Novel markers of cancer stem cells in head and neck squamous cell carcinoma

Takahito Fukusumi¹,², Hideshi Ishii², Jun Koseki², Atsushi Hanamoto¹, Susumu Nakahara¹, Yoshihumi Yamamoto¹, Hidenori Inohara¹

¹Department of Otorhinolaryngology - Head and Neck Surgery, Osaka University, Graduate School of Medicine, Suita, 2-2 Yamadaoka, Osaka 565-0871, Japan
²Department of Cancer Profiling Discovery, Osaka University, Graduate School of Medicine, Suita, 2-2 Yamadaoka, Osaka 565-0871, Japan

Correspondence: Hidenori Inohara
E-mail: hinohara@ent.med.osaka-u.ac.jp
Received: March 09, 2015
Published online: May 07, 2015

Introduction

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer, and its prognosis remains poor. The poor prognosis results from recurrence, metastasis, and therapeutic resistance. Recently, cancer stem cells (CSCs) were shown to result in treatment failure, but their function and identification are not well understood in HNSCC. Previously, several markers of CSCs in HNSCC have been reported from 2007 to 2009. However, whether these markers serve as true markers of CSCs remains controversial. Thus, there is a need to explore novel CSC markers in HNSCC. Since 2013, several novel HNSCC CSC markers have been reported, and in this review we describe and discuss these markers.

Keywords: cancer stem cells; CD44v; CD98; CD271; CD10; head and neck squamous cell carcinoma


Recently, the theory of cancer stem cells (CSCs) was developed[9]. CSCs are defined as cells that have tumorigenicity and self-renewal properties. In terms of clinical characteristics, CSCs are capable of metastasizing[10] and contribute to therapeutic resistance, such as chemoresistance and radio-resistance[11]. Thus, the identification of true CSC markers is the first step for the establishment of prognostic biomarkers and target-specific drugs. Previously, CD44[12], CD133[13], and aldehyde dehydrogenase 1 (ALDH1)[14] were reported as CSC markers in HNSCC from 2007 to 2009 (Table 1). Several reports also revealed that expression of these markers in HNSCC cells exhibit CSC properties. For example, CD44(+) cells from primary tumors can give rise to tumors more rapidly in xenograft models compared to CD44(−) cells, and these xenograft tumors subsequently reproduce the original tumor...
Table 1. Previous CSCs markers in HNSCC and the conflicting data

<table>
<thead>
<tr>
<th>Marker</th>
<th>Year</th>
<th>Contrary opinions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD44</td>
<td>2007</td>
<td>CD44 expression in normal head and neck epithelium is as same as in HNSCC. CD44(+) and CD44(−) cells derived from squamous-spheres are capable of regenerating these spheres from single cell suspensions. The CD44(−) cells have sphere-formation ability and tumor-initiating capacity similar to the CD44(+) cells.</td>
<td>18, 19, 20</td>
</tr>
<tr>
<td>CD133</td>
<td>2008</td>
<td>In clinical specimens of hypopharyngeal cancers, CD133 expression was negative, as determined by flow cytometry analysis and immunohistochemistry.</td>
<td>21</td>
</tr>
<tr>
<td>ALDH1</td>
<td>2009</td>
<td>Poor prognosis linked to decreased rather than increased expression of ALDH1</td>
<td>22</td>
</tr>
</tbody>
</table>

heterogeneity observed in the primary tumor. Importantly, CD44(+) cells have also been discovered to have a greater capacity to handle oxidative stress and exhibit radio-resistance[15]. This population has also been shown to have a significantly greater ability to metastasize to regional lymph nodes in animal models[16], and patients whose tumors have greater percentages of CD44(+) cells have significantly poorer clinical outcome[17].

However, whether these markers are true CSC markers remains controversial (Table 1). For example, CD44 expression in normal head and neck squamous epithelium is as same as in HNSCC[18]. Lim et al. found that both CD44(+) and CD44(−) cells derived from squamous-spheres are capable of regenerating these spheres from single cell suspensions[19]. Oh et al. showed that CD44(−) cells have stem-cell like traits. In their report, CD44(−) cells showed sphere-formation ability and tumor-initiating capacity similar to CD44(+) cells[20]. In clinical specimens from hypopharyngeal cancers, CD133 expression was negative, as determined by flow cytometry and immunohistochemistry[21]. Furthermore, poor prognosis linked to decreased rather than increased expression of ALDH1[22]. Because of these discrepancies, the novel CSCs marker in HNSCC is needed to explore. Since 2013, several novel HNSCC CSCs markers have been reported. The methods used to find these markers, and the phenotypes of these proposed CSCs differ. In this review, we explain and explore these markers in chronological order.

CD44v

Yoshikawa et al. reported that CD44v(+) cells have CSC properties in HNSCC[23]. CD44 is a major marker for CSCs in many epithelial tumors[24-26] and is implicated in tumor growth, invasion, metastasis[27, 28]. CD44 have variant isoforms generated by alternative mRNA splicing[29]. Previously, their group showed that CD44v8-10(+) gastrointestinal cancer cells have CSC phenotypes, and CD44v interacts with the cystine transporter subunit xCT. xCT reduces glutathione (GSH) and promotes reactive oxygen species (ROS) resistance in cancer cells[30]. Sulfasalazine is a specific inhibitor of cysteine transport that is mediated by xCT, and suppresses the proliferation, metastasis, and invasion of several cancers[31, 32]. They also showed that sulfasalazine treatment suppressed tumor growth and chemoresistance of CD44v(+) gastrointestinal cancer cells[30]. Similar to gastrointestinal cancer, Yoshikawa et al. explored whether the CD44v(+) cells in HNSCC have various CSC properties and investigated their relationship with xCT. To explore the clinical characteristics of CD44v(+) cells in HNSCC patients, CD44v9 expression in tumor tissues of patients treated with or without neoadjuvant chemotherapy were evaluated. CD44v(+) areas are increased in HNSCC tumors of patients who received neoadjuvant chemotherapy, suggesting that the CD44v(+) cells have chemoresistance compared to the CD44v(−) cells. Moreover, expression levels of the differentiation marker involucrin tended to be reduced in tumor tissue resected after neoadjuvant chemotherapy compared with patients not subjected to chemotherapy. The GSH-dependent antioxidant system promotes resistance of CD44v(+) gastrointestinal cancer cells[30]. Thus, to investigate the chemoresistant mechanism in HNSCC cells, they examined the effect of cisplatin, which induces cytotoxicity mediated by ROS[33]. The results show that CD44v expression status of HNSCC cell lines is related to cisplatin resistance. Additionally, the GSH-dependent system plays a key role in cisplatin resistance as a result of its ability to scavenge ROS[34]. Therefore, they also examined GSH levels in HNSCC cell lines. As expected, intracellular GSH levels correlated with cisplatin resistance. Given that xCT promotes GSH synthesis, and that CD44v stabilizes xCT and ROS defense in cancer cells[30], they next examined the effect of sulfasalazine on HNSCC cells. The results showed that CD44v(+) cells are sensitive to sulfasalazine in vivo. Moreover, the tumors formed by the CD44v(+) cell line comprised a heterogeneous population of CD44v(+) and CD44v(−) cells. Furthermore, CD44v(−) cells in established tumors expressed involucrin. Given that the expression level of the epidermal growth factor receptor (EGFR) affects the squamous cell carcinoma differentiation[35], Yoshikawa et al. investigated cellular
EGFR expression and revealed that it was lower in the CD44v(+) cells than in CD44v(−) cells. These results suggest that EGFR signaling is suppressed in CD44v(+) cells. Moreover, the effect of the EGFR inhibitor increased significantly followed CD44 ablation. They also investigated the effect of combined treatment consisting of the EGFR inhibitor cetuximab and sulfasalazine on mouse xenograft tumor; sulfasalazine significantly increased the anti-cancer effect of cetuximab. Therefore, they concluded that CD44v(+) HNSCC cells are undifferentiated, are chemoresistant, stabilize xCT, and maintain low ROS levels. However, CD44v(+) cells are sensitive to the xCT inhibitor sulfasalazine. Thus, they suggest targeting the CD44v(+) CSC population using sulfasalazine.

**CD98**

Squamous epithelium stem cells are hypothesized to exist in the basal cell layer,[36] Moreover, mutations of normal epithelium stem cells may be the first step of oncogenesis.[37] Kemp et al. generated a monoclonal antibody for squamous cell carcinoma surface antigens.[38] This monoclonal antibody K984 targeted undifferentiated cells in the basal layer. To determine what was recognized by K984, they performed immunoprecipitation. Analysis of this immunoprecipitation product obtained from this study indicated that this antibody combines CD98. CD98 expression has been shown to relate to the survival rate of several cancers[39−43]; these studies indicate that higher CD98 expression in tumors is related to poor prognoses. In head and neck carcinoma, CD98 expression is an independent prognostic marker.[44] However, the underlying mechanisms of this relationship is not clear. CD98 consists of heavy chains and light chains. The CD98 heavy chain was shown to mediate integrin signals,[45], leading to AKT phosphorylation and stimulate cell survival and metastasis. On the other hand, its light chain can import essential amino acids,[46], which activate the mTOR pathway. This pathway stimulates cell proliferation and protein synthesis. Kemp et al. reported that CD98 is specifically expressed on the basal layer of normal mucosa and HNSCC, and assumed that CD98 is a novel marker of HNSCC CSCs.[47]. To confirm this hypothesis, they first examined CD98 and CD44 expression by immunohistochemistry and found that the expression of CD98 overlaps with CD44v6 in HNSCC tumors. Next, they evaluated the tumorigenicity of the CD98(+) cells by means of transplantation into immunodeficient mice. Mice injected with unselected cells showed one tumor following six injections of 25,000 cells. CD98(−) cells did not result in any tumor growth, whereas CD98(+) cells grew into tumors in four out of six injections of 25,000 cells. These tumors arising from the CD98(+) cells, were passaged by injection of 25,000 sorted cells from the CD98(+) and CD98(−) population. The CD98(−) cells form no tumors, whereas CD98(+) cells developed tumors. From these observations, they concluded that CD98(+) cells have more tumorigenicity than the CD98(−) cells. To further investigate the difference between CD98(+) and CD98(−) cells at the molecular level, they isolated these cell populations from mice tumors made by a HNSCC cell line, and analyzed gene expression using a microarray. 292 genes were expressed significantly higher in CD98(+) cells than CD98(−) cells. They used a database and found that cell cycle and DNA integrity genes were significantly enriched in this dataset. In this result, CD98(+) cells were indicated to control cell cycle that is tightly regulated by CSCs. Conversely, 387 genes that were expressed significantly higher in CD98(−) cells than CD98(+) cells. Using a database, they found that the dataset was significantly enriched for genes related to cellular differentiation, including keratinocyte differentiation. This result implies that CD98(−) cells are differentiated and therefore not able to produce progeny. However, stem cell related genes, such as *OCT3/4, SOX2, KLF4*, and *Nanog*, did not show significant differences between CD98(+) and CD98(−) cell populations.

**CD271**

CD271 is known as the low affinity nerve growth factor receptor (NGFR) or p75 neurotrophin receptor. In the nervous system, CD271 has critical functions in cell survival,[48], differentiation,[49], and migration[50] of neuronal cells. Previously, CD271 was reported as a tissue stem cell marker of laryngeal[51], oral[52], and esophageal squamous epithelia.[53] CD271 is also reported to be a CSC marker in malignant melanoma[54, 55], and esophageal squamous cell carcinoma.[56] Given this background, Imai et al. speculated that CD271 is a marker of CSCs in HNSCC.[21]. To prove this hypothesis, they used tumor specimens resected from hypopharyngeal cancer (HPC) patients, and injected these specimens into immunodeficient mice. From this, they established three xenograft tumor lines. In these transplanted tumor lines, the CD271 expression was analyzed. The result showed that 2%−20% of cells were positive for CD271. Next, they analyzed the location of CD271(+) cells in transplanted tumors. The most of CD271(+) cells existed in the basal layer of the tumor, and in the peripheral zone of the tumor. CD271(+) cells were almost observed next to the stroma. CD271(+) cells were also moderately positive for CK8, which is a marker of undifferentiated squamous carcinoma cells. They also examined CD271 expression within clinical HPC specimens and normal hypopharyngeal mucosa. Similar to transplanted tumors, CD271(+) cells exist in the basal layer and in the invasive cancer front. These results suggest that CD271(+) HPC cells exist in the basal layer where CSC may reside, and they contain undifferentiated cells. They further examined whether sphere formation culture would
affect CD271 expression and showed that sphere formation caused enrichment of the CD271(+) population. Tumorigenicity analysis of the CD271(+)(-) cells was also performed; CD271(+) cells kept tumorigenic with as few as 30 cells, and possessed higher tumorigenicity than CD271(-) cells. They next asked whether the CD271(+) cells had characteristics associated with stemness, invasion, and metastasis. They examined the expression of stem cell related genes, Nanog, Oct3/4, and Sox2. Nanog expression was significantly higher in the three CD271(+) HPC lines compared to CD271(-) cells. However, Oct3/4 and Sox2 expression showed no significant difference between CD271(+) cells and CD271(-) cells. Next, they examined the expression of MMP1, MMP2, and MMP10 that were known as invasion related genes. The CD271(+) cells of the HPC lines showed a significantly higher expression of these genes than CD271(-) cells. They also examined immunohistochemistry staining of these MMPs, and showed the CD271(+) cells were positive for all of these genes. Considering the localization of the CD271(+) cells at invasive fronts, these results suggest that CD271(+) cells are affected by MMPs that enable tissue invasion. To determine whether CD271(+) cells have chemoresistant properties, the effect of cisplatin on these cells was analyzed. They treated mice bearing tumors with cisplatin. The tumors were resected and analyzed by flow cytometry analysis on day 7. The CD271(+) fraction increased from 16.3% to 35.2%. This result indicated that the CD271(+) cells had cisplatin resistance. The chemoresistance of CSCs is linked to higher expression of the ATP binding transporter. Therefore, they compared the expression of ABCC2, ABCB5, and ABCG2 between CD271(+) and CD271(-) cells; the expression of these ABC-transporter in CD271(+) cells was significantly higher than that in CD271(-) cells. Next, they investigated whether CD271 expression related to the prognosis of HPC patients. They classified 83 patients as strong or moderate-weak group according to the degree of CD271 expression. The 3-year survival rate of the CD271 strong group was significantly lower than that of the CD271 moderate-weak group. These data indicate that CD271 expression is associated with poor clinical outcome.

Murillo-Sauca et al. also indicated that CD271 is a novel HNSCC CSC marker. They mainly used surgical specimens and cell lines in oral squamous cell carcinoma. Similar to Imai et al., CD271 expression was located in the basal layer of malignant epithelium, and CD271(+) cells exhibited greater tumorigenicity than CD271(-) cells in oral squamous cell carcinoma. Additionally, they examined the interdependence between CD271 and CD44; the CD271(+) cells comprised a subpopulation among CD44(+) cells in primary tumors, but there was no appreciable CD44(-)CD271(+) subpopulation by flow cytometry. Thus, they sorted the CD44(+)CD271(+) cells, CD44(+)CD271(-) cells, and CD44(-)CD271(-) cells from primary human oral SCC specimens and implanted them into immunodeficient mice. Overall, the CD44(+) cells had a greater capacity to form xenograft tumors compared to CD44(-) cells, but when low numbers of cells were injected, it was clear that the CD44(+)CD271(+) cells had the greatest capacity to form tumors among these three populations. The tumors that formed in mice implanted with CD44(+)/CD271(+) cells recapitulated the heterogeneity seen in the parental tumor. To assess the function of CD271, they generated CD271 knockdown cells, and investigated subsequent tumor growth and cell cycle. Their results showed that CD271 significantly affected tumor growth and a partial cell cycle block during G2-M transition. Based on these results, they suggested that this molecule might be a therapeutic target in HNSCC. To address this hypothesis, they focused on the fact that CD271 is known as low affinity NGFR. They targeted the receptor with a monoclonal antibody specific for NGFR. Incubation of oral squamous cancer cells with this antibody resulted in a significant reduction in cell proliferation in vitro. Furthermore, cells treated with the anti-NGFR antibody resulted in a significant reduction in tumor growth in vivo.

CD271/NGFR activation has been previously shown to work through downstream activation of both the MAPK and PI3K pathways. To determine the functional role of CD271 in HNSCC, they treated cells with recombinant human NGF in vitro and assessed activation of the MAPK pathway. NGF treatment resulted in an increase in detectable Erk phosphorylation. Furthermore, pre-incubation with the monoclonal antibody against CD271 abrogated this increase in Erk phosphorylation. However, there was no apparent effect on the PI3K pathway, since p-Akt was not affected. Their data indicate that CD271/NGFR is functionally active in HNSCC and that targeting CD271 with a monoclonal antibody is a potential therapeutic strategy in this cancer. One of the significant adverse features seen with some HNSCC tumors is perineural invasion (PNI), which has been associated with local recurrence and metastases. Because Schwann cells around neurons and epithelium rich in neurons express NGF, they assumed that expression of CD271 might predispose tumor cells to PNI. Indeed, in malignant melanoma, CD271 expression has been associated with PNI, and in oral cancer, NGF expression has also been associated with PNI.

CD200

CD200 is a type-I transmembrane glycoprotein. CD200 is expressed in kidney glomeruli, endothelial cells, lymphocytes, neurons, hair follicle epithelial cells, and thymocytes. Several cancer cells and tissues express...
CD200, including melanoma, malignant mesothelioma, neuroblastoma, chronic lymphocytic leukemia, ovarian, testicular, renal cell carcinoma, prostate, breast, and colon cancers\cite{68}. CD200 is also interdependent on CSC markers of prostate, colon cancer, glioblastoma, and breast\cite{69}, but there are no studies focused on the role of CD200.

Jung et al. proposed CD200 as a HNSCC CSC marker\cite{70}. First, to assess the expression of CD200, several HNSCC cell lines of varying human papillomavirus (HPV) statuses were analyzed; CD200 was expressed in all HNSCC cell lines irrespective of HPV status. These results indicate that HPV status does not related to CD200 expression. Next, they analyzed the relationship between CD200 and the CSC related markers, such as sonic hedgehog (Shh) and B lymphoma Mo-MLV insertion region 1 homolog (Bmi-1). Their result showed that Shh signal intensity was related to CD200 signal intensity, and that overexpression of CD200 increased Shh and Bmi-1 expression. Next, they used tonsil epithelial cells of wild-type mouse. They transfected HPV E6, E7 protein and Ras, and immortalized these cells (MEER). MEER were transfected to overexpress CD200 to investigate whether CD200 expression results in phenotypic changes associated with oncogenesis. CD200 overexpressing cells demonstrated significantly slower proliferation than control MEER cells. However, colony formation assays after exposure to cisplatin and radiation revealed that CD200 did not induce resistance in vitro. To evaluate if the expression of CD200 affects the resistance of cisplatin and radiation in vivo, they injected CD200 overexpressing and control MEER cells (1 x 10⁶ cells) into C57BL/6 mice, and treated mice with cisplatin and radiation. CD200 overexpressed cells grew significantly faster than control cells. Moreover, the survival rate of CD200 overexpressed cells was significantly worse than control cells. These data indicated that CD200 expression related to the resistance of chemo-radiation in vivo. However, tumorigenicity without treatment demonstrated no difference between CD200 overexpressed cells and control cells when injected into wild type or immunodeficient mice.

**CD10**

CD10 was detected by an antigen array Lyoplate to identify antigens refractory to cisplatin and radiation. The BD Lyoplate Human Cell Surface Marker Screening Panel consists of 242 purified monoclonal antibodies against cell surface markers (CD1–CD340). This is the first research that attempted to identify the marker of therapeutic resistance and CSC properties using this array. The antigen expression levels of three HNSCC cell lines that survived cisplatin or radiation treatment were compared with untreated counterparts using this array. The result showed that four cell surface antigens, CD10, CD15s, CD146, and CD282, were upregulated in all cell lines after cisplatin or radiation exposure. To validate whether these antigens related to therapeutic resistance, they used a cisplatin-resistant cell line, and compared these four antigens expression. They found that only CD10 expression was higher in cisplatin-resistant cells than parental cells. Indeed, the CD10(+) subpopulation accounted for 22.5% of cisplatin-resistant cells compared with 1.4% of parental cells, respectively. They concluded that CD10 is the marker of therapeutic resistance in HNSCC cells\cite{71}.

CD10 is a zinc-dependent metalloendoprotease that cleaves signaling peptides\cite{72, 73}. CD10 has been shown to be expressed in various normal cells, and to be the tissue stem cell marker of the bone marrow\cite{74}. adipose tissue\cite{75}, lungs\cite{76}, and breasts\cite{77}. CD10 is also expressed in several types of cancers, such as the kidney, liver, skin, cervix, prostate, lung, breast, pancreas, stomach, and bladder\cite{78}. The association between CD10 and metastasis have been shown by several reports\cite{79}. In HNSCC, the role of CD10 in tumor differentiation and growth has been reported\cite{80}; however, the underlying mechanism is not clear. Fukusumi et al. focused on the cell cycle, because slow-cell cycling cells are resistant to chemotherapeutic agents\cite{81}, and most resistant to radiation during G0/G1 and S phases\cite{82}. Using Hoechst33342 staining, they revealed that CD10(+) cells were slow-cell cycling compared to CD10(−) cells. Given that CSCs are responsible for therapeutic resistance\cite{83}, and are slow-cell cycling and mainly in G0/G1 phase\cite{84}, they hypothesized that CD10 serve as a CSC marker of HNSCC. First, they compared the CD10 expression in spheroid cells and control adherent cells. In two HNSCC cell lines, CD10 expression in spheroid cells was significantly greater than in adherent cells. Next, they compared sphere formation ability between CD10(+) and CD10(−) cells; CD10(+) cells formed significantly more spheroid colonies than CD10(−) cells. Moreover, CD10(+) colonies were significantly larger than CD10(−) colonies. They also examined whether CD10(+) cells had more tumorigenicity than CD10(−) cells in vivo. They sorted CD10(+) cells and individually injected into immunodeficient mice. When 1,000 cells were injected, the CD10(+) cells formed tumors in all mice, while the CD10(−) cells formed tumors in one third of mice. Furthermore, CD10(+) cells kept tumorigenic with only 100 cells. Based on these results, they concluded that the CD10(+) cells had sphere formation ability in vitro and tumorigenicity in vivo compared to the CD10(−) cells. They also examined the interdependence between CD10 and other HNSCC CSCs markers, such as CD44 and ALDH1. They found no significant interdependence between CD44 and CD10. On the other hand, they revealed CD10(+) cells contained...
significantly more ALDH1(+) fraction compared to CD10(−)
cells. To determine the mechanisms of tumorigenicity and
the self-renewing properties of CD10(+) cells, the expression
of OCT3/4, a tissue stem cell gene[83], and CSCs[86] was
compared between CD10(+) and CD10(−) cells. Chen et
al.[18, 87] showed that HNSCC CSCs upregulated OCT3/4. As
expected, the expression of OCT3/4 in the CD10(+) cells was
significantly increased compared to the CD10(−) cells. Of
note, CD10 knockdown using siRNA resulted in decreased
OCT3/4 expression.

Discussion

CSCs were first identified in acute myeloid leukemia; acute
myeloid leukemia cells with CD34(+)/CD38(−) are
tumorigenic in immunodeficient mice[88]. Since this report,
several others have described CSC markers in several organs.
For instance, brain CD133(+) tumor cells[89] and breast
CD44(+)/CD24(−) cancer cells[24] were identified as CSCs.
From 2007 to 2009, CSC markers in HNSCC have been
reported. However, whether these markers are true CSCs
markers remains controversial (Table 1). Thus, novel CSC
markers in HNSCC require further exploration.

Since 2013, several CSC markers, such as CD44v[23],
CD98[87], CD271[21, 58], CD200[70], and CD10[71] were
reported HNSCC (Table 2). The strategies for finding these
markers vary, and include investigating a CSC marker of
other organs, staining the basal cell layer, and using a cell
surface antigen array. These markers have also been studied
with respect to known CSC properties, such as chemoresistance, radio-resistance, sphere formation assay, tumorigenicity, and the expression of stem cell related genes. However, to date, there is no report that performs all of these
experiments using these markers. There are also reports that
performed experiments to compare expression of potential
CSC markers with previous HNSCC markers. For example,
the CD10(+) cells expressed significantly more ALDH1 than
CD10(−) cells[71]. Again, however, there are no studies
investigating CSC properties using a combination of these
recent markers. These facts indicate that further studies
combining various markers are needed. In fact, CSC markers
of other organs, such as AML[88] and breast cancer[24], are
defined by a combination of several CD markers.

Table 2. Characteristics of current CSCs markers in HNSCC

<table>
<thead>
<tr>
<th>Marker</th>
<th>CD44v</th>
<th>CD98</th>
<th>CD271</th>
<th>CD271</th>
<th>CD200</th>
<th>CD10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Materials</td>
<td>cell line</td>
<td>cell line, clinical specimen</td>
<td>clinical specimen</td>
<td>cell line, clinical specimen</td>
<td>cell line</td>
<td>cell line</td>
</tr>
<tr>
<td>Chemoresistance</td>
<td>CDDP</td>
<td>-</td>
<td>CDDP</td>
<td>-</td>
<td>CDDP*</td>
<td>CDDP, 5-FU</td>
</tr>
<tr>
<td>Radioresistance</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Yes*</td>
<td>Yes</td>
</tr>
<tr>
<td>Sphere assay</td>
<td>Yes</td>
<td>-</td>
<td>Yes</td>
<td>-</td>
<td>-</td>
<td>Yes</td>
</tr>
<tr>
<td>Minimum cells of tumorigenicity</td>
<td>-</td>
<td>25,000</td>
<td>30</td>
<td>100 **</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td>Related genes</td>
<td>xCT genes related to cell cycle and DNA integrity</td>
<td>Nanog, MMPs, ABCC2, ABCB5, ABCG2</td>
<td>-</td>
<td>Shh, Bmi1</td>
<td>Oct3/4</td>
<td></td>
</tr>
<tr>
<td>Function</td>
<td>ROS resistance</td>
<td>-</td>
<td>-</td>
<td>cell cycle block</td>
<td>-</td>
<td>slow cell cycle</td>
</tr>
</tbody>
</table>

Reference 23, 47, 21, 58, 70, 71

CDDP: cisplatin; 5-FU: fluorouracil; ROS: reactive oxygen species. *mouse tonsil epithelial cell line; **CD44(+)/CD271(+) cells

Table 2 shows the genes related to the investigated
markers. Each CD marker is related to different genes, and
almost all of these genes are related to stemness. The
relationship between CSCs and squamous epithelium stem
cells also remains unknown. As for squamous epithelium
stem cells in the head and neck region, these cells reside in
the basal cell layer[36]. Bmi-1[90] and Lgr5[36] were reported as
markers of squamous epithelium stem cells. Bmi-1 has been
already been examined with respect to a HNSCC CSC context[87]; Bmi-1 overexpression ALDH1(+) HNSCC cells resulted
in increased colony formation, migration, and invasion.
Moreover, Bmi-1 over expression increased tumor
formation, radio-resistance, and distant metastasis[91].
CD98[84] and CD271[21, 58] also exist in the basal layer of
malignant epithelium; however, these reports did not show
the relationship or changes between normal epithelium and
cancerous tissue.

Conclusions

Recently, several HNSCC CSC markers have been
reported. Identification of accurate HNSCC CSC markers is
imperative to reveal this disease characteristic. But, further
study about the mechanisms and functions of these markers is needed. These studies may contribute to the diagnosis, prognosis, and treatment of HNSCC patients.

Conflicting interests

Institutional endowments were received partially from Taiho Pharmaceutical Co., Evidence Based Medical (EBM) Research Center, Chugai Co., Ltd, Yakult Honsha Co., Ltd., and Merck Co., Ltd.

Author contributions

All authors conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

References


58. Murillo-Sauca O, Chung MK, Shin JH, Karamboulas C, Kwok S,


90. Tanaka T, Komai Y, Tokuyama Y, Yanai H, Ohe S, Okazaki K, et
