

Correlation of CD133 and Oct-4 expression with clinicopathological and demographic parameters in oral squamous cell carcinoma patients

ABSTRACT

Objective: Squamous cell carcinoma of oral cavity is one of the most common cancers of Indian subcontinent with the 5-year survival rate of 50% despite the recent advances in the treatment. The aim of the present study was to study cancer stem cell markers CD133 and Oct-4 in oral squamous cell carcinoma (OSCC) patients and their correlation with clinicopathological variables.

Materials and Methods: This was a prospective study which included 50 cases of histopathologically proven squamous cell carcinoma of oral cavity. Expression of CD133 and Oct-4 was evaluated by immunohistochemistry (IHC) and their expression was correlated with various clinicopathological and demographic parameters.

Results: CD133 expression was seen in 20.6% cases of clinical Stage I–II and in 79.4% of clinical stage of III–IV OSCC patients, the difference being statistically significant with the $P = 0.048$. There was no statistically significant association between CD133 expression and any other clinicopathological or demographic variable. Oct-4 was expressed only in one case.

Conclusions: CD133 expression was significantly seen higher in Stage III–IV tumors, the stem cells may be responsible for the aggressiveness of the OSCCs and these stem cells can be potential prognostic markers and targets for the future targeted therapy.

Keywords: CD133, cancer stem cells, Oct-4, oral squamous cell carcinoma

INTRODUCTION


Oral squamous cell carcinoma (OSCC) is the sixth most common type of head-and-neck cancer, in the world. India is known as the capital of oral cancer, as OSCC is the most common cancer in man and the third most common carcinoma in females here.^[1] Its incidence has increased by 50% in the past decade^[2] and nearly 200,000 mortality cases occurs annually worldwide.^[3,4] The prognosis and the overall survival greatly depends on the stage of the tumor at the time of diagnosis. Despite the recent advances in the treatment, the 5-year survival rate is <50% in OSCC patients^[5,6] which could be attributed to the late stage diagnosis, high incidence of metastasis and suboptimal response to the current treatment modalities. An emerging concept of cancer stem cell (CSC) theory postulates that a group of tumor cells possess the ability of self-replication and tumorigenesis which may be responsible for suboptimal response to treatment,

metastasis, and recurrence of cancers such as head-and-neck squamous cell cancers.^[7] Identification of these CSCs is a major challenge. CD133 also called prominin-1 is one of the earliest and most widely used stem cell markers.^[8] The CD133 plays a major role not only as biomarkers for classification

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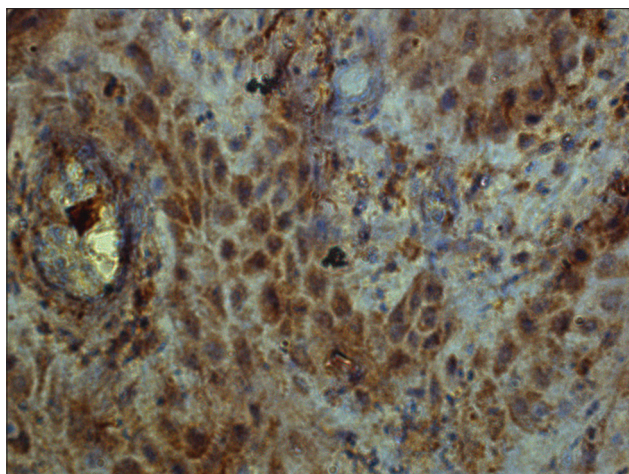


Figure 1: Immunohistochemical expression profile of CD133 (Cytoplasmic, x40)

and isolation of stem cells but also functions in tumor biology, growth, and development.^[9,10]

Expression of CD133 has been observed in normal as well as CSCs of tissues, comprising human OSCC.^[11,12] Many studies have revealed that CD133 expression in cancer cells shows resistance toward therapies and this fact predicts that they play a major role in defining the clinical course of OSCC.^[13]

Several other CSC markers such as Oct-4, Sox-2, Nanog, ABCG2 have been identified in many tumors, and the role of these cancer markers explains the involvement of CSCs in cancer progression.^[14] Genes of POU family regulate the Oct-4 transcription factor and are involved in the self-renewal of the stem cell. Oct-4 is expressed by pluripotent stem cells and not by differentiated cells. It has been validated as a CSC marker of stem cells in tumors of germ cell.^[15] The Oct-4 expression in tumor cells can be correlated with CSC behavior, tumorigenic potential, and aggressive clinical features such as metastasis and disease progression.^[16,17] In many studies, expression of Oct-4 was also found to be higher in aldehyde dehydrogenase (ALDH+) tumor cells. The ALDH is also a known CSC marker in HNSCC.^[18]

In the present study, we studied the expression of CD133 and Oct-4 in OSCC samples by immunohistochemistry (IHC) and correlated with various clinicopathological variables.

MATERIALS AND METHODS

Patients and tissue samples

The present study was approved by the Institutional review board and the stem cell ethics committee of the institute. Written informed consent was obtained from all the patients. Biopsies of all 50 patients with clinical diagnosis of OSCC

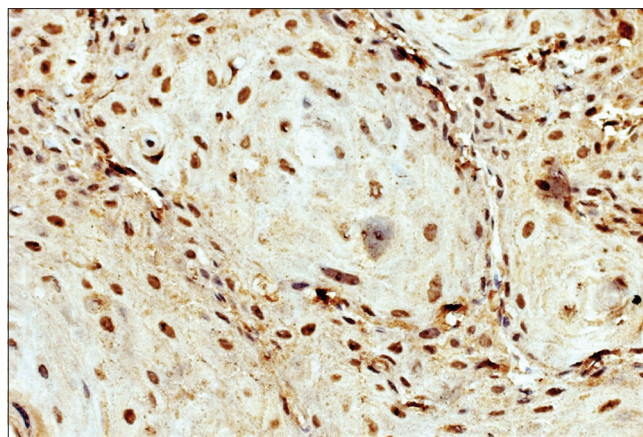


Figure 2: Immunohistochemical expression profile of Oct-4 (Nuclear staining, x40).

were done at the Department of Surgical Oncology, King George's Medical University (KGMU), Lucknow, India. OSCC patients underwent surgery, and samples were collected in formalin. Clinical and demographical history of patients was taken from the hospital records. The histological diagnosis was done at the Department of Pathology, Era's Lucknow Medical College and Hospital (ELMC andH), Lucknow, India (ELMCandH) by an experienced pathologist.

Inclusion criteria

1. Patients with histopathologically proven diagnosis of OSCC
2. Patients who did not receive any previous treatment for OSCC or other malignancy.

Exclusion criteria

1. Patients with any other malignancy either in present or past
2. Patients who have AIDS or any other known Immunodeficiency disorder.

Immunohistochemical analysis

All biopsy specimens were processed as formalin-fixed paraffin embedded (FFPE) tissue blocks. The cut sections of FFPE were 3–5 μm thick on tissue bond chemically coated slides for CD-133 as well as Oct-4 and de-waxed in xylene followed by rehydration in gradient alcohol (100%, 70%, and 50%) and distilled water. Endogenous peroxidase activity of tissue was blocked, using 3% H₂O₂ in methanol for 30 min. Antigen retrieval was done using high pH solution (DAKO, Denmark) in a pressure-cooker for one whistle after which the slides were cooled for 15–20 min. The slides were dipped in phosphate-buffered-saline (PBS) solution (DAKO, Denmark) for washing at RT thrice for 3 min each. CD-133 primary antibody (polyclonal, 1:50 dilution, Proteintech, USA) and Oct-4 (Monoclonal, DAKO, Denmark)

were incubated for 1.5 h at RT followed by washing with PBS thrice. Horseradish peroxidase (Enzyme linked secondary antibody) at RT for 30 min followed by washing with PBS buffer thrice. Slides were immersed in diaminobenzidine chromogen for 5–10 min followed by rinsing with water. The counterstained using hematoxylin for 3–5 min and then finally rinsed with water. The tumor sections were mounted using dibutylphthalate polystyrene xylene and covered using coverslips and examined under microscope (Leica, Germany). For the specificity of immunohistochemical staining, the experiment was performed using positive (known positive case) as well as negative (omit primary antibody) control slides in every batch of IHC.

Statistical analysis

Statistical analysis was carried out using the IBM-Statistical Package for Social Sciences (SPSS) version 16 (Chicago). Data were summarized as mean \pm standard error of the mean. Groups were compared by Chi-Square test/Fisher's exact test. Groups were also compared by Kruskal–Wallis (H) analysis of variance. A two-tailed $P < 0.05$ was considered statistically significant.

RESULTS

The study group comprised 50 cases of OSCC with 38 (76%) males and 12 (24%) female patients. These patients were classified according to their clinical parameters [Table 1] such as tumor size (<2, 2–4 and > 4 cm), node involvement, pathological grade, clinical stage, tumor stage, and demographical parameters in OSCC [Table 2]. CD133 expression was seen in 35 (68%) cases [Figure 1] while Oct-4 was seen only in one case [Figure 2]. Significant correlation of CD133 expression was seen only with clinical stage [Table 3] while no significant correlation was seen with any of the demographic parameters [Table 4]. Positive staining for CD133 [Figure 1] was detected in 20.6% samples between I–II stage and in 79.4% samples between stages III–IV. Consequently, the statistical analysis identified a significant correlation ($P = 0.04$) between clinical stage and immunostaining of CD133 marker.

In this study, as Oct-4 expression was seen in only one case so no correlation with clinicopathological or demographic parameters was possible.

DISCUSSION

OSCC is the most common malignancy in Indian subcontinent with the overall survival remaining at 50% unchanged for last four decades despite all the recent advances in treatment modalities. The main reason for treatment failure

Table 1: Clinicopathological parameters of 50 cases of oral squamous cell carcinoma

| | Number of cases (%) |
|--------------------------------|---------------------|
| Gender | |
| Male | 38 (76.0) |
| Female | 12 (24.0) |
| Tumor size (cm) | |
| <2 | 1 (2.0) |
| 2-4 | 34 (68.0) |
| >4 | 15 (30.0) |
| Node involvement | |
| Yes | 35 (70.0) |
| No | 15 (30.0) |
| Pathological grade | |
| WD | 26 (52.0) |
| MD | 22 (44.0) |
| PD | 2 (4.0) |
| Clinical stage | |
| I-II | 13 (26.0) |
| III-IV | 37 (74.0) |
| Tumor stage | |
| T ₁ -T ₂ | 33 (66.0) |
| T ₃ -T ₄ | 17 (34.0) |
| Lymph node status in OSCC | |
| N-N ₁ | 44 (88.0) |
| N ₂ | 6 (12.0) |

OSCC: Oral squamous cell carcinoma, WD: Well differentiated, MD: Moderately differentiated, PD: Poorly differentiated

Table 2: Demographical parameters of 50 cases of oral squamous cell carcinoma

| | Number of OSCC cases (%) |
|-----------------|--------------------------|
| Tobacco chewers | |
| Yes | 42 (84.0) |
| No | 16 (16.0) |
| Smoking | |
| Yes | 22 (44.0) |
| No | 28 (56.0) |
| Pan masala | |
| Yes | 17 (34.0) |
| No | 33 (66.0) |
| Alcohol | |
| Yes | 7 (14.0) |
| No | 43 (86.0) |

OSCC: Oral squamous cell carcinoma

and poor prognosis is due to its complex invasiveness and recurrence.^[15] There is a need to identify and understand the molecular markers in OSCC which play key role in proliferation and survival of tumor cells. With the origin of CSC theory in leukemias, it has been extended to solid organ malignancies and multiple studies have established their prognostic role in various malignancies such as non-small cell lung carcinoma, breast cancer, intrahepatic cholangiocarcinoma, hepatocellular carcinoma, esophageal squamous cell carcinoma, and glioblastoma.^[19,20]

Table 3: Immunohistochemical expression of CD133 in relation to clinicopathological parameters in a series of 50 oral squamous cell carcinoma cases

| | Total number of cases (%) | CD133 positive (%) | P |
|--------------------------------|---------------------------|--------------------|---------------|
| Sex | | | |
| Male | 38 (76.0) | 25 (73.5) | 0.551 (NS)* |
| Female | 12 (24.0) | 9 (26.5) | |
| Tumor size (cm) | | | |
| <2 | 1 (2.0) | 1 (2.9) | 0.355 (NS) |
| 2-4 | 34 (68.0) | 21 (61.8) | |
| >4 | 15 (30.0) | 12 (35.3) | |
| Node involvement | | | |
| Yes | 35 (70.0) | 25 (73.0) | 0.427 (NS) |
| No | 15 (30.0) | 9 (26.5.0) | |
| Clinical stage | | | |
| I-II | 13 (26.0) | 7 (20.6) | 0.048 |
| III-IV | 37 (74.0) | 27 (79.4) | (significant) |
| Tumor size | | | |
| T ₁ -T ₂ | 33 (66.0) | 20 (58.8) | 0.230 (NS) |
| T ₃ -T ₄ | 17 (34.0) | 14 (41.2) | |
| Nodal status | | | |
| N ₀ | 15 (30.0) | 8 (23.5) | 0.115 (NS) |
| N ₁ -N ₂ | 35 (70.0) | 26 (76.5) | |
| Metastatic status | | | |
| M ₀ | 48 (96.0) | 32 (94.1) | 0.322 (NS) |
| M ₁ | 2 (4.0) | 2 (5.9) | |
| Pathological grade | | | |
| WD | 26 (52.0) | 17 (50.0) | 0.600 (NS) |
| MP-PD | 24 (48.0) | 17 (50.0) | |

*Cm: Centimeter, WD: Well differentiated, MP: Moderately differentiated, PD: Poorly differentiated, NS: Not significant

The CSCs derived from oral cancer play a major role in tumor progression, metastasis, and also show the capacity of resistance toward radiotherapy and chemotherapy.^[21] Therefore, it is important to identify the mechanisms of CSCs activation and their therapeutic role in OSCC. Altered pathophysiology and differential expression of CSC markers (CD133) and transcription factor (Oct-4) participate in clonal proliferation of cells.^[22]

CSC marker CD133 (Prominin-1) has been found in epithelial cells and somatic stem cells of prostate, kidney, liver, skin, lung, colorectal, prostate, and neural tissue.^[23] Wu *et al.* (2009) reported that CD133 positive cells showed self-renewal, differentiation, proliferation, tumorigenicity, clonogenicity, and EMT phenotype in OSCC.^[24] Chiou *et al.* (2008) noticed positive correlation between Oct-4, Nanog and high expression of CD133 with poor prognosis outcome in patients of oral cancer.^[25] Further studies are needed to elucidate the positive correlation with other stem cell markers in cancers such as OSCC for prognosis and better therapeutic outcomes for early treatment of cancer.

Table 4: Immunohistochemical expression of CD133 in relation to demographical parameters in a series of 50 oral squamous cell carcinoma cases

| | Total number of cases (n=50) (%) | CD133 positive (%) | P |
|------------|----------------------------------|--------------------|------------|
| Tobacco | | | |
| Yes | 42 (84.0) | 29 (85.3) | 0.716 (NS) |
| No | 8 (16.0) | 5 (14.7) | |
| Smoking | | | |
| Yes | 22 (44.0) | 13 (38.2) | 0.321 (NS) |
| No | 28 (56.0) | 21 (61.8) | |
| Panmasala | | | |
| Yes | 17 (34.0) | 13 (38.2) | 0.357 (NS) |
| No | 33 (66.0) | 21 (61.8) | |
| Alcohol | | | |
| Yes | 7 (14.0) | 5 (14.7) | 0.834 (NS) |
| No | 43 (86.0) | 29 (85.3) | |
| Total | 50 (100.0) | 34 (100.0) | |
| Tobacco | | | |
| Yes | 42 (84.0) | 29 (85.3) | 0.716 (NS) |
| No | 8 (16.0) | 5 (14.7) | |
| Smoking | | | |
| Yes | 22 (44.0) | 13 (38.2) | 0.321 (NS) |
| No | 28 (56.0) | 21 (61.8) | |
| Pan masala | | | |
| Yes | 17 (34.0) | 13 (38.2) | 0.357 (NS) |
| No | 33 (66.0) | 21 (61.8) | |
| Alcohol | | | |
| Yes | 7 (14.0) | 5 (14.7) | 0.834 (NS) |
| No | 43 (86.0) | 29 (85.3) | |
| Total | 50 (100.0) | 34 (100.0) | |

NS: Not significant

The Oct-4 plays a major role in the self-renewal of embryonic stem cells and maintains their pluripotency through interaction with other transcription factors such as Stat3, Zic3 (Zinc family member 3) and HesX1 and some signaling factors such as LEFTY2 (Left-right determination factor 2), FGF2 (Fibroblast growth factor2), transcription factor 3.^[26,27] Siu *et al.* have found that Oct-4 in combination with Nanog and CD133 showed worst prognosis in survival of OSCC, which indicates these markers being as an invasive and predictive marker.^[28] These studies have motivated us to check the correlation between Oct-4 and CD133 with respect to clinical and demographical data of oral cancer.

Very few studies have been done to markers with clinical survival parameters in OSCC patients. Expression analysis of these two biomarkers using IHC may facilitate prognosis of OSCC patients using clinical factors (tumor, node, metastasis; clinical staging etc.). These factors might serve as valuable tools for clinicians for a better prognostic and therapeutic stratification of OSCC patients.

In the present study, it was found that the expression of CD133 was significantly associated with clinical stage [Table 3] of OSCC patients. Positive staining of CD133 was observed in 68% of the OSCC cases but Oct-4 staining was observed in only 2% of the poorly differentiated cases.

Till date, a number of studies have revealed CD133 as useful biomarker in identification of CSCs in different type of human cancers including oral cancer,^[29] also with an ability to identify cells which initiate tumor growth and are responsible for tumor recurrence and progression including colonization of distant organs and metastasis.^[26] The present study showed a significant association between CD133 expression and clinical stage but lack of any significant correlation between CD133 expression and age, sex, tumor, lymph node involvement in OSCC patients.^[11] The significant association of CD133 expression with clinical stage, points toward a major role of CD133 expressing cells in the progression of OSCC and signifies an involvement of CSCs in the progression of OSCC.^[13]

CONCLUSIONS

The expression of CD133 and Oct-4 in a cohort of 50 OSCC patients demonstrated their correlation with different demographical parameters and clinical prognostic indicators. These findings suggest that CD133 might represent a useful prognostic indicator with respect to clinical stage of OSCC patients, while Oct-4 findings are less conclusive. Hence, further studies are needed to find out the correlation of CD133 with other CSCs markers in the development and progression of OSCC and their suitability as prognostic and predictive biomarkers for oral early diagnosis of oral cancer. Studies on a larger cohort of patients may also help to identify patients at higher risk for cancer progression and targeting CSCs in these cases as for better therapeutic outcomes.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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