Role of epidermal growth factor receptor expression in recurrent cases of oral squamous cell carcinomas

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Abstract

Background: Oral squamous cell carcinoma (OSCC) is the most common head and neck cancers and locoregional recurrence in OSCC is related to lowest survival rate. The present study was undertaken to compare the clinicopathological findings and epidermal growth factor receptor (EGFR) expression in recurrent and non-recurrent cases of OSCC.

Methodology: A total of 64 cases of OSCC were studied and immunohistochemistry was done, of which, 33 cases recurred at end of follow up. Categorical variables for each case included age, gender, site of primary lesion, neck metastasis, AJCC stage, locoregional recurrence, histological grade and pattern of invasion.

Results: In quantitative analysis, 7 cases (21.2%) of recurrent OSCC and one case (3.2%) of non recurrent OSCC with EGFR extent score 4 was observed. In qualitative analysis, 5 cases (3.22%) of recurrent group and one case (0.64%) of non recurrent group with strong intensity was observed. Recurrent cases of OSCC showed higher score of EGFR expression [extent {p-value 0.001} and intensity {p-value 0.03}]. However, on clinicopathological correlation of EGFR expression, no significant correlation was observed. In TNM stage IV, out of 22 cases only 7 cases (4.48%) showed recurrence. Among 26 cases, 17 Cases (10.88%) of recurrent OSCC with H/P grade I showed recurrence. Recurrence was frequently observed in 15 cases (9.6%) with pattern of invasion (POI) type II.

Conclusion: The EGFR expression in between recurrent and non-recurrent OSCC can be exploited as prognostic /or predictive parameter despite its role as therapeutic marker.

Keywords: Epidermal growth factor receptor, Recurrence, Oral squamous cell carcinoma

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Introduction

Oral cancer is one of the ten most common cancers in the world; the incidence rate of oral cancer (per 100,000 cases) is 13.5 in India.[1] Approximately 94% of all oral malignancies are squamous cell carcinoma.[2] These tumors are usually present in advanced clinical stages and are associated with high rates of loco-regional recurrences.[3,4,5] The rates of loco-regional recurrences range from 25 to 48%, and distant metastasis rarely occur in an isolated manner.[5] Recurrent and/or metastatic patients have a poor prognosis, which has not changed significantly in 30 years.[6]

The cellular and biochemical factors that underlie oral squamous cell carcinoma (OSCC) dissemination are poorly understood. There is clearly an unmet therapeutic need for new active agents for the treatment of patients with recurrence and/or metastasis and to start with valid biomarker for prediction of disease course is required. Oral squamous carcinogenesis is a multistep process in which multiple genetic events occur that alter the normal function of oncogenes and tumor suppressor genes.[7,8] One of these oncogenes is epidermal growth factor receptor (EGFR) considered as a driver of oncogenesis.[9] EGFR activation induces activation of several downstream intracellular substrates, leading to mitogenic signaling and other tumor-promoting cellular activities. Increased rates of EGFR positivity and expression have generally been concluded to indicate a poor prognosis in laryngeal, gastric, cervical, esophageal and breast cancer.[9,10,11]

The aim of the present study was to compare the clinicopathological findings and EGFR expression in recurrent and non-recurrent cases of OSCC.

Methodology

The study comprised a total of 64 patients with OSCC in the study time period from November 2008 to January 2011; corresponding medical records were obtained through hospital electronic data base. The patients with incomplete recording, loss of at least 2 years follow up, concomitant primary malignant head and neck lesion, secondary neoplasm or cases with

distant metastasis were not included in the study. Categorical variables for each cases included age, gender, site of primary lesion, neck metastasis, AJCC stage[12], locoregional recurrence, histological grade, pattern of invasion. The locoregional recurrence arising in both primary site and neck was considered as recurrence. Histopathological grading of cases was done according to Broder's grading system[13] (Fig. 1) and pattern of invasion was graded according to modified Bryne's classification[14] (Fig. 2) (Table 1).

EGFR expression was checked using polyclonal EGFR antibody (6 ml of Ready-to-Use Antibody, Biogenix pvt ltd). This antibody helped to detect both EGFR phosphorylated on Tyrosine 1068 of the mature human isoform 1 (corresponding to Y1092 from the precursor form P00533-1/p170), and also unphosphorylated EGFR.

Immunohistochemistry staining procedure

All tests were carried out on 3 μm formalin fixed paraffin embedded sections. Slides were baked in hot air oven at 65°C overnight. Sections were sequentially dewaxed through a series of xylene, graded alcohol and water immersion steps. Antigen retrieving was done as recommended by the manufacturers using 1000 ml of Tris EDTA buffer: (retrieval solution) at pH 9 using E7 antigen retrieval machine (Biogenix) in 2 cycles (unmasking of antigen was done using by heat mediated antigen retrieval technique). Endogenous peroxidase activity was blocked followed by blocking the non specific staining. Primary antibody (100 ml) was applied for each section and samples were incubated in humid chamber. After washing with PBS secondary antibodies were applied to the sections, incubated and rinsed with a stream of PBS. Primary antibody was visualized with DAB chromogen. Sections were counterstained with Mayer's hematoxyline for 30 seconds, dehydrated and mounted. Both membranous and cytoplasmic (accentuated membranous) staining of EGFR in OSCC was considered positive. Histopathologically confirmed cases of EGFR positive breast carcinoma tissues were used as positive control (Fig. 3).

Assessment of IHC results

Evaluation of staining results was performed in accordance with modified Putti's method.[15] Immunoscoring for intensity & extent of EGFR expression in recurrent and non-recurrent cases of OSCC was done. Quantitative analysis of EGFR expression (percentage of cell positivity): 5 randomly representative fields were selected for EGFR positive tumour cells with each field containing minimum of 200 tumour cells per field at 40x objective (light microscopy) and scoring of extent as well as intensity was done (Table 1).

Statistical analysis

The data was statistically analyzed by using SPSS software version 22. The quantitative & qualitative data was compared by standard statistical tests as applicable (p-value <0.05 was considered as significant).

Results

Among 64 cases of OSCC, 33 cases recurred during follow up. The mean age of patients was 50 years (25-71 yrs). Recurrence was seen in 24 males and 9 females. Buccal mucosa (4.95%) was the most frequently affected site in locoregional recurrence while tongue (10.24%) was the most involved site in non recurrent group. Maximum cases in recurrent group were in TNM stage II and in non recurrent group in TNM stage IV [18 (54.5%) and 15 (9.6%) cases, respectively]. Mean time to recurrence was 5 months. Most frequently, histopathological grade I cases recurred [17 cases (51.5%)] while in non recurrent group, 23 cases (14.72%) were graded as histopathological grade II. No case of recurrent OSCC with TNM stage IV in histopathological grade III was present. Most frequently, pattern of invasion (POI) was of type 2 accounting for 15 (9.6%) cases each in the recurrent and non recurrent groups. It was observed that tumor with undifferentiated cells showed high score of POI in the recurrent group of OSCC.

Majority of recurrent cases were scored for EGFR extent as score 2 accounting for 11 (33.3%) cases while 16 (51.6%) cases presented as score 1 in the non-recurrent group. Among 64 cases of OSCC, there were 4 cases with no EGFR stain, 5 cases with weak intensity, 19 cases with moderate intensity and 5 cases with strong intensity that showed recurrence. Recurrent cases of OSCC showed higher score of EGFR expression [extent {p-value 0.001} and intensity {p-value 0.03}]. (Table 2) No significant statistical correlation of EGFR expression (extent & intensity) with TNM stage, histopathological grade and POI was observed.

Table 1: Histological grading, pattern of invasion, characteristics of immunoreactivity of staining extent and intensity of EGFR

Histological grading
Grade Degree of differentiation
1 well differentiated (<25% of undifferentiated cells)
II moderately differentiated (<50% of undifferentiated cells)
III poorly differentiated (<75% of undifferentiated cells)
Pattern of invasion
Type Pattern of invasion
1. Broad pushing manner
2. Fingers or separate large tumour islands with stellate like appearance.
3. Invading tumour islands greater than 15 cells per island
4. Invading tumour islands less than 15 cells per island or single cell invasion
5. Widely dispersed pattern of tumour infiltrate or tumour satellite of any size with 1 mm or greater distance (intervening at tumour host interface i.e. not fibrosis)
Characteristics of immunoreactivity of staining extent and intensity of EGFR
Score EGFR extent
0 negative staining of considered cells
1 10% of positive staining of considered cells
2 11-50% of positive staining of considered cells
3 51 -80% of positive staining of considered cells
4 >81% of positive staining of considered cells
Score EGFR intensity
0 No stain (-)
1 Weak stain (+)
2 Moderate stain (++)
3 Strong stain (+++)

Table 2: Characteristics of EGFR expression in patients with oral squamous cell carcinoma

	Total N=64	Recurrent OSCC N=33	Non recurrent OSCC N=31	p-value
Gender				
Male	52(33.92%)	24(15.36%)	28(17.92%)	
Female	12 (7.68%)	9(5.76%)	3(1.92%)	
Site				
Tongue	31(19.84%)	15(4.95%)	16(10.24%)	
Buccal mucosa	25(16%)	15(4.95%)	10(6.4%)	
Hard palate	3(1.92%)	1(1.56%)	2(1.28%)	
Alveolus	3(1.92%)	0	3(1.92%)	
Lower lip	1(1.56%)	1(1.56%)	0	
Floor of mouth	1(1.56%)	1(1.56%)	0	
TNM stage				
I	6(3.84%)	2(1.28%)	4(2.56%)	**0.005**
II	23(14.72%)	18(11.52%)	4(2.56%)	
III	14(8.96%)	6(3.84%)	8(5.12%)	
IV	22(14.8%)	7(4.48%)	15(9.6%)	
Histopathological grade				
I	26(16.64%)	17(10.88%)	8(5.12%)	**0.001**
II	33(21.12%)	10(6.4%)	23(14.72%)	
III	6(3.84%)	6(3.84%)	0	
POI Type				
1	7(4.48%)	3(1.92%)	4(2.56%)	0.27
2	30(19.2%)	15(9.6%)	15(9.6%)	
3	15(9.6%)	6(3.84%)	9(5.76%)	
4	4(2.56%)	2(1.28%)	2(1.28%)	

5	8(5.12%)	7(4.48%)	1(0.64%)	
EGFR extent				
0	7(4.48%)	4(2.56%)	3(1.92%)	**0.001**
1	18(11.52%)	2(1.28%)	16(10.24%)	
2	17(10.88%)	11(7.04%)	6(3.84%)	
3	14(8.96%)	9(5.76%)	5(3.2%)	
4	8(5.12%)	7(4.48%)	1(0.64%)	
EGFR intensity				
0	7(4.48%)	4(2.56%)	3(1.92%)	**0.003**
1	6(3.84%)	5(3.2%)	1(0.64%)	
2	28(17.92%)	19(12.16%)	9(5.76%)	
3	6(3.84%)	5(3.2%)	1(0.64%)	

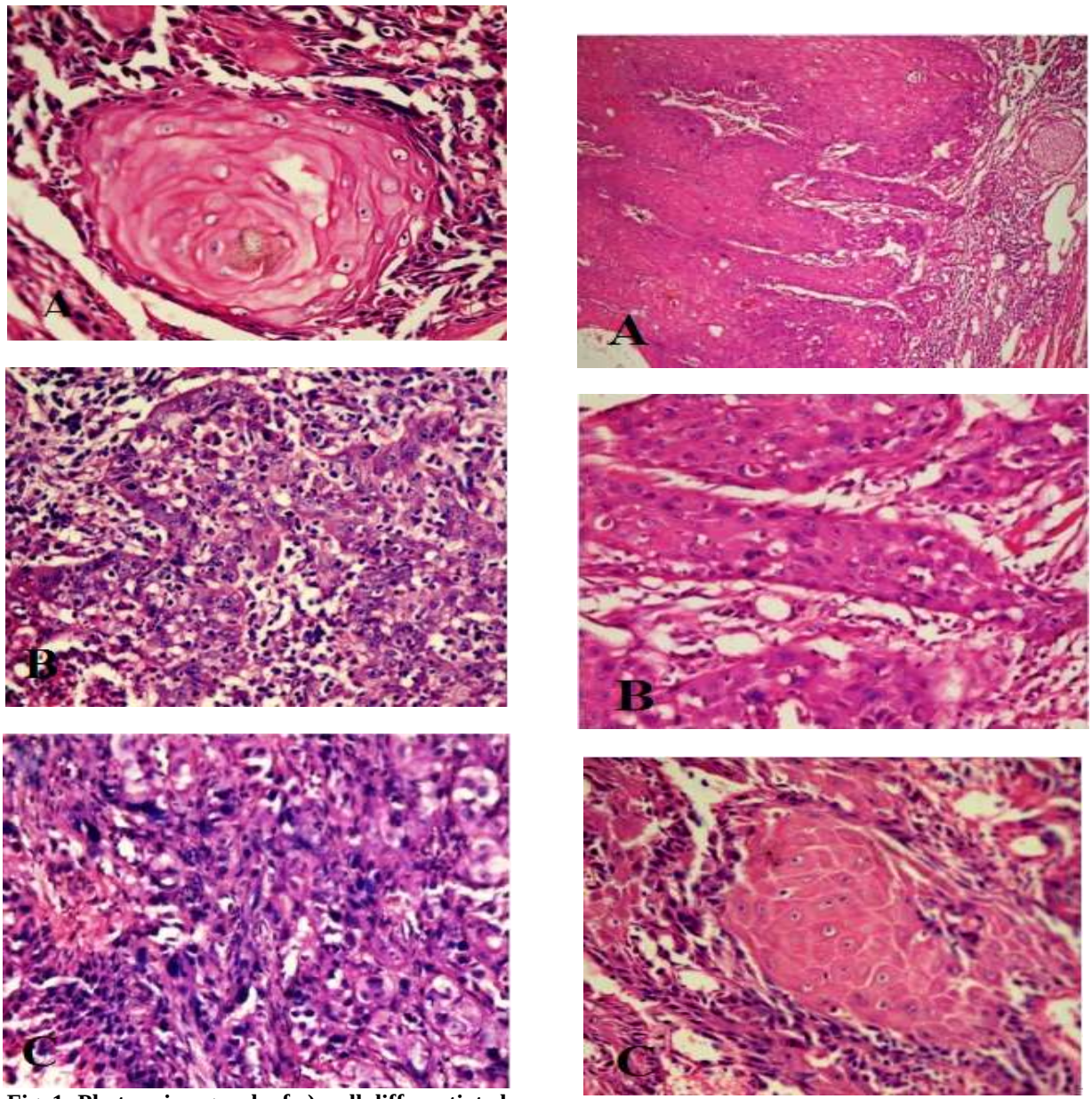
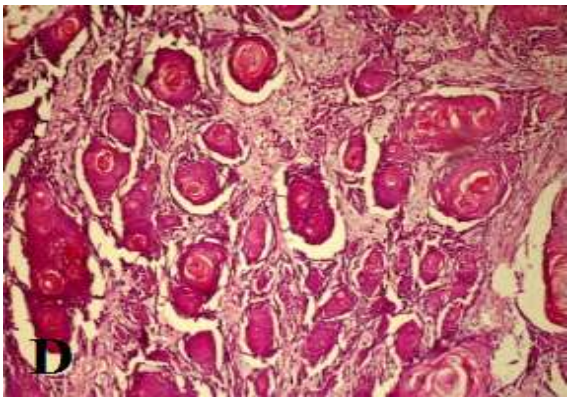


Fig. 1: Photo-micrograph of a) well differentiated OSCC, b) moderately differentiated OSCC, c) poorly differentiated OSCC (H&E, 40X)



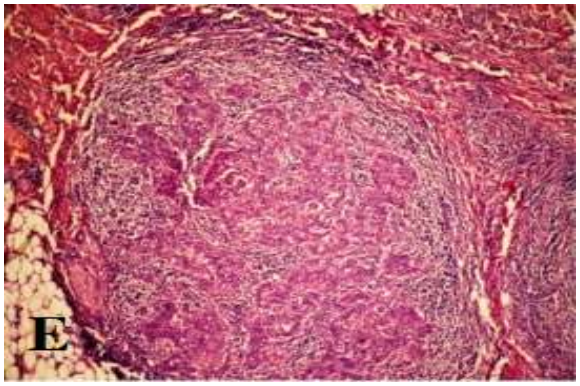
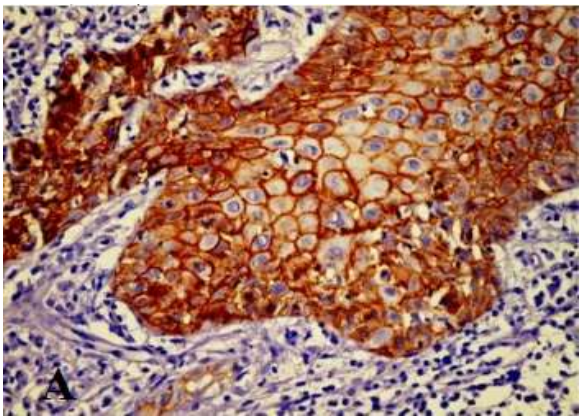
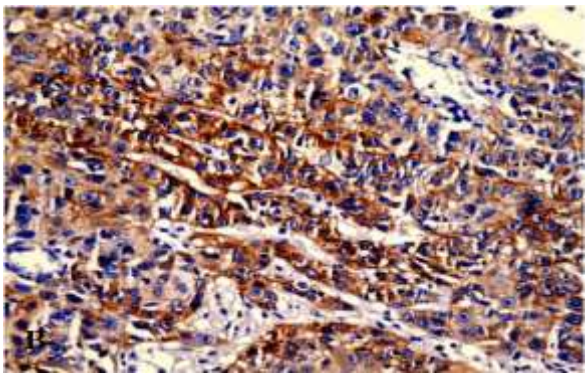


Fig. 2: Photo-micrograph of a) Pattern of invasion 1[Broad pushing manner] (H&E 10 X), b) Pattern of invasion 2 [Fingers or separate large tumor islands with stellate like appearance] (H&E 40 X), c) Pattern of invasion 3 [Invading tumour islands greater than 15 cells per island] (H&E 40 X), d) Pattern of invasion 4 [Invading tumour islands less than 15 cells per island or single cell invasion] (H&E 10 X), e) Photomicrograph of Pattern of invasion 5 [Widely dispersed pattern of tumor infiltrate or tumor satellite of any size with 1 mm or greater distance (intervening at tumor host interface i.e. not fibrosis)] (H&E 10X)





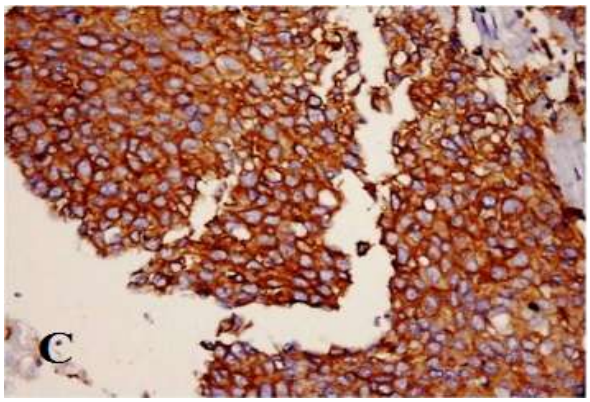


Fig. 3: Photo-micrograph of membranous staining of EGFR in a) well differentiated OSCC, b) poorly differentiated OSCC (40X), c) EGFR staining in poorly differentiated breast carcinoma [positive control] (40X)

Discussion

In the Indian subcontinent, OSCC is the second most common malignancy that accounts for more than 30-40% of all malignancies.[1] Rates of regional/locoregional recurrences range from 25-48% and distant metastases rarely occur in an isolated manner.[2] Early detection of second primary cancers or locoregional recurrence affords the best chance for disease control; suggesting that there is a need for valid tumor biomarker for predicting prognosis. Several biomarkers responsible for cell cycle progression and proliferation have been identified; a family of epidermal growth factor receptors (EGFRs), Cyclin D1, Ki-67.[16] EGFR activation, as its name indicates, can augment the malignant potential of epithelial cells.[17] There are many studies determining EGFR expression in SCC of other body parts like breast, colorectal, skin, lung etc; also the quantitative assessment of EGFR is reported to serve as a valid biomarker for predicting prognostic course of the disease.[18] However, to the best of our knowledge there is no earlier reported study depicting the role of EGFR in prediction of recurrence in OSCC. This study aimed to assess EGFR as a prognostic and predictive biomarker for recurrence in OSCC.

For many years, the TNM staging system has been used to estimate both recurrence and survival outcome. Earlier studies have reported that some patients with OSCC died with locoregional recurrence despite having TNM stage I or II and were treated accordingly.[19,20] In such patients, a combined assessment of clinicopathological staging is suggested as a precise measure of predicting prognosis. In our study, most frequently, cases in stage I and II had recurred. This observation highlights the fact that TNM stage may not be an important prognostic parameter and probably other parameters need to be defined.

The biological activity of OSCC is categorized on the basis of differentiation of tumor cells. Lack of correlation between histopathological grades and the prognosis of OSCC has been explained by the fact that SCC's usually exhibit a heterogeneous cell population with probable differences in invasiveness and metastasis behavior.[21] Dilana et al observed in their study that out of 7 cases with low scores of malignancy, 4 had recurrence.[22] In our study also, maximum cases of recurrence were well differentiated. This reverse prognostic significance may be due to the lesser response of better differentiated tumors to adjuvant therapy(s).[11]

Earlier studies reveal that higher the clinicopathological grading, higher the risk of cervical metastasis in OCC and indicates that neck dissection therapy is a significant prognostic factor for recurrence and survival.[23,24] In our study, statistical analysis did not show correlation between TNM classification and histological scores of malignancy demonstrating variation in disease biology and behavior that in turn cannot reflect definitive treatment regime. Dilana[22] et al in a clinicopathological study of 16 cases of OSCC reported significant correlation between TNM classification and prognosis but there was no correlation between the histological scores of malignancy and prognosis.

Besides clinical staging of tumor cells, the reaction of the host to the tumor and pattern of invasion needs to be graded to predict prognosis. It is intuitive that neoplasia infiltrating in a widely dispersed manner is more aggressive than those growing in a bulky pushing fashion. Over the past two decades, POI alone, and as part of weighted scoring systems, has been demonstrated to predict locoregional recurrence and decreased overall survival (OS).[14] However, various studies have reported that POI is not related to local recurrence in OSCC. In our study also, maximum cases with early POI showed recurrence. These observations reflect that the manner in which cancer infiltrates tissue at the tumor-host interface will not be enough to define the mode of invasion and probably other molecular factors need to be explored.[25] Because of the complex behavior of cancer, studies concerning different aspects of tumor dynamics through the immunohistochemical evaluation at molecular level are required in OSCC.

Although EGFR expression is considered universal, abnormally expressed EGFR expression representing heterogeneousity of tumor indicates altered proliferative potential of tumor cells. In the present study, recurrent cases of OSCC showed higher score of EGFR expression (extent & intensity) in comparison to the non-recurrent cases. Our study results were in concordance with Miyaguchi et al where they reported that EGFR intensity in 18 recurrent cases of glottic carcinomas increased in comparison to 15 non-recurrent carcinomas.[11] Although EGFR expression is proven as a predictive biomarker of local recurrence, it does not correlate with TNM staging,[18] H/P grade[26,27] and POI.[15] The increased expression of EGFR in tumors may influence the rate of recurrence. Numerous works attribute a negative independent prognostic value to EGFR and propose a lower survival rate if EGFR is present.[9]

Overall, this is the first study depicting the role of EGFR in prediction of recurrence in OSCC. The present study can orient the current classification system to use EGFR expression as a valid biomarker for prediction of locoregional recurrence in OSCC.

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