Acta Derm Venereol 2016; 96: 128–129

SHORT COMMUNICATION

Melanoma with *BRAF* Mutation in Circulating Cell-free DNA despite no Mutation in the Primary Lesion: A Case Report

Atsuko Ashida, Hisashi Uhara, Asuka Mikoshiba, Kaori Sakaizawa, Naomi Kumagai, Hiroshi Koga and Ryuhei Okuyama*
*Department of Dermatology, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto 390-8621, Japan. *E-mail: rokuyama@shinshu-u.ac.jp*
Accepted Jun 25, 2015; Epub ahead of print Jun 30, 2015

Substantial advances have been made recently in the treatment of metastatic melanoma, with the advent of therapies such as *BRAF* inhibitors (iBRAF), which have been shown to be effective in the treatment of *BRAF* mutation-positive melanoma (1). The frequency of *BRAF* mutations is 50–60% in Caucasians and 20–30% in Asian populations (1–3). Mutation is usually determined by Sanger sequencing and high-sensitivity PCR-based methods, such as the Cobas 4800 BRAF Mutation Test (Roche Molecular Diagnostics, Basel, Switzerland), using primary or metastatic lesions. However, the accuracy of these tests is grossly influenced by the quality and quantity of the DNA extracted from the tissues. Thus, *BRAF* mutation can be missed in cases of contamination, widespread necrosis, or when only small quantities of DNA are available. We describe here a case of advanced-stage melanoma with a $BRAF^{V600E}$ mutation. Although the $BRAF^{V600E}$ mutation was not detected in the DNA of the primary lesion, it was detected in circulating cell-free DNA (cfDNA) in peripheral blood. cfDNA includes DNA from apoptotic and necrotic cancer cells, it harbours the genetic alterations present in the tumour (4, 5), and is useful for evaluating tumour features in patients with advanced cancer.

CASE REPORT

A 47-year-old Japanese woman was diagnosed with stage IV melanoma. She had a history of limb pain for 3 months, and computed tomography (CT) scans showed multiple bone, lung and lymph nodes metastases. A pigmented macule, measuring 39×14 mm, with extensive regression, was situated on the right abdomen. Histopathologically, she was diagnosed with melanoma. Although tumour cells were positive for S-100, MART-1 and HMB45, the lesion contained < 10% tumour cells with numerous pigment-laden macrophages. After obtaining written informed consent, we analysed the tumour for *BRAF* mutation using the Sanger method (3), but did not detect *BRAF* mutation in the primary lesion (Fig. 1a). We also performed Competitive Allele-Specific TaqMan PCR (castPCR: Life Technologies, Carlsbad, CA, USA) for $BRAF^{V600E}$, which has higher specificity than Sanger sequencing. Assay sensitivity was established by the manufacturer at 0.1%. However, cast-

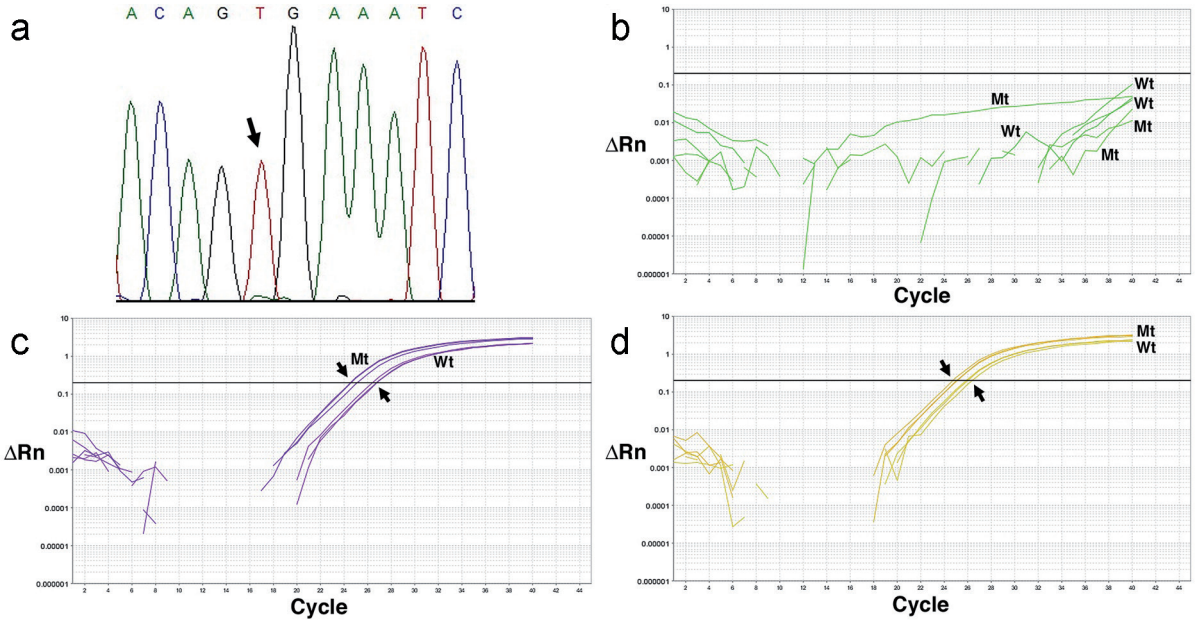

Fig. 1. (a) *BRAF* mutation analysis of the primary lesion using Sanger sequencing. The arrow indicates the wild-type profile. Three bases, GTG, encode the amino acid V at codon 600. (b) *BRAF* mutation analysis of the primary lesion using castPCR. Representative amplification of DNA from the primary lesion using *BRAFwild-type* and $BRAF^{V600E}$ probes. Analysis was performed in triplicate, and samples with a quantification cycle >37 were considered non-informative. Neither V600E-mutated nor wild-type curves crossed the threshold level (*black line*). BRAF mutation analysis of cfDNA derived from (c) plasma and (d) serum using Competitive Allele-Specific TaqMan PCR (castPCR: Life Technologies, Carlsbad, CA, USA). Patient samples were measured in triplicate. V600E mutated curves and wild-type curves crossed the threshold level (*arrows*). Mutation analysis was repeated 3 times, independently. MT: mutated-type; WT: wild-type.

 doi: 10.2340/00015555-2194
 ISSN 0001-5555

PCR amplified neither *BRAF*^{V600E} nor *BRAF*^{wild} from DNA of the primary lesion, suggesting that the abundant melanin in the tumour cells, which is known to inhibit PCR, interfered with the reaction (Fig. 1b). Since it was impractical to take biopsies of the metastatic lesions, we analysed cfDNA for the *BRAF* mutation. DNA was extracted from plasma and serum using the QIAamp Circulating Nucleic Acid Kit (Qiagen, Venlo, The Netherlands), and castPCR was performed using DNA from the plasma and serum. The results showed that *BRAF*^{V600E} comprised 63.2% and 49.8% of the total *BRAF* isolated from plasma and serum, respectively (Fig. 1c, d). In addition, no *BRAF* mutation was detected in samples from 5 healthy volunteers. Unfortunately, the general condition of the patient declined rapidly, and she died before iBRAF could be administered.

DISCUSSION

Apoptotic and necrotic cancer cells are a key source of cfDNA, harbouring the same genetic alterations present in the primary tumour (4). Detection of *BRAF* mutations in blood could represent a useful biomarker for non-invasive diagnosis. However, a crucial issue is that blood usually contains only a small amount of tumour-derived DNA. Most PCR methods developed to detect *BRAF*^{V600E} are limited by the presence of a high proportion of wild-type *BRAF* that interferes with cfDNA mutation assays. Several methods, including BEAMing (Beads, Emulsions, Amplification, and Magnetics) (6), droplet digital PCR (7), and next-generation sequencing (8), have been developed to facilitate mutation analysis from cfDNA in a highly specific and sensitive manner. However, these methods require specialized equipment. castPCR requires no specific instruments except a real-time PCR system, and is able to detect and quantitate rare mutations in samples containing large amounts of wild-type DNA. The high sensitivity of this method suggests that analysis of cfDNA isolated from blood for the *BRAF*^{V600E} mutation could help to identify those patients with melanoma who would benefit from iBRAF therapy.

The case described here shows that cfDNA is useful for identifying mutations in melanoma with regression, in which melanoma cells have disappeared or become reduced in number and have been replaced by pigment-laden macrophages with inflammatory infiltration. Regression in primary melanoma occurs in 10–35% of cases (9). Sanger analysis requires a tumour cell content of at least 40–50% to produce accurate results (10); analysis using samples with lower numbers of tumour cells can result in a failure to detect *BRAF* mutation and lead to inadequate treatment. In summary, in cases of melanoma with extensive regression, it may be beneficial to assess *BRAF* mutations in the blood as well as in the primary lesion.

ACKNOWLEDGEMENT

This study was supported by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of the Japanese government (AA).

The authors declare no conflicts of interest.

REFERENCES

1. Menzies AM, Long GV. Systemic treatment for BRAF-mutant melanoma: where do we go next? Lancet Oncol 2014; 15: e371–381.
2. Si L, Kong Y, Xu X, Flaherty KT, Sheng X, Cui C, et al. Prevalence of BRAF V600E mutation in Chinese melanoma patients: large scale analysis of BRAF and NRAS mutations in a 432-case cohort. Eur J Cancer 2012; 48: 94–100.
3. Ashida A, Uhara H, Kiniwa Y, Oguchi M, Murata H, Goto Y, et al. Assessment of BRAF and KIT mutations in Japanese melanoma patients. J Dermatol Sci 2012; 66: 240–242.
4. Schwarzenbach H, Hoon DS, Pantel K. Cell-free nucleic acids as biomarkers in cancer patients. Nat Rev Cancer 2011; 11: 426–437.
5. Thierry AR, Mouliere F, El Messaoudi S, Mollevi C, Lopez-Crapez E, Rolet F, et al. Clinical validation of the detection of KRAS and BRAF mutations from circulating tumor DNA. Nat Med 2014; 20: 430–435.
6. Taniguchi K, Uchida J, Nishino K, Kumagai T, Okuyama T, Okami J, et al. Quantitative detection of EGFR mutations in circulating tumor DNA derived from lung adenocarcinomas. Clin Cancer Res 2011; 17: 7808–7815.
7. Sanmamed MF, Fernandez-Landazuri S, Rodriguez C, Zarate R, Lozano MD, Zubiri L, et al. Quantitative cell-free circulating BRAFV600E mutation analysis by use of droplet digital PCR in the follow-up of patients with melanoma being treated with BRAF inhibitors. Clin Chem 2015; 61: 297–304.
8. Kukita Y, Uchida J, Oba S, Nishino K, Kumagai T, Taniguchi K, et al. Quantitative identification of mutant alleles derived from lung cancer in plasma cell-free DNA via anomaly detection using deep sequencing data. PLoS One 2013; 8: e81468.
9. Blessing K, McLaren KM. Histological regression in primary cutaneous melanoma: recognition, prevalence and significance. Histopathology 1992; 20: 315–322.
10. Warth A, Penzel R, Brandt R, Sers C, Fischer JR, Thomas M, et al. Optimized algorithm for Sanger sequencing-based EGFR mutation analyses in NSCLC biopsies. Virchows Arch 2012; 460: 407–414.