Original Article

Expression of p53 at invasive front of oral squamous cell carcinoma and negative histopathological surgical margins to establish correlation at 3-year survival

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Abstract

Background: Oral squamous cell carcinoma (OSCC) is the most common malignancy of the oral cavity. The histologic features of OSCC differ from area to area within same tumor, and most prognostic information can be revealed from the invasive front of tumor. The most accepted line of treatment is radical neck dissection. The boundaries of a resected specimen are the surgical margins (SMs), which are excised by the surgeon. The survival outcome is based on the status of these resected SMs. To avoid local recurrence and improve overall survival, it is necessary to attain negative SM. Apart from routine histopathology, the molecular assessment of resected margins has recently gained value which has a promising role for margin surveillance. The value of the use of molecular markers in the routine examination of resection specimens of OSCC has not yet established. It is crucial to identify the percentage of altered cells in SMs which go undetectable in the routine histopathology. It is essential to assess their role in recurrence and survival.

Materials and Methods: The study was divided into two groups, i.e., Group I (control group): ten cases of normal oral mucosa and Group II consisted of thirty cases, in which biopsy samples of invasive tumor front and histopathologically negative SM of OSCC were included. Both the groups were subjected to p53 immunohistochemical staining

Results: There was overexpression of p53 at the deep tumor invasive front of OSCC associated with different histologic grades of malignancy.

Conclusion: The overexpression of p53 at the invading tumor front with clear SMs is associated with poor survival. p53 expression at the tumor front can be a prognostic marker for OSCC.

Keywords: Oral squamous cell carcinoma, p53, survival, tumor-free margins

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INTRODUCTION

Oral squamous cell carcinoma (OSCC) ranks eighth in the frequency of cancers of the whole body, and an increase in the rate of mortality has been reported in developing countries.[1] Several reports have examined the usefulness of clinical and pathologic factors for the prognosis of OSCC. In particular, the histologic factors of OSCC may differ widely from area to area within the same tumor. It

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is believed that most useful prognostic information can be deduced from the invasive front of the tumor where most aggressive cells reside.[2] Oral carcinogenesis is a multistep process, in which the occurrence of a series of genetic events may lead to dysregulation of the cell cycle. p53 is the most commonly affected tumor suppressor gene.

p53 protein is a product of the tumor suppressor TP53 gene. The TP53 gene functions in cell cycle as to allow the repair of DNA damage in G1 arrest and to prevent the cell from entering the S phase of the cell cycle or ultimately to guide the unrepaired damaged cells to apoptosis, and thus it has been called "the guardian of the genome".[3] High frequency of mutation in TP53 gene and mutant p53 protein expression has been shown in a variety of human tumors such as breast, brain, rectum, colon, esophagus and lung cancers and OSCC.[4] The TP53 tumor suppressor gene mutation is well established in carcinogenesis of oral tumors as well as its progression and metastasis. In previous studies, it was found that it has a significant role as a prognostic marker in advanced oral tumors.[5] A low concentration of wild-type p53 protein is usually found in normal cells because of its relatively short half-life 20 min. Its concentration increases as its half-life is extended, which may occur due to TP53 gene mutation, association of wild-type p53 with other proteins or disruption of its degradation pathway.[6] This increased in wild-type p53 protein and mutated p53 protein detected in various cancerous cells by the simple, reliable and routine examination of immunohistochemistry, whereas normal p53 protein is undetectable.[7]

Molecular markers can be used to establish tumor-free surgical margins (SMs) and assist in the complete resection of the tumor, thereby preventing the recurrence. The molecular marker p53 has been established to predict for recurrence and survival of OSCC patients.[8] Keeping this in mind, in this study, p53 protein expression was evaluated in the invasive front of OSCC as well as in histologically negative SMs of resected OSCC and its correlation with 3-year survival of OSCC.

MATERIALS AND METHODS

This study was carried out at the Department of Oral Pathology and Microbiology, Sharad Pawar Dental College, DMIMS (DU), Wardha, India. The study was approved by the institute of the ethical committee. The study comprised two groups. Group I was considered as a control group which comprised of 10 cases of normal oral mucosa obtained from the patients undergoing third molar extraction. Group II consisted of thirty cases of OSCC. Archival cases of OSCC and the relevant data were retrieved from the archival of the department. Patients with clinical and histopathological evidence of OSCC and undergone surgical excision with or without radiation were included in the present study. The patients were staged according to the clinical TNM staging system as mentioned by the American Joint Committee of Cancer staging manual 2002.[9] Histopathological examination was used for the confirmation of nodal metastasis. Patients with a history of previous OSCC, recurrent or distant disease and preoperative chemotherapy, radiotherapy or surgery (other than a biopsy) were excluded from the study. All the included cases were then followed up prospectively for obtaining disease-free survival data of more than 3 years.

Immunohistochemistry

The immunohistochemistry was carried out on the formalin-fixed and paraffin-embedded blocks of histologically proven OSCCs and their negative SMs from the archives of the department of oral pathology as well as on normal oral mucosa. The standard immunohistochemistry procedure was carried out for p53 protein expression using p53 antibody (clone DO-7; Product code: N1581, Dako, Denmark) and the HRP-labeled polymer anti-mouse secondary antibody (DakoEnVision System, PC: K4000, DD.)

Assessment of p53-positive cells

Tissue sections positive for p53 protein expression were examined for the presence of brown-stained nuclei. The most heavily p53-labeled areas were located by scanning the sections at ×100 magnification. Cell counts were made at ×400 magnification with a compound light microscope in five randomly selected fields. The number of positively stained nuclei was expressed as a percentage of the total number counted in complete epithelium. The p53 labeling index was calculated as: number of immunohistochemical-positive cells ×100/total number of cells observed.

Statistical analysis

All the relevant data were collected as per pro forma and tabulated in Microsoft Excel and were analyzed using statistical software and Chi-square test applied between the different associations above mentioned. Immunoexpression of p53 was related to parameters of Bryne's grading system using Chi-square test. A two-sided $P < 0.05$ was considered statistically significant for all tests. Analysis of data was done using appropriate statistical software such as SPSS 24.0 version and GraphPad Prism 7.0 version.

RESULTS

Among the OSCC cases, it was observed that the

expression of p53 increased with the increasing grades of OSCC from well-differentiated squamous cell carcinoma (WDSCC) to poorly differentiated squamous cell carcinoma (PDSCC) [Figures 1 and 2].

Varied expression of p53 was observed in SMs of OSCC. WDSCC showed a negative expression for p53 [Figure 3].

Normal oral mucosa showed negative expression of p53

On comparing the survival status of various grades of OSCC, it was observed 8 out of 13 cases of WDSCC, 2 out of 11 cases of MDSCC and 0 out of 6 cases of PDSCC survived over a period of 3 years. The p53 expression was increased in patients who succumbed to the disease as compared to cases who were survived [Figure 4-6].

DISCUSSION

Carcinogenesis is a multifactorial and multidimensional disease process. The two major gene classes, i.e., the proto-oncogenes and the tumor suppressor genes, regulate the fate of the lesion.[10] In the present study, p53 expression was checked in forty cases to correlate it with the survival rate of the individuals suffering from OSCC. The p53 expression is normally limited to basal cells, and once the expression is seen beyond the basal level, it indicates the changes occurring in the epithelium and progression from normal toward dysplasia and later to frank carcinoma, thus suggesting a progressive accretion of mutational errors of p53 protein.[11]

Broder's classification system is the most widely accepted grading system based on the ratio of terminally differentiated cells. A two-factor grading system comprising the degree of cellular differentiation and the depth of tumor growth was subsequently introduced and has generally been accepted. However, Broder's classification of squamous cell carcinoma according to the differentiation or maturation of the tumor cell population alone remains of limited value regarding both the prognosis and choice of treatment.[12] Recent evidence suggests that cells present at the invasive tumor front of carcinomas have different molecular characteristics when compared with those in the superficial

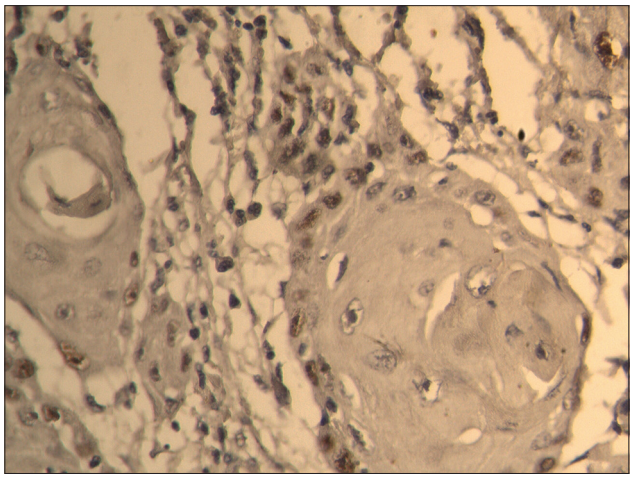

Figure 1: Mild staining (×10)

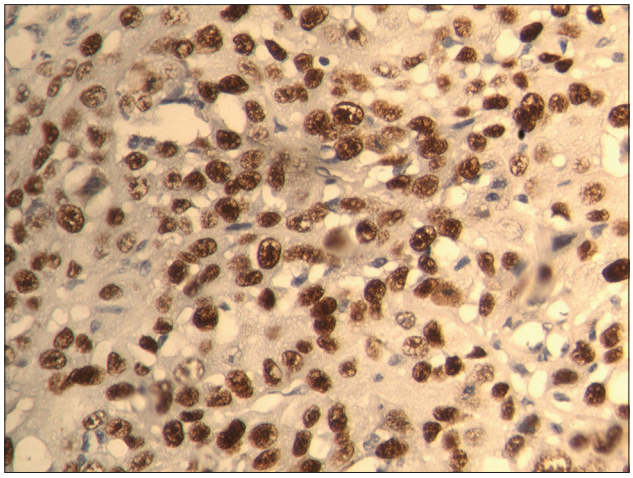

Figure 3: Strong staining (×10)

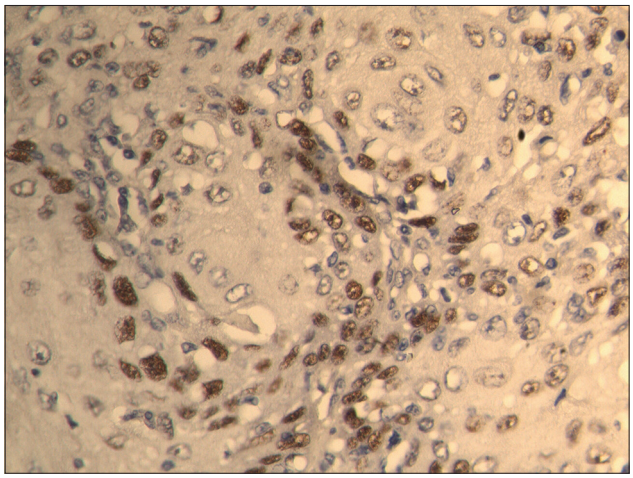

Figure 2: Moderate staining (×10)

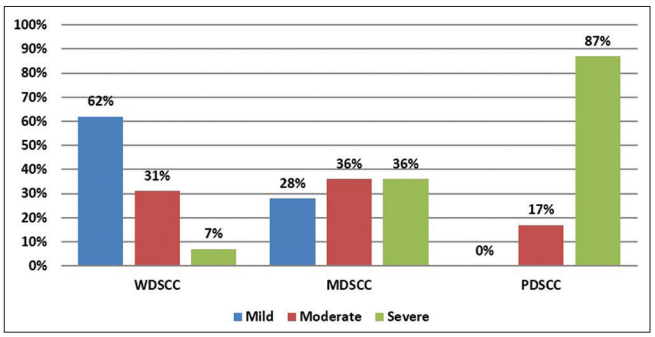

Figure 4: Correlation between immunohistological staining of p53 protein and invasive front grading score

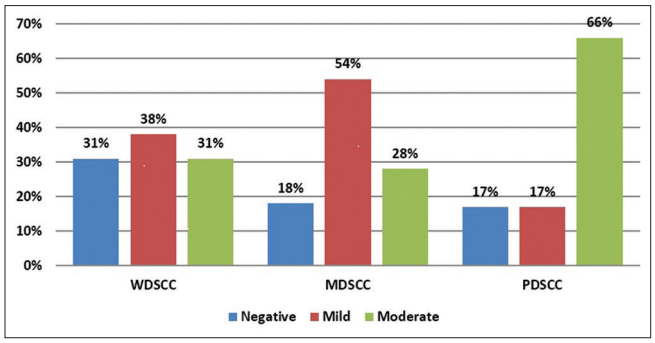

Figure 5: Correlation between immunohistological staining of p53 protein and surgical margins of oral squamous cell carcinoma

areas of the tumor, making the invasive front the most important area of the tumor for determination of the prognosis.[13] Tumor cell proliferation activity is believed to indicate the degree of aggressiveness of the tumor. The proliferative activity of carcinoma cells is generally considered related to the differentiation of the cells in the tissue. Recently, many studies on immunohistochemical detection of p53 have demonstrated the association of tumor cell proliferation and the prognosis of OSCC.[14]

In the present study, overexpression of p53 was found in 15 out of thirty cases, which directly correlated with the survival rate of patients.

The primary mode of treatment of OSCC till date is surgery, followed by chemotherapy and radiotherapy. To demarcate, the exact margin is a big challenge for oral surgeon. The histopathological SMs are prognostic indicators for tumor relapse and distant metastasis. However, relapse often occurs with clear SMs, so it becomes imperative to assess the status of SM by molecular marker.[8]

In our study, we found that 23 out of thirty cases showed positive p53 expression in histopathologically negative SM.

CONCLUSION

The findings of this study demonstrate that overexpression of p53 at the deep tumor invasive front of OSCC is associated with histologic grade of malignancy. Expression of p53 increases with the increasing grades of OSCC from WDSCC to PDSCC. Most of the margins from WDSCC showed a negative expression for p53. We conclude that patient's survival decreases with the increased expression of p53 at the advancing front of the tumor.

Ethical approval

The study was approved by the ethical guidelines committee.

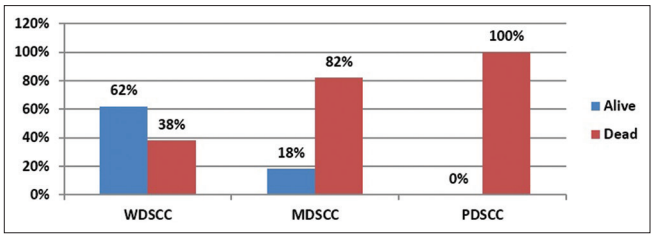

Figure 6: Survival of oral squamous cell carcinoma patients at different histological grades

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Conflicts of interest

There are no conflicts of interest.

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