RESEARCH LETTER

CK20- and Virus-negative Merkel Cell Carcinoma with Complete RB1 Loss and *TP53*/*APC* Truncations

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To the Editor,

Merkel cell carcinoma (MCC) is a rare, aggressive skin cancer arising primarily in the dermis. It typically occurs on sun-exposed areas, such as the head, neck and upper extremities of elderly patients (1). Major etiological factors include Merkel cell polyomavirus (MCPyV) and ultraviolet (UV) radiation (1). While over 95% of MCCs show dot-like CK20 positivity, CK20-negative cases are often MCPyV-negative (2) and characterized by frequent UV-induced mutations (3). We report a case of CK20-negative, MCPyV-negative MCC on the lower leg, a site with relatively low UV exposure. The tumour lacked RB1 expression and harboured several cancer-related gene mutations.

An 88-year-old woman presented with a 7-month history of a red nodule on her left lower leg, which had rapidly increased in size over the preceding 2 weeks. At the time of initial examination, a red nodule approximately 22 mm in diameter was observed without symptoms (**Fig. 1**A). With no significant medical or family history and no evidence of metastasis or lymphadenopathy on CT, the tumour was resected under a clinical diagnosis of cutaneous squamous cell carcinoma (cSCC). Histopathologically, the tumour was a dome-shaped lesion with atypical cells with enlarged nuclei and numerous mitotic figures in the dermis (Fig. 1B). Immunohistochemistry (IHC) showed that the tumour cells were positive for synaptophysin, chromogranin A, CD56 and CK5/6 but negative for CK20, MCPyV, RB1, p53 (**Fig. 2**), S100 and TTF-1 (data not shown). Based on previous research, it is considered necessary to use a combined assessment involving IHC and PCR with multiple primer sets to determine whether MCC is MCPyV-positive or negative (4). Therefore, we used validated LT3 and LT4 primer sets (Large T) and TPO primer (internal control) (5). As a result, no MCPyV Large T DNA was detected by PCR. Based on these results, the patient was diagnosed with stage IIA (T2N0M0) MCC which was CK20-negative and MCPyV-negative. Recent studies show some MCPyV-negative MCCs arise from keratinocytes, as combined MCC/cSCC lesions share identical mutations (6, 7). Our case's CK5/6 positivity may reflect partial keratinocytic marker expression occasionally observed in virus-negative MCC. The surgical margins were negative, and there was no recurrence at 26 months without additional treatment.

It has been reported that many CK20-negative MCCs are also MCPyV-negative and tend to have a high incidence of UV-induced gene mutations (3). Although our case is also CK20-negative and MCPyV-negative, the lower legs are relatively less exposed to UV radiation than the head and neck. Additionally, it is known that numerous somatic mutant clones accumulate in the normal skin of elderly individuals (8). To elucidate the underlying mechanism of onset in the MCC, we performed next generation sequencing (NGS) Cancer panel (Axen

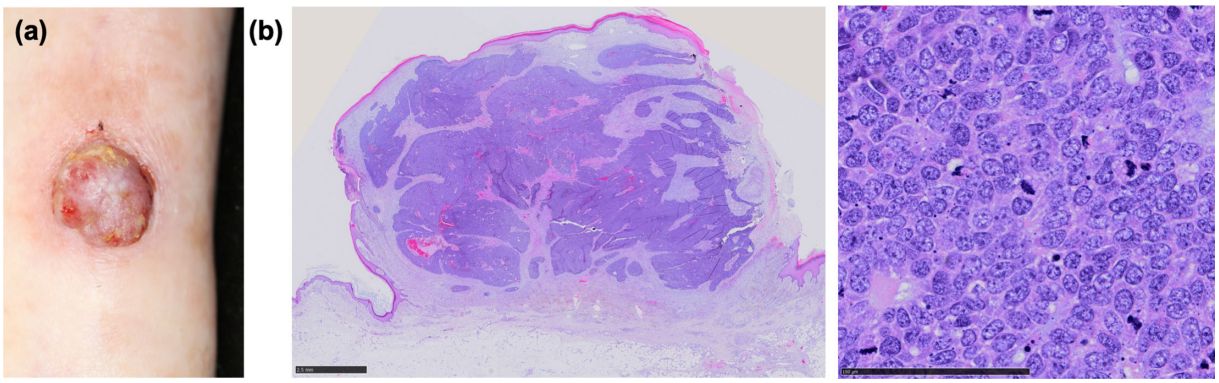

Fig. 1. [Clinical image and histopathological findings].

(A) Clinical image showing a red nodule on the left lower leg. (B) Histopathological findings demonstrated the tumour was composed of atypical cells with large nuclei and numerous mitotic figures in the dermis. Scale bars: left (low-magnification), 2.5 mm; right (high-magnification), 100 μm.

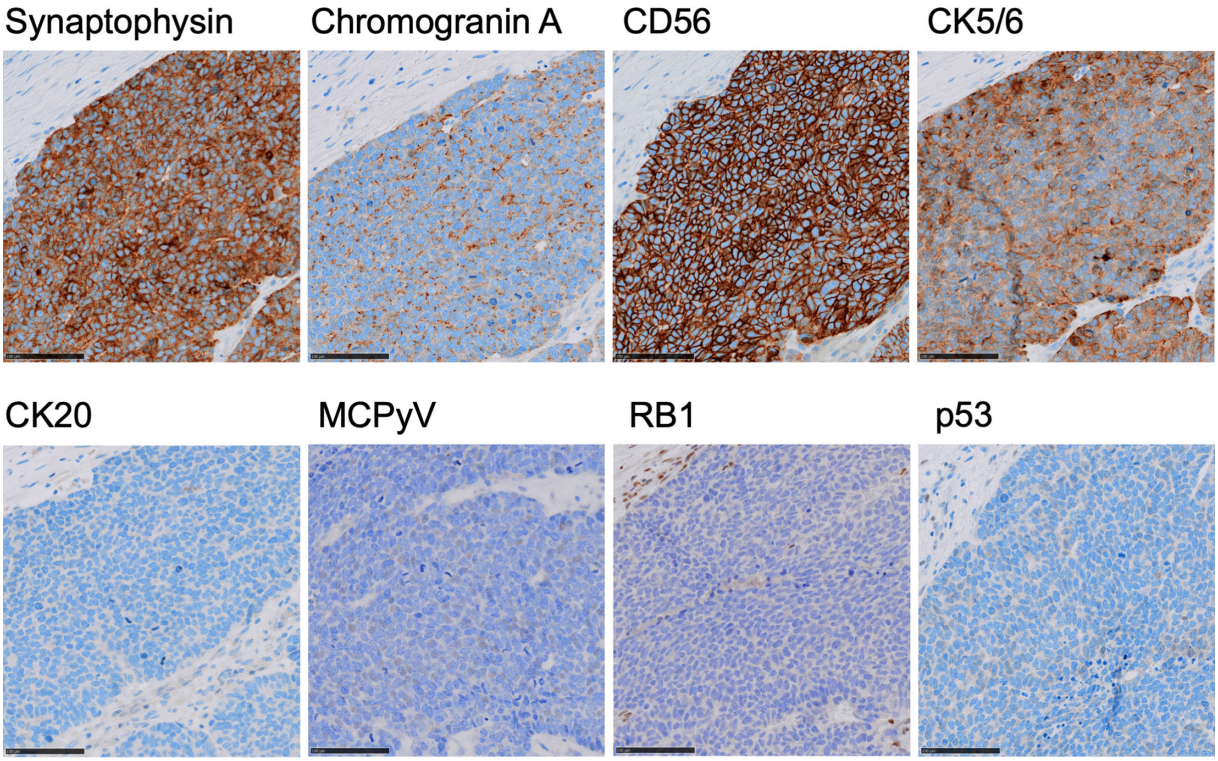

Fig. 2. [Immunohistochemistry findings].

Immunohistochemistry showed tumour cells were positive for synaptophysin, chromogranin A, CD56 and CK5/6 but negative for CK20, Merkel cell polyoma virus (MCPyV) Large T, RB1 and p53. Scale bars: 100 μm.

Cancer Panel 1, Macrogen). Targeted NGS with the tumour tissue identified truncating variants in *TP53* (c.902dupC, p.Gly302fs; allele frequency 66.9%) and *APC* (c.2063C>G, p.Ser688Ter; allele frequency 47.5%), and missense variant in *NOTCH1* (c.128C>T, p.Thr43Met; allele frequency 43.5%). This result is consistent with negative p53 expression in IHC (Fig. 2). The mutation of *NOTCH1* was predicted to be probably damaging by PolyPhen-2 (score=0.999), although its clinical significance remains uncertain. Notably, the detected truncating variants of *TP53* and *APC* are not typical UV-signature substitutions, indicating these mutations may be influenced by aging. However, the lack of mutational signature analysis in this panel makes it impossible to evaluate the presence of UV-related mutagenesis. Complete RB1 loss further supports RB pathway inactivation, a recurrent feature in virus-negative and CK20-negative MCC. While targeted NGS found no RB1 variants, cryptic alterations like copy-number loss or structural variants may exist. In this context, a truncating *TP53* alteration may further compromise checkpoint control and facilitate survival of RB-deficient clones, while the truncating *APC* variant could provide additional proliferative drive through dysregulated Wnt/β-catenin signalling acting in parallel to RB pathway loss. Notch signalling can intersect with cell-cycle regulation upstream of the RB pathway, including through modulation of the cyclin D-CDK4/6 axis; however, the contribution of the *NOTCH1* p.Thr43Met variant remains uncertain in this case. In conclusion, this case is consistent with previously reported virus-negative MCC characterized by RB pathway disruption. This case highlights a virus-negative MCC with complete RB1 loss occurring in the context of aging skin.

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IRB approval status: The patient was provided informed consent to participate in this study according to protocols approved by the Institutional Review Board at the recruiting institution (The University of Osaka, Osaka, Japan).

The authors have no conflicts of interest to declare.

REFERENCES

1. Albores-Saavedra J, Batich K, Chable-Montero F, Sagy N, Schwartz AM, Henson DE. Merkel cell carcinoma

demographics, morphology, and survival based on 3870 cases: a population based study. J Cutan Pathol 2010; 37: 20–27. https://doi.org/10.1111/j.1600-0560.2009.01370.x
2. Miner AG, Patel RM, Wilson DA, Procop GW, Minca EC, Fullen DR, et al. Cytokeratin 20-negative Merkel cell carcinoma is infrequently associated with the Merkel cell polyomavirus. Mod Pathol 2015; 28: 498–504. https://doi.org/10.1038/modpathol.2014.148
3. Harms PW, Collie AMB, Hovelson DH, Cani AK, Verhaegen ME, Patel RM, et al. Next generation sequencing of Cytokeratin 20-negative Merkel cell carcinoma reveals ultraviolet-signature mutations and recurrent TP53 and RB1 inactivation. Mod Pathol 2016; 29: 240–248. https://doi.org/10.1038/modpathol.2015.154
4. Starrett GJ, Thakuria M, Chen T, Marcelus C, Cheng J, Nomburg J, et al. Clinical and molecular characterization of virus-positive and virus-negative Merkel cell carcinoma. Genome Med 2020; 12: 30. https://doi.org/10.1186/s13073-020-00727-4
5. Moshiri AS, Doumani R, Yelistratova L, Blom A, Lachance K, Shinohara MM, et al. Polyomavirus-negative Merkel cell carcinoma: a more aggressive subtype based on analysis of 282 cases using multimodal tumor virus detection. J Invest Dermatol 2017; 137: 819–827. https://doi.org/10.1016/j.jid.2016.10.028
6. Walsh NM, Cerroni L. Merkel cell carcinoma: a review. J Cutan Pathol 2021; 48: 411–421. https://doi.org/10.1111/cup.13910
7. Kervarrec T, Appenzeller S, Samimi M, Sarma B, Sarosi EM, Berthon P, et al. Merkel cell polyomavirus–negative Merkel cell carcinoma originating from in situ squamous cell carcinoma: a keratinocytic tumor with neuroendocrine differentiation. J Invest Dermatol 2022; 142: 516–527. https://doi.org/10.1016/j.jid.2021.07.175
8. Martincorena I, Roshan A, Gerstung M, Ellis P, Van Loo P, McLaren S, et al. Tumor evolution. High burden and pervasive positive selection of somatic mutations in normal human skin. Science 2015; 348: 880–886. https://doi.org/10.1126/science.aaa6806