RESEARCH LETTER

EGFRvIII Expression in Squamous Cell Carcinoma of the Skin

Cutaneous squamous cell carcinoma (SCC) is a common cancer with an estimated 200,000 new cases annually. It is usually readily curable. However, a small subset of SCC tumors (approximately 10%, or 20,000 cases annually), termed “high-risk SCC,” carries an elevated risk of metastasis and death and accounts for nearly all the mortality associated with SCC. Epidermal growth factor receptor (EGFR) is overexpressed in a large percentage of SCC. Several therapeutic trials using EGFR blockade (with agents such as lapatinib, erlotinib, or panitumumab) to treat high-risk or metastatic SCCs are ongoing. Cetuximab, a chimeric IgG1 monoclonal antibody against EGFR, has been considered and used in the treatment of SCC; however, formal trials have not been performed, and good data are lacking. The most common form of mutant EGFR, called EGFRvIII, has been described in several cancers, including head and neck cancer. It has been shown that EGFRvIII contributes to enhanced growth of SCC and resistance to EGFR inhibitor drugs. The aim of this study was the assessment of wild-type EGFR (wtEGFR) and EGFRvIII expression in skin SCC and its correlation with tumor biology.

Methods | Ethical approval was obtained from the institutional review board for processing specimens from clinically indicated excisions. All participants provided their written consent to participate in the research study. A total of 73 SCC samples for the analysis of EGFR messenger RNA (mRNA) expression levels were derived from Mohs surgery. Total RNA was extracted using RNeasy Mini Kit (Qiagen) according to the manufacturer’s protocol. Previously described primers flanking the deletion of exons 2 to 7, which generate bands for both wtEGFR and EGFRvIII, were used for standard reverse transcriptase-polymerase chain reaction. Polymerase chain reaction products were separated and visualized on a 2% agarose gel containing ethidium bromide.

Immunohistochemical staining was performed on tissue microarray composed of 11 normal skin samples and 240 SCC samples with monoclonal anti-wtEGFR IgG rabbit antibody (EP38Y; Abcam) and rabbit monoclonal anti-EGFRvIII (L8A4) antibody. Immunoreactivity was rated as 1 for weakly positive (<25% of the tumor mass stained), 2 for moderately positive (25%-75% of the tumor mass stained), and 3 for strongly positive (>75% of the tumor mass stained). Statistical analysis was performed using GraphPad Prism 5.0 and Microsoft Excel 2000. \( P < .05 \) was considered statistically significant. Differences in the immunoreactivity between the groups were calculated using Mann-Whitney test or analysis of variance.

Results | High Expression of wtEGFR Protein in Skin SCC. The immunohistochemical staining of wtEGFR (Figure 1A–C) showed its high expression in cutaneous SCC as well as in the epidermis of normal skin. There was, however, no detectable difference in the expression between the 2 groups (Figure 1D;

Figure 1. Representative Immunohistochemical Staining of SCC Tumor Samples

Paraffin-embedded tumor samples were stained with mouse anti-epidermal growth factor receptor (EGFR) (31G7) antibodies, which recognize the wild-type EGFR (wtEGFR). Mouse monoclonal IgG1 was used as an isotype control. Immunoreactivity was rated as (A) 1 for weakly positive (<25% of the tumor mass stained), (B) 2 for moderately positive (25%-75% of the tumor mass stained), and (C) 3 for strongly positive (>75% of the tumor mass stained) staining. D, EGFR immunostaining scores of normal skin, in situ, and invasive squamous cell carcinoma (SCC). E, No significant differences in the expression of wtEGFR have been detected when comparing normal skin with SCC or when comparing well, moderately (mod), and poorly differentiated (diff) SCC. Error bars in D and E indicate standard deviations.
Although the expression varied within the group of SCC, no significant differences in the EGFR expression have been detected when compared to well, moderately, and poorly differentiated tumors to each other (Figure 1E; \( P = .09 \)).

**No Detection of EGFRvIII in Either Primary or Metastatic SCC.** None of the examined SCC samples expressed mRNA for EGFRvIII. We detected wtEGFR mRNA in all samples (data not shown). Similarly, in the immunohistochemical analysis of both invasive and in situ SCC, none of the examined samples showed detectable expression of the mutated EGFRvIII protein. The representative SCC staining is shown in Figure 2A-C. Since EGFRvIII has been previously detected in metastatic tumors, the staining was next extended to the metastases of cutaneous SCC; however, none of the metastatic SCC demonstrated EGFRvIII immunoreactivity (Figure 2D-F).

**Discussion** | Mutations in the EGFR gene are commonly found in many types of human tumors, and deletions in the extracellular domain are the most frequent. The most common of these is the type III EGFR deletion mutant (EGFRvIII).\(^5\) It has been shown that EGFRvIII contributes to enhanced growth and resistance in targeting wtEGFR. Since EGFR seems to be an attractive target for the therapy of high-risk SCC, it is of high importance to analyze the presence of the potentially resistant mutant variant (EGFRvIII) in the skin SCC.

In this study of the screening of a large number of samples, EGFRvIII has not been detected. Our data are consistent with those of previous studies performed on smaller numbers of samples. Previous studies showed only very low incidence of EGFR mutations overall.\(^6\) Interestingly, however, even the selected group of particularly high risk SCC (eg, metastatic SCC did not show expression of the mutated variant of EGFR).

The study confirms the high expression of the wtEGFR in the cutaneous SCCs. Similarly to previous findings, we did not find any correlation between EGFR expression level and the histologic differentiation of the tumors. EGFR is a validated therapeutic target in other cancers, and approved drugs targeting EGFR exist. These findings may provide a therapeutic opportunity for a small subset of patients, with advanced SCC refractory to conventional treatment.

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Vemurafenib-Induced DRESS

The BRAF inhibitor vemurafenib was approved by the US Food and Drug Administration in 2011 for the treatment of metastatic melanoma in individuals harboring the somatic BRAF V600E mutation. Cutaneous adverse events from vemurafenib are frequent and include skin eruption, pruritus, photosensitivity, hyperkeratosis, squamous cell carcinoma, and keratoacanthomas.1–3 We report herein a case of vemurafenib-induced drug reaction with eosinophilia and systemic symptoms (DRESS).

Report of a Case | A woman in her 80s with hypertension and chronic kidney disease presented with a 1-year history of an enlarging violaceous growth on her calf. A biopsy revealed nodular melanoma with a Breslow depth of at least 5.6 mm. Positron emission tomography/computed tomography identified 2 suspect lymph nodes in the groin, and fine-needle aspiration confirmed metastatic melanoma harboring the V600E BRAF mutation. The patient began treatment with vemurafenib, 960 mg, twice daily. Three weeks later, she developed scattered pustules and generalized pink, pruritic papules coalescing into plaques on the face, trunk, and extremities (Figure) with prominent facial edema. There was no mucosal involvement, and lymph node examination revealed stable inguinal lymphadenopathy.

The patient complained of fevers, chills, and bone pain but was afebrile on admission and throughout her hospitalization. However, 3 days earlier, a low-grade fever of 37.3°C was documented in her outpatient record, and she was advised to start treatment with antipyretic drugs. Laboratory workup showed a white blood cell count of 12 400/μL, with 26% eosinophils and 1% atypical lymphocytes; transaminitis (aspartate transaminase level, 107 U/L; alanine transaminase level, 132 U/L); and a creatinine concentration of 3.3 mg/dL (baseline, 1.8 mg/dL). (To convert white blood cells to ×109/L, multiply by 0.001; creatinine to micromoles per liter, multiply by 88.4.) A drug eruption was suspected, and after review of the patient’s medications (metoprolol and hydrochlorothiazide, which she had been taking for at least 9 months), vemurafenib treatment was discontinued.

Skin biopsy findings supported a diagnosis of DRESS, with specimens displaying a mild to moderate dermal lymphocytic infiltrate, erythrocyte extravasation, occasional eosinophils, and scattered necrotic keratinocytes. The patient was treated with intravenous methylprednisolone followed by oral prednisone. Over the subsequent 6 weeks she experienced desquamation and resolution of her skin eruption and laboratory abnormalities. During this time, the melanoma of the skin and the ipsilateral lymphadenopathy continued to decrease in size.

Discussion | DRESS, a hypersensitivity drug reaction with systemic symptoms, is most commonly associated with the use of aromatic anticonvulsants, but it has also been reported with a number of other medications.4 Although the pathogenesis of this condition is incompletely understood, a defect in the detoxification of certain drugs is thought to play a role in its development, producing toxic metabolites that cause cellular injury or trigger an immune response.