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BACHELOR THESIS

The Persistence of Human Polyomaviruses

Perzistence lidských Polyomavirů

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Author's declaration:

I declare that I have written this thesis independently with the help of literature and sources listed. Neither this thesis nor any substantial part of it was submitted with the aim to obtain another or the same academic degree.

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List of abbreviations:

BKV – BK virus

HIV – Human Immunodeficiency Virus

IFN-γ – Interferon gamma

JCV – JC virus

KIV – Karolinska Institute Polyomavirus

LPV – Lymphotropic Polyomavirus

LT-Ag – large tumour antigen

MCC – Merkel cell carcinoma

MCV – Merkel cell Polyomavirus

MT-Ag – middle tumour antigen

PBMC - peripheral blood mononuclear cells

PHFG - permissive human foetal glial cells

PCR – polymerase chain reaction

PML – progressive multifocal leukoencephalopathy

PyV – Murine Polyomavirus

ST-Ag – small tumour antigen

SV40 – simian virus 40

truncT-Ag – truncated large tumour antigen

TSV – Trichodysplasia Spinulosa-associated Polyomavirus

WUV – Washington University virus

Abstract

Despite years of research, even the most scrutinized Polyomaviruses - BK and JC - have not yet been thoroughly understood. With a number of new Polyomaviruses - KIV, WUV, MCV, HPyV6, HPyV7, TSV and HPyV9 described in the past few years, the need to understand how Polyomaviruses operate in their hosts has become even more urgent.

The probable route of transmission appears to be either respiratory or faecal-oral. The initial infection occurs most likely in the early childhood or early-adolescence and is followed by a life-long persistence. The seroprevalence of Human Polyomaviruses among healthy adult population is high: BKV (81-97 %), JCV (35-69 %), KIV (55 %), WUV (69 %), MCV (25-46 %) and TSV (70-80 %). Human Polyomaviruses can cause fatal diseases in immunocompromised patients.

The site of persistence in humans probably varies depending on the specific Polyomavirus. BK and JC are known to persist in kidneys and the urinary tract. Human Polyomaviruses have been detected in the lymphatic tissues, blood, respiratory, urinary, and gastrointestinal systems. It is not clear, however, if they persist in all of these sites.

Mechanisms which Polyomaviruses use to establish and maintain persistent infection could include the viral miRNA and viral agnoprotein, which would result in a modulation of viral proliferation and of the immune response. To some extent Polyomaviruses are capable of self-regulation.

Keywords: Human polyomaviruses, persistence, miRNA, alpha-defensins, seroprevalence, site of persistence

Abstrakt

Navzdory mnohaletému výzkumu jsou i ty nejznámější Polyomaviry – BK a JC – stále záhadou. Vzhledem k objevení mnoha nových Polyomavirů - KIV, WUV, MCV, HPyV6, HPyV7, TSV a HPyV9 v průběhu posledních pár let se ještě zdůraznila důležitost výzkumu mechanismů, které Polyomaviry využívají ve svých hostitelích.

Pravděpodobný způsob přenosu je buď skrze dýchací cesty nebo fekálně-orální. Počáteční infekcí lidé procházejí nejčastěji v raném dětství nebo v předpubertálním věku. Poté následuje celoživotní perzistence. Seroprevalence lidských Polyomavirů je mezi dospělou populací relativně vysoká: BKV (81-97 %), JCV (35-69 %), KIV (55 %), WUV (69 %), MCV (25-46 %) a TSV (70-80 %). Lidské polyomaviry mohou v imunosuprimovaných jedincích působit i smrtelná onemocnění.

Místo perzistence v lidech pravděpodobně závisí na konkrétním Polyomaviru. U BK a JC virů je prokázána perzistence v ledvinách a močových cestách. Lidské Polyomaviry byly dále detekovány v lymfatických tkáních, krvi, dýchacích, močových a trávicích cestách, není ale jasné, zda ve všech těchto místech i perzistují.

Mezi mechanismy, které Polyomaviry využívají k vytvoření a udržení perzistence, mohou patřit virové miRNA a virový agnoprotein, které modulují samotnou proliferaci viru i imunitní odpověď. Jsou tedy do určité míry schopné seberegulace.

Klíčová slova: Lidské Polyomaviry, perzistence, miRNA, alfa-defenziny, seroprevalence, místo perzistence

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1. Introduction

Polyomaviruses are small DNA viruses that belong to the family *Polyomaviridae*. While some viruses from this family like SV40, JCV or BKV have been known for decades, several new have been described in the past few years.

Polyomaviruses can infect a wide variety of avian, rodent and primate hosts. Contrary to this large scale of hosts particular Polyomaviruses are very host-specific and possess high cell tropism.

1.1. Human Polyomaviruses

Human Polyomaviruses include the nine species known to infect men. In 1971, BK virus (BKV) (Gardner et al., 1971; in Howley et al., 1975) and JC virus (JCV) (Padgett et al., 1971 in Howley et al., 1975) have been first described and they have been studied extensively since then. More recently a number of new Human Polyomaviruses has been discovered: Karolinska Institute Polyomavirus (KIV) (Allander et al., 2007), Washington University virus (WUV) (Gaynor et al., 2007) and Merkel Cell virus (MCV) (Feng et al., 2008), Human Polyomavirus-6 (HPyV6) and Human Polyomavirus-7 (HPyV7) (Schowalter et al., 2010), Trichodysplasia Spinulosa-associated virus (TSV) (van der Meijden et al., 2010) and Human Polyomavirus 9 (HPyV9) (Scuda et al., 2011).

Additionally it has been established that two other Polyomaviruses can be found in humans – Simian virus 40 (SV40) (Kean et al., 2009; Knowles et al., 2003) and Lymphotropic Polyomavirus (LPyV) (Takemoto et al., 1982; Viscidi and Clayman, 2006).

Following the initial exposure Polyomaviruses are known to establish long-term persistence. In spite of decades of research we still lack a detailed knowledge of interactions between

Polyomaviruses, host cells and the immune system. Shedding some light onto these will help us understand the mechanisms used by Polyomaviruses to establish and maintain this persistent infection.

This thesis therefore aims to review past findings and suggest promising new areas that could elucidate the mechanisms of Polyomavirus persistence in humans. Since very little is known about HPyV6, HPyV7, TSV and HPyV9, this work will focus mainly on the remaining 5 Human Polyomaviruses with the occasional addition of SV40.

2. Polyomaviruses

Polyomaviruses consist of non-enveloped virions with icosahedral symmetry and supercoiled double-stranded circular DNA genome, which put together with cellular histone lacking H1 creates a so called viral minichromosome.

2.1. Genome of Polyomaviruses

The genome is composed of the early region encoding the large tumour antigen (LT-Ag) and the small tumour antigen (ST-Ag) located on one strand. In rodent Polyomaviruses middle tumour antigen (MT-Ag) is also present nevertheless Human Polyomaviruses do not encode MT-Ag. On the other strand, we can find the late region encoding capsid proteins VP1, VP2 and VP3 and in BKV, JCV and SV40 also VP4. Between these two regions we can find a highly variable regulatory region (NCCR).

NCCR contains binding sites for the LT-Ag in all Human Polyomaviruses but PyV6, where only a LT-Ag-binding motif can be found (Van Ghelue et al., 2012). Possible binding-sites for a number of human transcription factors are also located in the NCCR region, however little is known about whether or not binding actually occurs (Van Ghelue et al., 2012).

The Human Polyomavirus genome encodes other mRNAs, but they are not present in every virus, some even encode miRNAs. For details about specific mRNAs, please see Table 1.

2.1.1. Agnoprotein

JC, BK, SV40 encode an agnoprotein in the late region, however that does not apply to any other Human Polyomaviruses (see Table 1 for details).

The agnoprotein might play a significant role in the release of viral particles as it increases the permeability of the membranes (Suzuki et al., 2010). It has also been shown to decrease T-

antigen-induced DNA replication of JCV and modulate gene expression, both possibly achieved through direct interaction with an early protein LT-Ag (Safak et al., 2001). Keeping JCV proliferation in check may be one of the mechanisms of Polyomavirus persistence (Safak et al., 2001). Further looking into the functions and impacts of agnoprotein on viral processes and persistence could provide valuable observations.

2.1.2. Truncated T-Ag

In some Human Polyomaviruses a third early protein, so-called truncated tumour antigen (truncT-Ag) is encoded. So far, it has been described in BKV (Abend et al., 2009) and MCV (Shuda et al., 2008) and the function seems to be very different in each virus. In BKV, it is a result of alternative splicing of LT-Ag with a predicted function in the regulation of the cell cycle (Abend et al., 2009). While in MCV, it is a genetic mutation, that is likely required for the creation of MCC (Shuda et al., 2008).

2.1.3. VP4

Recently a so-called very late viral protein VP4 has been found in SV40 (Daniels et al., 2007). VP4 is a nucleus-located non-structural protein necessary for the induction of the lysis of the host cell and the propagation of the viral replication (Daniels et al., 2007). Open reading frame for VP4 has also been found in JCV and BKV, however expression of VP4 has not yet been demonstrated in their case (Van Ghelue et al., 2012). Other Human Polyomaviruses do not encode VP4 (Van Ghelue et al., 2012).

Virus	Agnoprotein	Trunc T-Ag	VP4
BKV	Yes (Rinaldo et al., 1998)	Yes (Abend et al., 2009)	ORF, but expression not demonstrated (Van Ghelue et al., 2012)

JCV	Yes (Safak et al., 2001)	No data	ORF, but expression not demonstrated (Van Ghelue et al., 2012)
KIV	No (Allander et al., 2007)	No data	No (Van Ghelue et al., 2012)
WUV	No (Gaynor et al., 2007)	No data	No (Van Ghelue et al., 2012)
MCV	No (Van Ghelue et al., 2012)	Yes, truncT-Ag in MCC prevents viral activation (Shuda et al., 2008)	No (Van Ghelue et al., 2012)
TSV	No (van der Meijden et al., 2010; Van Ghelue et al., 2012)	No data	No (Van Ghelue et al., 2012)
SV40	Yes (Jay et al., 1981 in Rinaldo et al., 1998)	No data	Yes (Van Ghelue et al., 2012)

Table 1 – The Proteins of Human Polyomaviruses

2.1.4. miRNA

BKV and JCV were shown to encode miRNAs (Seo et al., 2008) similar to SV40 miRNA, which was first described in 2005 (Sullivan et al., 2005). MCV miRNA was also found (Lee et al., 2011) and studied (Seo et al., 2009). In case of other Human Polyomaviruses, miRNA presence has not yet been proven. Nevertheless the computer models (Kumar et al., 2009; Li et al., 2008) have predicted miRNA sequences in the genome of all other Human Polyomaviruses (Van Ghelue et al., 2012). Chen et al. (2011a) have not found a persuasive pre-miRNA candidate using the Vmir software in the case of WUV, but Van Ghelue et al. (2012) have been successful with more accurate software.

3. Seroprevalence

Human Polyomaviruses appear to be widespread in the population to a point of ubiquity.

3.1. Seroprevalence of BKV

Overall detected seroprevalence for BKV in healthy adults is 81 % - 97 % (Antonsson et al., 2010; Egli et al., 2009; Kean et al., 2009; Knowles et al., 2003). Similar rates were found in pregnant women over 25 years of age - 96 % (Stolt et al., 2003). For a more detailed view, please see Table 2.

Study	Test group	Seroprevalence
Knowles et al. (2003)	2 435 UK residents, 1 - 69 years old	81%
Antonsson et al. (2010)	458 Australians participating in a longitudinal skin cancer study, 27 – 76 years old	96 % or 97% based on the sample set
Egli et al. (2009)	400 healthy Swiss-based blood donors, 20-59 years old	82 %
Kean et al. (2009)	1 501 Americans over the age of 21	82%
	721 Americans under the age of 21	73 %
Stolt et al. (2009)	150 Finnish women in the first trimester of pregnancy, 14 – 31 years old	Up to 96%
	290 Swedish children aged 0 – 13	Significant increase from early childhood to 98 % in the 7 – 9 years of age group

Table 2 – The Seroprevalence of BKV

3.2. Seroprevalence of JCV

JCV seroprevalence appears to be lower than that of BKV with 35 - 69 % (Antonsson et al., 2010; Egli et al., 2009; Kean et al., 2009) and 72 % in pregnant women over 25 years of age (Stolt et al., 2003). For details, see Table 3.

Study	Test group	Seroprevalence
Antonsson et al. (2010)	458 Australians participating in a longitudinal skin cancer study, 27 – 76 years old	63 %

Egli et al. (2009)	400 healthy Swiss blood donors, 20–59 years old	58 %
Kean et al. (2009)	1 501 healthy US blood donors over the age of 21	39 %
Knowles et al. (2003)	2 435 UK residents, 1 - 69 years old	35 %
Stolt et al. (2003)	150 Finnish women in the first trimester of pregnancy, 14 – 31 years old	72 %

Table 3 – The Seroprevalence of JCV

3.3. Seroprevalence of Other Human Polyomaviruses and SV40

Even other Human Polyomaviruses are widespread in the serum of the adult population: MCV – 25-46 % (depending on the isolate), KIV – 55 %, WUV – 69 % (Kean et al., 2009) and TSV – 70-80% (Chen et al., 2011c; van der Meijden et al., 2011). The rates detected for SV40 are 3.2 - 9% (Kean et al., 2009; Knowles et al., 2003). The seropositivity for MCV IgG in pregnant women reached 45.9 % (Sadeghi et al., 2010). For a detailed view, see Table 4.

Study	Virus	Test Group	Seroprevalence
Sadeghi et al. (2010)	MCV	462 pregnant Finnish women in their first trimester	45.9 %
Kean et al. (2009)	MCV strain 350	1 501 healthy US adult blood donors over the age of 21	25 %
	MCV strain 339		42 %
	KIV		55 %
	WUV		69 %
	SV40		9 %
Chen et al. (2011c)	TSV	149 Finnish adults	70 %
Van der Meijden et al. (2011)	TSV	380 healthy Dutch adults, 20-79 years old	80 %
Knowles et al. (2003)	SV 40	2 435 UK residents, 1 - 69 years old	3.2 %

Table 4 – The Seroprevalence of other Human Polyomaviruses

3.4. Seroprevalence in regards to Gender

There has never been found any difference between the seropositivity for Polyomaviruses in regards to the gender (Antonsson et al., 2010; Chen et al., 2011c; Kean et al., 2009), with the exception of one study. In it, more men than women were found to have antibodies to JCV, however that could be attributed to the seroprevalence increasing more rapidly in men (Knowles et al., 2003). In the case of MCV, higher IgG seroprevalence rate and higher IgG seroconversion rates were seen in males in a study on MCV prevalence in children (Chen et al., 2011b). An intriguing find was the fact that BKV IgG activity is higher in women, but JCV IgG activity is the same in both genders (Egli et al., 2009).

Generally, it is possible to conclude that no significant distinction can be made between seroprevalence in men and women based on the current findings.

3.5. Age-Distribution of Seroprevalence

The initial assessment of Polyomavirus infection suggested two possible modi operandi – lifelong persistence or repeated re-infections. Long-term persistent infection has been proven for JCV (Kitamura et al., 1997) and appears to be most likely for BKV and possibly HPyV6 and HPyV7 (Schowalter et al., 2010) and other Human Polyomaviruses.

3.5.1. Differences in Polyomavirus Seroprevalence between Age-Groups

The exposure to BKV, MCV, KIV, WUV and SV40 happens in the early childhood, while the initial infection with JCV occurs in pre-adolescence (Chen et al., 2011b; Kean et al., 2009; Stolt et al., 2003). The age of primary infection with JCV appears to be more dependent on specific community with socioeconomic status suggested as a reason for this variability (Knowles et al., 2003). In the case of TSV, the increase in seroprevalence seems to be gradual, from early

childhood to around 30 years of age, and then the levels remain approximately the same (Chen et al., 2011c; van der Meijden et al., 2011).

In the case of BKV the seroprevalence for children around the age of 9 is very high reaching from 91 % (Knowles et al., 2003) to more than 96 % (Stolt et al., 2003). These levels of seroprevalence are more or less maintained up to the age of 60. Afterwards some describe a slight drop (from almost 100 % to 93 – 94 %) (Antonsson et al., 2010), while others see the change as more dramatic (from around 90 % to 68 %) (Knowles et al., 2003).

There is a significant change of IgG seroprevalence as well as IgG activity with increasing age. In case of BKV the trend is a decreasing one with the results ranging from 87 % (Egli et al., 2009) for the lower age group to 71 % in the higher age group. These findings may be a result of the decreasing ability of the ageing organism to produce antibodies.

For JCV the trend is quite the opposite. From young age the seroprevalence keeps increasing slowly and steadily (Egli et al., 2009; Knowles et al., 2003; Stolt et al., 2003) peaking between ages 50 and 70. Some studies suggested that the prevalence then slightly drops, while some described that it remained approximately the same. One study reported that the highest seroprevalence of 82 % was found in people older than 70 (Viscidi et al., 2011). While the absolute numbers for seroprevalence vary from study to another, the trends of increasing seropositivity with increasing age seems to apply generally. The differences found in the group of people older than 70 can be attributed to a relatively smaller number of samples from this age group in all studies. Therefore this would probably be clarified if there were a larger study focused on this age group.

Recently the relation between age and seroprevalence has been described for MCV as well. The levels in children under the age of 10 were 45 % and kept rising steadily till they peaked at 81 % between the ages 60 and 69. Then they slightly decreased to 73 % in people over 70 years of age (Viscidi et al., 2011).

4. The Pathology of Human Polyomaviruses

Human Polyomaviruses are known to be latently present in their hosts, yet in certain cases they can cause serious life-threatening diseases. This chapter illustrates the pathology of the three best scrutinized of Human Polyomaviruses – JCV, BKV and MCV.

4.1. JCV

JCV is a known causative agent of progressive multifocal encephalopathy (PML), a serious demyelinating disease that develops in immunocompromised individuals. PML is caused by the lytic replication of JCV in oligodendrocytes.

Recently, there have been two more diseases linked to the JC Polyomavirus - JC virus granule cell neuropathy (Koralnik et al., 2005) and JC virus encephalopathy (Dang et al., 2012; Wuthrich et al., 2009). Both these diseases require further research due to a very limited number of documented cases.

So far 3 types of JCV virus have been described based on their DNA (its sequence variation and restriction fragment length polymorphism) – type A prevalent only in Europe, type B prevalent in Asia and Africa and type C found in part of Africa (Guo et al., 1996; Yogo et al., 1991). Humans become persistently infected with a specific JCV type, according to the geographic location early in life, and the association remains even if they live in a different geographic region later in life

(Kitamura et al., 1997), which further supports the hypothesis of life-long persistent monoclonal infection rather than common repeated re-infections.

4.2. BKV

BKV has been identified as a cause of BKV-associated nephropathy (Hogan et al., 1980 in Leung et al., 2001). It has also been associated with the development of the heamorrhagic cystitis (Leung et al., 2001), a disease responsible for mortalities after a bone marrow transplant.

BKV isolates can be divided into 4 genotypes – I, II, III, IV, with the first genotype being further categorized into Ia, Ib1, Ib2 and Ic (Luo et al., 2009). The genotype I is both the most common and geographically widespread, while the other three genotypes appear to be far less common (Pastrana et al., 2012). There is an ongoing issue with the detection of genotypes III and IV, which are less sensitive to commonly used PCR primers, designed mainly for genotype I (Randhawa et al., 2011), therefore it is necessary to confirm these findings with a study designed to cope with this problem.

4.3. MCV

Even though MCV presence has been proven in 80 % of the Merkel cell carcinoma (MCC) samples (Feng et al., 2008), other study brought evidence of a chronic MCV shedding from the skin surface that occurs in 40 % of healthy humans (Schowalter et al., 2010). Which would point to a more common epidermal cell type than Merkel cells being the likely site of MCV persistence (Schowalter et al., 2010). The link between MCC and MCV is therefore still questioned.

There is evidence showing that the association of MCC with specific MCV genotypes is unlikely. As to the serotype of MCV, all evidence appears to support that there is just one for all MCV isolates (Schowalter et al., 2010).

4.3.1. The Causal Link between MCV and MCC

MCV has also been found in healthy individuals and other tissues than Merkel cells. Moreover there are MCCs which do not test positive for MCV, on the other hand it has been suggested that MCV could induce transformation to MCC after which the transformed cells would lose the viral DNA resulting in MCV-negative MCC lines (Houben et al., 2010).

The fact that MCV integration into the genome of the host cell occurs before the clonal replication of tumour cells (Feng et al., 2008; Sastre-Garau et al., 2009) and that only monoclonal copy of MCV can be found in MCC cells (Sastre-Garau et al., 2009; Shuda et al., 2008) supports the causality. It was proposed by Shuda et al. (2008) that since MCV in MCC have only truncated T-Ag, based on their findings, truncT-Ag prevents the activation and replication of viral DNA as well as it aids carcinogenesis. In this model both MCV insertion and LT-Ag mutation must occur for the development of MCC. These two uncommon events could explain why there is a very small number of MCC cases while MCV seropositivity is not rare at all (Shuda et al., 2008).

5. Site of Persistence

5.1. Kidney

JCV and BKV are both known to create lifelong persistence in the kidney (Chesters et al., 1983; in Sundsfjord et al., 1999). JCV presence in the kidney is found regardless of whether PML patients or healthy individuals are studied (Boldorini et al., 2005; White et al., 1992). JCV and BKV presence has been proven in the urinary tract – in the kidneys, the renal pelvis, the urinary bladder and the ureter (Boldorini et al., 2005).

5.1.1. Viruria

The viruria, presence of viruses in the urine, of JCV was found to be 19 %, compared to a lower 7 % viruria of BKV (Egli et al., 2009). Only 1 % of people have both BKV and JCV detectable (Egli et al., 2009).

In case of BKV, there is no correlation between urinary shedding and IgG level, whereas in JCV, antibody response was significantly higher in people with high levels of urinary shedding (Egli et al., 2009).

MCV was found by PCR in the urine of MCC patients, but not in the urine of healthy individuals (Mertz et al., 2010). The PCR might have detected only viral fragments or neutralized particles, therefore it remains unclear if MCV can persist in the urinary tract (Mertz et al., 2010). KIV and WUV were not detected in the urine (Bialasiewicz et al., 2009; Gaynor et al., 2007).

Kidneys are considered the site of persistence of BKV and JCV, while other Human Polyomaviruses are not believed to persist there. The likely persistence of BKV and JCV is further substantiated by the continuous urinary shedding (Kitamura et al., 1997).

5.2. Brain

Even though in a few studies JCV was not detected in non-PML brains, other researchers have found evidence of JCV genes and only low levels of viral proteins (Tan et al., 2010; White et al., 1992), which could suggest a latent infection with occasional expression of LT-Ag (Tan et al., 2010).

Even though one study has found KIV and WUV in the brain tissue of HIV-positive patients (Barzon et al., 2009) no one else did (Bialasiewicz et al., 2009; Focosi et al., 2009; Giraud et al.,

2009). MCV has been found neither in the childhood CNS tumours (Giraud et al., 2009) nor in immunocompetent adults (Lam et al., 2010).

Overall JCV is the only Polyomavirus DNA of which has been found in the human brain, even though many questions about its possible persistence in the brain are yet to be answered. BKV, MCV, WUV and KIV have not been detected.

5.3. Blood

Despite some suggestions that the peripheral blood mononuclear cells (PBMC) could be another site of persistence of BKV and JCV, it is not likely to be so in healthy children and adults (Comar et al., 2010; Dolei et al., 2000). Detection of viral DNA in PBMCs seems to point to a recent infection rather than persistent one (Dolei et al., 2000).

An interesting find was that there has been increased prevalence of BKV in peripheral blood mononuclear cells of operators of a blood transfusion centre, when they were compared with blood donor group of the same age characteristics. The difference was quite significant – BKV NCCR levels were 3 times higher and BKV VP1 were 9.4 times higher, pointing to a occupational hazard of re-infection or reactivation of BKV (Dolei et al., 2000).

MCV has been found in 10 % of non-neoplastic lymph node specimens of adults, which could suggest that lymphocytes and monocytes might be the site of MCV persistence in humans (Toracchio et al., 2010). Inflammatory monocytes were found to be harbouring MCV (Mertz et al., 2010). Even though it remains to be determined if the inflammatory monocytes are just digesting inactive virus or actually providing a hiding spot for MCV, Mertz et al. (2010) proposed a MCC creation mechanism. In it, the infected monocyte is directed to the already-

present inflammation of the skin, e.g. psoriasis, by chemokines, where it then releases the virus and helps create the MCC. If this is so is yet to be determined.

In plasma, it is impossible to detect either BKV or JCV (Egli et al., 2009). Plasma specimens, however, contained KIV and WUV in both healthy blood donors (3.1 % and 0.8 %) and HIV-positive patients (2.6 % and 4.6 %), with only WUV infection being slightly more likely in immunocompromised people.

5.4. Tonsils

In immunocompetent children and adults, rates of JCV infection were found to be 35 – 39 % with either tonsillar stromal-cells or tonsillar lymphocytes infected (Monaco et al., 1998).

Contrary to some studies (Kato et al., 2004; Monaco et al., 1998), JCV levels in tonsils of immunocompetent children were found to be low in one study (Patel et al., 2008). The result can be explained by the lower sensitivity of the methods used and possibly by the fact that primary JCV infection occurs at higher age than in the case of SV40 (Patel et al., 2008). Another study has not detected any JCV DNA in the tonsillar tissue of adults (Babakir-Mina et al., 2009) and attributed the fact to the high degree of JCV prevalence variation depending on the geographical region.

BKV presence in the tonsillar tissue was detected (Babakir-Mina et al., 2009; Goudsmit et al., 1982 in Goh et al., 2009). KIV and WUV were discovered in tonsils too, in contrast to MCV, which was not detected (Babakir-Mina et al., 2009). SV40 was found in tonsils of immunocompetent children suggesting that SV40 infects the lymphoid system and tonsils specifically (Patel et al., 2008).

5.5. Gastrointestinal Tract

Polyomavirus (BKV, JCV and SV40) shedding in the stool occurs in immunocompetent adults (BKV – 8.2%, JCV – 9.1%, SV40 – 1.8%) and leads some to believe that the probable site of Polyomavirus persistence is the gastrointestinal tract (Vanchiere et al., 2009). Polyomavirus presence was determined for healthy adults and those with a compromised immunity. Results have shown that there is no significant difference in the JCV shedding in the stool (18.1 % for immunocompromised compared to 18.2 % in immunocompetent) (Vanchiere et al., 2009), which further supports the hypothesis of a persistent infection/common repeated reinfection.

JCV LT-Ag presence has been proven in malignant and normal colorectal epithelium (Laghi et al., 1999) and generally in both the upper (70.6 %) and lower (81.2 %) gastrointestinal tract (Ricciardiello et al., 2000).

When the Polyomavirus shedding in stool (JCV, BKV and SV40) was compared for children and adults, significantly higher numbers were reached in the former (Vanchiere et al., 2009; Vanchiere et al., 2005). Both WUV and KIV have been detected in the gastro-intestinal tract (Bialasiewicz et al., 2009).

The evidence would point to a higher shedding during the initial infection followed by a weaker long-term persistence.

5.6. Respiratory Tract

No BKV or JCV was found in the salivary glands or oropharyngeal epithelial cells of both healthy and HIV-positive humans, which likely rules them out from being a potential site of persistence of Polyomaviruses in humans (Sundsfjord et al., 1994).

MCV was detected in 4.3 % of nasopharyngeal aspirates at low levels, mainly in samples from adults rather than children (Goh et al., 2009).

WUV and KIV were detected at 2.4 % and 0.5 % respectively in the nasopharyngeal aspirates of pediatric patients with acute respiratory tract infections, however, they are not thought to be the cause of the respiratory disease of the patients (Ren et al., 2008). No WUV or KIV were found in nasal and throat swabs from immunocompetent adults (Ren et al., 2008). Since WUV and KIV are found mainly in the respiratory tracts of children (Bialasiewicz et al., 2009; Ren et al., 2008), it has been proposed that respiratory tract is the place of initial infection, but not of persistence (Bialasiewicz et al., 2009).

6. Transmission

Even though the exact way is still unknown, there has been suggested a faecal-oral or a respiratory transmission of Human Polyomaviruses.

The initial infection is thought to occur early in childhood and is probably followed by the establishing of lifelong persistence. In the case of TSV persistent infection has not yet been proven, but in light of other Human Polyomaviruses, it is likely even in its case (van der Meijden et al., 2011).

6.1. Faecal-Oral Transmission

Environmental behaviour of JCV and BKV viruses was studied with interesting results. Virions of both viruses were observed with high prevalence (JCV: 93 % - 96 % with very high levels of JCV particles, BKV: 62 % - 77.8 %) in urban sewage (Bofill-Mas et al., 2000) at divergent geographical locations with similar results (Bofill-Mas et al., 2001) and in the sludge and solids generated in wastewater treatment plants (Bofill-Mas et al., 2006). JCV has also been proven to

have high t_{90} value (the time required to reduce the viral concentration by 90 %) of 63.9 days (Bofill-Mas et al., 2006). All this substantiates the argument that humans could become infected with JCV and possibly BKV through the consumption of contaminated water or food (Bofill-Mas et al., 2001).

Furthermore, recently it has been reported that MCV, KIV and WUV are also present in urban sewage in 7 out of 8, 1 out of 8 and 2 out of 8 samples respectively (Bofill-Mas et al., 2010), which would lead to a conclusion that Human Polyomaviruses presence in polluted waters is common. This together with the presence of *Polyomaviridae* in the gastrointestinal tract would suggest that Human Polyomaviruses are perpetuated through faecal-oral way.

Specifically the detection of KIV and WUV in the gastrointestinal tract could indicate that the faecal-oral transmission occurs even with these two Polyomaviruses, in contrast to the respiratory route of transmission, which has seemed more likely. However, their presence in the intestine does not prove that it is an active site of replication (Bialasiewicz et al., 2009), therefore further investigation into the matter is required.

6.2. Mother-to-Foetus Transmission

A small-scale study suggested a possibility of BKV transmission from mother to foetus with 80 % of maternal tissue samples and 80 % of placenta, brain and 60 % of kidney foetal samples testing positive for regulatory region of BKV. There was no such evidence for JCV in that study (Pietropaolo et al., 1998).

There has not been found any strong evidence to suggest that mother-to-foetus transmission is possible in KIV, WUV and MCV, as illustrated by only one case of MCV positivity (but not of KIV or WUV) in a miscarried foetus out of 535 foetal samples, the rest being seronegative for

KIV, WUV and MCV (Sadeghi et al., 2010). However most of the samples were acquired in the first trimester of pregnancy, therefore it might be possible that potential transmission occurs later on.

6.3. Respiratory Route of Transmission

Since some Human Polyomaviruses were found in respiratory tract, a respiratory route of transmission has been proposed. Most importantly it was the discovery of KIV and WUV in respiratory tract samples - 3 % of respiratory specimens positive for WUV (Gaynor et al., 2007) and 1% of nasopharyngeal aspirates for KIV (Allander et al., 2007). Moreover 4.3% samples of respiratory secretions tested positive for MCV (Goh et al., 2009). BKV(Goudsmit et al., 1982 in Goh et al., 2009) and JCV (Monaco et al., 1998) presence in the tonsillar tissue was also detected.

A study by Comar et al. (2010) that investigated Polyomavirus presence in children's tonsils, adenoids and PBMC found that BKV and SV40 were rarely present and JCV was not present at all. These results would argue against the respiratory route of transmission for these viruses, even though the results could have been influenced by the low age of the subjects (Comar et al., 2010).

However, KIV and WUV were often detected in the respiratory tract of children, but not of adult's (Bialasiewicz et al., 2009; Ren et al., 2008), which suggests that they are transmitted through the respiratory route (as stated in 5.6.).

With current information, it seems that either the respiratory or faecal-oral transmission is most likely for Human Polyomaviruses. Since there is no correlation between JC and BK virus infection and seropositivity, it can be assumed that these two are transmitted independent on one another and possibly even via different routes (Knowles, 2006). The same probably applies to other Human Polyomaviruses as well.

No conclusive evidence about the route of Polyomavirus transmission makes determining the mechanisms of the establishment of the persistent infection even harder.

7. Molecular Mechanisms

Little is known about the mechanisms that Polyomaviruses use to escape immunity in persistent infection. In the past few years, however, there have been some studies that can shed some light on the molecular interactions between the virus, the host cell and the immune system.

7.1. miRNA

MicroRNAs (miRNAs) are small RNA molecules, approximately 22-nucleotides in length, that can influence a number of processes in wide range of organisms including the cell cycle control, the transition from latent state to lytic cycle and many others (reviewed in Bartel, 2004).

Primary transcripts of RNA (pri-mRNA) are cleaved by an endonuclease, so that a hairpin structure from pri-mRNA can be transported to cytoplasm. This hairpin structure is called a pre-mRNA and is further cleaved into the final miRNA of 22 nucleotides, which is stabilized by the RNA induced silencing complex (RISC). MiRNA can then either cleave or block target mRNAs.

7.1.1. Homogeneity of miRNAs in Polyomaviruses

JCV and BKV encode pre-miRNAs that are homologous to SV40 pre-miRNA (Seo et al., 2008), while PyV pre-miRNA does not bear any sequence identity to SV40 pre-miRNA (Sullivan et al., 2009).

7.1.2. miRNA and LT-Antigen

Polyomavirus miRNA has been shown to target the transcription rates of the large T Antigen late during infection in BKV and JCV (Seo et al., 2008), SV40 (Sullivan et al., 2005) and MCV (Seo et al., 2009). Furthermore miRNAs with the same function were proven to exist in Bandicoot

Papillomatosis Carcinomatosis Viruses 1 and 2 (BPCV 1 and BPCV 2) (Chen et al., 2011a). BPCVs were only described recently and are likely hybrids of Polyomaviridae and Papillomaviridae. That would suggest that the polyomaviral or polyomaviral-like genomic organization, which includes miRNAs that regulate LT-Ag, is evolutionary advantageous and therefore maintained (Chen et al., 2011a).

In the case of MCC tumours, very low levels of miRNA expression were found (Lee et al., 2011). Since LT-Ag is necessary for MCC growth (Houben et al., 2010), it makes sense that miRNAs, which would inhibit LT-Ag and thereby the formation of MCC, are not expressed in high numbers (Lee et al., 2011).

On the contrary, in case of PyV no substantial effect on transcription rates of LT-Ag has been shown and the PyV miRNA is not essential for the infection of the host (Sullivan et al., 2009) and for WU virus Vmir software package failed to identify strong pre-miRNA candidates (Chen et al., 2011a), which could suggest that the importance of the regulatory function of miRNA is not as significant as it may seem (Chen et al., 2011a). Further research into the issue might help elucidate these questions.

In all cases mentioned above the Polyomavirus miRNA can be found antisense to the early transcripts, even though the exact position may vary (Chen et al., 2011a).

miRNAs have two arms – 3p and 5p. In SV40 both arms, which are encoded by one pre-miRNA, affect the same target mRNA. SV40 miRNAs were proven to cleave viral early mRNA and thereby downregulate LT-Ag levels in the host. The miRNAs activities did not influence the number of viral particles, but rather they decreased the susceptibility of the SV40-infected cells to

the cytotoxic T-cells (Sullivan et al., 2005). In contrast miRNA did not have a significant effect on the T-cell response to LT-Ag in case of murine PyV in vivo (Sullivan et al., 2009).

7.1.3. miRNA and Ligands for the NKG2D Receptor

The miRNA derived from the 3p arm of the pre-miRNA hairpin (miR-J1-3p) has been proven to downregulate the host gene ULBP3 that encodes one of the eight stress-induced ligands for the NKG2D receptor of NK cells and CD8+ T-cells (Bauman et al., 2011), which suggests a significant role of miRNA in modulating the immune response. Furthermore, it was shown that miR-J1-3p of JCV and BKV was expressed in levels significant enough for the downregulation of ULBP3 gene and that it lead to a decrease in NKG2D-mediated killing (Bauman et al., 2011).

It was suggested that since miRNA targets only the ULBP3 ligand and at the same time levels of expression of MICA, another ligand of NKG2D, are downregulated as well following the JCV infection, it might imply yet another way of targeting NKG2D ligands, presumably by a protein-based mechanism (Bauman and Mandelboim, 2011).

7.2. Polyomavirus Mechanisms Regarding Interferon and Apoptosis

JCV infection of permissive human foetal glial cells (PHFG) cells increases the transcription of interferon-responsive genes including myxovirus resistance 1 (MxA), 2’5’-oligoadenylate synthetase (OAS) and interferon alpha-inducible protein (IFI6 - 16). As a result products of these genes might restrict the spread of the virus and help establish a long-term persistence in the host (Verma et al., 2006).

The significance of the role of IFN-γ has been shown in the case of BKV (Abend et al., 2007) and murine Polyomavirus PyV (Wilson et al., 2011), where IFN-γ was effective in directly reducing the incidence of viral proteins and hinder host cell proliferation. IFN-γ-mediated inhibition takes

place at the level of transcription of viral proteins in BKV infected cells (Abend et al., 2007). On the other hand, the absence of IFN-γ receptor signaling lead to tumorigenesis in 100 % of mice, that had been neonatally infected with murine PyV (Wilson et al., 2011).

While it might not be possible to apply the effects of IFN-γ on PyV and BKV generally due to possible difference in mechanisms between specific Polyomaviruses, this interaction definitely presents an interesting opportunity for further research.

JCV LT-Ag down-regulates the expression of the BAG 3 gene and thereby has a pro-apoptotic effect (Basile et al., 2009). Polyomaviruses, however, were shown to possess strong anti-apoptotic mechanisms like the induction of survivin production, which proved to be a significant anti-apoptotic agent in PML (Pina-Oviedo et al., 2007) and seems likely to have the same effect in MCC (Kim and McNiff, 2008).

Finding the balance between the employment between anti-apoptotic and anti-inflammatory and pro-inflammatory (Verma et al., 2006) or pro-apoptotic mechanisms, which prevent the onset of PML in JCV, might be the key to establishing a long-term persistence in the host (Basile et al., 2009).

7.3. The Role of truncT-Ag

A third BKV early protein truncT-Ag originally thought to be a degradation product of T-Ag (Bollag et al., 1989) was recently characterized (Abend et al., 2009). TruncT-Ag is a result of alternative splicing of LT-Ag. It includes the J domain, the binding region for pRb proteins as well as E3 ubiquitin ligase, CUL7 and Bub1 (a mitotic spindle checkpoint protein), which lead to the prediction that it has impact on the regulation of cell growth and transformation (Abend et al., 2009).

The regulatory regions and regions coding early proteins are responsible for low transforming abilities of JCV (Bollag et al., 1989).

It seems that Polyomaviruses themselves have mechanisms that limit the viral infection, which helps them create persistence and escape the immune system.

7.4. Alpha-Defensins

Human alpha-defensin 5 (HD5) and alpha-defensin human neutrophil protein 1 (HNP1) limit the binding of BKV particles to the surface of kidney cells by direct interaction with the Polyomavirus. As a result virions aggregate and thereby the interaction with the host cells is limited. Similarly a dose-dependent inhibition has been observed in case of JCV and SV40 (Dugan et al., 2008). Alpha-defensins could be potentially important factor in controlling the Polyomavirus infection, persistence and onset of a disease. Since the number of copies of the genes for alpha-defensins HNP1, HNP2 and HNP3 and their expression varies significantly between individuals (Aldred et al., 2005), some speculate that this interaction could explain why 10 – 20 % of people never become infected with BK virus (Dugan et al., 2008). HD5 expression has been proven in the small intestine (Bevins 2006 in Spencer et al., 2012) and recently in the kidney and the urinary tract of healthy individuals as well (Spencer et al., 2012), which would further substantiate the possibility of the defensin impact on Polyomavirus infection.

So far there has not been any other research published on the interactions of human defensins and Polyomaviruses than Dugan et al. (2008), but further inquiries into the relation between Polyomavirus persistence and human alpha-defensins seems desirable.

8. Conclusion

With Polyomaviruses being so widespread in the human population, it is surprising that we are still not able to conclusively answer so many basic questions about their behaviour in the human host. The discoveries of several new Polyomaviruses in the past few years and more importantly the association of the MCV with an aggressive Merkel cell carcinoma have initiated many inquiries into their pathogenicity and persistence in humans.

Either the respiratory or faecal-oral route of transmission seems to be most likely for Human Polyomaviruses. The initial infection is followed by a life-long persistence in human host. Immunocompetent individuals have symptoms close to none, but a severe impairment of the immune system can cause the reactivation of the virus and lead to a fatal disease. Most common are PML associated with JCV and BK-associated renal nephropathy linked to BKV.

The most promising areas that could answer the questions about molecular mechanisms used by Polyomaviruses to persist are the study of viral miRNAs, the interactions with alfa-defensins and the influence of Polyomavirus infection on the induction of not only anti-apoptotic, but also pro-apoptotic mechanisms. The Polyomavirus proteins, like the agnoprotein, could provide interesting answers as well.

The recently discovered KIV, WUV, MCV, HPyV6, HPyV7, TSV and HPyV9 need to be studied extensively, so that our understanding of Polyomaviruses grows in width and depth.

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