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REVIEW

Role of the interaction between large T antigen and Rb family members in the oncogenicity of JC virus

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Human polyomaviruses (JC virus, BK virus and simian virus 40) are causative agents of some human diseases and, interestingly, are involved in processes of cell transformation and oncogenesis. These viruses need the cell cycle machinery of the host cell to complete their replication; so they evolved mechanisms that can interfere with the growth control of infected cells and force them into DNA replication. The retinoblastoma family of proteins (pRb), which includes pRb/p105, p107 and pRb2/p130, acts as one of the most important regulators of the G1/S transition of the cell cycle. Rb proteins represent an important target for viral oncoproteins. Early viral T antigens can bind all members of the pRb family, promoting the activation of the E2F family of transcription factors, thus inducing the expression of genes required for the entry to the S phase.The interaction between early viral antigens and cell cycle regulators represents an important mechanism through which viruses deregulate cell cycle and lead to cell transformation. In this review, we will discuss the effects of the interaction between large T antigen and Rb proteins in JC virus-mediated oncogenesis.
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Introduction

Nearly 15% of human cancer incidence can be attributed to viral infections. Although only two RNA viruses, hepatitis C and human T-cell lymphotropic virus type I, are considered as causative agents for cellular transformation in humans, most virus-induced tumors are attributed to the infection with DNA viruses. These viruses are capable of modulating cellular pathways, mainly by increasing cell survival, and by stimulating DNA replication (Cinatl and Doerr, 2001). Indeed, DNA viruses depend on the replication machinery of the host cell to complete viral replication cycle. As the enzymes for DNA replication are present in sufficient amounts only during the S phase of the cell cycle, the viruses have evolved mechanisms to meet this requirement. They encode proteins that interfere with growth control mechanisms of the infected cells, which allow them to drive cells from quiescence to DNA replication (Nemethova *et al*., 2004). The main contributors in dysregulating cellular equilibrium are E1A protein of adenovirus, large T antigens (T-Ag) of simian virus 40 (SV40) and polyomaviruses (Py), and the E7 protein of human papillomaviruses (Moran, 1993).

Human polyomaviruses, which include JC virus (JCV) and BK virus (BKV), as well as the SV40, have been associated with human tumors and have been shown to be highly tumorigenic in experimental animal models (Barbanti-Brodano *et al*., 1998; Howard *et al*., 1998; Khalili *et al*., 1999). Here, we focus our attention on JCV, because of its ability to induce a fatal demyelinating disease in the brain, the progressive multifocal leukoencephalopathy (PML); because of its presence in various tumors including brain tumors and colorectal cancer; and finally because of its oncogenic potential in several animals models. In particular, we are interested in the interactions between JCV and the Rb family of tumor suppressors, which are strongly involved in the regulation of normal cell growth. Better understanding of this molecular interaction could represent an essential step for the development of new clinical strategies for the prevention and treatment of virus-associated tumors.

Human polyomaviruses

Human polyomaviruses are icosahedral non-enveloped DNA viruses with capsid diameters of approximately 45 nm. Their genome consists of covalently bound, double-stranded, circular supercoiled DNA with an average length of 5 kb (Croul *et al*., 2003). All polyomaviral genomes have common structural features, which include an early region encoding for proteins involved in the regulation of viral replication,

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such as T-Ag, its isoforms and the small t antigen (t-Ag). A late region encodes for capsid proteins VP1, VP2 and VP3 and for agnoprotein that influences the assembly process. A regulatory region separates the coding regions and contains sequences that are necessary for the initiation of viral gene transcription and viral DNA replication. Transcription of the early and late genes proceeds in opposite directions around the viral DNA (Frisque and White, 1992; Bollag *et al*., 2000; Croul *et al*., 2003). The human JCV infects asymptomatically nearly 80% of human population. Primary infection leads to lifelong persistence in the kidney, in the central nervous system (CNS) and in lymphoid cells. Virus is shed into the urine and could be transmitted orally. During severe immunosuppression, virus replication is accompanied by extensive cytolytic damage of the host cell (Dorries, 1998). JCV is the etiologic agent of PML, a fatal demyelinating disease of the CNS leading to lytic destruction of myelin-producing oligodendroglial cells (Frisque and White, 1992; Berger *et al*., 1998). Although this rare disease was originally described in patients with systemic immunosuppression, later it has been associated and detected more frequently in acquired immunodeficiency syndrome (AIDS) patients. It is presently recognized that JCV infection accounts for a distinct rise in the incidence of PML in the AIDS population (Berger *et al*., 1998; Seth *et al*., 2003). In addition, there are many reports of JCV detection in multiple malignant astrocytomas, primary cerebral malignant lymphomas and oligoastrocytomas as well as in multiple gliomas in patients with PML (Sima *et al*., 1983; Ariza *et al*., 1994; Rencic *et al*., 1996).

JCV detection in human brain tumors

Different studies have been accomplished to investigate the presence of JCV in human brain tumors. Khalili *et al*. (1999) examined viral gene expression in 23 primitive neuroectodermal origin tumors, such as medulloblastomas. Twenty of them revealed the presence of N-terminal T-Ag sequences, 13 contained C-terminal T-Ag sequences and 20 had sequences from the VP1 region (Khalili *et al*., 1999; Krynska *et al*., 1999). Furthermore, a subset of 16 of these tumors revealed sequences that code for the viral accessory protein, agnoprotein (Del Valle *et al*., 2002). As in the case of SV40, JCV sequences have been detected in a variety of low- and high-grade glial tumors, including those of ependymal, astrocytic and oligodendroglial origin (Rencic *et al*., 1996; Boldorini *et al*., 1998; Caldarelli-Stefano *et al*., 2000; Del Valle *et al*., 2000, 2001). The transforming activity of JCV and other viruses has been well documented in cell cultures, and their oncogenicity has been documented in experimental animals. Inoculation of JCV intracerebrally into newborn Golden Syrian hamsters produces CNS tumors in more than 85% of the animals. The most common tumors found are primitive neuroectodermal tumors, including medulloblastomas and pineocytomas (ZuRhein, 1983). Astrocytomas, glioblastomas and peripheral neuroblastomas are also commonly seen in the hamster model. Of interest, JCV is the only human virus known to induce solid tumors in non-human primates, as owls and squirrel monkeys injected intracerebrally with JCV develop astrocytomas (London *et al*., 1978). In these experimental animals, T-Ag can be detected in the absence of viral capsid protein expression (Frisque and White, 1992). Although studies on JCV are based on its role in brain tumors, this virus can infect a variety of tissue types, and its presence has also been associated with other tumors such as colorectal and esophageal carcinomas (Casini *et al*., 2005; Del Valle *et al*., 2005). Despite its association with some tumors and its transforming activity in experimental models, a causative role of JCV in human cancer has not been proven to date. Nevertheless, it could have a potential role as cofactor in oncogenesis. The intracellular environment may permit the expression of early but not late gene of JCV not allowing the production of the complete virus. This may result in cell cycle dysregulation without cellular lysis leading to the expansion of these infected cells and their resulting transformation (Croul *et al*., 2003).

Polyomavirus T-Ag and its oncogenic potential

As mentioned above, polyomaviruses can induce DNA replication in host cells. Deregulatory effects of these viruses on cell growth control mechanisms and their transforming and oncogenic properties are generally attributed to the viral early gene products, T-Ag and, to a lesser extent, t-Ag (White and Khalili, 2004, 2006). T-Ag has the ability to associate with and affect the function of a large number of cellular proteins, including members of the Rb tumor suppressor family (pRb, p107 and pRb2/p130), insulin receptor substrate 1 and p53 (Brodsky and Pipas, 1998; Sullivan *et al*., 2000a; Lassak *et al*., 2002; Trojanek *et al*., 2006a, b). The binding of T-Ag to the Rb family members is critical for the transactivation of DNA synthesis enzymes, as well as cyclins E and A, which play a role in the initiation and propagation of the S phase of the cell cycle. In addition, t-Ag interferes with protein phosphatase 2A, causing a destabilization of the Cdk-2 inhibitor p27, and thus leading to strong cyclin E- and cyclin A-dependent Cdk2 activity (Klucky *et al*., 2004).

T-Ag

Polyomavirus T-Ags are highly phosphorylated multifunctional proteins that play a role in viral DNA replication, as well as cellular transformation (Simmons, 2000). Most of the T-Ag phosphorylation occurs after its traslocation into the nucleus, which is possible because T-Ag contains two different nuclear localization signals (Richardson *et al*., 1986; Howes *et al*., 1996). T-Ag is an essential factor for viral DNA replication owing to its ability to bind to the viral origin of replication, where it promotes unwinding of DNA double helix (through its helicase activity). This primary T-Ag action leads to the recruitment of proteins

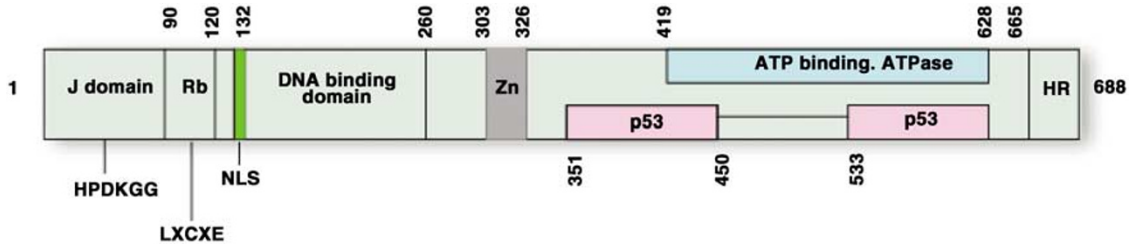

Figure 1 Linear structure of the JCV large T-Ag. The protein, whose size is 688 amino acids, share the N-terminal domain with the T′ proteins (T'_{135}, T'_{136} and T'_{165}), which are encoded by alternatively spliced early viral transcripts (Trowbridge and Frisque, 1995). The last 33 C-terminus amino acids of the T-Ag are shared with T'_{165}, and also include a host range domain (HR), required for virion assembly. The diagram illustrates the position of the DNA-binding domain, the zinc-finger motif, and the sequences required for nucleotide binding ATPase activity and p53 association. HPDKGG and LXCXE represent the conserved sequences for binding to heat-shock protein and pocket proteins, respectively.

required for DNA synthesis (White and Khalili, 2004). The ability of T-Ag to induce cells to enter the S phase of the cell cycle contributes to its ability to transform multiple cell types (Sullivan and Pipas, 2002).

The T-Ag functions have been attributed to different structural domains and functional motifs within its amino-acid sequence. The T-Ags of primate polyomaviruses contain at least three domains that influence their oncogenic potential: the LXCXE domain, the J domain and the p53-binding domain (Frisque, 2001). Proteolysis experiments separating the N- and C-terminal domains revealed that the N-terminal region is sufficient for immortalization by T-Ag and for stimulation of cell cycle progression in serum-starved cells (Gjoerup *et al*., 1994; Holman *et al*., 1994).

The N-terminal region carries both the LXCXE motif for binding to Rb family members and the J domain, containing an HPDKGG motif (J box) (Brodsky and Pipas, 1998; Nemethova *et al*., 2004). The LXCXE motif of viral oncoproteins interacts directly with the B domain of the pRb family members and this binding is indispensable for virus-mediated cellular transformation (Pipas, 1992; Chestukhin *et al*., 2002). The J box interacts with dnaK-type chaperones such as Hsc70, stimulating their ATPase activity, and consequently modulating their ability to bind and release protein complexes. As a result, the chaperone activity of T-Ag is required to modulate activities of multiprotein assemblies involved in the activity of the cellular protein machine stimulating their ATPase activity (Brodsky and Pipas, 1998; Sullivan and Pipas, 2002). Mutations in either one of these sites abolish the transactivation of S-phase-specific genes by T-Ag (Lin and DeCaprio, 2003; Nemethova *et al*., 2004). The C-terminus of the T-Ags of polyomaviruses contains a region involved in specific binding to the origin of replication of viral DNA, and it harbors the ATP-binding and helicase activities that are essential for viral DNA replication (Fanning and Knippers, 1992). It also contains a zinc-finger region known to function mainly in the replication of viral DNA, but which seems to modulate the DNA-binding specificity of the protein and it is known to interfere in the replication of viral DNA and transactivation (Pipas, 1992; Nemethova *et al*., 2004). A schematic representation of JCV T-Ag is reported in Figure 1. T-Ag from different viruses are highly homologous and share a number of functional domains, including the J domain and the LXCXE domain (Frisque *et al*., 1984; Trowbridge and Frisque, 1995). JCV, as well as BKV, has been shown to induce transformation in cultured cells, even though less efficiently than SV40 (Bollag *et al*., 1989, 2000), and their T-Ags also bind the Rb family of tumor suppressors less efficiently than SV40 T-Ag (Dyson *et al*., 1990). Interestingly, the transforming activity of JCV seems to be specific for cells of neural origin, as the JCV early promoter directs glial cell-specific transcription of T-Ag (White and Khalili, 2004, 2006).

pRb family of cell cycle regulators

pRbs is a group of nuclear proteins including pRb/p105, p107 and pRb2/p130. These proteins are the major regulators of cell proliferation and cell differentiation through their unique ability to suppress cell cycle progression (Mulligan and Jacks, 1998). They are regulated in a cell cycle-dependent manner and are critical targets for inactivation by transforming oncoproteins of DNA tumor viruses (Paggi *et al*., 1996; Stiegler *et al*., 1998). pRb/p105 has been identified as a tumor suppressor gene deleted or mutated in childhood Rb and in a variety of adult cancers (Friend *et al*., 1986; Weinberg, 1995; Chestukhin *et al*., 2002). Although the role of p107 and pRb2/p130 in tumor suppression is less clear than that of pRb/p105, there are several reports of pRb2/p130 inactivating mutations identified in human cancers (Cinti *et al*., 2000; Claudio *et al*., 2000a, b).

pRbs structure

All three pRb proteins share a high degree of sequence homology in conserved regions, such as the A and B domains. A spacer region separates the two domains that form a peculiar steric conformation, the 'pocket region', whose integrity is fundamental for most of the functional interactions of pRbs (Paggi *et al*., 1996). The

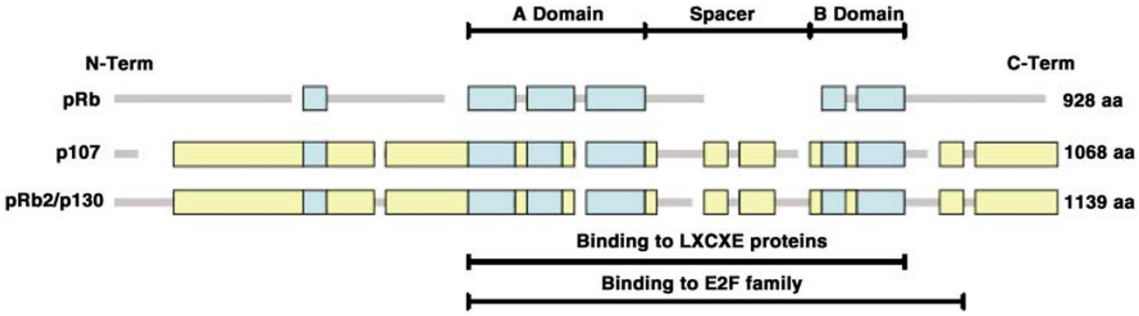

Figure 2 Amino-acid sequence homology among the pRbs. pRb/p105, p107 and pRb2/p130 contain multiple regions of homology throughout their length. The highly homologous regions correspond essentially to the A and B domains, which represent the 'pocket' (blue). p107 and pRb2/p130 share a higher sequence homology with respect to pRb/p105 (yellow). Non-homologous sequences are represented in gray.

A and B boxes define the minimal region essential for binding to the LXCXE-containing proteins. These include viral oncoproteins such as adenovirus E1A, polyomavirus T-Ag and E7 (Dyson *et al*., 1990; Lee *et al*., 1998), and a number of endogenous Rb-binding proteins such as histone deacethylase 1 and 2 (HDAC-1 and HDAC-2) (Brehm *et al*., 1998; Magnaghi-Jaulin *et al*., 1998), and ATPases BRG1 and BRM (Strober *et al*., 1996). The crystal structure of the pocket in complex with an LXCXE peptide revealed that the binding site for LXCXE is actually located in domain B. In addition, domain A is required for domain B to assume an active conformation, thus explaining the conservation of both domains (Lee *et al*., 1998; Harbour and Dean, 2000). The A and B domains are also involved in the formation of a second protein-binding site, a 'large pocket', which interacts with the E2F family of transcription factors (Knudsen and Wang, 1998). E2Fs do not contain an LXCXE motif. Therefore, they bind pRbs at a distinct site that appears to involve points of contact in both the pocket and the carboxy-terminal region (Lee *et al*., 1998; Xiao *et al*., 2003). Although the large pocket overlaps with the LXCXE-binding site, it seems to be functionally independent, allowing pRbs to bind E2Fs and to form transient regulatory complex with other proteins (Harbour and Dean, 2000). The pRb members contain another functional domain, located within the carboxy-terminal region, which has been shown to bind c-abl tyrosine kinase and MDM2. The c-abl/Rb interaction seems to be important for the role of pRb as proliferation suppressor (Harbour and Dean, 2000). Of interest, the c-abl-binding domain is absent in p107 and pRb2/p130 (Knudsen and Wang, 1998). The homology among pRb proteins is mainly concentrated in the pocket region. For instance, p107 and pRb2/p130 show a sequence homology of about 50%, and share about 35% homology with pRb/p105 (Lipinski and Jacks, 1999). In contrast to pRb/p105, p107 and pRb2/p130 share a highly conserved spacer domain between the A and B boxes. This connecting region is important for the binding of p107 and pRb2/p130 with cyclin E/Cdk2 and cyclin A/Cdk2, and has homology with Cdk inhibitors such as p21Cip1 and p27Kip. This function is absent in pRb/p105 (Woo *et al*., 1997; Lipinski and Jacks, 1999) (Figure 2).

pRb-mediated transcriptional repression

pRbs control cell proliferation acting in the G1 phase of the cell cycle: they repress the transcription of genes involved in the regulation of progression into the S phase. pRbs do not bind directly to DNA, but they interact and inhibit the activity of transcription factors. Among them, the most representative are the E2F family of transcription factors. Many promoters contain binding sites for E2F factors. These promoters are involved in cell cycle regulation, such as cyclin D1, E and A, p107, E2F-1, -4 and -5, Cdk2, cdc2; and in DNA synthesis, such as timidine kinase, dehydrofolate reductase, DNA polymerase α and cdc6 (Lipinski and Jacks, 1999). There are functional and structural differences within the E2F family. E2F-1, -2 and -3 are transcriptional activators and interact with pRb/p105. E2F-4 and -5 are transcriptional repressors and preferentially bind pRb2/p130 and p107. They are dispensable in quiescent cells, but necessary in cycling cells for the so-called pocket protein-mediated G1 arrest (Gaubatz *et al*., 2000). E2F-6 apparently does not have a pocket protein interaction domain, but it interacts with polycomb proteins and thus represses transcription (Cartwright *et al*., 1998; Trimarchi *et al*., 1998). The recently identified E2F-7 and E2F-8 are also believed to repress specific promoters (Di Stefano *et al*., 2003; Maiti *et al*., 2005). Although p107, pRb2/p130 and pRb/p105 are closely related members of the same family, they have different affinities for E2F members and exhibit different temporal regulation during the cell cycle. Whereas E2F-4/pRb2/p130 complexes are the most abundant in quiescent cells, E2F-4/p107 and E2F-4/pRb/p105 accumulate in G1 cells. In addition, whereas pRb2/p130 and p107 are bound to a number of promoters in asynchronously growing cells, only pRb2/p130 is recruited to promoters in quiescent or serum-restimulated human cells (Balciunaite *et al*.,

2005). A precise mechanism by which pRb family of proteins controls cell proliferation is not completely understood. Biochemical studies show, however, that pRb/p105 can repress transcription in at least three ways when recruited to the promoters (Frolov and Dyson, 2004). First, pRb/p105 binds directly to the activation domain of E2F and, in doing so, it physically blocks the activity of this domain (Helin *et al*., 1993). Second, the recruitment of pRb/p105 to the promoter blocks the assembly of preinitiation complexes, potentially allowing it to inhibit the activity of adjacent transcription factors (Ross *et al*., 1999). Third, pRb/p105 uses a protein interaction domain that is distinct from the E2F-binding site to associate with complexes that modify chromatin structure (Brehm *et al*., 1998; Magnaghi-Jaulin *et al*., 1998; Ross *et al*., 2001). Similar binding sites are found in both p107 and pRb2/p130 and, by forming these complexes, the pRb family of proteins serves as molecular adapters allowing chromatin-modifying enzymes to be recruited to E2F-regulated promoters (Frolov and Dyson, 2004). At present, there is no definitive evidence to explain which of these mechanisms of repression is the most important for cell cycle control (Frolov and Dyson, 2004). An interesting model involves the chromatin modification through histone acetylation/deacetylation process. Histones are generally hyperacetylated at the promoters of actively transcribed genes and are hypoacetylated at silenced genes. It has been proposed that E2F-bound acetyltransferases (HATs – histone acetyltransferases), acetylating histones, make chromatin accessible to E2F and promote transcription. In contrast, HDAC recruited by pRb/p105 appears to promote nucleosome assembly on the promoter, blocking access to transcriptional machinery (Harbour and Dean, 2000) (Figure 3). Similar to HDACs, pRb/p105 also binds to BRG1/BRM and forms a complex with E2F (Zhang *et al*., 2000). BRG1/BRM are two ATPases belonging to the human SWI/SNF nucleosome-remodeling complex, which reversibly alter the chromatin condensation in an ATPase-dependent manner. SWI/SNF complex ensures continuous oscillation of nucleosomes between a functional and disrupted structure. The disrupted nucleosomes may be targeted by HAT, which fixes them in an inactive conformation. Conversely, recruitment of HDAC removes the inhibitory acetylation, forcing SWI/SNF to assemble them back into functional nucleosomes. Thus, the balance between HDAC and HAT activity in the vicinity of SWI/SNF determines whether SWI/SNF will facilitate transcriptional repression or activation (Harbour and Dean, 2000).

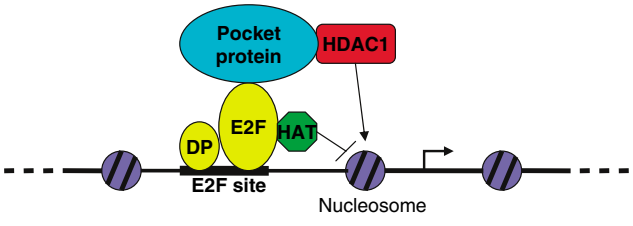

Figure 3 Model of transcriptional repression through E2F sites. The E2F-DP heterodimer recognizes the E2F site. HATs activity associated with E2F may promote the binding of E2F to the promoter and inhibit nucleosome formation, allowing further access of transcription factors to the promoter. E2F also forms complexes with pocket proteins. The HDAC recruited by the pocket protein may promote nucleosome assembly on the promoter, blocking the access to the transcription machinery.

pRb regulation

The protein-binding and growth suppression functions of pRb/p105, p107 and pRb2/p130 are modulated by cell cycle-dependent phosphorylation. In G0 and early G1 phase of the cell cycle, all three pRb family members are under- or hypophosphorylated. The phosphorylation process is progressive and is initiated in mid-to-late G1 by kinase complexes of Cdk4/6 and D-type cyclin (Canhoto *et al*., 2000). When cells are stimulated to enter the cell cycle, pRb/p105 and p107 become phosphorylated during late G1 and S phases by cyclin D/Cdk4, cyclin E/Cdk2 and cyclin A/Cdk2 (DeCaprio *et al*., 1989; Knudsen and Wang, 1998; Lundberg and Weinberg, 1998; Lin and DeCaprio, 2003). pRb2/p130 also becomes hyperphosphorylated during the G1 to S phase transition. However, unlike pRb/p105 and p107, pRb2/p130 is also phosphorylated during G0 and early G1 phase of the cell cycle (Canhoto *et al*., 2000). Cell cycle-dependent phosphorylation of the Rb family inhibits their growth suppression functions by reducing their interactions with E2F transcription factors and permits E2F-mediated transcription during proliferation (Chestukhin *et al*., 2002). The regulation of pRb/p105 by phosphorylation is well understood. Hyperphosphorylation of pRb/p105 results in a loss of binding to both E2F and to chromatin-remodeling factors, and reverses pRb-mediated cell cycle arrest (Dyson, 1998; Ferreira *et al*., 1998; Luo *et al*., 1998; Helt and Galloway, 2003). Similar to pRb/p105, p107 and pRb2/p130 regulate cell cycle progression via interactions with E2F and are Cdk substrates (Classon and Dyson, 2001). However, in contrast to pRb/p105, they also act as Cdk inhibitors, which appear to contribute to their function in controlling cell cycle progression (De Luca *et al*., 1997; Woo *et al*., 1997; Helt and Galloway, 2003). With regard to this, Howard *et al*. (2000), using an inducible system for pRb2/p130 expression in JCV-induced hamster brain tumor cells, demonstrated that one of the unique features of pRb2/p130 is its ability to specifically inhibit Cdk2-associated kinase activity *in vivo*. Different mechanisms control the kinases that phosphorylate pRbs. Among them, the most important involve two groups of inhibitory proteins: the INK4 and Cip/Kip families, including respectively $p16^{INK4a}$, $p15^{INK4b}$, $p18^{INK4c}$ and $p19^{INK4d}$ members and $p21^{Cip1}$, $p27^{Kip1}$ and $p57^{Kip2}$ members (Sherr and Roberts, 1999). pRb2/p130, for example, also increases the protein level of the Cdk inhibitor p27kip1. p27kip1 is a universal Cdk inhibitor, thus it may provide a positive feedback loop for enhancing the growth regulatory functions of pRb2/p130 (Howard *et al*., 2000). In addition to changes in

phosphorylation, pRb2/p130 levels fluctuate significantly throughout the cell cycle, with pRb2/p130 highly expressed during G0, p107 highly expressed in S phase and pRb/p105 expressed at a fairly steady level throughout the cell cycle (Classon and Dyson, 2001).

pRb—T-Ag interaction

Although the interaction between pRb/p105 and SV40 T-Ag is well characterized, very little is known about regulatory mechanisms that control the recruitment of JCV T-Ag to the pocket proteins. In addition, the binding of JCV to pRb family members is difficult to detect, owing to low expression levels of the viral protein (Helt and Galloway, 2003). There is evidence that JCV may alter the phosphorylation and expression levels of pocket proteins. In JCV-transformed primary human fetal glial (PHFG) cells, pRb2/p130 levels were reduced and, in contrast, steady-state levels of both pRb/p105 and p107 appeared to be increased and the proteins were hyperphosphorylated (Bollag *et al*., 2000; Helt and Galloway, 2003). Using a tetracycline-regulated Rb2/p130 expression system in hamster glioblastoma cells transformed by the JCV, Howard *et al*. (1998) demonstrated that JCV T-Ag binds the hypophosphorylated form of pRb2/p130. Furthermore, the stimulation of pRb2/p130 expression overcomes cellular transformation mediated by T-Ag and results in suppression of tumor formation both *in vitro* and *in vivo*. Studies on pRb/p105 have shown that it contains 16 phosphorylation sites and that phosphorylation of Thr 821/826 is required to inhibit pRb/p105 binding to T-Ags; the disruption of E2F binding follows the phosphorylation of Ser/Thr localized both in the insert domain and the C-terminal region of pRb/p105 (Knudsen and Wang, 1998). The E2F binding and growth suppression activities of p107 are also inactivated by phosphorylation (Xiao *et al*., 1996). It has been found that, unlike pRb/p105, p107 and pRb2/p130 retain their ability to bind to T-Ag in their phosphorylated state. Knudsen and Wang (1998) demonstrated that this lack of regulation of A/B activity is due to a critical regulatory sequence that is specifically found in pRb/p105 but is absent from the p107/pRb2/p130 sequence. Thus, p107/pRb2/p130 and pRb/p105 are differently regulated with respect to the LXCXE binding (Knudsen and Wang, 1998).

A general model of the T-Ag–pRbs interaction proposed by Sheng *et al*. (1997) and supported by more recent studies (Sullivan *et al*., 2000a; Sullivan and Pipas, 2002) predicts that polyomavirus T-Ags, through their LXCXE motif, bind to underphosphorylated forms of pRb/p105, p107 and pRb2/p130. These latter proteins may repress cellular transcription via their interaction with the E2F-DP family of transcription factors and, in doing so, prevent exit from the G0/G1 phases of the cell cycle. Once bound to the pRbs–E2F complex, T-Ag recruits, through its J domain, a member of DnaK family of molecular chaperones, Hsc70, and activates its intrinsic ATPase activity. By inducing a conformational change in the complex, Hsc70 is thought to affect the release of E2F-DP from pRb/p105, p107 and pRb2/p130, leading to the activation of genes involved in S-phase progression (Frisque, 2001; Sullivan and Pipas, 2002). It has been demonstrated that JCV T-Ag, like its simian counterpart SV40, binds pRb family members and influences their phosphorylation status and stability (Dyson *et al*., 1990; Howard *et al*., 1998). On the contrary, JCV T-Ag interaction with Hsc70 has only been shown using chimeric JCV-SV40 T-Ag (Sullivan *et al*., 2000b). Large T antigen J domains of JCV and SV40 are highly homologous (Pipas, 1992). Sullivan *et al*. (2000a) demonstrated that the T-Ag J domain of JCV can replace the SV40 T-Ag J domain for all the activities necessary for a complete SV40 life cycle in cell culture, including the ability to disrupt pRb–E2F complex. However, the JCV J domain is less efficient than the SV40 T-Ag J domain. One function of the T-Ag J domain is to act in *cis* with the pRb-binding motif to disable the growth inhibitory functions of the pRb family of proteins (Sullivan *et al*., 2000a, b).

In addition to T-Ag, a new series of viral early proteins have been discovered in JCV in the last decades. These proteins, generally called T′ proteins, contribute to the T-Ag viral activity and are suspected to contribute to the JCV-transforming properties. T′ proteins are expressed by JCV during lytic infections and in JCV-transformed cells (Trowbridge and Frisque, 1995). They represent products of post-transcriptional processing of the early mRNA and contribute to the pathogenic and oncogenic potential of the virus (Frisque, 2001). Each transcript is generated by the removal of two introns from the immature early mRNA. One of these introns is the same as that removed from the T-Ag mRNA. The second splicing reaction utilizes a new 5′-donor splice site shared by all three T′ transcripts that is joined to a different 3′-acceptor splice site for each message. These transcripts encode proteins of 135, 136 and 165 amino acids, with the first 132 residues overlapping those of T-Ag, resulting in their respective names T'_{135}, T'_{136} and T'_{165} (Frisque, 2001). The shared amino-terminal 132 amino acids of T-Ag, T'_{135}, T'_{136} and T'_{165} contain important functional domains that influence the ability of JCV to replicate its genome and to exhibit oncogenic activity. The J and LXCXE domains are preserved in the JCV T′ proteins, whereas the p53-binding domain is lost during removal of the second intron from the T′ mRNAs. The two preserved domains have been hypothesized to collaborate on the way of inducing viral DNA replication in permissive human cells, or unregulated cell proliferation in non-permissive rodent cells (reviewed by Brodsky and Pipas, 1998). Each T′ protein has a unique C-terminus. All three T′ antigens bind pRb family members, although with different affinities (Bollag *et al*., 2000). The T′ proteins ability to bind pRb members was assayed in a representative experiment where T-Ag, T'_{135}, T'_{136} and T'_{165} purified from insect cells infected with recombinant baculoviruses were mixed with extracts of MOLT-4 cells, a human cell line containing pRb/p105, p107 and pRb2/p130. The four viral proteins preferentially bound

hypophosphorylated species of the cellular proteins and exhibited highest binding affinity to p107 and lowest affinity to pRb/p105. Importantly, these T′ proteins exhibited unique binding properties and are not simply defective or less active form of T-Ag. For example, T-Ag and T'_{165} bound more pRb/p105 and less p107 than did T'_{135} and T'_{136}; T'_{165} also bound less pRb2/p130 than the other three early proteins. In general, most of the cellular p107 and pRb2/p130 was bound by the T′ antigens, as well as by JCV T-Ag, whereas only a fraction of the hypophosphorylated pRb/p105 present in the cell lysates was bound by them (Bollag *et al*., 2000; Frisque, 2001). In other experiments, extracts isolated from untransformed PHFG cells or JCV-transformed PHFG cells (expressing all five JCV early proteins) were used to examine the interactions between these viral proteins and pRb family members. It has been shown that at least one of the JCV proteins was bound to Hsc70, and that T′ proteins, like SV40 and JCV T-Ags, inhibit the phosphorylation of p107 and pRb2/p130 and promote pRb2/p130 degradation (Frisque, 2001; Frisque *et al*., 2003). On the basis of these and other data, Frisque (2001) proposed that the ability of JCV T′ proteins to interact with pRb family members may contribute to T-Ag's ability to disrupt cell cycle regulation. The ability to stimulate the entry into the S phase could, in turn, affect viral replication or transforming efficiency, depending on the type of the infected cell.

Perspectives

Many studies contributed to the knowledge on pRb-mediated cell cycle control and their role in cell transformation and cancer development. In this context, one interesting aspect is the involvement of polyomaviruses in the dysregulation of pRb functions. Although numerous efforts have been made to understand mechanisms by which polyomaviruses interfere with the members of pRb family, there are still many questions that remain to be answered. The observation that viral oncoproteins have different effects on pRb/p105, p107 and pRb2/p130 supports the idea that each pocket protein may imply a specific role in regulating DNA replication and cell cycle progression. Also, a challenging question concerns the significance of different binding affinities between T′ proteins and pRb proteins. It could allow JCV to target more efficiently specific pRb family members during different phases of the cell cycle or in different cell types. Therefore, the inactivation of the pRb family members by JCV may contribute to the pathogenesis associated with this polyomavirus. This could include astrocytomas, primary cerebral malignant lymphomas, oligoastrocytomas and gliomas. Better understanding of the functional interplay between T-Ags and pRb proteins could represent an important step to the identification of new therapeutic targets against CNS tumors.

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