No Evidence for ErbB4 Gene Amplification in Malignant Melanoma

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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 ZytoLight 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.

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 Prickett et al., (2) who transfected NIH-3T3 cells with mutated ErbB4. In fact, both oncogenic and tumour-suppressing

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).
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.

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