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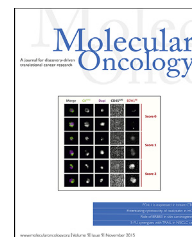

ERBB3 is required for tumor promotion in a mouse model of skin carcinogenesis

Maik Dahlhoff[a], *Matthias Schäfer*[b], *Sukalp Muzumdar*[b], *Christian Rose*[c], *Marlon R. Schneider*[a,*]

[a]*Institute of Molecular Animal Breeding and Biotechnology, LMU Munich, Munich, Germany*
[b]*Department of Biology, Institute of Molecular Health Sciences, ETH Zurich, Zurich, Switzerland*
[c]*Department of Dermatology, University Hospital Schleswig-Holstein, University of Lübeck, Lübeck, Germany*

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ABSTRACT

The epidermal growth factor receptor (EGFR) plays a key role in skin inflammation, wound healing, and carcinogenesis. Less is known about the functions of the structurally related receptor ERBB3 (HER3) in the skin. We assessed the requirement of ERBB3 for skin homeostasis, wound healing, and tumorigenesis by crossing mice carrying a conditional *Erbb3* allele with animals expressing cre under the control of the keratin 5 promoter. Erbb3^{del} mice, lacking ERBB3 specifically in keratinocytes, showed no obvious abnormalities. The EGFR was upregulated in *Erbb3^{del}* skin, possibly compensating the loss of ERBB3. Nonetheless, healing of full-thickness excisional wounds was negatively affected by ERBB3 deficiency. To analyze the function of ERBB3 during tumorigenesis, we employed the established DMBA/TPA multi-stage chemical carcinogenesis protocol. *Erbb3^{del}* mice remained free of papillomas for a longer time and had significantly reduced tumor burden compared to control littermates. Tumor cell proliferation was considerably reduced in Erbb3^{del} mice, and loss of ERBB3 also impaired keratinocyte proliferation after a single application of TPA. In human skin tumor samples, upregulated ERBB3 expression was observed in squamous cell carcinoma, condyloma, and malignant melanoma. Thus, we conclude that ERBB3, while dispensable for the development and the homeostasis of the epidermis and its appendages, is required for proper wound healing and for the progression of skin tumors during multi-stage chemical carcinogenesis in mice. ERBB3 may also be important for human skin cancer progression. The latter effects most probably reflect a key role for ERBB3 in increasing cell proliferation after stimuli as wounding or carcinogenesis.

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1. Introduction

Nonmelanoma skin cancer (NMSC), whose major subtypes are basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), shows a worldwide increasing incidence (Lomas et al., 2012). BCC is actually the most common human cancer and represents 2/3 of all skin cancers in patients of mixed European descent (Kasper et al., 2012). The epidermal

* *Corresponding author*. Institute of Molecular Animal Breeding and Biotechnology, Gene Center, LMU Munich, Feodor-Lynen-Str. 25, 81377 Munich, Germany.
E-mail address: marlon.schneider@lmu.de (M.R. Schneider).

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growth factor receptor family of tyrosine kinases (EGFR, also known as ERBB1/HER1, ERBB2/HER2/neu, ERBB3/HER3, and ERBB4/HER4) plays a major role in the regulation of keratinocyte proliferation and differentiation, with implications for wound healing, skin inflammation and carcinogenesis (Schneider et al., 2008). These receptors, in particular EGFR and ERBB2, are also the target of several therapeutic agents used clinically to treat a number of cancer types (Yarden and Pines, 2012). In this study, we provide evidence that ERBB3 may be a promising target against NMSC.

Unlike other ERBB receptors, ERBB3 lacks intrinsic catalytic activity due to changes in the kinase domain, and can therefore not auto-phosphorylate (Guy et al., 1994). Phosphorylation of ERBB3, which is indispensable for development, occurs by other family members, most commonly by ERBB2 (Yarden and Sliwkowski, 2001). The potential of ERBB3 as a target for cancer therapy, long clouded by its defective kinase activity, recently turned the object of intense investigation. Overexpression of ERBB3 has been detected in a variety of cancers, including those of the breast, lung, colon, ovary, and pancreas (Amin et al., 2010). More recent studies suggested that targeting ERBB3 may be of therapeutic value in colorectal (Lee et al., 2009) and luminal breast cancers (Morrison et al., 2013). Accordingly, considerable efforts have been put in the development of compounds that target this receptor either singly or in combination with other targets (Aurisicchio et al., 2012; Ma et al., 2014; Gaborit et al., 2015).

In the skin, ERBB3 was shown to be expressed in all epidermal layers, with highest levels in the suprabasal and spinous layers (Kiguchi et al., 2000; Piepkorn et al., 2003). However, apart from its potential involvement in wound repair (Okwueze et al., 2006; Forsberg and Rollman, 2010), little is known about its function in the skin. Here, we employed tissue-specific gene deletion to assess the role of ERBB3 in skin homeostasis, wound healing, and tumorigenesis.

2. Materials and methods

2.1. Mice

Mice carrying floxed *Erbb3* alleles (Lee et al., 2009) were obtained from the Mutant Mouse Regional Resource Center (MMRC, University of North Carolina). Mice expressing cre under the keratin 5 promoter (Ramirez et al., 2004) were a courtesy of A. Ramirez and J. Jorcano (CIEMAT, Madrid, Spain). Both mouse strains were maintained in the C57BL/6 background. Animals were maintained under specific pathogen-free conditions and had access to water and standard rodent diet (V1534, Ssniff, Soest, Germany) *ad libitum*. All experiments were approved by the Committee on Animal Health and Care of the state of Upper Bavaria (Regierung von Oberbayern, Germany) or by the local veterinary authorities of Zurich (Switzerland). Genotyping of mouse lines and detection of the floxed and deleted *Erbb3* alleles were performed according to the original publications.

2.2. Chemical skin carcinogenesis

Chemical carcinogenesis was carried out according to internationally accepted standards (Abel et al., 2009). Seven-week-old *Erbb3*^{del} females and control littermates were shaved on their backs and received a single application (400 nmol) of the initiating agent 7,12-dimethylbenz(a)anthracene (DMBA, Sigma–Aldrich, Germany). This was followed by two weekly applications (10 nmol each) of the promoting agent 12-O-tetradecanoylphorbol-13-acetate (TPA, Sigma–Aldrich) for 22 weeks, after which mice were euthanized. Tumor development was assessed weekly. After euthanasia, tumors and skin samples were fixed in 4% paraformaldehyde (Sigma–Aldrich), dehydrated, and embedded in paraffin or snap-frozen and stored at −80 °C. The following PCR primers were used for detecting *Hras* mutation: 5′-CAGGAGCTCCTG-GATTGGC-3′; 5′-GGTGGATATGAGCCAGCTAGC-3′; procedures have been described previously (Dahlhoff et al., 2012).

For TPA-induced hyperproliferation, shaved back skin was treated once with 10 nmol TPA or ethanol only (vehicle) and mice were sacrificed 24 h later. Epidermis thickness was measured on H&E-stained skin sections over a length of 4.33 mm on 125 single points for each animal. For proliferation, both Ki67-positive and negative nuclei were counted on pictures taken with a 200 magnification lens and a Leica DFC425C digital camera (Leica Microsystems GmbH, Wetzlar, Germany) covering a length of 4.33 mm.

2.3. Wound healing

Experimental wounding was performed as following: 8–10 week-old female mice were anaesthetized by intraperitoneal injection of ketamine/xylazine and the back was shaved. Subsequently, two anterior and two posterior excision wounds (left and right from the midline), 5 mm in diameter were generated on the back skin using a circular blade. 5 days after wounding, mice were euthanized by carbon dioxide inhalation, wounds were excised, fixed in 4% paraformaldehyde in PBS, and embedded in paraffin. Subsequently, 7 μm sections were cut from the middle of the wounds, stained with H&E, photographed and wound morphometry was analyzed. The boundary between the dermis and granulation tissue was assigned as the wound edge. Length and area of the thickened epithelium outside the wound (defined as outer thickened epidermis; OTE) and of the wound epithelium (WE) was measured. Wound diameter was defined as the distance between the left and right wound edges. Wound closure was defined as the ratio of the sum of the lengths of the left and right wound epithelium to the wound diameter. Proliferation was analyzed in 1 anterior and 1 posterior wound each of 4 control and 4 *Erbb3*^{del} mice by performing immunofluorescence on paraffin sections using an anti-PCNA primary antibody. Quantification was performed by counting the number of PCNA-positive nuclei per length of outer thickened epithelium, and wound epithelium separately.

2.4. Immunohistochemistry

After euthanasia, skin samples were fixed in 4% paraformaldehyde, dehydrated, and embedded in paraffin. For target

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