Lung Cancer Stem Cells and Their Therapeutic Targeting

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Abstract
Lung cancer is responsible for causing more than 1 million deaths worldwide each year making it the most common cancer in humans. It has now been established that lung cancers contain a subpopulation of cancer cells, responsible for tumor initiation, propagation, and metastasis, termed as cancer stem cells (CSCs). However, this stem cell population in lung cancers remains poorly characterized. The present review discusses on the novel cell surface markers that would be required for isolating lung CSCs, and characterization of these important cells. The discussion also elucidates the regulatory signalling pathways involved in their maintenance and the role of miRNA in lung cancer stem cells and prospects of using them as therapeutic targets.

ABBREVIATIONS

INTRODUCTION
LC is a deadly disease and has a mortality rate higher than that of prostate cancer, breast cancer and colon cancer combined [1]. It is mainly of two types, NSCLC and SCLC [1]. NSCLC accounts for about 85% of all lung cancers with mean survival rate of 15% [1], and is mainly divided into three subtypes: adenocarcinoma, squamous cell carcinoma, and large-cell carcinoma [2] out of which adenocarcinoma (30%-50% of NSCLC) and squamous cell carcinoma (30% of NSCLC) are most common [3]. SCLC is classified into Small cell carcinoma and combined small cell carcinoma (a combination of SCLC with neoplastic squamous and/or glandular components [4]. SCLC is more aggressive than NSCLC, fast growing and spreads rapidly while NSCLC grows slower. NSCLC has a high recurrence rate of 35%-50% among early stage NSCLC patients [2]. This property of post-therapy reoccurrence of lung cancer leads us to conclude that the minimal disease left has a sub-population of cells with enormous self-renewal capacity generally a property of somatic stem cells [3].

‘Cancer stem cells’ (CSC), cells with enormous self-renewal capacity are immortal tumor-initiating cells capable of self-renewal and are pluripotent in nature. They have been identified in multiple malignancies, and are thought to play an important role in tumor initiation, development, metastasis, and therapy-resistance leading to tumor relapse [5]. CSCs are the initial cell in carcinoma formation and they survive anti-cancer therapies (aimed at dividing cells) by hiding in the quiescent phase of growth and lead to tumor relapse. This provides a logical explanation about why anti-cancer therapies might even show initial results but are unable to fully cure cancer [6].

LC development, in a step wise manner, results in the progression of pre-malignant lesions to invasive LC. Though the exact mechanism of LC development is unknown, it is hypothesized that prevention of pre-malignant lesion formation formed from subpopulation of stem cells in the lung might help to prevent LC [6].Current therapeutic strategies involving chemotherapy, radiation and targeted therapies have failed to considerably increase survival in LC. CSCs are thought to be responsible for endowing tumors with therapeutic resistance [7]. Identification & targeting of these CSCs causing pre-malignant lung lesions will provide opportunities for their therapeutic interventions [8].

LC-SC markers
CSCs can be distinguished from other tumor cells and normal stem cells on the basis of certain distinct and specific biomarker
phenotypes [9]. The complete list of LC-SC markers is beyond the scope of discussion in this review; however the most recognized markers specific for LC-SC are discussed here Table 1.

### Key signalling pathways

1) Dysregulation of embryonic signalling pathways involved in organogenesis, plays an important role in enabling CSCs to retain stem cell properties [3]. The core stem cell pathways, including Hedgehog, Notch and WNT are involved in the renewal of normal stem cells and in maintenance of tissue homeostasis [22]. A deeper understanding of these dysregulated pathways is needed to establish therapeutic strategies targeting these pathways to eliminate LC-SC population [3]Hh pathway

The Hh pathway regulates development of vertebrate embryonic cells. Activation of the transmembrane domain protein SMO, followed by subsequent activation of the transcription factors (GLI1, GLI2 and GLI3) regulating downstream target genes, is responsible for activation of this pathway [3]. Hh signalling has been implicated in many cancers as a CSC regulatory pathway. Watkins et al. reported first on Hh pathway activation in SCLC in 2003 [23]. Hh signalling inhibition results in

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loss of tumourigenicity in SCLC which suggests that deregulation of Hh signalling might cause SCLC by acquiring neuroendocrine fate of airway progenitor cells in response to paracrine signals from adjacent epithelial cells [3].

2) Notch pathway

Notch signalling is an evolutionary conserved embryonic pathway in mammals involved in regulating cellular fate determination during development [3]. Activation of Notch signalling through cell-to-cell interaction causes asymmetric cell division thereby retaining stem cell viability [24]. Elevated levels of Notch transcripts have been reported in NSCLC cell lines wherein Notch activity promotes pro-apoptotic signals and inhibits anti-apoptotic signals [3]. Though active Notch pathway has been reported to be required for the clonogenic activity of ALDH+ cells in NSCLC, but there is no clear evidence that this pathway is involved in the maintenance of CSC phenotype [3].

3) WNT/β-CATENIN pathway

WNT/β-CATENIN pathway is an important pathway in human malignancies. Blockade of WNT signalling with monoclonal antibodies induces apoptosis in LC cell lines overexpressing WNT-2 protein [25]. However, a detailed investigation of this pathway in LC-SC is required to elucidate the therapeutic potential of targeting the WNT pathway [3].

Therapeutic targeting of LC-SC

CSCs are more resistant to cancer therapy than non-CSCs. Therefore, eliminating CSCs is crucial for the treatment of malignant diseases. Therapies targeting the subtle surface marker differences and alterations in signalling pathway can be used for killing CSCs and altering the microenvironment (niches) supporting these cells [9] Table 2.

miRNA & lung cancer stem cells

Recent studies have shown that miRNAs are useful in LC diagnosis and that specific miRNA profiles may predict prognosis, drug response and disease recurrence. Therefore, eliminating CSCs is crucial for the treatment of malignant diseases. Therapies targeting the subtle surface marker differences and alterations in signalling pathway can be used for killing CSCs and altering the microenvironment (niches) supporting these cells [9] Table 2.

**Table 2:**

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<td>1</td>
<td>Antitelomeric therapy</td>
<td>In NSCLC lines, the ALDH+ CSCs display longer telomeres than the non-CSC population. Treatment with MST312, a telomerase inhibitor could significantly reduce the ALDH+ CSC population and their telomeric length <em>in vivo</em> by inducing p21, p27 and apoptosis in the entire tumor population</td>
<td>[26]</td>
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<td>2</td>
<td>Mmp10</td>
<td>Mmps degrade the extracellular matrix thereby stimulating tumor invasion and metastasis. Mmp10 plays a critical role in the maintenance and tumorigenicity of mouse LC-SCs. Oncosphere cultures enriched in LC-SCs show high expression of Mmp10, and its RNAi-mediated knockdown led to loss of stem cell marker gene expression, inhibition of clonal propagation <em>in vitro</em>, suggesting Mmp 10 as a therapeutic target in lung cancer stem cells.</td>
<td>[27]</td>
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<td>3</td>
<td>Chk1</td>
<td>Chk1 is activated in CSCs (derived from primary NSCLC cultures) upon exposure to chemotherapeutic drugs, therefore co-administration of the Chk1 inhibitor AZD7762 with chemotherapy showed suppression of tumour growth.</td>
<td>[28]</td>
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<td>4</td>
<td>(i) Targeting CD44/CD133</td>
<td>The anti-psychotic drug trifluoperazine has been reported to inhibit CSC tumor, spheroid formation with downregulation of CSC markers (CD44/CD133). Combination of trifluoperazine with either gefitinib or cisplatin could also overcome drug resistance in LC-SCs by targeting the cell surface markers CD44/CD133</td>
<td>[29]</td>
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<td>5</td>
<td>(i) Notch signalling</td>
<td>Expression analysis of ALDH+ lung adenocarcinoma stem cells reveals elevated expression of Notch pathway transcripts. Suppression of the Notch pathway by treatment with either a gamma-secretase inhibitor (eg: DAPT, MRK-003 &amp; RO4929097) or stable expression of shRNA against NOTCH3 resulted in a significant decrease in ALDH+ LC cells, commensurate with a reduction in tumor cell proliferation and clonogenicity.</td>
<td>[3,8]</td>
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<td>(ii) Wnt signalling</td>
<td>In LC, dysregulated Wnt signalling contributes to tumorigenesis. Inhibition of Wnt signalling by a Wnt-2 monoclonal antibody results in induced apoptosis in NSCLC cells. Aberrant activation of this pathway in lung tumour prospects this pathway as a therapeutic target, however further investigations are required to elucidate the correlation of activated Wnt signalling with LC-SC.</td>
<td>[30]</td>
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<td>6</td>
<td>(iii) Hh signalling</td>
<td>Inhibition of Hh signalling with the <em>Veratum</em> alkaloid cyclopamine, a naturally occurring SMO antagonist, leads to the loss of tumourigenicity in SCLC. Inhibition of Hh signalling, using second-generation SMO antagonists such as LDE-225 (Novartis), can be used as an adjunct to chemotherapy to inhibit tumour recurrence. Also, Hh pathway inhibitor GDC-0449 shows dose-dependent growth inhibitory effects in the LC cell lines HCC (adeno-carcioma) and H1339 (SCLC) and also the cisplatin resistant LC cells, and the effects of GDC-0449 is mediated via SPs.</td>
<td>[3,31]</td>
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<td>(iv) PTEN signalling</td>
<td>The tumour suppressor gene, PTEN, an inhibitor of PI3K/AKT/mTOR signal transduction axis is involved in regulation of CSCs in NSCLC. Functional loss of PTEN activates this signalling cascade and leads to lung adenocarcinoma.</td>
<td>[32]</td>
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miRNAs are being considered as a promising technology for the development of LC therapeutics. More recent studies have also investigated the functional role of miRNAs in CSCs, as they bear significance in tumor maintenance, progression, metastasis and poor prognosis and regulatory roles in LC-SCs [36]. Therefore, a better understanding of the modulation of CSC gene expression by miRNAs could aid the identification of promising biomarkers and therapeutic targets [37] Table 3.

CONCLUSION

LC is the most lethal form of cancer for both the genders. A common cause of treatment resistance is the chemo-radio-resistant properties of these malignancies [49]. CSCs are thought to be responsible for therapy-resistance in malignant tumors due to their extensive ability for self-renewal and pluripotency thereby causing tumor-recurrence [50]. This heterogeneous population of CSCs is exclusively responsible for tumorigenicity [51]. Therefore targeting these cells is important for improving the efficacy of current therapeutic strategies by sensitizing the tumors towards conventional therapies and inhibiting recurrence [52]. For this purpose it is essential to establish distinct biomarkers to identify and isolate LC-SCs. Therapeutic strategies against LC-SCs include targeting of key signalling pathways involved in LC-SC formation and maintenance, targeting tumor microenvironment which

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<td>1</td>
<td>miR145</td>
<td>Its expression is reduced in metastatic lung adenocarcinoma and CSC-like tumor cells. This miRNA might be functionally regulated in de-differentiating or reprogramming processes in normal lung epithelial cells and LC cells suggesting miR-145 is a potential stemness-regulating factor for LC-SCs</td>
<td>[38]</td>
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<td>2</td>
<td>miR135a/b</td>
<td>This function in modulating the effect of the SNP in CD133 on the risk of LC in CD133+ cells. CD133 contributes to tumorigenesis and metastasis by virtue of CSC properties. Mimics of miR-135a/b can significantly suppress the miRNA expression of CD133 in peripheral blood lymphocytes containing the SNP. The SNP is inversely related to CD133 gene expression via the modulation of miR-135a/b.</td>
<td>[39,40]</td>
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<td>3</td>
<td>miR296</td>
<td>It regulates the function of LC-SCs via Klf4-Numbl-like signalling. Several types of human cancersshow decreased expression of miR-296, sometimes correlating with disease progression. miR-296 functions as a tumor repressor which actively represses the polarity protein, Numbl, that functions as a regulator of stem cell-related phenotypes including CSCs. Numbl inhibits Klf4-dependent transcription. Increased expression of Numbl is associated with multiple metastases in LC and enhanced cell invasion, resistance to therapy, maintenance of cancer initiation, progenitor-like cells, and metastatic competency in vivo.</td>
<td>[36,41-45]</td>
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<td>4</td>
<td>miR874</td>
<td>By regulation of MMP-2 and uPA proteins in LC cells, it has the potential to inhibit invasion, migration and CSC-phenotype of tumor cells, both in-vitro and in-vivo. The overexpression of miR-874 in the CD133+ CSC population leads to significant loss of the CSC phenotype with enhanced sphere-de-differentiation into epithelial-like cells, suggesting its role as tumour suppressor and a potential target in the treatment of NSCLC.</td>
<td>[36,46]</td>
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<td>let7 and miR31</td>
<td>SP cells are an enriched source of CSCs which drive and maintain many types of human malignancies. Both let-7 and miR-31 are significantly down-regulated in LC-SP cells as compared to LC non-SP cells, and this reduced expression is involved in maintaining the balance between differentiation and quiescence in SP cells</td>
<td>[47]</td>
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<td>6</td>
<td>miR34a</td>
<td>Its overexpression has been shown to inhibit NSCLC cell holoclone formation and clonogenic expansion in vitro and tumor regeneration in vivo. The inhibitory effects of miR-34a might be due to its effects on stem-like NSCLC cells, and research support rationale to develop it as a potential therapeutic agent.</td>
<td>[48]</td>
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creates a niche for CSCs and using ligands or antibodies against CSC cell surface markers [9]. miRNAs also play a crucial role in CSC maintenance by post-transcriptionally controlling CSC self-renewal and differentiation [53]. Certain miRNAs show either an upregulated or downregulated profile in LC-SCs and can be used as markers for stemness of these cells [9], suggesting targeting of LC-SC at transcriptional level itself by targeting the involved miRNAs [9]. This approach of targeting LC-SC holds great therapeutic potential.

REFERENCES


