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***BRAF*, *NRAS*, AND *KIT* SEQUENCING ANALYSIS OF SPINDLE CELL MELANOMA**

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Abstract

Background—Spindle cell melanoma represents a rare but distinct subset of melanoma, and its genomic spectrum has not been fully defined.

Methods—We searched our institutional database for patients with a diagnosis of pure spindle cell-type melanoma whose tumors had been analyzed for *BRAF*, *NRAS*, and *KIT* mutations using pyrosequencing technique.

Results—We identified 24 patients with spindle cell melanoma, including 10 patients with desmoplastic melanoma, whose tumors had been analyzed for at least one of the three genes. The median Breslow thickness was 2.6 mm, and the most common site of the primary melanoma was the trunk, followed by the head and neck region. *BRAF*, *NRAS*, and *KIT* genomic sequencing was performed successfully in 20, 18, and 14 patients, respectively. Among the 20 melanomas with completed *BRAF* sequencing analysis, 6 (30%) harbored a mutation, of which 5 (83%) had a V600E mutation and 1 (17%) had a V600R mutation. None of the melanomas harbored *NRAS* or *KIT* mutations.

Conclusion—As has been reported in other common types of melanoma, V600 *BRAF* mutation is the most common mutation of those tested in spindle cell melanoma. *NRAS* or *KIT* mutation appears to be rare, if not completely absent.

Keywords

spindle cell; melanoma; *BRAF*; *NRAS*; *KIT*

INTRODUCTION

Melanoma represents a malignancy showing evidence of melanocytic differentiation that can be identified histopathologically or ultrastructurally. Melanocytes are melanin-producing cells that originate from the neural crest and are generally situated in the basal layer of the epidermis but can also occur at different parts of the epidermis. The incidence rates vary throughout the world, and in the United States, the rate has increased over the past 30 years (1). In the United States alone, it is estimated that about 70,230 new melanomas

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will be diagnosed, and approximately 8,790 people are expected to die of melanoma in 2011 (2).

The most common histopathologic types of melanoma are superficial spreading melanoma (70%) (3), nodular melanoma (15-30%) (3), lentigo maligna melanoma (4-15%) (4), and acral lentiginous melanoma (2-8%) (5). Spindle cell melanoma constitutes a relatively uncommon variant of melanoma, characterized by tumor cells with distinctly spindled morphology. Desmoplastic melanoma, a subset within this morphological group of melanoma, known for its tendency for neurotropism, is characterized histopathologically by dermal and/or subcutaneous infiltrates of spindle cells arranged singly or in thin fascicles within a prominent collagenous or, less commonly, myxoid stroma (6, 7). These melanomas are known to arise most often in older individuals, particularly in the head and neck region. Local recurrences are common. Because of the unique features and behavior of desmoplastic melanoma, it is designated as a distinct clinicopathologic type of melanoma. Because of the rarity of melanoma showing purely spindle cell morphology, its biology and optimal therapy are not well known.

In the past decade, the understanding of the molecular basis of cancer development has advanced, and attempts to block the mediators of cancer-promoting genes and proteins are under way. In particular, melanoma was found to have the highest prevalence of mutations in the *BRAF* gene when compared with many kinds of human cancers (8). Although *BRAF* mutations were commonly found (59%) in non-chronic sun-induced melanomas, they were found less frequently in acral and mucosal melanomas (8-10). Ten to twenty percent of the four major types of melanomas harbor mutations in the *NRAS* gene, and *NRAS* and *BRAF* mutations rarely occur concurrently within a given melanoma's cells (11, 12). A recent meta-analysis showed that *BRAF* mutations were frequently detected in patients with superficial spreading melanoma and in melanomas arising in non-chronic sun-damaged skin while *NRAS* mutation was frequently found in patients with nodular melanoma and in melanomas arising in chronic sun-damaged skin (13). In addition to the *BRAF* and *NRAS* mutations found in melanoma, activating mutations and/or gene amplification of *KIT* are found in more distinct subsets of melanomas; they are found in 39% and 36% of mucosal and acral lentiginous melanomas, respectively (9). Recently, inhibitors of such mutations have been clinically tested and have shown great promise in clinical trials (14-19), underscoring the importance of selecting patients with relevant genomic mutations in order for targeted therapies to be successful.

Herein, we report the incidence rates of *BRAF*, *NRAS*, and *KIT* mutations in spindle cell melanoma. Our study shows that *BRAF* mutation, but not *NRAS* or *KIT*, is commonly detected in melanomas of pure spindle cell type.

MATERIAL AND METHODS

Experimental methods

The protocol for this retrospective study was approved by the institutional review committee at The University of Texas MD Anderson Cancer Center (MD Anderson). We searched the pathology database at MD Anderson to identify patients with a diagnosis of spindle cell melanoma whose tumors had been analyzed for genomic mutations. We included in our analysis patients with pure spindle cell-type melanoma only, including the desmoplastic melanoma variant. Tumors that had mixed histopathologic morphology were excluded even if they contained spindle cells to avoid the possibility of analyzing other types of melanoma cells. Genomic sequencing analyses were performed with pyrosequencing, a PCR-based DNA sequencing technique, using formalin-fixed paraffin-embedded tumor samples. For the *BRAF* gene, the analysis was performed on codons 595 to 600 of exon 15. For the *NRAS*

gene, the analysis was performed on codons 12, 13, and 61, and for the *KIT* gene, exons 11, 13, and 17 were assessed. These exons were selected for genomic analysis because they are the sites of the most common mutations observed in melanoma. The lower limit of detection of this assay was approximately one cell bearing the mutation per 10 cells (10%). In all but 1 patient, sequencing analysis was performed on metastatic tumor samples. The genomic analysis was performed in the Molecular Diagnostic Laboratory at MD Anderson.

We abstracted the following information from each patient's medical record: patient's sex and age, microscopic characteristics of the primary melanoma, and sites of primary tumors.

Statistical analysis

The main objective of this study was to estimate the frequency of genomic mutations in spindle cell melanoma. In our analysis, the rate of each genomic mutation (*BRAF*, *NRAS*, and *KIT*) among patients with completed sequencing analysis was described. Patient demographics were summarized using descriptive statistics.

RESULTS

We identified 476 patients whose melanoma lesions were analyzed for at least one of the three genes at MD Anderson between April 1, 2008 and July 30, 2010. Among these patients, a majority of patients had mixed melanoma cell types that included spindle cells; only 24 patients had microscopically-confirmed pure spindle cell melanoma. Table 1 describes patient characteristics. Ten of the 24 patients had a diagnosis of desmoplastic melanoma.

Among the 24 patients with spindle cell melanoma, 15 (62.5%) patients were male. The mean age was 57 years (range: 18 - 81 years) at the time of their primary diagnosis; a peak of occurrence was in the fifth decade, followed by the sixth decade. Breslow thickness was known in 14 of 18 patients with known site of primary melanoma, and the median thickness was 2.6 mm (range: 0.42 - 5.00 mm) for the primary melanoma. Among those with known site of primary melanoma, 5 (28%) patients had their primary lesion in the head and neck region, 7 (39%) in the trunk, 3 (17%) in the pelvic region, 1 (6%) in the upper extremities, and 2 (11%) in the lower extremities.

Among the 24 patients analyzed in our study, *BRAF*, *NRAS*, and *KIT* sequencing was performed successfully in 20, 18, and 14 patients, respectively. In the other patients, the sequencing analysis could not be performed because of inadequate tumor samples. Among the 20 melanomas with completed *BRAF* sequencing analysis, 6 (30%) harbored a mutation, of which 5 (83%) had a *V600E* mutation and 1 (17%) had a *V600R* mutation. None of the desmoplastic melanomas (n=8 with complete sequencing) carried a *BRAF* mutation. None of the melanomas harbored *NRAS* or *KIT* mutations.

DISCUSSION

This is the first report that describes the frequencies of genomic mutations of spindle cell melanoma. Our results showed that *V600* mutation in *BRAF* is the most common mutation (of the genes tested) in spindle cell melanomas, and *NRAS* or *KIT* mutation is rare, if not completely absent. The predominance of *V600 BRAF* mutation is similar to the mutation pattern of the major types of melanomas (8). Although the incidence rate was 30% for spindle cell melanoma in our analysis, compared with 50% reported in the other types of melanomas (8), it should be noted that the number of patients in our study is too small for a firm conclusion regarding the true incidence rate of *BRAF* mutation. Likewise, it is premature to conclude that *NRAS* or *KIT* mutation is absent in this type of melanoma. We

will need a larger number of patient samples to estimate the true incidence rates. If a larger series confirms our finding, it could have great financial implications, as patients with this type of melanoma could then be tested for only *BRAF*, but not *NRAS* or *KIT*.

Interestingly, none of the pure desmoplastic melanomas harbored a *BRAF* mutation. Our result is consistent with the report by Davison and colleagues who noted the absence of *BRAF* mutation among 12 desmoplastic melanoma specimens (20). These findings suggest that this particular type of melanoma, which has a tendency to exhibit prominent infiltration of peripheral nerves, may have a different molecular biology than other types of spindle cell melanomas without desmoplastic stromal response.

Our results suggest that *BRAF* may play a more important role in growth and survival of spindle cell melanoma than *NRAS* or *KIT*. However, whether growth and survival of this type of melanoma depends on *BRAF* signaling to the same extent as other types is unknown. This question may be answered more clearly by clinical investigation of selective *BRAF* inhibitors.

Desmoplastic or spindle cell melanoma is thought to occur most frequently in the head and neck region (21). In our study, primary spindle cell melanomas were found in the trunk more frequently than expected, and this observation may be explained by the small number of patients analyzed. However, the patterns of the sites of primary melanomas among men and women were similar to what was anticipated. Men had primary lesions concentrated to the head and neck and trunk regions, accounting for 73% of all sites. On the other hand, women showed dominant occurrence in the extremities (upper, 14%; lower, 29%) and pelvic region (43%).

In this study, we used pyrosequencing method to analyze the genomic mutations. Pyrosequencing technique detects bioluminescence signal caused by a release of pyrophosphate when a nucleotide is introduced in the DNA strand and this bioluminescence signal is quantitatively connected to the number of bases added (22). Pyrosequencing method has a higher sensitivity than Sanger sequencing method, a chain terminator sequencing technique, which is considered a classic standard (23); therefore, it is unlikely that we would have found higher number of genomic mutations using Sanger technique in our analysis. With recent development of high-throughput sequencing techniques, such as sequenom method, we may be able to find novel mutations in spindle cell melanomas in a more efficient way.

In conclusion, we demonstrated that *BRAF* mutation is commonly found in pure spindle cell-type melanomas. We hope that this report will inspire interest in evaluating genomic aberrations in atypical subsets of melanomas, including spindle cell melanoma.

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FIGURE 1. Spindle cell melanoma shows a diffuse proliferation of distinctly spindled cells often arranged in fascicles (A, 200X) while desmoplastic melanoma is characterized by spindle cells with less abundant cytoplasm interspersed within a distinctly fibrotic or sometimes myxoid stroma as depicted (B, 200X).

Table 1

Patient Characteristics

Characteristic	No. of patients (%) n=24	<i>BRAF</i> status*	
		Mutated n=6	Wild-type n=14
Sex			
Male	15 (62)	3 (50)	9 (64)
Female	9 (38)	3 (50)	5 (36)
Age (yrs)			
Mean	57	54.7	68.9
Range	18-81	30-67	55-87
Race			
White	23 (96)	6 (100)	14 (100)
Black	1 (4)	0	0
Type			
Desmoplastic	10 (42)	0	8 (57)
Breslow thickness (mm)			
0 – 1	2 (8)	0	1 (7)
>1 – 2	2 (8)	1 (17)	1 (7)
>2 – 3	3 (12)	1 (17)	2 (14)
>3 – 4	4 (17)	1 (17)	2 (14)
> 4	3 (12)	0	3 (21)
Unknown	10 (42)	3 (50)	5 (36)
Primary site of melanoma	Total (n=24)	Men (n=15)	Women (n=9)
Head and neck	5 (21)	5 (33)	0
Trunk	7 (29)	6 (40)	1 (11)
Pelvic region	3 (12)	0	3 (33)
Upper extremities	1 (4)	0	1 (11)
Lower extremities	2 (8)	0	2 (22)
Unknown	6 (25)	4 (27)	2 (22)

* *BRAF* mutation analysis was completed in 20 patient samples.