RESEARCH COMMUNICATION

Lack of Elevated HER2/neu Expression in Epithelial Dysplasia and Oral Squamous Cell Carcinoma in Iran

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Abstract

Purpose: The role of the HER family in oral squamous cell carcinomas (OSCCs) is not well-defined. This study was aimed to assess the frequency of HER2/neu overexpression in oral carcinogenesis. Materials and Methods: Expression of HER2/neu oncoprotein in OSCCs (N=18), oral epithelial dysplasia (N=18) and normal oral mucosa (N= 18) was assessed by immunohistochemistry using a cerbB2 antibody kit. Results: HER2/neu was almost undetectable in normal oral mucosa and only 1/18 (0/05) of cases was positive. In oral epithelial dysplasia, 2/18 (11.1%) demonstrated staining, as did 3/18 OSCCs. Membrane staining was observed in all cases and there was no significant variation in frequency/intensity between normal oral mucosa / oral epithelial dysplasia and OSCCs (p>0/05). Conclusions: Aberrant expression of HER2/neu apparently does not contribute to carcinogenesis in the oral epithelium. The lack of overexpression in OSCCs indicates that molecular targeting is not feasible for adjuvant treatment.

Key Words: Oral squamous cell carcinoma - dysplasia - HER2/neu immunohistochemistry - negative findings

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Introduction

Squamous cell carcinoma (SCC) is the most common of all head and neck cancers (Vockes et al.,1993). Particularly in the young (Rautava et al.,2008) its incidence is increasing in developing countries (Baez, 2000). The etiology and pathogenesis of oral squamous cell carcinomas (OSCCs) are influenced by environmental factors and carcinogen-metabolizing enzymes can increase the risk (Day and Blot, 1992). Despite combined treatment approaches, such as surgery, radiotherapy and chemotherapy, prognosis of OSCC is poor. The five-year survival rate is only about 40% and some patients suffer from multiple primary lesions as a result of field cancerization (Satoru et al., 2004). Management of such patients is still faced with high failure rates despite significant researches exploring the pathogenesis and management of these tumors (Mort Tefler and Shepherd,1993). Although surgery is still a suitable treatment approach, side-effects often result in chronic pain, difficulty in swallowing and speech and disfigurement (Chaturvedi et al., 2008). Radiotherapy and chemotherapy have anti-tumoral effects, but they cause damage to normal tissues (Vockes et al.,1993; Chaturvedi et al., 2008).

Despite advancements in diagnosis and treatment, survival rate of patients is not satisfactory. A better understanding of molecular mechanisms and identification of potential of oncogenes in OSCC may provide new therapeutic decisions such as target therapy in the treatment of patients with OSCC.(Yamamoto et al.,1986). Although molecular carcinogenesis of head and neck SCC is not yet clear,(Fong et al.,2008) target therapy is the newest therapeutic approach in the treatment of OSCC.(Yamamoto etal.,1986) which has fewer side-effects in comparison with other modes of treatment.

The oncoprotein ErbB2 (HER2/neu) is a ~185KD tyrosine kinase transmembrane receptor that belongs to the same family as epidermal growth factor receptor (Pauletti et al.,1998). It lacks a specific ligand and is encoded by a gene located on chromosome 17 (Press etal.,1997; Bossuyt et al., 2005). Overexpression of HER2/neu has been found in 15-20% of breast carcinomas and therefore anti HER2/neu is referred to as an anti-cancer medication.(Cavalot et al., 2007). Frequency of HER2/neu expression and its prognostic relevance in OSCC is still controversial. The present immunohistochemical study was therefore conducted.

Materials and Methods

Materials

In this retrospective study, paraffinized blocks of OSCCs and oral epithelial dysplasia from 36 patients were obtained from archives of Oral Pathology Department. Eighteen normal oral mucosa specimens were retrieved from specimens of normal margin of epithelium around OSCCs.

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Demographic data (Age, Sex) and location of the lesion were excluded from patients' papers and were recorded in tables. Then 4µm sections of paraffin blocks were made (Microtom) and stained with H&E .

Staining procedure: Immunohistochemistry

Sections, 3µm thick, were mounted on positive charged microscope slides. After dewaxing in xylene, sections were dehydrated in ethanol, rinsed in distilled water, placed in 3% H2O2 for 10 min and rinsed in distilled water for 15 min for antigen retrieval procedure. Slides were placed in citrate buffer solution, PH=6, in a microvave at 92˚C for 10 min. After cooling at room remperature for 20 min, slides were exposed to primary antibodies (anti-ErbB2 (CerbB2) DAKO Carpentaria 9 (A) 1/200 diluted in PBS made 30 min at 4˚C. Sections were washed again and incubated with biotinylated secondary antibody for 30 min followed by the streptavidin biotin- peroxidase (strept ABC complex, HRP duet kit, Dako) for 30 min at room temperature. Reactions were developed with a solution containing 0.6 mg/mL of 3,3' diaminobenzidine dihydrochloride (DAB, sigma) and 0.01% H_2O_2 and counter stained with Mayer's hematoxylin for about 2 min. Positive control was invasive ductal carcinoma of breast with positive membrane staining of tumoral epithelial cells. Negative control was remission of primary antibody and using non immunized serum of rat.

Histomorphometic evaluation of HER2/neu stained sections: Representative fields were randomly selected in each immunohistochemistry stained section. Ten fields were chosen for each section. We counted the total number and intensity of staining of positive cells for all 10 examined fields per case was calculated. This allowed calculation of the mean number and intensity of staining of HER2/neu positive cells per fields. Results are presented as the mean number of HER2/neu positive membrane cells per field for, normal oral epithelium , oral epithelial dysplasia and OSCC.

Immunohistochemistry was scored as follows (Lebeau et al.,2001) : 3+: complete and intense membrane staining of >10% tumor cells. 2+ :complete but moderate staining of >10% cells. 1+: weak and incomplete staining in >10% cells; 0=no membrane staining or staining in<10% cells. Score (0,1) was considered negative but score (2,3) were positive.

Statistical analysis:

Analysis of difference in the mean number and intensity of HER2/neu positive cells per field among all type of lesions and normal oral epithelium was done using ANOVA and Fishers exact test. Statistical significance was set at $p<0.05$. The statistical package for the social sciences (SPSS 13) software was used for computations.

Results

The results of IHC slides of HER2/neu are summarized in Table 2. The 18 cases of OSCC were well to moderately differentiated. There were 6 patients in each group of mild, moderate and severe dysplasia. Score 1 HER2 /neu positive membrane staining was seen in 1 case of normal oral mucosa and was found in 7 patients associated with oral epithelial dysplasia and 5 patients wih OSCC (Figure 1a). The cases for score 2 were 1 (see Figure 1b), 2 and 3, respectively and score 3 was not found in any cases.

Table 1. Immunohistochemical Assessment of HER2/neu Immunohistochemistry with the CB11 Antibody

Tissue Scores	0	1	2
Normal oral mucosa	16 (88.8)	1 (5.1)	1 (5.1)
Oral epithelial dysplasia	9 (50.0)	7 (38.8)	2 (11.1)
Oral squamous cell carcinoma	10 (55.5)	5 (27.7)	3 (16.8)

Discussion

In our study, HER2/neu expression in normal oral mucosa was almost undetectable and immunoreactivity of HER2/neu in oral epithelial dysplasia was (score2)positive membrane staining in 2 cases of dysplasia but in OSCC was found score 2 in 3 cases. overexpression of HER2/neu was not seen in these oral lesions and normal oral epithelium. This implies that abnormal expression of HER2/neu could not play a role in carcinogenesis process of OSCC. Other studies have reported overexpression of HER2/neu as a potential useful marker in distinguishing non cancer from cancer tissues (Lebeau et al.,2001; Cavalot et al., 2007). Fong et al (2008) suggested ,there are dynamic changes in HER2/neu expression in oral carcinogenesis process. In the present study, expression of HER2/neu could not used as a marker in distinguishing normal oral mucosa/ oral epithelial dysplasia from OSCC ($p>0.05$). Also, HER2/neu expression is not a useful marker in distinguishing normal oral mucosa from oral epithelial dysplasia.

HER2/neu overexpression is seen in many cancers such as breast carcinoma (Xia et al .,1999) but is controversial in OSCC in different studies (Mort Tefler and Sheferd,1993; Khan et al.,2002). Xia et al (1999) used immunohistochemistry in 111 patients with OSCC to examine levels of four epidermal growth factor receptor family members. They considered cytoplasmic and membrane staining as positive and reported HER2/neu to be the most significant single factor in predicting disease outcome, while River (1990) Field et al (1992), Khan et al (2002), Ekberg et al (2005) and Angiero et al (2008) could not use HER2/neu as a prognostic factor or treatment indicator in patients with OSCC.

Controversial results in different studies might be due to using different immunohistochemical methods (direct, indirect), type of antibody (clone CerbB2, CB11,ICR1b, polyclonal DAKO,monoclonal zymed) no specific criteria for positive staining of HER2/neu protein (Membrane and/or cytoplasmic) and /or using different techniques (immunosorbent assay, radioimmunoassay, IHC) or different locations of lesions and sex of patients with OSCC.

Many studies have used monoclonal antibody CB11, which has a tendency also to stain the cytoplasm and have considered cytoplasmic staining similar to membrane staining which is specific to the EGFR family members

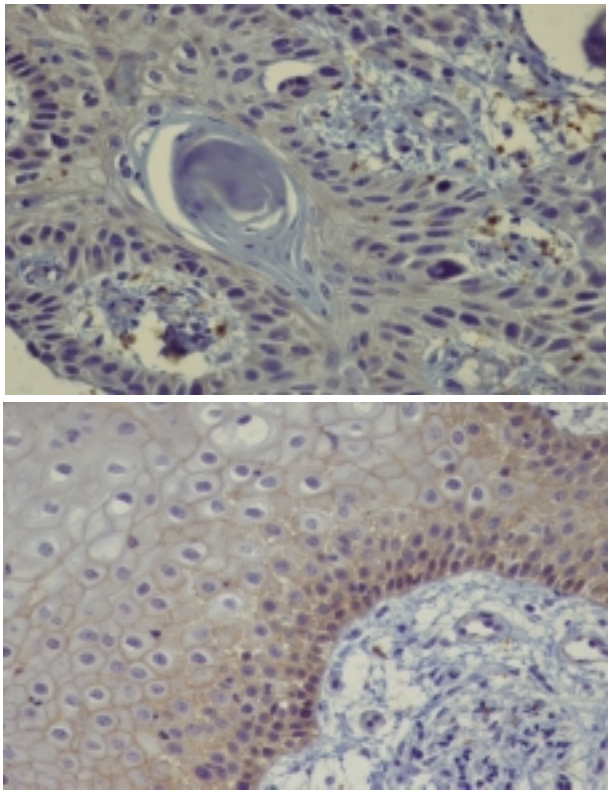

Figure 1. HER2/neu immunoreactivity. a) Oral squamous cell carcinoma (x40) (score1); b) Normal oral mucosa (x40) (score2)

(Xia et al.,1997; 1998). Silva et al (2004) reported that intracytoplasmic HER2/neu immunolabeling was able to predict the survival probability of OSCC, but other authors have stated that cytoplasmic staining is a technical artifact generated by a cross- reactive antibody or by antigen retrieval (De Potter et al.,1989; Paik et al., 2002). Angiero et al used IHC with 2 types of antibody and fluorescence in situ hybridization (FISH) with HER2/neu. In Hercept kit test was found only 1 positive case and with monoclonal antibody CB11 was seen 10 positive cases but FISH was not approved these IHC results with CB11 antibody.They suggested any role of HER2/neu in management of OSCC (Angiero et al .,2008). Our results are in agreement. It is suggested that the type of antibody that is used might be effective in the location of positive staining (cytoplasmic and/or membrane). Chung-Ho et al used ELISA and radioimmunoassay for evaluating HER2/neu expression in patients with OSCC pre- and post-treatment. They reported that HER2/neu mean levels were reduced significantly after treatment (Chung- Ho et al., 2007). Activation of EGFR family (HER2/neu) by a variety of ligands is necessary for normal growth and differentiation (Rautava et al., 2008).

These findings suggest that other EGFR member except HER2/neu might be role in normal growth and differentiation process of normal oral epithelium. Only 2 out of 16 studies that are available in the literature have identified the location of OSCC lesions (tongue) but this localization was not possible in our study due to the limited number of cases. However, the location of carcinoma might be an important factor in terms of therapy and prognosis. Breast cancers are more common in female. HER2/neu is a useful marker for immunotherapy in metastatic breast carcinoma.(Neville et al.,2009). HER2/neu is the main target of the monoclonal antibody Trastuzumab (Herceptin). Trastuzumab is effective in the treatment of breast cancer with overexpression of HER2/neu (Ciardiello et al.,2001).

In conclusion, the data suggest that HER2/neu is not an effective protein in carcinogenesis process of OSCC. HER2/neu is not a suitable marker that could play a role in differentiating normal oral epithelium/epithelial dysplasia from OSCC . Its role in differentiating normal oral epithelium from epithelial dysplasia is not well demonstrated (p>0/05). HER2/neu shows no overexpression in OSCC; therefore, target therapy is not an effective adjuvant treatment in these patients.These report suggest other EGFR member might be effective in treatment approach of OSCC.

References

Angiero F, Sordo RD, Dessy E, et al (2008). Comparative analysis of c-erbB2 (HER2/neu) in squamous cell carcinoma of the tongue: does over expression exit? And what is its correlation with traditional diagnostic parameters? *J Oral Pathol Med*, 37, 145-50.

Baez A (2008). Genetic and environmental factors in head and neck cancer genesis. *J Environ Sci Health C Environ Carcinogen Ecotoxical Rev*, **26**, 174-200.

Bossuyt V, Fadare O, Martel M, et al (2005). Remarkably high frequency of EGFR expression in breast carcinomas with squamous differentiation. *Int J Surg Pathol*, **13**, 319-27.

Cavalot A, Martone T, Roggero N, et al (2007). Prognostic impact of HER2/neu expression on squamous head and neck carcinoma. *Head Neck*, **29**, 655-64.

Chaturvedi P, Shan B, Gosselin BJ (2008). Combined modality molecular targeted therapy head/neck squamous cell carcinoma. *Head Neck Oncol*, **10**, 38-48.

Chung-Ho Ch, Sung-Lin T, Yi-Shin Y, et al (2007). Studies of serum HER2/neu and squamous cell carcinoma-related antigen expression in patients with oral squamous cell carcinoma. *J Oral Pathol Med*, **36**, 83-7.

Ciardiello F, Tortora G(2001). Anovel approach in the treatment of cancer: targeting the epidermal growth factor receptor. *Clin Cancer Res*, **7**, 2958-70.

Day GL, Blot WJ (1992). Second primary tumors in patients with oral cancer. *Cancer*, **70**, 14-9.

De Potter CR, Quatacker J, MAertens G, et al (1989). The subcellular localization of the neu protein in human normal and neoplastic cells. *Int J Cancer*, **44**, 969-74.

Ekberg T, Nestor M, Engstrom M, et al (2005). Expression of EGFR, HER2, HER3 and HER 4 in metastatic squamous cell carcinomas of the oral cavity and base of tongue. *Int J Oncol*, **26**, 1172-85.

Field JK, Spandidos DA, Yiagnisis M (1992). C-erbB-2 expression in squamous cell carcinoma of the head and neck. *Anticancer Res*, **12**, 613-20.

Fong Y, Chou SJ, Hung KF, et al (2008). An investigation of the differential expression of HER2/neu Gene expression in normal oral mucosa, epithelial dysplasia and oral squamous cell carcinoma in Taiwan. *J Clin Med Assoc*, **71**, 123 -7.

Khan AJ, King BL, Smith GL, et al (2002). Characterization of HER2/neu oncogene by immunohistochemical and fluorescence in situ hybridization analysis in oral and oropharyngeal squamous cell carcinoma. *Clin Res*, **8**, 540-48.

Lebeau A, Delming D, Kaltz C, et al (2001) .HER2/neu analysis ion archival tissue samples of human breast cancer.comparison of immunohistochemistry and fluorescence in situ hybridization. *J Clin Oncol*, **19**, 354-63.

Mort Tefler MR, Shepherd JP (1993). Psychological distress in patients attending an oncology clinic after definitive treatment for maxillofacial malignant neoplasia. *Int J Oral Maxillofac Surg*, **22**, 347-9.

Neville BW, Dam DD, Allen CM, et al (2009). Oral and Maxillofacial Pathology. 3rd ed Philadelphia: WB Saunders.

Paik S, Bryant J, Tan-Chiu E, et al (2002). Real world performance of HER2 testing: national surgical adjuvant breast and bowel project experience. *J Natl Cancer Inst*, **24**, 852-4.

Pauletti G, Godelphin W, Press MF, et al (1998). Detection and quantification of HER2/neu gene amplification in human breast cancer archival material using flourescence in situ hybridization. *Oncogene*, **13**, 63-72.

Press MF, Bernstein L, Thomas PA (1997). HER2/neu gene amplification characterized by fluorescence in situ hybridization poor prognosis in node negative breast carcinoma. *J Clin Oncol*, **15**, 2894-904.

Rautava J, Jee KJ, Miettinen PJ, et al (2008). ERBB receptors in developing, dysplastic and malignant oral epithelia. *Oral Oncol*, **44**, 227-35.

Rivere A, Wilckens C, Loning T (1990). Expression of C-erbB2 and C- myc in squamous epithelia and squamous cell carcinoma of the head and neck and the lower female genital tract. *J Oral Pathol Med*, **19**, 408-13.

Satoru SH, Yuuji N, Chunnan Li,et al (2004). HER2/neu expression in oral squamous cell carcinoma. *J Oral Maxillofac Surg*, **16**, 172-6.

Silva SD, Agostini M, Nishimoto In, et al (2004). Expression of fatty acid synthethase, ErbB2 and Ki67 iead and neck squamous cell carcinoma. A clinico- pathological study. *Oral Oncol*, **40**, 688-96.

Vockes EE, Weichselbaum RR, Lippman SM, et al (1993). Head and neck cancer. *N Engl J Med*, **328**, 184-94.

Xia W, Lau YK, Zhans HZ et al (1997). Strong correlation between c-erbB2 overxpression and overall survival of patients with oral squamous cell carcinoma. *Clin Cancer Res*, **3**, 3-9.

Xia W, Lau YK, Zhans HZ, et al (1999). Combination of EGFR, HER2/neu and HER3 is a stronger predictor for the outcome of oral SCC than any individual family members. *Clin Cancer Res*, **5**, 4164-74.

Yamamoto T, Kamata N, Kawano H, et al (1986). High incidence of amplification of the epidermal growth factor receptor gene in human squamous carcinoma cell lines. *Cancer Res*, **46**, 414-16.