ORIGINAL ARTICLE

Immunohistochemical study of BRAF V600E mutant protein expression in high-grade sarcomas

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Received: December 17, 2014 **Accepted:** February 27, 2015 **Online Published:** March 11, 2015
DOI: 10.5430/jst.v5n1p44 **URL:** http://dx.doi.org/10.5430/jst.v5n1p44

Abstract

BRAF V600E is a mutation present in numerous neoplasms, including melanomas, thyroid, colorectal and ovarian carcinomas, gastrointestinal stromal tumors, and Langerhans' cell histiocytosis. Vemurafenib, a BRAF V600E kinase inhibitor has been successfully used in the treatment of melanoma. The role of this mutation in unclassified high-grade sarcomas, malignancies with very limited treatment options, has not been widely studied. Because of the availability of a highly sensitive and specific antibody (VE1) against the BRAF V600E mutant protein, we tested 48 cases of unclassified high-grade sarcomas. Cytoplasmic expression intensity was graded as negative (0), or positive (2+ or 3+) by two pathologists and a pathology resident. Forty one out of 48 specimens remained intact in the cores after immunohisto chemistry (IHC) processing. Six of the 41 cases (15%) were scored as positive. In addition, non-specific nuclear staining was detected in 12/88 cores (14%). The 6 positive cases were tested for the BRAF V600E mutation by RT-PCR, and all were negative. Based on these results, we concluded that BRAF V600E mutation is rare in unclassified high-grade sarcomas, and because of the non-specific staining, results should be interpreted with caution.

Key words

BRAF, High-grade sarcomas, VE1 antibody, Immunohistochemistry

1 Introduction

V-raf murine sarcoma viral oncogene homolog B1 (BRAF), a serine-threonine protein kinase, is a member of the RAS-REF-MEK-ERK signaling pathway. The BRAF V600E has been considered as a cancer driving mutation in a variety of neoplasms, including melanomas, thyroid carcinomas, gastrointestinal stromal tumors, colorectal (but not gastroesophageal) malignancies [1], ovarian carcinomas, a small subset of non-small cell lung cancers, and Langerhans' cell histiocytosis [2]. Vemurafenib (Zelboraf), an FDA appproved BRAF V600E kinase inhibitor, has been used in the treatment of unresectable or metastatic melanoma harboring the BRAF V600E, with considerable response. The determination of BRAF mutation status is nowadays a requirement for treatment selection in melanoma [3], and an attractive target for other malignancies with the BRAF V600E. A few studies have also been done in sarcomas. Ahmed et al. found no expression of BRAF in 72 Ewing family tumors [4], and Cipriani et al. [5] evaluated 104 tumors, including 90 sarcomas, with no BRAF mutation identified in the sarcoma group. On the other hand, Shukla et al. [6] identified BRAF mutations in 1.3% of Ewing sarcomas and 1.7% of embryonal rhabdomyosarcomas. However, the status of BRAF V600E in

unclassified high-grade sarcomas, malignancies that have limited therapeutic options, still remains unclear. In this study, we tested 48 cases of unclassified high-grade sarcomas by immunohistochemistry (IHC) with antibody VE1 against BRAF V600E mutant protein, reported to be highly specific and sensitive for BRAF V600E mutation [3, 7, 8], and compared the results with PCR-based assay on those with positive or equivocal immunostaining.

2 Material and methods

2.1 Case selection

Prospective samples were identified from the laboratory information system by diagnosis, and the H&E slides were reviewed by a board-certified pathologist. Subsequently, forty-eight cases of undifferentiated high-grade sarcomas were selected, and the paraffin blocks that still had remaining tissue after immunohistochemistry (41 cases), were used in this study. Patient's age ranged from 1 to 96 years old, with a mean of 57 years. Twenty-two patients (53.7%) were male, and nineteen (46.3%) were female, with a male to female ratio of 1.16:1.0. The most common location for these malignancies was the thigh, followed by the arm/shoulder, hip and lower leg/foot (in decreasing order of frequency). Other locations included chest, abdominal wall, forearm/hand, pleura and lung. All these sarcomas were high-grade with an unclear line of differentiation, and therefore, with poor prognosis.

2.2 Microarray construction

Representative sections were sampled from the tissue blocks to construct three microarrays of one-millimeter cores in duplicate or triplicate.

2.3 Immunohistochemistry

Immunohistochemistry was performed on formalin fixed, paraffin-embedded (4 mm thick) sections ontissue micro-array with Ventana Benchmark UHra/XT automated immunostainers (Ventana Medical System, Tucson, AZ), with appropriate positive and negative controls. BRAF antibody (VE1) was purchased from Spring Bioscience (Pleasanton, CA) and diluted at 1:50 for application.

2.4 Real-time PCR

DNA in paraffin-embedded tissue was extracted using QIAamp DSP DNA FFPE Tissue Kit per manufacturer's instructtions (Qiagen, Valencia, CA). BRAF V600E (1799 T>A) mutation at exon 15 was detected by real-time PCR using allele-specific TaqMan probes. The mutant probe was labeled with FAM-fluorophore while the wild-type probe with VIC-fluorophore. The amount of fluorescent emissions rendered by specific probe hybridization was associated with the amounts of PCR product, and analyzed by Qiagen Rotor-Gene Q MDx real time instrument. Minimum percentage of mutant DNA (limit of detection) needed is 10%, given sufficient DNA input.

2.5 Evaluation

The microarrays were independently reviewed by two board-certified pathologists and one pathology resident. IHC staining intensity in the cytoplasm of neoplastic cells was graded from 0 to 3+. A score of 0 was considered as negative, 1+ as equivocal, and 2+ or greater as positive. Cases that scored at least 2+ were tested for BRAF V600E gene mutation by real-time PCR for comparison.

3 Results

Forty-one out of 48 specimens remained intact in the cores after IHC processing (see Table 1). Six of the 41 cases (15%), were scored as positive (2+ or 3+) in at least one core by consensus (see Table 1, Figure 1). These 6 cases were

subsequently tested for the BRAF V600E mutation by PCR, and they were all negative. Additionally, weak nuclear staining, which was considered as non-specific, was seen in 14% (12/88) of the cores examined (see Table 1, Figure 2).

Table 1. Demographics and Immunohistochemical/PCR Results of High-Grade Sarcoma Samples Surviving Processing

ID	Age	Sex	Diagnosis	Microarray Location	Immunohistochemistry Score	PCR Result
2	71	F	Olfactory neuroblastoma	3B5/3D2	2+/2+	Negative
3	8	M	Rhabdomyosarcoma, alveolar and embryonal types	1D4/2D4	Negative/1+	*
4	1	M	Neuroblastoma, differentiated	3E2/3F5	Negative/Negative	*
6	21	M	Ewing's sarcoma	3A5/3C2	Negative/Negative	*
7	21	M	High-grade sarcoma consistent with synovial sarcoma	3B6/3D3	3+/3+	Negative
8	67	F	Undifferentiated high-grade pleomorphic sarcoma	1A2/1C8	Negative/1+	*
9	53	M	Epithelioid/spindle cell sarcoma most consistent with malignant peripheral nerve sheath tumor	1A3/1C7	Negative/2+	Negative
11	85	F	High-grade spindle cell sarcoma	3A4/3D6	Negative/Negative	*
12	96	M	High-grade sarcoma	1A5/1C1	Negative/Negative	*
14	16	M	High-grade sarcoma consistent with osteosarcoma	3C3/3E5	1+/Negative	*
15	74	F	Pleomorphic high-grade sarcoma	1A7/1C4	1+/2+	Negative
16	44	M	High-grade sarcoma	1C5/1F6	Negative/2+	Negative
17	79	M	High-grade sarcoma consistent with malignant fibrous histiocytoma	1B1/1D8	Negative/Negative	*
18	67	M	High-grade pleomorphic spindle cell sarcoma	1B2/1E7	Nuclear/Nuclear	*
19	44	M	High-grade sarcoma	1B3/1E8	Nuclear/Nuclear	*
20	54	F	High-grade sarcoma consistent with malignant fibrous histiocytoma	1B5/1E3	1+/1+	*
21	88	F	Malignant fibrous histiocytoma	1B6/1E2	Negative/Nuclear	*
22	58	M	Synovial sarcoma, monophasic	3A7/3E3	Negative/Negative	*
23	76	F	High-grade sarcoma	1B7/1E1	1+/1+	*
24	70	M	High-grade sarcoma consistent with malignant fibrous histiocytoma	1B8/1D1	Negative/Nuclear	*
25	49	F	High-grade sarcoma consistent with malignant fibrous histiocytoma	1E6/1F3	Negative/Nuclear	*
26	82	F	High-grade sarcoma	1A8/1F4	Negative/Nuclear	*
27	15	M	Poorly-differentiated sarcoma	2A3/2C7	Negative/Negative	*
28	73	M	Pleomorphic sarcoma consistent with malignant fibrous histiocytoma	2A4/2C8	Negative/Negative	*
29	84	F	Malignant fibrous histiocytoma	2A5/2D1	Negative/Nuclear	*
31	53	M	High-grade myxoid sarcoma	2A8/2E1	Negative/Negative	*
32	42	F	High-grade pleomorphic sarcoma consistent with malignant fibrous histiocytoma	2B1/2D7	Negative/Negative	*
33	69	M	High-grade sarcoma	3C4/3C7	Negative/Negative	*
35	79	M	High-grade sarcoma	2B4/2E2	Negative/Nuclear	*
36	36	F	Synovial sarcoma	1D6/2D6	1+/3+	Negative
37	46	F	High-grade myxoid sarcoma	2B5/2E3	Negative/Negative	*
38	50	M	High-grade sarcoma most consistent with malignant peripheral nerve sheath tumor	2B6/2E4	Negative/Negative	*
39	19	F	Clear cell sarcoma-like tumor	1D3/2D3	1+/Negative	*
41	75	F	High-grade myxoid sarcoma	2C1/2E6	Negative/Negative	*
42	80	M	Pleomorphic sarcoma most consistent with malignant fibrous histiocytoma	2C2/2F3	Negative/Negative	*
43	60	M	Pleomorphic sarcoma	2C3/2F4	Negative/Negative	*
44	85	F	High-grade sarcoma	2C4/2F5	Negative/Nuclear	*
45	13	F	Myxoid liposarcoma	1D5/2D5	Negative/Negative/Negative	*
46	36	F	High-grade sarcoma	3B7/3C1/3D4	Negative/Negative/Negative	*
47	71	M	Pleomorphic undifferentiated sarcoma	3D7/3E4/3B1	Negative/Negative	*
48	57	F	High-grade sarcoma	3E1/3E6	Negative/Nuclear	*

*Not done

ISSN 1925-4067 E-ISSN 1925-4075

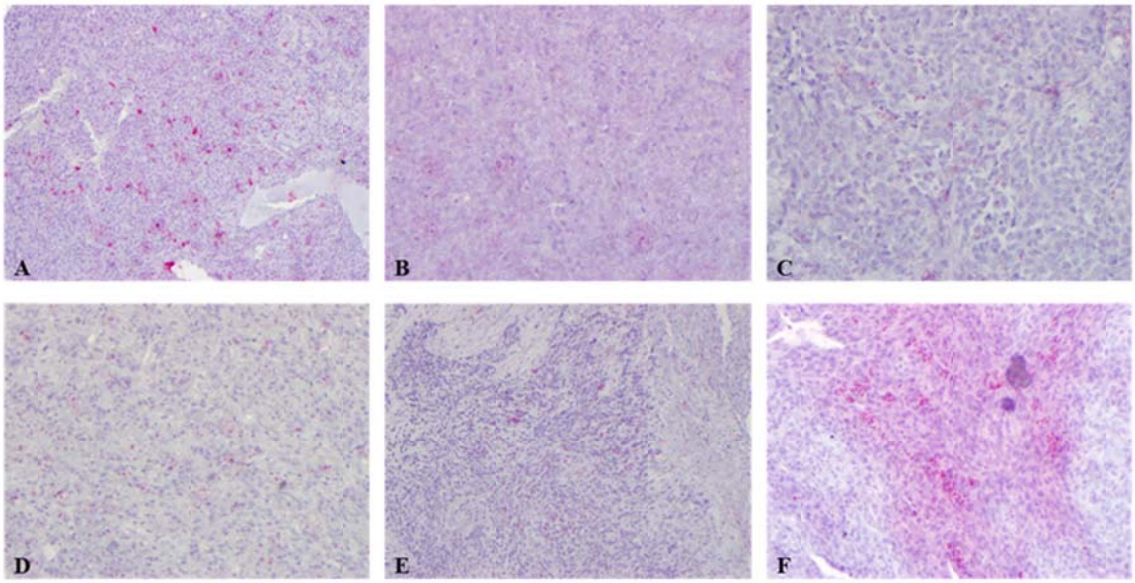

Figure 1. The six samples with positive immunohistochemistry for the VE1 antibody (20× magnification). Sample 2 demonstrates focal 2+ staining (A). Sample 7 demonstrates 3+ staining (B). Sample 9, 15 and 16 showed focal 2+ (C, D, and E respectively). Sample 36 demonstrates focal 3+ staining (F).

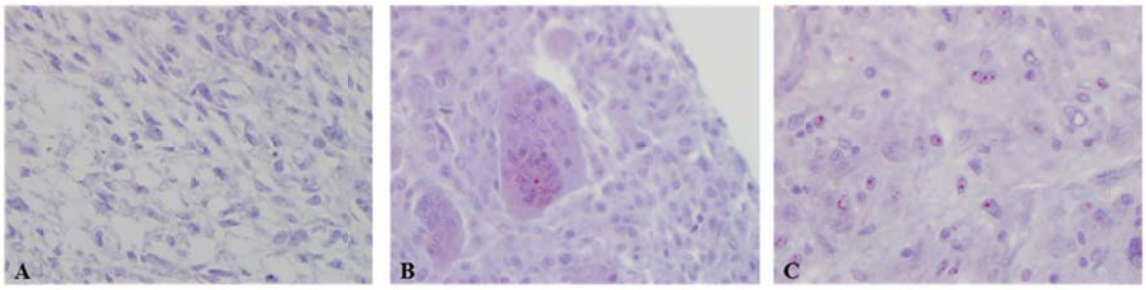

Figure 2. Examples of negative and nonspecific staining (40× magnification). Negative staining by the VE1 antibody (A). Giant cells staining positive for the VE1 antibody (B). Nonspecific nuclear positivity (C).

4 Discussion

BRAF V600E mutation in cutaneous melanoma is common (~50%) and has been used as a target for personalized therapy [3]. In colorectal adenocarcinomas, BRAF mutation status is increasingly being tested given its utility as a prognostic and predictive biomarker [8]. The mutation has also been reported in other neoplasms, such as thyroid carcinomas, gastrointestinal stromal tumors, ovarian and certain lung carcinomas. Although BRAF mutation in sarcomas was tested before [4, 5, 10, 11], it has not been widely studied with immunohistochemistry. Availability of a highly sensitive and specific monoclonal antibody (VE1) [3, 7, 9] against mutant BRAF V600E has allowed faster and cost-effective IHC detection [3], for potential targeted therapy [7]. In our study with appropriate positive controls, we performed IHC with VE1 antibody in 41 high-grade sarcomas, and found positive expression in 6 cases, with intensities ranging from 2+ to 3+. In addition, the presence of intratumoral staining intensity heterogeneity was a feature observed by all pathologists. All the positive cases by IHC were negative by our real time PCR, a validated laboratory developed assay, regarded as gold standard. Therefore, these positive results were considered as a non-specific immunoreaction, and results should not be used as a biomarker for targeted therapies or prognosis. Finally, based on these findings, we concluded that the BRAF V600E mutation is a rare event, if not at all, in unclassified high-grade sarcomas. At the same time, we concluded that due

to the significant non-specific expression of VE1 antibody in these sarcomas, caution should be exercised in the interpretation of the IHC results to avoid pitfalls.

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