# Human Polyomaviruses: the battle of large and small Tumor antigens.

**Camila Freze Baez[1], Rafael Brandão Varella[2],Sonia Villani[3], Serena Delbue[3]**

[1]Department of Preventive Medicine, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil
[2]Biomedical Institute, Universidade Federal Fluminense, Niteroi, Brazil
[3]Department of Biomedical, Surgical and Dental Sciences, University of Milano, Milano, Italy

Corresponding author

Serena Delbue,

Department of Biomedical, Surgical and Dental Sciences,

University of Milano, Via Pascal, 36,

20133 Milano, Italy; telephone: 0250315070; email: serena.delbue@unimi.it

**Abstract**

About forty years ago the Large and small Tumor Antigens (LT-Ag and sT-Ag) of the polyomavirus (PyVs) SV40 have been identified and characterized. To date, it is well known that all the discovered human PyVs (HPyVs) encode these two multifunctional and tumorigenic proteins, expressed at viral replication early stage. The two T-Ags are able to transform cells both *in vitro* and *in vivo* and seem to play a distinct role in the pathogenesis of some tumors in humans. Additionally, they are involved in viral DNA replication, transcription, and virion assembly. This short review focuses on the structural and functional features of the HPyVs LT-Ag and sT-Ag, with special attention to their transforming properties.

**Keywords**

**Polyomavirus; Large T antigen; small T antigen; neoplasia**

## Introduction

The *Polyomaviridae* family has been considered capable of transforming mammals' cells *in vitro*. In fact, since the first polyomaviruses were discovered in the 1950s, their tumorigenic properties observed in cell culture and animal models rendered the family name: "polyoma"-multiple tumors[1-5]. In spite of the transformative abilities of simian vacuolating virus 40 (SV40) and murine polyomavirus (MPyV), the human polyomaviruses BK (BKPyV)[6] and JC (JCPyV)[7]infections often are asymptomatic and mostly related to non-neoplastic diseases in immunocompromised individuals. For instance, BKPyV has been associated to nephropathy (PVAN) in renal transplant recipients and hemorrhagic cystitis in allogeneic hematopoietic stem cell transplantation[8-10], while JCPyV is the causative agent of progressive multifocal leukoencephalopathy (PML) in HIV/AIDS and oncologic patients, along with those under immunomodulatory therapies[11,12] .

Almost 40 years have elapsed since the discovery of the first two human polyomaviruses (HPyVs) until the third and fourth HPyVs identification took place, in 2007. The Washington University polyomavirus (WUPyV) and the Karoliska Institute polyomavirus (KIPyV) were detected in children with acute respiratory tract illness through molecular techniques, though their pathogenicity is still controversial[5,13-18]. In 2008, Feng et al.[19], using digital transcriptome subtraction, identified a polyomavirus clonally integrated in about 80% of Merkel cell carcinoma (MCC), a rare and aggressive neuroendocrine neoplasia and hence named the virus after the cancer. Several other research groups confirmed the Merkel cell polyomavirus (MCPyV) presence in MCC, although the viral prevalence in MCC varies around the world[20-34]. This HPyV was the first and, so far, the only human polyomavirus to be etiologically related to a neoplasia in humans. Since its discovery, the scientific curiosity upon polyomaviruses, especially regarding their relationship to human cancer, has been reignited. This resulted in new researches aiming for the identification of HPyVs in different biological samples through new sequencing technologies[35-41]. Consequently, there are 14 proposed HPyV species, but only six of them (BKPyV, JCPyV, MCPyV, HPyV-6,

HPyV-7 and the Trichodysplasia Spinulosa- associated human polyomavirus [TSPyV]) have been associated with human diseases[39,42,43].

To accommodate the fast growing number of new PyVs, an update in the family taxonomy was published in 2016. So far, the International Committee on Taxonomy of Viruses (ICTV) acknowledges 73 polyomavirus species, 13 of which are HPyVs, classified in four genera, according to their genomic properties and natural host[44]. A new nomenclature was proposed, grouping HPyV in three genera and using the official term "*Human polyomavirus*" followed by the viral discovery order, as follows: *Alphapolyomavirus* genus: MCPyV or HPyV-5, TSPyV or HPyV-8, HPyV-9, HPyV-12, NJPyV or HPyV-13; *Betapolyomavirus* genus: BKPyV or HPyV-1, JCPyV or HPyV-2, KIPyV or HPyV-3 and WUPyV or HPyV-4; and *Gammapolyomavirus*genus: HPyV-6, HPyV-7, HPyV-10 or MWPyV and HPyV-11 or TSPyV[44]. In spite of the taxonomical effort made to encompass all described polyomaviruses, three unclassified viruses and a recently described HPyV have not been classified yet[41,44].

HPyVs have a small, non-enveloped icosahedral capsid, presenting 72 pentameric capsomers formed mainly by VP1 associated with minor structural proteins such as VP2 and VP3[45-50]. Viral genome is formed by a double-stranded, histone-associated circular DNA functionally divided into three main regions: a non-coding control region (NCCR), an early coding region and a late coding region. PyVs have bidirectional genomes and therefore, the early and late coding regions are in opposite strands[19,51-55]. Most, if not all of the PyVs transforming capacities are derived from the expression of the early coding region, also known as tumor antigen locus. This region codes for multiple spliced transcripts that, despite the variations observed among HPyVs, generates two main proteins: the large Tumor antigen (LT-Ag) and small Tumor antigen (sT-Ag)[56-60]. These two T-Ags are involved in the coordination of viral replication and gene expression, as well as in the cell cycle progress and malignant transformation both *in vivo* and *in vitro*[57-65]. Hence, this review presents the principal interactions of HPyVs T-Ag involved in tumorigenesis.

**Human polyomaviruses Large Tumor Antigen**

The LT-Ag is a complex, multifunctional protein with many roles in viral replication and cell cycle progression. Due to its ability to replicate in and transform cell cultures, most of the knowledge about T antigens was obtained from SV40 studies[66], which can be extrapolated to other PyVs[55]. Indeed, genetic and protein analyses have shown that LT-Ag virtually belonging to all HPyVs have several common domains: DnaJ domain; origin-binding domain (OBD); zinc (Zn)-binding domain, and helicase/ATPase domain[55,66-68] (Figure 1, panels a-c). For viral DNA replication, six LT-Ag molecules interact as an hexameric structure through the recognition of three out of four pentanucleotide G(A/G)GGC(P1, P2 and P4) motifs in viral replication origin core, which also presents a highly conserved AT-rich region. This recognition is allowed by multiple and complex interactions between OBD, pentanucleotides motifs, GpC dinucleotides, Zn-binding domain, AT-rich region and histidine residues in the helicase/ATPase[69-72]. Two-hexameric LT-Ag unwind the viral genome in both directions through ATP hydrolysis, followed by the binding of the eukaryotic replication protein A (RPA) at the single-stranded viral DNA. The cellular topoisomerase I then releases the torsional stress, while alpha-primase and DNA polymerase initialize the viral DNA synthesis[55,72-76]. This replicative role of LT-Ag is frequently seen in lytic infections, and can be abrogated in neoplasic tissues.

***HPyVs integration and LT-Ag truncation***

As mentioned previously, the first HPyV associated with human cancer, MCPyV, was discovered clonally integrated in about 80% of Merkel cell carcinomas. The viral integration into the cellular genome is a well-known event that may lead to cancer through several pathways, such as functional loss or gain in cell cycle regulation genes, viral activation of human gene promoters, expression of viral oncogenes, and acquisition of mutational profile both in human or viral genes [77]. In fact, LT-Ag premature truncation in MCC samples is considered as a MCPyV tumor-specific

signature, since the LT-Ag from non-tumoral samples do not harbor LT-Ag truncated forms. Hence, it is suggested that after MCPyV integration into the cellular genome the infected cells undergo selection of LT-Ag mutations to prevent viral replication and the viral Ori bidirectional unwinding, which would lead to collision with cellular replication forks[57]. In 2013, Li and colleagues[78] showed that the intact helicase/ATPase domain activates DNA damage response, enhances p53 phosphorylation and cell cycle arrest, which corroborates the proposed explanation for the need of LT-Ag truncation for MCPyV tumorigenesis. Moreover, LT-Ag truncation mutations are unequivocally observed downstream the pRb binding domain, occurring near to the OBD and helicase/ATPase domains. Therefore, a critical role for either pRb binding domain or sT-Ag preservation in MCC pathogenesis has been suggested [62].

Although a strong causal association between other HPyVs with human tumors has not been confirmed yet, circumstantial data suggesting their transformation properties are growing. In fact, a recent meta-analysis revealed that the BKPyV prevalence was significantly higher in prostate cancer (PCa) tissues than in the control, considering as "prostate cancer tissues" samples ranging from very early onset of PCa to well-defined tumors and, as controls, non-PCa samples from PCa patients or benign prostate hyperplasia (BPH)[79]. There are also evidences linking BKPyV to bladder carcinoma in both immunocompetent[80] and renal transplant recipients, as well as in urothelial and renal carcinomas[5,77,81-83].

Furthermore, BKPyV DNA has been found integrated into a limited number of brain tumor[84], urothelial carcinoma[85] and, more recently, a case of renal allograft tumor[77]. BKPyV genome was found linearized and inserted into human chromosome 12 in a high-grade urothelial carcinoma from the allograft after almost 10 years of uneventful renal transplant. Viral genome sequencing revealed a new, undescribed variant of BKPyV subtype 1A, which was named Chapel Hill tumor-associated polyomavirus 1 (or CH-1)[77]. Similarly, the same group reported another undescribed variant of BKPyV 1A subtype, named Chapel Hill 2 (or CH-2) integrated into human chromosome 2 in a

poorly differentiated renal cell carcinoma[83]. Kenan and colleagues[83] suggest that BKPyV integration into host genome after linearization disrupts the expression of late viral transcripts, which would impair LT-Ag downregulation and could eventually lead to tumorigenesis.

Moreover, JCPyV genome has been found integrated into various brain tumors in animal models such as mice and owl monkeys[11,86,87]. Viral integration might also happen in human neoplasia, especially in colorectal cancer[88,89], in which JCPyV is considered a co-factor for chromosomal instability[90,91]. Interestingly, most of BKPyV and JCPyV integration studies did not report a truncated LT-Ag. Instead, the viral genome was disrupted at the VP1 region, although replicative impairment has also been observed[77,82,83]. Nonetheless, unregulated LT-Ag expression, loss of late protein expression and replicative arrest occurred following viral integration[83].

Whereas the role of BKPyV integration in human oncogenesis is still hazy, MCPyV integration into host genomes and its involvement at least in MCC carcinogenesis have been better characterized. Still, the low frequency of MCPyV integration in non-MCC tumor has raised attention. Pantulu and colleagues[92] identified LT-Ag truncation mutations in four chronic lymphocytic leukemia cases, which might indirectly testify the viral integration. Additionally, the deletion of a 90pb fragment in the MCPyV VP1 gene in non-melanoma skin cancer may also indicate incomplete viral integration[93]. Thus, the real role of low-frequency HPyVs integration into human cancers is yet to be established.

***HPyV LT-Ag domains for cellular proteins interaction***

LT-Ag contains several interaction domains with cellular proteins involved in cell cycle regulation. Some of these motifs are well conserved and most of them facilitate viral replication through activation of cellular proliferation and phase S entry, resulting in upregulation of enzymes involved in cellular DNA replication, DNA damage response and accessory replicative enzymes[55,65,66] . Indeed, LT-Ag expression itself induces viral replication and cellular transformation in animal models and in cell cultures, under specific conditions[62,64,66,79,91-96]. The first exon of LT-Ag

(approximately 1-70 amino acids, shared with sT-Ag) contains the DnaJ domain, which has a HPDKGG (His-Pro-Asp-Lys-Gly-Gly) motif conserved among HPyV. The DnaJ domain is able to bind to the Hsc70, a cellular chaperone and transcriptional repressor. HPyVs ability to replicate viral DNA *in vivo* and to promote cell transformation depends on the intact DnaJ domain, as well as capsid assembling *in vivo*[55,68,97-99]. In fact, the MCPyV LT-Ag interaction between its DnaJ domain and Hsc70 has been shown to be necessary for viral replication *in vitro*[65,68]. Moreover, DnaJ domain binding to Hsc70 promotes the chaperone ATPase activity, generating enough energy to dissociate pRb/E2F[55,100-102]. The HPyVs BKPyV, JCPyV and MCPyV also contain a CR1 motif, formed by the pentapeptide LXXLL (Leu-X-X-Leu-Leu), which apparently has an auxiliary role in pRb/E2F disruption and cell proliferation [100,103].

The LT-Ag linker domain is located downstream to the DnaJ domain. Almost all HPyVs (JCPyV, BKPyV, WUPyV, KIPyV, TSPyV, HPyV-6, HPyV-7, HPyV-9), but not MCPyV, present a conserved motif WXXWW (Trp-X-X-Trp-Trp) that can bind to the Bub1, as demonstrated for SV40 LT-Ag[66,104]. Bub1 is a mitotic checkpoint serine-threonine protein kinase that, when functionally impaired, may result in chromosomal instability, as observed in cells expressing SV40 LT-Ag[105]. Although MCPyV is oncogenic, it does not present the Bub1-binding motif. Thus, it is not conclusive whether Bub1:LT-Ag interaction is relevant for HPyVs transformation *in vivo.* Instead, at the nucleotide position where other HPyVs encode Bub1-binding domain, MCPyV has a sequence with little similarity with other PyVs, denominated unique region (MUR)[65,103]. MUR contains a minimal fragment (171–218 nucleotides), the Vam6P-binding domain, that sequestrates this cytoplasmatic protein to a nuclear location, resulting in lysosome clustering impairment, without disruption of TGF-β or mTOR signaling pathways[103]. The LT-Ag interaction with Vam6P seems to be regulatory, as its loss enhances viral replication; consequently, it is considered a mechanism for persistent infection establishment[65,94,95].

Another interesting MUR interaction was observed for other known oncoviruses, such as the bovine papillomaviruses (BPV). Wang and colleagues [106] investigated the interaction between LT-Ag and bromo domain protein 4 (Brd4), a member of BET family involved in cellular growth control, cell cycle progression, and cancer development. The Brd4 interacts with BPV's early protein 2 (E2) promoting the rightful partition of viral episomes to daughter cells during mitosis[106,107]. During the MCPyV infection, a similar interaction between LT-Ag and Brd4 is observed, promoting the MCPyV DNA replication through the cellular protein factor C (RFC) recruitment to viral Ori[106]. In addition, recent studies have shown that the human papillomavirus (HPV) are able to interact with Brd4, which is implicated in viral replication and E2 transcriptional activation function, since the use of Brd4 inhibitor reduces HPV transcription[108,109]. Likewise, the use of a specific peptide, named Brd4 410-730, which is homologous to the same region of the Brd4 but without other domains, successfully inhibited MCPyV replication *in vitro* as it disrupts Brd4:LT-Ag interaction [106].

JCPyV LT-Ag is also able to bind the cellular beta-catenin, a cellular protein belonging to the Wnt pathway, important for tissue development, polarity, differentiation and cell cycle control through cell-cell contact**.** When hypophosphorylated, beta-catenin complexes with LEF-1/TCF-4 transcription factors, and migrates to the nucleus, where it promotes cell cycle progress through c-myc and cyclin D1 expression. Phosphorylated beta-catenin undergoes degradation via ubiquitin-depent proteasome through the activation of a complex formed mainly by the glycogen synthase kinase-3 (GSK-3), Axin scaffold proteins and adenomatous polyposis coli protein (APC), which phosphorilates beta-catenin[91,110-112] In this context, JCPyV promotes beta-catenin stabilization through a LT-Ag central domain, which comprehends amino acid residues from 82 to 628. Besides, this interaction increases beta-catenin levels and promotes its nucleic localization, with subsequent enhancement of c-myc expression[61,113]. Although the JCPyV LT-Ag:beta-catenin interaction was also described in mouse medulloblastoma[114] and in glioblastoma cell lines[115], it has been recently described in human colorectal carcinoma (CRC), in which beta-catenin and Wtn pathway are

frequently increased (Figure 2, panel c). JCPyV LT-Ag and beta-catenin co-localization into the tumor cell nucleus, as well as c-myc and cyclin D1 activation in a subset of JCPyV-positive CRC has been described. This suggests a role of JCPyV in CRC pathogenesis, although JCPyV detection is frequent in both normal colorectal and CRC tissues[91,116]. JCPyV LT-Ag also interacts with the insulin-like growth factor/insulin receptor substrate 1 (IGF/IRS1) pathway, which has been proposed as a mechanism for malign transformation in medulloblastoma. It has been shown that JCPyV LT-Ag binds IRS1 through its C-terminal domain[117]. LT-Ag:IRS1 promotes the IRS1 nuclear translocation, followed by IRS1 interaction with Rad51, which indirectly and eventually prevents DNA damage response[90] (Figure 1, panels a-c).

Despite its recent discovery, it is already known that MCPyV is involved in different molecular regulatory mechanisms. For example, MCPyV detection in non-small cell lung cancer (NSCLC) was found to deregulate BRAF and Bcl2: the first is involved in cell cycle progression through MAP kinase signaling pathway, and was upregulated in MCPyV positive samples; the second is an anti-apoptotic mitochondrial protein that was downregulated in MCPyV infection. These findings were suggestive of MCPyV-mediated deregulation in NSCLC, mainly in smoker patients[118]. Furthermore, another NSCLC study reported a significant association between MCPyV DNA detection and LT-Ag expression with mutation on epithelial growth factor receptor (EGFR) mainly in non-smoker patients, supporting the hypothesis of MCPyV's participation in a subgroup of NSCLC oncogenesis[119].

A similar study investigating PIK3's mutational profile showed a higher frequency of PIK3 mutations in MCPyV-positive MCC than in MCPyV-negative MCC, although the Akt/mTOR expression was higher in MCPyV-negative tumor, supporting the employment of different mechanisms for MCC oncogenesis in distinct MCPyV backgrounds[120]. Furthermore, the expression of tumor-derived MCPyV T-Ag in mice stratified epithelium promotes gross epithelial phenotypes, consistent with neoplasic progression comparable with those observed for high-grade HPV-16 E6

and E7 oncoproteins. Further molecular studies demonstrated the LT-Ag interaction with BIRC5 survivin and increased E2F target gene expression, being both the pathways mediated by LT-Ag LXCXE motif [121].

Finally, BKPyV LT-Ag and other HPyV LT-Ag have been recently found in the upregulation of the DNA cytosine deaminase APOBEC3, an important innate immune system enzyme responsible for suppressing viral replication and cancer. Evidences suggest that APOBEC3 may be correlated with cytosine-based mutation patterns and in coincident sites of DNA rearrangement. As APOBEC3B was also upregulated in HPV-related cancers, it is suggested that HPyV LT-Ag upregulation may also contribute to carcinogenesis through host genomic mutations[122].

***HPyV LT-Ag binding with retinoblastoma protein (pRb)***

Indubitably, one of the most important LT-Ag interaction is that with retinoblastoma protein (pRb) family[55,66] (Figure 2, panel a). This tumor suppressor family comprises three main proteins, pRb, p107 and p130 encoded by the RB1, RBL1 and RBL2 genes, respectively. Although the pRb can regulate the cell cycle through many pathways, its interaction with the E2F transcription factors family is one of the most important, as its loss potentiates tumorigenesis[123,124]. In a hypophosphorylated state, pRb binds the E2F transcription factor, preventing the E2F-mediated gene expression and thus promoting cell cycle arrest. During normal cell cycle progress, cyclin-dependent kinases (CDKs) activation leads to hyperphosphorylation of serine and threonine residues at pRb phosphorylation-specific sites resulting in the release of the E2F transcriptional factors; their transcriptional activity promotes the cell cycle progress from G1 to S phase[125] The components of the pRb family are also known as “pocket proteins”, because of a conserved “pocket” domain that interacts with viral oncoproteins, notoriously the SV40 LTAg and human papillomavirus E7[126].

HPyV LT-Ag, as observed in SV40 LT-Ag prototype, has a highly conserved pRb binding domain in its linker region. The LXCXE (Leu-X-Cis-X-Glu) motif is responsible for the LT-Ag:pRb family

interaction. It has been hypothesized that the energy provided by the LT-Ag DnaJ domain binding to Hsc70 is used to disassociate pRb/E2F through pRb phosporilation, as corroborated by studies showing the DnaJ domain requirement for the SV40 LT-Ag growth activities[127-129]**.** LT-Ag is also capable of disrupting the transcriptional repressor complex formed by p130 and the repressive E2F4/5 proteins through p130 dephosporylation in a DnaJ domain dependent pathway[66,91,130]. The overall pRb:E2F disruption leads to uncontrolled cell cycle progress and, eventually, to malignant transformation. In fact, the pRb pathway deregulation is frequently observed in many tumors[127,128,131].

The SV40 LT-Ag interactions with pRb, p107 and p130 cause well known transformative effects in cell culture and animal models and have been recently reviewed[66]. However, HPyV LT-Ag also mediated cell transformation through pRb family binding. For instance, JCPyV LT-Ag binds the hypophosphorylated pRb in hamster glioblastoma cells and this interaction suppression successfully disables cell transformation, though JCPyV LT-Ag might be less efficient in cell transformation than SV40 LT-Ag[132]. Moreover, JCPyV LT-Ag might alter pRb expression pattern and cellular distribution in mouse primitive neuroectodermal tumors (PNET) and human medulloblastoma (HMB) cell lines, suggesting a cell-type specific LT-Ag-mediated tumorigenesis[133].

BKPyV LT-Ag can significantly reduce pRb, p107 and p130 levels and increase transcriptionally active E2F, requiring intact LT-Ag DnaJ and pRb binding domains in order to induce cell cycle deregulation[101,134]. Furthermore, BKPyV LT-Ag interaction with pRb and consequent E2F release leads to DNA methyltransferase 1 (DNMT1) gene expression, one of E2F target genes[135]. This enzyme adds methyl residues on cytosine located 5' to guanosine and is involved in cellular expression control, as methylation significantly reduces gene transcription [135]. Thus, DNMT1 expression promoted by BKPyV LT-Ag probably induces the inheritable silencing of tumor suppressor genes, and might contribute to carcinogenesis[135].

Likewise, MCPyV LT-Ag contains LXCXE motif and interacts with pRb family proteins[57]. In fact, this natural interaction is the decisive evidence for LT-Ag tumorigenesis pathway through pRb family, as pRb-binding domain is systematically maintained in MCPyV-positive MCC while the LT-Ag C-terminal is truncated [57]. Moreover, truncated LT-Ag is more efficient in transforming cells then full-length LT-Ag, mainly due to its LXCXE motif and to the disruption of the C-terminal region inhibitory activity on cell growth[94]. The MCPyV LT-Ag C-terminal inhibitory activity on cell growth might be mediated by the phosphorylation of a LT-Ag serine residue at the position 816, stimulated by the ATM pathway activation. This phosphorylation site might represent a negative selection mechanism to eliminate functional and growth-inhibitory C-terminal during tumorigenesis, which also preserves pRb domain[136]. In vitro studies showed that the ablation of MCPyV LT-Ag:pRb would lead to tumor regression, suggesting that the LT-Ag:pRb interaction is critical for cell proliferation and sustained tumor growth[137]. Also, a new interaction for MCPyV LT-Ag was observed when Arora et al.[138] found that the cellular oncoprotein survivin BIRC5a, an anti-apoptotic protein, was seven fold more expressed in MCPyV positive MCC than in MCPyV negative MCC. This study also identified the need for an intact pRb binding domain for the direct LT-Ag binding to BIRC5a gene promoter and hence proposed the survivin as a therapeutic target for MCC[137]. However, it was already known that the HPyV LT-Ag:survivin interaction might promote cell survival, as observed in oligodendrocyte and astrocyte cell lines infected *in vitro* and expressing LT-Ag[91].

The LT-Ag:pRb interaction has also been studied in the newly discovered HPyVs. The TSPyV LT-Ag mediates cells cycle progression in trichodysplasia spinulosa cases through pRb interaction. Kassem et al.[139] showed evidences of TSPyV LT-Ag clusters with phosphorylated pRb by histological immunofluorescence, and concluded that LT-Ag:pRb may induce cell proliferation as a potential driver of papule and spicule formation. In contrast, the MWPyV LT-Ag is less stable and has not been yet associated to transforming abilities in vitro, although it is able to bind to pRb, p107

and p130[139,140]. Likely, HPyV-7 LT-Ag expression in thymic epithelial tumors does not correlate with pRb expression[141], although HPyV detection and expression in thymomas was relatively frequent (62%) [142].

***HPyV LT-Ag binding with p53***

Finally, another important LT-Ag interaction in HPyV mediated tumorigenesis is that with p53, a tumor suppressing protein that regulates the gene expression in response to stressful events such as DNA damage, leading to cell apoptosis, cell cycle arrest or senescence, and is usually deregulated in cancer[143]. PyVs LT-Ag contain a p53 binding site on the helicase/ATPase domain. During SV40 carcinogenesis, LT-Ag binds and blocks p53 activity, preventing apoptosis and cell cycle arrest, induced by DNA damage, derived from pRb:E2F disruption. Instead of inducing p53 degradation through ubiquitinase, as seen for HPV E6 protein, SV40 LT-Ag stabilizes p53, which is sufficient for the abrogation of its transcriptional activities[66] (Figure 2, panel b).

BKPyV LT-Ag also complexes with p53 and, in BKPyV positive renourinary tumors, such association is considered an important oncogenic mechanism. The complex formation also promotes p53 kidnapping to cytoplasm, which is observed in both infected and transformed cell lines. Even if the sequestration is not a requirement for p53 inhibition, the p53 cytoplasmic accumulation correlates with an increased cellular mutational profile in LT-Ag expressing cells, and has been considered the main hallmark of BKPyV involvement in prostate cancer development[143]. Additionally, the preservation of the LT-Ag:p53 binding domain observed in tumors with BKPyV integration is suggestive for the LT-Ag:p53 mediated oncogenesis.

In the same way, as observed for the p53 cytoplasmic sequestration mediated by BKPyV LT-Ag, JCPyV LT-Ag is able to alter p53 expression and its cellular distribution in PNET and HMB cell lines[133]. A recent study conducted on paraffin embedded specimens reported a dramatic decrease in

p53 expression in JCPyV-positive glioblastomas when compared to JCPyV-negative gliobastomas, suggesting a potential role of JCPyV LT-Ag in p53 expression modulation and tumorigenesis[144]. Nevertheless, MCPyV mediated MCC tumorigenesis does not require p53 inactivation. In fact, p53 disruption in MCC is MCPyV LT-Ag independent, as demonstrated by Houben et al.[145] This experimental study corroborates with other previously published papers, showing that the C-terminal region of MCPyV LT-Ag mediates cell cycle progress inhibition[94], and that the truncated LT-Ag has a higher transformation potential compared to the full-length LT-Ag[62]. Finally, these findings are consistent with the MCPyV tumor-specific signature in MCC, where the truncated LT-Ag lacks the Helicase/ATPase and p53 binding domain[57].

**Human polyomaviruses small T antigen**

The small T antigen (sT-Ag) is another PyV protein with cell transformation properties. Although sT-Ag expression is not always a condition for viral replication or transformation, it is required for PyVs optimal functioning[68,146]. The SV40 sT-Ag is functionally comparable to HPV E7, in such a way that E7 is capable of successfully replace sT-Ag functions *in vitro*[147]. The PyV sT-Ag are encoded in the early region of PyVs genomes, superposed to the LT-Ag coding sequence. Produced by an alternative splicing of this region, sT-Ag shares about 80 amino acids in its N-terminus with LT-Ag[55]. Therefore, the sT-Ag presents the DnaJ domain with a conserved HPDKGG motif among all HPyV, which potentially affects viral replication, cell proliferation and transformation, although the functional importance of this domain for sT-Ag activity is unknown[65,148-150]. The BKPyV, JCPyV and SV40 sT-Ag share high amino acid similarities with each other, especially on the N terminus, although the sT-Ag middle region is more divergent among HPyVs [151], and its hydrophobicity and flexibility are important for sT-Ag functionality [152].

The sT-Ag transcript also contains a unique region, removed from LT-Ag during the intronic processing, which may have distinct features among HPyVs. For instance, JCPyV sT-Ag contains a

LYCKE and a LHCWE motif and BKPyV has only one LYCKE motif. Thus, these LXCXE motifs are potentially able to interact with pRb family proteins, whereas MCPyV sT-Ag does not present any LXCXE motif, as well as the other HPyVs.[55,153]. Regardless, all HPyVs sT-Ags have two conserved domains at their unique C-terminus region: two zinc-binding domains (Cys-X-Cys-X-X-Cys motif), which provide structural and functional stability for sT-Ag, and two domains for phosphatase 2A (PP2A), rich in cysteine and proline residues responsible for sT-Ag:PP2A interaction, that require the sT-Ag DnaJ domain and the second zinc-binding motif[55,151] (Figure 3, panels a-c).

***HPyV sT-Ag binding with PP2A***

The PP2A is a serine-threonine phosphatase that regulates, among many other important cellular processes, the cell cycle progression and apoptosis by dephosphorylating protein targets such as Akt, p53, c-Myc and β-catenin, and hence is considered a tumor suppressing protein. PP2A is composed by catalytic (PP2Ac), scaffold (PP2AA) and regulatory subunits (PP2AB), that interact to form an active enzymatic complex[154]. The SV40 sT-Ag prototype has a cysteine-proline rich and conserved domain (Cys-X-X-X-Pro-X-Cys) that interacts with PP2A, mainly through the sT-Ag cysteine residues[55,151] In fact, the ablation of only one cysteine from CXXXPXC motif dramatically reduces sT-Ag:PP2A binding rate[155]. The sT-Ag interacts with the PP2AA (scaffold) subunit by the HEAT repeats3-7, displacing the PP2AB (regulatory) subunit, that also binds HEAT repeats 3-7, and thus inhibiting PP2AAc phosphatase activity[152] (Figure 4, panel a-b).

The sT-Ag:PP2A interaction triggers several pathways related to cellular transformation. The entry on cell cycle S phase may be stimulated by sT-Ag:PP2A, as PP2A no longer dephosphorylates the cyclin-dependent kinase (CDK) inhibitor p27, leading to its degradation. Additionally, sT-Ag:PP2A also induces the phase S entry by promoting cyclin A/CDK2 and cyclin E/CDK2

expression through a cell cycle regulated E2F site[156,157]. Furthermore, sT-Ag:PP2A has been linked to human fibroblast transformation by CDK2 activation and DNA synthesis induction[158].

The SV40 sT-Ag:PP2A interaction might induce oncogenesis *in vitro* through the activation of the MAP kinase cascade[159]. The mitogen-activated protein kinases (MAPK) are involved in cell proliferation via the ERK1 and ERK2 (extracellular signal related kinases 1 and 2) upregulation of cyclin D1, leading to phosphorylation of pRb and the subsequent releasing of the E2F transcription factor. MAPK pathway activation has been linked to increased BKPyV replication in cell cultures, although induced by LT-Ag[160]. However, Wu et al.[161] demonstrated that TSPyV sT-Ag overexpression activates the MAPK pathway by enhancing the MEK/ERK phosphorylation. Once more, it is suggested that sT-Ag:PP2A interaction prevents the PP2A dephosphorylation activity and ultimately, the suppression of the MAPK cascade.

It has been demonstrated that PyV sT-Ag are capable of upregulating and stabilizing the myelocytomatosis transcription factor (Myc), which requires intact DnaJ and PP2AsT-Ag domains. Studies have demonstrated that sT-Ag:c-Myc upregulation is related with later increase of human telomerase and cyclin D1, suggestive for Myc targeted expression[162]. The c-Myc and cyclin D1 promoter activity was also increased by JCPyV sT-Ag and beta-catenin, both separated and associated, the latter found to potentiate the effect[116]. Moreover, MCPyV sT-Ag is capable of increasing c-Myc activity. Normally, the Fbw7 ubiquitin ligase protein complex promotes the degradation of proto-oncogenes products such as cyclin E, c-MYc, c-Jun, mTOR and NF-kB by a phosphorylation-dependent mechanism. Recent findings support the idea that the sT-Ag can inhibit the LT-Ag, c-Myc and cyclin E proteasomal degradation through their stabilization, thus regulating several cell cycle proliferation pathways[163].

Furthermore, human cells expressing SV40 sT-Ag have shown upregulation of anti-apoptotic targets of NF-kB[164], despite most viral proteic interactions with NF-kB pathway have been shown to have inhibitory effects. For instance, MCPyV sT-Ag downregulates NF-kB by targeting the NEMO

adaptor protein and, thus, disrupting the inflammatory pathway[165]. Moreover, NF-kB regulation pathway by MCPyV sT-Ag involves the regulatory sub-unit 1 of the protein phosphatase 4 (PP4R1), which is required for NEMO adaptor protein interaction[166]. NF-kB has also been described as a promoter of JCPyV replication during DNA damage response induced by JCPyV itself[167]. These sT-Ag inhibitory activities on NF-kB pathway, responsible for innate immune response, might be related to host immune response evasion, whose impact on tumorigenesis still remains unclear.

***sT-Ag/PP2A independent pathways***

Despite the principal PyVs sT-Ag oncogenic pathway is associated with the PP2A deregulation, MCPyV sT-Ag may be able to induce cell transformation in a PP2A independent way. For instance, it has been shown that MCPyV sT-Ag may induce cell proliferation depending on the Akt-mTOR signaling without PP2A deregulation[168]. The mTOR (mammalian target of rapamycin) is a serine threonine kinase that controls cellular functions such as transcription and translation. When activated, mTOR phosphorylates some translational control proteins, such as the initiation factor 4E binding protein 1 (4E-BP1), preventing its inhibitory activity and releasing the initiation factor 4 (eIF4E), which may then promote translation and further cell cycle progression[151]. The PP2A domain of MCPyV sT-Ag domain is not required for epithelial transformation in transgenic mice[169]. Furthermore, it has been demonstrated that MCPyV sT-Ag is able to dislocate a restrict number of PP2AB subunits, which lead to a sT-Ag:PP2A interaction insufficient to promote tumorigenesis *in vitro*. Instead, MCPyV sT-Ag induces oncogenesis through the so-called large T stabilization domain (LSD)[163,170]. The LSD domain is located at residues 91-95 and inhibits MCPyV LT-Ag proteasomal degradation, since LT-Ag is the target for the Fbw7 E3 ubiquitin-ligase. Mutations at LSD disrupt LT-Ag stabilization, prevent sT-Ag cell transformation and viral replication, as well as reduce sT-Ag induction of cellular oncoprotein, still in a PP2A independent manner[65,170].

Another MCPyC sT-Ag interaction related to *in vitro* tumorigenesis is that with c-Jun, a transcription factor that regulates cellular differentiation, and proliferation. When hyperphosphorylated, c-Jun is capable of inducing tumorigenesis, especially in keratinocytes[171]. A recent study[172] observed that c-Jun hyperphosphorylation increased after MCPyV sT-Ag overexpression in HEK293 cell line. Additionally, although c-Jun phosphorylation status in *in vivo* MCC is currently unknown, its activation by MCPyV sT-Ag may contribute to the MCC aggressive pattern, thus demanding further investigations[172]. Finally, it has also been demonstrated that TSPyV sT-Ag overexpression is associated with c-Jun phosphorylation and activation, indicating a role for TSPyV sT-Ag in the TS pathogenesis[161].

**Conclusions**

Indubitably, among the proteins encoded by the HPyVs, the principal agent involved in cell transformation and tumor development is the LT-Ag, which is also the most studied. Interestingly, the structure and function of this protein are quite conserved among the different HPyVs, testifying that it is indispensable for both viral replication and interaction with the host cells. However, this review underlined also the strategic role of the other early protein encoded by the HPyVs, the sT-Ag. Additionally, it should be taken into account that some of the classic and newly discovered HPyVs are also able to produce agnoprotein, which may have transforming activities itself[91]. Most probably, these proteins act synergically, orchestrated by LT-Ag, fighting a battle against the infected host, trying to evade from the immune system and targeting multiple cellular pathways. To this particular regard, the evasion of the innate immune system by HPyV has been so far studied for MCPyV. Both MCPyV LT-Ag and sT-Ag, and also BKPyV LT-Ag, are able to interact with the Toll-like receptor 9 (TLR9) and to inhibit it, causing the subsequent lack of transcription of C/EBPβ. This last transcription factor plays several roles in the suppression activity of the tumor proliferation, i.e. it regulates IL-6, IL-8, TNF-a and E2F expressions. Consequently, the suppression of

C/EBPβ expression by the HPyVs T-Ag induces modification in the immune system reactions against the viruses and also triggers the cell proliferation[173-176]

Not all of the involved mechanisms, neither the interactions among the viral proteins have been fully understood and the continuous discovery of new HPyVs might favor the understanding of cell transformation mediated by the HPyVs.

## References


1. Sweet BH, Hilleman MR. The vacuolating virus, S.V. 40. *Proc Soc Exp Biol Med.* 1960;105:420-427.
2. Gross L. A filterable agent, recovered from Ak leukemic extracts, causing salivary gland carcinomas in C3H mice. *Proc Soc Exp Biol Med.* 1953;83(2):414-421.
3. Stewart SE, Eddy BE, Borgese N. Neoplasms in mice inoculated with a tumor agent carried in tissue culture. *J Natl Cancer Inst.* 1958;20(6):1223-1243.
4. Butel JS, Lednicky JA. Cell and molecular biology of simian virus 40: implications for human infections and disease. *J Natl Cancer Inst.* 1999;91(2):119-134.
5. Dalianis T, Hirsch HH. Human polyomaviruses in disease and cancer. *Virology.* 2013;437(2):63-72.
6. Gardner SD, Field AM, Coleman DV, Hulme B. New human papovavirus (B.K.) isolated from urine after renal transplantation. *Lancet.* 1971;1(7712):1253-1257.
7. Padgett BL, Walker DL, ZuRhein GM, Eckroade RJ, Dessel BH. Cultivation of papova-like virus from human brain with progressive multifocal leucoencephalopathy. *Lancet.* 1971;1(7712):1257-1260.
8. Bogdanovic G, Priftakis P, Giraud G, et al. Association between a high BK virus load in urine samples of patients with graft-versus-host disease and development of hemorrhagic cystitis after hematopoietic stem cell transplantation. *J Clin Microbiol.* 2004;42(11):5394-5396.
9. Kato J, Mori T, Suzuki T, et al. Nosocomial BK Polyomavirus Infection Causing Hemorrhagic Cystitis Among Patients With Hematological Malignancies After Hematopoietic Stem Cell Transplantation. *Am J Transplant.* 2017.
10. Hirsch HH, Randhawa P. BK polyomavirus in solid organ transplantation. *Am J Transplant.* 2013;13 Suppl 4:179-188.
11. Hirsch HH, Kardas P, Kranz D, Leboeuf C. The human JC polyomavirus (JCPyV): virological background and clinical implications. *Apmis.* 2013;121(8):685-727.
12. Wollebo HS, White MK, Gordon J, Berger JR, Khalili K. Persistence and pathogenesis of the neurotropic polyomavirus JC. *Ann Neurol.* 2015;77(4):560-570.
13. Gaynor AM, Nissen MD, Whiley DM, et al. Identification of a novel polyomavirus from patients with acute respiratory tract infections. *PLoS Pathog.* 2007;3(5):e64.
14. Allander T, Andreasson K, Gupta S, et al. Identification of a third human polyomavirus. *J Virol.* 2007;81(8):4130-4136.
15. Rao S, Lucero MG, Nohynek H, et al. WU and KI polyomavirus infections in Filipino children with lower respiratory tract disease. *J Clin Virol.* 2016;82:112-118.
16. Dehority WN, Eickman MM, Schwalm KC, et al. Complete genome sequence of a KI polyomavirus isolated from an otherwise healthy child with severe lower respiratory tract infection. *J Med Virol.* 2017;89(5):926-930.
17. Siebrasse EA, Pastrana DV, Nguyen NL, et al. WU polyomavirus in respiratory epithelial cells from lung transplant patient with Job syndrome. *Emerg Infect Dis.* 2015;21(1):103-106.
18. Siebrasse EA, Nguyen NL, Willby MJ, Erdman DD, Menegus MA, Wang D. Multiorgan WU Polyomavirus Infection in Bone Marrow Transplant Recipient. *Emerg Infect Dis.* 2016;22(1):24-31.
19. Feng H, Shuda M, Chang Y, Moore PS. Clonal integration of a polyomavirus in human Merkel cell carcinoma. *Science.* 2008;319(5866):1096-1100.
20. Paolini F, Donati P, Amantea A, Bucher S, Migliano E, Venuti A. Merkel cell polyomavirus in Merkel cell carcinoma of Italian patients. *Virol J.* 2011;8:103.
21. Jung HS, Choi YL, Choi JS, et al. Detection of Merkel cell polyomavirus in Merkel cell carcinomas and small cell carcinomas by PCR and immunohistochemistry. *Histol Histopathol.* 2011;26(10):1231-1241.
22. Mangana J, Dziunycz P, Kerl K, Dummer R, Cozzio A. Prevalence of Merkel cell polyomavirus among Swiss Merkel cell carcinoma patients. *Dermatology.* 2010;221(2):184-188.

23. Kassem A, Schopflin A, Diaz C, et al. Frequent detection of Merkel cell polyomavirus in human Merkel cell carcinomas and identification of a unique deletion in the VP1 gene. *Cancer Res.* 2008;68(13):5009-5013.
24. Sastre-Garau X, Peter M, Avril MF, et al. Merkel cell carcinoma of the skin: pathological and molecular evidence for a causative role of MCV in oncogenesis. *J Pathol.* 2009;218(1):48-56.
25. Santos-Juanes J, Fernandez-Vega I, Fuentes N, et al. Merkel cell carcinoma and Merkel cell polyomavirus: a systematic review and meta-analysis. *Br J Dermatol.* 2015;173(1):42-49.
26. Andea AA, Patel R, Ponnazhagan S, Isayeva T, Kumar S, Siegal GP. Detection of Merkel cell polyomavirus in formalin-fixed, paraffin-embedded tissue of Merkel cell carcinoma and correlation with prognosis. *Rom J Morphol Embryol.* 2014;55(3 Suppl):1057-1062.
27. Mizuno Y, Kato G, Shu E, et al. Merkel cell polyomavirus-positive Merkel cell carcinoma in a patient with epidermodysplasia verruciformis. *Acta Derm Venereol.* 2015;95(1):98-99.
28. Vaira F, Nazzaro G, Pesapane F, et al. Detection of Polyomavirus in Merkel cell carcinoma by immunohistochemistry: report of three cases. *G Ital Dermatol Venereol.* 2015;150(5):617-621.
29. Erovic BM, Al Habeeb A, Harris L, Goldstein DP, Ghazarian D, Irish JC. Significant overexpression of the Merkel cell polyomavirus (MCPyV) large T antigen in Merkel cell carcinoma. *Head Neck.* 2013;35(2):184-189.
30. Leitz M, Stieler K, Grundhoff A, Moll I, Brandner JM, Fischer N. Merkel cell polyomavirus detection in Merkel cell cancer tumors in Northern Germany using PCR and protein expression. *J Med Virol.* 2014;86(10):1813-1819.
31. de Biase D, Ragazzi M, Asioli S, Eusebi V. Extracutaneous Merkel cell carcinomas harbor polyomavirus DNA. *Hum Pathol.* 2012;43(7):980-985.
32. Matsushita M, Iwasaki T, Kuwamoto S, et al. Merkel cell polyomavirus (MCPyV) strains in Japanese merkel cell carcinomas (MCC) are distinct from Caucasian type MCPyVs: genetic variability and phylogeny of MCPyV genomes obtained from Japanese MCPyV-infected MCCs. *Virus Genes.* 2014;48(2):233-242.
33. Garneski KM, Warcola AH, Feng Q, Kiviat NB, Leonard JH, Nghiem P. Merkel cell polyomavirus is more frequently present in North American than Australian Merkel cell carcinoma tumors. In: *J Invest Dermatol.* Vol 129. United States2009:246-248.
34. Becker JC, Houben R, Ugurel S, Trefzer U, Pfohler C, Schrama D. MC polyomavirus is frequently present in Merkel cell carcinoma of European patients. In: *J Invest Dermatol.* Vol 129. United States2009:248-250.
35. Schowalter RM, Pastrana DV, Pumphrey KA, Moyer AL, Buck CB. Merkel cell polyomavirus and two previously unknown polyomaviruses are chronically shed from human skin. *Cell Host Microbe.* 2010;7(6):509-515.
36. Scuda N, Hofmann J, Calvignac-Spencer S, et al. A novel human polyomavirus closely related to the african green monkey-derived lymphotropic polyomavirus. *J Virol.* 2011;85(9):4586-4590.
37. Siebrasse EA, Reyes A, Lim ES, et al. Identification of MW polyomavirus, a novel polyomavirus in human stool. *J Virol.* 2012;86(19):10321-10326.
38. Lim ES, Reyes A, Antonio M, et al. Discovery of STL polyomavirus, a polyomavirus of ancestral recombinant origin that encodes a unique T antigen by alternative splicing. *Virology.* 2013;436(2):295-303.
39. Korup S, Rietscher J, Calvignac-Spencer S, et al. Identification of a novel human polyomavirus in organs of the gastrointestinal tract. *PLoS One.* 2013;8(3):e58021.
40. Mishra N, Pereira M, Rhodes RH, et al. Identification of a novel polyomavirus in a pancreatic transplant recipient with retinal blindness and vasculitic myopathy. *J Infect Dis.* 2014;210(10):1595-1599.
41. Gheit T, Dutta S, Oliver J, et al. Isolation and characterization of a novel putative human polyomavirus. *Virology.* 2017;506:45-54.
42. Ho J, Jedrych JJ, Feng H, et al. Human polyomavirus 7-associated pruritic rash and viremia in transplant recipients. *J Infect Dis.* 2015;211(10):1560-1565.

43. Nguyen KD, Lee EE, Yue Y, et al. Human polyomavirus 6 and 7 are associated with pruritic and dyskeratotic dermatoses. *J Am Acad Dermatol.* 2017;76(5):932-940.e933.
44. Calvignac-Spencer S, Feltkamp MC, Daugherty MD, et al. A taxonomy update for the family Polyomaviridae. *Arch Virol.* 2016;161(6):1739-1750.
45. Stehle T, Gamblin SJ, Yan Y, Harrison SC. The structure of simian virus 40 refined at 3.1 A resolution. *Structure.* 1996;4(2):165-182.
46. Liddington RC, Yan Y, Moulai J, Sahli R, Benjamin TL, Harrison SC. Structure of simian virus 40 at 3.8-A resolution. *Nature.* 1991;354(6351):278-284.
47. Li TC, Takeda N, Kato K, et al. Characterization of self-assembled virus-like particles of human polyomavirus BK generated by recombinant baculoviruses. *Virology.* 2003;311(1):115-124.
48. Stroh LJ, Neu U, Blaum BS, Buch MH, Garcea RL, Stehle T. Structure analysis of the major capsid proteins of human polyomaviruses 6 and 7 reveals an obstructed sialic acid binding site. *J Virol.* 2014;88(18):10831-10839.
49. Li TC, Iwasaki K, Katano H, et al. Characterization of self-assembled virus-like particles of Merkel cell polyomavirus. *PLoS One.* 2015;10(2):e0115646.
50. Hurdiss DL, Morgan EL, Thompson RF, et al. New Structural Insights into the Genome and Minor Capsid Proteins of BK Polyomavirus using Cryo-Electron Microscopy. *Structure.* 2016;24(4):528-536.
51. Fiers W, Contreras R, Haegemann G, et al. Complete nucleotide sequence of SV40 DNA. *Nature.* 1978;273(5658):113-120.
52. Reddy VB, Thimmappaya B, Dhar R, et al. The genome of simian virus 40. *Science.* 1978;200(4341):494-502.
53. Frisque RJ, Bream GL, Cannella MT. Human polyomavirus JC virus genome. *J Virol.* 1984;51(2):458-469.
54. Hirsch HH, Steiger J. Polyomavirus BK. *Lancet Infect Dis.* 2003;3(10):611-623.
55. Van Ghelue M, Khan MT, Ehlers B, Moens U. Genome analysis of the new human polyomaviruses. *Rev Med Virol.* 2012;22(6):354-377.
56. Prins C, Frisque RJ. JC virus T' proteins encoded by alternatively spliced early mRNAs enhance T antigen-mediated viral DNA replication in human cells. *J Neurovirol.* 2001;7(3):250-264.
57. Shuda M, Feng H, Kwun HJ, et al. T antigen mutations are a human tumor-specific signature for Merkel cell polyomavirus. *Proc Natl Acad Sci U S A.* 2008;105(42):16272-16277.
58. Abend JR, Joseph AE, Das D, Campbell-Cecen DB, Imperiale MJ. A truncated T antigen expressed from an alternatively spliced BK virus early mRNA. *J Gen Virol.* 2009;90(Pt 5):1238-1245.
59. van der Meijden E, Kazem S, Dargel CA, van Vuren N, Hensbergen PJ, Feltkamp MC. Characterization of T Antigens, Including Middle T and Alternative T, Expressed by the Human Polyomavirus Associated with Trichodysplasia Spinulosa. *J Virol.* 2015;89(18):9427-9439.
60. Carter JJ, Daugherty MD, Qi X, et al. Identification of an overprinting gene in Merkel cell polyomavirus provides evolutionary insight into the birth of viral genes. *Proc Natl Acad Sci U S A.* 2013;110(31):12744-12749.
61. Gan DD, Khalili K. Interaction between JCV large T-antigen and beta-catenin. *Oncogene.* 2004;23(2):483-490.
62. Borchert S, Czech-Sioli M, Neumann F, et al. High-affinity Rb binding, p53 inhibition, subcellular localization, and transformation by wild-type or tumor-derived shortened Merkel cell polyomavirus large T antigens. *J Virol.* 2014;88(6):3144-3160.
63. Gou WF, Zhao S, Shen DF, et al. The oncogenic role of JC virus T antigen in lens tumors without cell specificity of alternative splicing of its intron. *Oncotarget.* 2015;6(10):8036-8045.
64. Houben R, Angermeyer S, Haferkamp S, et al. Characterization of functional domains in the Merkel cell polyoma virus Large T antigen. *Int J Cancer.* 2015;136(5):E290-300.
65. Wendzicki JA, Moore PS, Chang Y. Large T and small T antigens of Merkel cell polyomavirus. *Curr Opin Virol.* 2015;11:38-43.
66. An P, Saenz Robles MT, Pipas JM. Large T antigens of polyomaviruses: amazing molecular machines. *Annu Rev Microbiol.* 2012;66:213-236.

67. Topalis D, Andrei G, Snoeck R. The large tumor antigen: a "Swiss Army knife" protein possessing the functions required for the polyomavirus life cycle. *Antiviral Res.* 2013;97(2):122-136.
68. Kwun HJ, Guastafierro A, Shuda M, et al. The minimum replication origin of merkel cell polyomavirus has a unique large T-antigen loading architecture and requires small T-antigen expression for optimal replication. *J Virol.* 2009;83(23):12118-12128.
69. Harrison CJ, Meinke G, Kwun HJ, et al. Asymmetric assembly of Merkel cell polyomavirus large T-antigen origin binding domains at the viral origin. *J Mol Biol.* 2011;409(4):529-542.
70. Harrison C, Jiang T, Banerjee P, et al. Polyomavirus large T antigen binds symmetrical repeats at the viral origin in an asymmetrical manner. *J Virol.* 2013;87(24):13751-13759.
71. Chang YP, Xu M, Machado AC, Yu XJ, Rohs R, Chen XS. Mechanism of origin DNA recognition and assembly of an initiator-helicase complex by SV40 large tumor antigen. *Cell Rep.* 2013;3(4):1117-1127.
72. Meinke G, Phelan PJ, Kalekar R, et al. Insights into the initiation of JC virus DNA replication derived from the crystal structure of the T-antigen origin binding domain. *PLoS Pathog.* 2014;10(2):e1003966.
73. Mahon C, Liang B, Tikhanovich I, et al. Restriction of human polyomavirus BK virus DNA replication in murine cells and extracts. *J Virol.* 2009;83(11):5708-5717.
74. Tikhanovich I, Nasheuer HP. Host-specific replication of BK virus DNA in mouse cell extracts is independently controlled by DNA polymerase alpha-primase and inhibitory activities. *J Virol.* 2010;84(13):6636-6644.
75. Deyerle KL, Sajjadi FG, Subramani S. Analysis of origin of DNA replication of human papovavirus BK. *J Virol.* 1989;63(1):356-365.
76. Liang B, Tikhanovich I, Nasheuer HP, Folk WR. Stimulation of BK virus DNA replication by NFI family transcription factors. *J Virol.* 2012;86(6):3264-3275.
77. Kenan DJ, Mieczkowski PA, Burger-Calderon R, Singh HK, Nickeleit V. The oncogenic potential of BK-polyomavirus is linked to viral integration into the human genome. *J Pathol.* 2015;237(3):379-389.
78. Li J, Wang X, Diaz J, Tsang SH, Buck CB, You J. Merkel cell polyomavirus large T antigen disrupts host genomic integrity and inhibits cellular proliferation. *J Virol.* 2013;87(16):9173-9188.
79. Delbue S, Ferrante P, Provenzano M. Polyomavirus BK and prostate cancer: an unworthy scientific effort? *Oncoscience.* 2014;1(4):296-303.
80. Weinreb DB, Desman GT, Amolat-Apiado MJ, Burstein DE, Godbold JH, Johnson EM. Polyoma virus infection is a prominent risk factor for bladder carcinoma in immunocompetent individuals. *Diagn Cytopathol.* 2006;34(3):201-203.
81. Alexiev BA, Randhawa P, Vazquez Martul E, et al. BK virus-associated urinary bladder carcinoma in transplant recipients: report of 2 cases, review of the literature, and proposed pathogenetic model. *Hum Pathol.* 2013;44(5):908-917.
82. Roberts IS, Besarani D, Mason P, Turner G, Friend PJ, Newton R. Polyoma virus infection and urothelial carcinoma of the bladder following renal transplantation. *Br J Cancer.* 2008;99(9):1383-1386.
83. Kenan DJ, Mieczkowski PA, Latulippe E, Cote I, Singh HK, Nickeleit V. BK Polyomavirus Genomic Integration and Large T Antigen Expression: Evolving Paradigms in Human Oncogenesis. *Am J Transplant.* 2017;17(6):1674-1680.
84. Dorries K, Loeber G, Meixensberger J. Association of polyomaviruses JC, SV40, and BK with human brain tumors. *Virology.* 1987;160(1):268-270.
85. Bialasiewicz S, Cho Y, Rockett R, et al. Association of micropapillary urothelial carcinoma of the bladder and BK viruria in kidney transplant recipients. *Transpl Infect Dis.* 2013;15(3):283-289.
86. Wold WS, Green M, Mackey JK, Martin JD, Padgett BL, Walker DL. Integration pattern of human JC virus sequences in two clones of a cell line established from a JC virus-induced hamster brain tumor. *J Virol.* 1980;33(3):1225-1228.
87. Del Valle L, White MK, Khalili K. Potential mechanisms of the human polyomavirus JC in neural oncogenesis. *J Neuropathol Exp Neurol.* 2008;67(8):729-740.

88. Hori R, Murai Y, Tsuneyama K, et al. Detection of JC virus DNA sequences in colorectal cancers in Japan. *Virchows Arch.* 2005;447(4):723-730.
89. Coelho TR, Almeida L, Lazo PA. JC virus in the pathogenesis of colorectal cancer, an etiological agent or another component in a multistep process? *Virol J.* 2010;7:42.
90. Theodoropoulos G, Panoussopoulos D, Papaconstantinou I, et al. Assessment of JC polyoma virus in colon neoplasms. *Dis Colon Rectum.* 2005;48(1):86-91.
91. Delbue S, Comar M, Ferrante P. Review on the role of the human Polyomavirus JC in the development of tumors. *Infect Agent Cancer.* 2017;12:10.
92. Pantulu ND, Pallasch CP, Kurz AK, et al. Detection of a novel truncating Merkel cell polyomavirus large T antigen deletion in chronic lymphocytic leukemia cells. *Blood.* 2010;116(24):5280-5284.
93. Kassem A, Technau K, Kurz AK, et al. Merkel cell polyomavirus sequences are frequently detected in nonmelanoma skin cancer of immunosuppressed patients. *Int J Cancer.* 2009;125(2):356-361.
94. Cheng J, Rozenblatt-Rosen O, Paulson KG, Nghiem P, DeCaprio JA. Merkel cell polyomavirus large T antigen has growth-promoting and inhibitory activities. *J Virol.* 2013;87(11):6118-6126.
95. Stakaityte G, Wood JJ, Knight LM, et al. Merkel cell polyomavirus: molecular insights into the most recently discovered human tumour virus. *Cancers (Basel).* 2014;6(3):1267-1297.
96. Trabanelli C, Corallini A, Gruppioni R, et al. Chromosomal aberrations induced by BK virus T antigen in human fibroblasts. *Virology.* 1998;243(2):492-496.
97. Whalen KA, de Jesus R, Kean JA, Schaffhausen BS. Genetic analysis of the polyomavirus DnaJ domain. *J Virol.* 2005;79(15):9982-9990.
98. Berjanskii MV, Riley MI, Xie A, Semenchenko V, Folk WR, Van Doren SR. NMR structure of the N-terminal J domain of murine polyomavirus T antigens. Implications for DnaJ-like domains and for mutations of T antigens. *J Biol Chem.* 2000;275(46):36094-36103.
99. Chromy LR, Pipas JM, Garcea RL. Chaperone-mediated in vitro assembly of Polyomavirus capsids. *Proc Natl Acad Sci U S A.* 2003;100(18):10477-10482.
100. Sheng Q, Denis D, Ratnofsky M, Roberts TM, DeCaprio JA, Schaffhausen B. The DnaJ domain of polyomavirus large T antigen is required to regulate Rb family tumor suppressor function. *J Virol.* 1997;71(12):9410-9416.
101. Harris KF, Christensen JB, Radany EH, Imperiale MJ. Novel mechanisms of E2F induction by BK virus large-T antigen: requirement of both the pRb-binding and the J domains. *Mol Cell Biol.* 1998;18(3):1746-1756.
102. Bollag B, Prins C, Snyder EL, Frisque RJ. Purified JC virus T and T' proteins differentially interact with the retinoblastoma family of tumor suppressor proteins. *Virology.* 2000;274(1):165-178.
103. Spurgeon ME, Lambert PF. Merkel cell polyomavirus: a newly discovered human virus with oncogenic potential. *Virology.* 2013;435(1):118-130.
104. Cotsiki M, Lock RL, Cheng Y, et al. Simian virus 40 large T antigen targets the spindle assembly checkpoint protein Bub1. *Proc Natl Acad Sci U S A.* 2004;101(4):947-952.
105. Hu L, Filippakis H, Huang H, Yen TJ, Gjoerup OV. Replication stress and mitotic dysfunction in cells expressing simian virus 40 large T antigen. *J Virol.* 2013;87(24):13179-13192.
106. Wang X, Helfer CM, Pancholi N, Bradner JE, You J. Recruitment of Brd4 to the human papillomavirus type 16 DNA replication complex is essential for replication of viral DNA. *J Virol.* 2013;87(7):3871-3884.
107. You J, Croyle JL, Nishimura A, Ozato K, Howley PM. Interaction of the bovine papillomavirus E2 protein with Brd4 tethers the viral DNA to host mitotic chromosomes. *Cell.* 2004;117(3):349-360.
108. Gauson EJ, Donaldson MM, Dornan ES, et al. Evidence supporting a role for TopBP1 and Brd4 in the initiation but not continuation of human papillomavirus 16 E1/E2-mediated DNA replication. *J Virol.* 2015;89(9):4980-4991.
109. Helfer CM, Yan J, You J. The cellular bromodomain protein Brd4 has multiple functions in E2-mediated papillomavirus transcription activation. *Viruses.* 2014;6(8):3228-3249.
110. Voronkov A, Krauss S. Wnt/beta-catenin signaling and small molecule inhibitors. *Curr Pharm Des.* 2013;19(4):634-664.

111. Ikeda S, Kishida S, Yamamoto H, Murai H, Koyama S, Kikuchi A. Axin, a negative regulator of the Wnt signaling pathway, forms a complex with GSK-3beta and beta-catenin and promotes GSK-3beta-dependent phosphorylation of beta-catenin. *Embo j.* 1998;17(5):1371-1384.
112. Xing Y, Clements WK, Kimelman D, Xu W. Crystal structure of a beta-catenin/axin complex suggests a mechanism for the beta-catenin destruction complex. *Genes Dev.* 2003;17(22):2753-2764.
113. Enam S, Del Valle L, Lara C, et al. Association of human polyomavirus JCV with colon cancer: evidence for interaction of viral T-antigen and beta-catenin. *Cancer Res.* 2002;62(23):7093-7101.
114. Krynska B, Del Valle L, Gordon J, Otte J, Croul S, Khalili K. Identification of a novel p53 mutation in JCV-induced mouse medulloblastoma. *Virology.* 2000;274(1):65-74.
115. Bhattacharyya R, Noch EK, Khalili K. A novel role of Rac1 GTPase in JCV T-antigen-mediated beta-catenin stabilization. *Oncogene.* 2007;26(55):7628-7636.
116. Ripple MJ, Parker Struckhoff A, Trillo-Tinoco J, et al. Activation of c-Myc and Cyclin D1 by JCV T-Antigen and beta-catenin in colon cancer. *PLoS One.* 2014;9(9):e106257.
117. Khalili K, Del Valle L, Wang JY, et al. T-antigen of human polyomavirus JC cooperates withIGF-IR signaling system in cerebellar tumors of the childhood-medulloblastomas. *Anticancer Res.* 2003;23(3a):2035-2041.
118. Lasithiotaki I, Antoniou KM, Derdas SP, et al. The presence of Merkel cell polyomavirus is associated with deregulated expression of BRAF and Bcl-2 genes in non-small cell lung cancer. *Int J Cancer.* 2013;133(3):604-611.
119. Xu S, Jiang J, Yu X, Sheng D, Zhu T, Jin M. Association of Merkel cell polyomavirus infection with EGFR mutation status in Chinese non-small cell lung cancer patients. *Lung Cancer.* 2014;83(3):341-346.
120. Iwasaki T, Matsushita M, Nonaka D, et al. Comparison of Akt/mTOR/4E-BP1 pathway signal activation and mutations of PIK3CA in Merkel cell polyomavirus-positive and Merkel cell polyomavirus-negative carcinomas. *Hum Pathol.* 2015;46(2):210-216.
121. Spurgeon ME, Cheng J, Bronson RT, Lambert PF, DeCaprio JA. Tumorigenic activity of merkel cell polyomavirus T antigens expressed in the stratified epithelium of mice. *Cancer Res.* 2015;75(6):1068-1079.
122. Verhalen B, Starrett GJ, Harris RS, Jiang M. Functional Upregulation of the DNA Cytosine Deaminase APOBEC3B by Polyomaviruses. *J Virol.* 2016;90(14):6379-6386.
123. Di Fiore R, D'Anneo A, Tesoriere G, Vento R. RB1 in cancer: different mechanisms of RB1 inactivation and alterations of pRb pathway in tumorigenesis. *J Cell Physiol.* 2013;228(8):1676-1687.
124. Chinnam M, Goodrich DW. RB1, development, and cancer. *Curr Top Dev Biol.* 2011;94:129-169.
125. Bertoli C, Skotheim JM, de Bruin RA. Control of cell cycle transcription during G1 and S phases. *Nat Rev Mol Cell Biol.* 2013;14(8):518-528.
126. Chellappan S, Kraus VB, Kroger B, et al. Adenovirus E1A, simian virus 40 tumor antigen, and human papillomavirus E7 protein share the capacity to disrupt the interaction between transcription factor E2F and the retinoblastoma gene product. *Proc Natl Acad Sci U S A.* 1992;89(10):4549-4553.
127. Stubdal H, Zalvide J, Campbell KS, Schweitzer C, Roberts TM, DeCaprio JA. Inactivation of pRB-related proteins p130 and p107 mediated by the J domain of simian virus 40 large T antigen. *Mol Cell Biol.* 1997;17(9):4979-4990.
128. Brown VD, Gallie BL. The B-domain lysine patch of pRB is required for binding to large T antigen and release of E2F by phosphorylation. *Mol Cell Biol.* 2002;22(5):1390-1401.
129. Sullivan CS, Cantalupo P, Pipas JM. The molecular chaperone activity of simian virus 40 large T antigen is required to disrupt Rb-E2F family complexes by an ATP-dependent mechanism. *Mol Cell Biol.* 2000;20(17):6233-6243.
130. Lin JY, DeCaprio JA. SV40 large T antigen promotes dephosphorylation of p130. *J Biol Chem.* 2003;278(47):46482-46487.
131. Pilon AA, Desjardins P, Hassell JA, Mes-Masson AM. Functional implications of mutations within polyomavirus large T antigen Rb-binding domain: effects on pRb and p107 binding in vitro and immortalization activity in vivo. *J Virol.* 1996;70(7):4457-4465.

132. Caracciolo V, Reiss K, Khalili K, De Falco G, Giordano A. Role of the interaction between large T antigen and Rb family members in the oncogenicity of JC virus. *Oncogene.* 2006;25(38):5294-5301.
133. Caracciolo V, Macaluso M, D'Agostino L, et al. Cross-talk between T-Ag presence and pRb family and p53/p73 signaling in mouse and human medulloblastoma. *J Cell Biochem.* 2010;110(1):182-190.
134. Tognon M, Corallini A, Martini F, Negrini M, Barbanti-Brodano G. Oncogenic transformation by BK virus and association with human tumors. *Oncogene.* 2003;22(33):5192-5200.
135. McCabe MT, Low JA, Imperiale MJ, Day ML. Human polyomavirus BKV transcriptionally activates DNA methyltransferase 1 through the pRb/E2F pathway. *Oncogene.* 2006;25(19):2727-2735.
136. Li J, Diaz J, Wang X, Tsang SH, You J. Phosphorylation of Merkel cell polyomavirus large tumor antigen at serine 816 by ATM kinase induces apoptosis in host cells. *J Biol Chem.* 2015;290(3):1874-1884.
137. Houben R, Adam C, Baeurle A, et al. An intact retinoblastoma protein-binding site in Merkel cell polyomavirus large T antigen is required for promoting growth of Merkel cell carcinoma cells. *Int J Cancer.* 2012;130(4):847-856.
138. Arora R, Shuda M, Guastafierro A, et al. Survivin is a therapeutic target in Merkel cell carcinoma. *Sci Transl Med.* 2012;4(133):133ra156.
139. Kazem S, van der Meijden E, Wang RC, et al. Polyomavirus-associated Trichodysplasia spinulosa involves hyperproliferation, pRB phosphorylation and upregulation of p16 and p21. *PLoS One.* 2014;9(10):e108947.
140. Berrios C, Jung J, Primi B, et al. Malawi polyomavirus is a prevalent human virus that interacts with known tumor suppressors. *J Virol.* 2015;89(1):857-862.
141. Keijzers M, Rensspiess D, Pujari S, et al. Expression of pRb and p16INK4 in human thymic epithelial tumors in relation to the presence of human polyomavirus 7. *Diagn Pathol.* 2015;10:201.
142. Rennspiess D, Pujari S, Keijzers M, et al. Detection of human polyomavirus 7 in human thymic epithelial tumors. *J Thorac Oncol.* 2015;10(2):360-366.
143. Liu J, Zhang C, Hu W, Feng Z. Tumor suppressor p53 and its mutants in cancer metabolism. *Cancer Lett.* 2015;356(2 Pt A):197-203.
144. Malekpour Afshar R, Mollaei HR, Zandi B, Iranpour M. Evaluation of JC and Cytomegalo Viruses in Glioblastoma Tissue. *Asian Pac J Cancer Prev.* 2016;17(11):4907-4911.
145. Houben R, Dreher C, Angermeyer S, et al. Mechanisms of p53 restriction in Merkel cell carcinoma cells are independent of the Merkel cell polyoma virus T antigens. *J Invest Dermatol.* 2013;133(10):2453-2460.
146. Tsang SH, Wang R, Nakamaru-Ogiso E, Knight SA, Buck CB, You J. The Oncogenic Small Tumor Antigen of Merkel Cell Polyomavirus Is an Iron-Sulfur Cluster Protein That Enhances Viral DNA Replication. *J Virol.* 2015;90(3):1544-1556.
147. White EA, Kramer RE, Hwang JH, et al. Papillomavirus E7 oncoproteins share functions with polyomavirus small T antigens. *J Virol.* 2015;89(5):2857-2865.
148. Srinivasan A, McClellan AJ, Vartikar J, et al. The amino-terminal transforming region of simian virus 40 large T and small t antigens functions as a J domain. *Mol Cell Biol.* 1997;17(8):4761-4773.
149. Whalen B, Laffin J, Friedrich TD, Lehman JM. SV40 small T antigen enhances progression to >G2 during lytic infection. *Exp Cell Res.* 1999;251(1):121-127.
150. Kolzau T, Hansen RS, Zahra D, Reddel RR, Braithwaite AW. Inhibition of SV40 large T antigen induced apoptosis by small T antigen. *Oncogene.* 1999;18(40):5598-5603.
151. Khalili K, Sariyer IK, Safak M. Small tumor antigen of polyomaviruses: role in viral life cycle and cell transformation. *J Cell Physiol.* 2008;215(2):309-319.
152. Cho US, Morrone S, Sablina AA, Arroyo JD, Hahn WC, Xu W. Structural basis of PP2A inhibition by small t antigen. *PLoS Biol.* 2007;5(8):e202.
153. Bollag B, Hofstetter CA, Reviriego-Mendoza MM, Frisque RJ. JC virus small T antigen binds phosphatase PP2A and Rb family proteins and is required for efficient viral DNA replication activity. *PLoS One.* 2010;5(5):e10606.

154. Seshacharyulu P, Pandey P, Datta K, Batra SK. Phosphatase: PP2A structural importance, regulation and its aberrant expression in cancer. *Cancer Lett.* 2013;335(1):9-18.
155. Mungre S, Enderle K, Turk B, et al. Mutations which affect the inhibition of protein phosphatase 2A by simian virus 40 small-t antigen in vitro decrease viral transformation. *J Virol.* 1994;68(3):1675-1681.
156. Schuchner S, Wintersberger E. Binding of polyomavirus small T antigen to protein phosphatase 2A is required for elimination of p27 and support of S-phase induction in concert with large T antigen. *J Virol.* 1999;73(11):9266-9273.
157. Skoczylas C, Henglein B, Rundell K. PP2A-dependent transactivation of the cyclin A promoter by SV40 ST is mediated by a cell cycle-regulated E2F site. *Virology.* 2005;332(2):596-601.
158. Sotillo E, Garriga J, Kurimchak A, Grana X. Cyclin E and SV40 small T antigen cooperate to bypass quiescence and contribute to transformation by activating CDK2 in human fibroblasts. *J Biol Chem.* 2008;283(17):11280-11292.
159. Frost JA, Alberts AS, Sontag E, Guan K, Mumby MC, Feramisco JR. Simian virus 40 small t antigen cooperates with mitogen-activated kinases to stimulate AP-1 activity. *Mol Cell Biol.* 1994;14(9):6244-6252.
160. Seamone ME, Wang W, Acott P, Beck PL, Tibbles LA, Muruve DA. MAP kinase activation increases BK polyomavirus replication and facilitates viral propagation in vitro. *J Virol Methods.* 2010;170(1-2):21-29.
161. Wu JH, Simonette RA, Nguyen HP, Rady PL, Tyring SK. Small T-antigen of the TS-associated polyomavirus activates factors implicated in the MAPK pathway. *J Eur Acad Dermatol Venereol.* 2016;30(6):1061-1062.
162. Klucky B, Wintersberger E. Polyomavirus small T antigen transactivates genes by its ability to provoke the synthesis and the stabilization of MYC. *Oncogene.* 2007;26(43):6356-6360.
163. Kwun HJ, Shuda M, Feng H, Camacho CJ, Moore PS, Chang Y. Merkel cell polyomavirus small T antigen controls viral replication and oncoprotein expression by targeting the cellular ubiquitin ligase SCFFbw7. *Cell Host Microbe.* 2013;14(2):125-135.
164. Sablina AA, Hahn WC. SV40 small T antigen and PP2A phosphatase in cell transformation. *Cancer Metastasis Rev.* 2008;27(2):137-146.
165. Griffiths DA, Abdul-Sada H, Knight LM, et al. Merkel cell polyomavirus small T antigen targets the NEMO adaptor protein to disrupt inflammatory signaling. *J Virol.* 2013;87(24):13853-13867.
166. Abdul-Sada H, Muller M, Mehta R, et al. The PP4R1 sub-unit of protein phosphatase PP4 is essential for inhibition of NF-kappaB by merkel polyomavirus small tumour antigen. *Oncotarget.* 2017;8(15):25418-25432.
167. White MK, Bellizzi A, Ibba G, Pietropaolo V, Palamara AT, Wollebo HS. The DNA damage response promotes polyomavirus JC infection by nucleus to cytoplasm NF- kappaB activation. *Virol J.* 2017;14(1):31.
168. Shuda M, Kwun HJ, Feng H, Chang Y, Moore PS. Human Merkel cell polyomavirus small T antigen is an oncoprotein targeting the 4E-BP1 translation regulator. *J Clin Invest.* 2011;121(9):3623-3634.
169. Verhaegen ME, Mangelberger D, Harms PW, et al. Merkel cell polyomavirus small T antigen is oncogenic in transgenic mice. *J Invest Dermatol.* 2015;135(5):1415-1424.
170. Kwun HJ, Shuda M, Camacho CJ, et al. Restricted protein phosphatase 2A targeting by Merkel cell polyomavirus small T antigen. *J Virol.* 2015;89(8):4191-4200.
171. Zenz R, Scheuch H, Martin P, et al. c-Jun regulates eyelid closure and skin tumor development through EGFR signaling. *Dev Cell.* 2003;4(6):879-889.
172. Wu JH, Simonette RA, Nguyen HP, Rady PL, Tyring SK. Merkel cell polyomavirus in Merkel cell carcinogenesis: small T antigen-mediates c-Jun phosphorylation. *Virus Genes.* 2016;52(3):397-399.
173. Shahzad N, Shuda M, Gheit T, et al. The T antigen locus of Merkel cell polyomavirus downregulates human Toll-like receptor 9 expression. *J Virol.* 2013;87(23):13009-13019.
174. Li H, Gade P, Xiao W, Kalvakolanu DV. The interferon signaling network and transcription factor C/EBP-beta. *Cell Mol Immunol.* 2007;4(6):407-418.

175. Johnson PF. Molecular stop signs: regulation of cell-cycle arrest by C/EBP transcription factors. *J Cell Sci.* 2005;118(Pt 12):2545-2555.
176. Nerlov C. The C/EBP family of transcription factors: a paradigm for interaction between gene expression and proliferation control. *Trends Cell Biol.* 2007;17(7):318-324.

**Acknowledgements**

The authors would like to thank Mrs. Rosalia Ticozzi for her technical support.

**Funding sources**

None

**Author Contributions**

Wrote the first draft of the manuscript: CFB. Contributed to the writing of the manuscript: RFV, SV, SD. Agree with manuscript results and conclusions: CFB, RFV, SV, SD. Jointly developed the structure and arguments for the paper: CFB, RFV, SV, SD. Made critical revisions and approved final version: CFB, RFV, SV, SD. All authors reviewed and approved of the final manuscript.

**Disclosures and Ethics**

As a requirement of publication author(s) have provided to the publisher signed confirmation of compliance with legal and ethical obligations including but not limited to the following: authorship and contributorship, conflicts of interest, privacy and confidentiality and (where applicable) protection of human and animal research subjects. The authors have read and confirmed their agreement with the ICMJE authorship and conflict of interest criteria. The authors have also confirmed that this article is unique and not under consideration or published in any other publication, and that they have permission from rights holders to reproduce any copyrighted material. Any disclosures are made in this section. The external blind peer reviewers report no conflicts of interest.

**Figure 1.** Scheme of the functional domains of the HPyVs' large T antigen (L-Tag). The L-Tag consists of several functional domains: DnaJ domain, linker domain, origin binding domain (OBD), zinc ($Zn^{++}$)-binding domain and helicase/ATPase domain. (a) The DnaJ domain contains: a HPDKGG motif, conserved among HPyVs, which is able to bind to the Hsc70, a cellular chaperone and transcriptional repressor; a CR1 motif (LXXLL), present only in BKPyV, JCPyV and MCPyV. (b) The linker domain contains: WXXWW motif, conserved in the majority of HPyVs but not in MCPyV, for binding Bub1, a mitotic checkpoint serine-threonine protein kinase; a unique region (MUR), only present in MCPyV, that binds the Vamp6P-protein and the Bromodomain protein4 for the recruitment of cellular protein factor C (RFC); a LXCXE motif, conserved in the majority of HPyVs, crucial for the interaction with the retinoblastoma protein (pRb) family. (c) The helicase/ATPase domain comprises: a p53 binding domain, conserved in the majority of HPyVs; a C-terminal domain, only present in JCPyV, that binds the insulin receptor substrate 1 (IRF1).

**Figure 2.** HPyVs' large T antigen (L-Tag) oncogenic mechanisms. (a) In physiological conditions, the retinoblastoma proteins (pRbs) are in a hypophosphorylated state, which allows them to bind and inhibit the E2F transcription factors, preventing the E2F-mediated gene expression and consequently the transition from G1 into S phase. HPyVs' L-Tag is able to bind the pRb proteins promoting their hyperphosphorylation, thus pRb is unable to bind E2F, leading to its transcriptional activity; at the same time the hyperphosphorylation of p130 disrupts the transcriptional repressor complex (p130-E2F4/5), leading to uncontrolled cell cycle progression and sometimes to malignant transformation. (b) Multiple cellular stress, normally, raise the levels of p53, which promotes the DNA repair and cell cycle arrest. HPyVs' L-Tag is able to bind and block the activity of p53 protein, preventing apoptosis and cell cycle arrest induced by DNA damage. (c) In physiological conditions, the phosphorylated β-catenin undergoes degradation via ubiquitin-dependent proteasome. JCPyVs' L-Tag binds the β-catenin protein promoting its hypophosphorylation, thus β-catenin complexes with

LEF-1/TCF-4 transcription factors, promoting the cell cycle progression by c-myc and cyclin D1 expression.

**Figure 3.** Scheme of the functional domains of the HPyVs' small T antigen (s-Tag). The s-Tag presents a DnaJ domain, followed by an unique domain, that is removed from LT-Ag during the splicing process. (a) The DnaJ domain contains a LYCKE motif (JCPyV and BKPyV) and a LHCWE motif (only JCPYV), able to interact with pRb family proteins. (b) The unique domains contains two $Zn^{++}$ binding sites and, additionally and only in MCPyV, a PPAR1/NEMO binding site and a large T stabilization domain (LSD), that is involved in the oncogenesis process. (c) The binding site for the PP2A is conserved in the majority of the HPyVs and triggers several pathways related to cellular transformation.

**Figure 4.** HPyVs' small T antigen (s-TAg) oncogenic mechanisms. (a) In physiological conditions, the Akt, p53, c-Myc and β-catenin proteins are in a phosphorylated state; the subsequent dephosphorilation due to the PP2A serine-threonine phosphatase regulates the cell cycle progression and the apoptosis process. (b) The binding between s-TAg and PP2A avoids the dephosphorilation of Akt, p53, c-Myc and β-catenin proteins, and the subsequent deregulation of the cell cycle progression and apoptosis process drives the cell to a malignant transformation.

**Figure 1.**

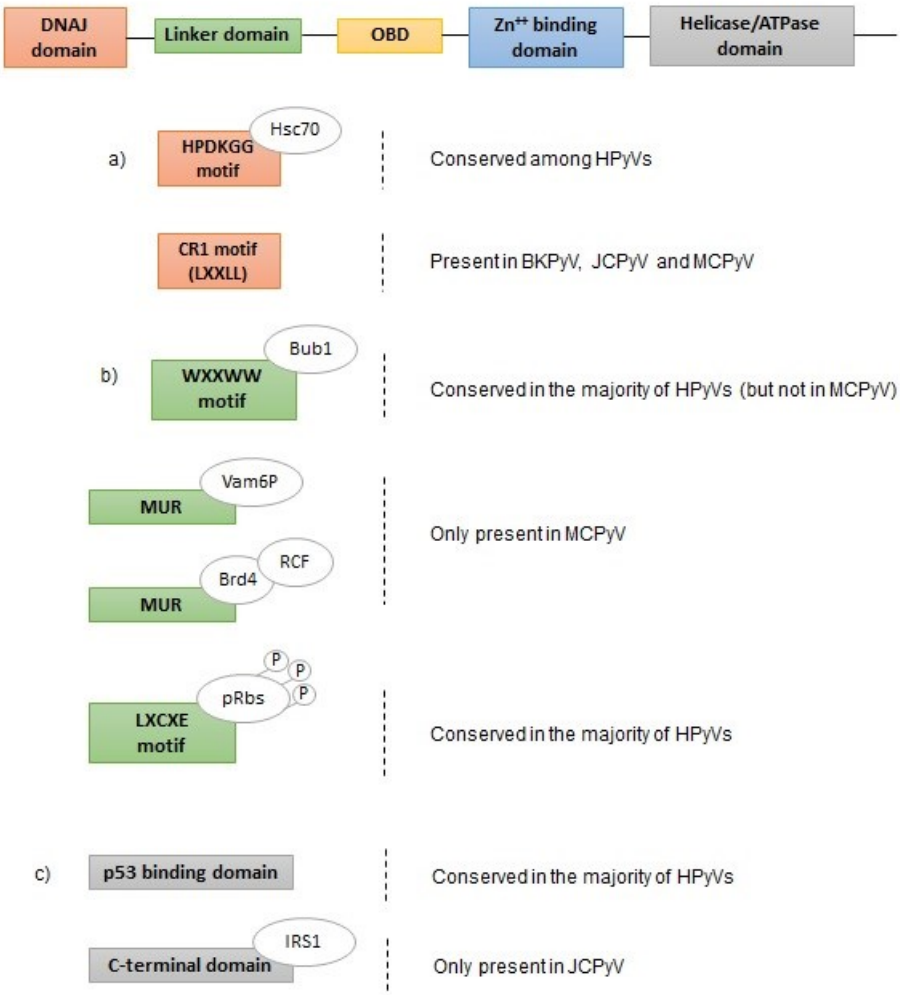

DNAJ domain
Linker domain
OBD
Zn++ binding domain
Helicase/ATPase domain
a)
Hsc70
HPDKGG motif
Conserved among HPyVs
CR1 motif (LXXLL)
Present in BKPyV, JCPyV and MCPyV
b)
Bub1
WXXWW motif
Conserved in the majority of HPyVs (but not in MCPyV)
Vam6P
MUR
Only present in MCPyV
RCF
Brd4
MUR
P
P
P
pRbs
LXCXE motif
Conserved in the majority of HPyVs
c)
p53 binding domain
Conserved in the majority of HPyVs
IRS1
C-terminal domain
Only present in JCPyV

**Figure 2.**

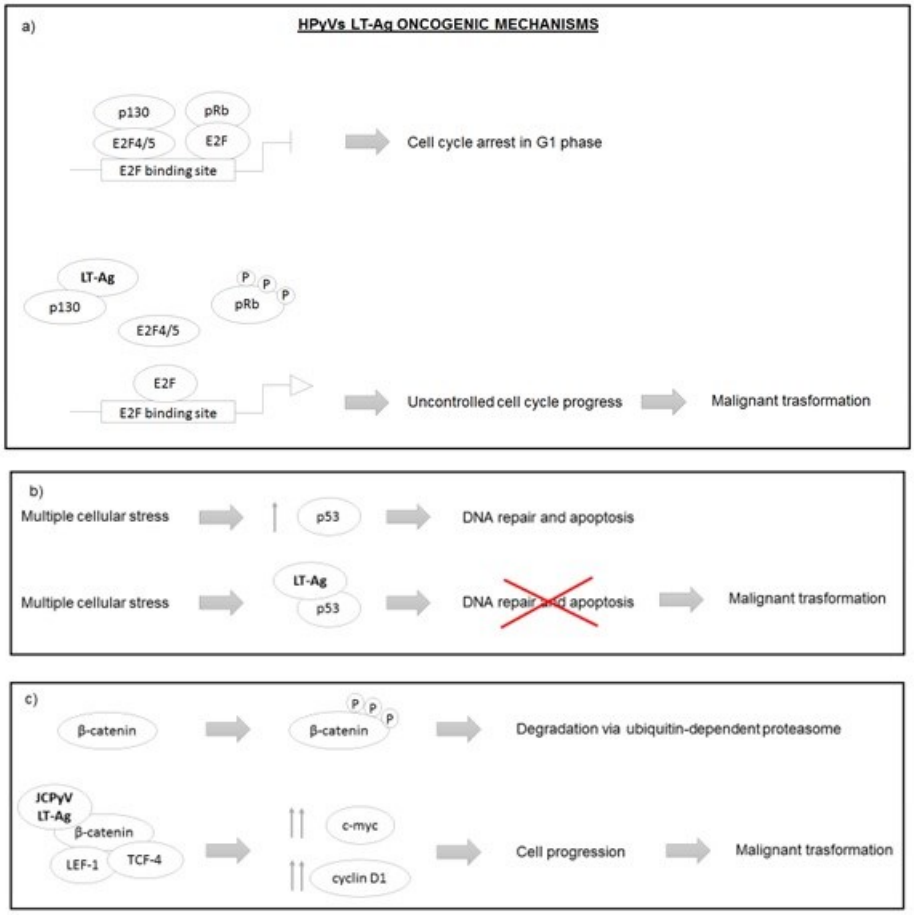

a)
HPyVs LT-Ag ONCOGENIC MECHANISMS
p130
pRb
E2F4/5
E2F
E2F binding site
Cell cycle arrest in G1 phase
LT-Ag
p130
E2F4/5
P
P
P
pRb
E2F
E2F binding site
Uncontrolled cell cycle progress
Malignant trasformation
b)
Multiple cellular stress
p53
DNA repair and apoptosis
Multiple cellular stress
LT-Ag
p53
DNA repair and apoptosis
Malignant trasformation
c)
β-catenin
P
P
P
β-catenin
Degradation via ubiquitin-dependent proteasome
JCPyV
LT-Ag
β-catenin
LEF-1
TCF-4
c-myc
cyclin D1
Cell progression
Malignant trasformation

**Figure 3.**

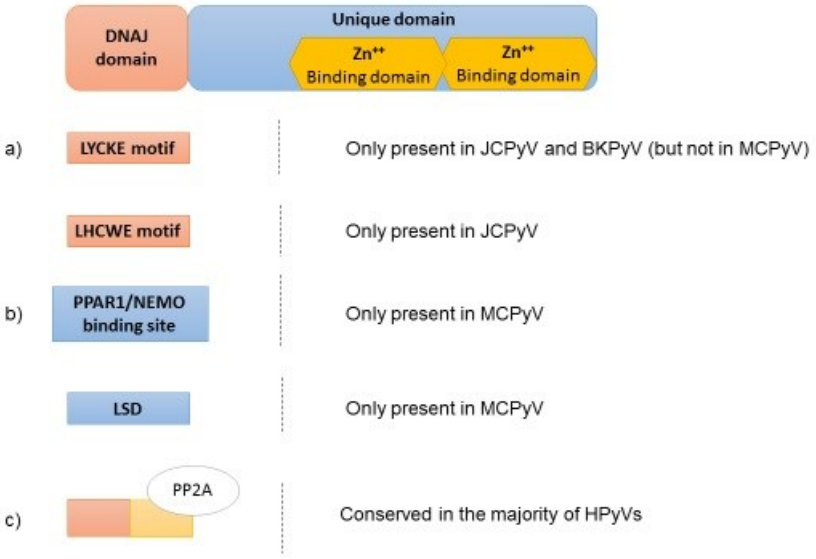
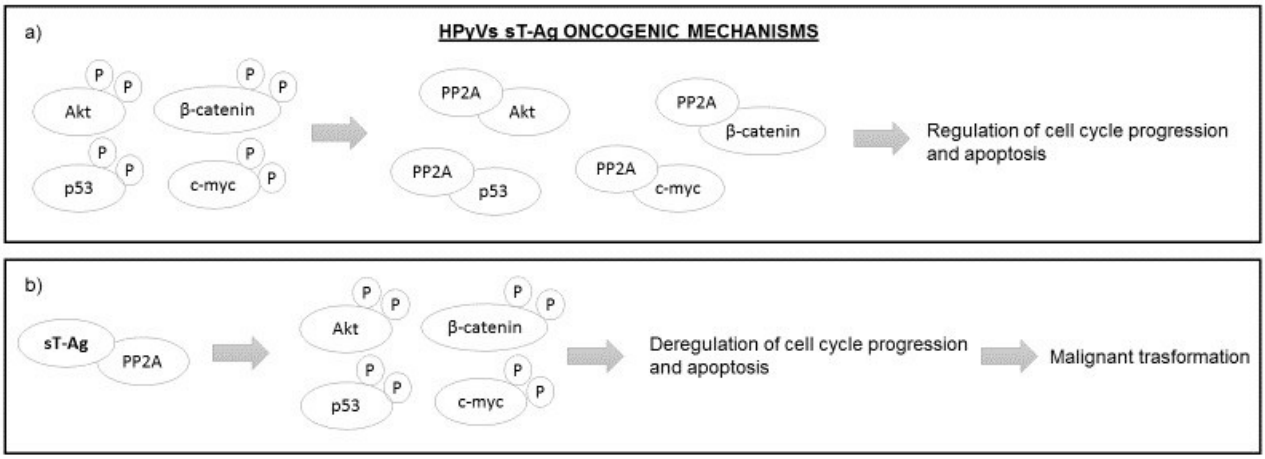

DNAJ domain
Unique domain
Zn++ Binding domain
Zn++ Binding domain
a)
LYCKE motif
Only present in JCPyV and BKPyV (but not in MCPyV)
LHCWE motif
Only present in JCPyV
b)
PPAR1/NEMO binding site
Only present in MCPyV
LSD
Only present in MCPyV
PP2A
c)
Conserved in the majority of HPyVs

**Figure 4.**


a)
HPyVs sT-Ag ONCOGENIC MECHANISMS
Akt
β-catenin
p53
c-myc
PP2A
Akt
PP2A
β-catenin
PP2A
p53
PP2A
c-myc
Regulation of cell cycle progression and apoptosis
b)
sT-Ag
PP2A
Akt
β-catenin
p53
c-myc
Deregulation of cell cycle progression and apoptosis
Malignant trasformation