Immunohistochemical Analysis of EGFR and HER-2 in Patients with Metastatic Squamous Cell Carcinoma of the Skin

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Abstract. Background: Metastatic squamous cell carcinoma (SCC) of the skin often raise difficult therapeutic problems. Few data are available about the expression of EGFR and HER-2 in SCC of the skin. Overexpression of EGFR and of HER-2 proteins has been reported. The purpose of this study was to investigate the expression of EGFR and HER-2 in a series of metastatic SCC of the skin. Patients and Methods: EGFR and HER-2 expression was studied by immunohistochemistry on 13 specimens of metastatic recurrence and on 2 primary lesions of these tumours. Results: EGFR had a strong membranous expression in all specimens. HER-2 was weakly expressed in 4 specimens, with a membrane expression in 2 cases. Conclusion: In the present study, EGFR was overexpressed in all samples of metastatic SCCs of the skin. Therefore, these metastatic tumours appear to be suitable targets for treatment with tyrosine kinase inhibitors. Additional studies are warranted to establish whether or not HER-2 is expressed in SCC of the skin.

Squamous cell carcinoma of the skin (SCC) accounts for 20% of cutaneous malignancies. Most of these SCCs are cured by surgery and/or radiotherapy. However, dramatic courses may be observed with the development of regional or distant metastases. Metastatic SCC of the skin often raise difficult therapeutic problems, since chemotherapy is not consistently efficient. Therefore, more effective treatments are needed.

Tyrosine kinases have emerged as clinically useful drug target molecules for the treatment of some cancers (1). The epidermal growth factor receptor (EGFR) family of proteins includes 4 transmembrane tyrosine kinase receptors: EGFR, HER-2, HER-3 and HER-4. Several ligands, such as TGFα and amphiregulin, can bind to the extracellular domain of EGFR, whereas there is to date no known ligand for HER-2. EGFR and HER-2 can interact with other members of the family by heterodimerization resulting in activation of their intrinsic kinase activity. Activation of the EGFR tyrosine kinase enzyme results in autophosphorylation, which drives the signal transduction pathway leading to tumour growth and malignant progression. EGFR and HER-2 are overexpressed or aberrantly activated in the most common tumours including head and neck cancers (2). This overexpression has been correlated with the risk of local and/or regional relapses (3) and with a poor overall survival (4, 5). Strategies have been developed in clinical trials to target HER-2 and EGFR by monoclonal antibodies or tyrosine kinase specific inhibitors (6).

Few data are available about the expression of EGFR and HER-2 in SCC of the skin. Overexpression of EGFR (7) and of HER-2 (8) proteins has been reported.

The purpose of the present study was to investigate the expression of EGFR and HER-2 in a series of metastatic SCC of the skin.

Patients and Methods

Cases. Patients, who had undergone surgery for a metastatic recurrence of SCC of the skin at our institute between 1997 and 2002, were considered for inclusion. Tissues blocks were retrieved and slides were reviewed to confirm the diagnosis. From the 15 consecutive cases which had been identified by the clinical records, 13 patients were included in the study because sufficient pathological material was available for the study. There were 9 men and 4 women. The mean age at diagnosis of metastatic SCC was 63 years (29 to 85 years).

Clinical features with locations of the primary SCCs and of the removed metastatic relapses, adjuvant therapy administered after surgical excision of the recurrences and survival were recorded for...
all the patients and are presented in Table I. Primary SCCs were located on the face \( (n=7) \), on the limbs \( (n=4) \), and on the penis \( (n=2) \). Recurrences occurred in regional lymph nodes \( (n=11 \) including one located in the parotid gland), or were in transit metastases \( (n=1) \).

Three patients received a treatment prior to surgical excision of the recurrence, respectively radiotherapy in 2 cases (patients 4 and 7) and chemotherapy in one case (patient 4).

Adjuvant treatment, after surgical excision of the recurrences, consisted of radiotherapy delivered to the regional lymph node area \( (n=7) \), a combination of radiotherapy and chemotherapy \( (n=4) \) or chemotherapy alone \( (n=2) \). The follow-up period after surgical excision of the metastatic lesions, ranged from 1 to 72 months \( (\text{mean} = 24 \text{ months}) \). Seven of the patients subsequently died of SCC progression.

**Immunohistochemistry.** A tissue specimen was available for 13 metastatic SCCs of the skin, which had recurred. In addition, pathological material was also available for 2 primary SCC which were also studied (cases 10 and 12).

For the immunohistochemical detection of EGFR on the alcohol formaldehyde and acetic acid (AFA) -fixed, paraffin-embedded material, tissue sections 4-\( \mu \)m thick, were deparaffinized and rehydrated using xylene and graded alcohols. The slides were treated with protease (Protease 1, Ventana, Tucson, AZ). EGFR immunostaining was performed by incubating sections for 32 minutes with a mouse monoclonal antibody \( (1:1000) \) against the external domain of the EGFR (Zymed Laboratories; clone 31G7, South San Francisco, CA, USA) on a Ventana /Nexes automate system (Avidin-biotin/HRPO). The slides were counterstained in Mayer’s hematoxylin and covered by a mounting medium.

For the immunohistochemical detection of HER-2 on the AFA-fixed, paraffin-embedded material, sections \( (4-\mu \text{m}) \) thick, were deparaffinized and rehydrated using xylene and graded alcohols. The sections were treated at 98°C for 30 minutes in a citrate buffer \( (\text{ph} \ 7.3 \ / \ 10 \ \text{mM}) \), then incubated for 20 minutes at room temperature. Endogenous peroxidase activity was blocked with 3 % \( \text{H}_2\text{O}_2 \) for 10 minutes. After a wash with 1 % tween-phosphate-buffered saline (PBST) solution, the sections were incubated for one hour with an antibody diluent solution \( \text{(A0485, Dako Corporation, Carpenteria, California, USA)} \) containing a HER-2 monoclonal antibody \( (1:1000) \) raised against the internal domain of the p185\( ^{c-erbB-2} \) protein \( \text{(NCL- \text{CB11, Novocastra, Newcastle, UK)} \). Anti-mouse HRP-labelled polymer (Dako) was applied for 45 minutes and the slides were washed with tween-PBS solution. The sections were incubated for 10 minutes with DAB and substrate (Dako), washed in water and counterstained with haematoxylin.

On each slide, specimens of normal placenta and of a breast infiltrating carcinoma were used as positive control for EGFR and HER-2. Overlying skin, normal adjacent salivary ducts and skin appendages were used as internal controls for EGFR and HER-2, respectively, in 2 and 4 examined slides.

The proportion of the stained cells was scored as well as the percentage of positively-stained cells from the entire tumour cell population per high-power field in the tissue sections. The intensity of the membrane and cytoplasmic staining was expressed semiquantitatively as values of between + and +++.

**Results**

All metastatic SCCs of the skin had a strong membranous expression of EGFR. (Figure 1 and Table II). EGFR was also expressed in the two primary SCCs which were studied. The primary SCCs and metastatic SCCs had a similar staining pattern.

HER-2 was weakly expressed in only four specimens. Two metastatic SCCs had a membrane expression of HER-2 protein in some well-differentiated keratinocytes (Figure 2). A cytoplasmic expression in 2 cases and a nuclear expression in 1 case were also observed (Figure 3). Among the 4
positive cases, there were 2 well-differentiated cases and 2 poorly-differentiated cases. The 2 primary SCCs and their metastases were similar in the staining pattern. One specimen was not interpretable for HER-2 staining because its internal control was not stained (patient 5). Three of the HER-2-negative tumours had a positive internal control; and on each slide, the control specimen was positive.

In normal skin close to the tumour, the EGFR expression was weak and limited to the basal layer in the epidermis and was also observed in sebaceous and eccrine glands (Figure 4a). HER-2 protein expression was observed only in the upper epidermal layers (Figure 4b). Cells of the sebaceous and eccrine glands stained for HER-2.

**Discussion**

In the present study, EGFR and HER-2 were differently expressed in metastatic SCCs of the skin. Our results demonstrated a massive and constant expression of EGFR and a weak expression of HER-2 in about one-third of the studied specimens only.

In primary SCCs of the skin, the expression of EGFR has been established by radio-labelled ligand binding studies (9, 10) and by immunohistochemical studies (7, 11-13). The intensity of the staining is often weak and comparable with staining in the basal layers of normal epidermis, except in poorly-differentiated SCCs which showed a loss of membrane staining and appearance of a cytoplasmic staining (12). Liu reported that the intensity of EGFR expression had a negative correlation with the degree of SCC differentiation. In the single publication which studied both primary tumours and metastases in five patients with SCCs of the skin, an overexpression of EGFR was found in 4 out of 5 cases; the intensity of the staining was lower in the primary tumours, which were weakly and focally positive (+), than in the metastatic lesions (7). Our results in 13 metastatic SCCs which all strongly expressed EGFR, confirm this previous work. They are also in accordance with the EGFR expression in squamous cell carcinoma of the head and neck (HNSCC), since EGFR was found expressed in 80% to 100% of these tumours (14) and was found overexpressed at various levels (15-17). As EGFR overexpression in HNSCC was associated with poor survival (2), the prognosis significance of EGFR expression in SCC of the skin would be worth studying. In clinical trials, standard treatment of HNSCC has recently been combined with antibodies against EGFR or with small molecule EGFR tyrosine kinase inhibitors (18). These studies have entered phase III clinical trials because of the enhanced anti-tumoural effect of anti-EGFR drugs compared to conventional treatments.

Few data are available about the use of anti-EGFR drugs in SCCs of the skin. In vitro, ZD1839 (Iressa®), an EGFR tyrosine kinase inhibitor, inhibited EGFR activation and blocked invasiveness of human cutaneous SCC cells (19). Stable disease was recently reported for 4 patients with advanced SCC of the skin treated with a pan-erbB tyrosine
Figure 1. Metastatic SCC of the skin: strong membranous staining of keratinocytes with EGFR antibody (original magnification x 200).

Figure 2. Metastatic SCC of the skin: weak membranous staining of keratinocytes with HER-2 antibody; original magnification x 100 (Figure 2a) and 400 (Figure 2b).

Figure 3. Metastatic SCC of the skin: weak cytoplasmic staining of keratinocytes with HER-2 antibody (original magnification x 200).

Figure 4. a. Weak staining of normal adjacent eccrine glands and ducts with EGFR antibody (original magnification x 100). b. Normal skin close to the tumour: weak staining of the upper epidermal layers with HER-2 antibody (original magnification x 100).
kinase inhibitor (20). Such treatments with EGFR-targeted agents might be of interest in metastatic SCCs of the skin.

HER-2 expression was found positive in 14 out of 17 primary tumours and 6 out of 7 metastases of SCC of the skin by Ahmed et al. (8) who observed a high expression with a tendency to more positive cells in metastatic lesions and in poorly-differentiated cells. Staining was observed exclusively in the cytoplasm, with or without membranous accentuation, but the proportion of cases with membranous staining was not detailed. An expression of HER-2 was found by Liu et al. (13) in 22 out of 26 SCC and the intensity of this expression was positively correlated to the degree of SCC differentiation. Our results are not consistent with these data since 2 cases only (17%) had a weak membranous expression of HER-2. Two other cases had a weak cytoplasmic staining. The significance of the cytoplasmic staining of HER-2 is still controversial in breast cancer cells. Some authors did not observe a correlation between the cytoplasmic protein and the mRNA levels (21). Moreover, in HNSCC, HER-2 is not always expressed (22) and the cytoplasmic protein and the mRNA levels (21). Moreover, in HNSCC, HER-2 is not always expressed (22) and the clinical relevance of HER-2 expression is unknown (2, 23).

Co-expression of HER-2 and EGFR was observed in about one-third of our cases. This co-expression of HER-2 and EGFR or other receptors might be a prognostic factor for survival in some SCC (24). In a series of 47 patients with oral SCC, the expression of all EGFR members was significantly associated with shortened patient survival and the combination of HER-2, HER-3 and EGFR, but not HER-4, significantly improved the predictability. However, the number of patients in our series was too small to allow such an analysis. Co-expression of EGFR and HER-2 mRNA, but also of HER-2 and HER-3 and of EGFR, HER-2 and HER-3, was found by conventional reverse transcriptase polymerase chain reaction in a small series of 5 SCCs of the skin, which excludes any definitive conclusion (25). We did not observe by immunohistochemistry the trend to an inverse relationship between the expression of EGFR and HER-2 that was found by Liu et al. (13).

In conclusion, in our study, EGFR was overexpressed in all samples of metastatic SCCs of the skin which confirms the results of the pilot study of Shimizu et al. (7). Therefore, these metastatic tumours appear to be suitable targets for treatment with tyrosine kinase inhibitors. Additional studies are warranted to establish whether or not HER-2 is expressed in SCC of the skin.

References