Chinese Journal of Cancer

Review

The role of stem cells in airway repair: implications for the origins of lung cancer

Michael S. Mulvihill, Johannes R. Kratz, Patrick Pham, David M. Jablons and Biao He

Abstract

Lung cancer is the leading cause of cancer-related deaths worldwide. Recently, advancements in our ability to identify and study stem cell populations in the lung have helped researchers to elucidate the central role that cells with stem cell-like properties may have in lung tumorigenesis. Much of this research has focused on the use of the airway repair model to study response to injury. In this review, we discuss the primary evidence of the role that cancer stem cells play in lung cancer development. The implications of a stem cell origin of lung cancer are reviewed, and the importance of ongoing research to identify novel therapeutic and prognostic targets is reiterated.

Key words: Lung tumorigenesis, cancer stem cells, airway repair, adenocarcinoma, squamous cell carcinoma

The Discovery of Stem Cells

In the 1960's, McCulloch and Till[1] proposed three functional characteristics that came to form the definition of stem cells. Their findings supported the hypothesis that a specific population of cells was responsible for propagation and sustained growth in a hierarchical manner, a theory first proposed in the middle of the 19th century by Virchow[2]. The three characteristics they identified were as follows. First, stem cells have the ability to make identical copies of themselves (self-renewal). Second, stem cells have the ability to divide rapidly (proliferation). Third, stem cells can give rise to multiple cell types (multipotency).

Assessment of a cell's ability to proliferate extensively and self-renew requires the induction of these behaviors. Thus, the cell must be isolated and the behaviors induced *in vitro* for the cell to be classified as a stem cell. To more efficiently isolate candidate stem cells, much work has turned to the use of cell surface proteins unique to stem cells, which permit isolation by flow cytometry, magnetic bead isolation, fluorescent protein tagging, and immunostaining. These markers also enable stem cells to be tracked and their functions to be characterized.

Advances in stem cell biology have provided insight into the role of stem cells in the lung. Due to the relative quiescence of the lung epithelia, with a proliferative fraction of just 0.06% to 1.3% in the bronchiolar and tracheal epithelia, identification of putative stem cells has been more difficult than in tissues with higher rates of turnover, such as the epithelia of the gut or skin[3]. Despite these challenges, populations of stem cells have been identified throughout the lung by way of their role in the response to injury of the airway. In each case, the functional tenants of stem cell behavior—namely, self-renewal, proliferation, and multipotency—have been observed. A growing body of evidence now indicates that the earliest stages of lung malignancies may be related to a deregulation of this stem cell-based repair mechanism.

Stem Cells in Repair of the Airway and Initiation of Lung Tumors

The epithelial lining of the adult airway progresses from proximal to distal in a stereotyped fashion. Proximal airways consist of pseudostratified epithelium, middle airways consist of a simple columnar epithelium, and the most distal airways consist of a cuboidal epithelium. Multiple studies indicate that cells capable of stem-like

Authors' Affiliation: Thoracic Oncology Program, Department of Surgery, Helen Diller Family Comprehensive Cancer Center, University of California San Francisco, San Francisco, CA 94115, USA.

Corresponding Author: Biao He, Thoracic Oncology Program, Department of Surgery, Helen Diller Family Comprehensive Cancer Center, 2340 Sutter Street, Box 1724, University of California San Francisco, San Francisco, CA 94115, USA. Tel: +1-415-476-6907; Email: biao.he@ucsfmedctr.org.

doi: 10.5732/cjc.012.10097

behavior in response to airway injury are present throughout the airway.

In proximal airways, studies of airway repair in the trachea have identified two cellular populations that act as stem cells in response to injury. Borthwick *et al.*[4] identified a population of tracheal basal cells that are pluripotent, retain bromodeoxyuridine, and express keratin-5 (K5). A second study using a naphthalene inhalation injury model identified an additional population of tracheal basal cells, this time expressing the keratin-14 (K14) marker[5]. These $K14^+$ cells were able to proliferate to give rise to cells of multiple phenotypes in response to airway injury. It appears that in the trachea, basal cell populations expressing either K5 or K14 are capable of the functional aspects of stem cell niches. This finding has implications for the origins of squamous cell carcinoma. Squamous cell carcinoma frequently arises from the more proximal tracheobronchial airways, yielding centrally located tumors that often exhibit elevated levels of $K5^+$ basal cells[6]. As such, it appears that cancers with histology consistent with squamous cell carcinoma are more likely to arise from $K5^+$ stem cells. Although there is no targeted therapy available against $K5^+$ cells to date, this stem cell niche of squamous cell carcinoma may provide a worthwhile direction for targeted research, providing a unique biomarker that can be used to target the cell of origin in these tumors.

In the middle airways, two potential stem cell niches have also been identified. A rat model of nitrogen dioxide (NO_2)/ozone (O_3) inhalation injury revealed that mature rat Clara cells could proliferate into phenotypically diverse progeny[7]. This ability to move from quiescent and fully differentiated epithelial cells to actively proliferating multipotent cells led Evans *et al.*[7] to deem these Clara cells "facultative progenitor cells," as they are capable of but not limited to behavior indicative of a stem cell. In the setting of injury to a Clara cell–depleted airway, as is the case with the naphthalene model of injury, Clara-cell secretory protein (CCSP)–positive cells located within neuroepithelial bodies (NEB) have been shown to proliferate to repair the airway[8]. These NEB $CCSP^+$ cells are able to independently repopulate the middle airways with phenotypically diverse progeny after naphthalene injury, characteristic of true bronchial airway stem cells. Small cell lung carcinomas, frequently arising in the mid-level bronchioles, have been shown to contain fractions of cells expressing cell surface markers associated with mid-airway stem cell subpopulations. In particular, the cell surface markers CD44 and multidrug resistance 1 (MDR1) have previously been identified in a series of small cell lung carcinoma cell lines[6]. Cells expressing CD44 have also very recently been implicated in lung cancer progression and metastasis by the epithelial-to-mesenchymal transition (EMT)[9]. In their 2011 study, Tellez *et al.*[9] found that bronchial epithelial cells exposed to carcinogens gained stem-like properties and that EMT led to a progressively motile and invasive phenotype. Again, while no targeted therapies are presently available to target cells expressing CD44 and MDR1, the potential exists for improved delivery by using agents directed at this cell or origin in conjunction with the current standard of care.

For the most distal airway, there is no consensus on the cell surface marker phenotype of representative lung stem cells. Stripp *et al.*[10] first identified pluripotent, NEB-independent, $CCSP^+$ cells in the bronchoalveolar duct junction (BADJ) that could, in response to naphthalene injury, yield diverse populations of cells. Cells expressing surface markers surfactant protein C (SP-C), stem cell antigen-1 (SCa-1), and CD34—but not expressing CD45 or Pecam—were shown to act as pluripotent and to have the ability to proliferate and self-renew in culture. However, others have argued that these $CCSP^+$/$SP\text{-}C^+$ cells are insufficient as the distal lung stem cell population because they are unable to differentiate into type II alveolar cells. Rawlins *et al.*[11] instead promoted $Id2^+$ as a more suitable cell surface marker for distal lung epithelial stem cells. Thus, while there is consensus that stem cells form a niche population in the BADJ, the cell marker phenotype remains under debate. Carcinomas of the distal airway—most frequently lung adenocarcinoma and bronchioloalveolar carcinoma—have been associated with stem cells from the BADJ region[10].

Implications of a Stem Cell Origin of Lung Cancer

The hierarchical or cancer stem cell hypothesis has proven to be an important theory with which to understand lung tumorigenesis, and may have important implications for future management of lung tumors. In this model, relatively rare, multipotent cells gain unlimited proliferative potential that may then drive tumor growth. The multipotent nature of these cells leads to heterogeneity within the tumor by aberrant differentiation and epigenetic changes in the progeny. It follows, then, that a stem-like cell, with the ability to rapidly proliferate into phenotypically diverse progeny, could act as this cancer stem cell. Stem cells are also known to express high levels of anti-apoptotic and drug-resistant proteins, which may explain why chemotherapy is extremely effective in shrinking the size of primary tumors but frequently ineffective against localized recurrence and metastatic disease. Proponents of the theory of lung cancer stem cells propose that although chemotherapy is able to effectively kill non–stem cell cancer cell types in these settings, chemoresistant lung cancer stem cells to continue to proliferate unchecked. This understanding has sparked new energy in the development of targeted

therapies against specific cell types within the heterogeneous tumor.

As introduced above, common tumors from each representative airway zone have been associated with stem cell niches based on shared cellular expression of surface markers. In addition, recent studies have successfully identified cells within lung cancer specimens that exhibit stem-like properties. Ho *et al*.[12] identified tumor cell subpopulations that exhibited several key functional properties—multidrug resistance, increased telomerase expression, and self-renewal—that are characteristic of stem cells. In addition, $CD133^+$ cells isolated from freshly resected human lung cancer specimens can grow indefinitely in culture, a characteristic usually only seen in transformed cell lines[13]. $CD133^+$ cells are also able to proliferate into phenotypically diverse tumors, both in culture and when injected into immunodeficient mice. Of high clinical interest are recent observations that patients with lung tumors enriched with these $CD133^+$ lung cancer stem cells tend to have worse prognosis than those with fewer $CD133^+$ lung cancer stem cells. In addition, these $CD133^+$ subpopulations exhibit increased resistance to cisplatin-based therapies, suggesting that the presence of $CD133^+$ cell populations may warrant changes to chemotherapeutic regimens in these patients[14]. That lung cancer stem cells impact prognosis raises the possibility, then, that major therapeutic advances in lung cancer treatment, through the specific targeting of lung cancer stem cells or the pathways that confer stem cell-like properties to lung cancer cells, are possible in the near future. In addition, measurement of the lung cancer stem cell fraction in a tumor could prove a useful modality for better differentiating between transient tumor debulking effects of chemotherapy and longer lasting therapies.

Conclusions and Next Steps

The study of the lung epithelial response to airway injury has shown compelling evidence for the role of stem cells as a component of airway repair[10]. While there is still controversy in the field, there is also a growing body of evidence to suggest that these same lung stem cells may play a key role in the initiation and proliferation of human lung cancers. Two primary pieces of evidence reviewed here support the hypothesis that lung stem cells may play a role in lung tumorigenesis: 1) cells sharing the functional properties of stem cells are present in many tumors, and 2) many tumor sites of origin match known stem cell niches. Because characterization of putative stem cells requires manipulation of the cells themselves to induce a specific phenotype, it remains exceptionally difficult to discriminate between potential or facultative and true stem cells. On account of the inducible abilities that form the critical features of stem cells, it remains possible that these cancer stem cells may exist on a continuum of inducible features. That is, if genetic and epigenetic changes can confer stem cell characteristics upon a given tumor cell, then any tumor cell may in fact have the potential to drive tumor growth and resistance. This spectrum of qualities may yield a mixed model, as proposed by Rahman *et al*.[15], in which stem-like cells may have variable potential to drive tumor growth, depending on the conditions presented to the cell.

Several key questions remain unanswered with respect to the role of stem cells in lung tumorigenesis. It remains to be conclusively determined whether healthy adult stem cells undergo oncogenic transformation leading to the initiation of tumorigenesis, or whether healthy airway cells undergo oncogenic transformation leading to their de-differentiation and subsequent adoption of stem cell-like properties. In addition, the relationship between the tumor microenvironment and the lung cancer stem cell remains poorly characterized. However, the sum of the available evidence to date, in conjunction with ever-improving modalities for the identification and isolation of putative stem cell populations, should provide a wealth of future directions that hold great promise in improving the management of patients with lung tumors.

Received: 2012-04-18; revised: 2012-08-31;
accepted: 2012-09-11.

References

[1] McCulloch EA, Till JE, Siminovitch L. The role of independent and dependent stem cells in the control of hemopoietic and immunologic responses. Wistar Inst Symp Monogr, 1965,4:61–68.
[2] Bataille R, Drouin E. Human tumor stem cells, the petri dish and the patient. A tribute to Sydney E. Salmon. Vesalius, 2011,17:42–44.
[3] Snyder JC, Teisanu RM, Stripp BR. Endogenous lung stem cells and contribution to disease. J Pathol, 2009,217:254–264.
[4] Borthwick DW, Shahbazian M, Todd Krantz Q, et al. Evidence for stem-cell niches in the tracheal epithelium. Am J Respir Cell Mol Biol, 2001,24:662–670.
[5] Hong KU, Reynolds SD, Watkins S, et al. Basal cells are a multipotent progenitor capable of renewing the bronchial epithelium. Am J Pathol, 2004,164:577–588.
[6] Giangreco A, Groot KR, Janes SM. Lung cancer and lung stem cells. Am J Respir Crit Care Med, 2007,175:547–553.
[7] Evans M, Cabral-Anderson L, Freeman G. Role of the Clara

cell in renewal of the bronchiolar epithelium. Lab Invest, 1978,38:648–653.
[8] Reynolds SD, Giangreco A, Power JH, et al. Neuroepithelial bodies of pulmonary airways serve as a reservoir of progenitor cells capable of epithelial regeneration. Am J Pathol, 2000,156: 269–278.
[9] Tellez CS, Juri DE, Do K, et al. EMT and stem cell-like properties associated with miR-205 and miR-200 epigenetic silencing are early manifestations during carcinogen-induced transformation of human lung epithelial cells. Cancer Res, 2011,71:3087–3097.
[10] Stripp BR, Maxson K, Mera R, et al. Plasticity of airway cell proliferation and gene expression after acute naphthalene injury. Am J Physiol, 1995,269:L791–L799.
[11] Rawlins EL, Clark CP, Xue Y, et al. The Id2+ distal tip lung epithelium contains individual multipotent embryonic progenitor cells. Development, 2009,136:3741–3745.
[12] Ho MM, Ng AV, Lam S, et al. Side population in human lung cancer cell lines and tumors is enriched with stem-like cancer cells. Cancer Res, 2007,67:4827–4833.
[13] Eramo A, Lotti F, Sette G, et al. Identification and expansion of the tumorigenic lung cancer stem cell population. Cell Death Differ, 2007,15:504–514.
[14] Bertolini G, Roz L, Perego P, et al. Highly tumorigenic lung cancer CD133+ cells display stem-like features and are spared by cisplatin treatment. Proc Natl Acad Sci USA, 2009,106: 16281–16286.
[15] Rahman M, Deleyrolle L, Vedam-Mai V, et al. The cancer stem cell hypothesis: failures and pitfalls. Neurosurgery, 2011,68: 531–545.