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PERSPECTIVES

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

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Judong Luo[1,2,*]
Xifa Zhou[1,*]
Juan Sebastian Yakisich[3]

[1]Changzhou Tumor Hospital, Soochow University, Changzhou, [2]School of Radiation Medicine and Protection, Jiangsu Province Key Laboratory of Radiation Medicine and Protection, Soochow University, Suzhou, People's Republic of China; [3]Department of Clinical Neuroscience, Karolinska University Hospital Stockholm, Sweden

*These authors contributed equally to this work.

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 death 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[8] 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 differentiation[13,14] 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

Correspondence: Juan Sebastian Yakisich
Department of Clinical Neuroscience R54, Karolinska Institute, Karolinska University Hospital, Sweden,
S-141 86, Stockholm, Sweden
Tel +46 8 585 89 533
Fax +46 8 585 89533
Email yakisich@gmail.com

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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 CSCT, the stemness phenotype model (SPM)[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",[20] the "reprogramming model",[21] the "dynamic CSC model",[22] and the "plasticity model".[23] The idea that CSCs possess constantly evolving features and are "moving targets" rather than fixed entities is gaining acceptance.[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 cells 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[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[26] and,[19] respectively). LCSCs have been associated with radioresistance[27] and chemoresistance.[8–11] Similar to findings in other tumors LCSCs are able to form spheres[28] and express stem cell markers such as CD133, CD44, ALDH1, and β-catenin and were found to be associated with higher recurrence rates.[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[26] in order to be used as a more rational guideline 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.[4] An activated Wnt/beta-catenin pathway, which in A540 cells up-regulates the stem marker OCT-4,[30] predicts increased risk of tumor recurrence.[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/PI3K/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.

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

Cell type	Microenvironmental factor	Effect on stemness	Reference
H-146 (small-cell lung carcinoma)	Hypoxia	↑	70
NSCLC cell lines, PC9 and HCC827	Hypoxia	↑	71
A549, NCI-H358	CAF likely via (TGF)-β1	↑	72
A549, PC-14, and CRL-5807	VAF	↑	73
A549	TGF-β1	↑	74
A549	IL-8	↑	75
A549	VEGF	↑	75
A549 and HTB177	tMVs	↑	76
CMT167	Matrix metalloproteinase-10	↑	77

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.

inhibits proliferating cells, is frequently downregultated in lung cancer cells.[32] Hedgehog is also linked to lung cancer development[33] 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 cells[11] 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 gene[34] 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]

Stemness modulation of LCSCs by the microenvironment

The tumor microenvironment contains a variety of malignant and non-malignant cells[36] and plays a key role in the regulation of the epithelial-mesenchymal transition (EMT)[37] that is associated with the acquisition of stem cell traits.[38] Specifically, NSCLC induction of EMT by TGFβ-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, cancer 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.[41] There is evidence that tumor associated macrophages (TAMs) play an important role in cancer progression and metastasis in NSCLC.[42] TAMs depending 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 commonly associated with resistance to radiation and chemotherapy in lung cancer[44] 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 ability 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 hyerachically organized and can interconvert into each other.[19] This property expands the plasticity of cancer cells (that can undergo both differentiation and dedifferentiation) 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 conversion 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 subpopulations 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,[51] 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.[52] In the cell line LC-42 expression of the stem cell marker CD133 does not correlate with tumorigenic potential.[53] The recent observation that committed epithelial cells can differentiate in vivo into stem cells[54] provides supporting evidence that stemness may be a general porperties of all cells.[55]

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.[56] 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.[57] 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.

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