Low incidence of EGFR and HRAS mutations in cutaneous squamous cell carcinomas of a German cohort

Andreas Mauerer1, Eva Herschberger1, Wolfgang Dietmaier2, Michael Landthaler1 and Christian Hafner1

1Department of Dermatology, University of Regensburg, Franz-Josef Strauss-Allee, Regensburg; 2Molecular Pathology Diagnostic Unit, Institute of Pathology, University of Regensburg, Franz-Josef Strauss-Allee, Regensburg, Germany

Correspondence: Christian Hafner, MD, Department of Dermatology, University of Regensburg, Franz-Josef-Strauss-Allee 11, D-93053 Regensburg, Germany, Tel.: +49 9419449606, Fax: +49 9419449544, e-mail: christian.hafner@klinik.uni-regensburg.de

Abstract: Epidermal growth factor receptor (EGFR) is highly expressed in squamous cell carcinoma (SCC). The response of patients with lung cancer to EGFR inhibitors is significantly associated with the presence of EGFR mutations. Although these drugs have already been used for the treatment of advanced cutaneous SCC, the knowledge about EGFR mutations in this cancer is limited to one previous study in the US population. We analysed the presence of EGFR and concomitant HRAS mutations in a German cohort of 31 patients with cutaneous SCC by direct sequencing of EGFR and SNaPshot analysis of concomitant RAS mutations. We found a low prevalence of EGFR mutations (1/31; 3%) and HRAS mutations (1/31; 3%). The detected P741L EGFR mutation was proven to be somatic. Our results indicate that both EGFR and HRAS mutations are rare events in the carcinogenesis of cutaneous SCC, and therefore, only a small subgroup of patients will benefit from the screening for EGFR mutations in the run-up to targeted therapies with EGFR inhibitors.

Key words: cutaneous squamous cell carcinoma – EGFR – HRAS – skin – somatic mutation

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Background

The incidence of cutaneous squamous cell carcinoma (SCC) of the skin in the Caucasian population is increasing. Risk factors for the development of SCC comprise chronic UV light exposure, fair skin, immunosuppression, HPV infection, chronic wounds, scarring dermatoses and various genodermatoses (1). Several molecular genetic alterations have been identified in the pathogenesis of cutaneous SCC, including chromosomal aberrations, mutations in the p53 tumor suppressor gene and oncogenic HRAS mutations (2). In addition, cutaneous SCC express the epidermal growth factor receptor (EGFR), a transmembrane type I receptor tyrosine kinase (3). Overexpression of EGFR was recently described in 25 of 32 cutaneous SCC, and EGFR amplification was observed in seven of 35 SCC (4). Impaired EGFR signalling showed decreased incidence of papillomas in a multistage skin chemical carcinogenesis mouse model (5). Previous case reports suggested that inhibition of activated EGFR by antibodies or small molecule inhibitors might be a therapeutic option in advanced cutaneous SCC (6–8). Activation of the EGFR pathway may be caused by both gene amplification and activating somatic mutations (9). The presence of EGFR mutations is of relevance for the efficiency of EGFR-based molecular targeted therapies in lung and colon cancer (10–12). EGFR mutations are also strong predictors of a better outcome with gefitinib, an EGFR tyrosine kinase inhibitor, among patients with non-small-cell lung cancer in East Asia (13).

Questions addressed

The aim of our study was to investigate the presence of oncogenic EGFR mutations and concomitant RAS mutations in a cohort of German patients with cutaneous SCC.

Experimental design

Sample and DNA acquisition

Thirty-one formalin-fixed paraffin-embedded cutaneous SCC (19 from men and 12 from women) were retrieved from the histological files of the Department of Dermatology, University of Regensburg. Written informed consent has been obtained from the patients, and the study was performed according to the guidelines of the local Ethics Committee of the University Clinic of Regensburg and the declaration of Helsinki. All material at first underwent routine histopathological diagnosis, and only material not needed for further diagnostic procedures was used for this study. DNA was isolated after manual microdissection following standard protocols as described previously (14).

Mutational analysis of EGFR and RAS genes

Possible EGFR mutations were studied by sequence analysis as described earlier (15). In brief, we used a nested PCR approach. The first multiplex PCR contained all forward and reverse primers for exons 18 (fwd: GCATGGTGAGGGCTGAGGTGA; rev: CCCCTCAGCAGCTGACACG), 19 (fwd: TGCCAGTTCAGCTCGCTTCTTCT; rev: CCACACAGCAAGAGCACAAAC) and 21 (fwd: AGCTTCTCCCATGATGATCTGTCC; rev: GGCAGCCCTGTCCTGGTGTCT) of EGFR. For the second multiplex PCR (exon 18 fwd: ACCTTGCTCTGTGTTCTTCTTCTGCTCC; rev: GCCCAGGCCAGGCTCGTG; exon 19 fwd: AAAGCTCTCTCATCTGTCTCTCTCTGCT; rev: CCAACACGCAGCAGAACAG; and exon 21 fwd: TCTCTGCGGATCTTCACACA; rev: CAGGAAAATGCTGGTCAGCCTACAAAG), 1 μl from the first multiplex PCR was used as a template. Sequencing was performed in forward and reverse directions for each exon. Hot spot mutations of HRAS, KRAS and NRAS genes were screened using a highly sensitive SNaPshot® multiplex assay (Applied Biosystems, Carlsbad, CA, USA) as described earlier (16). In brief, two exons containing the codons 12, 13 and 61, which represent hot spot mutation loci of RAS genes in human cancer, were amplified in a multiplex PCR. The hot spot loci were then analysed by mutation-specific SNaPshot® probes, followed by the separation of SNaPshot® products on the basis of size by gel electrophoresis. Possible mutations were confirmed by a second independent PCR.
Results

An *EGFR* mutation was found only in 1/31 (3%) SCC (Table 1). This c.2222C>T transition of exon 19 resulted in a p.P741L substitution. Analysis of normal skin adjacent to this SCC showed a wild-type *EGFR* sequence (Fig. 1), confirming the somatic nature of this mutation and excluding a germline mutation. Interestingly, the patient with the *EGFR* mutation is an organ transplant recipient. The P741L mutation has not yet been described for SCC, but 2/81 glioblastomas previously showed this missense mutation (17).

Analysis of *RAS* genes showed a c.182A>T transversion in 1/31 (3%) SCC, resulting in a p.Q61L substitution. According to the COSMIC database (http://www.sanger.ac.uk/genetics/CGP/cosmic/), the Q61L mutation has been described in 67 tumors, most frequently in the skin (two SCC, one basal cell carcinoma, three malignant melanomas, 10 Spitz nevi and nine keratoacanthomas), the urinary tract and prostate cancer. No further mutations of *HRAS*, *KRAS* and *NRAS* genes were found at the investigated loci (Table 1).

Table 1. Results of *EGFR* and *HRAS* mutation analyses in cutaneous SCC

| No. | Sex | Age | IS | Thickness (mm) | EGFR  | HRAS  | KRAS  | NRAS  |
|-----|-----|-----|----|---------------|-------|-------|-------|-------|
| 1   | F   | 93  | No | 4.5           | wt    | wt    | wt    | wt    |
| 2   | F   | 67  | Yes| 5.1           | wt    | wt    | wt    | wt    |
| 3   | F   | 86  | No | 2.4           | wt    | wt    | wt    | wt    |
| 4   | M   | 81  | Unknown | 3.0 | wt    | wt    | wt    | wt    |
| 5   | F   | 87  | Unknown | 6.0 | wt    | wt    | wt    | wt    |
| 6   | M   | 74  | No | 2.3           | wt    | wt    | wt    | wt    |
| 7   | F   | 68  | No | 4.0           | wt    | na    | na    | na    |
| 8   | M   | 74  | Unknown | 2.1 | wt    | wt    | wt    | wt    |
| 9   | M   | 78  | No | 2.8           | wt    | wt    | wt    | wt    |
| 10  | M   | 83  | Unknown | 2.5 | wt    | wt    | wt    | wt    |
| 11  | M   | 71  | No | 3.0           | wt    | wt    | wt    | wt    |
| 12  | M   | 77  | No | 3.5           | wt    | wt    | wt    | wt    |
| 13  | M   | 77  | No | 7.0           | wt    | wt    | wt    | wt    |
| 14  | F   | 87  | Unknown | 4.0 | wt    | wt    | wt    | wt    |
| 15  | M   | 73  | Unknown | 2.3 | wt    | wt    | wt    | wt    |
| 16  | M   | 80  | Unknown | 2.0 | wt    | wt    | wt    | wt    |
| 17  | M   | 69  | Unknown | 2.0 | wt    | wt    | wt    | wt    |
| 18  | F   | 92  | Unknown | 3.0 | wt    | wt    | wt    | wt    |
| 19  | M   | 82  | No | 1.2           | wt    | p.Q61L| wt    | wt    |
| 20  | M   | 75  | No | 5.0           | wt    | wt    | wt    | wt    |
| 21  | M   | 64  | Yes | 4.0           | p.P741L| wt     | wt    | wt    |
| 22  | F   | 81  | Unknown | 2.4 | wt    | wt    | wt    | wt    |
| 23  | F   | 87  | No | 7.5           | wt    | wt    | wt    | wt    |
| 24  | F   | 44  | Yes | 3.0           | wt    | wt    | wt    | wt    |
| 25  | M   | 95  | No | 2.3           | wt    | wt    | wt    | wt    |
| 26  | M   | 74  | No | 6.0           | wt    | wt    | wt    | wt    |
| 27  | F   | 80  | Unknown | 2.0 | wt    | wt    | wt    | wt    |
| 28  | M   | 92  | No | 5.2           | wt    | wt    | wt    | wt    |
| 29  | F   | 86  | No | 7.0           | wt    | wt    | wt    | wt    |
| 30  | M   | 65  | No | 2.4           | wt    | wt    | wt    | wt    |
| 31  | M   | 72  | No | 3.5           | wt    | wt    | wt    | wt    |

wt, wild type; na, not available; IS, immunosuppression; *EGFR*, Epidermal growth factor receptor; SCC, squamous cell carcinoma.

 getSupportFragmentManager()
Letter to the Editor

Author contributions
Andreas Mauzer analysed the data and prepared the manuscript, Eva Hirschberger performed the mutation analyses, Wolfgang Dietmaier analysed the EGFR data, Michael Landthaler discussed the data and contributed to the preparation of the manuscript and Christian Hafner designed the study, analysed the data and contributed to the manuscript preparation.

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Conflict of interest
The authors declare no conflict of interest.

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Ex vivo demonstration of a synergistic effect of Adapalene and benzoyl peroxide on inflammatory acne lesions

Thomas Zuliani¹, Amir Khammari¹,², Hélène Chaussy², Anne Chantal Knol¹ and Brigitte Dréno¹,²

¹Laboratory of Immuno-Dermatology, CIC biotherpay INSERM 0503, University Hospital, Nantes, France; ²Unit of Dermato-Cancerology, CHU de Nantes, CIC biotherpay INSERM 0503, University Hospital, Nantes, France

Correspondence: Prof Brigitte Dréno, Department of dermatology, CHU, Place A. Ricordeau, 44095 Nantes, France, Tel.: +33-24-00-83-118, Fax: +33-24-00-83-117, e-mail: brigitte.dreno@wanadoo.fr

Abstract: Acne is a chronic inflammatory disease of the pilosebaceous follicle. Thanks to its ability to reduce both comedones and inflammatory lesions, the association of a retinoid and benzoyl peroxide (BPO) is now recommended for the treatment of acne. However, the mechanisms of action of this combined therapy on inflammatory acne lesions are not well understood. In an ex vivo immunohistochemistry study, we investigated the potential synergistic modulator effect of Adapalene associated with BPO on keratinocytes proliferation/differentiation and innate immunity in inflammatory acne lesions. We demonstrated that proliferation (Ki-67), adhesion/differentiation (integrin α2, α5 and ζ4) and innate immunity (TLR-2, β-defensin 4, IL-8) markers are overexpressed in inflammatory acne skin compared with uninvolved acne skin. Association of Adapalene and BPO significantly decreased expression of Ki67, α2 and ζ4 integrins, TLR-2, β-defensin 4 and IL-8 in inflammatory acne skin, whereas single treatments with Adapalene or BPO alone were less effective. These results contribute to explain the comedolytic and anti-inflammatory activities of this combined therapy observed in recent clinical trials.

Key words: acne – Adapalene – benzoyl peroxide – epidermal proliferation/differentiation – innate immunity

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Background
Acne is a chronic multifactorial disease of the pilosebaceous follicle involving seborrhea, abnormal follicular differentiation, microbial proliferation and inflammation (1,2).

International guidelines recommend the combination of retinoids and benzoyl peroxide (BPO) for treating acne because of their complementary mechanisms of action (3). Therapeutic activity of retinoids relies mainly on their lytic action on the