A Review on Merkel Cell Polyomavirus - A Subclinical Infection or a Sinisterical Carcinogen?

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Abstract

Viral infections can be attributed to a significant percentage of the global cancer burden. In this review, we will delve into the principles of the molecular mechanism underpinning Merkel cell polyomavirus associated oncogenesis. Merkel cell polyomavirus leads to highly aggressive and relatively rare skin cancer known as Merkel cell carcinoma. This virus was first detected in 2008 when it was secluded from tumors of patients with Merkel cell carcinoma. The presence of Merkel cell polyoma virus in tumors was confirmed by Southern blot analysis and sequencing. Of the 10 tumors tested, 8 were positive for virus, confirming a strong association, which led to the WHO IARC classification as a Group 2A, "carcinogenic to humans". There is little evidence which shows the association of Merkel cell polyomavirus and lung cancer. The plethora of diagnostic and predictive biomarkers that are evolving alongside therapeutic and preventive strategies for Merkel cell polyomavirus associated cancers and the evidence of its role in tumourigenesis.

Keywords: Merkel cell polyoma virus • Viral infection • Carcinogen

Introduction

Merkel cell polyomavirus (MCPyV) is a small mammalian polyomavirus belongs to the Polyomaviridae family. The term Polyomavirus has been derived from the initial observation of cells infected with a murine variant of polyomavirus which induced poly-multiple tumours Omas in immunosuppressed mice [1]. It is a double-stranded DNA virus that is small non-enveloped with a genome of approximately 5386 bp (Figure 1).

It consists of 2 transcriptional units with an early region and a late region which encodes for large T antigen, small T antigen, an alternate frame of large T open reading frame and two viral coat proteins respectively [2].

Literature Review

Merkel cell polyomavirus is the first known polyomavirus to be linked to cancer. It is ubiquitously expressed and is an innocuous infection [3]. Feng and associates during their search for an oncogenic virus identified that Merkel cell carcinoma (MCC) disproportionately occurs in the immunosuppressed elderly and hypothesized the potential role of an infectious agent to the pathogenesis of MCC. The authors compared the human genome sequence with the transcriptomic sequence of patients with MCC tumour and substracted the non-viral sequence as well as the background and identified an integrated MCPyV sequence and further found that prior to metastasis there is monoclonal integration of MCPyV into the host genome. This study provided evidence of viral integration as a key milestone in the tumourigenesis of MCC [4].
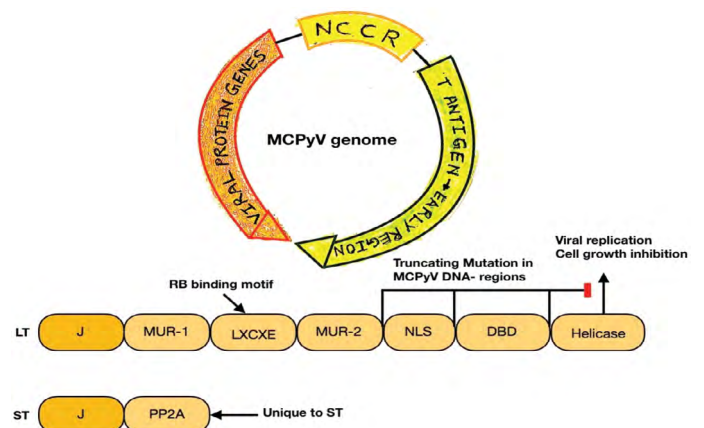
Epidemiology

As per the International Agency for Research, on Cancer (IARC), MCPyV

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Figure 1. MCPyV viral genome. (NCCR: Non Coding Control Region, which consists the viral origin of replication and bidirectional promoter; PP2A: Protein Phosphatase 2A; LT: Large T-antigen; ST: Small Tantigen; Truncating mutations of MCC which occurs in LT after LXCXE has been shown) [2].

has been categorized to be Group 2A carcinogen which indicates that it is probably carcinogenic to humans. Serological surveys show that the exposure to MCPyV occurs during early childhood and the agent is ubiquitously prevalent in the human skin microbiota [3]. Merkel cell carcinoma (MCC) is more prevalent among Caucasians which raised suspicion of an oncogenic European strain of MCPyV but a study contradicted the suspicion by showing five genotypes of MCPyV based on geography and showed a very close link between the African sequence and the European sequence of MCPyV [5].

Transmission of the virus can occur via direct skin contact or saliva or fecal-oral route [5,6]. Incidence of MCC in the UK has been increasing with an estimate of about 12 fold increases from previous reports and a threefold increase in the past 10 years [7]. The annual incidence in the US is around 0.7 cases per 100,000 and is expected to exceed 3000 cases by 2025. The incidence rises with age and is predominant in the immunosuppressed [8].

Merkel Cell Carcinoma (MCC)

MCC is an aggressive cutaneous neuroendocrine malignancy that is predominant in the elderly especially in the head and neck region of white males [9]. Immunohistochemistry staining of the neuroendocrine marker

cytokeratin 20 has significantly improved the diagnosis of MCC. There is an exponential increase in incidence per 100,000 person-years from 1.0 among the 60 year old to 9.8 among those aged more than 85. With an increase in the aging population, the incidence of MCC in between 2000 to 2013 raised to 95% which is far more than the increase in the incidence of solid cancers and melanoma [10].

Evidence of association of MCC with MCPyV

i.   The majority of MCC patients are positive for the presence of MCPyV genome integration into tumour genome, integration appears to occur prior to the clonal expansion of tumour [5].

ii. MCC patients have higher MCPyV specific antibody titers in comparison to the general public [11].

iii. Metastases of MCC also harbor the same integration pattern as per the primary tumour [12].

iv. IHC detection of MCPyV T antigen protein in tumour sections [13].

v.   Knockdown of such T antigen using shRNAs resulted in impeded proliferation and survival of MCC in cell lines [14].

MCPyV is found to be positive in 80% of MCC. Studies have shown integration of the virus into the human genome and expression of viral oncoproteins viral T antigens that promote cell proliferation, suppression of retinoblastoma protein (Figure 2) which is a tumour suppressor along with impaired immunity as seen in the aged and immunosuppressed aids in the transformation of asymptomatic infection to an aggressive malignancy [14]. Although MCPyV infection is quite prevalent among humans it does not cause cancer in the majority, a potential explanation perceivable from the stance of virus, is that integration into human genome is not a regular part of the virus life cycle and results in a deficiency in replicative ability among the virus and reduced DNA damage is favourable for the continuous proliferation of the host as well as the virus [3].

Molecular genetics

Studies of whole-genome sequencing of tumour samples reveal an overlap between the integration sites and the genome amplifications which draws the conclusion that even with a low mutational burden the virus can drive cell division mechanism to facilitate tumourigenesis in MCC [15]. The site of integration of viral DNA into the tumour cell remains consistent among all the tumour cells suggesting viral integration as an early step in the tumourigenesis of MCC [16]. Studies using PCR to amplify the MCPyV sequence showed a vast majority of MCC to be positive for the presence of MCPyV and the detection of the virus in a skin tumour which has a neuroendocrine morphology proves to be a marker of relative specificity to MCC [17].

Molecular detection techniques for MCC in tumour tissue

IHC can be used to detect MCPyV in MCC using CM2B4 clone which is specific to MCPyV. But some studies have shown amplification of the 2nd exon of LT-Ag sequence using PCR to have more sensitivity to detect MCPyV than IHC [18]. Moshiri and his team did a direct comparison between CM2B4 antibody clone and Ab3 antibody clone alongside PCR, the study revealed that CM2B4 was by far more sensitive and highly specific in detecting MCPyV in MCC. The study also showed improved survival in MCPyV positive MCC cases probably due to the ability to detect at early stages as well as limited mutations in comparison to less common MCPyV negative tumours caused by UV induced mutation which has higher mutational burden and numerous chromosomal aberrations [19,20].

The role of CD99 as a potential biomarker

IHC revealed a CD99 dot-like expression which was synchronous with MCPyV positive MCC and can serve as a useful biomarker of viral positivity as well as disease aggressiveness [21].

Micro RNA

Level of miRNAs such as miR-34 and miR-30a has been shown in studies
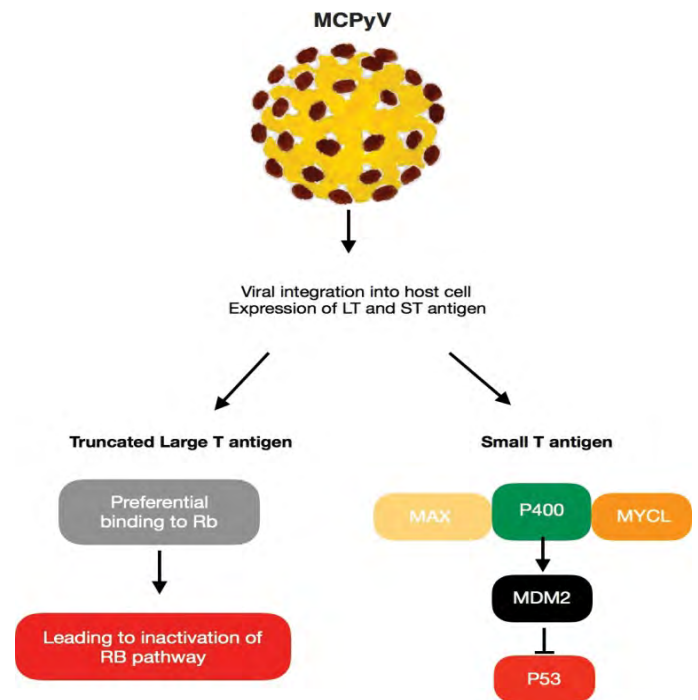
Figure 2. Viral integration and the tumour suppressor pathways affected by the viral T antigens which facilitate tumourigenesis [16].

to be higher among MCPyV positive MCC which can be used to distinguish them from MCPyV negative MCC. Whereas miR-375 has given mixed results [22]. Another study has shown a correlation between the MCPyV genome copy number and miRNA expression in tumours [23].

PCR with miRNA?

The present technique of using PCR to amplify a specific sequence of MCPyV from the patient's tumour can be replaced with real-time PCR that involves primers which are specific towards miRNAs that can detect MCPyV positive MCC. The unique advantage of this technique is that we can collect patient samples such as urine or blood which are much easier to collect than a biopsy of the MCC lesion. RNA in situ hybridization can help exclude background infection by aiding in visual correlation with tumour morphology, Next-generation sequencing can be an effective way of detecting MCPyV but the cost and expertise make it difficult [24].

Treatment

Surgery with a margin of about 1 cm to 2 cm is one of the main stay in the management of MCC [25]. Adjuvant radiotherapy is also given in some cases as the beneficial abscopal effect has been observed due to immune response modulation following irradiation [26]. There is a high risk of metastasis so sentinel lymph node biopsy is often done. Systemic therapy includes platinum-based regimens which are often administered in a palliative setting due to limited efficacy [27].

In MCPyV positive cases there are a plethora of viral antigens which gives scope for the use of immunotherapy. Anti-PD-L1 Avelumab has been approved by the Food and Drug Administration (FDA) in the US for use in MCC. Anti-PD-1 Pembrolizumab has also shown efficacy in patients with advanced disease. But given the fact that MCPyV positive MCC arises in patients with immunosuppression for example in those who need immunosuppression post organ transplantation, it is difficult to use immunotherapy in such clinical scenarios as well as there is an additional issue of resistance. Reports from the trial in the UK showed some benefit from Pazopanib which is a multi-kinase inhibitor [27].

DNA vaccine which encodes MCPyV LT aa1-258 has been developed to target the regions surrounding the stop codons that truncate the protein which is likely to be prevalent among all MCPyV positive tumours. The DNA vaccine showed protection against MCPyV induced MCC as well as therapeutic

efficacy against MCPyV LT- expressing tumour models in B16 melanoma cell lines in mice. If the vaccine can have similar efficacy in humans then it can be implemented in the setting of immunocompromised elderly as well as in elderly patients prior to organ transplantation [28].

Are dermal fibroblasts the natural host of MCPyV?

Several aspects of the MCPyV lifecycle remain to be determined. A study has suggested human dermal fibroblasts to be the natural host of MCPyV. Epidermal Growth Factor (EGF) and Fibroblast Growth Factor (FGF) has been shown to stimulate viral entry in culture and such growth factors are generally induced by fibroblasts during wound healing process providing the suitable environment (Figure 3) that facilitates MCPyV infection [29].

MMP and Wnt signaling

The study further showed using chemical screening that Wnt signaling can facilitate the transduction of MCPyV to dermal fibroblasts in culture, suggesting the role of Wnt signaling in MCPyV induced tumourigenesis. Furthermore *MMP* gene expression has also been shown to contribute to MCPyV infection by disruption of the extracellular matrix, in humans following UV damage and wound healing process, *MMP* expression and Wnt signaling are activated [30]. A key aspect to be noted is MCPyV induced MCC has been seen in the aged population and studies have shown aging skin to be associated with an increase in *MMP* expression and decreased collagen thereby enabling degradation of extracellular matrix and stimulation of MCPyV infection [31]. In 2015, Richards and his associates who found the initial association of MCPyV with MCC further showed the induction of numerous *MMP* genes in
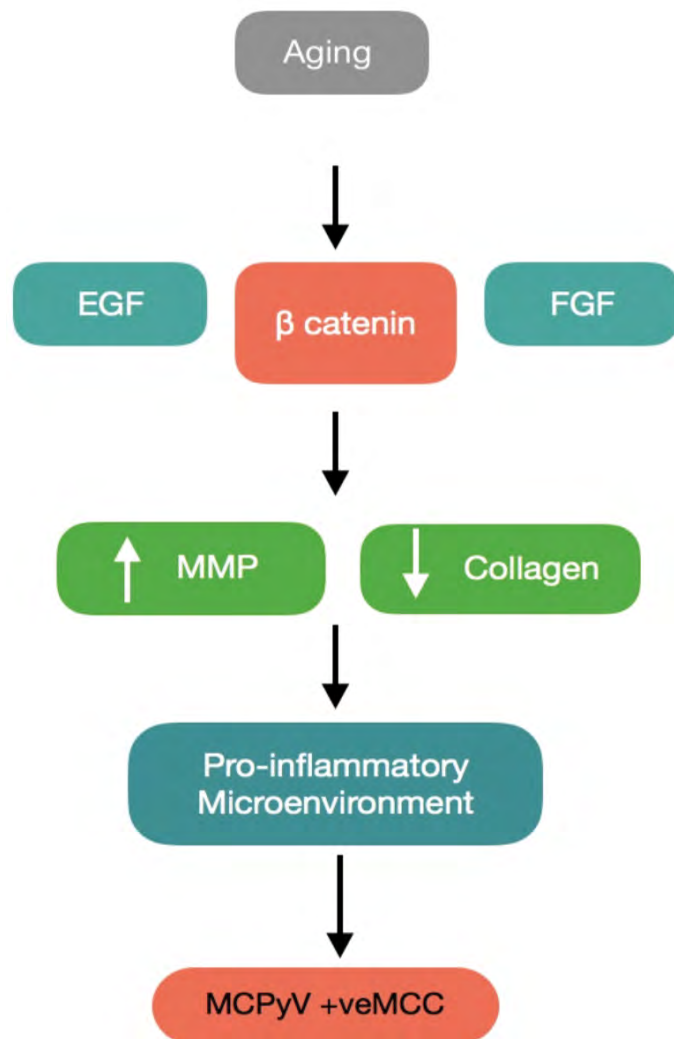
MCC derived tumour cells which expressed T antigens [32]. A study by Liu and colleagues also showed the ability of Trametinib which is a MEK inhibitor in the inhibition of MCPyV infection in cultures, suggesting the role of the MAP kinase pathway in MCPyV infection [29]. Since treatment options are limited for MCC, further study is necessary to determine the potential role of Trametinib to decrease the viral load in the immunosuppressed elderly to prevent possible MCPyV induced MCC.

MCPyV association with lung cancer

Studies have shown an association between MCPyV and Non-small cell lung cancer (NSCLC) in various ethnic groups, especially among non-smokers. The prevalence of MCPyV has been reported in NSCLC patients about 16.7% among Europeans, 16.9% among Chinese and 17.9% among Japanese [33-35].

A study has shown a decrease in *BCL-2* gene expression as well as an association with EGFR mutations among MCPyV positive lung tumour specimens [36]. MCPyV LT antigen transcripts were detectable in NSCLC tumour samples consistent with earlier studies which found MCPyV LT antigen to be pivotal for oncogenesis in MCPyV positive MCC. Similarly, integration of the virus into the host genome also appears to be a pivotal step in the process of oncogenesis and the integration site was found to be localised within the LT gene similar to MCPyV positive MCC [36].

Although the viral load found in NSCLC patients among such studies were low compared to MCPyV load in MCC, prolonged exposure of MCPyV in the lower respiratory tract might over time lead to its integration and facilitate tumourigenesis [37-40]. If this hypothesis can be validated by future studies then the factors that enable MCPyV to remain persistent in the lower respiratory tract and facilitate its integration need to be deciphered to provide clues to its association to NSCLC which can enable the development of targeted therapy.

Discussion and Conclusion

In the era of gradual transition from systemic therapy such as chemotherapy to more targeted therapies. Evidence of MCPyV association with tumourigenesis provides a lustrous target, but establishing concrete causality has been an issue. Chang and Moore who initially established the causality quoted, "Given the near-ubiquity of human MCV infection, establishing cancer causality does not fit comfortably within traditional infectious disease epidemiological models".

Nevertheless, the ability to target MCPyV requires further studies to give a better understanding of the critical pathways involved in MCPyV induced tumourigenesis. It is unclear as to whether hair follicles, keratinocytes, B lymphocytes or dermal fibroblasts support viral replication in the skin. The rare specific integration pattern of the MCPyV genome with the expression of N terminus of LT and early region genes ST along with specific mutation to prevent unlicensed LT initiated DNA replication which can result in cell death via replication fork collision explains the rarity of tumour development despite the ubiquitous presence of MCPyV. A significant challenge in terms of diagnosis is that how does one catch the infection early enough prior to the actual development of MCC? As detection of MCPyV infection using real- time qPCR can be sensitive but cannot be specific enough to determine whether a MCPyV infection will progress to MCC. Nevertheless, with the rising number of cases of MCC and doubts surrounding MCPyV association with other cancers such NSCLC it can prove to be a valuable Achilles heel if future studies can decipher the Waddington landscape surrounding MCPyV which changes MCPyV from a ubiquitous asymptomatic infection to a sinisterical carcinogen.

Figure 3. The pro-inflammatory micro environment in the elderly that favours MCPyV induced oncogenesis. (MMP: Matrix Metallo Proteinase; EGF: Epidermal Growth Factor; FGF: Fibroblast Growth Factor) [29].

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