HER3 Expression in Cutaneous Tumors

EVA WIMMER[1*], GERTRAUD KRAEHN-SENFTLEBEN[2*] and WOLFGANG J. ISSING[3]

[1]*Department of Otorhinolaryngology, Head and Neck Surgery, Ludwig-Maximilians-University, Munich;*
[2]*Department of Dermatology, Ulmer Strasse 26, 89143 Blaubeuren, Germany;*
[3]*Department of Otorhinolaryngology, The Freeman Hospital, Newcastle-upon-Tyne NE7 7DN, United Kingdom*

Abstract. *Background: In order to elucidate the role of the receptor tyrosine kinase HER3, the expression characteristics in different tissues of cutaneous malignancies and in normal skin were compared. Materials and Methods: In this study HER3 expression was evaluated by RT-PCR analysis and immunohistochemistry from different tissue specimens of cutaneous tumors like nevi, primary malignant melanomas, basal cell carcinoma, squamous cell carcinoma and malignant melanoma metastases and normal skin samples and graded into weak, moderate and strong expression. Associations of tumor thickness in these specimens with HER3 expressions were also analyzed. Results: HER3 expression was found in 63% (10/16) of the basal cell carcinomas, in 4/5 of squamous cell carcinomas and in one Merkel cell carcinoma. Within the group of different malignant melanomas, HER3 expression was detected in 35% of the nodular malignant melanomas (6/17) and in 9/19 of the superficial spreading melanomas, including 2 lentigo malignant melanomas. The majority of melanomas with a higher tumor thickness expressed HER3, and 85% of melanoma metastasis were HER3-positive. Conclusion: HER3 expression was associated with hyperproliferate tumor stages and suggested that HER3 expression could reflect an increased malignant potential in cutaneous lesions.*

Due to the increasing incidence rates (1) of melanomas, basal cell carcinomas and squamous cell carcinomas and the potentially lethal outcome of these tumors, cutaneous neoplasias have attracted growing interest regarding the molecular mechanisms leading to malignant transformation of normal epidermis (3). In recent years, a variety of proto-oncogenes, oncogenes themselves and tumor suppressor genes have been studied in order to elucidate pathogenetic pathways leading to malignancies (4).

The type I receptor tyrosine kinases have been implicated in the development and progression of multiple human malignancies (5-7). This family of receptor tyrosine kinases consists of the epidermal growth factor receptor (EGFR) (8), HER2 (9, 10), HER3 (11, 12) and HER4 (13, 14). The sequence identity between these polypeptides ranges between 40-60% in their extracellular domains and 60-80% in their intracellular domains (15, 16).

EGFR was shown to cause or contribute to malignant cell transformation in gastrointestinal, urinary (17) and reproductive tract malignancies, brain tumors, squamous cell carcinomas (18) and melanomas (4, 19). HER2 overexpression caused by gene amplification and/or transcriptional upregulation occurs in human breast and ovarian carcinomas, and seems to be correlated with reduced patient survival (7-11, 20). Equal amounts of HER2 mRNA transcripts were found in benign nevi and melanoma, with even higher expression levels in normal epidermis (21), suggesting a function in proliferation and differentiation of normal epidermal cells. The presence of HER2 protein was detected in 12/30 primary melanomas, 8/10 already metastasized melanomas and 6/15 breast carcinomas (20).

Human HER3 cDNA was sequenced, cloned and mapped to the chromosomal locus 12q13 (11). Elevated mRNA levels and constitutive tyrosine phosphorylation were first described in human mammary carcinoma cell lines (11). In adult tissue the 6.2 kb mRNA was detected in human placenta, skin, stomach, lung, kidney and brain, but not in skin fibroblasts, skeletal muscle or lymphoid cells. Protein expression was found in gastrointestinal, urinary, reproductive, and respiratory tracts, as well as in endocrine tissue and the nervous system. Moreover, HER3 protein is detectable in human skin (12, 20, 21). In malignant tissue HER3 protein was detected in 13% to 29% of breast carcinomas (22, 23). HER3 overexpression has been observed in a range of malignant tumors, such as pancreatic and gastrointestinal tumors (7), lung (24), bladder (25), cervical, and prostate cancer (17). Moderate expression

*Both authors contributed equally to this study.

Correspondence to: Eva Wimmer, MD, Department of Otorhinolaryngology, Head and Neck Surgery, Ludwig-Maximilians-University, Klinikum Grosshadern, Marchioninistrasse 15, 81377 Muenchen, Germany. Tel: +49 89 70953892, e-mail: ehilgert@gmx.net

Key Words: HER3, cutaneous tumors, squamous cell carcinoma, nevus, PCR.

of the *erbB3* transcript was detected by northern blotting analysis in cell lines derived from two squamous cell carcinomas of the tongue and one pharyngeal squamous cell carcinoma (26-28). However, PCR analysis of 59 tissue specimens of squamous cell carcinomas of the head and neck detected amplification neither of HER2 nor of HER3 (27). In oral squamous cell carcinomas, immunohistochemically detected overexpression of HER3 protein was associated with an elevated malignant potential (28).

In human skin, HER3 expression has to date only been studied immunohistochemically (29). Positive reactions using the RTJ1 antibody were found in 60% of nevi and 25% of melanomas (30). In a further immunohistochemical-based study, HER3 expression was identified in 14/30 primary melanomas, 7/10 metastasized melanomas and 6/15 breast carcinomas (20). A specific role of HER3 expression during progression of cutaneous malignancies therefore still remains to be elucidated.

The aim of this study was to determine the differential expression of HER3 *via* RT-PCR and by immunostaining in basal cell carcinomas, squamous cell carcinomas, primary melanomas and melanoma metastases in comparison to normal skin and benign nevi.

Materials and Methods

Samples. Shock-frozen tissue specimens taken from 60 primary malignant melanomas and melanoma metastases (17 nodular malignant melanomas, 19 superficial spreading melanomas, 2 lentigo maligna melanomas, 2 melanomas on nevus, 2 recurrent locoregional melanoma metastases and 18 distant cutaneous and lymph node metastases) were studied by conventional RT-PCR. The tumor thicknesses of primary carcinomas ranged from 0.2 to 12.1 mm. Patients with a family history of hereditary malignant melanoma were excluded. Histologically, one Merkel cell carcinoma, 16 basal cell carcinomas and 5 cutaneous squamous cell carcinomas were found, among them a nine-year-old girl with xeroderma pigmentosum. RNA was isolated from 11 samples of normal skin, one seborrhoic keratosis and 22 melanocytic nevi and analyzed by RT-PCR. Immunohistochemistry and examination of the proliferation markers PCNA (proliferating cell nuclear antigen) and Ki-67 was carried out on 4 Merkel cell carcinomas, 13 nevi, 10 primary melanomas and 29 melanoma metastases.

RNA isolation and RT-PCR analysis. Tissue specimens were shock frozen in liquid nitrogen immediately after surgical excision. Tissue was minced; total mRNA was isolated from homogenates using the RNA-Clean™ System (Angewandte Gentechnologie Systeme GmbH, Germany) according to the manufacturer's instructions.

RNA transcription was performed immediately by and adding 1 μl Oligo and 1 μl dNTP to 10 μl of each cell sample and starting the cDNA transcription program (Promega, Madison, USA). Afterwards, each cDNA was phenol extracted, precipitated by ethanol and redissolved in aqua bidest. The cDNA concentration was evaluated by absorbance at 260 nm. In subsequent 25 μl polymerase chain reactions, 100 ng cDNA template extracted as above were used to investigate the HER3 gene expression applying the following primers: HER3 sense: 5'-CUA CUA CUA CUA GAG GCT GAG CTC TAG GAG AA-3'; antisense: 5'-CAU CAU CAU CAU CTG GGA CCT GGG AGA GAG AG-3'; 813 bp. The PCR mixtures contained 100 ng of template cDNA, 1.5 mM $MgCl_2$, 1 μM primer (sense and antisense), 0.2 mM dNTPs (Promega) and 0.2 μl TaqPolymerase (5U/μl, Boehringer Mannheim, Germany). Cycling parameters were: an initial denaturation step (95˚C, 5 min), followed by 94˚C 1 min; 52˚C 1 min, 72˚C 1.5 min for 35 cycles and one final extension step (72˚C, 2 min). Controls without DNA as well as GAPDH primers (sense: 5'-CCA CCC ATG GCA AAT TCC ATG GCA-3'; antisense: 5'-TCT AGA CGG CAG GTC AGG TCC ACC-3'; 598 bp) were performed for each PCR set. Cycling parameters for GAPDH: an initial heating step (95˚C, 5 min), followed by 94˚C 1 min, 60˚C 1 min, 72˚C 1.5 min for 29 cycles and one final extension step (72˚C, 2 min). The DNA molecular weight standard (PhiX 174RF/HaeIII) was purchased from Boehringer Mannheim. As a positive control, cDNA known to express HER3 and GAPDH was amplified; water instead of cDNA was applied as a negative control template.

Identical aliqots (5 μl) of PCR products were loaded onto 2% agarose gels and visualized by ethidium bromide staining. The optical density of GAPDH and HER3 bands were analysed by a computerized densitometric analysis device (Image master VDS, Pharmacia, Freiburg, Germany).

PCR was performed in a GeneAmp 9700 thermocycler (Applied Biosystems, Weiterstadt, Germany). The final PCR mixture contained 10 ng of targeted cDNA, 2.5 mM MgCl2, 10 mM TRIS, 50 mM KCL, 0.5 μM of each primer, 200 μM each of dNTPs and 25 mU/μl Taq Polymerase. The downstream-primer was 5'-labeled with digoxigenin. Each PCR tube contained the same amount of internal controls (=mimics), consisting of the identical primer sequences as the target but of a different intermediate sequence. PCR consisted of an initial heating step (95˚C, 5 min), 40 amplification cycles for HER3 (94˚C 1 min, 72˚C 1.5 min for 35 cycles and one final extension step (72˚C, 2 min).

DNA-mimics were constructed using the PCR MIMIC Construction Kit (Clontech, Palo Alto, CA, USA) following the manufacturer's protocol. These mimic fragments were stabilized by cloning them into a plasmid using the T/A Cloning Kit (Invitrogen, San Diego, CA, USA). Plasmid DNA was extracted using the High Pure Plasmid Isolation Kit (Boehringer, Mannheim) and quantified by reading the optical density (OD) at 260 nm. The molar concentration was calculated and the mimics were appropriately diluted.

Quantification by PCR ELISA. This assay was performed using a commercially available system (PCR ELISA, Boehringer). In brief, a part of the respective PCR product was denatured with the same amount of an alkaline solution for 10 min at room temperature. Five-fold dilutions of the denatured PCR product of each sample were hybridized with 1.5 pmol of a 5'-biotinylated HER3-specific oligonucleotide probe in rows A to D of a streptavidin-coated microtiter plate, corresponding to 10.0 μl, 2.0 μl, 0.4 μl and 0.08 μl of denatured PCR product. Rows E to H contained 10.0 μl, 2.0 μl, 0.4 μl and 0.08 μl of denatured PCR product hybridized with the mimic-specific biotinylated oligonucleotide probe. The total reaction volume was 200 μl. The plate was incubated at 55˚C for one hour, washed five times, incubated with 200 μl of a peroxidase-conjugated anti-digoxigenin antibody at 37˚C for 30 min, washed again and incubated with 200 μl of the substrate for peroxidase, 3,3'-5,5'-tetramethylbenzidine (TMB) at 37˚C for 15 min. The

reaction was stopped by adding 75 μl of 2M HCl, and the optical density was measured at a wavelength of 450 nm. By subtraction of the OD of the negative control net ODs were calculated.

Quantification was performed using the principle of a commercially available system (HIV Monitor; Roche Diagnostics Systems, Branchburg, NJ, USA). For each sample, the lowest net OD for mimic and target over 0.100 was chosen for calculating the target-copy number using the formula:

target-copies = (target-net OD x dilution factor): (mimic-net OD x dilution factor) x input copy number of mimic.

Detection of PCR-products. First the tissue samples were screened by internally controlled PCR-ELISA for the presence of GAPDH cDNA as a housekeeping gene and for HER3 cDNA. Subsequently, 10 μl of the target and mimic product were denatured with 20 μl denaturation solution, and the PCR ELISA was continued as described above. Fragment lengths were 598 bp for GAPDH and 604 bp for the GAPDH mimic, 813 bp for HER3 and 596 bp for the HER3 mimic.

In samples positive for HER3 cDNA, copy numbers of GAPDH and HER3 were quantified as described above. To correct for different amounts of starting mRNA and for differing reverse-transcription, efficiency copy numbers for HER3 were expressed in relation to GAPDH copies. A HER3/GAPDH copy ratio >0 as weak overexpression, a HER3/GAPDH ratio <1 as moderate overexpression but a ratio ≥1 was regarded as strong overexpression.

Immunohistochemistry. Specimens were cut into 4 μm sections formalin fixed and paraffin embedded. All tissue samples were stained with hematoxylin/eosin and dermatohistopathology was performed by two independent dermatopathologists.

Slides were deparaffinized in xylene and ethanol followed by antigen retrieval using the citric acid process. Slides were then stained with the monoclonal antibodies directed at HER3, PCNA and Ki-67 (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The ImmunoCruz™ staining system kit was used to complete staining. Protein products were visualized with HRP-streptavidin complex binding to biotinylated secondary antibody, using goat anti-rabbit secondary antibodies. After optimal color development, sections were counterstained with Mayer's hematoxylin and coverslipped. As negative controls, tissue sections were treated identically omitting the addition of the primary antibody. Interpretation of the antibody staining was performed independently by two dermatopathologists without knowledge of the patient's background or diagnosis. Antibody staining was graded as negative (0), weak (w), moderate (+), or strong (++).

Results

HER3 expression frequencies. Overall, 162 tissue samples from patients with melanocytic nevi, basal cell carcinoma, squamous cell carcinoma and melanomas were studied by RT-PCR using the house keeping gene *GAPDH* as a positive control. Gene expression was categorized into three groups (weak positive expression, moderate overexpression, strong overexpression) and compared to the intensity of the genomic DNA bands.

Non-UV exposed gluteal skin was chosen for examination in order to avoid modification of gene expression levels by ultraviolet radiation influence. Only 3/11 of the samples taken from healthy skin showed weak HER3 expression and none of the benign verruca seborrhoica skin (0/1) samples showed HER3 expression. Accordingly, there was no positive HER3 gene expression in benign skin at a comparable level to the genomic DNA bands.

This was considerable dissimilar in benign nevi: of the 22 cases of nevi, including diagnosis of compound nevi, congenital nevi, junctional nevi, dermal nevi and nevocellular nevi, 15 revealed moderate HER3 expression, only one of the positive 15/22 cases exhibited a weak expression status. Seven (7/22) nevi demonstrated no HER3 expression. In semi-malignant basal cell carcinomas (comprising superficial multicentric and sclerodermiform variants), HER3 was positive in 10/16 and weakly positive in 2. Furthermore, HER3 was shown in 4/5 of the squamous cell carcinomas, but in the single case of the xeroderma pigmentosum patient with a squamous cell carcinoma the quantity of amplified HER3 cDNA was especially low. The single Merkel cell carcinoma sample exhibited positive expression of HER3. Seventeen out of 40 primary melanomas (42.5%) expressed HER3; but 2 lentigo maligna melanomas (2/2), one superficial spreading and one nodular melanoma, among them were only weakly positive for HER3 (Figure 1).

Seven HER3-positive melanomas were located on the lower leg, 6 melanomas were found on the back. Both lentigo maligna melanomas were excised from the facial skin. Primary nodular malignant melanomas and superficial spreading melanomas were analyzed separately, since the average tumor thickness at the time of diagnosis was higher in nodular malignant melanomas than in superficial spreading melanomas in general.

Of the HER3-positive melanomas, 12/14 were detected on UV-exposed body sites, whereas 15 of the HER3-negative melanomas (n=23) were on UV-exposed and 8 on non-UV-exposed skin sites. (Table I) Both locoregional cutaneous melanoma metastases and 15/18 of the melanoma metastases were HER3-positive.

Primary nodular and superficial spreading melanomas were examined separately due to their different tumor thicknesses. A median tumor thickness of 4.95 mm was found in HER3-positive nodular malignant melanomas (range from 0.9 mm to 12.1 mm). In contrast, HER3-negative nodular melanomas showed a significant lower median tumor thickness of 2.22 mm (range from 1.85 mm to 3.6 mm). These results were similar to the expression profile in superficial spreading melanomas: HER3-positive tumors presented a somewhat higher tumor thickness (1.00 mm; range from 0.2 mm to 4.20 mm) than HER3-negative melanomas (0.73 mm; range from 0.4 mm to 1.36 mm), (Figure 2) which is known as the strongest aspect of prognosis in melanoma disease.

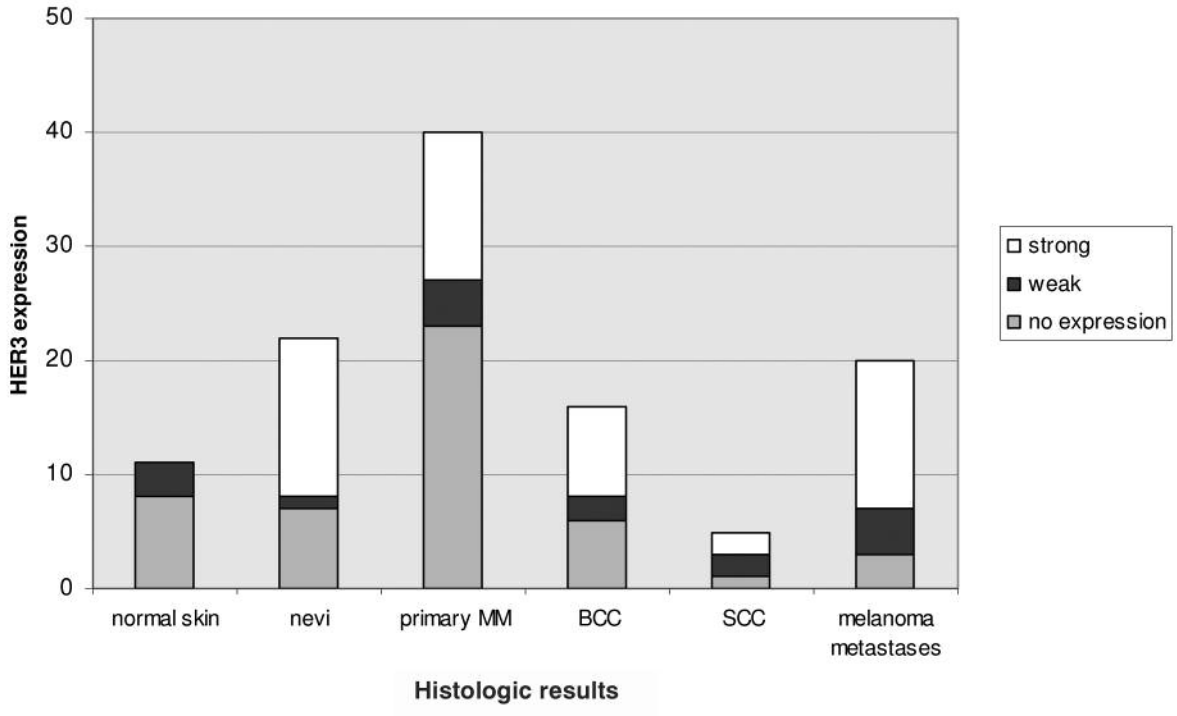

Figure 1. *HER3 expression in conventional RT-PCR. y-axis: Amount of tissue samples of normal skin, nevi, primary malignant melanomas, basal cell carcinoma, squamous cell carcinoma and malignant melanoma metastases specimens. x-axis: Listing of all different specimen entities (normal skin, nevi, primary melanoma, basal cell carcinoma, squamous cell carcinoma and malignant melanoma metastases).*

Table I. *HER3 expression results concerning different UV-exposed body sites in primary melanomas.*

HER3 positive primary melanomas, n=14				HER3 negative primary melanomas, n=23			
female, n=8		male, n=6		female, n=8		male, n=15	
UV-exposed	not UV-exposed	UV-exposed	not UV-exposed	UV-exposed	not UV-exposed	UV-exposed	not UV-exposed
7 (88%)	1 (13%)	5 (83%)	1 (17%)	5 (63%)	3 (38%)	10 (67%)	5 (33%)

In quantitative RT-PCR, normal skin showed only weak expression of HER3, while nevi (2/7) revealed a higher proportion of HER3-positive tissue than primary melanomas (2/12). All nodular melanomas (NMM) 17/17 were tested as HER3-positive (3/17 strong, 14/17 weak), only 3/21 of the lentigo malignant melanoma (LMM) and superficial spreading malignant melanoma (SSM) cohort were negative, 13/18 demonstrated weak and 5/18 strong expression of HER3. All melanoma metastases (14/14) were tested HER3-positive: 5/14 showed strong and the remaining 9/14 weak expression of the tyrosine kinase receptor. The median ratio of HER3/GAPDH was highest in nodular malignant melanoma (NMM) (0.5) compared to LMM and SSM (each 0.2). The highest median (1.1) and absolute (5.1) HER/GAPDH ratio was detected in metastases. Furthermore, all squamous cell carcinomas (4/4) showed weak expression of HER3, while strong expression was found in 2/6 of all basal cell carcinomas, while the remaining basal cell carcinomas weakly expressed HER3.

Immunohistochemistry results. HER3 expression in immunohistochemistry was absent in 5 out of 13 nevi tissue samples, 4/13 showed a weak expression status and 4/13 a moderate HER3 expression. Furthermore, all HER3-positive

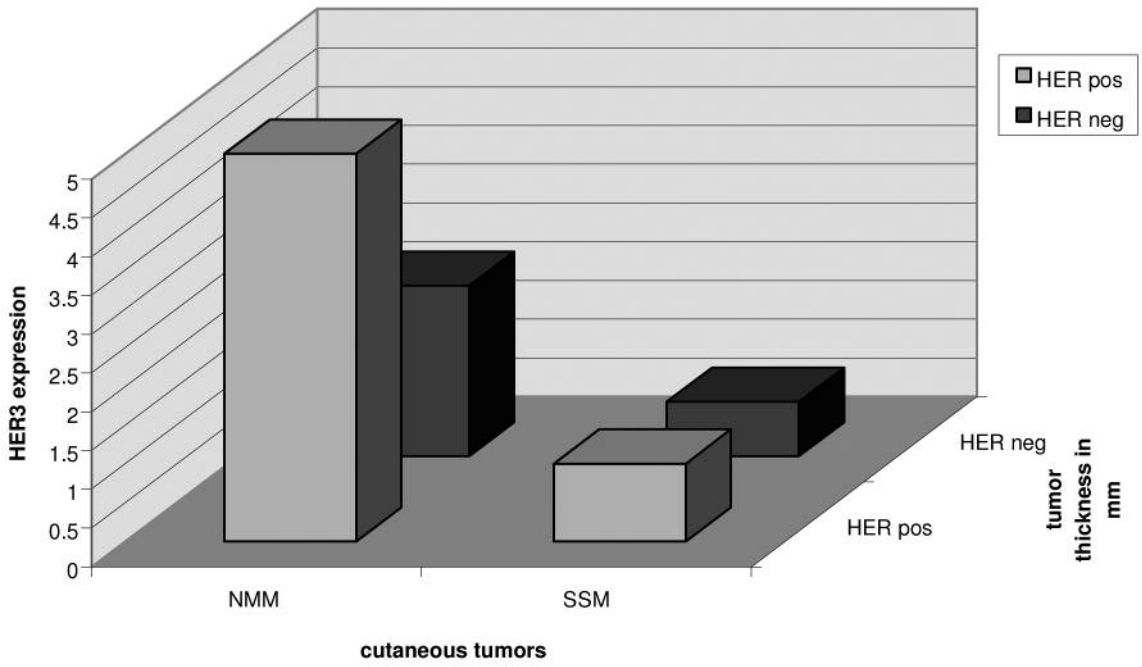

Figure 2. *Tumor thickness in HER positive/ negative skin cancer. y-axis: Tumor thickness in millimetres (mm). x-axis:Listing of different malignant melanoma specimens; nodular malignant melanoma (NMM) and superficial spreading melanoma (SMM). z-axis: Expression status of HER3 by conventional RT-PCR.*

samples were either expressing the proliferation marker PCNA. Ki-67 was found less frequently, with only 4 moderate and 2 strong expressions in HER-positive nevi. In immunohistochemistry results, one of the 10 primary melanomas was weakly positive, 7 were moderately and 2 were strongly positive for HER3 expression. In the cases of melanoma metastases (n=29), 2 out of 29 were negative, 13 were moderately and 5 were strongly positive with regard to HER3 expression. All Merkel cell carcinomas (n=4) showed moderate immunostaining results.

Discussion

In this report, we investigated the role of HER3 expression in normal skin, basal cell carcinomas, malignant melanomas and their metastases, cutaneous squamous cell carcinomas and in Merkel cell carcinomas. Although constitutive activation of the RTK members (epidermal growth factor receptor/erbB1/HER1, neu/erbB2/HER2, erbB3/HER3, and erbB4/HER4) (31) frequently occurs in various types of cancer (32), owing to overexpression, (33), this does not seem to be universal for other cancer entities. In most studies, HER3 protein expression as evaluated by immunohistochemical techniques was found to be related to poor prognosis in malignant diseases (34). However many results have proven to be controversial. In oral squamous cell carcinomas, positive staining results for HER3 in immunohistochemistry seem to correspond to a worse prognosis (35). Conversely, other studies report on a loss (27) or only weak expression of HER3 (26) in head and neck squamous cell carcinomas, which again corresponds to the low HER3/GAPDH ratio of the cutaneous cell carcinoma found in this study.

Nevertheless, the exact role of HER3 in the molecular pathway of carcinogenesis has not yet been completely clarified. In normal tissue, HER2 and HER3 are expressed and upregulated by confluence, concurrent with the induction of epidermal differentiation (36).

Since HER3 overexpression at the protein level, shown in immunohistochemistry results, might be the result of increased gene transcription, any proof for *erbB3* gene amplification in head and neck cancer or other cell lines is still outstanding.

Positive staining of HER3 was identified immunohistochemically in 14/30 primary melanomas, 7/10 metastasized melanomas and 6/15 breast carcinomas and in 46% of oral squamous cell carcinomas (20). Another recent study investigated HER2 and HER3 expression in 13 melanoma cell lines at the protein level by Western blotting and at the mRNA level by RT-PCR. Similar amounts of HER3 protein were found in melanocytes of 11/13 examined cell lines and evidence of only low HER3 mRNA levels in the remaining 2 cell lines was found (37).

In quantitative RT-PCR, nevi and melanoma metastases demonstrated the strongest expression levels with the highest number of positive samples. In general, primary melanoma showed lower expression levels and less positive samples determined both in quantitative RT-PCR and in immunohistochemistry. Results at the mRNA level were quite analogous to the expression pattern of immunohistochemistry. Primary melanomas and their metastases revealed a positive staining result in more than 90% of the cases (49/52), while nevi showed no strong expression, but a weak/moderate expression in 15/22.

Regarding the melanoma body sites in this study, HER3-positive cells were found both in tissue samples of characteristic melanoma localizations of the back and the lower extremities, related to only intermittent sun exposure, and in the case of lentigo malignant melanomas on the sun-exposed facial skin expressing HER3 only very weakly. There appears to be no association between UV radiation exposure and induction of HER3 expression. These HER3-negative or weakly expressing melanomas might have expressed HER3 previously, but developed HER3 loss over the course of time. More investigation is required to elucidate this phenomenon of HER3 loss and HER3 expression in UV-exposed skin cancer and nevi (38-41).

In 17/40 of the primary melanomas, positive HER3 expression was detected. It is already known that the heregulin (HRG)/HER system is functional in melanocytes and in the majority of melanoma cell lines, leading to growth stimulation (37). Consequently, a loss of HER3 protein expression prevents melanoma cells from growth stimulation by HRG. Seeing as induction of HER3 up-regulation depends on differentiation in normal keratinocytes (36), predominately non-differentiated melanoma cancer cells may show high expression levels unlike well-differentiated cells. Furthermore, it is important to note that the HER3-positive samples of both most common melanoma subtypes (superficial spreading melanoma and nodular malignant melanoma) were of a considerably higher tumor thickness than the HER3-negative tumors. This reflects the additional prognostic impact on the tumor's thickness according to its aggressiveness, considering that the tumor size has been proven to be the best suited prognostic parameter in melanoma. 14/14 HER3 positive samples in melanoma metastases confirm our postulations. On the other hand, metastases supposedly have a high HER3 expression, because of their proliferation status and their possibly induction by an autocrine HRG loop.

At first sight the relatively high proportion of HER-positive nevi 15/22 does not underline our suggestions towards an increased malignant potency in HER-positive cells. However, nevi excised in the clinical setting are most likely those that were expected to be suspicious in their enlargement or their colour pattern, criteria which are caused by proliferation of these nevus cells. Additionally, the investigated proliferation markers, PCNA and Ki-67, were positive in these samples.

HER3 expression at the mRNA level was detected in only 27% of the normal epidermis tissue samples (3/11), whereas 4/6 of the basal cell carcinomas and 4/5 of the cutaneous squamous cell carcinomas expressed the *erbB3* oncogene. These different findings in basal cell and squamous cell carcinomas may indicate the role of HER3, especially in squamous cell carcinoma's aggressiveness and progression in comparison to the basal cell carcinoma.

References

1 Thomas JM and Giblin V: Cure of cutaneous melanoma. BMJ *332*: 987-998, 2006.

2 Lasithiotakis K, Leiter U, Kruger-Krasagakis S, Tosca A and Garbe C: Comparative analysis of incidence and clinical features of cutaneous malignant melanoma in Crete (Greece) and southern Germany (central Baden-Wurttemberg). Brit J Dermatol *154*: 1123-1127, 2006.

3 Kraehn G, Schartl M and Peter RU: Malignant melanoma: a genetic disease? Cancer *75*: 1228-1237, 1995.

4 Kraehn G, Leiter U, Kaskel P, Udart M, Utikal J, Bezold G and Peter RU: Coexpression patterns of EGFR, HER2, HER3 and HER4 in non-melanoma skin cancer. Eur J Cancer *37*: 251-259, 2001.

5 Ferby I, Reschke M, Kudlacek O, Knyazev P, Pante G, Amann K, Sommergruber W, Kraut N, Ullrich A, Fassler R and Klein R: Mig6 is a negative regulator of EGF receptor-mediated skin morphogenesis and tumor formation. Nat Med *12*: 568-573, 2006.

6 Soung YH, Lee JW, Kim SY, Wang YP, Jo KH, Moon SW, Park WS, Nam SW, Lee JY, Yoo NJ and Lee SH: Somatic mutations of the *ERBB4* kinase domain in human cancers. Int J Cancer *118*: 1426-1429, 2006.

7 Lee JW, Soung YH, Seo SH, Kim SY, Park CH, Wang YP, Park K, Nam SW, Park WS, Kim SH, Lee JY, Yoo NJ and Lee SH: Somatic mutations of *ERBB2* kinase domain in gastric, colorectal, and breast carcinomas. Clin Cancer Res *12*: 57-61, 2006.

8 Ullrich A, Coussens L, Hayflick JS, Dull TJ, Gray A, Tam AW, Lee J, Yarden Y, Liberman TA, Schlessinger J, Downward J, Mayes ELV, Whittle N, Waterfield MD and Seeburg PH: *Human epidermal growth factor receptor* cDNA sequence and aberrant expression of the amplified gene in A431 epidermoid carcinoma cells. Nature *309*: 418-425, 1984.

9 Jackson-Fisher AJ, Bellinger G, Shum E, Duong JK, Perkins AS, Gassmann M, Muller W, Kent Lloyd KC and Stern DF: Formation of Neu/ErbB2-induced mammary tumors is unaffected by loss of *ErbB4*. Oncogene *25*: 5664-5672, 2006.

10 Seth RM, Burger AM and Kahn HJ: Analysis of the *HER2/neu* gene amplification in microdissected breast cancer tumour samples. Anticancer Res *26*: 927-931, 2006.

11 Kraus MH, Issing W, Miki T, Popescu NC and Aaronson SA: Isolation and characterization of ERBB3, a third member of the ERBB/epidermal growth factor receptor family: evidence for overexpression in a subset of human mammary tumors. Proc Natl Acad Sci USA *86*: 9193-9197, 1989.

12 Bei R, Budillon A, Masuelli L, Cereda V, Vitolo D, Di Gennaro E, Ripavecchia V, Palumbo C, Ionna F, Losito S, Modesti A, Kraus MH and Murano F: Frequent overexpression of multiple ErbB receptors by head and neck squamous cell carcinoma contrasts with rare antibody immunity in patients. J Pathol *204*: 317-325, 2004.
13 Plowman GD, Whitney GS, Neubauer MG, Green JM, McDonald VL, Todaro GJ and Shoyab M: Molecular cloning and expression of an additional epidermal growth factor receptor-related gene. Proc Natl Acad Sci USA *87*: 4905-4909, 1990.
14 Rimon E, Sasson R, Dantes A, Land-Bracha A and Amsterdam A: Gonadotropin-induced gene regulation in human granulosa cells obtained from IVF patients: modulation of genes coding for growth factors and their receptors and genes involved in cancer and other diseases. Int J Oncol *24*: 1325-1338, 2004.
15 Roskoski R Jr: The ErbB/HER receptor protein-tyrosine kinases and cancer. Biochem Biophys Res Commun *319*: 1-11, 2004.
16 Downward J, Yarden Y, Mayes E, Scarce G, Totty N, Stockwell P, Ullrich A, Schlessinger J and Waterfield MD: Close similarity of epidermal growth factor receptor and v-erb-B oncoprotein sequences. Nature *307*: 521-527, 1994.
17 Koumakpayi IH, Diallo JS, Le Page C, Lessard L, Gleave M, Begin LR, Mes-Masson AM and Saad F: Expression and nuclear localization of *ErbB3* in prostate cancer. Clin Cancer Res *12*: 2730-2737, 2006.
18 Bei R, Pompa G, Vitolo D, Moriconi E, Ciocci L, Quaranta M, Frati L, Kraus MH and Muraro R: Co-localization of multiple ErbB receptors in stratified epithelium of oral squamous cell carcinoma. J Pathol *195*: 343-348, 2001.
19 Palmieri G, Ascierto PA, Cossu A, Colombino M, Casula M, Botti G, Lissia A, Tanda F and Castello G: Assessment of genetic instability in melanocytic skin lesions through microsatellite analysis of benign naevi, dysplastic naevi, and primary melanomas and their metastases. Melanoma Res *13*: 167-170, 2003.
20 Bodey B, Bodey B Jr, Groger AM, Luck JV, Siegel SE, Taylor CR and Kaiser HE: Clinical and prognostic significance of the expression of the *c-erbB2* and *c-erbB3* oncoproteins in primary and metastatic malignant melanomas and breast carcinomas. Anticancer Res *17*: 1319-1330, 1997.
21 Peris K, Cerroni L, Chimenti S, Soyer HP, Kerl H and Hofler H: Proto-oncogene expression in dermal naevi and melanomas. Arch Dermatol Res *283*: 500-505, 1991.
22 Gaci Z, Bouin-Pineau MH, Gaci M, Daban A, Ingrand P and Metaye T: Prognostic impact of cathepsin D and c-erbB-2 oncoprotein in a subgroup of node-negative breast cancer patients with low histological grade tumors. Int J Oncol *18*: 793-800, 2001.
23 Abd El-Rehim DM, Pinder SE, Paish CE, Bell JA, Rampaul RS, Blamey RW, Robertson JF, Nicholson RI and Ellis IO: Expression and co-expression of the members of the epidermal growth factor receptor (EGFR) family in invasive breast carcinoma. Br J Cancer *91*: 1532-1542, 2004.
24 Shigematsu H and Gazdar AF: Somatic mutations of epidermal growth factor receptor signaling pathway in lung cancers. Int J Cancer *118*: 257-262, 2006.
25 Memon AA, Sorensen BS, Melgard P, Fokdal L, Thykjaer T and Nexo E: Expression of HER3, HER4 and their ligand heregulin-4 is associated with better survival in bladder cancer patients. Br J Cancer *91*: 2034-2041, 2004.
26 Issing WJ, Heppt WJ and Kastenbauer ER: *ErbB3*, a third member of the erbB/epidermal growth factor receptor gene family: its expression in head and neck cancer cell lines. Eur Arch Otorhinolaryngol *250*: 392-397, 1993.
27 Rodrigo JP, Ramos S, Lazo PS, Alvarez I and Suarez C: Amplification of *ERBB* oncogenes in squamous cell carcinomas of the head and neck. Eur J Cancer *32A*: 2004-2010, 1996.
28 Kiyota A, Shintani S, Mihara M, Nakahara Y, Ueyama Y, Matsumura T, Todd R and Wong DT: Expression of a truncated epidermal growth factor receptor in oral squamous cell carcinomas. Cancer Lett *161*: 9-15, 2000.
29 Lebeau S, Masouye I, Berti M, Augsburger E, Saurat JH, Borradori L and Fontao L: Comparative analysis of the expression of ERBIN and Erb-B2 in normal human skin and cutaneous carcinomas. Br J Dermatol *152*: 1248-1255, 2005.
30 Korabiowska M, Mirecka J, Brinck U, Hoefer K, Marx D and Schauer A: Differential expression of cerbB3 in naevi and malignant melanomas. Anticancer Res *16*: 471-474, 1996.
31 Olayioye MA, Neve RM, Lane HA and Hynes NE: The ErbB signalling network: receptor heterodimerization in development and cancer. EMBO J *19*: 3159-3167, 2000.
32 Révillion F, Bonneterre J and Peyrat JP: *ERBB2* oncogene in human breast cancer and its clinical significance. Eur J Cancer *34*: 791-808, 1998.
33 Kountourakis P, Pavlakis K, Psyrri A, Rontogianni D, Xiros N, Patsouris E, Pectasides D and Economopoulos T: Prognostic significance of HER3 and HER4 protein expression in colorectal adenocarcinomas. BMC *6*: 46, 2006.
34 Salomon DS, Brandt R, Ciardiello F and Normanno N: Epidermal growth factor-related peptides and their receptors in human malignancies. Crit Rev Oncol Hematol *19*: 183-232, 1995.
35 Shintani S, Funayama T, Yoshihama Y, Alcade RE and Matsumura T: Prognostic significance of erbB3 overexpression in oral squamous cell carcinoma. Cancer Lett *95*: 79-83, 1995.
36 De Potter IY, Poumay Y, Squillace KA and Pittelkow MR: Human EGF receptor (HER) family and heregulin members are differentially expressed in epidermal keratinocytes and modulate differentiation. Exp Cell Res *271*: 315-328, 2001.
37 Stove C, Stove V, Derycke L, Van Marck V, Mareel M and Bracke M: The heregulin/human epidermal growth factor receptor as a new growth factor system in melanoma with multiple ways of deregulation. J Invest Dermatol *121*: 802-812, 2003.
38 Scoggins CR, Ross MI, Reintgen DS, Noyes RD, Goydos JS, Beitsch PD, Urist MM, Ariyan S, Sussman JJ, Edwards MJ, Chagpar AB, Martin RC, Stromberg AJ, Hagendoorn L and McMasters KM: Gender-related differences in outcome for melanoma patients. Ann Surg *243*: 693-700, 2006.
39 Bell M, Beyl CM, Schopf RE and Schramm P: Light exposure of the lower leg as a pathogenic factor in the occurrence of malignant melanoma. Dermatol *185*: 257-261, 1992.
40 Elder DE: Pathology of melanoma. Clin Cancer Res *12*: 2308s-2311s, 2006.
41 Hallberg O and Johansson O: Malignant melanoma of the skin – not a sunshine story! Med Sci Monit *10*: CR335-340, 2004.

Received September 25, 2007
Revised December 27, 2007
Accepted February 1, 2008