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Merkel Cell Polyomavirus is not detected in Mesotheliomas

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Abstract

Background—Merkel cell polyomavirus (MCPyV) is the first polyoma virus consistently linked to the etiology of a human cancer. Serological studies indicate that the virus is commonly acquired in childhood, with seroprevalence reaching 50% or higher among young adults. The modes of MCPyV transmission are still unclear, but it has been identified in respiratory tract samples. Given its respiratory tropism, we examined whether MCPyV could be detected in mesothelioma tissue, a malignancy induced in animal models by another polyomavirus, SV40.

Objective—To determine if MCPyV DNA can be detected in mesothelioma.

Study design—DNA was extracted from 45 fresh-frozen mesothelioma samples. PCR was used to detect and quantify the abundance of MCPyV DNA, and a human control gene, in duplicates of the tissues. DNA from a sequence verified MCC tumor was used as a positive control.

Results—The human control gene was detected at high levels in all but three mesothelioma tissues. MCPyV DNA was detected in only one mesothelioma, and the level of viral DNA was very low.

Conclusions—These results are inconsistent with the hypothesis that MCPyV is etiologically linked to meosthelioma.

Keywords

Mesothelioma; polyomavirus; Merkel cell carcinoma and MCPyV

1. Background

Malignant mesotheliomas are most often associated with asbestos exposure [1, 2]. However, the majority of individuals exposed to asbestos do not develop this malignancy [3, 4]. Moreover, of the 2000–3000 cases of mesotheliomas reported annually in the US, as many as 400–600 occur in persons who are apparently asbestos naïve, suggesting the possibility of other etiological factors. The likelihood that additional causative or risk factors might exist is also supported by the smaller than predicted decline in the incidence of mesothelioma following asbestos control measures [5].

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Among agents of potential etiological interest in mesothelioma, the SV40 polyomavirus has featured prominently. Several reports support a role for SV40 in mesothelioma, while many other reports have questioned or even negated a role for this virus [6, 7, 8, 9]. A biological rationale for a role for SV40 is that it efficiently induces transformation of mesothelial cells and that asbestos and transforming proteins of the virus synergize to enhance proliferation and survival and to counteract apoptosis of mesothelial cells. The oncogenic effects of SV40 are attributed to up-regulation of oncogenic pathways, including activator protein-1 and NF-κB transcription factors and the prosurvival Akt pathway in human and murine mesotheliomas [10, 11, 12, 13, 14, 15].

In contrast, epidemiological studies have generally failed to support a role for SV40 [16, 17]. Perhaps the dichotomy between the positive mechanistic laboratory models of viral induced mesothelioma and the negative epidemiological studies relates to a different or unidentified human virus with similar potential oncogenic attributes.

The Merkel cell polyomavirus (MCPyV) was recently discovered and tightly linked to a rare neuroendocrine cancer, Merkel cell carcinoma (MCC) [18]. Like SV40, this virus is dependent upon its Large T (LT) antigen to affect transformation19. Conclusive data from several reports support an etiological link between MCPyV and MCC 20, 21, 22, [23]. These data support the concept that, unlike the classical human polyomaviruses (JC, BK, KI and WU), MCPyV is the first human polyomavirus with clear oncogenic potential. Like JC, BK, KI and WU, MCPyV is quite prevalent. Seroepidemiology data indicate that humans are commonly exposed to MCPyV during childhood, with seroprevalence reaching 50% or more by early adulthood. In one report, the virus was detected in skin swabs from 62% of healthy adults [24], 25, 26. The route of MCPyV transmission is not yet established, but MCPyV has been detected in respiratory tract specimens 27, [28, 29]. Higher levels of the virus have been reported in the upper aerodigestive tract, digestive system, and saliva, with lower levels in lung and genitourinary system samples [30]. MCPyV was not detected in 48 lung tumors of neuroendocrine subtypes [31, 32].

2. Objective

The overall objective of this study was to test the hypothesis that the human carcinogenic polyomavirus MCPyV might contribute to malignant mesothelioma.

3. Study design

3.1 Tissues

We assembled 45 fresh frozen independent mesothelioma samples from The National Mesothelioma Virtual Bank (NMVB). All samples were histologically confirmed. As control material, we obtained DNA from 20 peripheral blood lymphocyte specimens from an unrelated set of individuals. DNA from 3 MCC tumors previously found to contain the virus with an abundance of > 1 MCPyV genome per tumor cell were used as positive controls. The National Institutes of Health, Office of Human Subjects Research approved the use of these de-identified specimens.

3.2 DNA extraction and PCR

DNA from frozen tissue samples was extracted using the Quick DNA extraction kit (Bioserve Biotechnologies Ltd, Laurel, MD). Details of the real time PCR assay used to detect and quantify MCPyV has been described previously [20]. In brief, the MCPyV forward primer was GCAAAAAAACTGTCTGACGTGG and the reverse primer was CCACCAGTCAAAACTTTCCCA. The probe sequence was TATCAGTGCTTTATTCTTTGGTTTGGATTTCCTCCT. Each reaction contained genomic

DNA in 10μl, 1x TaqMan™ Master Mix, dual-labeled probe (100nM each), and PCR primers (900nM each). Amplification was carried out at 50°C for 15 minutes and 95°C for 10 minutes, followed by 50 cycles of 95°C for 15 seconds and 60°C for 1 minute. Viral quantification was estimated by normalization to the concentration of each tissue's human DNA by using a probe to detect the single copy human gene RNase P. All MCPyV and RNaseP reactions were performed in duplex, with means used for quantification. Data were analyzed by SDS software developed by Applied BioSystems, Foster City, CA.

4. Results

Forty two of the 45 samples and all 20 PBL samples contained amplifiable human DNA and yielded ample product when tested for cellular control gene (RNase P). None of PBL DNAs yielded a MCPyV amplicon. In one and only one of the duplex runs, one of the 42 mesotheliomas was positive for MCPyV, and estimated viral abundance in this tissue was low. Relative amount of viral DNA present in this mesothelioma was calculated as less than 150 MCPyV copies per million cells.

5. Discussion

Various laboratory studies indicate that early gene products of a polyomavirus[7–13] such as SV40 can induce mesothelioma in animal models. Human epidemiological data, however, have failed to support a link directly with SV40[8,9,13]. The recent discovery of a new polyomavirus, MCPyV, in a human cancer suggested that the discrepancy between the epidemiology studies and the laboratory models might be a result of a different virus with functional similarities to SV40. The long latent period for development of mesothelioma and the small proportion of asbestos exposed individuals who develop this malignancy both indicate the possibility of additional exogenous or endogenous factors[3]. It is possible that exposure to both asbestos and a virus is necessary for development of mesothelioma.

There is growing molecular pathology evidence that MCPyV infection can be oncogenic and that the oncogenicty of this virus, like that of SV40, is linked to early gene products[23]. MCPyV has been detected in multiple human tissues [34]. To test if there is a direct association between MCPyV and mesothelioma we screened by quantitative PCR analyses a panel of high-quality mesothelioma samples for presence of MCPyV. Our data do not support the hypothesis that MCPyV is directly associated with mesothelioma. Given that some MCC cases have had detectable MCPyV antibodies despite the inability to detect the viral genome in their tumors [24], MCPyV serological studies of mesothelioma cases and appropriate controls would be useful. Nonetheless, our findings strongly suggest that MCPyV, the only human polyomavirus with known carcinogenic potential, is not etiologically linked to mesothelioma. The finding of viral genome at very low levels in 1/42 samples tested is similar to the prevalence and levels of MCPyV DNA in various non-tumor samples including nasopharyngeal aspirates and bronchoalveolar lavage.[28,29] These might represent infected bystander or other normal cells in the tumor milieu. If a polyomavirus is indeed linked to human mesothelioma, it probably is not MCPyV.

Abbreviations

MCPyV	Merkel cell polyomavirus
MCC	Merkel Cell Carcinoma
LT	Large T antigen
FFPE	formalin-fixed, paraffin-embedded

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