

SPECIAL REPORT:

Merkel Cell Polyomavirus: The Seventh Human Cancer Virus?

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The recent discovery of a new human polyomavirus in Merkel cell carcinoma (MCC) has markedly changed the directions and opportunities for research on this enigmatic cancer.¹ MCC has several epidemiologic features that suggest it might have an infectious etiology, the most important of which is the strong association between MCC and immunosuppression.

Feng, et al developed a direct sequencing approach called *digital transcriptome subtraction* (DTS) to discover new viral infectious agents in human cancers.² This technique is based on creating complementary DNA (cDNA) from RNA extracted from fresh tumor tissue through reverse transcription and exhaustively sequencing the cDNA library. All sequences matching known human sequences are then computationally subtracted. The remaining nonmatching — presumably non-human — sequences are candidates that may represent pathogen cDNAs (**Figure 1**).

Given that hundreds of thousands of sequences are screened in DTS, even miniscule error rates in sequencing or analysis can lead to dozens of potential candidate sequences that do not match the human genome. Furthermore, highly polymorphic human sequences such as mitochondrial DNA and immunoglobulin hypervariable regions can also pass through computational screens to become part of the pool of candidate sequences. Thus, the bulk of the effort in DTS involves careful screening and selection of high-quality sequences prior to computational subtraction.

Discovery of Merkel Cell Polyomavirus

Feng and colleagues applied DTS to RNA extracted from four human MCC tumors. After examining 400,000 cDNA sequences, one cDNA was found with homology to a large tumor (T) antigen sequence belonging to polyomaviruses—a group of viruses known to cause cancers in experimental animals, particularly rodents. Flanking viral DNA regions were then isolated, allow-

ing identification of the entire 5.4-kilobase genome of a new human polyomavirus, termed Merkel cell polyomavirus (MCV or MCPyV).

The MCV genome includes an early coding region encoding the large tumor (T) and small tumor (t) antigen proteins named for their ability to induce tumors and cell transformation. MCV also possesses a late coding region, which encodes the viral capsid proteins VP1 and VP2/3.¹ MCV large T antigen, like large T antigens from other polyomaviruses, is multifunctional and possesses highly conserved domains that bind known host cell tumor suppressor proteins. Other large T antigen domains are involved in viral DNA replication. The MCV genome is normally a circular double-

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stranded DNA; T antigen binds to the viral replication origin, then a helicase domain in the T antigen carboxyl region opens the double-stranded DNA to allow the viral DNA to replicate.

Evidence of a Causative Role

Polyomaviruses are controversial candidates for causing human cancer.³ Two of them, BK virus and JC virus, have been isolated from human cancers, but proof of an etiopathogenic role has been elusive. Similarly, simian virus 40 (SV40) induces tumors in experimental animals and has been proposed to cause human cancer, but there is no clear evidence for involvement of this virus in human disease.

In their initial report, Feng, et al found 7 of 10 MCC tumors from different patients positive for MCV by Southern (blot) hybridization, a technique that is highly

specific and not prone to contamination. An additional tumor was positive for the virus but only with PCR, a less reliable but highly sensitive method. This high rate of MCV positivity has been subsequently confirmed in over a dozen studies around the world that included several hundred MCC patients.⁴⁻⁶

Extensive surveys have also been performed on other tumors, including cutaneous melanomas, basal cell carcinomas, and squamous cell carcinomas. Most,⁷⁻¹⁰ but not all,¹¹⁻¹² studies have found that MCV infection is specific for MCC. In fact, MCV is clonally integrated into the Merkel cell carcinoma genome.¹ This suggests that MCV infection preceded initial cancer development and that the virus integrated into the host genome prior to clonal tumor cell expansion, a pattern analogous to that of high-risk human papillomavirus integration into cervical carcinoma cells. Since MCV cannot “excise” itself from the human genome, the virus is in a non-transmissible, dead-end state in MCC tumor cells. Integration occurs at different sites in the genome in different individual cases;^{1,13} in one case, metastatic tumor had the same monoclonal integration pattern as the primary tumor, showing that the metastasis arose from a single cancer cell already infected with the virus.¹ Although this does not rule out insertional mutagenesis playing a role in MCV-related cancer, no obvious patterns for virus integration into the human genome have been described to date.

A peculiar set of mutations are also present in tumor-derived viruses, which supports MCV having a direct etiopathogenic role in MCC.¹⁴ These tumor-specific signature mutations truncate the helicase portion of T antigen and are not present in viruses isolated from morphologically normal tissues. In the polyomavirus, the large T antigen regulates the life cycle of the virus as well as the cell cycle of the host cell. The latter occurs via interaction with the tumor suppressor gene p53 and the members of the retinoblastoma protein (Rb) gene family. The mutated viruses retain their ability to inhibit the retinoblastoma protein but can no longer initiate replication and would be lost without integration into the cellular genome.¹⁴ For this reason, the virus cannot be a

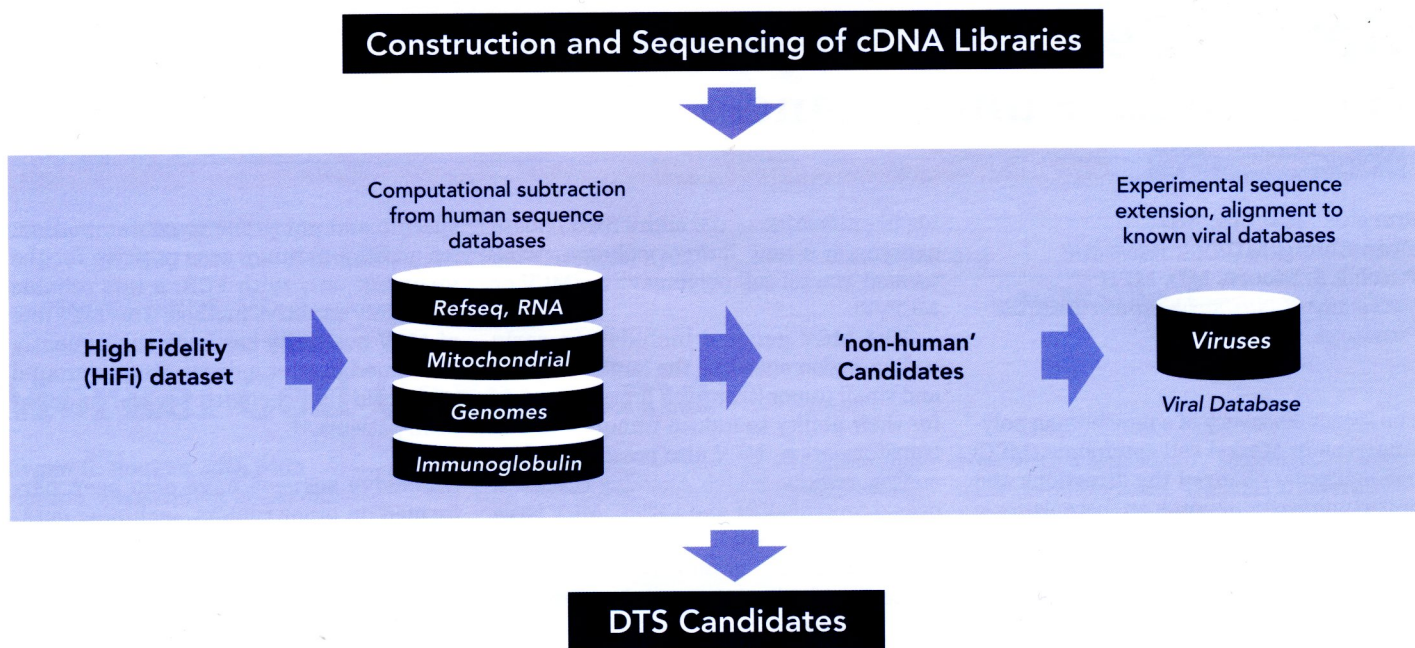


Figure 1: Digital transcriptome subtraction (DTS) relies on alignment of high throughput sequencing of cDNA from tumors to the human genome databases. Sequences that do not align are candidate “nonhuman” sequences that can be examined using molecular techniques to determine if they belong to a foreign virus.

passenger virus that infects the tumor after the tumor has emerged. The mechanistic requirement for two independent mutation events to occur during tumorigenesis (virus integration and T antigen truncation) may explain why MCC is rare. Ongoing studies are attempting to ascertain whether the viral mutations result from pyrimidine dimer substitutions that could arise from ultraviolet irradiation from sun exposure.

Direct in situ evidence for MCV infection in MCC tumor cells also points to the virus playing a direct role in carcinogenesis. (See **Figure 2**.)⁸ Several MCC cell lines have been found to harbor the viral genome stably, allowing functional studies and examination of virus gene expression.¹⁴ A monoclonal antibody raised against a T antigen shows uniform staining for MCV cells in a nuclear pattern. Quantitative PCR (qPCR) studies also show that MCV is generally present at > 1 copy per cell in MCC tumors positive for the virus, whereas uninvolved tissues can harbor virus but at 2-3 logs lower levels.^{8,15} Thus, there is a biological gradient for detecting the virus in MCC that is consistent with the virus causing the tumor.

While most studies thus far show MCV associated only with MCC, individual reports of infection using PCR have described MCV in small cell lung cancers¹⁶ and various skin cancers.^{12,17} These results are not replicated elsewhere,^{7,18,19} so further studies

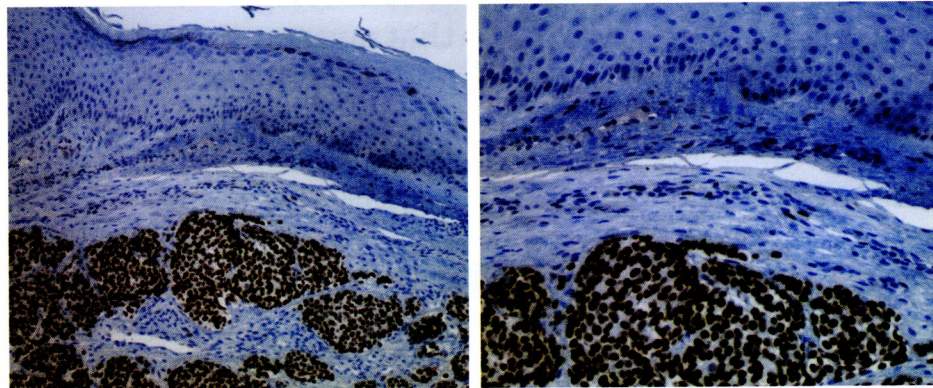


Figure 2: Merkel cell carcinoma infiltrating the skin stained for MCV T antigen using the CM2B4 antibody. Note on high power view (right) that viral T antigen is localized to nuclei of tumor cells and is not found in surrounding non-neoplastic tissues.

are needed to determine whether technical errors account for these results or whether subpopulations of these tumors harbor the virus. Low-level MCV infection can be found in non-MCC tissues, such as some hematolymphoid malignancies and skin,^{1,8} which is consistent with coincidental infection.

Is MCV a Common Human Infection?

These findings raise the question whether MCV is a common infection of humans that rarely causes MCC, or a rare infection that commonly causes MCC. Serologic testing

suggests the former. Kean, et al developed an antibody assay based on recombinant MCV VP1 protein, finding that 42 percent of adults have evidence for past MCV exposure.²⁰ A substantial fraction of children under age 15 have MCV antibodies, indicating that infection can be acquired early in life. This is consistent with several studies that have found MCV in the upper aerodigestive tract, digestive system, and respiratory secretions, indicating that respiratory and/or gastrointestinal transmission may be a common route of infection.^{21,22,15}

We confirmed this using a serologic

assay made from virus-like particles (VLP) generated by expression of the MCV VP1 and VP2 proteins.²³ No cross-reactivity was present for MCV VLP with VLP from other polyomaviruses, suggesting it is a suitable antigen for a blood test.²³ All MCC patients whose tumors are known to be positive for the virus have significantly higher IgG (but not IgM) antibodies against MCV. In a small set of MCC patients with MCV-negative tumors, approximately half show evidence of past MCV exposure — a rate nearly identical to that of various control populations without MCC. This is consistent with the view that MCC tumors without MCV infection have a separate etiology from MCV-positive tumors, rather than simply having lost the virus as the tumor progresses. Evidence for MCV infection was found among children under age 5, and MCV seropositivity rates increased with age, reaching 80 percent by age 50.²³

Unanswered Questions

We are just beginning to investigate this important new human pathogen. Fortunately, MCV's close relatives, SV40 and mouse polyomavirus, have been studied for over 50 years and form part of the bedrock of modern molecular and cancer biology. Applying knowledge gained from these viruses to MCV will rapidly accelerate basic research on this new human polyomavirus. Since direct evidence for MCV causing cell transformation has not been established, investigation into the mechanisms used by this virus to initiate carcinogenesis has become critical to MCC pathology. Although MCV appears to be relatively specific for MCC tumors, the search for MCV in other cancers is far from over. The conflicting results on the presence of MCV in non-MCC cancers needs to be resolved. Moreover, the natural history of MCV infection is largely unknown, and efforts to determine whether MCV is linked to non-neoplastic diseases have only just begun.

It is important to answer whether or not MCV infection affects the clinical course of MCC and its treatment. One retrospective study with long-term follow-up showed that the presence of MCPyV DNA in MCC tumor samples predicted better overall survival.⁶ This raises the possibility that virally mediated MCC may be more immunogenic and less genetically complex than MCV-free MCC. Future therapies targeting the MCV T antigen or augmenting large T antigen-specific immune response may prove beneficial for these patients. The availability of MCV-specific monoclonal antibodies provides an immediately available clinical benefit in differentiating MCC

from other round cell tumors of the skin.¹⁸

Finally, a substantial minority of MCC tumors are not infected with MCV. How do these tumors arise, what is their clinical course, and how should they be treated? The answers to these questions will be particularly important for Merkel cell carcinoma, but may shed light on other cancers as well.

Conclusions

Does MCV cause MCC? Evidence already firmly indicates that MCV is the infectious cause of a portion of MCCs. Current data suggest that MCC actually includes two different diseases, one caused by MCV and another having an unknown etiology. There is remarkable consistency among many studies performed in different settings showing that virus is present at high levels in most but not all MCC tumors. This is supported by detection of viral DNA using a variety of techniques, by serologic studies, by monoclonal antibody-staining, and by *in situ* hybridization. Among tumors that are MCV-positive, the virus is clonally integrated and present at high copy number. Further viruses isolated from tumors contain signature mutations and can no longer independently replicate, whereas virus isolated from normal tissues retains the wild-type genetic sequence. While MCV is a common human infection, most studies agree that MCV infection in tumors is largely specific to Merkel cell carcinoma. Taken together, there is already strong, convincing evidence that MCV is the cause of most but not all MCC tumors.

Despite the good news that a new virus is the probable fundamental cause of most Merkel cell carcinomas, it is sobering to remember another virus that causes an important skin cancer, Kaposi's sarcoma (KS). KS is the most commonly reported cancer in sub-Saharan Africa and remains the most common malignancy among AIDS patients in the US. In the 15 years since Kaposi's sarcoma herpesvirus (KSHV) was first described, a wealth of basic, translational and clinical research on KSHV has accumulated, but thus far none of this data has been used to improve KS clinical care. One hopes that enthusiasm for investigating MCV and Merkel cell carcinoma will take a different path, with the recent basic science findings being actively applied to new methods to diagnose, treat, and prevent this dismal cancer.

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