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Review

SV40 early region oncoproteins and human cell transformation

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Summary. We now understand neoplastic transformation to be the consequence of multiple acquired genetic alterations. The combination of these acquired changes confer the various phenotypes that constitute the clinical features of cancer. Although only rare human cancers derive from a viral etiology, the study of DNA tumor viruses that transform rodent and human cells has led to a greater understanding of the molecular events that program the malignant state. In particular, investigation of the viral oncoproteins specified by the Simian Virus 40 Early Region (SV40 ER) has revealed critical host cell pathways, whose perturbation play an essential role in the experimental transformation of mammalian cells. Recent work has re-investigated the roles of two SV40 ER oncoproteins, the large T antigen (LT) and the small t antigen (ST), in human cell transformation. Co-expression of these two oncoproteins, together with the telomerase catalytic subunit, *hTERT*, and an oncogenic version of the *H-Ras* oncoprotein, suffices to transform human cells. LT inactivates two key tumor suppressor pathways by binding to the retinoblastoma protein (pRB) and p53. The ability of ST to transform human cells requires interactions with PP2A, an abundant family of serine-threonine phosphatases. Here we review recent developments in our understanding of how these two viral oncoproteins facilitate human cell transformation.

Key words: SV40 Early Region, small t antigen, large T antigen, human cell transformation, tumor suppressor genes

Introduction

Although viruses are the etiologic agents responsible for only a small portion of human cancers, the study of how DNA tumor viruses transform mammalian cells in experimental models of cellular transformation has revealed a number of critical intracellular signaling pathways that contribute to the spontaneously arising cancers. Unlike transforming retroviruses that acquire and express mutated versions of cellular genes to accomplish cell transformation, DNA tumor viruses encode specific proteins, which disrupt host cell regulatory circuits and enhance the host cell environment for viral replication. The perturbation of these host cell pathways, which often control cell proliferation and survival, sometimes also leads to cell transformation.

The polyomavirus simian virus 40 (SV40) was discovered in secondary rhesus monkey kidney cultures used for the production of poliovirus vaccines and was subsequently shown to induce tumors in newborn hamsters (Girardi et al., 1962). Studies from several laboratories revealed that the expression of SV40 Early Region (ER) proteins was required for cell transformation (Martin et al., 1979; Chang et al., 1985; Nachtigal et al., 1990). The SV40 ER encodes three distinct proteins by alternative splicing, the large T antigen (LT), the small t antigen (ST), and the 17 kT antigen (Fig. 1). These three proteins share the aminoterminal 82 amino acids (aa) but each carries unique carboxyterminal domains (reviewed in Saenz-Robles et al., 2001). Two of these SV40 early proteins, LT and ST, have been intensively studied not only because they play important roles in viral transcription and replication, but also because they participate in cell immortalization and transformation (reviewed in Fanning and Knippers, 1992). The 17 kT protein consists of the 131 amino terminal residues of LT with four additional aa derived from alternative splicing of ER. Although one report suggested that transformation of 17 kT induces changes in rat cell morphology (Zerrahn et al., 1993), the precise function of 17 kT protein in cell transformation remains unclear.

Here we focus on the SV40 LT and ST oncoproteins since the study of how these oncoproteins interact with specific host cell proteins provides clues to the mechanisms that lead to cell transformation. Indeed, identification of the cellular targets of these viral

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oncoproteins has played a key role in identifying tumor suppressor pathways whose loss participates in most, if not all, human cancers.

SV40 LT

LT binds to and inactivates the retinoblastoma protein (pRB) and p53

Upon viral infection, SV40 ER proteins interact with several host cell proteins involved in DNA transcription and cellular proliferation. In particular, the 708 aa LT oncoprotein plays a critical role in establishing a permissive environment for viral replication, and specific functional domains of LT have been identified which mediate this process (reviewed in Syed and DeCaprio, 2001). In addition, several lines of investigation implicate at least three distinct domains in LT that contribute to the process of cellular transformation (Zhu et al., 1992; Conzen and Cole, 1995; Beachy et al., 2002). These domains permit specific LT-host cell protein interactions and consist of (1) A bipartite region within the carboxy-terminal portion of LT (residues 351-450 and 533-626) that governs interactions with p53 (Kierstead and Tevethia, 1984; Srinivasan et al., 1989a); (2) A region containing the LxCxE motif (residues 103-107) that permits binding of pRB protein family (Srinivasan et al., 1989b); and (3) The J domain (residues 1-82) which is homologous to the DnaJ family of molecular chaperones and binds to hsc70 (Butel et al., 1997) (Fig. 1).

The tumor suppressor protein p53 was one of the first cellular proteins to be identified as a binding partner of LT (Carroll and Gurney, 1982). p53 monitors genomic stability and activates the cellular response to many agents that induce DNA damage by both halting cell cycle progression and inducing apoptosis (Finlay et al., 1989). p53 mediates this repression, at least in part, by acting as a transcription factor (Pipas and Levine, 2001). The p53 protein is itself regulated by the HDM2 protein (MDM2 in the mouse), which controls p53 stability. HDM2 is regulated by the $p14^{ARF}$ ($p19^{ARF}$ in the mouse) protein (Fig. 2). Inactivating mutations or loss of expression of members of this protein network are often associated with human cancers (reviewed in Sherr, 2001). For example, deletion of either the p53 (Donehower et al., 1992) or $p14^{ARF}$ (Kamijo et al., 1997) genes from the murine genome results in animals with a high predisposition for the cancer, confirming the essential role of this pathway in the pathogenesis of cancer. By binding to p53, LT abrogates p53-mediated transcriptional activity (Jiang et al., 1993). This interaction inactivates p53 and permits uncontrolled cell proliferation (Bargonetti et al., 1992). Using a series of LT deletion mutants, several laboratories have demonstrated that LT binds p53 through two distinct domains (bipartite regions described above), both of which are required to bind to, stabilize, and inactivate p53 (Kierstead and Tevethia, 1993).

A second tumor suppressor protein targeted by LT is pRB. pRB plays a critical role in regulating passage through the cell cycle at the G1 restriction point by binding specific members of the E2F transcription factor

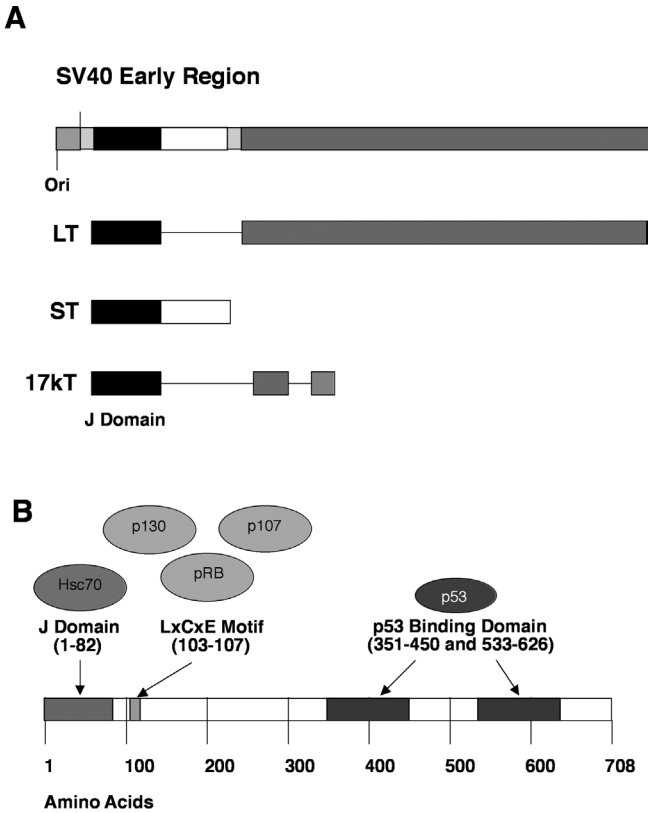

Fig. 1. A. The SV40 Early Region encodes three proteins, LT, ST and 17kT as a result of alternative splicing of viral transcripts. Origin of replication is indicated as Ori. **B.** Three domains of the SV40 LT required for cell transformation. LT: large T antigen; ST: small T antigen.

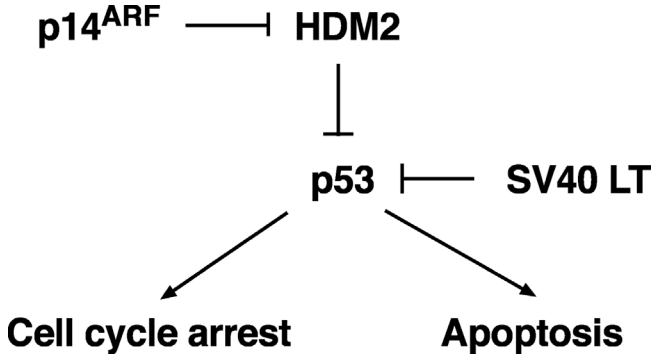

Fig. 2. The p53 tumor suppressor pathway. p53 plays a critical role in regulating cell cycle progression and apoptosis. HDM2 (the human homologue of MDM2) binds directly to p53 and antagonizes p53 function. The *CDKN2A* protein, $p14^{ARF}$ activates p53 by binding to and inhibiting HDM2. Similarly, SV40 LT binds to and inhibits p53.

family. The binding of pRB to E2F serves to repress E2F transcriptional activity (reviewed in Dyson, 1998). Similar to p53, pRB is regulated by a network of proteins (Fig. 3). Sequential hyperphosphorylation of pRB by D-type cyclin-cyclin dependent kinase 4 (CDK4)/CDK6 and E-type/CDK2 complexes releases E2F from pRB-mediated repression and leads to the activation of several genes, many of which serve to stimulate cell proliferation (Eckner et al., 1996). Notably LT binds to hypophosphorylated forms of pRB through the LxCxE motif (DeCaprio et al., 1988; Ludlow et al., 1989) and disrupts pRB-mediated repression of E2F family members, promoting progression into the S phase of the cell cycle (Zalvide and DeCaprio, 1995).

LT also binds to two additional members of pRB family, the p107 and p130 proteins, through the LxCxE motif (Zalvide and DeCaprio, 1995). In murine cells expressing wild type LT, p107 and p130 are hypophosphorylated (Stubdal et al., 1996) while in cells expressing a LT protein carrying an altered LxCxE domain, p107 and p130 are heavily phosphorylated, suggesting that the interaction of LT with these proteins affects their phosphorylation state and function. Although the precise roles of p107 and p130 in cell transformation may depend on the particular cell type studied, the binding of LT to each of these family members may play a role in the transformation of mammalian cells (reviewed in Syed and DeCaprio, 2001).

The aminoterminal 82 aa domain (J domain) shared by SV40 LT, ST and 17kT is homologous with the DnaJ family of molecular chaperones (Butel et al., 1997). J domains consist of four α-helices arranged in a manner that allows the antiparallel second and third helices to form a finger-like projection (Pellecchia et al., 1996; Qian et al., 1996). A short sequence consisting of histidine-proline-aspartic acid (HPD) is located in the loop connecting the second and third helices. This motif permits LT to form a complex with heat shock 70 (hsc70) protein (Tsai and Douglas, 1996; Sullivan et al., 2000). Both LT and ST exhibit J domain-dependent binding to hsc70 and stimulate hsc70 ATPase activity. This hydrolysis of ATP alters the folding of the associated p107 and p130 proteins, suggesting a mechanism by which the J domain affects the function of these proteins (Marsilio et al., 1991; Srinivasan et al., 1997).

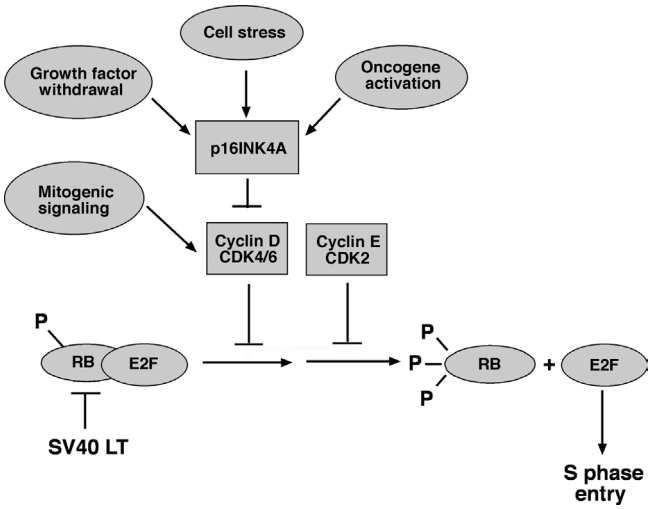

Fig. 3. The pRB tumor suppressor pathway. D-type cyclins and the cyclin-dependent kinases 4/6 (CDK4/6) form an active complex in response to mitogenic stimulation. This complex activates hypophosphorylated RB by mediating hyperphosphorylation of pRB. This phosphorylation leads to the release of the E2F family of transcriptional regulators, permitting E2F-dependent transcription which drives cells into S phase. The *CDKN2A* protein $p16^{INK4A}$ stabilizes RB-E2F by binding to CDK4/6 and disrupting cyclin D-CDK4/6 complexes. $p16^{INK4A}$ is induced by growth factor withdrawal, oncogene activation, and cell stress. SV40 LT binds to pRB and disrupts its function.

The role of LT in cell immortalization and transformation

The expression of the LT oncoprotein suffices to permit transformation of many cell types and cooperates with other oncogenes in most models of experimental transformation (Zhu et al., 1992; Syed and DeCaprio, 2001). However, both the particular species and type of cells used in such experimental systems play a critical role in determining whether LT is sufficient to accomplish immortalization and transformation. Most normal mammalian cells exhibit a limited lifespan in culture (Hayflick and Moorhead, 1961). In contrast, unlimited replicative potential or immortalization is a prequisite step to achieve transformation (Newbold and Overell, 1983). Dissecting the roles of LT in permitting immortalization in specific cell types will inform our understanding of cell transformation by LT.

Although many regulatory pathways that control cell lifespan are shared between human and rodent cells, recent evidence indicates that there are several notable differences in the requirements for cell immortalization. In murine cells, loss of the p53 or ARF tumor suppressor proteins suffices to immortalize most types of cells (reviewed in Levine, 1997; Sherr, 1998). Consistent with these observations, expression of LT in primary MEFs is sufficient to achieve immortalization (Zhu et al., 1991). In contrast, primary human cells must bypass two proliferative barriers, replicative senescence and crisis, in order to achieve immortalization (Shay et al., 1991). Expression of LT permits human cells to avoid replicative senescence; such LT-expressing cells then proliferate until they reach crisis (Ryan et al., 1992). Similarly, ablation of the pRB and p53 pathways with the human papillomavirus (HPV) E6 and E7 oncoproteins or by other methods allows human cells to bypass replicative senescence (Rhim et al., 1994; Sashiyama et al., 2001; Hahn et al., 2002). In contrast to murine cells, LT mutants that bind to only pRB or p53 fail to immortalize human cells (Hahn et al., 2002). Similarly, expression of a dominantly acting, inactive p53 mutant extends cell lifespan (Bond et al., 1996) but

also fails to immortalize human fibroblasts (reviewed in Hahn and Weinberg, 2002). These observations indicate that the requirements for human cell immortalization are more complex than those required in murine cells since even the simultaneous disruption of the p53 and pRB pathways by LT fails to immortalize human cells.

Since immortalization is a prerequisite for cell transformation (Newbold and Overell, 1983), these observed differences between human and rodent cells with regard to LT-mediated immortalization also extend to transformation by LT. Using retroviral vectors, two groups have demonstrated that the introduction of LT into rodent cells is sufficient for cell transformation in primary and established rodent cells (Kriegler et al., 1984) and NIH 3T3 murine fibroblasts (Brown et al., 1986). Moreover in primary rodent cells, continuous expression of LT is required for transformation (Conzen and Cole, 1995).

Several groups have identified three portions of LT (the LxCxE motif, the bipartite p53 binding domain, and the J domain) that are required for murine cell transformation (Zhu et al., 1992; Conzen and Cole, 1995). Expression of LT truncation mutants consisting only of the aminoterminal end of LT are sufficient to transform established secondary rat embryo fibroblasts and established 10T1/2 cells (Sompayrac and Danna, 1991; Zhu et al., 1992), since the process of establishment encompassed the immortalization process. These observations suggest that immortalization by other means substitutes for the binding of LT to p53 in murine cell transformation. Similarly, mutation of the LxCxE motif renders LT is defective in transforming mouse C3H 10T1/2 cells and REF-52 cells (Zhu et al., 1992). Thus the binding of LT to pRB and p53 are both required for murine cell transformation.

Previous studies showed that the J domain of LT plays an important role in the transformation of MEFs (Montano et al., 1990; reviewed in Peden and Pipas, 1992; Syed and DeCaprio, 2001). Since the J domain is dispensable for these phenotypes in MEFs doubly deficient for the pRB-related proteins p107 and p130, the J domain appears to alter the ability of LT to inactivate these pRB-related proteins (Stubdal et al., 1997). Thus, the J domain activity cooperates with the LxCxE motif of T antigen to disable pRB function, although mecahnistic basis for these observations remains undetermined (Srinivasan et al., 1997). Moreover, the J domain of ST is functional since expression of ST can complement LT J domain mutant form to transform MEFs (Montano et al., 1990).

Consistent with these studies in cultured rodent cells, expression of LT and LT mutants as transgenes in mice leads to murine strains with an increased susceptibility to cancer. For example, transgenic mice expressing the dl1137 LT mutant protein, which contains the pRB-binding domain and the J domain but lacks the p53 binding region, develop brain tumors slowly while mice expressing wild type LT develop brain tumors rapidly (Saenz-Robles et al, 1994). When mice expressing the dl1137 mutant were bred with mice lacking p53, the resulting doubly transgenic mice exhibited a phenotype similar to that observed in mice expressing transgenic full length LT (Saenz-Robles et al., 1994). These studies indicate that each of the regions identified in murine cell transformation studies also cooperate to transform murine cells *in vivo*. Furthermore, transgenic mice expressing SV40 LT in particular target tissues have provided model systems with which to study specific types of cancer. For example, expression of LT under the control of the 5' flanking region of the insulin gene leads to expression of LT in the beta-cells of the endocrine pancreas and the formation of beta cell tumors (Hanahan, 1985). Moreover expression of LT under the control of an albumin enhancer-promoter results in the development of hepatocellular carcinomas, as well as benign and malignant biliary neoplasms (Cullen et al., 1993). At present, the tissue specific expression of LT has been used to create several other animal models of cancer. These studies confirm that the expression of LT alone is able to transform a wide variety of rodent cells.

In contrast, LT expression fails to transform primary human cells. Recent work has elucidated several reasons for this discrepancy in LT-induced transformation ability between human and rodent cells. As mentioned above, the inability of LT to transform human cells is, in part, due to the additional steps required to immortalize human cells. Expression of LT permits cells to bypass senescence but the majority of LT-expressing cells die by apoptosis at crisis (Macera-Bloch et al., 2002). Recently, several groups have demonstrated that expression of the catalytic subunit of telomerase, *hTERT*, in such pre-crisis cells permits cells to avoid crisis by elongating telomeres (Counter et al., 1998; Halvorsen et al., 1999; Zhu et al., 1999). One reason that this requirement for telomerase expression is not observed in murine cells is that telomere biology differs considerably between human and murine cells (Wright and Shay, 2000). Specifically, murine telomeres are maintained at lengths 5-15 times longer than those observed in human cells (Kipling and Cooke, 1990; Zijlmans et al., 1997), and murine cells constitutively express telomerase in all somatic tissues (Prowse and Greider, 1995; Greenberg et al., 1998). While the co-introduction of LT and *hTERT* suffices to immortalize human cells, this combination fails to transform most human cells (Hahn et al., 1999). Thus, although LT facilitates the transformation of human cells, further alterations are necessary for full malignant transformation.

SV40 ST

Role of ST in transformation

SV40 ST is formed from alternative splicing from the SV40 ER (Sompayrac and Danna, 1982) (Fig. 1). The ST transcript encodes for 174 aa, of which the 82 aminoterminal residues are shared with LT, while the remaining 92 carboxyl-terminal aa residues are unique

Rubin et al., 1982). These ST-specific aa are rich in cysteines, specifically forming two CxCxxC clusters motifs that bind zinc ions, necessary for ST conformational stability (Friedman et al., 1978; Goswami et al, 1992; Turk et al., 1993).

Animal and *in vitro* cell-based experiments support the concept that ST stimulates cell proliferation and facilitates the transformation of human and rodent cells by LT (Shenk et al., 1976; Crawford et al., 1978). In murine cells, the expression of ST facilitates transformation by LT under conditions when LT is expressed at low levels or under particular growth conditions such as low serum and density-dependent arrest (Sleigh et al., 1978; Sugano et al., 1982; Bikel et al., 1987). Furthermore, transgenic animals created with a mutant version of SV40, which is unable to express functional ST, develop tumors in rapidly dividing tissues, but the spectrum of tumors in these animals is markedly decreased compared to transgenic mice expressing a wild type SV40 (Choi et al., 1988). Subcutaneous injection of wild type SV40 into Syrian hamsters induces fibrosarcomas, while expression of SV40 mutants carrying ST deletions leads to the induction of lymphomas with prolonged latencies (Matthews et al., 1987). Taken together, these observations provide evidence that ST perturbs cellular targets that participate in tumor development in murine cells.

A similar result is seen in human cells, where infection of established cell lines with wild type SV40 resulted in cell transformation, while infection of these same types of cells with SV40 mutants defective in their ability to produce ST were unable to induce focus formation (de Ronde et al., 1989). Recent work indicates that many types of primary human cells are efficiently transformed by the introduction of LT, ST, hTERT, and H-Ras (Hahn et al., 1999). Further experiments demonstrated that the contribution of LT in this system of cell transformation was due only to its ability to inactivate the pRB and p53 tumor suppressor pathways (Hahn et al., 2002). Although cells expressing LT, *hTERT*, and *H-Ras* were morphologically transformed, such cells did not grow in an anchorage-independent manner or form tumors in animals without the additional introduction of ST. Furthermore, expression of a ST mutant lacking a functional J domain in combination with cyclin D1, a CDK4 mutant resistant to regulation by p16INK4A, a dominant negative version of p53, the human telomerase catalytic subunit hTERT, and an oncogenic allele of the *H-ras* oncogene is able to transform primary fibroblasts to a tumorigenic state (Hahn et al., 2002). These observations indicate that ST is required for transformation independently of LT and that an intact LT J domain is not required for human cell transformation. In cells derived from a patient with an inherited deletion in the tumor suppressor locus CDKN2A, the introduction of *hTERT* and *H-Ras* permitted soft agar growth but such cells did not form tumors in animals, again suggesting a functional role for ST in transformation (Brookes et al., 2002). In mesothelial cells, the introduction of LT, ST, and hTERT sufficed to transform such cells (Yu et al., 2001); it remains unclear whether certain cell types will require fewer genetic alterations for full transformation.

Interaction of ST with PP2A

Several laboratories have demonstrated that the major cellular binding protein for ST is the serine-threonine phosphatase PP2A (Yang et al., 1991; Sontag et al., 1993; Mateer et al., 1998). PP2A is an abundantly expressed phosphatase that targets serine and threonine residues (Cohen et al., 1989). PP2A is a heterotrimer composed of a conserved catalytic subunit C, a structural A subunit, and one of several B subunits. The A and C subunit each exist as two isoforms, whereas the 16 B subunits fall into four families (Cohen, 1989). PP2A A subunits are composed of 15 non-identical tandem repeats of a 39 amino acids sequence, termed a HEAT (named after proteins that contain them: huntingtin, elongation factor, A subunit , TOR kinase) motif. The B subunits as well as ST bind to repeats 1-10, and the C subunits binds to repeats 11-15 of the A subunit (Kremmer et al., 1997). PP2A regulates a large number of cellular events, including DNA replication, cell cycle regulation, cell morphology and development (reviewed in Janssens and Goris, 2001). The diversity of PP2A heterotrimers suggests that the various regulatory subunits may mediate specific physiological functions in vivo, however, the precise roles of PP2A or specific PP2A heterotrimers in cellular growth control has not yet been thoroughly established.

ST forms stable complexes with PP2A and this interaction leads to partial inhibition of PP2A *in vitro* (Pallas et al., 1990). ST associates with both free A subunits and the AC core enzyme of PP2A (Yang et al., 1991). In vitro, some investigators reported that ST associates with PP2A by displacing the Bα subunit, resulting in inhibition of phosphatase activity (Pallas et al., 1990; Sontag et al., 1993). However the particular binding sites on the PP2A A subunit used by each of the B subunits and ST are incompletely understood, and thus it is unclear whether the interaction of ST with PP2A displaces some or all of the B subunits.

In any case, the interaction of ST with PP2A is essential for its ability to transform mammalian cells (Rundell and Parakati, 2001). ST mutants with aa substitutions at residues 97, 101, and 103 not only ablate the ability of ST to bind PP2A but also eliminate the ability of such ST mutants to cooperate to transform of growth-arrested rat F111 cells (Mungre et al., 1994). Recent work using similar ST mutants confirms that the interaction with PP2A is necessary for ST to cooperate to transform human cells (Hahn et al., 2002). Thus, PP2A appears to be the relevant ST cellular target necessary for cell transformation.

These observations raise the question of whether alterations of PP2A function or expression also occur

during the pathogenesis of spontaneously arising human tumors. Recent studies reveal that PP2A A subunit mutations are found in a subset of human cancers. Aß subunit mutations were found in 15% of primary lung tumors, 6% of lung tumor derived cell lines and 15% of primary colon tumors (Wang et al., 1998). In addition, both Aα and Aß subunit isoforms of PP2A were found to be genetically altered in a variety of primary human cancers (Calin et al., 2000). Four cancer associated mutants of Aa subunit have also been described: Glu64→Asp in lung carcinoma, Glu64→Gly in breast carcinoma, Arg418→Trp in melanoma, and Δ171-589 in breast carcinoma (Ruediger et al., 2001a). Expression of these PP2A mutants in vitro demonstrated that all of these PP2A A subunit mutants are defective in binding either PP2A B or B and C subunits (Ruediger et al., 2001a,b). Taken together, these observations suggest that regulation of PP2A activity by loss or mutation of particular A and B subunits plays an important role in tumorigenesis; however, further study is required to determine whether such mutations in PP2A affect a common pathway altered in a majority of human cancers.

Potential targets of ST-PP2A interaction

Since ST complexes with PP2A and inhibits PP2A activity *in vitro*, one can infer that the inhibition of PP2A phosphatase activity by ST plays an important role in cell transformation. Unfortunately, PP2A is an ubiquitiously expressed enzymatic complex consisting of multiple combinatorial associations of A, B, and C subunit isoforms. At present, a large number of potential substrates for PP2A have been reported, many of which suggest a role in cell proliferation or apoptosis (reviewed in Lechward et al., 2001). ST exerts its effects by inhibiting PP2A, thereby preventing dephosphorylation of multiple proteins including protein kinases, transcription factors and cell cycle regulators (Table 1). For example, ST promotes cell cycle progression by activating kinases such as members of the MAPK family (Sontag et al., 1993). In some cell types, ST cooperates with ERK to stimulate AP-1 activity (Frost et al., 1994). In addition ST is responsible for the reduction of the expression of p27KIP1, which cooperates with LT and confers cells with ability to escape the growth arrest checkpoint and drives quiescent cells into S phase (Porras et al., 1999). Although the interaction of ST with PP2A may serve to alter the regulation of diverse protein families, only some of those interactions are responsible for the transforming function of ST (summarized in Table 1).

Conclusion and future directions

The observation that SV40 transforms mammalian

Table 1. Potential targets of the interaction of ST with PP2A.

TARGET	CELL TYPE	SPECIES	REFERENCE
1. Transcriptional activators			
AP-1	CV-1P	Monkey	Frost et al., 1994
CREB	HepG2/rat liver cells	Human/rat	Wheat et al., 1994
NF-kappaB	CV-1/NIH 3T3 cells	Monkey/mouse	Sontag et al., 1997
Sp1	CV-1	Monkey	Garcia et al., 2000
2. Protein kinases			
MEK1, ERK1	CV-1	Monkey	Sontag et al., 1993
PKCξ	HepG2	Human	Sontag et al., 1997
Shc	Rat fibroblasts	Rat	Ugi et al., 2002
JNK/SAPK	JEG-3 and CV-1	Human/Monkey	Watanabe et al., 1996
PEPCK	H4IIE	Rat	Wheat et al., 1994
3. Cell cycle regulator			
p27KIP1	Human fibroblasts	Human	Porras et al., 1999
4. Transcriptional targets			
Cyclin D1 promoter	JEG-3	Human	Watanabe et al., 1996
Cyclin A promoter	CV-1	Monkey	Porras et al., 1996
Adenovirus E2A promoter	CV-1	Monkey	Loeken, 1992
Adenovirus VA-I promoter	CV-1	Monkey	Loeken, 1992
HPV type16 enhancer-promoter	Human fibroblasts	Human	Smits et al., 1992
c-fos promotor	CV-1P	Monkey	Wang et al., 1994
5. Others			
Na^+/H^+ antiporter	CV-1	Monkey	Howe et al., 1998

Protein phosphatase 2A (PP2A) is the major serine-threonine specific phosphatase in eukaryotic cells. PP2A directly regulates the activity of multiple protein kinase cascades by dephosphorylation. This table only lists the potential targets of PP2A which affected by ST-PP2A interaction. AP-1: activator protein-1; CREB: cAMP response element-binding protein; NF-KappaB: nuclear factor-KappaB; Sp1: stimulating protein 1; MEK1: MAPK (mitogen-activated protein kinase)-/ERK(extracellular signal-regulated kinase)-kinase; PKCξ: protein kinase Cx; Shc: SH2-containing adaptor protein; JNK/SAPK: jun N-terminal kinase/stress-activated kinase; PEPCK: phosphoenolpyruvate carboxykinase; HPV: Human Papillomavirus.

cells has facilitated the study of critical pathways involved in cell transformation and continues to be an important tool to understand malignant transformation. Two Early Region proteins, LT and ST, play critical roles in perturbing cellular pathways essential for malignant cell proliferation. Although important differences exist between human and murine models of transformation by these SV40 ER proteins, understanding these differences will permit us to understand the unique cancer biology of these model systems.

Several reports have documented the presence of SV40 sequences in a subset of brain tumors and mesotheliomas (reviewed in Klein et al., 2002). Although epidemiological studies failed to observe an increase in the incidence of cancer in birth cohorts exposed to the SV40 contaminated vaccines (Mortimer et al., 1981; reviewed in Carbone et al., 1997), the presence of these sequences raises the possibility that SV40 may play an etiologic role in a subset of human cancers (reviewed in Jasani et al., 2001; Lednicky and Butel, 2001). Since it is clear that the expression of LT and ST are required for SV40-mediated transformation in cell and animal models, future studies will need to investigate whether these viral oncoproteins are expressed in human tumor samples. Whether or not SV40 plays a role in some human cancers, the understanding of the molecular mechanisms by which SV40 transforms human cells has been and will continue to be served as a critical tool to understanding how cancer develops.

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