Human Polyomaviruses and Other Human Viruses in Neuroendocrine Tumors

Markus Schmitt1, Daniela Höfler1, Nadezda Koleganova2, and Michael Pawlita1

Abstract

Background: While the association of the Merkel cell polyomavirus (MCV) with the neuroendocrine Merkel cell carcinomas (MCC) has been shown recently, it is unknown whether other human polyomaviruses (HPyV) may be associated with neuroendocrine tumours (NETs) of distinct entities.

Methods: Using novel, highly sensitive polyomavirus genotyping assays, we evaluated the prevalence of eight distinct HPyVs in a selection of 51 NETs from different entities. In addition, we analyzed these NETs for the presence of DNA from 12 adeno-associated virus (AAV) genotypes, adeno virus-5, 27 mucosal human papillomavirus (HPV) genotypes, hepatitis B (HBV), 8 human herpes viruses (HHV), and xenotropic murine leukemia virus-related virus (XMRV).

Results: 43 of the 50 (86%) NETs were positive for the DNA integrity control. Of these, 2 of 3 MCCs (67%) were positive for MCV. NETs from other entities, however, were negative for all HPyVs. Only a small subset of lung and appendix NETs were positive for EBV, HHV-6, and -7.

Conclusion: While the association of MCV with MCC was confirmed, other human viruses could not be identified as potentially causative agents of other NETs.

Impact: Our findings suggest that the human viruses tested for in this study do not play a comparable role in NETs like the polyomavirus MCV in MCC.

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Background

Neuroendocrine tumors (NETs) (previously referred to as carcinoids) are rare malignancies that, although slow-growing, can behave aggressively. In 2004, they comprised 1.25% of all malignancies. Their incidence is increasing by approximately 6% per year (1). NETs occur most frequently in the gastrointestinal tract (66%), with the second most common location in the bronchopulmonary system (31%), followed by less frequent locations including the ovaries, testes, hepatobiliary system, and pancreas (2). Within the gastrointestinal tract, most NETs occur in the small intestine (41.8%), rectum (27.4%), and stomach (8.7%).

NETs show distinct biologic and clinical characteristics, however, share some common histologic features, such as neuroendocrine granules, the paranuclear ball of intermediate filaments, and intercellular junctions (3, 4). With the potential exception of Merkel cell carcinomas (MCC), NETs have a common embryonic origin from the neuroectoderm.

Recently, the Merkel cell polyomavirus (MCV) was identified as a possibly causative agent of 80% of MCC (5). Consequently, MCV is the first human polyomavirus (HPyV) to be considered as a carcinogen. HPyV are small (40–50 nm in diameter) double-stranded DNA viruses with a circular genome that encodes several proteins, among them the large tumor (T) antigen (6). The large T antigen regulates the life cycle of the virus as well as stimulates the cell cycle of the host cell.

Eight HPyV have now been identified: BKV (7), JCV (8), KIV (9), WUV (10), MCV (5), and very recently HPyV6 (11), HPyV7 (11), and the trichodysplasia spinulosa-associated polyomavirus (TSV) (12). Serologic studies showed that BKV, JCV, KIV, WUV, and MCV are apparently ubiquitous infecting over 50% to 80% of some populations (13–15). Since the discovery of the mouse polyomavirus (MPyV) in 1953 (16), polyomaviruses have been suspected as possible causes of cancer in humans. With the exception of MCV, ultimate proofs for the other types are still missing.

Based on the similarities observed between NETs, we analyzed whether polyomaviruses may be present in NETs other than MCC. To this end, we used a Luminex-based polyomavirus genotyping assay for detecting all eight HPyVs, and the monkey PyVs, SV40, and LPV in NETs from a variety of distinct occasions.
entities. In addition, these NETs were subjected to the analysis for other human, tumourogenic and nontumourogenic, viruses.

Materials and Methods

Tissue samples
Two 5 μm sections of formalin-fixed paraffin-embedded (FFPE) tissue samples of 50 neuro-endocrinal tumor from different entities (Table 1), collected between 1997 and 2008, were obtained from the tissue bank of the National Center for Tumor Diseases (NCT) Heidelberg. The work was covered by a votum of the Ethical Committee of the University of Heidelberg. Written consent was obtained from each patient.

Virus detection
DNA from FFPE sections was extracted as published previously (17). Two μL of purified DNA were analyzed for the human PyVs BKV, JCV, KIV, WUV, MCV, HPyV6, HPyV7, and TSV, as well as the monkey PyVs SV40 and LPV by a novel polyoma virus genotyping assay. This assay was based on multiplex PCR targeting less than 100 bp of the large T-antigen sequence, followed by hybridization to type-specific and universal Luminex-based coupled PyV probes as described by the manufacturer (Steinbeis Transfer Centre Multiplexion). For HPyV6, HPyV7, and TSV singleplex PCR and hybridization were carried out. Universal PyV probes were included in the assay to detect potentially new polyoma virus sequences. The assay as well as the cutoff definition has been developed analogously to recently reported human papillomavirus (HPV) (18, 19), bovine papillomavirus (BPV; 20) and adeno-associated virus (AAV; 21) genotyping assays. The sensitivity was between 10 and 100 for the detection of all PyVs. No cross-reactivity was observed. In addition, the specimens were further analyzed for the following viruses: 12 AAV genotypes (21), adenovirus-5 (22), 27 mucosal HPV genotypes, (18, 19), hepatitis B (HBV; 22), human herpes virus-1–8 (HHV-1 to –8; 22), and xenotropic murine leukemia virus-related virus (XMRV) (unpublished assay). DNA integrity was confirmed by analyzing the presence of the human β-globin DNA (22).

Results

A series of 50 FFPE biopsies and was subjected to the novel polyomavirus genotyping assays. Human β-globin DNA was amplifiable in 43 extracted DNAs. Among three MCCs with intact DNA, two were positive for MCV DNA with strong signals (67%; Table 1). All other NETs were negative for polyomavirus DNA using type-specific as well as universal hybridization probes.

We further analyzed these NETs for the presence of DNA from 12 AAVs, adenovirus-5, 27 HPVs, HBV, and XMRV. DNA of these viruses was not detected. Among the herpes viruses, only HHV-6 was detected in two out of five appendix carcinoids with high signals (Table 1). All remaining positive reactions of EBV and HHV-7 were weak signals (Table 1). Due to the strong HHV-6 reactions in appendix carcinoids, a larger set of appendix carcinoids was retested (n = 30), of which 22 were positive for human β-globin. Moderate signals of HHV-6 and HHV-7 DNA were detected in three (13.6%) and two (9.1%) tumors, respectively.

Discussion

Increased knowledge about NET cell biology and the genetic characteristics of the tumors are the key to develop early diagnostic tests and to establish a targeted therapeutic strategy. However, NETs are rare malignancies which make it difficult to collect large-scale studies.

We tested whether other HPyVs may play a similar role in NETs like it has been found for MCV in MCC. Due to insufficient DNA quality, only 3 of 5 MCCs could be analyzed for MCV presence. Of these, two were positive (67%) which is in good agreement with the prevalence in larger studies (5). Consequently, it seems justified to state that our study has enough power to detect virus-cancer associations of a similar strength than found for MCV and MCCs.

Mutations and deletions frequently affecting the helicase domain of the T-Ag gene from MCV have been described in MCCs with integrated virus (23). In our assay, HPyV primers targeted T-Ag sequences upstream of this domain. As the prevalence of MCV in MCC was similar in our study compared with others, mutations do not seem to influence the performance of our assay. Nonetheless, we have to state that in a minority of MCCs, these mutations could also affect the primer binding regions, thus, leading to false negative result. For the other PyVs except LPV, the target regions were located more upstream than in MCV, thereby reducing the risk of false-negative results. Thus, it is very unlikely that the general absence of PyVs in NETs was due to impaired amplification. Consequently, we trust to state that NETs of the brain, digestive system, lung, and thyroid glands are truly not associated with the 8 HPyV types currently known.

Taking advantage of the availability of a broad spectrum of Luminex-based multiplex virus genotyping assays in our laboratory, we further screened all NET DNAs for the presence of 12 AAV genotypes, adenovirus-5, 27 mucosal HPV genotypes, HBV, 8 HHV types, and XMRV. HPV, HBV, EBV, and HHV8 are casually associated with certain tumors (24–27). XMRV has been suspected to be associated with prostate tumors (28). None of these viruses have been detected in this study, except for EBV with weak signals in two lung NETs. HHV-6 was detected in 13.6% of appendix carcinoids, which could hint at the detection of latent HHV-6 in lymph nodes close to the carcinoid site. An active invol-
### Table 1. Site, tumor type, number of available tumors (n), percentage of tumor tissue to total tissue, number of tumors with sufficient DNA quality, and frequency of detected viruses

<table>
<thead>
<tr>
<th>Specimens</th>
<th>Malignancy</th>
<th>Tumors with sufficient DNA quality</th>
<th>Mean of tumor to total tissue</th>
<th>Polyoma-virus</th>
<th>Herpes virus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n</td>
<td>N</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>Brain</td>
<td>Kranipharyngeom</td>
<td>4</td>
<td>4</td>
<td>100</td>
<td>91</td>
</tr>
<tr>
<td>Thymus</td>
<td>Carcinoid tumor of the thymus</td>
<td>1</td>
<td>1</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>Digestive system</td>
<td>Gastrinom of the duodenum</td>
<td>1</td>
<td>1</td>
<td>100</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>Carcinoid tumor of the duodenum</td>
<td>1</td>
<td>1</td>
<td>100</td>
<td>70</td>
</tr>
<tr>
<td>Lung</td>
<td>Typical bronchial carcinoid</td>
<td>5</td>
<td>4</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>Atypical bronchial carcinoid</td>
<td>6</td>
<td>4</td>
<td>67</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>Large-cell neuroendocrine carcinoma of the lung</td>
<td>6</td>
<td>6</td>
<td>100</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>Small-cell neuroendocrine carcinoma of the lung</td>
<td>5</td>
<td>5</td>
<td>100</td>
<td>76</td>
</tr>
<tr>
<td>Skin</td>
<td>Merkel cell carcinoma</td>
<td>5</td>
<td>3</td>
<td>60</td>
<td>82</td>
</tr>
<tr>
<td>Thyroid gland</td>
<td>Medullary thyroid carcinoma</td>
<td>6</td>
<td>4</td>
<td>67</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>50</td>
<td>43</td>
<td>86</td>
<td>86</td>
</tr>
</tbody>
</table>

<sup>a</sup>Initial NET study/additional appendix carcinoids.
vement of HHV-6 in the development of the tumor is not implicated.

In conclusion, all viruses tested for in this study do not play a similar role in neuro-endocrine tumors as the polyomavirus MCV in MCC.

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References