Stemness and plasticity of lung cancer cells: paving the road for better therapy

Abstract: Lung cancer is a devastating disease that is responsible for around 160,000 deaths each year in United States. The discovery that lung cancer, like most other solid tumors, contains a subpopulation of cancer stem cells or cancer stem-like cells (CSCs/CS-LCs) that if eliminated could lead to a cure has brought new hope. However, the exact nature of the putative lung CSCs/CS-LCs is not known and therefore therapies to eliminate this subpopulation have been elusive. A limited knowledge and understanding of cancer stem cell properties and tumor biology may be responsible for the limited clinical success. In this review we discuss the stemness and plasticity properties of lung cancer cells that are critical aspects in terms of developing effective therapies. We suggest that the available experimental evidence obtained from lung cancer cell lines and patients' derived primary cultures does not support a tumor model consistent with the classical CSC model. Instead, all lung cancer cells may be extremely versatile and new models of cancer stem cells may be better working models.

Keywords: cancer stem cells, chemotherapy, interconversion, plasticity, phenotype

Introduction

Lung cancer is the most common malignancy in the United States and is responsible for around 160,000 deaths each year.1 Tumor recurrence after resection is very common and accounts for the majority of mortality.2 The cell of origin of lung cancer has been the subject of considerable debate since its elucidation and may lead to new and perhaps more effective therapies. Histopathologically, lung cancer is divided into two main subtypes: non-small-cell lung cancer (NSCLC) and small-cell lung cancer (SCLC). Each subtype may arise from distinct cells of origin localized in defined microenvironments.3–5 It was found that both subtypes contain a subpopulation of rare undifferentiated cells expressing CD133, a cancer stem cells marker.6 Cancer stem cells (CSCs) or cancer stem-like cells (CS-LCs) have been found in the majority of cancers and are usually related to chemoresistance and recurrence.7,8 Lung cancer with stem cell signatures have been associated with resistance to several anticancer drugs, such as, cisplatin,9,10 Epidermal growth factor receptor (EGFR) inhibitors such as gefitinib,11 docetaxel and gemcitabine.12 In a simplistic explanation the classical cancer stem cell theory (CSCT) states that CSCs are: a) rare, b) highly resistant to conventional therapies, c) similar to normal stem cells capable of unrestricted self-renewal and multipotent differentiation13,14 and thus responsible for tumor recurrence.14–16 From a clinical point of view the idea that the elimination of this subpopulation will lead to a cure or at least to dramatic improvement has become a new dogma in the cancer field.17,18 It is then not surprising that considerable efforts and resources are being allocated to identify and
eliminate this fraction. As our knowledge of CSCs improves, the acceptance of the classical CSC theory as a universal model has been questioned and gave rise to alternative models that have different clinical implications.

In contrast to the CSC theory, the stemness phenotype model (SPM)\(^\text{19}\) proposes that all cancer cells may have stem cell properties and that the stemness of cancer cells depend on the microenvironment. According to the SPM all cancer cells are potentially tumorigenic and any cancer cell could be responsible for tumor recurrence. Thus, from the clinical point of view, to cure cancer, all cancer cells should be targeted and eliminated at once. Models closely resembling the SPM with similar clinical implications have also been proposed, amongst them: the “complex system model”,\(^\text{20}\) the “reprogramming model”,\(^\text{21}\) the “dynamic CSC model”,\(^\text{22}\) and the “plasticity model”.\(^\text{23}\) The idea that CSCs possess constantly evolving features and are “moving targets” rather than fixed entities is gaining acceptance.\(^\text{24}\)

This mini-review will focus on the current knowledge of lung cancer stem cells in order to summarize the findings supporting alternative models of cancer stem cells. Such knowledge is crucial in order to better design new therapies that actually benefit patients.

Search method

Literature data of relevant studies were conducted using the PubMed (http://www.pubmed.com) and ScienceDirect databases for articles published up to January, 2014 (additional searches were done for a revised version). Relevant terms such as “lung cancer stem cells”, “lung cancer stem cells plasticity”, “lung cancer stem cell stemness”, and many other variants including keywords relevant to the minireview (eg, microenvironment, signaling pathways; see Table 1 and 2) were used.

Since this article is a minireview/perspective article, only selected relevant references were included.

**Lung cancer stem cells**

Probably the first observation of LCSCs came from the work published by Carney et al in 1982\(^\text{25}\) at a time when the CSC hypothesis was not prominent. Later on, putative LCSCs were isolated from a variety of cell lines and tumor specimens. Recent reviews has summarized this findings (see Table 1 and 2 in\(^\text{26}\) and\(^\text{19}\) respectively). LCSCs have been associated with radioresistance\(^\text{27}\) and chemoresistance.\(^\text{8-11}\) Similar to findings in other tumors LCSCs are able to form spheres\(^\text{28}\) and express stem cell markers such as CD133, CD44, ALDH1, and β-catenin and were found to be associated with higher recurrence rates.\(^\text{29}\) In summary, there is overwhelming evidence that lung cancers have cells with traits of stem cells. However, there are controversies regarding which model of CSC fits better\(^\text{26}\) in order to be used as a more rational guide-line to develop new therapies for this disease.

**Modulation of stemness by signaling pathways**

Multiple signaling pathways such as Wnt/beta-catenin, Hedgehog and Notch that appear to be involved in the regulation of stemness in other solid tumors have already been implicated in lung cancer development.\(^\text{4}\) An activated Wnt/beta-catenin pathway, which in A540 cells up-regulates the stem marker OCT-4,\(^\text{30}\) predicts increased risk of tumor recurrence.\(^\text{31}\) SOX17, which acts as a Wnt signaling inhibitor and

| Table 1 Stemness modulation of LCSCs by signaling pathways |
|-----------------|-----------------|-----------------|-----------------|
| **Cell type**     | **Signaling pathway** | **Effect on stemness** | **Reference**  |
| A549             | Wnt/beta-catenin  | ↑                | 30,51          |
| A549, H1299      | Hedgehog         | ↑                | 58,59          |
| HCC, H1339       | Hedgehog         | ↑                | 60             |
| Primary LSCC tumor cells | Hedgehog | ↑                | 61             |
| A549, H1299 and H1755 | Notch-1  | ↑                | 62             |
| H460 and H661    | Notch-1          | ↑                | 9              |
| A panel of primary NSCLC | Notch-3 | ↑                | 63             |
| NSCLC cell lines: NCI-H1299, NCI-H358, NCI-H441, NCI-H460, and A549 | Notch | ↑ | 64 |
| H1650, H1975, A549 | EGFR/Src/Akt     | ↑                | 65             |
| Several lung AD cell lines including H1975 and PC-3 | Akt/Sox2 | ↑ | 66 |
| A549             | pAkt             | ↑                | 67             |
| A549             | IGF1R/P3K/AKT/GSK3β | ↑ | 68             |
| Gefitinib-resistant A549 cells | CXCR4-mediated STAT3 pathway | ↑ | 69 |

**Abbreviations:** NSCLC, non-small-cell lung cancer; EGFR, epidermal growth factor receptor; AD, adenocarcinoma; LSCC, laryngeal squamous cell carcinoma; HCC, hepatocellular carcinoma.
inhibits proliferating cells, is frequently downregulated in
lung cancer cells.32 Hedgehog is also linked to lung cancer
development33 and plays a role in the maintenance of lung
cancer cells stemness.

Increased Notch activity enhances epithelial-mesenchymal
transition in gefitinib-acquired resistant lung cancer cells11 and
has been correlated with poor clinical outcome in NSCLCs
patients without TP53 mutations. Approximately 30% of
NSCLCs showed increased Notch activity due to loss of the
counteracting function of Numb. In approximately 10% of the
cases a gain of function mutation of the NOTCH-1 gene24 was
observed. Numb acts as an inhibitor of the Notch receptor
signaling pathway but it is also connected to Hedgehog- and
TP53-activated pathways, regulating multiple functions such
as maintenance of stem cell compartments, regulation of cell
polarity and adhesion, and migration.35

**Table 2** Microenvironmental factors implicated in stemness modulation of lung cancer cells

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Microenvironmental factor</th>
<th>Effect on stemness</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-146 (small-cell lung carcinoma)</td>
<td>Hypoxia</td>
<td>†</td>
<td>70</td>
</tr>
<tr>
<td>NSCLC cell lines, PC9 and HCC827</td>
<td>Hypoxia</td>
<td>†</td>
<td>71</td>
</tr>
<tr>
<td>AS49, NCI-H358</td>
<td>CAF likely via (TGF)-β1</td>
<td>†</td>
<td>72</td>
</tr>
<tr>
<td>AS49, PC-14, and CRL-5807</td>
<td>VAF</td>
<td>†</td>
<td>73</td>
</tr>
<tr>
<td>AS49</td>
<td>TGF-β1</td>
<td>†</td>
<td>74</td>
</tr>
<tr>
<td>AS49</td>
<td>IL-8</td>
<td>†</td>
<td>75</td>
</tr>
<tr>
<td>AS49</td>
<td>VEGF</td>
<td>†</td>
<td>75</td>
</tr>
<tr>
<td>AS49 and HTB177</td>
<td>tMVs</td>
<td>†</td>
<td>76</td>
</tr>
<tr>
<td>CMT167</td>
<td>Matrix metalloprotease-10</td>
<td>†</td>
<td>77</td>
</tr>
</tbody>
</table>

Abbreviations: CAF, cancer associated fibroblasts; VAF, vascular adventitial fibroblasts; TGF, transforming growth factor; IL-8, interleukin 8; VEGF, vascular endothelial growth factor; NSCLC, non-small cell lung cancer; CAF, cancer-associated fibroblasts; VAF, vascular adventitial fibroblasts; tMVs, tumor microvesicles.

**Stemness modulation of LCSCs by the microenvironment**

The tumor microenvironment contains a variety of malignant
and non-malignant cells16 and plays a key role in the regula-
tion of the epithelial-mesenchymal transition (EMT)37 that is
associated with the acquisition of stem cell traits.38 Specifi-
cally, NSCLC induction of EMT by TGF-β-1 has been shown to
increase stemness.39 Interactions between tumor cells and the
stroma cells are therefore considered candidate targets for
therapeutical interventions.40 In particular, in lung cancer, can-
cer associated fibroblasts (CAFs) have been found to promote
the stemness of cancer cells (Table 2). It seems that fibroblasts
in general have a promoting effect as they has been used as
feeder cells to establish LCSC cultures.31 There is evidence
that tumor associated macrophages (TAMs) play an important
role in cancer progression and metastasis in NSCLC.42 TAMs
dependent on the influence of various stimuli in the tumor
microenvironment can develop into a tumor-inhibitory (M1)
or tumor-promoting (M2) phenotype.36,43 Hypoxia that is com-
monly associated with resistance to radiation and chemotherapy
in lung cancer44 is also a known promoter of stemness in LCSCs
most likely via activation of the Notch pathway.37,38

**Plasticity of cancer cells: interconversion between CSCs and non-CSCs in lung tumors**

Cellular plasticity can be defined as the property or abil-
ity of cells to reversible change their phenotype.45 There is
an increasing acceptance that cancer cells display variable
degrees of plasticity.46–48 The classical cancer stem cell theory
proposed a hierarchical and unidirectional organization where
CSCs can give origin to more differentiated cells. Due to the
unidirectional organization, differentiated cells have limited
plasticity and are unable to originate new CSCs.20,22 In contrast,
the stemness phenotype model initially suggested that cancer
cells are not hierarchically organized and can interconvert into
each other.19 This property expands the plasticity of cancer
cells (that can undergo both differentiation and dedifferen-
tiation) since in theory a single non-CSC can originate a new
tumor and re-establish a new pool of CSCs. Perhaps the more
convincing argument for a lack of hierarchical organization in
lung cancer cells would be a direct observation of the conver-
sion from a non-CSCs phenotype to a CSCs phenotype and
vice versa as has been recently observed in other systems.49
In fact recently Akunuru et al.50 provided direct experimental
evidence of interconversion between different phenotypic sub-
populations of non-small cell lung adenocarcinoma (NSCLA).
In that study, interconversion was observed not only between
CSCs that were phenotypically different but also between
CSCs and non-CSCs. This is consistent with the prediction
of the SPM. Evidence that the culture conditions alters the
phenotype of lung cancer cells was reported in 1984,31 long
before the isolation of putative LCSCs.
More surprising, the plasticity of lung cancer stem cells seems to be not limited only to specific tissues. Zhang et al, found that the SCLC cell line NCI-H446 can also differentiate to neurons, adipocytes, and osteocytes. In the cell line LC-42 expression of the stem cell marker CD133 does not correlate with tumorigenic potential. The recent observation that committed epithelial cells can differentiate in vivo into stem cells provides supporting evidence that stemness may be a general property of all cells.

Implication for cancer therapy
Both extreme models of LCSC have also extreme clinical implications. In the classical CSC model, the hierarchical organization gives CSCs a predominant role in cancer resistance and tumor recurrence. Therefore, eliminating this fraction is considered a crucial target and considerable resources are being used in identifying this rare subpopulation and developing strategies to eliminate them. On the other hand, the SPM and similar alternative models propose that virtually all cancer cells are potentially tumorigenic. Thus, to have a significant impact on cancer treatment all cancer cells should be eliminated at once to prevent tumor progression and relapse. One aspect of tumor biology that is poorly investigated is the potential dynamic of the microenvironment due to external influences. In the classical CSC model, due to its hierarchical nature, CSCs can produce non-CSCs but not in the other way. It is then expected that microenvironmental changes in tumor regions with non-CSCs will have little therapeutic impact but similar changes in tumor regions with CSCs are potential promising avenues to explore for therapies targeting the CSC-microenvironment.

Conclusion
A better understanding of cancer stem cell biology in lung cancer is essential to develop effective therapies. At present there is increasing evidence suggesting that LCSCs are a dynamic subpopulation harboring a high degree of plasticity and not fixed entities. The complex interaction between a) a dynamic cancer cell phenotype that can interconvert from a pure non-CSC phenotype to a pure CSC phenotype in combination with b) a dynamic microenvironment that can either promote or suppress cancer stemness adds a significant challenge to the development of novel treatment for lung cancer. A similar scenario has been recently recognized in ovarian cancer. This complex interaction should be taken into consideration at the early stages of preclinical research to increase the chances of a successful translation into clinical practice.

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Disclosure
The authors report no conflicts of interest.

References


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