# An Investigation of the Differential Expression of Her2/neu Gene Expression in Normal Oral Mucosa, Epithelial Dysplasia, and Oral Squamous Cell Carcinoma in Taiwan

Yao Fong[1], Shan-Ju Chou[2,3], Kai-Feng Hung[4], Ho-Tai Wu[2,3], Shou-Yen Kao[2,3]*

*[1]Department of Surgery, Chi-Mei Medical Center, Tainan, [2]Oral and Maxillofacial Surgery, Department of Dentistry, Taipei Veterans General Hospital and [3]School of Dentistry, National Yang-Ming University, Taipei, and [4]Department of Dentistry, Buddhist Tzu Chi General Hospital, Sindian, Taiwan, R.O.C.*

**Background:** Her2/neu was thought to be a proto-oncogene with sequence homology to epidermal growth factor receptor (EGFR). Its overexpression was seen in many cancers and referred to regimens of anticancer therapy. The aim of this study was to investigate whether the abnormal expression existed in oral carcinogenesis.

**Methods:** Immunohistochemistry (IHC) was used to detect Her2/neu expression in normal oral mucosa (NOM) ($n = 20$), oral precancerous lesions of epithelial dysplasia (ED) ($n = 20$), and oral squamous cell carcinoma (OSCC) ($n = 30$). The association of clinicopathologic covariates of areca use, tumor size, neck lymph node metastasis, differentiation and stages of cancer with the expression of Her2/neu was examined. The significance of Neu immunoreactivity in different groups or with different covariates was investigated using Fisher's exact test.

**Results:** Her2/neu immunoreactivity was very low with Her2/neu(+) in 10% (2/20) of NOM cases and in 25% (5/20) of ED cases, respectively. The Her2/neu expression was high in OSCC cases, with 40% (12/30) of Her2/neu(+) and 10% (3/30) of Her2/neu(++). Significant difference was observed between NOM/ED and OSCC cases ($p < 0.05$). All clinicopathologic covariates showed no significant relation to the expression of Her2/neu in OSCC cases.

**Conclusion:** These findings suggested a dynamic change in Her2/neu expression during the development of OSCC. The overexpression of Her2/neu can be used as a marker in distinguishing NOM/ED from OSCC. [*J Chin Med Assoc* 2008;71(3):123–127]

**Key Words:** areca, differential expression, Her2/neu, oral cancer

## Introduction

Oral squamous cell carcinoma (OSCC) is one of the leading cancers worldwide and is becoming a great health threat in Taiwan.[1] Although advanced surgical techniques and new anticancer drugs are developed constantly, the overall postoperative survival of OSCC patients has not improved much, with a low 5-year survival rate of 25–30%. The overall poor prognosis of OSCC patients is highly related to the advanced stages (II & IV) of cancers comprising 50% of fresh OSCC cases in medical centers. This highlights the importance of early detection and early treatment of oral precancerous lesions and OSCC. Yet, unlike many other cancers such as colorectal carcinoma, hepatocellular carcinoma, and prostate carcinoma, which possess specific molecular markers useful for detection, one of the pivotal tasks is to find useful markers for distinguishing cancer or precancerous lesions from normal oral mucosa.[2–6]

Since OSCC exhibits aggressive biologic behavior, the development of new treatment modalities for the primary tumor, as well as for metastatic lesions, remains a challenge.[1–7] Previous studies of cancer biology have

*Correspondence to: Dr Shou-Yen Kao, Oral and Maxillofacial Surgery, Department of Dentistry, Taipei Veterans General Hospital, 201, Section 2, Shih-Pai Road, Taipei 112, Taiwan, R.O.C.
E-mail: sykao@vghtpe.gov.tw • Received: October 19, 2007 • Accepted: January 25, 2008

revealed that the activation of oncogenes plays an important role in the development and progression of malignancies. Her2/neu (also know as c-erbB-2) is a proto-oncogene located on human chromosome 17 and encodes a 185-kD transmembrane glycoprotein with tyrosine kinase activity. This glycoprotein demonstrates extensive homology to the epidermal growth factor receptor (EGFR).[8–11] Amplification of Her2/neu oncogene or overexpression of its protein has been demonstrated in several malignant neoplasms.[7,12–18] Yet, diverse results regarding the overexpression of Her2/neu in OSCC have been reported.[19–25] This highlights the importance of further examining the role of Her2/neu in Taiwan, where high prevalence of areca-related OSCC stands as the fourth highest incidence of malignancies in males.[1–3] In this study, we investigated the expression of Her2/neu in human tissues by the immunohistochemistry (IHC) method in order to primarily explore its potential role in the malignant transformation of OSCC.

## Methods

### *Samples*

All specimens were harvested from patients being treated in Taipei Veterans General Hospital (TVGH). This project was approved by the institutional review board of TVGH, with informed consent signed by patients. Specimens were collected between January 2002 and January 2005. All samples were fixed and sectioned following standard protocols.[2,6] The samples included 20 normal oral mucosa (NOM) lesions, 20 epithelial dysplasia (ED) lesions and 30 pathologically proven OSCCs, which had undergone wide excision of the primary lesions. The mean ages of the 3 groups (NOM, ED, OSCC) were 46 ± 7 years, 51 ± 6 years, and 53 ± 9 years, respectively. The gender ratios of male/female were 7:3, 13:7, and 22:8, respectively. The clinicopathologic features, including areca use, tumor size, neck lymph node (LN) metastasis, differentiation, and stages of OSCC subjects, were recorded.

### *IHC*

Sections were deparaffinized in xylene and rehydrated by immersion in a graded series of ethanol dilutions. All slides were immersed in 10 mm sodium citrate solution, in a microwave oven, to retrieve antigenicity. Sections were quenched with 3% fresh $H_2O_2$ for 10 minutes to inhibit endogenous tissue peroxidase activity, and rinsed with 1 × PBS for 5 minutes twice. Sections were further incubated in blocking serum for 30 minutes and then 2.5 hours with primary Neu (ICR12) sc-57378 rat monoclonal IgG2a antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) in a humid chamber. After washing with 1 × PBS for 5 minutes twice, sections were then incubated with biotinylated secondary antibody solution for 30 minutes. LSAB2® streptavidin-peroxidase detection reagent (DAKO, Santa Barbara, CA, USA) was subsequently added evenly over the sections and incubated for 30 minutes. The sections were washed with 1 × PBS for 5 minutes twice, incubated with freshly prepared aminoethylcarbazole (AEC; Zymed, South San Francisco, CA, USA) substrate solution for 15 minutes and then washed with 1 × PBS. All IHC staining was performed at room temperature. The sections were finally counterstained with hematoxylin, washed with 1 × PBS and de-ionized distilled water, and then mounted. The percentage of immunoreactive cells were estimated in 5 random 400 microscopic fields.[2] The tissue sections were categorized as Her2/neu(++), Her2/neu(+) or Her2/neu(−) when over 50%, 10–50%, or < 10% of cells were positive for immunostaining, respectively.

### *Statistical analysis*

The significance of Her2/neu immunoreactivity in different groups or with different covariates was investigated using Fisher's exact test. A $p$ value of less than 0.05 was considered to be statistically significant.

## Results

### *Characteristics of cases*

The mean age of the 30 OSCC cases was 52 ± 7 years (range, 36–69 years). A marked gender difference between males and females (25 *vs.* 5) was consistent with a previous report on OSCC in Taiwan.[1] The mean ages of the NOM and OPL groups were 47 ± 7 years and 50 ± 9 years, respectively, showing no significant difference in age among the different groups. The tissue samples of the NOM and ED groups were mainly from buccal mucosa. The tissue samples of OSCC were harvested from buccal mucosa (13 cases), tongue (5 cases), gingiva (5 cases), mouth floor (6 cases) and palate (1 case). The immunoreactivity in OSCC cases was analyzed. Twenty-four (24/30, 80%) cases were areca users and 6 (6/30, 20%) were non-users. Twelve cases (12/30, 40%) had an extensive T4 tumor mass. Eighteen (18/30, 60%) had tumor size ranging from T1 to T3. Twenty-two (22/30, 73%) cases were neck node negative. Eight (8/30, 27%) had neck nodal metastasis. Eighteen (18/30, 60%) were well differentiated OSCCs. Twelve (12/30, 40%) were moderately

**Table 1.** Analysis of the association of clinicopathologic covariates with Her2/neu expression in oral squamous cell carcinoma

| | Her2/neu(–) | Her2/neu(+) | *p** |
|---|---|---|---|
| Areca use | | | 0.35 |
| User ($n=24$) | 12 | 12 | |
| Non-user ($n=6$) | 3 | 3 | |
| Size | | | 0.36 |
| T1–T3 ($n=18$) | 10 | 8 | |
| T4 ($n=12$) | 5 | 7 | |
| Lymph node | | | 0.34 |
| N = 0 ($n=22$) | 12 | 10 | |
| N > 0 ($n=8$) | 3 | 5 | |
| Differentiation | | | 0.36 |
| Well differentiated ($n=18$) | 10 | 8 | |
| Moderately & poorly differentiated ($n=12$) | 5 | 7 | |
| Stage | | | 0.14 |
| I–III ($n=16$) | 10 | 6 | |
| IV ($n=14$) | 5 | 9 | |

differentiated to poorly differentiated OSCCs. Stage IV tumors were present in 14 (14/30, 47%) cases. Stage I–III tumors were present in 16 (16/30, 53%) cases (Table 1).

### *Her2/neu immunoreactivity*

Her2/neu immunoreactivity was scarcely observed in the membranous or cytosolic compartments of NOMs and in the majority of EDs. Her2/neu immunoreactivity was scored according to the number of cells exhibiting immunostaining. All except 2 NOM cases exhibited Her2/neu(–) (Figure 1A). Her2/neu(+) and (++) only appeared in 5 (5/20, 25%) ED cases (Figure 1B). In contrast, 12 (12/30, 40%) OSCCs were Her2/neu(+) and another 3 (3/30, 10%) OSCCs were (++) (Figures 1C and 1D). The remaining 15 OSCCs showed scattered Neu immunoreactivity and were scored as Her2/neu(–). Significant difference in Neu immunoreactivity was noted between ED and OSCC groups ($p<0.05$, Fisher's exact test; Figure 2).

While most clinical features of OSCC including areca use, tumor size, neck LN metastasis, and differentiation showed no correlation to Neu gene expression by IHC examination, tumor stage was noted to have significant correlation to Neu expression. In all, no significant difference in Neu differential expression was associated with clinicopathologic covariates (Table 1).

## Discussion

In our study, Her2/neu expression in NOM was almost undetectable and very low in ED. Significantly higher Her2/neu expression in the OSCC group than the NOM and ED groups was also noted. Furthermore, Her2/neu expression was significantly higher in the advanced stage IV cases than the stage I–III cases. This implied that the aberrant expression of Her2/neu could play a potential role in the progression of malignant transformation of OSCC instead of the early promotion stages. In the OSCC group, purely tumor size, neck nodal metastasis or differentiation did not have a statistically unique result as staging of the tumor in correlation to Her2/neu expression. Whether this was due to the sample size or its role in the transformation being irrelevant to the above covariates needs further exploration. Obviously, the overexpression of Her2/neu could be a potential useful marker in distinguishing non-cancer and cancer, as shown in this study. Once the overexpression of Her2/neu is found in cases with benign or precancerous lesions in the oral cavity, care should be taken in the follow-up of such patients. Early treatment with excision of the ED showing expression of Her2/neu may be required. An attractive target for receptor-mediated immunotherapy has been conducted in several large clinical multicenter trials as first-line therapy for metastatic breast carcinomas that exhibited Her2/neu overexpression. In this clinical trial, trastuzumab (Herceptin; Genentech, San Francisco, CA, USA), a humanized monoclonal antibody directed against the extracellular domain of the Her2/neu protein, was studied.[15–17] Whether Her2/neu could be treated as a molecular target for anticancer therapy in OSCC exhibiting overexpression of Her2/neu remains unknown and certainly warrants attention and value as an approach for clinical trials.

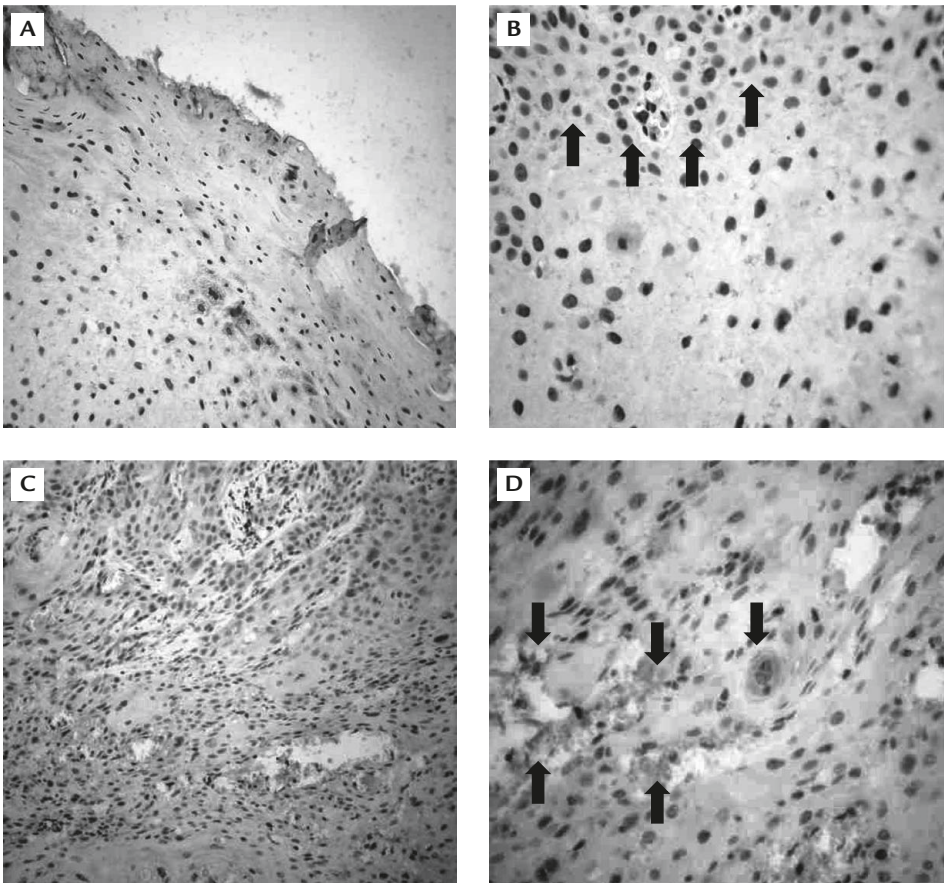


**Figure 1.** Her2/neu immunoreactivity in normal oral mucosa (NOM), epithelial dysplasia (ED) and oral squamous cell carcinoma (OSCC). Immunohistochemistry (IHC) showed differential expression between NOM/ED and OSCC. (A) NOM (Her2/neu(–), 100×). (B) Black arrows indicate the area of ED (Her2/neu(–), 400×). (C) Weak positive staining in most area of OSCC (Her2/neu(+), 100×). (D) Black arrows indicate some strong focal cytoplasmic staining in OSCC (Her2/neu(++), 400×).

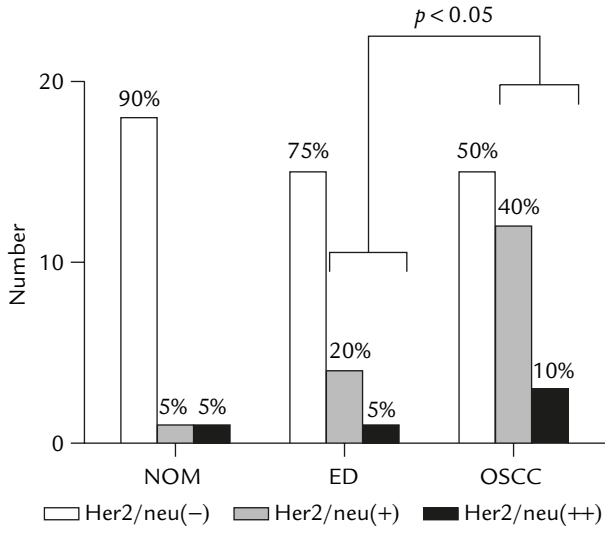


**Figure 2.** Elevated Her2/neu expression in stepwise carcinogenesis. Significant difference in Neu immunoreactivity was noted between the epithelial dysplasia (ED) and oral squamous cell carcinoma (OSCC) groups. NOM = normal oral mucosa.

Alterations of the Her2/neu proto-oncogene have been implicated in the carcinogenesis and prognosis of many cancers, especially breast cacner.[7,12–18] Its overexpression is correlated with a poor prognosis for breast and ovarian cancer patients. Aberrant expression of Her2/neu has been frequently observed in OSCC, but the reported results are controversial because of their wide range (between 0% and 88%).[7,19–25] The lack of a unique marker of OSCC has long been a problem in the early detection of OSCC. It would be necessary to discover more reliable and efficient markers to characterize the malignant transformation of oral epithelia.[1–4] Thus, the aim of this study was primarily to examine the expression of Her2/neu in the human tissues of NOM, ED and OSCC cases to validate the controversial results of various studies and to determine whether Her2/neu could be considered as a useful marker for oral cancer.

Her2/neu overexpression was seen in many cancers, but was controversial in OSCC from different reports.

We investigated its association of expression in oral carcinogenesis by IHC. Significant difference was observed between NOM/ED and OSCC cases. Significant increase of expression from stages I to III to the advanced stage IV OSCC cases was observed. Her2/neu can potentially be a useful marker in distinguishing NOM/ED from OSCC.

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