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Research Paper

The prognostic value of HER2 oncogene expression in human cutaneous squamous cell carcinoma.

Medical Science

KEYWORDS : immunohistochemistry, squamous cell carcinoma, HER2, oncogene

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ABSTRACT

BACKGROUND: Cutaneous squamous cell carcinoma is the second most common skin cancer, after basal cell carcinomas. HER2, a member of the Erb family of transmembrane receptor tyrosine kinases (RTK), is responsible for communicating extracellular signals to the nucleus and is implicated in tumorgenesis in several malignancies with potentially poor outcome. To date, the expression of HER2 has not been evaluated in primary cutaneous squamous cell carcinoma (SCC).

AIMS: The aim of this study is to (1) investigate the expression of HER2 in primary cutaneous SCC (2) relate the results to the established clinicopathological prognostic factors

PATIENTS AND METHODS: This study was conducted on about 40 skin specimens from patients with non melanoma skin cancer (squamous cell carcinoma) diagnosed and confirmed by biopsy. Immunohistochemical analysis for HER2 was carried out on formalin-fixed paraffin-embedded sections of bladder tissues using avidin-biotin peroxidase method.

RESULTS: 28 cases (70%) of cuaneous SCC evaluated for the presence of HER2 were immunoreactive for the receptor. There was a statistically significant correlation between HER2 expression and each of the tumor grade, lymph node and distant metastasis.

CONCLUSIONS: HER2 is overexpressed in 70% of SCC cases; this is cognate with the prior reports demonstrating that SCC possesses high metastatic potential. These results can have both prognostic and therapeutic implications.

INTRODUCTION

Cutaneous squamous cell carcinoma (SCC) accounts for 20% of all dermatologic malignancies and has been defined as a proliferation of keratinocytes with potential for local and metastatic disease(1).

Recent reports demonstrated that epidermal growth factor receptor (EGFR) overexpression is significantly associated with metastatic potential in head and neck cutaneous squamous cell carcinomas (HNcSCC). The EGFR family of transmembrane proteins, which includes HER2, plays an integral role in tumor proliferation. HER2 is highly expressed in many solid tumors and associated with poor prognosis in tumors from various sites, including lung, breast, and head and neck (2).

In non–small cell lung carcinoma, high copy numbers of the HER2 gene has been demonstrated to be of significance in predicting therapeutic response to EGFR-targeted tyrosine kinase inhibitors, such as gefitinib (3).Cohen et al (4) also suggest that mutations in the HER2 gene may play a role in predicting therapeutic response to EGFR-targeted therapy in some cases of head and neck cancers.

In a previous study, 83% of cases of SCC had elevated expression, with positive staining seen in the cytoplasm. Fewer positive cells and less staining were detected in Basal cell carcinoma (BCC) compared with SCC (5). In oral invasive SCCs, no HER2/neu immunostaining was detectable (6). In contrast, HER2 oncoprotein expression was associated with poor prognosis in cervical SCC (7).

Regulation of ErbB receptor activation is very complex. A number of different ligands have been described: e.g. epidermal growth factor (EGF), heparin-binding EGF-like growth factor (HB-EGF) and epiregulin (8-9). These ligands show different affinities to the ErbB receptors and upon binding induce heterodimerisations of the receptors. Thus, by binding to specific receptors and by forming specific heterodimers, differentiated signal transduction processes can be initiated. Hansen and colleagues (10-11) have shown that HER2 signaling is also involved in the actions of UV in inducing epidermal proliferation and hyperplasia, and that inhibition of HER2 using AG825 blocked UV promotion of skin tumors in TG:AC mice (12). Therefore, the knowledge of expression patterns of HER2 in cutaneous SCC will help in the understanding of malignant transformation and might have prognostic or therapeutic significance.

In the current study, the aim was to investigate the expression of HER2 in primary cutaneous SCC and relate the results to the established clinicopathological prognostic factors.

PATIENTS AND METHODS

Histopathology:

This study was carried out with co-operation of Pathology Department, King Fahd Specialist Hospital, Buraidah, and other hospitals in Qassim region. The study was conducted on skin specimens obtained from non melanoma Cutaneous SCC patients. Specimens were examined and diagnosed. The normal skin tissues were obtained from areas adjacent to the tumor tissues. The age and sex of the patients, the main complaints, the clinical presentations, the tumor size, site were recorded and analyzed. The tissue specimens were fixed in 10% formalin, dehydrated and embedded in paraffin and stained with hematoxylin and eosin (H&E). The slides were then examined for histological diagnosis and assessment.

Immunohistochemistry:

From the formalin fixed paraffin-embedded tissues, 4µ sections were immunostained with monoclonal antibodies against HER2 using labeled streptavidin biotin (LSAB) method. In brief; Sections were cut and dried at 37°C, dewaxed in xylene and rehydrated using serial concentrations of ethanol and washed in running water. Sections were washed with Tris buffer and preincubated with normal rabbit serum (10%) for 20 minutes. Slides were incubated with rabbit monoclonal primary antibody; c-erbB-2 / HER-2 / neu (Clone EP1045Y) (1:50; RM-2112-RQ, Thermo Fisher Scientific, Cheshire, UK) then exposed to avidin biotin peroxidase. Sections were then incubated in diaminobenzidine (DAB). The slides were then counterstained with Mayer's hematoxylin for 10 min and reviewed. For every batch a positive control was run simultaneously. For negative controls, the primary antibody was replaced with PBS. Positive staining for HER2 was defined as any IHC staining of tumor cell membranes or cytoplasm above background level. A grading system of weak

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(1) <10%, moderate (2) 10% to 50%, or strong (3) >50%, was then applied.

Statistical analysis:
Statistical calculations were performed by using Statview programme package. Data were compared using Chi-square test, and the differences between the means of continuous data were compared by using paired t-test. The percentage of expression of HER2 in epithelial cells of SCC; were used to assess the role of WHO's classification, differentiation, age, sex, and tumor size.

RESULTS
HER2 expression and correlation with the clinicopathological parameters of the 40 patients with cutaneous SCC enrolled in this study is shown in table (1) and Fig.1. Ten cases were grade 1, 18 cases were grade 2, and 12 cases were grade 3. Adjacent normal skin has low levels of expression and can serve as an internal positive control for comparison with squamous cell carcinoma cases.

Immunohistochemical Staining of HER2 Expression in cutaneous Squamous cell carcinoma:

Nearly all normal stratified squamous epithelium of the skin were negative, whereas cases of SCC showed positivity to HER2. HER2 expression was observed in approximately 70% of SCC cases. There was statistically significant correlation between HER2 expression histologic grade, lymph node metastasis and distant metastasis (P = 0.006, 0.009, 0.043, respectively) (Table 1).

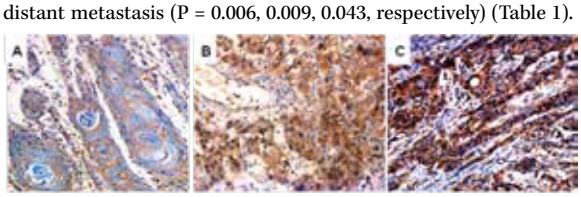


Fig.1: Immunohistochemical HER2 staining of cutaneous SCC (diaminobenzidine chromogen and hematoxylin counter stain). A: Weak HER2 expression in well differentiated SCC (+). B: Moderate HER2 expression in moderately differentiated SCC (++). C: Strong HER2 expression in poorly differentiated SCC (+++) (IHC, original magnification, ×400).

Table 1: The correlation between HER2 expression in cutaneous SCC and the clinicopathological parameters.

		HER2 Expression				
Samples	Total	Negative (%)	Weak (%)	Moderate (%)	Strong (%)	p*
SCC	40	12 (30)	4 (10)	11 (27.5)	13 (32.5)	
1. Age (Years)						
< 40	12	2 (16.6)	3 (25)	5 (41.7)	2 (16.7)	0.059
> 40	28	10 (35.7)	1 (0.3)	6 (21.4)	11 (39.2)	
2. Gender						
Male	30	9 (30)	3 (10)	8 (26.7)	10 (33.3)	0.997
Female	10	3 (30)	1 (10)	3 (30)	3 (30)	
3. Pathologic tumor size						
< 2cm	10	3 (30)	2 (20)	3 (30)	2 (20)	0.734
2-5cm	25	8 (32)	2 (8)	7 (28)	8 (32)	
>5cm	5	1 (20)	0 (0)	1 (20)	3 (60)	
4. Histologic Grade						
I (WD)	10	3 (30)	3 (30)	4 (40)	0 (0)	0.006 (HS)
II (MD)	18	5 (27.8)	1 (5.5)	7 (38.9)	5 (27.8)	
III (PD)	12	4 (33.3)	0 (0)	0 (0)	8 (66.7)	
5. LN metastasis						
Negative	32	12 (37.54)	4 (12.5)	8 (25)	8 (25)	0.009 (HS)
Positive	8	0 (0)	0 (0)	3 (37.5)	5 (62.5)	
6. Distant metastasis						
Negative	37	12 (32.44)	4 (10.8)	11 (29.7)	10 (27.1)	0.043 (S)
Positive	3	0 (0)	0 (0)	0 (0)	3 (100)	

(S): Significant statistical correlation (HS): Highly significant statistical correlation (WD): well differentiated SCC (MD): moderately differentiated SCC (PD): poorly differentiated SCC

DISCUSSION
As the second most common form of nonmelanoma skin cancer, SCC is clinically defined by its prevalent nature, multifactorial causal factors, numerous histological subtypes, and variable biologic behavior, including metastasis. With a Caucasian male predominance, the average age at the time of diagnosis is 70 (13). The reported lifetime risk of developing SCC is 4% to 14% (14). The causal factors considered important in the development of SCC have been divided into intrinsic (age, skin pigmentation, and health of the immune system) and extrinsic (UV light, radiation, chemicals, chronic inflammation, and infections). Ninety percent of all SCC are located in sun-exposed skin. UV exposure is the single most significant risk factor in the development of SCC (15-16). The continued annual increase in incidence of SCC may be attributed to an increase in the elderly and immunocompromised populations.

There is ongoing debate concerning specific histopathologic features as they relate to prognosis in SCC. Such features include size, depth of invasion, histologic grade. The American Joint Committee on Cancer (17) and the International Union Against Cancer (18) staging systems are based specifically on tumor size, despite overwhelming evidence demonstrating the importance of histopathologic features in predicting prognosis. Overall, prognostic factors are not completely defined or universally accepted in regards to SCC. Thickness and depth of invasion have been regarded by some as one of the most important determinants of prognosis, yet only 8% of dermatopathologists in a national survey actually measured tumor depth (19). The classification system was based on the degree of tumor differentiation (well, moderately, and poorly differentiated) is commonly used by most pathologists. As reported, the less differentiated the tumor, the greater potential for metastasis.

In this study, nearly all normal stratified squamous epithelium of the skin were negative, whereas cases of SCC showed positivity to HER2. HER2 expression was observed in approximately 70% of SCC cases. There was statistically significant correlation between HER2 expression histologic grade, lymph node metastasis and distant metastasis (P = 0.006, 0.009 , 0.043. respectively) HER2 overexpression, as a consequence of gene amplification, was initially seen to be present in breast cancer patients, and more recently in a similar percentage of stomach and esophagogastric junction tumors (20). Since HER2 alterations may predict a successful response to HER target specific therapy, and cutaneous SCC has a poor prognosis with currently available treatments, it is essential to analyze possible alterations of these receptors in cutaneous SCC, to evaluate the potential of use of anti-HER therapy to treat cutaneous SCC patients. A previous study showed HER2 to be expressed only in 25% of metastatic SCC of the skin. To date, however, HER2 has not been evaluated in primary cutaneous SCC (21). There are significant clinical im-

plications associated with the expression of this receptor; each is an independent prognostic indicator of poor outcome. Herceptin, directed to the HER2 receptor, is also used in the treatment of malignancies expressing this protein. This relatively new, tumor-targeted therapeutics have fewer side effects than do conventional chemotherapeutics, but they are not without risk. A clinical trial using Cetuximab in patients with cutaneous SCC is currently underway (clinical trial # NCT00240682). Kiguchi K. et al., reported that HER2 play an important role in the development of skin tumors during two-stage skin carcinogenesis, especially during the tumor promotion stage (22). Therefore, it is essential to fully understand the potential expression of HER2 receptor among the SCC cases.

In this study, 40 cases of primary cutaneous SCC were evaluated for the expression of HER2. IHC demonstrated (28/40) 70% immunoreactivity for HER2. Though a limited series, our results suggest that HER2 are overexpressed in SCC. Our findings indicate that metastatic potential of cutaneous SCC are cognate with the association of increased HER2 expression inferring a worse prognosis and greater metastatic potential. Further studies are needed to examine the expression of HER2 in the different subtypes of primary and metastatic SCC to allow a true understanding of prognosis and treatment options. Furthermore, a larger, more comprehensive study should be performed, looking at mRNA expression to help explain the mechanism behind our findings.

CONCLUSIONS

The present study demonstrated that HER2 expression in cutaneous SCC correlated strongly with, a well-established negative prognostic marker. HER2 expression correlated with high tumor grade, lymph node, and distant metastasis, which indicates that HER2 can be used as an independent prognostic marker indicative of poor prognosis and a probable target in the development of novel therapies. Thus, we recommend that HER2 be routinely added as a new negative prognostic marker in all cutaneous SCC patients.

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