Merkel Cell Polyomavirus and HPV-17 Associated With Cutaneous Squamous Cell Carcinoma Arising in a Patient With Melanoma Treated With the BRAF Inhibitor Dabrafenib

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Importance: Approximately 10% to 25% of patients treated with BRAF inhibitors develop cutaneous squamous cell carcinoma (SCC), but the mechanism responsible has not yet been determined. We report what we believe to be the first case in which Merkel cell polyomavirus (MCPyV) and human papillomavirus subtype 17 (HPV-17) were associated with cutaneous SCC that developed during treatment with the BRAF inhibitor dabrafenib.

Observations: A 62-year-old woman with V600E BRAF-mutant metastatic melanoma enrolled in a phase 1 trial of dabrafenib, a selective inhibitor of V600-mutant BRAF kinase. During the first 6 weeks of treatment, the patient developed multiple skin lesions, including a 6-mm crusted papule on the left eyebrow, which was resected and, on pathology examination, revealed SCC. The DNA extracted from paraffin-embedded tissue was amplified by polymerase chain reaction for detection of MCPyV and epidermodysplasia verruciformis HPV (EV-HPV) types. Analysis of the cloned and sequenced polymerase chain reaction products revealed the presence of MCPyV and HPV-17 DNA. Other EV-HPV subtypes were not detected.

Conclusions and Relevance: To our knowledge, this is the first report demonstrating the coexistence of MCPyV and HPV-17 in cutaneous SCC. Because both viruses have oncogenic potential, their role in the development of BRAF inhibitor–related SCC merits further investigation.


INHIBITION OF BRAF IS A PROMISING therapeutic strategy for the treatment of patients with BRAF-mutant cancer (NCBI Entrez Gene NM_004333.4). Clinical trials of tyrosine kinase inhibitors that are highly selective for V600 BRAF mutations have demonstrated high response rates (50%-80%) at therapeutic doses, as well as improvement in overall and progression-free survival.1-3 Approximately 10% to 30% of patients who are treated with BRAF inhibitors develop squamous cell carcinoma (SCC) of the skin.1-3 A prevailing hypothesis suggests that compensatory CRAF activation in the setting of BRAF inhibition and in the presence of a RAS mutation plays a role in the development of these squamous cell tumors, but the underlying causes of these tumors are not definitively elucidated.1-3 Indeed, approximately 60% of SCC developing in patients treated with BRAF inhibitors harbor HRAS and KRAS mutations.4 Several viruses are associated with the development of cancer, including human papillomavirus (HPV), human herpes virus, Epstein-Barr virus, hepatitis B virus, hepatitis C virus, and human T-lymphotrophic virus I.10-11 Merkel cell polyomavirus (MCPyV) was first described in 2008 and is present in approximately 80% of Merkel cell carcinoma tumors, which is a primary neuroendocrine tumor of the skin.12,13 In addition, MCPyV is found in approximately 13% to 25% of cutaneous SCCs among immunocompetent individuals14-16 and less than 1% of nonmelanoma skin cancers among organ transplant recipients.17 Human papillomavirus infection, usually subtypes 16 and 18, is associated with SCCs of the oropharynx, cervix, penis, anus, and skin.18-22 Squamous cell carcinoma of the skin associated with HPV-17 has been reported in epidermodysplasia verruciformis (EV), a disorder in which flat wartlike lesions frequently evolve to SCC, and HPV-17 is present in approximately 22% of cutaneous SCCs. Coexistence of HPV-17 and MCPyV has not been previously reported. We report a case in which coexisting MCPyV and...
HPV-17 were found in a cutaneous SCC that developed in a patient with melanoma during treatment with the BRAF inhibitor dabrafenib.

**REPORT OF A CASE**

A 62-year-old woman with V600E BRAF-mutant melanoma metastatic to the lungs and in-transit lesions involving the left thigh and lower leg enrolled in a phase 1 trial of dabrafenib, 200 mg twice daily, which is a selective inhibitor of V600 mutant BRAF kinase. During the first 3 weeks, the patient developed a mildly pruritic, grade 2, maculopapular, folliculitis-like rash involving the face, scalp, chest, abdomen, and axillary area. The rash improved after treatment with oral diphenhydramine hydrochloride and methylprednisolone. The patient also developed keratoderma on the soles of both feet during this time, which worsened during weeks 4 to 6, and was treated with topical carbamide, 20%, cream.

Several skin lesions not present at the baseline examination were noted at the end of 6 weeks of treatment, including a 6-mm crusted papule on the left eyebrow in the midline approximately 2 cm superior from the eyelid margin (Figure 1), a 4-mm crusted hyperkeratotic papule on the right scalp vertex, a 3-mm crusted papule on the anterior neck, several scattered pedunculated papules on the neck consistent with acrochordons, and many 2- to 3-mm pink hyperkeratotic papules scattered on the chest, back, arms, and legs. The lesions on the scalp vertex, left lateral arm, and left eyebrow were biopsied and reviewed by a University of Texas MD Anderson Cancer Center dermatopathologist (V.G.P.). The right scalp vertex biopsy contained verruca vulgaris. The left lateral arm biopsy contained hyperplastic actinic keratosis. Dermatopathology evaluation of the left eyebrow lesion demonstrated invasive SCC, moderately differentiated, at least 3.3 mm thick, and present at the deep tissue edge (Figure 2). Perineural invasion was not identified. Because of the positive margin on the left eyebrow SCC biopsy, the patient subsequently underwent Mohs surgery. Histologic tumor clearance was obtained after 1 stage and 2 sections.

The patient’s multiple hypertrophic actinic keratoses and verruca vulgaris lesions were treated with cryotherapy. At the end of cycle 5 (each cycle being 3 weeks), results of a biopsy of a right forearm lesion revealed verruca vulgaris with focal cytologic atypia of keratinocytes, and results of a biopsy of a right temple lesion demonstrated actinic keratosis, present at tissue edges, with focal cytologic atypia of keratinocytes. A biopsy of normal skin from the left gluteal area was later performed at the end of cycle 23 for comparison. At the time of writing, the patient’s melanoma lesions had decreased in size by 22% according to Response Evaluation Criteria in Solid Tumors 1.1. In addition, she had completed 22 cycles (66 weeks) of treatment and continued to receive dabrafenib.

**RESULTS**

This study and treatment were performed in accordance with the guidelines of The University of Texas MD Anderson Cancer Center Institutional Review Board. The DNA was extracted from paraffin-embedded tissue biopsies (SCC of the left eyebrow, actinic keratosis of the left arm, and normal skin of the left gluteal area) (Genta Puregene Genomic DNA Purification Kit; Qiagen Inc).

![Figure 1. Left eyebrow squamous cell carcinoma before resection.](image1)

![Figure 2. Invasive squamous cell carcinoma, moderately differentiated, 3.3 mm thick. A, Marked cytologic atypia at the interface between the tumor and the dermis (hematoxylin-eosin, original magnification ×20). B, Notice the presence of large nuclei, prominent nucleoli, and mitotic figures (original magnification ×40).](image2)
Polymerase chain reaction was used for MCPyV detection. The assay was performed twice on tissue acquired from different tissue blocks of the left eyebrow lesion. The forward PCR primer sequence was 5'TGCGTTGGTATTAGCTGAAGTGT3', and the reverse primer sequence was 5'ACCAGTCAACTTCTCCCGAGTAG3'. These primers were derived from the small T antigen viral gene region of MCPyV and were expected to generate a 150-base pair (bp) MCPyV-PCR product.22 The PCR steps were 1 minute at 94°C, followed by 40 cycles each at 94°C for 1 minute, 63°C for 1 minute, and 72°C for 1 minute, and final extension of 10 minutes at 72°C. The PCR products were run on 2.0% agarose gel electrophoresis and visualized on a UV transilluminator. The obtained MCPyV-PCR fragment was excised from agarose gel, cloned (TOPO TA cloning kit for sequencing; Invitrogen Co), and sequenced. The obtained DNA sequences were subjected to computer-assisted alignment by the NCBI-BLAST program using sequence information from NCBI-GenBank.

Expected 150-bp putative MCPyV PCR fragments were generated in the SCC and actinic keratosis samples (Figure 3C). The cloning and sequencing of PCR products, as well as the computer analysis of the obtained sequencing data, confirmed the presence of MCPyV DNA in these samples. Subsequent testing at a different time and performed on a second distinct piece of the SCC sample again demonstrated the presence of MCPyV DNA. No MCPyV DNA was detected in the normal skin sample.

**HRAS AND KRAS MUTATION ANALYSIS**

Mutation detection was performed by amplifying purified DNA with primers designed to amplify coding regions of the gene. Primers were designed using a variety of software applications (Primer Express v3.0 [Applied Biosystems], Primer Quest [Integrated DNA Technologies], and Primer Blast [National Center for Biotechnology Information]). The PCR products were purified (Exo-SAP-IT; USB) and sequenced in both directions (BigDye Terminator; Applied Biosystems) and run on a DNA analyzer (3730 DNA Analyzer; Applied Biosystems). The sequence data files were aligned and compared with a reference sequence (from Ensembl) in commercial software (SeqScape Software v2.5; Applied Biosystems), and mutations were analyzed.

In the SCC sample, HRAS analysis revealed no mutations in codons 12, 13, or 61 but did identify a silent base change in codon 27 (81T>C) and noncoding base changes in introns 3 (11-22delGCTGGCCCTGG) and 6 (55G>T). The HRAS analysis included only exons 1 and 2 because the sample did not amplify other exons. The KRAS analysis revealed no mutations in codons 12 or 13. The KRAS codon 61 could not be evaluated because the sample did not amplify exon 2. The KRAS analysis included only exon 1 because the sample did not amplify other exons. Analysis of HRAS and KRAS mutation was attempted in the actinic keratosis, verruca vulgaris, and normal skin samples, but the available samples were inadequate for analysis.
The BRAF inhibitors have demonstrated significant antitumor activity in clinical trials. Therefore, further investigation is needed to determine why 10% to 30% of patients receiving these treatments develop cutaneous SCC. One hypothesis suggests that, in BRAF wild-type cells, binding of the BRAF inhibitor to BRAF induces RAS-dependent BRAF/CRAF dimerization, resulting in CRAF activation.4,9

In addition to SCC, a spectrum of keratinizing SCCs has been observed during treatment with BRAF inhibitors, including keratoacanthomas, actinic keratoses, and verruca vulgaris, and many of these lesions demonstrate focal cytologic atypia of keratinocytes. Indeed, our patient developed actinic keratosis and verruca vulgaris in addition to SCC of the skin. Similar findings have been reported among patients receiving sorafenib tolvaptate, a multikinase inhibitor that inhibits intracellular RAF kinases (CRAF, BRAF, and mutant BRAF) and cell surface kinase receptors (vascular endothelial growth factor receptor 2 [VEGFR-2], VEGFR-3, platelet-derived growth factor receptor β, c-Kit [CD117], and Fms-like tyrosine kinase [FLT-3]).

We report what we believe to be the first case of HPV-17 and MCPyV co-infection associated with SCC of the skin in a patient with melanoma treated with a BRAF inhibitor. It is conceivable that some cells have some degree of transformation due to HPV or MCPyV and that the transformation processes are progressed and/or accelerated by paradoxical CRAF activation. Another possibility is that viral reactivation caused by BRAF inhibitor occurs. Indeed, β-HPVs occur in normal skin, including HPV-17 in 13% to 31% of normal eyebrow hair samples, and MCPyV is found in 0% to 17% of normal skin tissue samples. Of interest in this regard, a recent large study demonstrated a correlation between genus β-HPV infection (including HPV-17) and the incidence of SCC of the skin in the general population, as well as potential enhancement of risk by immunosuppression. Furthermore, the eruption of verruca vulgaris, also known to be associated with HPV, in the BRAF inhibitor–treated patient described herein further supports a viral role for the simultaneous SCC of the skin. In addition, MCPyV has been detected frequently in SCCs from both immunocompetent and immunocompromised patients. In the general population, UV radiation is the major risk factor for SCC of the skin, and HPV may be a cofactor, working by disturbing cellular DNA repair and/or apoptosis in sun-exposed keratinocytes. Preclinical models show that activation of the mitogen-activated protein kinase (MAPK) pathway by RAF inhibitors requires concurrent RAS activation, and one potential cause of RAS activation is occult viral infection of keratinocytes. Therefore, BRAF inhibitor–mediated CRAF activation could accelerate transformation of previously infected cells. Preliminary results of a trial combining the BRAF inhibitor dabrafenib with the MAPK kinase inhibitor trametinib demonstrated a markedly lower incidence of cutaneous SCC (<1%) and other hyperproliferative skin lesions, further supporting a role for activated CRAF in the development of cutaneous SCC. The silent base change in codon 27 of HRAS and the noncoding base changes in introns 3 and 6 that were identified in our patient have not been reported previously in association with BRAF inhibitor–induced SCC, and their potential relationship to this patient’s SCC is unknown.

Limitations of this study include the single sample size. It remains unknown whether co-viral infection in SCCs of patients not receiving BRAF inhibitors occurs. If further studies demonstrate evidence of a viral role in such patients, then therapeutic strategies to prevent cell transformation could be investigated. However, since existing HPV vaccines (eg, Cervarix, GlaxoSmithKline; and Gardasil, Merck Inc) do not cross-react with HPV-17 and do not eliminate HPV in patients with latent infections, it seems unlikely that a vaccination strategy with these vaccines would be effective.

In conclusion, our study demonstrates MCPyV in an actinic keratosis and coexisting HPV-17 and MCPyV in a cutaneous SCC, developing within 3 weeks after starting a BRAF inhibitor in a patient in whom verruca vulgaris simultaneously appeared. To our knowledge, this is the first report demonstrating the coexistence of these viruses in these lesions and, taken together with their potentially oncogenic nature, suggests that their role in the development of BRAF inhibitor–related SCC merits further investigation. Still, the active role of MCPyV in SCC needs to be proven by study of expression of the potential viral oncoproteins, specifically T antigens. In addition, larger quantitative viral copy number determination studies are needed to better evaluate the role of HPV-17 and other β-HPVs, as well as MCPyV, in SCC of the skin that develops during treatment with a BRAF inhibitor and to determine definitively whether these viruses play a driver rather than a passenger role.
REFERENCES