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REVIEW ARTICLE

Lung cancer stem cells: Tumor biology and clinical implications

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Abstract

The concept of cancer stem cells (CSC) has drawn great attention from researchers in both molecular and clinical fields as has brought a new perspective to the way we manage cancer. CSC have several characteristics that are shared by the properties of normal stem cells, such as differentiation, self-renewal and homeostatic control. However, CSC have the capacity to both divide and expand the CSC pool and to differentiate into heterogeneous non-tumorigenic cancer cells. Even more, CSC have an inherent high resistance to chemotherapeutic agents that leads to recurrence and poor long-term survival, especially in lung cancer patients. CSC-targeting agents are now undergoing *in vitro* and *in vivo* studies, some of which have provided promising results for further clinical studies setting. In this article we review the concept of CSC from the perspective of tumor biology, including the origin of CSC and its biomarkers. As lung cancer is the leading cause of cancer-related deaths worldwide, we focus on the properties and clinical implications of lung CSC.

Key words: clinical implication, lung cancer stem cells, tumor biology.

INTRODUCTION

Lung cancer is the leading cancer site in men, comprising 17% of all new cancer cases and 23% of all cancer deaths.[1] Furthermore, the mortality burden for lung cancer among women in developing countries is as high as the burden for cervical cancer, with each accounting for 11% of all female cancer deaths.[1]

Even with aggressive therapy, lung cancer patients with locally advanced disease who are not surgically resectable on the basis of the extent of the primary disease or regional nodal involvement, have an extremely poor long-term survival, in the order of 15 to 40%.[2] Despite improvement with combined modality therapy, both local control and survival still remain poor.[2]

These facts have led researcher to investigate other possible mechanism that may play role in the progression of lung cancer. In recent years cancer stem cells (CSC) have emerged as the focus of intense investigations in cancer research. This theory is now well accepted and the results of experiments from many research centers support the existence of this type among various cancers.[3]

STEM CELLS

Distinct types of stem cell have been established from studying embryos and have been identified in the fetal tissues and umbilical cord blood, as well as in specific niches in many adult mammalian tissues and organs, such as bone marrow, brain, skin, eyes, heart, kidneys, lungs, gastrointestinal tract, pancreas, liver, breast, ovaries, prostate and testis.[4] All stem cells are undifferentiated cells that exhibit unlimited self-renewal and can

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generate multiple cell lineages or more restricted progenitor populations that can contribute to tissue homeostasis by the replenishment of cells or the regeneration of tissue after injury.[4]

By definition, stem cells have three main properties: (i) differentiation – the ability to give rise to a heterogeneous progeny of cells, which progressively diversify and specialize according to a hierarchical process, constantly replenishing the tissue of short-lived mature elements; (ii) self-renewal – the ability to form new stem cells with an identical and intact potential for proliferation, expansion and differentiation, thus maintaining the stem cell pool; and (iii) homeostatic control – the ability to modulate and balance differentiation and self-renewal according to environmental stimuli and genetic constraints.[5]

Embryonic stem cells

Embryonic stem cells (ESC) have the ability to form any fully differentiated cells of the body.[6] Ginis e*t al*. have compared the expression of almost 400 genes in human and mouse ESC that have been maintained in an undifferentiated state.[7] The results showed that mouse and human cells share similarities in expressing markers of the pluripotent state.[7] However, human ESC are unique in their abilities to maintain pluripotency and a normal diploid karyotype over long periods in culture.[8] These properties make human ESC leading candidates for use in cell therapy and for studies of early human development.[8]

Mesenchymal stem cells

Mesenchymal stem cells are non-hematopoietic stromal cells that are capable of differentiating and contribute to the regeneration of mesenchymal tissue, such as bone, cartilage, muscle, ligament, tendon and adipose.[9]

Kim *et al*. have isolated a regional lung stem cell population, termed bronchioalveolar stem cells (BASC).[10] BASC are a stem cell population that maintains bronchiolar Clara cells and alveolar cells of the distal lung, but their transformed counterparts give rise to adenocarcinoma.[10] Although stem cells are present in the lungs and become activated following injury to restore lung function, lung stem cells do not contribute to normal tissue homeostasis and the airways are instead maintained by Clara cells.[11]

CSC

CSC have the capacity both to divide and expand the CSC pool and to differentiate into heterogeneous non-tumorigenic cancer cell types that in most cases appear to constitute the bulk of the cancer cells in the tumor.[12] To date, the practical translation of this definition, and the gold standard for showing "stemness" of cancer cells, is the ability to generate a phenocopy of the original malignancy in immunecompromised mice.[13]

A subset of stem cells, termed the "side population" (SP), has been identified in several tissues in mammalian species. An SP was also detected in breast cancer, lung cancer and glioblastoma cell lines, suggesting that this phenotype constitutes a class of CSC with inherently high resistance to chemotherapeutic agents that should be targeted during the treatment of malignant disease.[14] There is evidence for the ability of SP to regenerate a population resembling the original one.[15] SP displayed an elevated expression of adenosine triphosphate (ATP)-binding cassette transporter G2 (ABCG2) as well as other ATP-binding cassette transporters and showed resistance to multiple chemotherapeutic drugs.[15]

However, there are opposing arguments to the CSC hypothesis. Kelly *et al*. have argued that the recent approach to studying the existence of CSC has not been thoroughly criticized.[16] They suggested that the frequency of cells that can sustain tumor growth, and thus the generality of the CSC hypothesis, can best be tested by the transfer of titrated numbers of mouse tumor cells into non-irradiated histo-compatible recipient mice. Primary pre-B/B lymphoma cells were injected into non-irradiated congenic mice, which then developed fatal lymphoma on day 35. A small fraction (~2 to 5%) of these cells that displayed the characteristic stem cell markers (Sca-1 and/or AA4.1) was also transplanted and the recipient also had fatal lymphoma between days 17–40.

Nevertheless, the evidence that supports the role of CSC in carcinogenesis seems to keep coming and exceeds the studies that challenge it.

Origin of CSC

The exact origin of pluripotent stem cells in tumors might vary. They could arise from the malignant transformation of a normal stem cell that has accumulated oncogenic insults over time.[17] In rapidly dividing tissues such as the blood, gut and skin, stem cells persist throughout an individual's life and can easily acquire numerous oncogenic mutations.[18]

In the context of the lungs, oncogenic mutations by themselves should not efficiently promote lung tumorigenesis because airway stem cells exhibit a limited contribution to lung homeostasis.[11] In contrast, severe injury, epithelial cell loss, stem cell activation and clonal cellular expansion subsequent to oncogenic mutation

may more efficiently promote lung carcinoma formation.[11] Ooi *et al*. identified a population of keratin 14 (K14)-expressing progenitor epithelial cells that were involved in repair after injury.[19] The dysregulated repair mechanism results in the persistence of K14+ cells in the airway epithelium in potentially premalignant lesions. This suggests that repairing K14+ progenitor cells may be tumor-initiating cells in this subgroup of smokers with non-small cell lung cancer (NSCLC).[19]

CSC-like cells can be generated *in vitro* by the oncogenic reprogramming of human somatic cells during neoplastic transformation.[20] *In vitro* transformation confers stem cell properties to primary differentiated fibroblasts, including the ability to self-renew and to differentiate along multiple lineages. These findings have established an experimental system to characterize the cellular and molecular properties of human CSC and demonstrate that somatic cells have the potential to de-differentiate and acquire the properties of CSC.[20] Epithelial to mesenchymal transitions (EMT) are trans-differentiated programs that occur in tissue morphogenesis during embryonic development. EMT refers to a complex molecular and cellular program by which epithelial cells shed their differentiated characteristics, including cell–cell adhesion, planar and apical–basal polarity, and lack of motility, and acquire mesenchymal features, including motility, invasiveness and heightened resistance to apoptosis.[21] Studies have shown that EMT induction in cancer cells results in the acquisition of invasive and metastatic characteristic.[22] EMT-induced non-tumorigenic, immortalized human mammary epithelial cells by ectopic expression of either Twist or Snail transcription factors, both of which are capable of inducing EMT in epithelial cells, results in a high CD44/low CD24 expression pattern, a neoplastic mammary stem cell marker.[23]

It is important to remember that demonstrating one model for the formation of CSC in a given system does not necessarily exclude other mechanisms.[24] The possible origins of CSC are not mutually exclusive.[24]

PRESENCE OF LUNG CSC AND CLINICAL CONSEQUENCES

Until now, there has been no single marker that can be used to identify the CSC of all cancers. This results from the heterogeneity associated with tumor biology at various levels. Different tumors may express different molecules; different patients suffering from the same tumor type may have variant expression patterns because of variant genetic, epigenetic and environmental factors; and in a single patient, distinct populations of tumor cells may co-exist in primary and metastatic tumors, including a heterogeneous population of CSC.[25]

The aldehyde dehydrogenase 1 (ALDH1)-positive cancer cells exhibit important CSC properties: *in vitro* self-renewal; differentiation; multiple-drug resistance capacities; the expression of stem cell markers; *in vivo* tumor initiation and occurrence of a heterogeneous population of cancer cells.[26] Relatively high ALDH1 protein levels were positively associated with the stage and grade of the tumors and inversely related to the patients' survival.[26] Other studies suggest that ALDH1 expression may be used to detect both normal and malignant mammary stem cells *in situ*, in fixed paraffin-embedded sections.[27]

Li *et al*. have identified a subpopulation of highly tumorigenic cancer cells in human pancreatic adenocarcinomas using a xenograft model in which primary human pancreatic adenocarcinoma cells were implanted in immuno-compromised mice.[28] These highly tumorigenic cancer cells were identified by the expression of the cell surface markers CD44, CD24 and ESA.[28]

By using both *in vitro* systems and implemented *in vivo* models of direct xenografts of human primary lung cancers in mice, Bertolini *et al*. provided evidence that lung tumor CD133+ cells are highly tumorigenic, are endowed with stem-like features and, importantly, are spared by cisplatin treatment.[29] Moreover, lung cancer CD133+ cells were able to grow indefinitely as tumor spheres in a serum-free medium containing epidermal growth factor and basic fibroblast growth factor.[30] The injection of 104 lung cancer CD133+ cells in immune-compromised mice readily generated tumor xenografts phenotypically identical to the original tumor.[30] Translated into the clinical level, the study has shown that CD133 in NSCLC represents a resistance phenotype and evidence of metastatic cells, but CD133 expression in NSCLC does not correlate with patients' survival.[31]

However, other studies suggest that CD133 is a temporary marker of CSC in small cell lung cancer but not in NSCLC. In their study Cui *et al*. investigated the CD133 expression in human lung cancer cell lines A549, H157, H226, Calu-1, H292 and H446.[32] The results of a real-time polymerase chain reaction analysis after chemotherapy drug selection and a fluorescence-activated cell sorting analysis showed that CD133 functioned as a marker only in the small cell lung cancer line H446.[32]

Possession of multiple-drug resistance is an additional property of normal stem cells and one that contributes to their longevity by permitting them to survive toxic insults, including many of the drugs currently used to

treat cancer.[33] By using ATP hydrolysis energy, ABC transporter is involved in drug resistance by pumping out various structurally unrelated agents.[34] ABCG2 was elevated in the SP of all cell lines.[15] The attenuation of ABCG2 expression dramatically reduced the SP population in lung cancer cell lines.[35] Taking into account the fact that the ABCG2(+) subset of tumor cells are often enriched with cells with cancer stem-like phenotypes, it has been proposed that ABCG2 activity underlies the ability of cancer cells to regenerate post-chemotherapy.[36]

CLINICAL APPLICATION OF LUNG CSC

Markers for CSC could be used for predicting treatment responses by identifying the presence of specific CSC subtypes that are selectively sensitive to specific agents, particularly biological agents such as antibodies against specific CSC targets.[37] Until now, most available chemotherapy eradicates only the bulk of cancer cells and fails to overcome drug resistant cancer-initiating stem cells.[38] The concept of CSC has radically changed our understanding of cancer therapy as CSC are thought to be responsible for the failure of current chemotherapy of lung cancer.[38] That explains why individuals with cancer cannot generally be considered cured, even when their initial response to radiation or chemotherapy is encouragingly robust.[39] Therefore, the acquisition of stemness by NSCLC tumors is a negative prognostic and predictive factor in overall survival.[40]

The fact that many cancers are driven by CSC has important clinical implications. Clinical treatment regimens operate under the assumption that all cancer cells have an equally malignant potential.[41] These treatments suffer from their lack of specificity for only tumorigenic cells.[41] Therefore, active substances targeting CSC are becoming more important. Some of the so-called CSC-inhibitory mechanisms such as blocking stem cell factor (SCF), antagonists of ABCG2 pumping activity, and Notch inhibitors are discussed below.

c-kit belongs to the receptor tyrosine kinases superfamily, specifically to subclass III of thyrosine kinase superfamily. c-kit probably contributes to cancer formation and progression by inappropriately promoting survival and proliferation.[42] Research has shown that SCF–c-kit signaling is sufficient to inhibit CSC proliferation and survival promoted by chemotherapy. In contrast with other tumor cells, CSC expressed c-kit receptors and produced SCF.[43] The proliferation of CSC was inhibited by SCF-neutralizing antibodies or by imatinib, an inhibitor of c-kit.[43]

The Notch pathway is one of the most intensively studied putative therapeutic targets in CSC, and several investigational Notch inhibitors are being developed. Notch blockade suppressed expression of the pathway target Hes1 and caused cell cycle exit, apoptosis and differentiation in medulloblastoma cell lines.[44] The blockade of Notch signaling using pharmacological and genomic approaches prevented sphere formation, proliferation and colony formation in soft agar.[45] Notch-1 activates Akt-1 through the repression of phosphatase and tensin homologue expression and induction of the insulin-like growth factor 1 receptor that stimulates the survival of lung adenocarcinoma cells during hypoxia in lung adenocarcinoma cell lines.[46] Reducing Notch activities in CSC may promote their differentiation, thus reducing their ability to repopulate the cells forming the tumor mass.[47] Moreover, Notch inhibitors may be used in the clinic to target CSC and reverse or prevent chemoresistance or radioresistance.[48]

The SP of NSCLC cell lines, which comprised 24% of the total cell population, totally disappeared after treatment with the fumitremorgin C, a selective ABCG 2 inhibitor.[49] Furthermore, there was an increased mRNA expression of ABCG2 in SP cells.[49] In another study, flow cytometry analysis showed that lapatinib (10 μmol/L) inhibited the efflux of mitoxantrone, a specific substrate of the ABCG2 pump, in a manner similar to fumitremorgin C, confirming that lapatinib is an ABCG2 inhibitor.[50] Moreover, lapatinib reverses ABCG2-mediated multiple-drug resistance by inhibiting its transport function but not by blocking the AKT or ERK1/2 pathway or downregulating ABCG2 expression.[51] There are several other active substances with ABG2 antagonist activity undergoing *in vitro* and *in vivo* studies targeting lung CSC. However, the design of clinical trials of CSC-targeted agents will have to consider that anti-CSC effects will not necessarily translate into rapid changes of tumor volume.[48] It is important to remember that CSC constitute only a minority of the cells in a solid tumor.[39]

CONCLUSION

Cancers are perpetuated by a small population of tumor-initiating cells that exhibit numerous stem cell-like properties. The clinical implication of this concept is that CSC are thought to be involved in the failure of current chemotherapy of lung cancer and metastasis that leads to cancer recurrence. Understanding the properties of lung CSC will lead to progress in therapy and intervention, and improvement of the prognosis of patients with

lung cancer. The exploration of cell markers and signaling pathways specific to lung CSC have contributed to the development of novel CSC-targeted therapies, at least in *in vitro* and *in vivo* studies. Nevertheless, the clinical application of these novel approaches requires further research to confirm some important issues: how to target specific CSC without interfering with normal mesenchymal stem cells, and how to reduce the bulk of the tumor, and eventually, patients' symptoms, considering that CSC-targeting agents only work on a small population of CSC. Therefore, it might be worthwhile to study the efficacy of a combination of chemotherapy and CSC-inhibiting agents in clinical trials.

In future, CSC evaluation may be part of practical diagnostic pathology because it may provide information regarding biological aggressiveness and the prediction of prognosis.[52] Currently CD133, an established lung CSC marker, is available for detection using immunohistochemistry in histological specimens.

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