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Mini-review

Lung cancer stem cells: The root of resistance

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ABSTRACT

In the absence of specific treatable mutations, platinum-based chemotherapy remains the gold standard of treatment for lung cancer patients. However, 5-year survival rates remain poor due to the development of resistance and eventual relapse. Resistance to conventional cytotoxic therapies presents a significant clinical challenge in the treatment of this disease. The cancer stem cell (CSC) hypothesis suggests that tumors are arranged in a hierarchical structure, with the presence of a small subset of stem-like cells that are responsible for tumor initiation and growth. This CSC population has a number of key properties such as the ability to asymmetrically divide, differentiate and self-renew, in addition to having increased intrinsic resistance to therapy. While cytotoxic chemotherapy kills the bulk of tumor cells, CSCs are spared and have the ability to recapitulate the heterogenic tumor mass. The identification of lung CSCs and their role in tumor biology and treatment resistance may lead to innovative targeted therapies that may ultimately improve clinical outcomes in lung cancer patients. This review will focus on lung CSC markers, their role in resistance and their relevance as targets for future therapies.

Introduction

Lung cancer and resistance

Lung cancer is the leading cause of cancer-related death worldwide and is classified into two main subtypes; non-small cell lung cancer (NSCLC) which accounts for approximately 85% of all lung cancers and small-cell lung cancer (SCLC) which is diagnosed in 15% of cases [1,2]. Histologically, NSCLC is further divided into three subtypes; adenocarcinoma, squamous-cell carcinoma and large cell carcinoma [3]. Smoking is a major risk factor for lung cancer and is associated with all histological subtypes, in particular, squamous cell carcinomas. Adenocarcinoma on the other hand, is the predominant lung cancer subtype commonly seen in never smokers. Despite improved advances in the treatment of lung cancer, the 5-year survival rate remains low, largely due to the emergence of resistance prior to and during the course of treatment with chemotherapy and radiation therapy. This resistance to therapy represents a significant clinical challenge in the treatment of lung cancer and contributes largely to disease progression, recurrence and mortality [4,5]. Despite intense efforts to overcome such resistance in lung cancer and other cancer types using novel agents, alone and in combination with chemo- and radiotherapy, the underlying mechanisms conferring this resistant phenotype in lung cancer remain largely unknown [6]. It is now well established that CSCs constitute a unique subset of cells which are distinct from the bulk of tumor cells by their exclusive ability to perpetuate the growth of a malignant population of cells, indefinitely. This may explain the ineffectiveness of many conventional therapies and patient relapse [7]. These important clinical observations have stimulated intense interest in experimental approaches for further investigation of CSCs and their role in the treatment of drug resistant lung cancer.

The cancer stem cell hypothesis

The CSC hypothesis is now a well-accepted and widely studied field within oncology. Cellular heterogeneity is a histological hallmark of many solid tumors and the CSC hypothesis suggests that this heterogeneity within a tumor is due to a hierarchical cell dynamic and the presence of a small subpopulation of cells that display the properties of normal somatic stem cells [8]. These CSCs are multipotent, and have two key characteristics of stem cells; the abilities to self-renew and to differentiate, properties necessary for tumor initiation and progression (Fig. 1A). CSCs can

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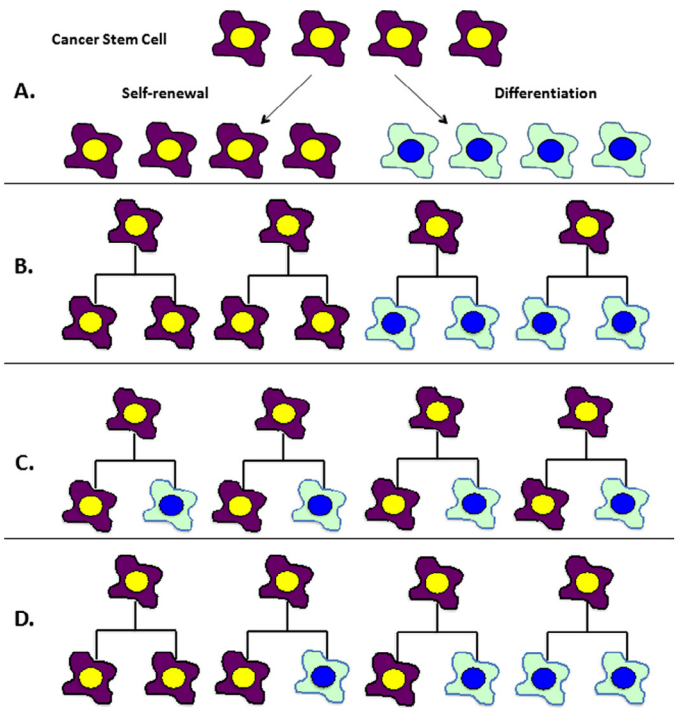

Fig. 1. Division strategies of cancer stem cells. (A) Cancer stem cells (purple) must accomplish the dual task of self-renewal and generation of differentiated progeny (blue). (B–D) Strategies that maintain a balance of stem cells and differentiated progeny. (B) Symmetric cell division. Each cancer stem cell can give rise to two cancer stem cells or to two differentiated progeny. (C) Asymmetric cell division, each cancer stem cell gives rise to one cancer stem cell and one differentiated cell. (D) Cancer stem cell maintenance may be a combination of symmetric and asymmetric division strategies. A population strategy provides dynamic control over the balance between stem cells and differentiated progeny, a capacity necessary for the maintenance or expansion of cancer stem cells.

asymmetrically divide (Fig. 1C), which enables them to simultaneously self-perpetuate and to generate differentiated progeny and give rise to heterogenic tumor, with a consistently maintained CSC subpopulation [9,10]. Although asymmetric division allows for both self-renewal and differentiated progeny, it does not allow the CSC subpopulation to expand. However, the stem cell niche has been shown to expand during development. Similarly, the CSC population expands during periods of stress, suggesting that they can also symmetrically divide (Fig. 1B) [11–13]. CSCs can rely on either asymmetric or symmetric division or a combination of the two (Fig. 1D). Previous studies have shown that a pure lung CSC population is capable of giving rise to a heterogenic progeny of CSCs and non-CSC cells through asymmetric cell division, whereas non-CSC lung cancer cells undergo only symmetric division resulting in a pure non-CSC cell population [14].

Cellular stress, such as chemotherapy treatment, can induce symmetric division of rare CSC populations and apoptosis of non-CSC cells, therefore enriching the population [15]. Symmetric division of the CSC population during initial cycles of chemotherapy may trigger relapse in the form of a chemoresistant tumor [16]. Despite an initial response to anti-cancer treatments, chemotherapeutic treatment can lead to the symmetric propagation of a small subpopulation of drug-tolerant cells with stem cell features [17–20]. Cisplatin treatment causes the lung CSC population to expand in patient explants and in H460-xenografted nude mice. Similar studies have also shown side-population CSC expansion following treatment with cytotoxic drugs such as 5'fluorouracil, an anti-metabolite commonly used in the treatment of several tumor types [21,22].

Lung cancer stem cells

Conventional anti-cancer therapies kill the bulk of the tumor, however, CSCs exhibit robust intrinsic resistance and survive therapy due to increased telomere length, activation of anti-apoptotic pathways, increased membrane transporter activity and their ability to migrate and metastasize [23].

Telomeres are comprised of a repetitive G-rich sequence and an abundance of associated proteins that form an effective cap that protects chromosome ends. Telomerase, also known as telomere terminal transferase, is a ribonucleoprotein complex that adds the telomere DNA sequence repeats to lengthen the telomere. It confers limitless proliferative capacity to cells through its ability to elongate telomeres [24,25]. Telomerase activity is readily detected in most cancers but not in somatic tissues and is essential for stem cell integrity and longevity [26]. Increased telomere length has previously been shown to confer resistance to a number of anti-cancer therapies; inversely, shortened telomere length is associated with drug sensitivity [27]. Telomerase inhibition enhanced the pro-apoptotic response to anti-cancer therapies resulting in delayed relapse following chemotherapeutic treatment [27–29]. Lung cancer stem cells display longer telomeres than their non-CSC counterparts. Treatment with the specific telomerase inhibitor, MST312, has a strong and preferential anti-proliferative effect on the lung CSC population *in vitro* and *in vivo* [14]. Inhibition of telomerase using Imetelstat, either alone or in combination with Trastuzumab, decreases breast CSCs and inhibits their capacity to self-renew in HER2 positive breast cancer cells [30].

Several mechanisms have been postulated to play a role in resistance, including impaired apoptotic machinery, increased DNA-repair mechanisms, up-regulation of multidrug resistance proteins and membrane efflux transporters [31]. Such mechanisms may be responsible, at least in part, for driving CSCs into a phenotype of reduced apoptotic cell death, which in turn forms the basis of tumor progression. ATP-binding cassette (ABC) transporters, such as p-glycoprotein and multidrug resistant associated protein (MRP1), are membrane transporters that can pump structurally unrelated small molecules, such as cytotoxic chemotherapeutic drugs out of the cell. Normal stem cells and lung CSCs express high levels of ABC-transporters resulting in low intracellular drug concentrations [32,33]. For example, the ABCG2 gene is highly expressed in hematopoietic and lung cancer stem cells but is switched off in most terminally differentiated progeny [34].

Cancer stem cells are chemoresistant and metastatic, two features that correlate with poor prognosis and tumor recurrence [35]. Lung adenocarcinoma CSCs have increased expression of galectin-1, a multifunctional protein which promotes invasiveness and metastasis and is associated with poor overall survival and lymph node metastasis [36]. Epithelial to mesenchymal transition (EMT) is an evolutionary conserved developmental process. Studies indicate that metastatic cancer cells, cells which have undergone EMT, exhibit a CSC phenotype [37,38]. Numerous pathways, including the Wnt/β-catenin [39–42], Notch [43,44], Hedgehog [45–47] and NFκB [48] regulate CSC maintenance as well as EMT and may prove to be key targets in eradicating the CSC population and therefore the root of resistance and metastasis [37]. Induction of EMT confers resistance of NSCLC cells to EGFR-tyrosine kinase inhibitors, however, enforced inhibition of the Hedgehog pathway re-sensitizes the NSCLC cells to EGFR-tyrosine kinase inhibitors through mediation of the EMT process [47].

Previously identified lung CSC subsets have been shown to confer resistance to conventional chemotherapeutics, biological molecules, targeted therapies and radiotherapy used in the current management of lung cancer. The elimination of lung CSCs is of utmost importance at the time of therapeutic intervention in order to prevent CSC expansion and subsequent tumor recurrence, relapse