The novel KI, WU, MC polyomaviruses: possible human pathogens?

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SUMMARY

Recently, three novel human polyomaviruses KIPyV, WUPyV and MCPyV were uncovered in biological specimens of patients with different underlying clinical conditions. Although it is too early to draw firm conclusions on their role in human pathology, this finding has revitalized the scientific debate on the *Polyomaviridae* family and their relation to human disease.

Seroepidemiological studies showed that, similarly to BKPyV and JCPyV, benign primary exposure to these new viruses occurs early in childhood. The viruses then remain latent in the body, and reactivate in immunosuppressed patients with possible pathological consequences. Furthermore, the discovery of MCPyV in a rare and aggressive skin cancer named Merckel cell carcinoma and its clonal integration within the tumor genome suggests that MCPyV infection may represent an early event in the pathogenesis of this disease.

This review describes the general aspects of human polyomavirus infection and pathogenesis. Current topics of investigation and future directions in the field are also discussed.

KEY WORDS: Novel polyomaviruses, Immunocompromised patients, Phylogenetic analysis, Oncogenic viruses

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INTRODUCTION

Until 2007, only two human polyomaviruses were known to infect human beings: BKPyV and JCPyV. BKPyV was identified in the urine of a renal transplant patient (Gardner *et al.,* 1971), while JCPyV was found in the brain of a patient with progressive multifocal leukoencephalopathy (Padgett *et al.,* 1971). Recently, three additional human polyomaviruses have been uncovered. The novel polyomaviruses KI and WU identified in the respiratory tract specimens of children with acute respiratory symptoms (Allander *et al.,* 2007; Gaynor *et al.,* 2007) and MCPyV found in a rare skin tumor named Merkel cell carcinoma (Feng *et al.,* 2008). The report by Allander *et al.* identified KIPyV by screening a library constructed from 20 randomly selected nasopharyngeal aspirates. A circular genome of 5,040 bp was cloned and sequenced. The same DNA was found in six out of 637 nasopharyngeal aspirates and in one out of 192 stool samples tested (Allander *et al.,* 2007). The virus, named after the Karolinska Institute, was only partly related to JC and BK viruses. Its DNA would not have been amplified by primer sequences used for the detection of JCPyV, BKPyV, or SV40 (Allander *et al.,* 2007).

Almost contemporaneously, a fourth novel human polyomavirus was reported by Gaynor *et al.* (2007). The virus, named WU polyomavirus (WUPyV), was also identified by screening respiratory secretions. Its genome is a circular double stranded DNA of 5,229 bp in length (Gaynor *et al.,* 2007) and it is geographically widespread. Although related to KIPyV, the two viruses differ substantially from each other as shown by the percentage of amino acid identity and phylogenetic analysis. Yet they are phylogenetically more closely related to each other than to SV40, BK,

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and JC viruses (Gaynor *et al.,* 2007). Seroepidemiological studies showed that similarly to BKPyV and JCPyV, primary exposure occurs early in life and in the adult population the seroprevalence of KIPyV and WUPyV is 55 and 69%, respectively (Kean *et al.,* 2009). Given the high seroprevalence of these two viruses, it may be difficult to find an etiological support to specific diseases. However, they appear to be significant human pathogens in immunosuppressed people. Indeed, in some cases they were the only viruses detected in patients with acute respiratory symptoms (Mourez *et al.,* 2009; Han *et al.,* 2007).

MCPyV was the third novel polyomavirus identified in a rare but aggressive skin cancer of neuroendocrine origin named Merkel cell carcinoma (Feng *et al.,* 2008). This tumor occurs typically in elderly and immunosuppressed patients. The virus was identified using the digital transriptome subtraction technique. Viral DNA has been detected in eight out of ten Merkel tumors, in the majority of them in an integrated clonal form with an identical clonal pattern in one of the metastases investigated. Weak signals for viral DNA were also found in five out of 59 control tissues from various body sites and in four out of 25 control skin tissues (Feng *et al.,* 2008). The presence of MCPyV DNA in Merkel cell tumors does not prove a causal involvement of this virus in Merkel carcinomas. The clonal pattern of integration in tumor tissue as well as in its metastasis, however, could favour this interpretation. The preferential occurrence of this cancer in immunosuppressed patients might also support a viral etiology. Merkel cell tumors seem to represent the first human malignancy with a relatively consistent presence of integrated sequences of a specific type of polyomavirus.

After these discoveries, the new polyomaviruses have been investigated in a variety of clinical samples (Table 1). This review summarizes the recent findings on the novel human polyomaviruses and discuss the possible pathogenic role of these viruses in human diseases.

Presence of KI, WU and MC polyomaviruses in respiratory tract

Recently, three novel human polyomaviruses, KIPyV, WUPyV, and MCPyV were detected in the respiratory tract specimens of patients with respiratory symptoms (Allander *et al.,* 2007; Gaynor *et al.,* 2007; Goh *et al.,* 2009; Babakir-Mina *et al.,* 2010a). KIPyV DNA was detected in 6/637 (1%), 24/951 (2.5%), and 1/222 (0.45%) nasopharyngeal specimens, respectively (Allander *et al.,* 2007; Bialasiewicz *et al.,* 2007; Babakir-Mina *et al.,* 2008). WUPyV DNA was amplified in 43/2135 (2%) nasopharyngeal aspirates (Gaynor *et al.,* 2007). A similar prevalence was reported by Abed *et al.* (2007) in children with respiratory tract infection, though the matched asymptomatic group shed the virus at higher frequency (6.4%).

Since KIPyV and WUPyV were first identified, viral sequences have been confirmed in respiratory specimens worldwide (Bialasiewicz *et al.,* 2008; Abed *et al.,* 2007; Abedi Kiasari *et al.,* 2008; Han *et al.,* 2007; Le *et al.,* 2007; Lin *et al.,* 2008; Neske *et al.*, 2008; Norja *et al.,* 2007; Payungporn *et al.*, 2008; Babakir-Mina *et al.,* 2008; Foulongne *et al.,* 2008a), suggesting that both viruses are widespread among human populations with respiratory tract diseases (Table 1). Nonetheless, the pathogenicity of KIPyV and WUPyV remains speculative (Norja *et al.,* 2007). The proposed association of KIPyV and WUPyV with respiratory disease is tenuous because up to now the majority of studies have not included specimens from asymptomatic patients. The three studies where these control groups were included detected viral sequences at similar or higher frequencies in asymptomatic patients (Han *et al.,* 2007; Norjaet *et al.,* 2007; Abed *et al.,* 2007). Further, the link between KIPyV and WUPyV infection and respiratory disease is complicated by the high rate of co-infection with other respiratory viruses (Babakir-Mina *et al.,* 2008; Allander *et al.,* 2007; Gaynor *et al.,* 2007; Bialasiewicz *et al.,* 2007; Abedi Kiasari *et al.,* 2008; Han *et al.,* 2007; Neske *et al.,* 2008). However, in some cases KIPyV or WUPyV were the only pathogens detected despite the wide screening performed including viruses, bacteria and fungi (Mourez *et al.,* 2009; Han *et al.,* 2007). These preliminary studies led to the hypothesis that these new viruses may have an etiological role in childhood respiratory disease.

MCPyV DNA was detected at a variable frequency in three out of 140 (2.1%) and 27 of 635 nasopharyngeal aspirates (NPAs) (4.25%), respectively (Kantola *et al.,* 2009; Goh *et al.,* 2009); two of 106 (1.9%) nasal swabs (Kantola *et al.,* 2009), and seven (1.3%) of 526 respiratory tract samples

TABLE 1 - *Detection of the novel KI, WU and MC polyomaviruses in biological specimens around the world.*

Polyomavirus	*Sample type*	*No. Positive samples /total*	*Country*	*References*
KIPyV	RTS	6/637	Sweden	Allander *et al.*, 2007
KIPyV	Blood	4/153	Italy	Babakir-Mina *et al.*, 2010b
KIPyV	Blood	4/130	Italy	Babakir-Mina *et al.*, 2010b
KIPyV	Feces	1/192	Sweden	Allander *et al.*, 2007
KIPyV	RTS	10/371	United Kingdom	Abedi Kiasari *et al.*, 2008
KIPyV	RTS	24/951	Australia	Bialasiewicz *et al.*, 2007
KIPyV	RTS	3/537	France	Foulongne *et al.*, 2008a
KIPyV	RTS	1/222	Italy	Babakir-Mina *et al.*, 2008
KIPyV	RTS	2/415	China	Ren *et al.*, 2008
KIPyV	Lung cancer tissue	9/20	Italy	Babakir-Mina *et al.*, 2009a
KIPyV	RTS	6/302	Thailand	Payungporn *et al.*, 2008
KIPyV	RTS	5/486	South Korea,	Han *et al.*, 2007
KIPyV	RTS	17/265	France	Mourez *et al.*, 2009
KIPyV	RTS	11/406	China	Yuan *et al.*, 2008
KIPyV	RTS	24 / 951	Australia	Bialasiewicz *et al.*, 2007
KIPyV	RTS	75/2866	Australia	Bialasiewicz *et al.*, 2007
KIPyV	Stool	1/9	France	Mourez *et al.*, 2009
KIPyV	Stool	2 /75	Finland	Kantola *et al.*, 2009
KIPyV	Nasal swabs	4/106	Finland	Kantola *et al.*, 2009
KIPyV	RTS	14/983	United Kingdom	Norja *et al.*, 2007
KIPyV	Plasma	2/62	Italy	Babakir-Mina *et al.*, 2009d
KIPyV	Tonsil	11/91	Italy	Babakir-Mina *et al.*, 2009b
KIPyV	Stool	10/86	Italy	Babakir-Mina *et al.*, 2009c
WUPyV	Blood	7/153	Italy	Babakir-Mina *et al.*, 2010b
WUPyV	Blood	1/130	Italy	Babakir-Mina *et al.*, 2010b
WUPyV	Stool	2/377	China	Lin *et al.*, 2008a
WUPyV	RTS	1/278	China	Lin *et al.*, 2008a
WUPyV	Stool	7/86	Italy	Babakir-Mina *et al.*, 2009c
WUPyV	Serum HCV pos	2/79	USA	Miller *et al.*, 2009
WUPyV	Serum HIV pos	10/121	USA	Miller *et al.*, 2009
WUPyV	Tonsils	4/91	Italy	Babakir-Mina *et al.*, 2009b
WUPyV	Plasma	1/62	Italy	Babakir-Mina *et al.*, 2009d
WUPyV	Cerebral spinal fluid	1/60	Italy	Barzon *et al.*, 2009a
WUPyV	RTS	10/983	United Kingdom	Norja *et al.*, 2007
WUPyV	RTS	4/371	United Kingdom	Abedi Kiasari *et al.*, 2008
WUPyV	RTS	37/1245	Australia,	Gaynor *et al.*, 2007
WUPyV	RTS	13/537	France	Foulongne *et al.*, 2008a
WUPyV	RTS	128/2866	Australia	Bialasiewicz *et al.*,2007
WUPyV	Tonsils	5/229	Finland.	Kantola *et al.*, 2009
WUPyV	Nasal swab	1/106	Finland.	Kantola *et al.*, 2009
WUPyV	RTS	10/415	China	Ren *et al.*, 2008
WUPyV	Stool	2/ 377	China	Ren *et al.*, 2009
WUPyV	RTS	19/302	Thailand	Payungporn *et al.*, 2008
WUPyV	RTS	34/486	South Korea,	Han *et al.*, 2007
WUPyV	RTS	2/79	Canada	Abed *et al.*, 2007
WUPyV	RTS	5/78	Canada	Abed *et al.*, 2007
WUPyV	RTS	62/1326	Germany	Neske *et al.*, 2008
WUPyV	RTS	2/265	France	Mourez *et al.*, 2009
WUPyV	RTS	34/486	South Korea,	Han *et al.*, 2007
MCPyV	MCC	8/9	France	Foulongne *et al.*, 2008b
MCPyV	RTS	7/526	Australia	Bialasiewicz *et al.*, 2009
MCPyV	RTS	27/635	Sweden	Goh *et al.*, 2009
MCPyV	MCC	8/10	USA	Feng *et al.*, 2008
MCPyV	MCC	8/9	France	Foulongne *et al.*, 2008b
MCPyV	Tonsils	8/229	Finland.	Kantola *et al.*, 2009
MCPyV	NPAs	3/140	Finland.	Kantola *et al.*, 2009
MCPyV	Nasal swabs	2/106	Finland.	Kantola *et al.*, 2009
MCPyV	Sera	1/840	Finland.	Kantola *et al.*, 2009
MCPyV	RTS	27/635	Sweden	Goh *et al.*, 2009
MCPyV	RTS	7/526	Australia	Bialasiewicz *et al.*, 2009
MCPyV	RTS	15/87	Italy	Babakir-mina *et al.*, 2010a

RTS: respiratory tract secretions; MCC: Merkel cell carcinoma; NPAs: nasopharyngeal aspirates

from Australian patients with upper respiratory symptoms (Bialasiewicz *et al.,* 2009a). Finally, MCPyV DNA was amplified in 15 (17.24%) of 87 lower respiratory tract samples from hospitalized Italian patients with lower respiratory tract symptoms (Babakir-Mina *et al.,* 2010a). Sequence data showed that MCPyV found in respiratory secretions is similar to the virus identified in Merkel cell carcinoma (Babakir-Mina *et al.,* 2010a). The presence of MCPyV in the respiratory tract raises questions about the mode of transmission and respiratory pathogenicity of this newly described polyomavirus. Further studies are needed before the role of these three new polyomaviruses as respiratory pathogens can be confirmed.

Detection of KI, WU and MC polyomaviruses in the blood

The discovery of KIPyV, WUPyV in respiratory secretions and MCPyV in Merkel cell carcinoma prompted researchers around the world to investigate the presence of these viruses in other body compartments.

Plasma samples from 62 HIV-1 positive patients revealed both KIPyV and WUPyV in two (3.2%) and one (1.6%) cases, respectively (Babakir-Mina *et al.,* 2009d). When the presence of KIPyV and WUPyV was correlated to the HIV-1 viral load and CD4+ cell counts, no significant correlation was found (Babakir-Mina *et al.,* 2010b). In addition, the prevalence of KIPyV in HIV-1 positive patients was similar to that of healthy blood donors (Babakir-Mina *et al.,* 2010b). Phylogenetic analysis did not suggest circulation of specific KIPyV and WUPyV strains in HIV-1–positive patients. Although firm conclusions cannot be drawn, our data seem to exclude an active role for KIPyV and WUPyV in HIV-1–positive patients (Babakir-Mina *et al.,* 2010b).

We detected WUPyV and KIPyV in healthy persons as well as immunocompromised persons. BK and JC polyomaviruses persist in peripheral blood mononuclear cells in healthy persons. Thus, detection of KIPyV and WUPy in blood cells of immunocompetent persons is needed to identify a possible hematologic reservoir.

MCPyV has been detected in inflammatory monocytes, but not in resident monocytes of patients with Merkel cell carcinoma leading to the hypothesis that MCPyV persists in inflammatory monocytes and spreads along the migration routes of inflammatory monocytes (Mertz *et al.,* 2010). In addition, MCPyV was detected in 27.1% of patients with chronic lymphocytic leukemia (CLL). Mutational analyses revealed a novel 246 bp Large T antigen (LT-Ag) deletion in the helicase gene in six of 19 MCPyV-positive CLL cases (Pantulu *et al.,* 2010).

The detection of MCPyV, including LT-Ag deletions and LT-Ag expression in CLL cells argues for a potential role of MCPyV in a subset of CLL cases (Pantulu *et al.,* 2010). By contrast, Tolstov *et al.* (2010) showed a lack of evidence for direct involvement of MCPyV in chronic lymphocytic leukaemia.

Finally, Toracchio *et al.* (2010) detected MCPyV in lymphomas as well as in normal lymph nodes. This finding seems to support the notion that lymphocytes and monocytes may serve as a tissue reservoir for MCPyV infection.

Detection of KIPyV and WUPyV in the gastrointestinal tract

The novel KI and WU polyomaviruses have also been detected in the digestive tract of children with respiratory and/or gastrointestinal symptoms (Gaynor *et al.,* 2007; Ren *et al.,* 2009). We have found KIPyV and WUPyV in the stool of patients with haematological disorders, but often in combination with other viruses involved in gastrointestinal disorders (CMV, adenovirus, BKPyV) (Babakir-Mina *et al.,* 2009c). Because of frequent co-infections, a clear correlation between novel polyomaviruses and clinical symptoms could not be established.

However, it was observed that diarrhea occurred frequently in patients infected by KIPyV compared to patients not infected by this virus ($P<0.02$); a trend of association between detection of KIPyV and vomiting ($P<0.06$) was also noted. There was no correlation between demographic variables and detection of KIPyV and WUPyV. Phylogenetic analysis of the small t-antigen (ST-Ag) gene of KIPyV and WUPyV isolates showed that the novel polyomaviruses identified in feces clustered with those identified in the respiratory tract suggesting an oral–fecal transmission of these viruses (Babakir-Mina *et al.,* 2009c). To obtain insights into the pathogenesis of these two polyomaviruses, it would be interesting to study patients with diarrhea but without respiratory symptoms.

Detection of KI, WU and MC polyomaviruses in the central nervous system

It has been established that reactivation of JCPyV in immunosuppressed patients such as AIDS patients can cause a demyelinating disease known as progressive multifocal leukoencephalopathy (PML). A recent study reported the case of a patient with AIDS and signs of PML but JCPyV negative. An extensive PCR screening disclosed WUPyV DNA in the CSF of this patient (Barzon *et al.,* 2009b). The same group also described the presence of KIPyV and WUPyV in the brains of patients with and without PML (Barzon *et al.,* 2009c).

These results contrast with those reported by other groups that did not detect KIPyV, WUPyV and MCPyV in the central nervous system (Giraud *et al.,* 2009; Bialasiewic *et al.,* 2009b; Focosi *et al.,* 2009; Lam *et al.,* 2010). These conflicting results may be linked to ethnic differences in patient populations, and to the different laboratory methods used in the various studies. Indeed, sequence variations in the viral target and the sensitivity of the PCR method used can affect the PCR results. Standardization of the methods can help to define the prevalence of the novel polyomaviruses in the central nervous system.

Detection of KIPyW, WUPyV and MCPyV in cancer tissues

Polyomaviruses are suspected to be causative agents of human cancer. *In vitro* studies and experimental animal models have shown that the early viral proteins LT-Ag and ST-Ag of SV40, BKPyV, and JCPyV possess oncogenic potential (Barbanti-Brodano *et al.,* 2006; Martini *et al.,* 2007). The LT-Ag acts mainly by blocking the function of the cellular tumor suppressor proteins pRb and p53 and by inducing chromosomal aberration and instability in the host cell genome. The ST-Ag instead binds the regulatory and catalytic subunits of the protein phosphatase PP2A, thereby constitutively activating the β-catenin pathway which drives the cells into proliferation. Recently, Feng and co-workers (2008) described the presence of a novel polyomavirus in a rare but aggressive skin tumor named Merkel cell carcinoma. The virus has been identified by digital transcriptome subtraction technique and has been named MCPyV. Viral DNA has been found clonally integrated in eight out of ten Merkel cell carcinomas, suggesting that viral infection may be an early event in the pathogenesis of Merkel cell carcinoma (Feng *et al.,* 2008). Interestingly, the LT-Ag gene of MCPyV found in Merkel cell carcinomas harbors mutations that prematurely truncate the MCPyV LT helicase. In contrast, four presumed episomal viruses from nontumor sources did not possess this T antigen signature mutation. These mutations do not affect the binding of LT-Ag to the pRb, but do prevent viral DNA replication capacity, thereby sparing the life of tumoral cells harbouring the mutant virus (Shuda *et al.,* 2008).

We reported the presence of KIPyV VP1 DNA in the lung tissue of 9/20 lung cancer patients and in the respiratory tissue of 2/2 transplanted patients. However, amplification of the early region genome of KIPyV performed on the 11 positive cases was successful only in two malignant lung tissues, one surrounding normal tissue, and one paranasal tissue of the transplanted patient (Babakir-Mina *et al.,* 2009a). In addition, KIPyV DNA was detected in one tonsil malignant lesion diagnosed as lymphoma (Babakir-Mina *et al.,* 2009b). Considering the oncogenic potential of polyomaviruses, it would be worth further investigating the presence of KIPyV in respiratory cancer tissue.

DISCUSSION

Recently, three additional human polyomaviruses have been uncovered: KIPyV, WUPyV, and MCPyV. This finding has revitalized the scientific debate on polyomaviruses and their relation to human disease. Similarly to BKPyV and JCPyV, infection by these three viral agents is widespread among human population. Indeed, the seroprevalence among healthy blood donors is 55% for KIPyV, 69% for WUPyV, 25% for MCPyV strain 350, and 42% for MCPyV strain 339 (Kean *et al.,* 2009). So, it is hypothesized that a benign primary infection occurs early in childhood, probably by respiratory or oral-fecal route (Babakir-Mina *et al.,* 2009c). They will persist in a latent state in a yet unidentified body location, and reactivate in a setting of immune suppression due to medications (immunosuppressive drugs) or other underlying medical conditions (Babakir-Mina *et al.,* 2009a, b, c and d). KIPyV, WUPyV

and MCPyV have been detected in respiratory specimens worldwide, however, the number of patients positive to any given virus is still low and there is no convincing evidence at this time for an etiological role of these viruses in respiratory disease. Furthermore, the frequent co-detection with other known human pathogens and often the lack of control groups makes it difficult to establish a causal link with the clinical symptoms. It might be possible that KIPyV, WUPyV and MCPyV play a role in at least a subset of pneumonia in immunocompromised patients.

The role of human polyomaviruses in human cancer is also debated. SV40, BKPyV, JCPyV, KIPyV and MCPyV have been detected in human malignancies (Giuliani *et al.,* 2007; Babakir-Mina *et al.,* 2009a; Feng *et al.,* 2008), but so far only MCPyV appears to be the primary candidate as human oncogenic polyomavirus. It was found monoclonally integrated into the genome of Merkel cell carcinomas (Feng *et al.,* 2008), and interestingly, all the integrated forms present mutations that truncate the LT helicase prematurely. Such mutations prevent viral replication but retain the capacity of LT-Ag to bind the pRb oncosuppressor protein which is involved in cell cycle control. So two mutagenic events (integration and truncation of LT-Ag) occur and appear to be necessary for the development of Merkel cell carcinoma.

KIPyV was detected in lung cancer tissue (Babakir-Mina *et al.,* 2009a), but the meaning of this finding remains unclear. Is it capable of transforming activity? Are the oncogenic viral proteins expressed in this anatomic site?

Many questions remain open. The lack of experimental models for KIPyV and MCPyV make answers to them problematic.

Thanks to the advances in molecular biology, new viruses have been uncovered and many others will be uncovered in the years to come. These discoveries will greatly improve our understanding of viral diseases, viral diagnosis, and the management of viral diseases. New viruses are welcome.

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