No Evidence for *ErbB4* Gene Amplification in Malignant Melanoma

Gero Brockhoff[1], Marietta Bock[1], Susanne Gantner[2] and Christian Hafner[2]
Departments of [1]Gynecology and Obstetrics and [2]Dermatology, University of Regensburg, Franz-Josef-Strauss-Allee 11, DE-93053 Regensburg, Germany.
E-mail: christian.hafner@klinik.uni-regensburg.de
Accepted January 10, 2011.

Malignant melanoma (MM) represents the most severe skin cancer (1). Somatic *ErbB4* gene mutations have been identified in 19% of individuals with MM (2). These mutations result in hyperactivation of the ErbB4 receptor (2, 3). This finding indicates a pivotal role of the ErbB4 receptor tyrosine kinase (RTK) in the tumourigenesis of MM. However, oncogenic receptor activation of ErbB RTKs in human cancer is not limited to activating point mutations. Amplifications of *ErbB* receptor genes *(ErbB1–4)*, resulting in receptor overexpression and ligand-independent activation have been observed in a variety of human malignancies (4). Based on this rationale we analysed potential *ErbB4* gene amplification in 28 melanoma samples using fluorescence-*in-situ*-hybridization (FISH).

PATIENTS AND METHODS

In total, 28 MM samples were analysed (17 superficial spreading melanomas, 9 nodular MMs, 2 secondary nodular MMs on the basis of a superficial spreading melanoma). The melanomas derived from 11 male and 17 female patients (mean age 60 years, age range 27–87 years). The study was performed according to the guidelines of the local ethics committee and the Declaration of Helsinki.

Sections 4 µm thick were prepared from formalin-fixed paraffin-embedded melanoma samples, as described in detail elsewhere (5, 6). In brief, for each tumour a representative tumour section was selected from a haematoxylin and eosin-stained section of the donor block. Core cylinders with a diameter of 1.5 mm each were punched from this area and deposited into a recipient paraffin block. Tissue microarray (TMA) sections were mounted on charged slides (SuperFrost™Plus; Menzel GmbH, Braunschweig, Germany). Haematoxylin and eosin stained TMA sections were used for reference histology. FISH was performed with the use of directly labelled Zyto*Light* SPEC HER4/2q11 dual-colour probe (ZytoVision Ltd, Bremerhaven, Germany) (Fig. 1). After probe hybridization nuclei were counterstained with anti-fading 4',6-diamidino-2-phenylindole (DAPI) Vectashield (Vector Laboratories, Burlingame, CA, USA) and analysed by epifluorescence microscopy using the AxioImager-Z1 (Zeiss, Göttingen, Germany). Hybridization signals of 25 nuclei were manually counted on single cell basis by two independent observers.

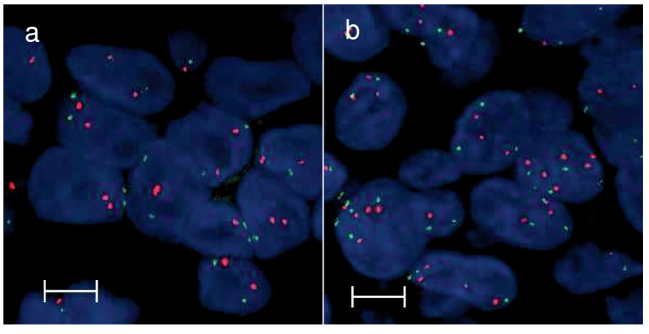

Fig. 1. Example images showing human ErbB4 (HER4) centromere (CEN2) fluorescence-*in-situ*-hybridization signals, (a) without, and (b) with low degree of chromosome 2 polysomy, respectively; (*red spots* = centromere signals; *green spots* = gene signals).

RESULTS AND DISCUSSION

Dual-colour FISH, revealed no evidence for *ErbB4* gene amplification (Table I). The highest *ErbB4* gene/centromer-2 ratio found was 1.17. Three cases showed a low degree of chromosome-2 polysomy (nos. 5, 6 and 20). Only one specimen appeared suspicious, showing a low degree of chromosome-2 loss (no. 19). Overall we did not observe any significant alterations in *ErbB4* gene copy number. Although the quantity of tissues investigated in this study is limited to 28 samples, the results indicate that, in contrast to somatic mutations, *ErbB4* gene amplifications do not play a major role in MM.

A number of somatic mutations scattered throughout the total coding region of the *ErbB4* receptor gene have been identified in 19% of patients with MM (2). All functionally analysed mutations, even those not located in the kinase encoding region, cause constitutive ligand-independent receptor activation; a finding that emphasizes the role of *ErbB4* as a pivotal cancer gene in MM (7). In a screen of 19 phosphotyrosine kinases, the *ErbB4* gene was found to be the most highly mutated gene, and the frequency of non-synonymous mutations has been found to be significantly higher than predicted for unselected passenger mutations. Therefore, one might conclude that the *ErbB4* gene represents a hotspot for a variety of mutations in melanomas, which in turn might act synergistically (2).

The contribution of wild-type and mutated *ErbB4* to tumourigenesis and progression of MMs, however, has not been elucidated in detail. Tvorogov et al. (8) demonstrated that ErbB4 kinase malfunction does not necessarily result in loss of function of the receptor protein. A kinase-defective ErbB4 receptor might still be able to heterodimerize (for example with ErbB2) and to trigger intracellular signalling in an ErbB2 kinase-dependent manner.

Hyperactivated ErbB4 receptor does, however, not necessarily lead to enhanced cell proliferation or increased anchorage-independent growth, as shown by Pricket et al., (2) who transfected NIH-3T3 cells with mutated *ErbB4*. In fact, both oncogenic and tumour-suppressing

Table I. *ErbB4 FISH analysis of melanoma*

Patient data						HER4 FISH data					
No.	Sex	Age (years)	Subtype	Thickness (mm)	Localization	ErbB4 gene signals	CEN2 signals	ErbB4/ CEN2 ratio	CEN2 signals ≥ 3	CEN2 signals = 2	CEN2 signals = 1
1	F	54	SSM	1.4	Trunk left	47	48	0.98	0	23	2
2	F	75	SSM	0.7	Left arm	51	49	1.04	1	22	2
3	F	68	SSM	0.7	Head right	55	55	1.00	6	16	3
4	F	76	SSM	0.8	Left lower arm	52	49	1.06	1	21	3
5[a]	M	70	NMM	3.0	Right lower leg	73	71	1.03	16	7	2
6[a]	F	84	NMM	2.4	Head left	59	66	0.89	13	11	1
7	M	71	NMM	2.0	Left lower arm	50	54	0.93	9	10	6
8	F	73	NMM	1.4	Nose	48	49	0.98	0	24	1
9	F	46	SSM	0.7	Left thigh	60	59	1.02	8	16	1
10	M	56	SSM	0.7	Right thigh	50	52	0.93	0	24	1
11	F	63	SSM	0.5	Back	50	54	0.93	5	18	2
12	F	87	NMM	6.0	Back left	49	48	1.02	0	24	1
13	M	45	SSM	0.7	Abdomen	52	51	1.02	1	22	2
14	M	27	SSM	1.1	Left lower leg	45	41	1.10	0	16	9
15	M	48	NMM	3.0	Back	51	53	0.96	4	20	1
16	F	65	SSM	0.9	Right elbow	45	50	0.90	4	16	5
17	F	45	NMM	12.0	Right elbow	51	50	1.02	3	19	3
18	M	54	sNMM	7.0	Back left	39	44	0.89	5	12	3
19[b]	F	34	SSM	0.6	Right thigh	41	35	1.17	0	10	15
20[a]	F	81	sNMM	4.0	Left arm	52	58	0.90	11	11	3
21	M	48	SSM	0.5	Back left	51	52	0.98	6	15	4
22	F	62	SSM	0.5	Right thigh	53	56	0.95	6	17	2
23	F	48	SSM	0.5	Abdomen	52	51	1.02	2	22	1
24	M	51	SSM	0.8	Left ear	47	48	0.98	0	23	2
25	F	83	SSM	1.9	Right back	48	46	1.04	1	19	5
26	M	74	NMM	2.2	Left arm	54	55	0.98	6	18	1
27	F	30	SSM	1.5	Left neck	50	48	1.04	3	17	5
28	M	60	NMM	2.7	Right knee	49	49	1.00	1	22	2

[a]Low polysomic tissue samples; [b]low degree of chromosome-2 loss.
SSM: superficial spreading melanoma; NMM: nodular malignant melanoma; sNMM: secondary NMM on the basis of a SSM; CEN: centromere.

signalling capacity has been attributed to differentially expressed ErbB4 isoforms, which result from alternative ErbB4 mRNA splicing (9–11). Four ErbB4 isoforms, which have been shown to be differentially expressed in malignancies of, for example, the breast (12) and the bladder (13), have not yet been analysed in MM. Even though ErbB4 receptor activity might not directly be affected by *ErbB4* gene mutations, some intronic mutations might impinge on splicing of the ErbB transcript and thereby result in either pronounced survival or cell death promoting ErbB4 signalling (11).

In summary, *ErbB4* gene amplification does not play a major role in MM. Nevertheless, ErbB4 represents an interesting drug target in MM, as this receptor has been shown to confer oncogenic properties by alternative genetic alterations (3, 14, 15). The role of both mutated and wild-type ErbB4, as well as the importance of differentially expressed ErbB isoforms in tumourigenesis and progression of MM still needs to be addressed.

ACKNOWLEDGEMENTS

The authors thank Dr Sven Hauke (ZytoVision GmbH, Bremerhaven, Germany) for kindly providing the dual colour Zytolight HER4/CEN2 FISH probe. We thank Eva Herschberger for excellent technical support.

The authors declare no conflicts of interest.

REFERENCES

1. Tsao H, Atkins MB, Sober AJ. Management of cutaneous melanoma. N Engl J Med 2004; 351: 998–1012.
2. Prickett TD, Agrawal NS, Wei X, Yates KE, Lin JC, Wunderlich JR, et al. Analysis of the tyrosine kinome in melanoma reveals recurrent mutations in ERBB4. Nat Genet 2009; 41: 1127–1132.
3. Kurppa K, Elenius K. Mutated ERBB4: a novel drug target in metastatic melanoma? Pigment Cell Melanoma Res 2009; 22: 708–710.
4. Kamath S, Buolamwini JK. Targeting EGFR and HER-2 receptor tyrosine kinases for cancer drug discovery and development. Med Res Rev 2006; 26: 569–594.
5. Sassen A, Diermeier-Daucher S, Sieben M, Ortmann O, Hofstaedter F, Schwarz S, et al. Presence of HER4 associates with increased sensitivity to Herceptin in patients with metastatic breast cancer. Breast Cancer Res 2009; 11: R50.
6. Sassen A, Rochon J, Wild P, Hartmann A, Hofstaedter F, Schwarz S, et al. Cytogenetic analysis of HER1/EGFR, HER2, HER3 and HER4 in 278 breast cancer patients. Breast Cancer Res 2008; 10: R2.
7. Futreal PA, Coin L, Marshall M, Down T, Hubbard T, Wooster R, et al. A census of human cancer genes. Nat Rev Cancer 2004; 4: 177–183.
8. Tvorogov D, Sundvall M, Kurppa K, Hollmén M, Repo S, Johnson MS, et al. Somatic mutations of ErbB4: selective loss-of-function phenotype affecting signal transduction pathways in cancer. J Biol Chem 2009; 284: 5582–5591.
9. Määttä JA, Sundvall M, Junttila TT, Peri L, Laine VJ, Isola J, et al. Proteolytic cleavage and phosphorylation

of a tumor-associated ErbB4 isoform promote ligand-independent survival and cancer cell growth. Mol Biol Cell 2006; 17: 67–79.
10. Sundvall M, Peri L, Määttä JA, Tvorogov D, Paatero I, Savisalo M, et al. Differential nuclear localization and kinase activity of alternative ErbB4 intracellular domains. Oncogene 2007; 26: 6905–6914.
11. Sundvall M, Veikkolainen V, Kurppa K, Salah Z, Tvorogov D, van Zoelen EJ, et al. Cell death or survival promoted by alternative isoforms of ErbB4. Mol Biol Cell 2010; 21: 4275–4286.
12. Junttila TT, Sundvall M, Lundin M, Lundin J, Tanner M, Härkönen P, et al. Cleavable ErbB4 isoform in estrogen receptor-regulated growth of breast cancer cells. Cancer Res 2005; 65: 1384–1393.
13. Junttila TT, Laato M, Vahlberg T, Söderström KO, Visakorpi T, Isola J, et al. Identification of patients with transitional cell carcinoma of the bladder overexpressing ErbB2, ErbB3, or specific ErbB4 isoforms: real-time reverse transcription-PCR analysis in estimation of ErbB receptor status from cancer patients. Clin Cancer Res 2003; 9: 5346–5357.
14. Hollmén M, Määttä JA, Bald L, Sliwkowski MX, Elenius K. Suppression of breast cancer cell growth by a monoclonal antibody targeting cleavable ErbB4 isoforms. Oncogene 2009; 28: 1309–1319.
15. Rudloff U, Samuels Y. A growing family: adding mutated Erbb4 as a novel cancer target. Cell Cycle 2010; 9: 1487–1503.