Pathology (April 2014) **46(3)**, pp. 193–198

ANATOMICAL PATHOLOGY

Concordant *BRAF*^{V600E} mutation status in primary melanomas and associated naevi: implications for mutation testing of primary melanomas

Hojabr Kakavand[1,2], Oana Crainic[3,4], Trina Lum[3], Sandra A. O'Toole[1,3,5], Richard F. Kefford[1,2], John F. Thompson[1,2,6], James S. Wilmott[2], Georgina V. Long[1,2] and Richard A. Scolyer[1,2,3]

[1]The University of Sydney, Sydney, [2]Melanoma Institute Australia, North Sydney, [3]Department of Tissue Pathology and Diagnostic Oncology, Royal Prince Alfred Hospital, Camperdown, NSW, Australia; [4]Canterbury Health Laboratories, Christchurch, New Zealand; [5]Kinghorn Cancer Centre, Garvan Institute of Medical Research, Darlinghurst, and [6]Department of Melanoma and Surgical Oncology, Royal Prince Alfred Hospital, Camperdown, NSW, Australia

Summary

There is concern that *BRAF* mutant naevus cells admixed with melanoma cells could cause false positive mutation tests in *BRAF* wild-type melanomas. We sought to assess the frequency of *BRAF*^{V600E} mutations in primary melanomas arising with/without associated naevi and determine *BRAF*^{V600E} concordance between melanomas and associated naevi. Formalin fixed, paraffin embedded (FFPE) tissue from 57 patients with primary melanomas with/without associated naevi was immunohistochemically stained to detect *BRAF*^{V600E} mutation. In a subset of patients ($n=29$), molecular mutation testing was also carried out using a panel of 238 known genetic variants. Of the primary melanomas with an associated naevus ($n=29$), 55% were *BRAF*^{V600E} mutant with 100% concordance between the melanoma and associated naevus. In contrast, only 21% of the primary melanomas unassociated with naevi were *BRAF*^{V600E} mutant ($p=0.009$). Our results suggest that melanomas with associated naevi have a higher frequency of *BRAF*^{V600E} mutations than melanomas unassociated with naevi. Furthermore, melanomas and their associated naevi were concordant in *BRAF*^{V600E} status, which suggests that false positive mutation tests occurring as a consequence of admixed *BRAF* mutant naevus cells in *BRAF* wild-type primary melanomas are unlikely to be a problem in clinical practice. The findings have important implications for adjuvant clinical trials of targeted therapies.

Key words: BRAF, diagnosis, immunohistochemistry, melanoma, mutation testing, naevus, pathology, targeted therapy, treatment.

Received 8 September, revised 15 October, accepted 17 October 2013

INTRODUCTION

The constitutive activation of the mitogen-activated protein kinase (MAPK) pathway driven by mutant BRAF signalling leads to proliferation and cell cycle dysfunction in many solid tumours, including melanocytic tumours.[1] In melanoma, the most common *BRAF* mutation causes a valine to glutamic acid substitution at position 600 (*BRAF*^{V600E}) and occurs in 70–95% of patients.[1–6] *BRAF* mutations are an early event in the development of melanocytic lesions with reported rates of up to 82% in benign naevi (including congenital, intra-dermal, compound and dysplastic naevi)[7] and rates of approximately 40% in metastatic melanomas.[8–10] Approximately 20–30% of melanomas arise in association with a naevus, although reported rates can vary from less than 20% to 70% depending on case selection and pathological interpretation of samples.[11,12]

Selective BRAF inhibitors are a standard of care for treating *BRAF*^{V600} mutant metastatic melanoma, with high response rates and a prolonged progression-free and overall survival compared with chemotherapy.[13,14] These inhibitors are only effective in patients with *BRAF* mutant melanoma and are potentially detrimental to patients with *BRAF* wild-type melanoma,[15–17] so the presence of a *BRAF* mutation in the melanoma must be verified prior to initiation of therapy. Mutation testing is routinely undertaken on archival formalin fixed, paraffin embedded (FFPE) tumour tissue.

The current conventional methods for mutation testing have a variety of limitations and costs.[18,19] The development of a monoclonal antibody (VE1) targeted to bind specifically to the mutant protein has provided a new method for detecting *BRAF*^{V600E} mutations[20,21] and in one study was reported to be more accurate than molecular mutation testing for detecting *BRAF*^{V600E} mutations in melanomas.[18] Immunohistochemistry (IHC) has the added advantage of allowing visualisation of individual antigen-bearing tumour cells, e.g., single metastatic tumour cells in lymph nodes or melanoma cells admixed with naevus cells. Furthermore, immunohistochemical detection of *BRAF*^{V600E} is an ideal method for mutation testing small biopsy samples and is a readily available tool in nearly all pathology laboratories. Therefore, it can efficiently expedite the determination of the *BRAF*^{V600E} mutation status, decrease the amount of tissue required and provide useful translational information to assess potential correlations between response, heterogeneity and intensity of staining.[18,19]

There is concern that if standard approved molecular genetic mutation testing is performed using a specimen with *BRAF* wild-type primary melanoma cells admixed with *BRAF* mutant naevus cells, the latter would lead to a false positive mutation result with subsequent adverse effects on the patient if they are

Print ISSN 0031-3025/Online ISSN 1465-3931
DOI: 10.1097/PAT.0000000000000077

treated with a BRAF inhibitor. In this study, we performed BRAF IHC on primary melanomas arising in association with compound/dermal naevi to determine the rates of *BRAF*^{V600E} mutations in primary melanoma with an associated naevus as well as the concordance in mutation status between the melanoma and naevus. Furthermore, we also compared the *BRAF*^{V600E} mutation rates in melanomas occurring with and without associated naevi.

METHODS

Patient selection

Patients presenting with primary melanoma associated with naevi between May 2000 and May 2013 were identified from the Melanoma Institute Australia Melanoma Research Database. Available archival FFPE tissue samples were retrieved from the archival files of the Department of Tissue Pathology and Diagnostic Oncology at the Royal Prince Alfred Hospital, Sydney, Australia, and outside institutions (in cases where the pathology of the specimen had been originally reported elsewhere). Twenty-nine patients were identified. The FFPE tissue blocks of an unselected cohort of 28 primary melanomas without an associated naevus diagnosed and treated during the same time period were also retrieved. This study was conducted with Human Ethics Review Committee approval.

Immunohistochemistry

Two 4-μm thick sections were cut and a haematoxylin and eosin (H&E) slide was analysed in each case to confirm the presence of a primary melanoma and the presence or absence of an associated naevus. IHC was performed using an automated IHC system (Ventana BenchMark Ultra; Ventana Medical Systems, USA) and OptiView DAB IHC Detection Kit (Ventana). Following deparaffinisation of FFPE sections, heat-induced epitope retrieval (HIER) was applied using CC1 for 64 min. The sections were incubated with anti-BRAF mouse antiserum VE1 (1:50 dilution; Spring Bioscience, USA) for 1 h followed by incubation with haematoxylin II counterstain for 4 min and then with bluing reagent for 4 min

Histological assessment

All histology slides were evaluated independently by three observers (HK, OC, and RAS) blinded to all clinical information. Melanoma and naevus cells were scored separately in each case. The staining was scored as positive when the melanoma and naevus cells showed definitive cytoplasmic staining and negative when there was no staining or focal faint staining. Positive cases were scored using a semi-quantitative scale (0–3), with 0 for absent staining, 1 for low/weak staining, 2 for moderate staining, and 3 for high/strong staining.

Molecular testing

BRAF mutation testing was performed on sections from archival FFPE tissue blocks of 29 patients at the Royal Prince Alfred Hospital (RPAH), Department of Tissue Pathology and Diagnostic Oncology (Sydney, Australia). From the FFPE sections, the melanoma was carefully macro-dissected using an H&E section as a guide. The DNA was extracted and amplified for 238 variant targets in a 24 multiplex polymerase chain reaction (PCR) using the OncoCarta Panel v1.0 Kit and analysed based on the matrix-assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF) technology on the MassArray platform (Sequenom, USA).

Statistical analysis

Categorical comparisons between patient cohorts were tested with the Pearson's chi-square. Continuous variables were tested with the Mann–Whitney U test for difference across two groups. SPSS statistic v21.0 (IBM, USA) was used to run all the statistical analyses and a p value less than 0.05 was considered statistically significant.

RESULTS

Immunostain for *BRAF*^{V600E} mutation in primary melanomas with/without an associated naevus

Sixteen of the 29 (55%) primary melanomas arising in association with a naevus had a *BRAF*^{V600E} mutation in both the melanoma and the naevus. In contrast, six of 28 (21%) melanomas unassociated with naevi had a *BRAF*^{V600E} mutation. Melanomas associated with naevi were more likely to have the *BRAF*^{V600E} mutation than those without an associated naevus ($p = 0.009$). There were no statistically significant differences in clinical and pathological characteristics (i.e., age, gender, Breslow thickness, ulceration, melanoma subtype and anatomical site) in the patients with primary melanoma arising in association with a naevus and primary melanomas unassociated with a naevus (Table 1). As expected, patients with a *BRAF*^{V600E} mutation were younger than *BRAF* wild-type patients ($p = 0.007$).

Concordance in *BRAF*^{V600E} mutation status between primary melanomas and the associated naevus

In the 29 primary melanomas with an associated naevus, there was 100% concordance in the *BRAF*^{V600E} mutation status between the primary melanoma component and the associated dermal/compound remnant naevus component of the lesion (Fig. 1 and 2).

Differential intensity of BRAF^{V600E} protein expression between primary melanomas and their associated naevus

Immunostaining for BRAF^{V600E} was strongly and diffusely positive (3+) in 82% of the positive primary melanomas (18/22) and moderately positive (2+) in 18% (4/22). The three independent observers were concordant in the assessment of the BRAF^{V600E} staining intensity for all cases. The intensity of the staining for BRAF^{V600E} was weaker (1+ to 2+) in a proportion (5/16) of associated naevi compared to the strong diffuse staining (3+) in the primary melanoma (Table 2; Fig. 3). However, there was no intratumoural heterogeneity in the intensity of staining; in the *BRAF*^{V600E} mutant melanomas, all melanoma cells showed strong BRAF^{V600E} protein expression.

Molecular mutation testing

In a subset of patients (29/57) that were tested using the BRAF^{V600E} immunohistochemistry in our study, molecular mutation testing of the primary melanoma was also undertaken. *BRAF*^{V600E} mutations were detected in 24% (7/29) of the cases. There was 100% concordance between the *BRAF* molecular mutation testing results and the BRAF^{V600E} immunohistochemistry results. One of the 29 cases that had molecular mutations testing performed had an associated naevus, with the *BRAF*^{V600E} mutation being the only one positive in the panel. The molecular mutation testing results also showed that there were 17% (5/29) non-V600E *BRAF* mutations [G469R, L597Q, V600M, V600R, and K601N), 34% (10/29) *NRAS* mutations (G12D, G13R, Q61H, Q61K, Q61L(X2), and Q61R(X4)], 3.4% (1/29) *HRAS* (G12D) and 3.4% (1/29) *cKIT* (L576P). As expected, all of these cases were negative with BRAF^{V600E} immunohistochemistry. No *BRAF*^{V600K} mutations were detected in this patient cohort.

DISCUSSION

The current literature on *BRAF* mutation status in primary melanomas arising in association with naevi is very limited, with these reports only including small numbers of cases and mostly using mutation analysis techniques that do not allow visualisation of the mutation status of individual cells