2003) Biological properties and gene expression associated with metastatic potential of human osteosarcoma. Clin Exp Metastasis 20(7):665–674

Ng JM, Curran T (2011) The Hedgehog's tale: developing strategies for targeting cancer. Nat Rev Cancer 11(7):493–501

Nicolas M, Wolfer A, Raj K, Kummer JA, Mill P, van Noort M, Hui CC, Clevers H, Dotto GP, Radtke F (2003) Notch1 functions as a tumor suppressor in mouse skin. Nat Genet 33(3):416–421

Ohira T, Gemmill RM, Ferguson K, Kusy S, Roche J, Brambilla E, Zeng C, Baron A, Bemis L, Erickson P, Wilder E, Rustgi A, Kitajewski J, Gabrielson E, Bremnes R, Franklin W, Drabkin HA (2003) WNT7a induces E-cadherin in lung cancer cells. Proc Natl Acad Sci USA 100(18):10429–10434

Olive KP, Jacobetz MA, Davidson CJ, Gopinathan A, McIntyre D, Honess D, Madhu B, Goldgraben MA, Caldwell ME, Allard D, Frese KK, Denicola G, Feig C, Combs C, Winter SP, Ireland-Zecchini H, Reichelt S, Howat WJ, Chang A, Dhara M, Wang L, Ruckert F, Grutzmann R, Pilarsky C, Izeradjene K, Hingorani SR, Huang P, Davies SE, Plunkett W, Egorin M, Hruban RH, Whitebread N, McGovern K, Adams J, Iacobuzio-Donahue C, Griffiths J, Tuveson DA (2009) Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. Science 324(5933):1457–1461

Osanyingbemi-Obidi J, Dobromilskaya I, Illei PB, Hann CL, Rudin CM (2011) Notch signaling contributes to lung cancer clonogenic capacity in vitro but may be circumvented in tumorigenesis in vivo. Mol Cancer Res 9(12):1746–1754

Park KS, Martelotto LG, Peifer M, Sos ML, Karnezis AN, Mahjoub MR, Bernard K, Conklin JF, Szczepny A, Yuan J, Guo R, Ospina B, Falzon J, Bennett S, Brown TJ, Markovic A, Devereux WL, Ocasio CA, Chen JK, Stearns T, Thomas RK, Dorsch M, Buonamici S, Watkins DN, Peacock CD, Sage J (2011) A crucial requirement for Hedgehog signaling in small cell lung cancer. Nat Med 17(11):1504–1508

Pasca di Magliano M, Sekine S, Ermilov A, Ferris J, Dlugosz AA, Hebrok M (2006) Hedgehog/Ras interactions regulate early stages of pancreatic cancer. Genes Dev 20(22):3161–3173

Qiu X, Wang Z, Li Y, Miao Y, Ren Y, Luan Y (2012) Characterization of sphere-forming cells with stem-like properties from the small cell lung cancer cell line H446. Cancer Lett 323(2):161–170

Rawlins EL, Ostrowski LE, Randell SH, Hogan BL (2007) Lung development and repair: contribution of the ciliated lineage. Proc Natl Acad Sci USA 104(2):410–417

Rawlins EL, Okubo T, Xue Y, Brass DM, Auten RL, Hasegawa H, Wang F, Hogan BL (2009) The role of Scgb1a1+ Clara cells in the long-term maintenance and repair of lung airway, but not alveolar, epithelium. Cell Stem Cell 4(6):525–534

Real PJ, Ferrando AA (2009) NOTCH inhibition and glucocorticoid therapy in T-cell acute lymphoblastic leukemia. Leukemia 23(8):1374–1377

Retera JM, Leers MP, Sulzer MA, Theunissen PH (1998) The expression of beta-catenin in non-small-cell lung cancer: a clinicopathological study. J Clin Pathol 51(12):891–894

Reya T, Morrison SJ, Clarke MF, Weissman IL (2001) Stem cells, cancer, and cancer stem cells. Nature 414(6859):105–111

Reya T, Duncan AW, Ailles L, Domen J, Scherer DC, Willert K, Hintz L, Nusse R, Weissman IL (2003) A role for Wnt signalling in self-renewal of haematopoietic stem cells. Nature 423(6938):409–414

Reynolds SD, Giangreco A, Power JH, Stripp BR (2000) Neuroepithelial bodies of pulmonary airways serve as a reservoir of progenitor cells capable of epithelial regeneration. Am J Pathol 156(1):269–278

Riely GJ, Kris MG, Rosenbaum D, Marks J, Li A, Chitale DA, Nafa K, Riedel ER, Hsu M, Pao W, Miller VA, Ladanyi M (2008) Frequency and distinctive spectrum of KRAS mutations in never smokers with lung adenocarcinoma. Clin Cancer Res 14(18):5731–5734

Rock JR, Onaitis MW, Rawlins EL, Lu Y, Clark CP, Xue Y, Randell SH, Hogan BL (2009) Basal cells as stem cells of the mouse trachea and human airway epithelium. Proc Natl Acad Sci USA 106(31):12771–12775

Rossi A, Maione P, Sacco PC, Sgambato A, Casaluce F, Ferrara ML, Palazzolo G, Ciardiello F, Gridelli C (2014) ALK inhibitors and advanced non-small cell lung cancer (review). Int J Oncol 45(2):499–508

Saitoh T, Mine T, Katoh M (2002) Frequent up-regulation of WNT5A mRNA in primary gastric cancer. Int J Mol Med 9(5):515–519

Salnikov AV, Gladkich J, Moldenhauer G, Volm M, Mattern J, Herr I (2010) CD133 is indicative for a resistance phenotype but does not represent a prognostic marker for survival of non-small cell lung cancer patients. Int J Cancer 126(4):950–958

Schnidar H, Eberl M, Klingler S, Mangelberger D, Kasper M, Hauser-Kronberger C, Regl G, Kroismayr R, Moriggl R, Sibilia M, Aberger F (2009) Epidermal growth factor receptor signaling synergizes with Hedgehog/GLI in oncogenic transformation via activation of the MEK/ERK/JUN pathway. Cancer Res 69(4):1284–1292

Schoenwolf GC, Bleyl SB, Brauer PR, Francis-West PH (2009) Larsen's human embryology, 4th edn. Elsevier, New York

Schulte G, Bryja V (2007) The Frizzled family of unconventional G-protein-coupled receptors. Trends Pharmacol Sci 28(10):518–525

Sekulic A, Migden MR, Oro AE, Dirix L, Lewis KD, Hainsworth JD, Solomon JA, Yoo S, Arron ST, Friedlander PA, Marmur E, Rudin CM, Chang AL, Low JA, Mackey HM, Yauch RL, Graham RA, Reddy JC, Hauschild A (2012) Efficacy and safety of vismodegib in advanced basal-cell carcinoma. N Engl J Med 366(23):2171–2179

Shan J, Shi DL, Wang J, Zheng J (2005) Identification of a specific inhibitor of the dishevelled PDZ domain. Biochemistry 44(47):15495–15503

Shigemitsu K, Sekido Y, Usami N, Mori S, Sato M, Horio Y, Hasegawa Y, Bader SA, Gazdar AF, Minna JD, Hida T, Yoshioka H, Imaizumi M, Ueda Y, Takahashi M, Shimokata K (2001) Genetic alteration of the beta-catenin gene (CTNNB1) in human lung cancer and malignant mesothelioma and identification of a new 3p21.3 homozygous deletion. Oncogene 20(31):4249–4257

Shu W, Jiang YQ, Lu MM, Morrisey EE (2002) Wnt7b regulates mesenchymal proliferation and vascular development in the lung. Development 129(20):4831–4842

Snyder JC, Teisanu RM, Stripp BR (2009) Endogenous lung stem cells and contribution to disease. J Pathol 217(2):254–264

Stanton BZ, Peng LF, Maloof N, Nakai K, Wang X, Duffner JL, Taveras KM, Hyman JM, Lee SW, Koehler AN, Chen JK, Fox JL, Mandinova A, Schreiber SL (2009) A small molecule that binds Hedgehog and blocks its signaling in human cells. Nat Chem Biol 5(3):154–156

Staudt MR, Buro-Auriemma LJ, Walters MS, Salit J, Vincent T, Shaykhiev R, Mezey JG, Tilley AE, Kaner RJ, Ho MW, Crystal RG (2014) Airway basal stem/progenitor cells have diminished capacity to regenerate airway epithelium in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 190(8):955–958

Stecca B, Mas C, Clement V, Zbinden M, Correa R, Piguet V, Beermann F, Ruiz IAA (2007) Melanomas require HEDGEHOG-GLI signaling regulated by interactions between GLI1 and the RAS-MEK/AKT pathways. Proc Natl Acad Sci U S A 104(14):5895–5900

Sterlacci W, Savic S, Fiegl M, Obermann E, Tzankov A (2014) Putative stem cell markers in non-small-cell lung cancer: a clinicopathologic characterization. J Thorac Oncol 9(1):41–49

Stupp R, Monnerat C, Turrisi AT 3rd, Perry MC, Leyvraz S (2004) Small cell lung cancer: state of the art and future perspectives. Lung Cancer 45(1):105–117

Sullivan JP, Spinola M, Dodge M, Raso MG, Behrens C, Gao B, Schuster K, Shao C, Larsen JE, Sullivan LA, Honorio S, Xie Y, Scaglioni PP, DiMaio JM, Gazdar AF, Shay JW, Wistuba II, Minna JD (2010) Aldehyde dehydrogenase activity selects for lung adenocarcinoma stem cells dependent on notch signaling. Cancer Res 70(23):9937–9948

Sunaga N, Kohno T, Kolligs FT, Fearon ER, Saito R, Yokota J (2001) Constitutive activation of the Wnt signaling pathway by CTNNB1 (beta-catenin) mutations in a subset of human lung adenocarcinoma. Genes Chromosomes Cancer 30(3):316–321

Sutherland KD, Proost N, Brouns I, Adriaensen D, Song JY, Berns A (2011) Cell of origin of small cell lung cancer: inactivation of Trp53 and Rb1 in distinct cell types of adult mouse lung. Cancer Cell 19(6):754–764

Taipale J, Chen JK, Cooper MK, Wang B, Mann RK, Milenkovic L, Scott MP, Beachy PA (2000) Effects of oncogenic mutations in Smoothened and Patched can be reversed by cyclopamine. Nature 406(6799):1005–1009

Takebe N, Ivy SP (2010) Controversies in cancer stem cells: targeting embryonic signaling pathways. Clin Cancer Res 16(12):3106–3112

Takebe N, Harris PJ, Warren RQ, Ivy SP (2011) Targeting cancer stem cells by inhibiting Wnt, Notch, and Hedgehog pathways. Nat Rev Clin Oncol 8(2):97–106

Teixeira VH, Nadarajan P, Graham TA, Pipinikas CP, Brown JM, Falzon M, Nye E, Poulsom R, Lawrence D, Wright NA, McDonald S, Giangreco A, Simons BD, Janes SM (2013) Stochastic homeostasis in human airway epithelium is achieved by neutral competition of basal cell progenitors. Elife 2, e00966

Travis WD (2011) Pathology of lung cancer. Clin Chest Med 32(4):669–692

Tsao PN, Chen F, Izvolsky KI, Walker J, Kukuruzinska MA, Lu J, Cardoso WV (2008) Gamma-secretase activation of notch signaling regulates the balance of proximal and distal fates in progenitor cells of the developing lung. J Biol Chem 283(43):29532–29544

Ueda M, Gemmill RM, West J, Winn R, Sugita M, Tanaka N, Ueki M, Drabkin HA (2001) Mutations of the beta- and gamma-catenin genes are uncommon in human lung, breast, kidney, cervical and ovarian carcinomas. Br J Cancer 85(1):64–68

Uematsu K, He B, You L, Xu Z, McCormick F, Jablons DM (2003a) Activation of the Wnt pathway in non small cell lung cancer: evidence of dishevelled overexpression. Oncogene 22(46):7218–7221

Uematsu K, Kanazawa S, You L, He B, Xu Z, Li K, Peterlin BM, McCormick F, Jablons DM (2003b) Wnt pathway activation in mesothelioma: evidence of Dishevelled overexpression and transcriptional activity of beta-catenin. Cancer Res 63(15):4547–4551

Varjosalo M, Taipale J (2008) Hedgehog: functions and mechanisms. Genes Dev 22(18):2454–2472

Veeman MT, Axelrod JD, Moon RT (2003) A second canon. Functions and mechanisms of beta-catenin-independent Wnt signaling. Dev Cell 5(3):367–377

Von Hoff DD, LoRusso PM, Rudin CM, Reddy JC, Yauch RL, Tibes R, Weiss GJ, Borad MJ, Hann CL, Brahmer JR, Mackey HM, Lum BL, Darbonne WC, Marsters JC Jr, de Sauvage FJ, Low JA (2009) Inhibition of the hedgehog pathway in advanced basal-cell carcinoma. N Engl J Med 361(12):1164–1172

Wang W, Chen Y, Deng J, Zhou J, Zhou Y, Wang S, Zhou J (2014) The prognostic value of CD133 expression in non-small cell lung cancer: a meta-analysis. Tumour Biol 35(10):9769–9775

Watkins DN, Berman DM, Burkholder SG, Wang B, Beachy PA, Baylin SB (2003) Hedgehog signalling within airway epithelial progenitors and in small-cell lung cancer. Nature 422(6929):313–317

Westhoff B, Colaluca IN, D'Ario G, Donzelli M, Tosoni D, Volorio S, Pelosi G, Spaggiari L, Mazzarol G, Viale G, Pece S, Di Fiore PP (2009) Alterations of the Notch pathway in lung cancer. Proc Natl Acad Sci USA 106(52):22293–22298

Wissmann C, Wild PJ, Kaiser S, Roepcke S, Stoehr R, Woenckhaus M, Kristiansen G, Hsieh JC, Hofstaedter F, Hartmann A, Knuechel R, Rosenthal A, Pilarsky C (2003) WIF1, a component of the Wnt pathway, is down-regulated in prostate, breast, lung, and bladder cancer. J Pathol 201(2):204–212

Xu X, Rock JR, Lu Y, Futtner C, Schwab B, Guinney J, Hogan BL, Onaitis MW (2012) Evidence for type II cells as cells of origin of K-Ras-induced distal lung adenocarcinoma. Proc Natl Acad Sci USA 109(13):4910–4915

Yamaguchi TP, Bradley A, McMahon AP, Jones S (1999) A Wnt5a pathway underlies outgrowth of multiple structures in the vertebrate embryo. Development 126(6):1211–1223

Yang X, Yang K, Kuang K (2014) The efficacy and safety of EGFR inhibitor monotherapy in non-small cell lung cancer: a systematic review. Curr Oncol Rep 16(6):390

You L, He B, Xu Z, Uematsu K, Mazieres J, Fujii N, Mikami I, Reguart N, McIntosh JK, Kashani-Sabet M, McCormick F, Jablons DM (2004a) An anti-Wnt-2 monoclonal antibody induces apoptosis in malignant melanoma cells and inhibits tumor growth. Cancer Res 64(15):5385–5389

You L, He B, Xu Z, Uematsu K, Mazieres J, Mikami I, Reguart N, Moody TW, Kitajewski J, McCormick F, Jablons DM (2004b) Inhibition of Wnt-2-mediated signaling induces programmed cell death in non-small-cell lung cancer cells. Oncogene 23(36):6170–6174

Zhang WC, Shyh-Chang N, Yang H, Rai A, Umashankar S, Ma S, Soh BS, Sun LL, Tai BC, Nga ME, Bhakoo KK, Jayapal SR, Nichane M, Yu Q, Ahmed DA, Tan C, Sing WP, Tam J, Thirugananam A, Noghabi MS, Pang YH, Ang HS, Mitchell W, Robson P, Kaldis P, Soo RA, Swarup S, Lim EH, Lim B (2012) Glycine decarboxylase activity drives non-small cell lung cancer tumor-initiating cells and tumorigenesis. Cell 148(1–2):259–272

Zheng D, Limmon GV, Yin L, Leung NH, Yu H, Chow VT, Chen J (2013) A cellular pathway involved in Clara cell to alveolar type II cell differentiation after severe lung injury. PLoS One 8(8), e71028