Lung cancer stem cells research

Clues from ontogeny

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ABSTRACT

تدعمنا نظرية خلايا جذع السرطان (CSC) مع الطريقة الجديدة لتفهم الجينات السرطانية، والاستراتيجيات الوقاية والعلاجية. في السنوات الأخيرة، تمت دراسة المصدر والصفات الحيوية لخلايا جذع السرطان (CSC) في الأورام الصلبة. من المهم معرفة الربط النسبي بين (CSC) من سلف خلايا جذع النخاع (ESC) خلال تطور المصدر. في هذه المراجعة، نقترح أن بعض الطرق الرئيسية خلال نمو وتطور سرطان الرئة باتت أمراً حاسما في التجديد الذاتي غير الطبيعي وتمييز خلايا جذع سرطان الرئة وكذلك محاولة تنظيم (CSCs) للرئة من وجهة نظر تطور الفرد بالمقارنة مع السلالة.

Cancer stem cell (CSC) hypothesis provides us with a new approach to the understanding of carcinogenesis, therapeutics, and prevention strategies. In recent years, the origin and biological characteristics of CSC were widely studied in solid tumors; it is astonishing to find out the delicate relevancy between CSC and committed progenitors evolved from embryonic stem cells (ESC) during organ development. In this review, we propose that some key molecular signal pathways during lung development are crucial for abnormal self-renewal and differentiation of lung cancer stem cell as well as try to elaborate the lung CSCs from the point view of ontogeny.

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Lung cancer is a great threaten to the health of human especially those who smoke. Figures provided by the National Cancer Institute of National Institutes of Health (NIH) showed an estimated 219,440 new cases and 159,390 deaths from lung cancer in the United States in 2009.[1] The traditional clonal evolution model for lung cancer cannot explain the high risk of recurrence and drug resistance. Cancer stem cell (CSC) hypothesis brings new dawn to the "bottleneck" of lung cancer research. Cancer stem cell theory advocates hierarchy exist in cells within tumor population, only a small proportion of cell exhibits the distinct proliferative and differentiative capacities, this part of cells are potential CSCs or cancer stem like cells.[2]

This review summarizes and evaluates the current evidence for the existence of CSCs in lung cancer. We compare the CSCs that have been prospectively isolated from different subtypes or cell lineages, with emphasis on the cell origin and micro-environment. We try to elucidate the molecular signals for lung development during embryonic period and its potential roles in CSCs activation which was demonstrated in other tumor types.[3] It is obvious that the progenitors cells committed to form lung differ from the lung CSCs, although they both exhibit proliferative and differentiative capacities. The former is regulated by a delicate procedure with molecular signals involved and spatial axis dominated, result in the airway branching and subsequent development;[4] the latter show excessive uncontrollable self-renewal and lead to neoplasm formation. This difference may attribute to a distinct genetic, epigenetic phenotype or diverse micro-environments. This article tries to elucidate silhouette of lung CSCs in comparison with its normal counterparts, hoping to get some suggestions on precise isolation and proliferation regulation or probably target therapy aiming at CSCs.

Cancer stem cells isolated from lung cancer. Cancer stem cells refer to a subset of tumor cells that has the ability to self-renew and generate the diverse cells which comprise the tumors. Cancer stem cells was first discovered in acute myeloid leukemia (AML), a minute

proportion made up of approximately 1/106 of the total cells that can seed tumor growth when transplanted into sublethally irradiated non-obese diabetic (NOD) with severe combined immunodeficient (SCID) mice.[5] During the following years, the CSCs research were confined to the hematopoietic system for the convenience of isolation and identification. However, solid tumors account for the major cancer burden, and epithelial cancers arising in tissues that include breast, lung, colon, prostate, and ovary constitute approximately 80% of all cancer. Therefore, it is necessary to find a new way to settle the high risk of recurrence and drug resistance in solid tumors. Later, as marker for CSCs were subsequently discovered in different solid tumor,[5] the research on CSCs developed.

The importance of CSCs in tumorigenesis has been demonstrated for several tumor types.[7-9] An important criterion of CSCs is that they enable serial propagation of tumors that retain the diverse marker profile of the primary tumor.[10] Depending on how the cells look under the microscope, Lung cancer is divided into 2 main categories; and the therapeutic method is quite different. The research on CSCs may start from the origin of each subtype (**Figure 1**). Are they pluripotent stem cells, which can differentiate into committed stem cells of each subtype, or are they independent

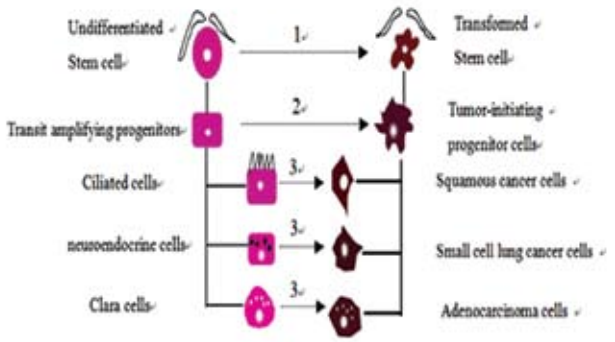

Figure 1 - Hierarchy of lung cancer stem cells (CSCs) and its normal counterpart. Model of the lung cancer stem cell hierarchy compared to the "classical" hierarchy of undifferentiated epithelial stem cell. The constructions of lung CSCs hierarchy resembles the differentiation process of adult stem cells reside along the airway. Malignant transformation may happens in any step from undifferentiated stem cell to differentiated cells such as ciliated cells. If the transformation happens in premature stage (dashed arrow 1 & 2), the transformed cells acquired the self-renewal and differentiated properties and initiated lung cancer. Whether the subtypes are determined this step is unknown since only CD133 is shown to expressed in the Tumor-initiating progenitor cells (Eramo[18]). The direct transformations in more differentiated cells offend the theory of CSCs (dashed arrow 3). However, the point-to-point relationship of histology and pathology may suggest the existence of a set of more differentiated progenitor cells.

without hereditary correlation? Injury models have suggested the existence of distinct epithelial stem cell population that located in the same position, which adenocarcinomas often arise.[11] Kim et al[12] discovered a group of cells at bronchioalveolar duct junction carrying clara cell and alveolar cell markers refractory to naphthalene treatment and stared to divide after naphthalene-induced damage. This cluster of cells can enriched FACS-sorted Sca-1pos/CD34pos and showed enhanced capacity for both self-renewal and differentiation in vitro. Activation of oncogenic protein K-ras boosted the proliferation of the double-positive cells and accelerates tumorigenesis. Those Sca-1pos/CD34pos cells were termed as bronchioalveolar stem cells which maybe the origin of adenocarcinomas.[12] Small cell lung cancer has a complicated histological origins of high-grade malignancy; its cell origin is still enigmatic. The discovery of co-expression of stem cell factor and receptor may stimulate the autocrine growth of small cell lung cancer, making the possibilities the existence of stem-like cells.[13] Stem cell markers PODXL-1 and Bmi-1 were found widely expressed in small lung cancer, however, lacking of known surface maker making it impossible to isolate potential CSCs by FACs.[14] The same disappointment was the discovery of ALDH as a potential marker for NSCLC precursors.[15] Stem cell subpopulation was gathered from human lung cancer A549 cells using FAC/Hoechst 33342 and show a unique ability to resist doxorubicin (DOX) and methotrexate (MTX) treatment,[16] making it easier to segregate potential CSCs. However, ascribing CSC characteristics to a cell population without evidence for differentiation and self-renewal in vivo may weaken the term "stem cells",[17] and tumor initiating cells may not always represent CSCs.[18] Eramo[19] proposed the existence of a precise hierarchical model with CD133+ at the highest rank. CD133+ cells were found in all the histological specimen derived from lung cancer biopsy, although generally infrequent, but consistently detectable compared with in control normal lung tissue specimen. Moreover, the in vitro cell culture with isolated CD133+ displayed the ability to generate differentiated lung cancer cells phenotypically similar to the major cancer cell population present in the original tumor. These observations indicate that the different types of lung cancers are maintained by aberrant immature cells committed to different lineages.[19]

The currently existed isolation protocols for lung CSCs are far from perfect (**Figure 2**). Cluster of differentiation antigen stands for the same surface antigens recognized by antibodies from different laboratories.

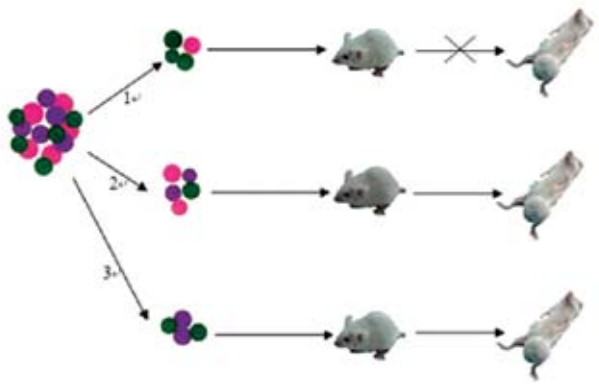

Figure 2 - Conventional isolation of cancer stem cell (CSCs) by FCM. Tumor cells suspension consist of various heterogeneous cells including CSCs (purple). When those cells are marked with different surface markers as shown above, subclone of cells are collected after FCM cell sorting based on their specific antigen. Case 1 showed a failure of xenograft model owing to negative selecting of CSCs, Case2 and 3 formed xenograft tumors by positive selecting of CSCs. Cases 2 and 3 subclone cells are not pure CSCs since the surface marker are not exclusive to CSCs. However, those clones are arbitrarily defined as tumor CSCs.

It was successfully been used in hematopoietic system for the description of blood cell lineage maturation; different CD positive cell clusters showed unique development stages. This method of isolation targeted cells seems to be quick and precise. However, the CD used for isolation of CSCs by FACs lack sufficient proof, are used to isolation targeted CSCs as they expressed in other tumor stem cell. Species difference is another problem. In Kim et al[12] experiment, mouse lung CSCs express Sca-1 and high levels of CD34. Sca-1 is not expressed in humans, while CD34 expression does not seem to characterize similar stem cell population in mice and human.[12,20] Furthermore, some studies that include in vivo data relied on expansion of putative stem cells in culture prior to engraftment, raising the possibility that culturing changed the potency of the isolated cell population.[21] Drug-resistant property of CSCs were utilized recently for the enrichment and was testified by the ability to form tumor spheres,[22,23] suggesting another effective way of isolating CSCs in solid tumors.

Potential niche left for lung CSCs. The mammalian lung develops a lateral bud from the ventral foregut endoderm between liver and the thymus. Lung development initiates at 5 weeks of gestation in the human and at 9 days of gestation in the mouse, and it is understood as proceeding through 4 discrete, subsequent stages: pseudoglandular, canalicular, saccular, and alveolar. Each stages is characterized morphologically, and each encompasses distinct structural, cellular, and regulatory features.[24] Through the process of development, epithelial cells lay along trachea, bronchi stretched to distal end of alveoli. Distinct patterns of epitheliums lying along the respiratory tubes are determined by proximal-distal axis during the process of branching, with BMP-4 playing an important regulatory role.[25] BMP-4 may participate in the signaling that is necessary to maintain developing lung epithelium in either an undifferentiated or distal committed state as defined by SPC.[26] Additionally, the inhibition of BMP-4 promotes a proximal airway phenotype as defined by CCSP expression. Lineage analysis suggests that the progenitor cells of the trachea and proximal lung differ in origin from those that will form the distal region of the lung.[27] This phenomenon may highlight the possibility that niches for lung somatic stem cell which response to lung epithelium damage may adjacent to the location of progeny. At least 2 populations of progenitor cells, giving rise respectively to the larynx and trachea versus the peripheral bronchi and alveolar surface. Current evidence supports the existence of multiple stem cell niches in the lung.[28] Bronchioalveolar stem cells named by Kim et al[12] are a stem cell population for distal lung epithelia with potentiality limited to Clara, AT2, and AT1 cells. Their location in the BADJ places BASCs next to each of the niches in which their putative progeny reside. BASCs were expanded at the early stages of tumorgenesis in vivo and exhibited the first proliferative response following K-ras G12D activation in culture. Normally, the microenvironment provided by niche will keep somatic stem cells in quiescent states until it will be activated by injury or some oncogenic signaling. It implies that CSCs might share a niche with normal stem cells, the identification of normal stem cell niches will provide us information on CSCs residence. Potential niches for CSCs located in the basal layer of the upper airways, within or near pulmonary neuroendocrine cell rests as well as at the bronchoalveolar junction.[12,29-31]

Cancer stem cells are quit different from normal stem cells with distinct characteristics of excessive self-renew and uncountable proliferation. Under normal circumstances, growth inhibitory signals from the niche prevent uncontrolled self-renewal or proliferation of progenitor cells.[32] However, loss of communication between the stem cells and the primary niche can lead to constitutive growth inducing signals and dysfunction of stem cell division.[33,34] Consequently, the deregulated stem cells can escape all inhibitory signaling and migrate to the secondary niches.[35] New niches create more suitable microenvironment for the maintaining of CSCs. Further studies should be focus on the cellular interaction, which helps CSCs to find a new home. Homing receptors, intergrin, and other adhesion molecules may play significant roles in the relocation

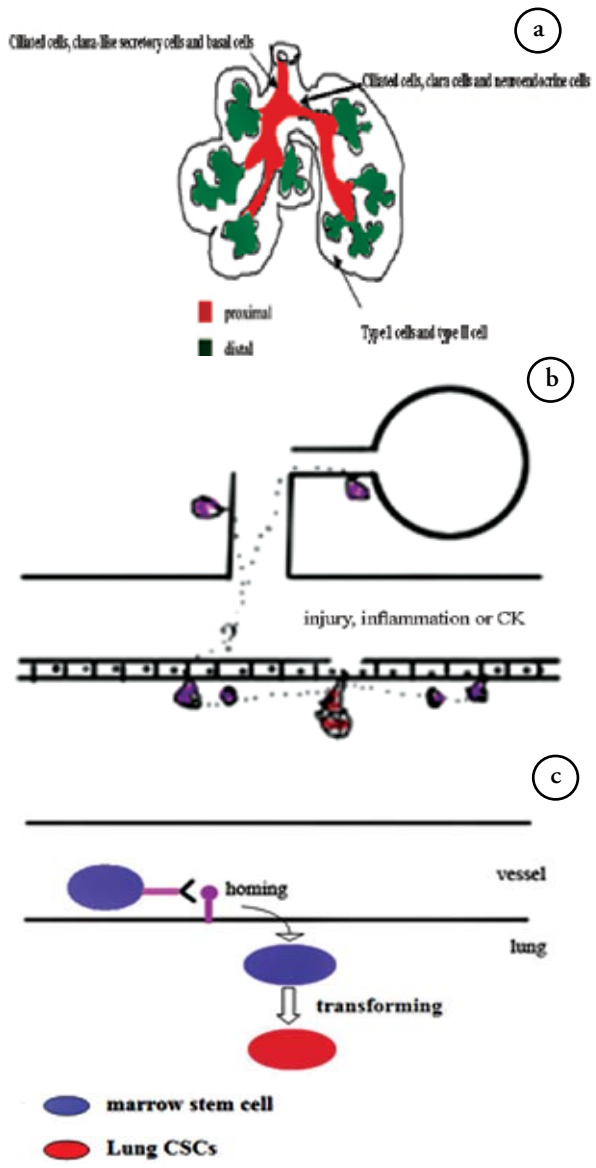

Figure 3 - Potential niches for epithelial-originated lung stem cells in physical and pathological state. **a**) During the lung development, a proximo-distal axis was engaged. Proximal airways contains mostly ciliated cells, Clara-like secretory cells, basal cells and neuroendorine cells, while distal region contains Clara cells and AT1 and AT2 cells. Dozens of signaling molecules and transcription factors were involved in this progress with BMP4 and Nkx2.1 playing pivotal roles in the correct differentiation of epithelium. Small fractions of stem cells may be kept quiescent along the airway. **b**) the primary(purple) niche consist of normal stem cells, epithelial injury made by inflammation or chemical components will lead to the recruitment of stem cell, not only one stem cells were gathered in the area, some are used for regeneration and others are located there. However, the persistence of injury made a quite different microenvironment for stem cell, the activation of some signaling molecules and transcription factors lead to the formation cancer stem cell and the second niche(red). **c**) exogenous originated stem cells such as marrow stem cells transfer to the lung by homing receptor, and transformed to a kind of stem cell adapt to the microenvironment in lung.

of CSCs. Exogenous stem cells such as bone-marrow cells may contribute to lung tissue after damage,[36,37] the possibility of recruitment stem cell from other tissue or bone marrow is still under debate (**Figure 3**).

Embryonic signal pathways in lung development and lung cancer. Because normal stem cells and CSCs share the capacity of self-renewal, it is reasonable to propose that some molecular pathways guiding the development of lung may be re-activated (**Table 1**). Evidence shows that some pathways that are classically associated with lung morphogenesis were also detected in lung oncogenesis.[38-40] During the lung development, the emphasis was put on budding as well as interacting between epithelium and mesenchyme. However, during the development of lung cancer, budding or sacculation are not seen. This means that the molecular pathways for epithelium self-renew are dominant in adult subject lung oncogenesis compared with those that guiding lung morphogenesis.

One particularly interesting pathway that has also been shown to regulate both self-renewal and oncogenesis in different organs is the Wnt signaling pathway. Wnt proteins are intercellular signaling molecules that regulate development in several organisms and contribute to cancer when disregulated.[41] During lung development, Wnt signaling appears to have a major role in the regulation of pulmonary vascular development as well as cleft formation.[42] The expression of Wnt proteins in the bone marrow suggests that they may influence HSCs as well. Consistent with the findings of Wnt/ß-catenin, mediated stem cell proliferation, ß-catenin stabilizing mutations have been identified as early events in many cancers. The abnormal activation of Wnt signaling in non-small-cell lung was also discovered.[43] Aberrant activation is usually caused by mutations and/or deregulation of many different Wnt signaling components, which is demonstrated in other cancers. Mutations in Wnt pathway components are rarely found in lung cancer. Instead, nongenetic events appear to be the major cause of aberrant activation of Wnt signaling in lung cancer. The disregulated ligands, antagonists is frequently in lung cancer, Wnt-1[44] and Wnt-2[45] overexpression were demonstrated in non-small cell lung cancer (NSCLC) and Wnt-7a,[46,47] downregulation has been reported in most lung cancer cell lines and tumor samples. Wnt-5a which has a controversial role in carcinogenesis is found significantly higher in squamous cell carcinoma than that in adenocarcinoma from 123 patients with NSCLC.[48] Although the histopathlogical type is still not clearly related to Wnt pathway, squamous cell carcinoma is prone to be more susceptible to disregulated Wnt pathway.[49] This correlation needs further investigation.

Sonic hedgehog (sHh), a mammalian hedgehog pathway ligand, mediates epithelial-mesenchymal

Table 1 - The activation of some embryonic signaling pathways in tumor.

Signaling pathways	Receptors	Targeted genes	Tumor types detected
Wnt	Frizzled	C-myc, Survivin, Cyclin-D	Mammary gland carcinoma, hepatocellular cancer, urothelial cancer, lung cancer, hair shaft tumors in the skin
sHh	Patched (Ptch)	Wnt, IGF2, PDGF receptor alpha	Glioblastoma, lung cancer, basal cell, carcinoma of skin, prostate cancer
Notch	Delta 1, Delta 3, Delta 4, Jagged 1 & 2	HES family member	Hematologic neoplasms, breast cancer, prostate cancer, lung cancer, melanomas, ovarian cancer
TGF- ß	TßR-ll, ActR-llA,B	CDK inhibitor, EMT, PDGF-B	Lung cancer, pancreatic cancer, breast cancer
Bmi-1		P16INK4, P19ARF	Hematologic Neoplasms, brain cancer, breast cancer, lung cancer

sHh - Sonic hedgehog, TGF-ß - Transforming growth factor beta, IGF2 - insulin growth factor 2, PDGF - platelet derived factor, HES - hairy/enhancer of split, EMT - Epithelial-mesenchymal transition

interactions in lung development by signaling to adjacent lung mesenchyme, as indicated by expression of the hedgehog receptor and pathway target Patched (Ptch).[38] Loss of sHh function results in severe lung defects associated with failure of branching morphogenesis.[50,51] Sonic hedgehog-null mutant mice display pulmonary hypoplasia as well as tracheoesophageal fistulas, however, proximal-distal differentiation of lung epithelium was normal in sHh-/- mice. Over-expression of sHh in distal lung epithelium resulted in the absence of functional alveoli and an increase in interstitial tissue caused by an increased proliferation of both epithelial and mesenchymal cells. The finely regulated sHh expression in designated region is crucial for normal lung development, abnormal sHh reactivation in adult individual lung epithelium may likewise end up be detrimental. Persistent hedgehog pathway is seen in small-cell lung cancer, manifested by a high level of expression of sHh and its receptors. The over-expressed sHh is thought to lead to malignant change by repeatedly expanding the airway progenitor pool.[52] Interestingly, sHh pathway is exclusively expressed in small cell lung cancer and dominantly in in vivo sample, suggesting sHh may act up on a subpopulation of cells within small-cell-lung cancer which may probably be cancer stem cell with SCLC-oriented differentiation capacity.[53]

Notch as an embryonic signal is frequently seen in a variety of developmental settings involving binary cell fate determination,[54] and stem cell renewal.[55] During mammalian lung development, the balance between endocrine and non-endocrine airway epithelial cells appears to follow such a binary notch regulation pattern. Ligands, receptors and regulators in this pathway have been studied using transgenic mice, abnormal HES - hairy/enhancer of split (HES)1 is thought to be causative of lung hypoplasia as well as reduced Clara cells.[56] Together suggest that Notch may play a role in normal lung growth, especially in Clara cell precursors. Non-small cell of lung cancers, especially adenocarcinoma, appear to actively utilize this conserved developmental pathway, elevated notch ligand, receptor and HES1 levels have been demonstrated in NSCLC lines.[57,58] The underlying relevance of Notch signaling and NSCLC is suggested by data demonstrating increased apoptosis and serum, and reduced in vitro and in vivo NSCLC tumor growth when Notch pathway is blocked by gamma-secretase inhibitor. Controversially, the total reversed effect of Notch signaling have also been seen in A549 cell line as a tumor repressor,[59] and blockade of notch signaling in tumor-bearing mice may lead to tumor regression, progression, or metastasis in several tumor cell types including lung cancer.[60] The paradox of notch signaling may attribute to the different subtypes of cells in the experiment which may responses differently to the signal, the proportion and characteristics of CSCs in specific tumor might determine the function of Notch signaling.

Transforming growth factor beta (TGF-ß), superfamily is cytokine family whose members regulate organism development and control stem-cell fate. Transforming growth factor beta evolved to regulate the expanding system of epithelial and neural tissues, the immune system, and wound repair. Virtually all human cell types are responsive to TGF-ß and this reasons the extensive effects of this pathway in a lot of

physiological and pathological process. All 3 isoforms of TGF-ß are expressed at high levels during normal lung development, being particularly important for branching morphogenesis and epithelial cell differentiation with maturing of surfactant synthesis.[61,62] Small amount of TGF-ß are still present in the adult lung, and TGF-ß is involved in normal tissue repair following lung injury. In a variety of forms of pathology, the expression of TGF-ß is increased. However, the relationship between TGF-ß and lung cancer is still elusive. Mice hemizygous for TGF-ß showed an increased incidence of adenocarcinoma compared to their normal littermate.[63] Samples from NSCLCs showed distinctive degrees of decrease in TGF-ß type ß receptor (TbetaRII) expression, and totally no expression in lung adenocarcinoma cell line (VMRC-LCD). Stable expression of TbetaRII in these cells restored TGF-ß pathway and inhibited cell proliferation and increased apoptosis.[64] Controversially, high concentration of TGF-ß was detected in bronchoalveolar lavage fluid from patients with primary lung cancer.[65] A549 cells were induced to becoming resistance to gefitinib by the epithelial to mesenchymal transition effects of TGF-ß.[65] The complexity is usually explained as the tumor-suppression effects of TGF-ß is dominant by inhibiting some oncogene expression such as c-Myc,[66] ID1,[67] while the tumor-promoting effect is the result of immunity deficiency harnessed by cancer cells using tumor-derived TGF-ß as a shield against anti-tumor immunity.[68] It is disappointing to find any data on the effects of TGF-ß on lung CSC, which maybe a potential molecular pathway in lung CSC self-renew or proliferation. This still needs further investigation.

Bmi-1, a member of the polycomb gene family, is a transcription repressor that targets p16INK4A and p19ARF, both of which suppress cell proliferation. Bmi-1 promotes self-renewal in HSC,[69,70] neuronal stem cells[71] and head squamous cell CSCs.[72] The increased expression of Bmi-1 was detected using immunohistochemistry in 58% samples from NSCLC patients, illustrating the tumor-promoting role of Bmi-1.[71] Further studies were set out to find out a possible histogenetic link developing lung, adult bronchial mucosa and SCLC, Bmi-1 were expressed ubiquitously in stromal as well as epithelial cells from the fifth week until maturity, a ubiquitous nuclear staining in normal respiratory epithelial cells, and 98.2% in SCLC cases. This study shows that Bmi-1 in the lung is not a phenotypic marker for stem cell but, instead, stains self-renewing cells in normal bronchial epithelium, proliferation cells in SCLC, and proliferating cells during fetal lung development.[14]

It can be deduced from above that embryonic signaling pathways can both exist in embryonic lung during development as well as lung neoplasms,[3] the abnormal reactivation of embryonic signaling pathways may be a result of epigenetic modification, rather than a mutation.[73,74] The concrete mechanism of the complicated signal pathways network remains illusive.[75] However, we can get some information from previous work that one particular embryonic signaling pathway may present in a specific subtypes of lung cancer, and contribute to the formation of this subtype. This may raise a question that does the embryonic signaling pathway trigger the malignant transformations of normal stem cells or merely a fuel in keeping self-renewal of cancer stem cell. Further studies are required to focus on those abnormal embryonic signaling pathways using gene chips[76] or analyzing microRNA expression,[77,78] and try to find some molecular targets for clinical prospect.

In conclusion, the development of effective, safe CSC-based therapies for the treatment of lung cancer remains a tantalizing prospect rather than a practicable possibility.[79] For CSC-based treatments to be effective, it will be imperative to understand the radical impetus in promoting CSCs self-renewal, proliferation and differentiation.[80] For it to be safe, the target should aimed exclusively at CSCs and shun normal adult stem cells. This article highlights recent progress in the identification of lung CSC and their normal counterpart. Generally, there are 3 ways to isolate CSCs and each way utilize one characteristic of stem cells. Surface markers which are most frequently used to isolate CSCs are also expressed in immature normal stem cell, side population cells utilized the property of stem cells to pump out the toxics drugs, and in vitro sphere forming is a sign of stem cells self-renew. This work enumerates general questions about the isolation and assessment of lung cancer stem cells. The committed orientation of lung CSCs are the reason of multiple histological subtypes, however, the hierarchical organization of lung CSCs is still elusive. To achieve a clearer understanding of lung CSCs, it will be important to study the normal counterpart and the possible mechanism of its malignant transformation. First, which cells are indeed CSCs during tumor progression? As the work of Kim et al,[12] co-expression of Sca and CD34 both exist in BASCs and malignant counterpart which lead to adenocarcinoma. The traditional ways of isolating CSCs may consist of a mixture of cells which attenuate the tumorigenesis potency. A more precise method should be performed to discriminate CSCs and normal adult stem cells. Second, do CSCs come from pluripotent adult stem cells or more differentiated committed stem cells? If CSCs originated from pluripotent adult stem cells, it seems CSCs can differentiated into all pathological types of lung cancer with more primitive characteristics, contrary, if CSCs come from more differentiated committed stem cells, the fate is already determined as it can only differentiated along a particular lineage.

To answer this, the lineage tracing technique should be used to testify the actual origination. Third, which signals promote proliferation, is there any lineage predisposition? Niches along the trachea for normal adult stem cells may be harnessed by CSCs, aberrant signals within the niches is utilized for abnormal proliferation of CSCs. The embryonic development of lung made a clear proximal-distal margin. Is there any possibility that this line may already determined lineage differentiation by remaining extinctive niches with potential re-activation of a particular signal pathway? The answers to these questions will not only improve fundamental knowledge but also may convert the concept of treating lung cancer by targeting CSCs from a desirable idea to a realistic possibility.

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