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

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 et al., 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 et al., 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 (Microtome) 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 microwave at 92°C for 10 min. After cooling at room temperature 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% H2O2 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.

Histomorphometric 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 with 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.

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 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.
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.

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