# Combined squamous cell carcinoma and Merkel cell carcinoma of the vulva: Role of human papillomavirus and Merkel cell polyomavirus

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***Key words:*** human papillomavirus; Merkel cell carcinoma; Merkel cell polyomavirus; squamous cell carcinoma; vulva.

Merkel cell carcinoma (MCC), an uncommon and highly aggressive cutaneous malignancy, usually occurs on the sun-damaged skin of the elderly and is characterized by coexpression of neuroendocrine markers and CK20, a discriminant from other types of visceral neuroendocrine neoplasias. Since the discovery of Merkel cell polyomavirus (MCV), many researchers have confirmed its presence in about 80% of cutaneous MCCs.[1] Although some cutaneous MCCs were reported to be associated with squamous cell carcinomas (SCCs), such combined cases accounted for only a minor portion and the viral status appeared to be different from pure MCC.[1-4] Rarely, primary MCCs occur on the female vulva,[5] with or without combined SCCs,[6,7] the latter is often human papillomavirus (HPV) related.

*Abbreviations used:*

| | |
|---|---|
| HPV: | human papillomavirus |
| MCC: | Merkel cell carcinoma |
| MCV: | Merkel cell polyomavirus |
| PCR: | polymerase chain reaction |
| SCC: | squamous cell carcinoma |

## CASE REPORT

A 63-year-old woman had a vulvar tumor over the right labium majus for 5 years, with recent enlargement and pain. The erosive exophytic tumor measured 5 cm in its greatest dimension. No other cutaneous lesions were found. The excision specimen consisted of a nodular dermal tumor measuring 3.6 × 2.5 × 1.3 cm in size with an eroded surface and irregular tumor borders. Microscopically, the tumor was biphasic and composed of: (1) a moderately differentiated malignant squamous cell component with keratin pearl formation; and (2) a poorly differentiated basophilic malignancy consisting of cells in sheetlike pattern with high nucleocytoplasmic ratio, vesicular nuclei, and small to occasionally prominent nucleoli. Comedo necrosis was common, without peripheral palisading. There was an abrupt transition from the squamous component to the blue cell component (Fig 1). Immunohistochemically, the basophilic tumor cells expressed an immunoprofile of synaptophysin$^+$/chromogranin$^+$/CK$^+$ (dotlike)/CK20$^+$/CD56$^+$/CK5$^-$/p63$^-$/p16$^+$ (Fig 2), whereas the squamous part was synaptophysin$^-$/chromogranin$^-$/CK$^+$ (diffuse)/CK20$^-$/CD56$^-$/CK5$^+$/p63$^+$/p16$^+$, consistent with a primary tumor of combined SCC and MCC. Both parts were negative to MCV large T-antigen (CM2B4) antibody (sc-136172, Santa Cruz Biotechnology, Dallas, TX) (dilution 1:50). P53 was overexpressed in both components.

Genomic DNA from both components was collected from paraffin blocks via manual microdissection for HPV and MCV detection by polymerase chain reaction (PCR) and *TP53* gene mutation by direct sequencing. To increase the chance for MCV

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Funding sources: None.
Conflicts of interest: None declared.
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JAAD Case Reports 2015;1:196-9.
2352-5126

http://dx.doi.org/10.1016/j.jdcr.2015.04.005

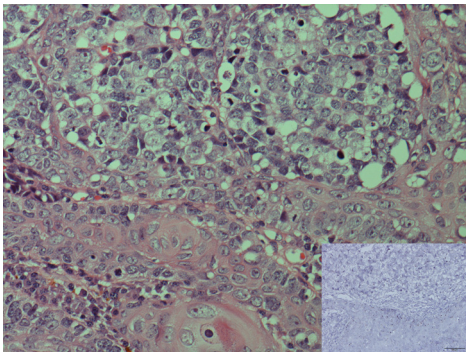

**Fig 1.** Combined squamous and Merkel cell carcinoma lesion. Area of transition between squamous (*lower*) and Merkel cell (*upper*) carcinoma. (Hematoxylin-eosin stain; original magnification: ×200.) *Inset*, Human papillomavirus E6/E7 messenger RNA expression in both components by RNAscope ISH.

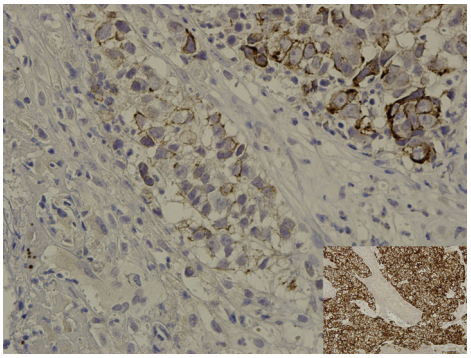

**Fig 2.** CK20 and synaptophysin immunostains. Right upper area with CK20$^{+}$ immunostaining is Merkel cell carcinoma (MCC) and left lower area is squamous cell carcinoma. (Hematoxylin-eosin stain; original magnification: ×400.) *Inset*, Synaptophysin$^{+}$ immunostaining in MCC area.

detection, 5 primer sets were used[8] and 2 ascertained MCV$^{+}$ MCCs were included as the biologic positive controls. No MCV DNA (Fig 3) or inactivating mutation in the *TP53* was detected. By contrast, an identical clone of type-16 HPV in both components was identified (Fig 3). To eliminate possible cross-contamination, we validated the result by RNAscope in situ hybridization to detect HPV E6/E7 messenger RNA expression on slides and identified cytoplasmic punctate dots in both components (Fig 1).

## DISCUSSION

MCC, known as a primary neuroendocrine carcinoma of the skin, tends to affect the elderly and is associated with chronic sun damage, immunosuppression, or both. Feng et al[9] first demonstrated the clonal integration of MCV DNA in the cutaneous MCC tumor cells. Later, several studies indicated that no MCV DNA was detected in other visceral high-grade neuroendocrine carcinomas. These discoveries stressed the oncogenic role of MCV in cutaneous MCCs. However, there are insufficient data regarding the role of MCV in MCCs arising from unusual sun-protected regions of the body. In the current case, a combined SCC component raises the suspicion of HPV DNA integration, rather than MCV DNA, as the pathogenesis of vulvar MCC development. We have determined this by PCR demonstrating the 2 malignant components (squamous and neuroendocrine) incorporating the same high-risk HPV DNA in their genome, but no MCV DNA. Moreover, combined squamous and neuroendocrine carcinomas of skin in sun-damaged regions share ultraviolet light–related *TP53* mutations in both components,[10] whereas in the current case the *TP53* mutation is lacking, further supporting the concept of MCV-independent and HPV-related tumorigenesis in vulvar MCCs.

Busam et al[1] reported 7 cutaneous SCC-MCC combined lesions, immunonegative to CM2B4 antibody, which was against an antigenic epitope on the MCV T antigen. Paik et al[3] reported another 15 SCC-MCC combined lesions, which were all immunonegative to CM2B4 antibody. Kuwamoto et al[2] reported 4 MCV$^{-}$ cutaneous SCC-MCC combined lesions, investigated by CM2B4 antibody and real-time PCR. Later, Mitteldorf et al[4] reported 2 patients with cutaneous SCC-MCC combined lesions, and detected MCV DNA in both cases and HPV type-6 DNA in one of them by PCR. It seems that by current definition, MCCs are a heterogeneous group of diseases that share similar morphological and immunohistochemical features and are related to miscellaneous conditions including MCV infection, ultraviolet light damage, arsenic intoxication, immunosuppression, and on the vulva, HPV infection.

Vulvar MCC is extremely rare with fewer than 20 cases reported in the English-language medical literature.[6,7] However, neuroendocrine carcinoma of the uterine cervix is a well-established entity that is highly correlated with HPV type-18 infection.[11] Generally, 2 major pathogenetic routes are linked to the development of vulvar carcinoma, ie, HPV infection and inflammatory dermatoses. The result from this study suggests that a portion of vulvar MCC is HPV related (cervical type), whereas the other portion is more akin to usual cutaneous MCV-related MCC (cutaneous type). An association with basaloid-type (or moderately to poorly differentiated) SCC may argue for the former, whereas an association

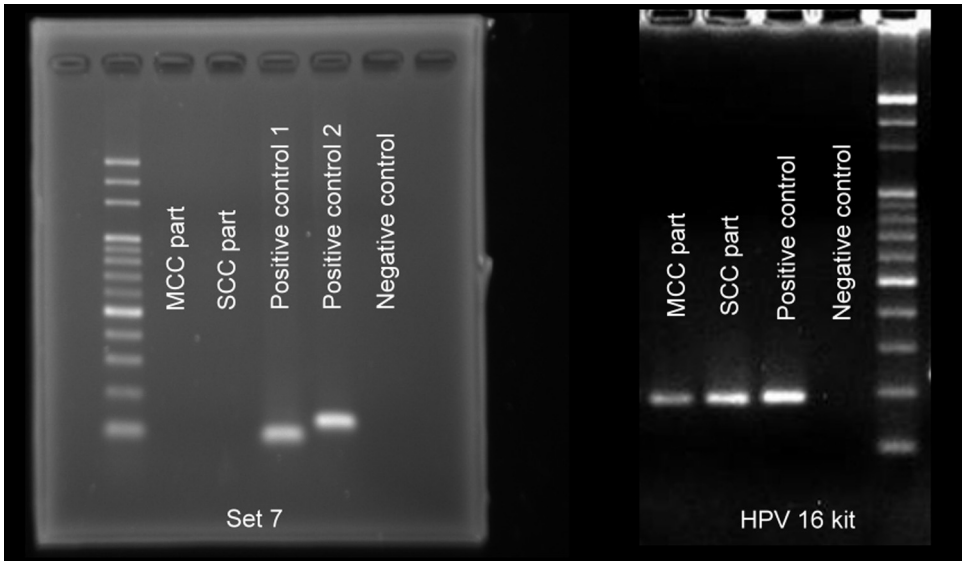


**Fig 3.** Polymerase chain reaction. Set 7 primer[8] showed no Merkel cell polyomavirus (*MCV*) DNA detected in squamous cell carcinoma (*SCC*) and MCC parts; human papillomavirus (*HPV*) 16 was detected in both SCC and MCC parts of the tumor (HPV gene chip revert blot hybridization, primers MY09/GP6$^{+}$ and GP5$^{+}$/GP6$^{+}$).

**Table I.** Merkel cell carcinoma of the vulva

| References | Age, y | Location | Size, cm | Combined SCC | MCV test | HPV test | *TP53* test |
|---|---|---|---|---|---|---|---|
| Tang et al,[13] 1982 | 67 | Labium minus | 1.5 | SCC in situ | ND | ND | ND |
| Bottles et al,[14] 1984 | 73 | Labium majus | 3 × 2 | SCC in situ | ND | ND | ND |
| Copeland et al,[15] 1985 | 59 | Labium majus | 8 × 6 | Absent | ND | ND | ND |
| Husseinzadeh et al,[16] 1988 | 47 | Labium minus | 4.2 × 3 | Absent | ND | ND | ND |
| Chandeying et al,[17] 1989 | 28 | Labium majus | 4 | Absent | ND | ND | ND |
| Cliby et al,[18] 1991 | 35 | Vulva | <1 | Absent | ND | ND | ND |
| Loret de Mola et al,[19] 1993 | 49 | Fourchette | 2 | Absent | ND | ND | ND |
| Chen,[20] 1994 | 68 | Vulva | 3 × 2.5 | Absent | ND | ND | ND |
| Scurry et al,[21] 1996 | 68 | Labium minus | 4 × 3 | Squamous differentiation | ND | ND | ND |
| Fawzi et al,[22] 1997 | 78 | Vulva | 5.5 | Absent | ND | ND | ND |
| Gil-Moreno et al,[23] 1997 | 74 | Labium majus | 9 | Absent | ND | ND | Polymorphism p53PIN3 without loss of heterozygosity |
| Hierro et al,[24] 2000 | 79 | Labium minus | 2.5 | Absent | ND | ND | ND |
| Khoury-Collado et al,[25] 2005 | 49 | Bartholin gland | 2 | Absent | ND | ND | ND |
| Pawar et al,[26] 2005 | 35 | Labium majus | 6 × 4 | Absent | ND | ND | ND |
| Mohit et al,[27] 2009 | 50 | Labium majus | 12 × 10 | Absent | ND | ND | ND |
| Sheikh et al,[6] 2010 | 63 | Labium majus | 7 × 5 | Absent | ND | ND | ND |
| Iavazzo et al,[7] 2011 | 63 | Vulva | 9 | Absent | ND | ND | ND |
| Current case | 63 | Labium majus | 3.6 × 2.5 | Present | Negative | Positive | Negative |

*HPV*, Human papillomavirus; *MCV*, Merkel cell polyomavirus; *ND*, not done; *SCC*, squamous cell carcinoma.

with well-differentiated SCC or dermatoses may suggest the latter. Unfortunately, the reported vulvar MCC cases in English-language literature seldom included this information, and tests for HPV and MCV were rarely done (Table I). The current case suggests that a subset of vulvar MCC is HPV related. This also suggests that the phenotype of MCC might represent a distinctive pathway of HPV-related tumorigenesis in certain body regions. Interestingly, Schrama et al[12] recently found the coexistence of MCV and HPV DNA in mutation-specific BRAF inhibitor-induced

epithelial proliferations. This raises the suspicion that an epigenetic scenario of MCV "hit-and-run tumorigenesis" might also be considered in the current case.

Based on the experiences from cutaneous MCCs, patients with combined SCC and MCC matched pure MCC in clinical aggressiveness. They tended to progress rapidly and have metastatic foci with pure neuroendocrine features. As for the patients with vulvar MCC, most of the patients died within the first 2 years after diagnosis. Our patient died of cancer-related cachexia and infection 6 months after initial diagnosis. Chemotherapy and radiotherapy provided only limited benefits. However, our case implicates that prophylactic vaccination against oncogenic HPV could prevent not only anogenital SCC, but also certain cases of HPV-associated vulvar/genital MCC. With efforts to elucidate the pathogenesis of vulvar MCCs (eg, HPV, MCV, ultraviolet related), further individualized therapy could be expected.

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