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Review

# Cancer stem cells with increased metastatic potential as a therapeutic target for esophageal cancer

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ABSTRACT

Esophageal cancers (EC) are highly aggressive tumors, commonly presented in a locally advanced stage with a poor prognosis and survival. Up to 50% of the patients are eligible for treatment with curative intent and receive the standard treatment with neoadjuvant chemoradiotherapy (nCRT) and surgery. Currently, pathologic complete response to nCRT is 20–30%, with a partial or no response in about 50% and 20%, respectively. EC recurrences occur frequently even after successful anti-cancer treatment, suggesting high aggressiveness with increased metastatic potential. A tumor sub-population of so-called cancer stem cells (CSCs), is known to display a high metastatic potential and resistance to conventional anti-cancer therapy, hereby being responsible for the unbeneficial clinical features. In this review, a concise overview will be given of the current literature on esophageal CSCs and related metastases. Esophageal CSC markers will be discussed followed by the pathways that initiate and sustain these cells. In addition, the cellular processes, epithelial-mesenchymal transition (EMT), hypoxia and autophagy, known to contribute to cancer stemness and metastasis will be explained. Finally, potential options for treatment also related to cancer genome atlas (TCGA) data on EC will be discussed.

## 1. Introduction

Esophageal cancer (EC) is currently the 8th most common malignancy worldwide and the 6th leading cause of cancer related death, accounting for more than 490 000 new cases and 400 000 deaths in 2014 (world cancer report 2014). The 5-year survival of this highly aggressive tumor is approximately 20% (www.cancer.org). At diagnosis, patients often present with locally advanced tumors, including lymph node involvement in more than 75%. Usually symptoms occur when the tumor has infiltrated over half of the circumference of the esophagus or has spread by direct local growth in the adventitial tissues, via lymph vessels to surrounding nodes and distantly through hematogenous dissemination. Distant metastases are frequently observed in the liver, lungs, bones, adrenal glands, kidney and brain [1]. There are two typical esophageal cancers, esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC). ESCC, predominantly present in the Eastern and Central Asian world, derives from dysplastic squamous cell epithelium that usually occurs in the upper two-third of the esophagus. EAC mainly develops in the distal esophagus, where ongoing gastroesophageal reflux esophagitis potentially transforms squamous epithelium into columnar intestinal epithelium that further evolves through low and high grade dysplasia into EAC [1,2]. Alcohol and tobacco are the most important risk factors of ESCC whereas EAC is associated with obesity, smoking and chronic gastroesophageal reflux disease (GERD) with premalignant Barrett's esophagus [3,4]. Nodal metastases occur frequently in the mid and upper mediastinum in ESCC and abdominal metastases in EAC [1]. The treatment of choice for locally advanced resectable tumors, both ESCC and EAC, is neoadjuvant chemoradiation (nCRT) followed by radical surgery [5]. Regretfully, around 20% of the tumors will not respond at all, more than 50% do not respond adequately, and even after pathologic complete response early and distant recurrences occur in most patients [6]. Therefore, it is necessary to investigate the subpopulation of cells with increased treatment resistance and metastatic potential, the so-called cancer stem cells (CSCs) [7].

CSCs were first proposed by Virchow and Conheim; a subpopulation of cancer cells resembles the same traits as embryonic cells such as the ability to proliferate, and cancer is derived from the activation of dormant cells of the same tissue [8]. One of the first experiments confirming the existence of CSCs, showed indeed that only a limited percentage of transplanted primary tumor cells could initiate a secondary tumor [9]. Subsequent research used FACS and cell surface

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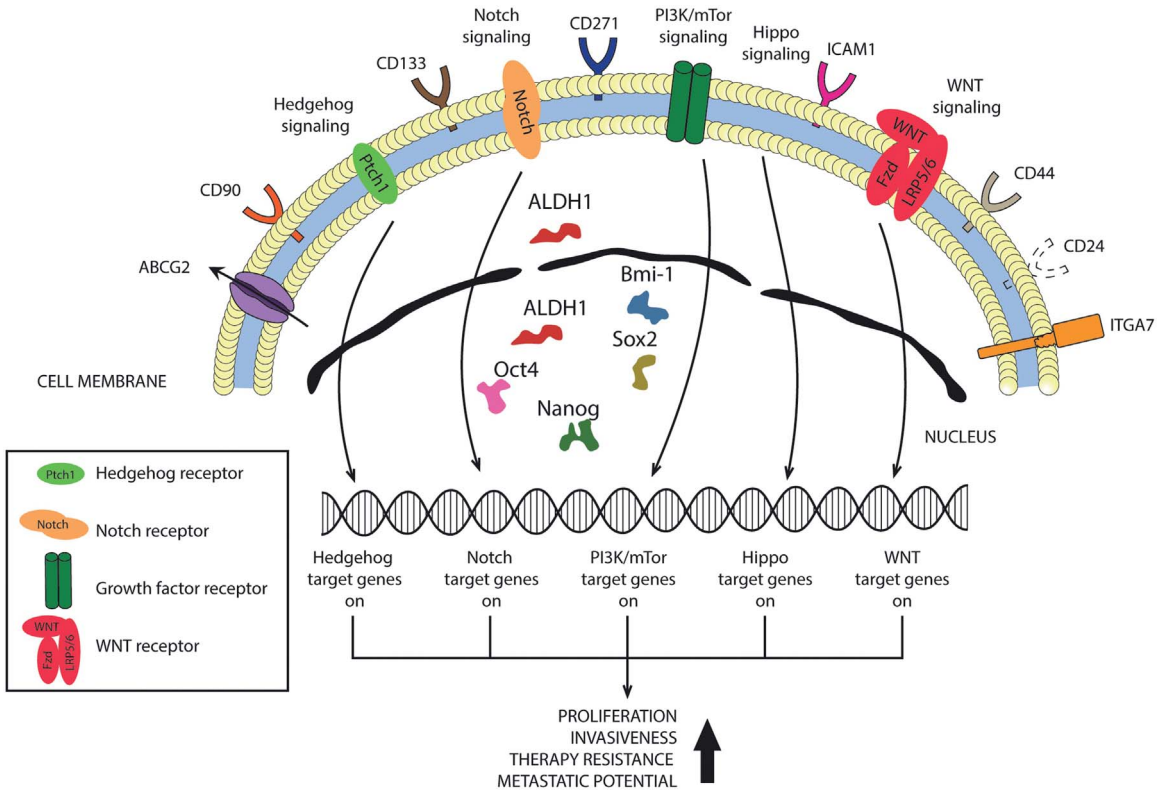


**Fig. 1.** Overview of markers and pathways defining esophageal CSC populations. Cell surface markers ABCG2, CD90, ITGA7 and CD44 are used as single markers while CD44 can be used in combination with CD24 ($CD44^+/CD24^-$), CD133 ($CD44^+/CD133^+$), the intracellular marker ALDH1 ($CD44^+/ALDH1^+$) and ICAM1 ($CD44^+/ICAM1^+$) to identify CSCs. ALDH1 can also be used as a single marker. CD133 can be used alone or in combination with ABCG2 and CXCR4. CD271 is another CSC marker. Other read-outs for cancer stemness are the transcription factors Bmi-1, Nanog, Sox2 and Oct4. Hedgehog, Notch, WNT, PI3K/mTOR and Hippo pathways are implicated to regulate CSC populations leading to more proliferation, invasiveness, therapy resistance and higher metastatic potential.

markers to further characterize CSCs and investigate mechanisms involved in the regulation of cancer stemness [10,11]. CSCs are, in contrast to non-CSCs, thought to be dormant or quiescent [12,13] and therefore therapy resistant but when re-entering the cell cycle are able to form recurrences or metastases [11,13,14]. In vitro cancer sphere forming potential and in vivo tumor initiating potential are often used as read-outs for cancer stemness [15–17]. It is believed that CSCs represent a small percentage of all EC cells with the majority part consist of more differentiated cells [18], albeit this has recently been challenged by studies showing plasticity of differentiated tumor cells [19,20].

This review will focus on EC CSCs as a target for eliminating resistant and highly metastatic cell populations and the role of tumor microenvironment in facilitating this process.

## 2. Markers to identify esophageal CSCs

Although the use of markers to select CSC enriched populations is disputed due to the lack of universal markers owing to tumor heterogeneity, it tremendously contributed to current knowledge, including that of EC [20–22] (Fig. 1).

CD44, a lymphocyte homing receptor that has a role in adhesion, motility, proliferation and cell survival [23] has extensively been studied both as a single and combined marker for CSCs. Interestingly, several CD44 variants were suggested to be a prognostic marker for adenocarcinoma of Barrett's esophagus [24] and ESCC [25]. Li et. al. [26] first suggested tumor stem-like cells to express CD44, being enriched in culture and highly expressed after irradiation. Next, Zhao et al. [27] showed increased colony formation, drug resistance and ESCC tumor initiation of CD44 cells. Regretfully, CD44 is being expressed by the majority of ESCCs in KYSE30 cells [28]. Combining CD44 with other markers greatly enhances its discriminative properties. As such, we [17] identified a $CD44^+/CD24^-$ subpopulation with CSC-like characteristics in esophageal cell lines OE33 (EAC), OE21 (ESCC), and in EC tumor biopsies. CD24, a heat-stable cell surface antigen, has a role in cell–matrix and cell–cell interactions [17,29]. $CD44^+/CD24^-$ cells had higher sphere forming potential, were more resistant to irradiation, formed tumors more aggressively, resided in hypoxic niches and the proportion of $CD44^+/CD24^-$ cells correlated with the tumor growth rate [17]. Furthermore, $CD44^+/CD24^-$ were present in half of the pretreatment biopsies of patients with residual EAC but not at all in biopsies of patients with complete pathologic response after nCRT. These results suggest that $CD44^+/CD24^-$ cells have CSC-like features and may be a target for therapy [17]. Based only on in vitro data CD44 in combination with aldehyde dehydrogenase 1 (ALDH1) was suggested to identify EC stem-like cells [30,32]. Moreover, ALDH1 expression in ESCCs was correlated with poor histological differentiation, lymph node metastasis and pathologic TNM classification [31–33], Even as a single marker, ALDH1 seems to be enriched in ESCC cell line derived tumor spheres and solely $ALDH1^{high}$ cells formed lung metastasis [34–36], had high EMT potential, were more invasive, showed increased metastic potential and were related to poor patient outcome [37,38]. Therefore, ALDH1 seems to be a good candidate CSC marker alone or in combination with CD44 [30]. Other combinations published are $CD44^+/ICAM1^+$ [39] showing all major CSC-like phenotypes, and $CD44^+/CD133^+$ that predict recurrence and prognosis of ESCC [40]. So compiling evidence indicates that CD44 in combination with other markers may enrich for EC CSCs and is of at least some prognostic value.

Another cell surface marker potentially identifying EC CSCs is ABCG2, member of group G in the ATP-binding cassette (ABC) transporter family [41]. In healthy tissue ABCG2 transporter functions as a first line defense mechanism against cytotoxic substances. In the gastrointestinal tract, including the esophagus, ABCG2 is abundantly