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A Monoclonal Antibody to SV40 Large T Antigen (PAb416) Does Not Label Merkel Cell Carcinoma

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Abstract

Aims—Merkel cell carcinoma represents poorly differentiated neuroendocrine carcinoma of cutaneous origin. In most studies the vast majority of Merkel cell carcinomas are Merkel cell polyomavirus (MCPyV)-associated. SV40 polyomavirus immunohistochemistry is typically used in the diagnosis other polyomavirus-associated diseases including tubulointerstitial nephritis and progressive multifocal leukoencephalopathy, given cross-reactivity with BK and JC polyomaviruses. MCPyV-specific immunohistochemistry is commercially available, but if SV40 immunohistochemistry also cross-reacted with MCPyV that would be advantageous from a resource-utilization perspective.

Methods and results—Tissue microarrays were constructed from 39 Merkel cell carcinomas, 24 small cell lung carcinomas, and 18 extrapulmonary visceral small cell carcinomas. SV40 large T antigen immunohistochemistry (clone PAb416) was performed; MCPyV large T antigen immunohistochemistry (clone CM2B4) had been previously performed. UniProt was used to compare the amino acid sequences of the SV40, BK, JC, and Merkel cell polyomavirus large T antigens, focusing on areas recognized by the PAb416 and CM2B4 clones. SV40 immunohistochemistry was negative in all tumors; MCPyV immunohistochemistry was positive in 38% of Merkel cell carcinomas and 0% of non-cutaneous poorly differentiated neuroendocrine carcinomas. UniProt analysis revealed a high degree of similarity between SV40, BK, and JC viruses in the region recognized by PAb416. There was less homology between SV40 and MCPyV in this region, which was also interrupted by 2 long stretches of amino acids unique to MCPyV. The CM2B4 clone recognizes a unique epitope in one of these stretches.

Conclusions—SV40 large T antigen immunohistochemistry does not cross react with MCPyV large T antigen and, thus, does not label Merkel cell carcinoma.

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Keywords

Merkel cell carcinoma; polyomavirus; large T antigen; SV40; PAb416; CM2B4; immunohistochemistry

Introduction

Merkel cell carcinoma represents poorly differentiated neuroendocrine carcinoma of cutaneous origin.(1, 2) The disease is uncommon, though sharply increasing in incidence, with the 1600 cases diagnosed in the United States each year representing a 400% increase over the last 25 years.(3) Its 33% associated mortality exceeds that of melanoma.(4) Given clinical associations with older age and immunosuppression, investigators hypothesized a possible infectious etiology. Utilizing an approach they dubbed “digital transcriptome subtraction,” in which diseased tissue is subjected to high-throughput sequencing, alignment and “subtraction” of known human sequences, and alignment of residual sequences to known infectious agents, Feng and colleagues identified a novel polyomavirus, which they named Merkel cell polyomavirus (MCPyV).(5) They showed that the virus is clonally integrated into most Merkel cell carcinomas and that the virus undergoes mutations that truncate its large T antigen, which are critical to oncogenesis.(6)

The same group developed a mouse monoclonal antibody (CM2B4) to a unique 14 amino acid sequence in exon 2 of the large T antigen.(7) The antibody subsequently became commercially available and was shown to be a sensitive and specific marker for Merkel cell carcinoma.(8) CM2B4 was only available from a single vendor. After we had optimized staining in our research laboratory but before CM2B4 was onboarded in our clinical laboratory, we learned that the vendor had reached an agreement (May 2016) with the United States Department of Agriculture (USDA) for alleged violations of the Animal Welfare Act (AWA) over a time period from 2012–2015.(9) Our diagnostic immunohistochemistry laboratory (along with many others) worked to immediately find alternatives for all assays utilizing primary antibodies from this vendor.

Merkel cell polyomavirus is one of thirteen known human polyomaviruses (8 have been discovered subsequent to MCPyV). Polyomavirus infection is ubiquitous and typically asymptomatic. The most familiar human polyomaviruses are BK virus and JC virus. BK (and occasionally JC) virus causes tubulointerstitial nephritis in ~5% of kidney transplant patients (and rarely in other settings), while JC virus is the etiologic agent in progressive multifocal leukoencephalopathy, usually in AIDS patients. Polyomavirus immunohistochemistry is used in urine cytology to confirm the presence of viral cytopathic effect in “decoy cells” (the presence of which may precede the development of nephropathy), is gold standard for the diagnosis of BK virus nephropathy in transplant kidney core biopsies, and is typically used in brain biopsies or at autopsy to support a diagnosis of progressive multifocal leukoencephalopathy. Antibodies to the monkey polyomavirus SV40, which is known to have a high degree of sequence homology to the BK and JC viruses, are typically utilized clinically.

Given known cross-reactivity of monoclonal antibodies to SV40 with other human polyomaviruses, we hypothesized that they might also label Merkel cell carcinoma, which would obviate the need to onboard CM2B4, desirable from both resource utilization and ethical perspectives. We demonstrate here that the most commonly utilized anti-SV40 immunostain does not, in fact, label Merkel cell carcinoma, which appears to relate to large T antigen sequence divergence in the antigenic region of interest.

Materials and methods

Poorly differentiated neuroendocrine carcinomas were identified from the surgical pathology archives of the University of Iowa Hospitals and Clinics, including Merkel cell carcinoma and small cell carcinoma of lung or extrapulmonary visceral origin. Original glass slides from 81 tumors were reviewed, the diagnosis was confirmed, and a “best tumor block” was identified. Tissue microarrays (TMAs) were constructed using the Manual Tissue Arrayer MTA-1 (Beecher Instruments; Sun Prairie, WI), with tumors arrayed as triplicate 1 mm cores.

SV40 large T antigen immunohistochemistry on an Autostainer Link 48 (Agilent Dako; Santa Clara, CA) was performed on 4- μ m-thick tissue sections after deparaffinization, rehydration, and PT Link (Agilent Dako) heat-induced epitope retrieval in Target Retrieval Solution (pH 9; Agilent Dako) using the mouse monoclonal antibody PAb416 (Calbiochem/EMD Millipore; Temecula, CA; 1:200 dilution; 30 minute incubation) and the polymer-based EnVision FLEX+ detection system (Agilent Dako; 30 minute incubation). BK polyomavirus nephropathy served as the positive control. MCPyV large T antigen immunohistochemistry (mouse monoclonal CM2B4; 1:200 dilution) had been performed previously, with a whole section of Merkel cell carcinoma serving as the positive control. (10) SV40 and CM2B4 immunostains were considered positive given any staining.

We subsequently explored SV40, BK, JC, and MCPyV amino acid homology in the regions recognized by the SV40 and MCPyV large T antigen primary antibodies. PAb416 recognizes an epitope within amino acids 83–128 of the SV40 large T antigen,(11) while CM2B4 was raised against amino acids 116–129 of the MCPyV large T antigen.(7) The UniProtKB protein knowledgebase was queried for the amino acid sequences of the relevant polyomavirus large T antigens and then compared using the UniProt Align tool (<http://www.uniprot.org/>).(12) This research was conducted with University of Iowa Institutional Review Board approval.

Results

SV40 large T antigen immunohistochemistry was negative in all 39 Merkel cell carcinomas tested, as well as 24 and 18 small cell carcinomas of lung and extrapulmonary visceral origin. CM2B4 immunohistochemistry had been positive in 15/39 (38%) Merkel cell carcinomas and 0 (0%) of the 42 visceral small cell carcinomas (Figure 1).

In the 46 amino acid stretch recognized by the PAb416 monoclonal antibody, BK and JC viruses demonstrated 70% amino acid identity and 91% identity or similarity to SV40. MCPyV demonstrated 41% identity and 74% identity or similarity to SV40; in addition,

areas of homology within the 46 amino acid SV40 sequence were interrupted by stretches of 110 and 66 amino acids unique to the MCPyV large T antigen. The CMB24 antibody was raised against a peptide based on a 14 amino acid sequence contained within that 110 amino acid stretch and, thus, would not be expected to cross react with these other polyomaviruses.

Discussion

Although antibodies purportedly specific to various early or late antigens of BK and JC viruses are commercially available, “pan-polyomavirus” immunohistochemistry using antibodies to SV40, especially to its large T antigen, are most commonly utilized in diagnostic pathology. Among 30 monoclonal antibodies to the SV40 large T antigen evaluated in a series of JC virus transformed or infected cells and tissues, PAb416 performed best.(13) While many of the antibodies gave strong signal in an SV40-transformed cell line, only PAb416 and PAb108 also labeled a JC virus-transformed cell line, a JC-induced hamster tumor xenograft, and human progressive multifocal leukoencephalopathy tissue, with much stronger signal from the former. In the Banff Initiative for Quality Assurance in Transplantation (BIFQUIT) study on reproducibility of polyomavirus immunohistochemistry in kidney allografts, 68% of 60 participating laboratories utilized the PAb416 clone, which was associated with more reproducible results than those seen from the minority of laboratories using alternative antibodies.(14) As demonstrated in our study, though, SV40 large T antigen immunohistochemistry utilizing this clone will not detect MCPyV large T antigen.

Our results corroborate those of Shuda and colleagues.(7) In the initial study that described the generation and evaluation of the CM2B4 monoclonal antibody, they performed immunofluorescence and immunoblotting experiments with MCPyV, JC, and BK virus large T antigen-transfected human embryonic kidney 293 cells. While CM2B4 was specific for MCPyV large T antigen, PAb416 recognized JC and BK and not MCPyV large T antigen. They also examined 22 other SV40 large T antigen monoclonal antibodies by immunoblotting, none of which reacted with MCPyV large T antigen: PAb101, 108, 204, 210, 211, 216, 405, 407, 409, 419, 423, 430, 431, 433, 441, 442, 602, 603, 605, 606, 901, 902.

Merkel cell polyomavirus is generally detected in 80% of Merkel cell carcinomas by PCR and at a similar rate by immunohistochemistry, though the frequency has been significantly lower in a few outlier studies.(8) Notably, in one Australian study CM2B4 immunohistochemistry was only positive in 21% (19/89) of pure Merkel cell carcinomas. (15) That all Merkel cell carcinomas are not MCPyV positive and that the frequency of MCPyV-positivity appeared much lower in Australian patients led investigators to hypothesize that Merkel cell carcinoma actually represented two diseases, one attributable to viral oncogenesis and another to UV-induced damage. This hypothesis has subsequently been supported by the results of next generation sequencing experiments, which have highlighted the mutational landscape of MCPyV-positive versus negative cases (see Table). (16, 17) The frequency of CM2B4 positivity in our cohort (38%) is lower than in other Western cohorts. Although this is perhaps due, at least in part, to unique characteristics of our patient population (Iowa is distinctly agricultural and thus sun-exposed, though it would

never be mistaken for Australia), it is quite possible that the actual frequency of MCPyV-positivity is higher, with false negatives attributable to a suboptimal immunohistochemical staining protocol or use of TMA rather than whole sections. Although these concerns led us to hold off on publishing an initial abstract, we chose to include this datapoint here as evidence that at least some of our Merkel cell carcinomas are virus-associated, and, thus, if PAb416 cross-reacted with MCPyV large T antigen, at least some of the Merkel cell carcinomas should have been positive.

As mentioned in the introduction, from the time it became commercially available, CM2B4 was only offered by a single vendor. That vendor had run afoul with the Animal and Plant Health Inspection Service of the USDA on numerous occasions, which culminated in a settlement with the United States government in which the vendor agreed to pay a \$3.5 million fine, had their AWA license revoked, and was ordered to cease activities as a “research facility” (as defined by the AWA). Despite this agreement, the company is still in business. In close reading of the Animal Welfare Act, while the term “animal” includes dogs, cats, primates, guinea pigs, hamsters, and rabbits, it specifically excludes “birds, rats of the genus *Rattus*, and mice of the genus *Mus*, bred for use in research.”

The ability to identify MCPyV has diagnostic, appears to have prognostic, and, in the future, may have predictive implications. While an immunopanel including TTF-1 and CK20 is generally useful in distinguishing small cell lung carcinoma (TTF-1+/CK20-) from Merkel cell carcinoma (TTF-1-/CK20+), small cell carcinomas of extrapulmonary visceral origin variably express TTF-1 and occasionally express CK20, leading to diagnostic ambiguity. While in our experience CK20 is typically expressed by only 5–10% of extrapulmonary visceral small cell carcinomas overall, primary parotid tumors are well-known to be TTF-1-/CK20+ (60–75% of cases).⁽⁸⁾ The distinction of a primary small cell carcinoma of salivary gland origin from Merkel cell carcinoma, which is a diagnostic dilemma 2 or 3 times a year in our practice, is the diagnostic setting in which the availability MCPyV immunohistochemistry would be most desirable. Entirely analogous to head and neck squamous cell carcinoma, in which there are viral (high-risk human papillomavirus) and non-viral tumors, MCPyV-positivity appears to connote a more favorable prognosis, possibly attributable to a lower mutational burden and/or to intrinsic viral-tumor-induced immunity. In a Finish population-based study, with a MCPyV-positivity rate of 80%, the 5-year-survival rate in virus vs. non-virus-associated tumors was 45% vs. 13%, respectively. (18) Given distinct tumor-associated immune complements and genetic profiles, MCPyV status will likely be incorporated into future clinical trial designs.

Conclusions

Most Merkel cell carcinomas are polyomavirus-associated. The ability to detect MCPyV has diagnostic and prognostic implications. BK and JC viruses are readily detectable using SV40 large T antigen immunohistochemistry. MCPyV large T antigen immunohistochemistry is commercially available, but if SV40 immunohistochemistry could be used instead, it would be advantageous from a resource-utilization perspective. Furthermore, ethical concerns have been raised surrounding the single vendor from which CM2B4 was commercially available. We have shown here that SV40 large T antigen immunohistochemistry using the PAb416

mouse monoclonal antibody does not label Merkel cell carcinoma. Although unfortunate, we have further shown that this is likely attributable to large T antigen amino acid sequence divergence between SV40 and MCPyV. Subsequent to our performing this study CM2B4 immunohistochemistry has been offered by a second vendor (Merck/Millipore Sigma), and we plan to onboard it in our diagnostic laboratory.(19)

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Figure 1.

A–D, Immunohistochemistry in MCPyV-Associated Merkel Cell Carcinoma. Tumor is disposed as sheets of large cells with powdery chromatin (A); CK20 is strongly expressed, with some dot-like staining (B); MCPyV large T antigen is diffusely, strongly expressed, detected with the CM2B4 clone (C); SV40 large T antigen immunostain (PAb416) is negative (D) (original magnification of each image 400x).

Table 1

Characteristics of MCPyV-Positive and Negative Merkel Cell Carcinomas

	MCPyV-Positive	MCPyV-Negative
Etiopathogenesis	Viral oncoprotein-associated	UV radiation-associated
Frequency	80%	20%
RB status	Inactivated by large T antigen	Inactivated by mutation
<i>TP53</i> status	Wild type	Mutated
Mutational burden	Very low	Very high
C>T transitions (characteristic of UV-associated DNA damage)	Less frequent	Very frequent
Tumor-associated lymphocytes	More frequent	Less frequent
Prognosis	More favorable	Less favorable