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Mini review

Cancer stem cell markers in lung cancer

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ABSTRACT

Purpose: Lung cancer is responsible for most cancer-related deaths. There are two broad types of lung tumors, usually classified as small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). Current clinicopathological staging systems provide the advantage of standardized criteria for assessing tumor stage, and a relationship between advancing tumor stage and poor prognosis has been established for NSCLC. However, these staging systems have not led to clear criteria for selection of therapy for individual patients with NSCLC. The concept of therapy based on anatomical location, as used in staging systems, is poorly associated with the cancer stem cell (CSC) characteristics of individual tumor tissues. CSCs may have self-renewal and multipotent differentiation abilities and be responsible for tumor initiation, progression, and metastasis; they are highly resistant to chemoradiotherapy. Therefore, research into CSCs will provide the basis for developing of novel diagnostic and therapeutic strategies. We review aldehyde dehydrogenase isoform 1 (ALDH1), CD133, CD44 and CD166 as CSC markers, as weel as the Wnt/β-catenin pathway, KRAS, and the embryonic stem cell (ESC) signature.
Study selection: PubMed databases were searched for relevant articles.
Results: The positivity rate for ALDH1 immunohistochemical (IHC) staining is 19% in patients with stage I NSCLC [1]. The positivity rates for CD133 are 19–48.9% [2–4] and for CD44 are 50.4–67.3% in patients with NSCLC [5,6].
Conclusions: CSCs have been identified in lung cancer and will provide new therapeutic targets for lung cancer. Research on these cells could improve the prognosis of lung cancer.

1. Background

Lung cancer is the most common cause of cancer-related deaths worldwide [7]. It is a heterogeneous disease with two distinct pathological classes: small cell lung cancer (SCLC, making up 20% of all lung cancers) and non-small cell lung cancer (NSCLC, which makes up 80% of cases). The most common forms of NSCLC are adenocarcinoma (AdenoCA, 30–50% of NSCLC) and squamous cell carcinoma (SCC, 30% of NSCLC) [8]. Although approximately 20% of cases of NSCLC are operable at presentation, recurrence rates remain high at 30–50% [9]. Locally advanced NSCLC can be treated with radical chemoradiotherapy with intent to cure; however, overall 5-year survival rates remain low at around 7–20% [10].

Cancer stem cells (CSCs) constitute a relatively rare subpopulation of tumor cells. These cells have the unique ability to initiate and perpetuate tumor growth [11–21]. CSCs also share various characteristics with embryonic and somatic stem cells including

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self-renewal and multipotent differentiation abilities [22]. CSCs may be highly resistant to radiation or chemotherapy [23,24] and may be responsible for tumor initiation, progression and metastasis [25]. Therefore, the development of more effective therapies for cancer requires effective targeting of CSCs [26–33]. Research into CSCs will provide the basis for the development of novel diagnostic and therapeutic strategies. CSCs could be new therapeutic targets for the management of lung cancer.

In recent years, an increasing amount of evidence has been provided to define the CSCs phenotype in human lung cancer [1,25,34,35]. Many of the markers of CSCs in lung cancer have also been found in other tumors and indeed in normal stem cells. Table 1 shows the tumor types in which the metabolic marker aldehyde dehydrogenase isoform 1 (ALDH1) and the cell surface markers CD133, CD44, and CD166 have been documented as markers of CSCs. The rates of positivity for ALDH1, CD133 and CD44 immunohistochemical (IHC) staining in patients with lung cancer are shown in Table 2. We reviewed ALDH1, CD133, CD44 and CD166 as CSC markers, as well as the Wnt/β-catenin pathway, KRAS, and the embryonic stem cell (ESC) signature.

2. Aldehyde dehydrogenase isoform 1 (ALDH1)

Aldehyde dehydrogenase (ALDH) enzymes are a family of intracellular enzymes that participate in cellular detoxification, differentiation and drug resistance through the oxidation of cellular aldehydes [36]. ALDH1 enzyme activity has emerged as a promising marker of CSCs and indeed of normal stem cells [37–39]. It has been known for some time that ALDH1 is highly expressed in normal hematopoietic stem cells, in addition to being a putative stem cell marker. ALDH1 activity also has a known role in drug resistance [40–44]. ALDH1 catalyzes the irreversible oxidation of a range of aliphatic and aromatic aldehydes to their corresponding carboxylic acids [36,45,46]. High ALDH activity is detected in stem and progenitor cells of various lineages including hematopoietic [39,47,48], mesenchymal [49], neural [47], mammary [50,51] and prostate [52] lineages. ALDH1 is expressed in putative lung epithelial stem cell niches, and is overexpressed in tumors compared to normal lung. ALDH1 expression was found to be associated with poor survival in a cohort of stage I NSCLC patients [1,53]. Furthermore, Patel et al. investigated the expression of ALDH1 isozyme in lung cancer tissue samples from patients with one of the following primary lung cancers: AdenoCA, SCC, or SCLC. The results indicate that NSCLC expresses very high levels of ALDH1 in comparison with SCLC [38].

IHC staining for ALDH1 was performed on 3-μm-thick paraffin sections using a mouse monoclonal antibody (anti-ALDH1: Abcam Inc., Tokyo, Japan). Fig. 1 shows ALDH1 IHC staining in lung AdenoCA. The intensity of cytoplasmic staining was scored as negative (0), weak (1), intermediate (2), or strong (3) immunoreactivity. The percentage of positive cells was graded as 0–100%. The ALDH1 score was calculated as follows: Multiplying the staining intensity (0–3) and the percentage of positive cell staining(0–100%). The ALDH1 score = 90 (intensity, 2 × percentage of positive cells, 30) + (intensity, 3 × percentage of positive cells, 10) (Fig. 1).

Table 1
The metabolic maker (ALDH1) and the cell surface makers (CD133, CD44 and CD166) as Cancer Stem Cells markers.

CSCs marker	Expression	Cancer
ALDH1	Cytosol [45,46]	Lung cancer [1] Leukemia [95,96] Glioblastoma [97] Head and neck cancers [98] Breast cancer [50]
CD133	Transmembrane glycoprotein	Lung cancer [34,60,62,63] Central nervous system tumors [18,19] Breast cancer [11] Colon cancer [16,17] Prostate cancer [61] Ovarian cancer [59]
CD44	Cell membrane glycoprotein	Lung cancer [6] Head and neck cancers [66] Breast cancer [11] Pancreatic cancer [27] Prostate cancer [65]
CD166	Cell membrane glycoprotein	Lung cancer [99] Breast cancer [76] Colorectal cancer [13,79] Ovarian cancer [78] Melanoma [77]

3. CD133

The CD133 antigen, sometimes referred to as prominin 1 (PROM1), is a 120 kDa five-pass transmembrane glycoprotein [34,54,55]. However, a few papers have shown CD133 expression in the nucleus [56–58]. The biochemical function of CD133 currently remains unclear, but its expression on the cell surface has been demonstrated to be a specific marker for CSCs in a number of malignancies, including central nervous system tumors as well as colon, breast, prostate, ovarian and lung cancers [1,11,16–19,34,59–63]. It has also been found to have prognostic value in many cancers. Wu et al. performed a systematic review and meta-analysis to evaluate the association of CD133 with prognosis and clinicopathological features of NSCLC patients, and found that a high level of CD133 expression tended to correlate with a worse prognosis and a higher rate of lymph node metastasis in these patients [64].

IHC staining for CD133 was performed on 3-μm-thick paraffin sections using a mouse monoclonal antibody (anti-CD133: Millipore Inc., Temecula, CA, USA). Fig. 2 shows CD133 IHC staining in lung AdenoCA. The CD133 expression score was defined as the proportion of cells with strong expression levels on membranous staining in the tumor section. The percentage of positive cells was graded as 0–100%. The percentage of positive cells for CD133 was 30% (Fig. 2).

4. CD44

CD44 is a cell membrane glycoprotein. In normal cells such as lymphocytes, monocytes and granulocytes, it plays important roles in cell to cell adhesion, interactions with the extracellular matrix, and cell migration. It has also been found to be an important identifier of CSCs in many cancers including breast cancer [11], prostate, pancreatic, and head and neck cancers [27,65,66]. Recently, Leung et al. suggested that CD44 may also plays a role in identifying lung cancer CSCs [6,67]. Wang et al. demonstrated the expression of CD44 variant exon 6 (CD44v6) in 79 lung cancers [68]. The expression rate of CD44v6 was 67.6% (48/71) in NSCLC and 0% (0/8) in SCLC. This finding suggests that the expression of CD44v6 is correlated with histologic type [68]. Sterlacci et al. explored the expression of multiple alleged stemness-associated markers in samples from 371 surgically resected NSCLCs; CD44 may hold promise in the future for ongoing targeted therapies [69].

5. CD166

CD166, also known as activated leukocyte cell adhesion molecule, is a membrane glycoprotein that has been implicated as a potential marker of CSCs. It has a variety of functions in normal tissues, such as intravasation of leukocytes into the central nervous