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Merkel Cell Polyomavirus Is More Frequently Present in North American than Australian Merkel Cell Carcinoma Tumors

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TO THE EDITOR

Merkel cell carcinoma (MCC) is an increasingly common neuroendocrine cancer of the skin. MCC is an aggressive malignancy that is a significant cause of nonmelanoma skin cancer deaths. Although the genetics of MCC are poorly characterized, it is well established that MCC is associated with advanced age and UV exposure. MCC is also linked to immune suppression; 8% of MCC patients are chronically

immunosuppressed, which is a 16-fold overrepresentation (Heath et al., 2008). In particular, MCC is linked to T-cell dysfunction associated with malignant, infectious, and iatrogenic causes (Miller and Rabkin, 1999; Engels et al., 2002; Heath et al., 2008).

Recently, Feng et al. (2008), described a novel polyomavirus that is associated with MCC. This virus, named Merkel cell polyomavirus (MCPyV), was present in 8 of 10 MCC

tumors as compared to 1 of 15 normal skin controls. Several clues suggest a possible functional role for this virus in cancer. MCPyV shares key features with the SV40 polyomavirus, an oncogenic virus in animals, such as the predicted ability of a viral T antigen protein to bind and inactivate the tumor suppressor Rb. Furthermore, MCPyV DNA was reported to be monoclonally integrated into six of the tumors, which implies that viral integration was an early event in MCC carcinogenesis and occurred before tumor expansion.

Abbreviations: MCC, Merkel cell carcinoma; MCPyV, Merkel cell polyomavirus

To confirm this recent discovery, we used quantitative real-time PCR to detect the presence or absence of MCPyV DNA in 37 MCC tumor specimens originating from 37 patients in North America and Australia. Two primer sets targeting MCPyV, but not other known human polyomaviruses, were used. One set amplifies the region corresponding to the common portion of large and small T antigen (nucleotides 236–374 of the “MCV350” sequence (EU375803)) and the second set amplifies a portion of the large T antigen surrounding a *Bam*H1 restriction site (nucleotides 1083–1181). Products of the second set were digested with *Bam*H1 and resolved on an acrylamide gel to verify product specificity. For a comparison control gene, primers were designed to amplify a gene on chromosome 2 (the *TPO* gene) because chromosome 2 is usually stable and only rarely gained or lost in MCC tumors (Van Gele *et al.*, 1998). Serial dilutions were employed to calibrate the relative efficiency of the PCR reactions and ensure that the $\Delta\Delta C_T$ method could be used to determine relative amounts of DNA between the viral and control genomic targets. The sensitivity of the real-time platform allowed us to detect levels of the viral gene as low as 1/5,000th the level of the control gene. All reactions were carried out in triplicate, and water and pooled human male lymphocyte DNA were included as negative controls. We also performed the PCR under less stringent conditions (lower annealing temperature). Under these conditions, we did not detect additional MCPyV-positive tumors, but cannot rule out the possibility of presence of a strain variant in the negative tumors.

We found that 16 of 37 MCC tumor tissues were positive for MCPyV DNA (43%). Interestingly, the proportion of tumors positive for MCPyV was much higher in tumors originating from North America than those originating from Australia (Figure 1). Among North American MCC specimens, 11 of 16 tumors (69%) were positive, which is similar to the initial report of 8 of 10 tumors (Feng *et al.*, 2008). In contrast, only 5 of 21 Australian MCC specimens

(24%) were positive for MCPyV. In addition, we tested 15 primary squamous-cell carcinoma tumors, and 15 apparently normal sun-exposed skin samples, from 30 North American patients without MCC. These patients had a similar age and gender distribution to the MCC patients. MCPyV was not detected in any of the normal skin DNA, but was present in 2 of 15 squamous-cell carcinoma tumors (13%).

The MCC tumor tissues tested included primaries and metastases. We observed MCPyV in 5 of 8 primary tumors, 3 of 13 recurrences, 7 of 15 nodal metastases, and in the single-studied distant metastasis. Outcome data were available for 30 of the 37 patients. In this subset, no survival difference was noted between virus-positive and virus-negative tumors using Kaplan–Meier analysis (data not shown). We found wide variation in the amount of viral DNA present. Indeed, the amount of viral DNA relative to control DNA varied over five orders of magnitude among the virus-positive MCC tumors (Figure 2).

These data confirm the important discovery of Feng *et al.*, that a polyomavirus is associated with MCC. Virus was present in MCC tumors, and was present in a similar percentage of North American tumors as previously reported.

Our data further present the interesting observation that North American MCC tumors are more frequently

MCPyV positive than Australian MCC tumors. This difference may be due to the increased sun exposure in Australia, making a possible viral contribution less frequent. We cannot exclude the possibility that a different and unknown MCPyV strain, not detectable with our specific assay, or perhaps an alternate virus is present in the Australian MCCs. The majority of Australian samples were nodal metastases, whereas most North American samples were primaries. However, we believe this is unlikely to account for the observed difference in viral prevalence because in two cases we had both primary and nodal tissues and all tissues were virus positive (data not shown). It is important to note that most of the Australian tumor specimens were collected in the 1990s and most North American specimens were collected after the year 2000. However, DNA quality from the Australian specimens was excellent both by spectrophotometry and PCR of control genes. Our observed percentages may over- or under-represent the true prevalence of MCPyV in MCC tumors because our left over surgical specimens are biased toward more advanced MCC than average. This was true of both the Australian and North American specimens.

Of 15 squamous-cell carcinoma samples 2 had detectable MCPyV DNA. A larger study is required to determine whether MCPyV is reproducibly more often present in squamous-cell carcinoma tumors than in normal skin.

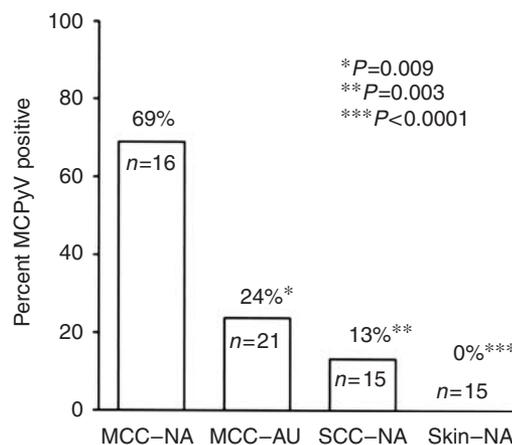


Figure 1. Prevalence of MCPyV DNA in samples from North America (NA) and Australia (AU).

Quantitative PCR was carried out targeting the T antigen region of the MCPyV genome. MCC, Merkel cell carcinoma; SCC, squamous-cell carcinoma; skin, UV-exposed skin from age- and sex-matched controls. *P*-values calculated using Fisher's exact test.

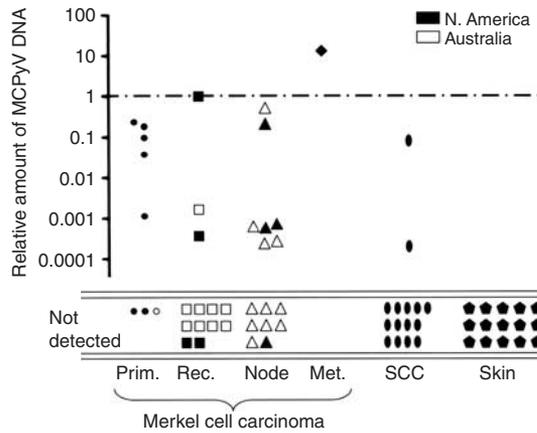


Figure 2. Marked variability of Merkel cell polyomavirus DNA quantity among MCC tumors. The amount of MCPyV DNA was compared to the amount of DNA of a control gene on chromosome 2 (*TPO*). Note the wide range in levels of MCPyV DNA. MCC primaries ($n=8$, circles), recurrences ($n=13$, squares), nodal metastases ($n=15$, triangles), and distant metastases ($n=1$, diamond) are compared. Also shown are squamous-cell carcinomas (ovals) and sun-exposed skin controls (pentagons). Tumors originating from North America are represented with closed symbols and those originating from Australia are represented with open symbols. Tumors represented as not detected had good amplification of control gene but no detectable MCPyV by both primer sets.

MCPyV represents an exciting direction for future studies in two major areas. The first area is functional characterization of the MCPyV and determination if it expresses biologically active viral proteins and whether it is in fact involved in the generation or maintenance of MCC tumors. The second major direction is an epidemiologic characterization of the prevalence of MCPyV in healthy people and in various tumors as this virus may plausibly be associated with other human diseases or malignancies.

Merkel cell carcinoma specimens were from the “Repository of Data

and Specimens for Merkel Cell Carcinoma Research” at the Fred Hutchinson Cancer Research Center. Normal sun-exposed skin and squamous-cell carcinoma tumors were from the “Main Biomarker Research Specimen Repository” at the University of Washington. The relevant Institutional Review Boards approved this study, and this study was performed with strict adherence to the Declaration of Helsinki Principles.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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MC Polyomavirus Is Frequently Present in Merkel Cell Carcinoma of European Patients

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TO THE EDITOR

Merkel cell carcinoma (MCC) is a rare, but highly aggressive neuroendocrine

cancer of the skin that occurs primarily in elderly immune-suppressed individuals. Little is known about the patho-

genesis of MCC. Now Feng *et al.* (2008) have identified a new polyomavirus present in MCC (MCPyV). In this regard, viral proteins expressed by polyomaviruses are known to initiate

Abbreviations: MCC, Merkel cell carcinoma; MCPyV, Merkel cell polyomavirus