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Varying Mutational Alterations in Multiple Primary Melanomas

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In melanoma, the mitogen-activated protein (MAP) kinase pathway plays a crucial oncogenic role. Recent studies identified additional genetic alterations, eg, *TERT*-promoter mutations. Up to 8% of melanoma patients present with multiple primary melanomas (MPMs). The pathogenesis is not fully understood, and data on the genetic diversity of MPMs are limited. To identify putative diagnostic and therapeutic consequences, we assessed the mutational status of the *BRAF* and *NRAS* genes and *TERT* promoter in patients with MPMs. The study cohort consisted of 96 patients with 237 malignant melanomas. The *BRAF*, *NRAS*, and *TERT*-promoter genotypes were assessed in all MPMs and were correlated with patients' clinicopathological characteristics. *BRAF* mutations were found in 84 melanomas (35.4%), *NRAS* mutations, in 33 (14.0%); and *TERT*-promoter mutations, in 112 (47.3%). Mutation patterns were concordant between first and subsequent primary tumors in 23.9% of patients and were discordant in 61.4% of patients. The genetic alterations were partially different in 14.7% of patients. By Cox regression analysis, only the *NRAS* mutation had a significant negative prognostic impact on time to progression to stage III ($P = 0.016$) and on distant metastasis–free survival ($P = 0.032$). In the majority of primary melanomas in patients with MPMs, *BRAF*, *NRAS*, and *TERT*-promoter genotypes were discordant. Thus, molecular testing for targeted therapy should be performed on metastatic tissue and not on primary tumors. *(J Mol Diagn 2016, 18: 75–83; http://dx.doi.org/10.1016/j.jmoldx.2015.07.010)*

Multiple primary melanomas (MPMs) were first described by Pack et al[1] in 1952 and account for 0.2% to 8.6% of all patients with cutaneous melanoma.[2] In the majority of MPM patients, a second melanoma develops within the first year after diagnosis of the primary tumor. However, subsequent melanomas have been reported to be significantly thinner than the first one.[2–5]

Several risk factors have been identified and include a positive family history (ie, having two or more affected relatives), the presence of atypical nevi, and a personal history of dysplastic nevi.[6–8] Furthermore, germline mutations of the melanoma-predisposing genes *CDKN2A* and *CDK4* may be associated with MPM development,[9,10] and somatic mutations of key regulator genes involved in the pathogenesis of melanoma have been reported.[5]

In 2005, Curtin et al[11] described four types of melanomas at different sites of the body and with different levels of sun exposure that could be distinguished by distinct patterns of somatic mutations. Activating oncogenic mutations of *BRAF* were reported in about 47% of all cutaneous melanomas, and somatic mutations of *NRAS*, in 20%.[12]

Recently, telomerase reverse-transcriptase gene (*TERT*)–promoter mutations were identified with a high prevalence in malignant melanoma. These mutations mostly are indicative of ultraviolet (UV) light–induced DNA damage and lead to an increased transcriptional activation of this gene.[13,14]

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The frequency of *TERT*-promoter mutations varies depending on the melanoma subtype and differs between primary and metastatic tumor samples.[15] Horn et al[16] demonstrated that these mutations also occur in familial types of malignant melanoma.

Here, we assessed the mutational status of candidate genes involved in the pathogenesis of melanoma (*BRAF*, *NRAS,* and *TERT* promoter) in patients with MPM, explored possible correlations with patients' clinicopathological characteristics and outcomes, and putative diagnostic and therapeutic consequences.

Materials and Methods

Patients and Data Collection

Our retrospective study cohort consisted of 96 patients with MPMs, treated from 1995 through 2013 at the Departments of Dermatology at the University Hospital of Schleswig-Holstein, Campus Kiel (Kiel, Germany), and at the Municipal Hospital Kassel (Kassel, Germany). Formalin-fixed, paraffin-embedded samples from 237 separate tumors were investigated.

Data on basic clinical tumor-specific treatment and follow-up, including survival after diagnosis, were collected from clinical records. Follow-up information was obtained from patient interviews or questionnaires sent to general practitioners. All patients had provided written informed consent before inclusion in the study.

Histological Examination

Tissue sections were stained with hematoxylin and eosin (H&E). All tumor samples were reviewed before their inclusion in the study by two board-certified histopathologists (F.E. and C.R.), and the diagnosis of a malignant melanoma was confirmed in every tumor sample.

Mutation Testing and DNA Sequence Analysis

Genomic DNA was extracted from formalin-fixed, paraffin-embedded tissue with the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. To ensure a tumor cell content of >40% in the analyzed specimens, the tissue sections were manually microdissected before DNA extraction. Mutational analyses of codon 600 of *BRAF* and of codons 12, 13, and 61 of *NRAS* were performed by pyrosequencing as described previously.[17] The genomic region containing the *TERT*-promoter mutational hotspots (chr5, 1,295,228 C>T; 1,295,242-243 CC>TT; 1,295,250 C>T, and 1,295,253 C>T) was analyzed as described previously.[17]

External Quality Assurance

The *BRAF* and all-*RAS*-mutational assays were certified successfully by the quality-assurance program of the German Society of Pathology and the *Bundesverband Deutscher Pathologen e.V.*

Statistical Analyses

The data were analyzed using IBM SPSS Statistics version 20.0 (IBM Corp., Armonk, NY). Baseline descriptive statistics included proportions and mean or median values, as appropriate by data distribution. Estimated survival curves were constructed by the Kaplan-Meier method. Differences between the curves were evaluated using the log-rank test. Melanoma-related deaths were considered as events in overall survival (OS). Progression-free survival and OS were calculated from the day of first melanoma diagnosis until progression of the disease (locoregional/distant) or death, respectively. A multivariate analysis using the Cox proportional hazards model including the mutational status was performed to evaluate prognostic relevance. $P < 0.05$ was considered statistically significant. There were no adjustments for multiple testing in this exploratory study.

Results

Patient Characteristics

Ninety-six patients [41 women (42.7%), 55 men (57.3%); median age, 64.7 years (range, 25.9 to 94.6 years)] harbored 237 primary melanomas (mean, 2.5 melanomas per patient). Fifty-nine patients (62%) had two, 20 (21%) had three, 10 (10%) had four, and 7 (7%) had more than four melanomas. A family history was obtained from 75 patients. Six patients (8%) showed a positive family history (presence of melanoma in first-degree relatives); in another 8 patients (11%), second- or third-degree relatives with melanoma were identified.

Synchronous melanomas occurred in 52 patients (54%); metachronous melanomas, in 44 (46%). Forty melanomas (16.9%) were located in the head and neck region; 91 (38.4%), on the trunk; and 106 (44.7%), on the extremities. Sixty melanomas (25.4%) were melanomas *in situ*; 113 (47.9%) had a tumor thickness of <1 mm; 52 (22.0%), 1 to 4 mm; and 11 (4.7%), >4 mm. The patients' characteristics are summarized in Table 1.

Genotype

Genotype was analyzed in all 237 primary melanomas. *BRAF* mutations were found in 84 melanomas (35.4%) and included the following genotypes: $BRAF_{V600E}$ [75 melanomas (89.3%)], $BRAF_{V600K}$ [8 (9.5%)], $BRAF_{V600R}$ [1 (1.2%)], and $BRAF_{L597S}$ [1 (1.2%)]. A total of 153 melanomas harbored the *BRAF* wild type. Thirty-three samples (14.0%) carried an *NRAS* mutation in codon 61. *NRAS* wild type was found in 202 melanomas. In two samples, the *NRAS* analysis failed. *BRAF* and *NRAS* mutations were mutually exclusive in the same melanomas.