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Cell polarity proteins: common targets for tumorigenic human viruses

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Abstract

Loss of polarity and disruption of cell junctions are common features of epithelial-derived cancer cells, and mounting evidence indicates that such defects have a direct function in the pathology of cancer. Supporting this idea, results with several different human tumor viruses indicate that their oncogenic potential depends in part on a common ability to inactivate key cell polarity proteins. For example, adenovirus (Ad) type 9 is unique among human Ads by causing exclusively estrogen-dependent mammary tumors in experimental animals and in having *E4 region*-encoded open reading frame 1 (E4-ORF1) as its primary oncogenic determinant. The 125-residue E4-ORF1 protein consists of two separate protein-interaction elements, one of which defines a PDZ domain-binding motif (PBM) required for E4-ORF1 to induce both cellular transformation *in vitro* and tumorigenesis *in vivo*. Most notably, the E4-ORF1 PBM mediates interactions with a selected group of cellular PDZ proteins, three of which include the cell polarity proteins Dlg1, PATJ and ZO-2. Data further indicate that these interactions promote disruption of cell junctions and a loss of cell polarity. In addition, one or more of the E4-ORF1-interacting cell polarity proteins, as well as the cell polarity protein Scribble, are common targets for the high-risk human papillomavirus (HPV) E6 or human T-cell leukemia virus type 1 (HTLV-1) Tax oncoproteins. Underscoring the significance of these observations, in humans, high-risk HPV and HTLV-1 are causative agents for cervical cancer and adult T-cell leukemia, respectively. Consequently, human tumor viruses should serve as powerful tools for deciphering mechanisms whereby disruption of cell junctions and loss of cell polarity contribute to the development of many human cancers. This review article discusses evidence supporting this hypothesis, with an emphasis on the human Ad E4-ORF1 oncoprotein.

Keywords

virus; polarity; tumor suppressor; PDZ; migration

Introduction

Cancer is a leading cause of death in developed countries, and viruses are associated with an estimated 15–20% of human malignancies worldwide (Flint *et al.*, 2000). Research directed at determining how viruses promote tumors in experimental animals has also contributed greatly to our understanding of molecular events associated with human cancer (Flint *et al.*, 2000). Illustrating this fundamental principle, studies of RNA tumor viruses led to the seminal concept of the oncogene, whereas studies of DNA tumor viruses led not only to the

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identification of the p53 tumor suppressor protein, but also proved instrumental in deciphering functions for the pRb tumor suppressor protein. Thus, tumor viruses are proven powerful tools for revealing mechanisms responsible for the development of all human cancers. This article reviews accumulating evidence suggesting that blocking functions of key cell polarity proteins represents an important new theme among tumorigenic human viruses.

Human adenovirus type 9

The *E1 region* codes for the oncogenic determinants of most human adenoviruses

Adenovirus (Ad) is a nonenveloped virus with an approximately 36-kb linear double-stranded DNA genome. The 51 different serotypes of human Ads are organized into six subgroups (A through F) on the basis of physical and immunological criteria and cause acute infections of the respiratory and gastrointestinal tracts, as well as the eye (Green *et al.*, 1979; Horwitz, 2001; Shenk, 2001). Although lacking a recognized association with human cancers, all human Ads can transform cultured rodent cells (Shenk, 2001), and a subset of the viruses, including all subgroup A and B Ads and two subgroup D Ads, also induces tumors in experimentally infected rodents (Graham, 1984). As opposed to productive lytic replication in human cells, Ad instead causes an abortive, nonpermissive infection in rodent cells (Graham, 1984). Thus, in Ad-induced rodent tumors or transformed cells, all or part of the Ad genome is maintained in the host cell by rare non-homologous recombination events occurring at random chromosomal integration sites (Graham, 1984). Expression of the viral *E1 region*, coding for the *E1A* and *E1B* oncogenes, is both necessary and sufficient for transformation and tumorigenesis induced by subgroup A and B Ads (Graham, 1984; Stillman, 1986; Shenk and Flint, 1991). *E1A* alone immortalizes cells (Houweling *et al.*, 1980), whereas *E1B* alone lacks transforming potential (Van den Elsen *et al.*, 1983). Together, however, *E1A* and *E1B* cooperate to oncogenically transform primary rodent cells or established rodent lines (Graham, 1984). The tumorigenic potential of E1A and E1B stems in part from their ability to bind and inactivate the pRb and p53 tumor suppressor proteins, respectively (Levine, 1990; Dyson *et al.*, 1992).

Unique tumorigenic properties of human adenovirus type 9

Adenovirus type 9 (Ad9) is a member of the subgroup D Ads (Green *et al.*, 1979), some of which cause epidemic outbreaks of keratoconjunctivitis, a painful and highly contagious eye infection that may lead to corneal opacities (Horwitz, 2001). Following infection of newborn rats, however, Ad9 is tumorigenic (Ankerst *et al.*, 1974; Javier *et al.*, 1991). Unlike subgroup A and B Ads that induce undifferentiated sarcomas in both male and female animals (Trentin *et al.*, 1962), Ad9 instead elicits exclusively estrogen-dependent mammary tumors in female rats (Ankerst *et al.*, 1974; Ankerst and Jonsson, 1989; Javier *et al.*, 1991). These females develop multiple tumors in several different mammary glands by 3-months post-infection, whereas males fail to develop tumors of any kind (Javier *et al.*, 1991). Similar to other Ad-induced tumors, the Ad9 genome is found integrated into the chromosomal DNA of mammary tumor cells and has a copy number varying from one to multiple genomes per cell (Javier *et al.*, 1991). As each mammary tumor exhibits a unique viral DNA integration site, the neoplasms are monoclonal (Javier *et al.*, 1991). Therefore, Ad9 is distinct from other tumorigenic Ads in generating only estrogen-dependent mammary tumors in animals.

Ad9-induced mammary tumors

Several histologically distinct types of mammary tumors arise in Ad9-infected animals, with fibroadenoma occurring most frequently (Javier *et al.*, 1991). Fibroadenoma, the most prevalent benign breast tumor of young women (Cotran *et al.*, 1994), has a sparse cellular stroma embedded in a dense extracellular matrix and contains varying amounts of glandular

mammary epithelium. Phyllodes tumor and solid sarcoma are two less common types of mammary tumor induced by Ad9. Phyllodes tumor histologically resembles fibroadenoma, yet the stroma shows higher cellularity or is malignant, whereas solid sarcoma is a malignant mammary tumor composed of highly atypical stromal cells with significant mitotic activity but devoid of glandular epithelial components. Phyllodes tumor and solid sarcoma of the breast are also occasionally observed in women (Javier and Shenk, 1996).

By *in situ* hybridization, Ad9 mRNAs are detected in fibroblasts of mammary fibroadenomas or in myoepithelial cells of mammary phyllodes tumors and solid sarcoma (Javier *et al.*, 1991), indicating that the latter two tumors do not arise from fibroadenomas by malignant conversion. Studies of human breast fibroadenoma and phyllodes tumor are consistent with this observation (Mechtersheimer *et al.*, 1990). Given that Ad9-induced mammary solid sarcoma histologically resembles malignant phyllodes tumor without the epithelial component, it has been proposed that the solid sarcomas likely derive from malignant phyllodes tumors through loss of the glandular epithelial component (Javier *et al.*, 1991). In addition, the development of Ad9-induced mammary tumors strictly depends on estrogen (Javier *et al.*, 1991). The fact that Ad9-induced mammary tumors express estrogen receptor mRNA (Javier *et al.*, 1991) suggests a direct function for estrogen in stimulating proliferation of tumor cells. It is possible that estrogen receptor, which activates transcription in the nucleus and cell signaling from the plasma membrane (Levin, 2002; Luconi *et al.*, 2002; McDonnell and Norris, 2002), cooperates with Ad9 gene functions to oncogenically transform mammary cells *in vivo*.

The Ad9 E4-ORF1 oncoprotein

E4-ORF1 is the primary oncogenic determinant of Ad9

Contrary to Ad9, most subgroup D Ads (for example, Ad26) fail to generate tumors of any kind in rats (Javier *et al.*, 1992). Exploiting this observation, analyses of Ad9-Ad26 hybrid viruses led to the surprising discovery of an Ad tumorigenic determinant genetically mapping to the viral *E4 region* rather than to the viral *E1 region* (Javier *et al.*, 1992). The Ad *E4 region* is a complex transcription unit containing six different open reading frames (ORFs) (Figure 1) (Herisse *et al.*, 1981; Hogenkamp and Esche, 1990; Javier and Shenk, 1996). Proteins encoded by *E4 region* ORFs regulate viral and cellular gene expression, viral DNA replication and host cell shutoff (Halbert *et al.*, 1985; Cutt *et al.*, 1987; Bridge and Ketner, 1989, 1990; Huang and Hearing, 1989a, b; Muller *et al.*, 1992; Stracker *et al.*, 2002) and also inactivate not only p53 but also Mre11, a component of the double-stranded DNA break–repair pathway (Flint and Gonzalez, 2003; Endter and Dobner, 2004; Weitzman, 2005; Weitzman and Ornelles, 2005). Several E4 proteins, including E4 region-encoded ORF1 (E4-ORF1) (Javier, 1994), E4-ORF3 (Nevels *et al.*, 1999b), E4-ORF6 (Moore *et al.*, 1996; Nevels *et al.*, 1997, 1999a, 2000a, 2001) and E4-ORF6/7 (Yamano *et al.*, 1999), also have the capacity to transform cultured cells. The transforming activities of E4-ORF6 and E4-ORF6/7 stem from an ability to inactivate p53 (Dobner *et al.*, 1996) or to stimulate the activity of the *E2F* protooncogene, respectively (Huang and Hearing, 1989b; Schaley *et al.*, 2000).

The major *E4 region* oncogenic determinant of Ad9 was found to be the *E4-ORF1* gene. For instance, disruption of *E4-ORF1*, but not *E1A* or *E1B* (Thomas *et al.*, 1999, 2001a), abolishes the ability of Ad9 to generate mammary tumors in rats, despite the fact that Ad9 *E4-ORF1* mutant viruses display no appreciable replication defects in permissive human cell lines (Javier, 1994). The E4-ORF1 protein is expressed in all Ad9-induced mammary tumors and, among the six isolated Ad9 *E4 region* ORFs, only *E4-ORF1* is capable of inducing transformed foci on cells (Javier, 1994). Such E4-ORF1-transformed cells also form tumors in immunocompetent animals and display morphological changes, anchorage-independent

growth and elevated saturation densities (Weiss *et al.*, 1996). Furthermore, substitution of the *E1 region* in otherwise nontumorigenic subgroup C Ad5 with an Ad9 *E4-ORF1* expression cassette confers a tumorigenic phenotype virtually identical to that of Ad9 (Thomas *et al.*, 2001a), indicating that *E4-ORF1* likewise controls the oncogenic tropism of Ad9 for mammary tissue *in vivo*. Although the sequence of the subgroup D Ad9 E4-ORF1 protein shares 50% identity and 70% similarity with E4-ORF1 proteins encoded by subgroup A–C Ads (Weiss *et al.*, 1997), the Ad9 E4-ORF1 protein is uniquely tumorigenic (Javier, 1994). In transformed cells, the 14-kDa Ad9 E4-ORF1 protein accumulates in cytoplasmic punctae (Weiss *et al.*, 1996) representing membrane vesicles (Chung *et al.*, 2007), and some protein also localizes at the plasma membrane (Frese *et al.*, 2003, 2006).

Evidence supports the idea that Ad *E4-ORF1* genes evolved from a cellular *dUTP pyrophosphatase* (*dUTPase*) gene (Weiss *et al.*, 1997), which codes for an essential enzyme of nucleotide metabolism. Polypeptides encoded by *dUTPase* genes are comparable in length to E4-ORF1 proteins and form homo-trimeric enzymes that hydrolyse dUTP to prevent detrimental incorporation of this nucleotide into replicating cellular DNA (Mol *et al.*, 1996). Although results indicate that E4-ORF1 and dUTPase have functionally diverged (Weiss *et al.*, 1997), the two polypeptides are predicted to share a conserved protein fold, and both form a homo-trimer dependent on a related trimerization element (Chung *et al.*, 2008). E4-ORF1 differs from dUTPase, however, by additionally existing as a monomer in cells (Chung *et al.*, 2008).

Ad9 E4-ORF1 consists of two separate protein-interaction elements: domain 2 and a PDZ domain-binding motif

The isolation of Ad9 E4-ORF1 mutants expressed at wild-type levels yet unable either to transform cultured cells (Lee *et al.*, 1997; Weiss and Javier, 1997; Chung *et al.*, 2007) or to promote mammary tumors in the context of Ad9 virus (Thomas *et al.*, 2001a) proved instrumental for identification of crucialE4-ORF 1 functional elements. The residues altered in transformation- defective E4-ORF1 mutants cluster either within a central region of the linear E4-ORF1 polypeptide (G40A, V41A, D65A, L89Q, F91S, H93A, F97A) or at the extreme carboxyl-terminus (T123D, V125A, IIIA) (Figure 2). The central and carboxyl-terminal mutations disrupt the association of E4-ORF1 with distinct subsets of cellular factors (Weiss and Javier, 1997; Chung *et al.*, 2007), indicating that E4-ORF1 is composed of two separate crucial protein-interaction elements.

Recent evidence has revealed that critical centrally located E4-ORF1 mutations define one functional element, designated domain 2 (D2), which mediates binding to several unidentified cellular phosphoproteins (p70, p150, p190) and which is sufficient to promote association of E4-ORF1 with membrane vesicles (Chung *et al.*, 2007). On the other hand, E4-ORF1 carboxyl-terminal mutations disrupt a separate functional element that was identified as a PSD-95, Dlg, ZO-1 (PDZ) domain-binding motif (PBM) (Lee *et al.*, 1997). This element, which represented the first functional PBM identified in a viral protein, was found to mediate interactions with a selected group of cellular PDZ domain-containing proteins, including Dlg1, Multi-PDZ domain protein 1 (MUPP1), PATJ, MAGI-1 and ZO-2 (Lee *et al.*, 1997, 2000; Glaunsinger *et al.*, 2000, 2001) (Figures 2 and 3). In polarized epithelial cells, Dlg1 localizes to the adherens junction (AJ) (Laprise *et al.*, 2004; Stucke *et al.*, 2007), whereas MUPP1, PATJ, MAGI-1 and ZO-2 localize to the tight junction (TJ) (Jesaitis and Goodenough, 1994; Hamazaki *et al.*, 2002; Patrie *et al.*, 2002; Shin *et al.*, 2005). These PBM-mediated interactions are highly specific because Ad9 E4-ORF1 fails to bind other cellular PDZ proteins (Glaunsinger *et al.*, 2000, 2001; Latorre *et al.*, 2005), including FAP-1, ZO-1, ZO-3, AF-6, hINADL, Par-6 and Scribble (Prasad *et al.*, 1993; Willott *et al.*, 1993; Sato *et al.*, 1995; Philipp and Flockerzi, 1997; Haskins *et al.*, 1998;

Joberty *et al.*, 2000; Nakagawa and Huibregtse, 2000). Similar to D2, the PBM is also sufficient to target E4-ORF1 to cytoplasmic membrane vesicles (Chung *et al.*, 2007).

High-risk human papillomaviruses E6 and human T-cell leukemia virus type 1 Tax oncoproteins also possess a carboxyl-terminal PBM

PDZ domains are approximately 90 amino-acid modular units that mediate protein–protein interactions (Kim, 1995; Kornau *et al.*, 1995; Songyang *et al.*, 1997), similar to Sarcoma (Src) homology region-2 and Src homology region-3 or phosphotyrosine-binding domains. The term PDZ derives from names of the first three proteins recognized to contain these domains (postsynaptic density protein (PSD-95), discs-large tumor suppressor (dlg) and zonula occludens protein 1 (ZO-1)). Suggesting coevolution with multicellularity, PDZ proteins are encoded primarily by metazoans, where these numerous proteins constitute 0.2–0.5% of ORFs (Harris and Lim, 2001). Such proteins typically function as scaffolds to assemble receptors and cytosolic factors into supramolecular signaling complexes and to localize them to specialized membrane regions of cell–cell contact, such as the AJ and TJ (Saras and Heldin, 1996; Sheng and Kim, 1996; Sheng, 1996).

PDZ domains bind to a specific sequence motif, or PBM, typically present at the extreme carboxyl-terminus of target proteins (Songyang *et al.*, 1997). A class I PBM having the consensus sequence -(S/T)-X-(V/I/L)-COOH (X denotes any amino acid) (Saras and Heldin, 1996; Sheng and Kim, 1996; Sheng, 1996) was identified at the carboxyl-terminus of Ad9 E4-ORF1 (Lee *et al.*, 1997). This observation, together with the fact that otherwise unrelated viral oncoproteins frequently share common mechanisms of cellular transformation, prompted an immediate search for additional viral oncoproteins having a carboxyl-terminal PBM. This search led to the discovery of a class 1 PBM at the carboxyl-terminus of E6 oncoproteins encoded by high-risk human papillomaviruses (HPV) and the Tax oncoprotein encoded by human T-cell leukemia virus type 1 (HTLV-1) (Lee *et al.*, 1997). The significance of this observation is illustrated by the fact that, in humans, HTLV-1 and high-risk HPV are the causative agents of adult T-cell leukemia and cervical cancer, respectively.

Functions of the HPV E6 PBM in cellular transformation and tumorigenesis

In accordance with the demonstrated oncogenic function of the Ad9 E4-ORF1 PBM, the E6 PBM was shown to be required for E6-mediated transformation of cultured cells and tumorigenesis in transgenic mice, as well as HPV-induced pathogenesis in organotypic raft cultures of human keratinocytes (Kiyono *et al.*, 1997; Mantovani and Banks, 2001; Watson *et al.*, 2003; Nguyen *et al.*, 2003a; Lee and Laimins, 2004; Simonson *et al.*, 2005; Shai *et al.*, 2007). Supporting an additional function of the E6 PBM in HPV-induced cervical cancer of women, E6 proteins encoded by low-risk HPVs, which are not associated with this disease, lack the carboxyl-terminal PBM (Kiyono *et al.*, 1997; Lee *et al.*, 1997). Also worth mentioning is that HPV E6 synergizes with E7, an additional crucial HPV oncogenic determinant, to trigger cervical carcinogenesis in transgenic mice by a mechanism requiring both the E6 PBM and estrogen (Shai *et al.*, 2007). The dual dependence of both Ad9- and HPV-induced tumors on estrogen and a viral oncoprotein PBM hints that dysregulation of cellular PDZ proteins may commonly contribute to the development of estrogen-dependent malignancies. A detailed discussion of functions for cellular PDZ proteins in HPV-induced cancers can be found in the accompanying review by Thomas *et al.* (2008) in this issue.

Functions of the HTLV-1 Tax PBM in cellular transformation and tumorigenesis

Human T-cell leukemia virus type 1 is the etiological agent of adult T-cell leukemia, a rapidly progressing, clonal malignancy of $CD4^+$ T lymphocytes in humans. An estimated 10–20 million people worldwide are infected with HTLV-1, and approximately 3% of these

individuals will develop adult T-cell leukemia. The primary oncogenic determinant of HTLV-1 is the *Tax* gene, which encodes a nuclear and cytoplasmic phosphoprotein with pleiotropic functions that promote cell survival, cell-cycle progression, multipolar mitosis, aneuploidy and DNA damage. In addition, Tax-induced cellular transformation depends in part on its ability to activate the cyclic AMP pathway, as well as the nuclear factor κB (NFκB) and phosphatidylinositol 3-kinase (PI3K) cell survival pathways (for review, see Matsuoka and Jeang, 2007). Pertinent to this review is that the Tax PBM mediates binding to multiple cellular PDZ proteins (Lee *et al.*, 1997; Rousset *et al.*, 1998; Ohashi *et al.*, 2004; Arpin-Andre and Mesnard, 2007) and that mutational disruption of the Tax PBM substantially decreases Tax-mediated cellular transformation and micronuclei formation, as well as the ability of HTLV-1 to stimulate T-cell proliferation and to cause a persistent viral infection *in vivo* (Suzuki *et al.*, 1999; Endo *et al.*, 2002; Hirata *et al.*, 2004; Tsubata *et al.*, 2005; Ishioka *et al.*, 2006; Kondo *et al.*, 2006; Xie *et al.*, 2006; Higuchi *et al.*, 2007). Results demonstrating that the HTLV-1 Tax PBM cooperates with Tax-induced NFκB activation to transform cells (Higuchi *et al.*, 2007) and that HPV E6 mediates NFkB activation in a PBM-dependent manner (James *et al.*, 2006) further hint at a potentially important interplay between the NFkB and PDZ-protein pathways in triggering cellular transformation.

As with the differences between high-risk and low-risk HPV E6 proteins, a natural example also lends strong support to the idea that the Tax PBM has an important function in promoting adult T-cell leukemia. In this regard, unlike HTLV-1, the closely related HTLV-2 is not associated with any malignant lymphoproliferative diseases in people (Mahieux and Gessain, 2003), despite the fact that the Tax proteins encoded by these two different retroviruses share the ability to activate the cyclic AMP and NFκB pathways (Wang *et al.*, 2000). Strikingly, HTLV-2 Tax differs from HTLV-1 Tax in lacking the carboxyl-terminal PBM (Rousset *et al.*, 1998; Suzuki *et al.*, 1999; Hirata *et al.*, 2004), and this difference explains the more efficient induction of cellular transformation by HTLV-1 Tax compared with HTLV-2 Tax (Endo *et al.*, 2002; Hirata *et al.*, 2004).

Taken together, findings with Ad9 E4-ORF1, high-risk HPV E6 and HTLV-1 Tax indicate that functional perturbation of cellular PDZ proteins is a common theme among tumorigenic human viruses and that this activity has an important function in their oncogenic potential.

The E4-ORF1 monomer and trimer possess distinct functions

In a PBM-dependent and D2-independent manner, E4-ORF1 affects the subcellular localization of its PDZ protein targets in one of two strikingly different ways by inducing either (1) translocation of AJ-associated Dlg1 to the plasma membrane (Frese *et al.*, 2006) or (2) sequestration of TJ-associated MUPP1, PATJ, MAGI-1 and ZO-2 within insoluble complexes associated with cytoplasmic punctae (Glaunsinger *et al.*, 2000, 2001; Lee *et al.*, 2000; Latorre *et al.*, 2005). The former and latter effects were recently shown to be specifically mediated by the E4-ORF1 trimer or monomer, respectively (Chung *et al.*, 2008). In addition to selective binding to Dlg1, the E4-ORF1 trimer also specifically possesses D2 activity (Chung *et al.*, 2007), consistent with the fact that molecular modeling of E4-ORF1 to the crystal structure of human dUTPase predicts that E4-ORF1 trimerization brings six out of seven D2 residues together at each of the three subunit interfaces (Chung *et al.*, 2007). These molecular modeling analyses further suggest that the Ad9 *E4-ORF1* gene evolved from an ancestral *dUTPase* gene by events that transformed the dUTPase catalytic cleft and carboxyl-terminal nucleotide-binding P-loop motif into the E4-ORF1 D2 or PBM element, respectively. The data support the idea that E4-ORF1 trimers bind both Dlg1 and D2-interacting cellular factors and promote their translocation to the plasma membrane, whereas monomers instead bind TJ-associated PDZ protein targets and sequester them within insoluble complexes associated with cytoplasmic membrane vesicles. Oligomerization of

E4-ORF1 is unlikely controlled by a posttranslational modification(s) because, thus far, none has been detected. Evidence rather hints at the possibility that cholesterol present in cellular membranes triggers E4-ORF1 monomers to assemble into trimers (Chung *et al.*, 2008). These collective findings expose a novel strategy wherein the oligomerization state of a protein not only determines the capacity to bind different cellular targets but also couples each type of interaction to a different functional consequence.

The E4-ORF1 cellular targets Dlg1, PATJ and ZO-2 are key polarity proteins

Polarity is a fundamental property of all cells and signifies the asymmetry in function, shape or content of a cell created through differential distribution of its macromolecular constituents. In metazoans, this asymmetry is central to the establishment of apical–basal polarity, planar cell polarity and anterior–posterior polarity, which have important functions in proper functioning of cells and development of organisms (Siegrist and Doe, 2007). The fundamental importance of cell polarity is further underscored by the requirement for proper polarization in a wide range of cellular processes, including morphogenesis, asymmetric division and directed migration (Siegrist and Doe, 2007). Notably, loss of apical–basal cell polarity and disruption of cell–cell junctions, such as the AJ and TJ, are common features of epithelial-derived cancer cells (Cochand-Priollet *et al.*, 1998; Soler *et al.*, 1999). Accumulating evidence indicates that such defects directly contribute to carcinogenesis by dysregulating normal proliferation and differentiation programs in cells (Cochand-Priollet *et al.*, 1998; Soler *et al.*, 1999; Bilder, 2003, 2004; Humbert *et al.*, 2003; Lallemand *et al.*, 2003; Matter and Balda, 2003; Aranda *et al.*, 2006; Curto *et al.*, 2007), suggesting that loss of cell polarity has an important function in the pathology of cancer.

Most relevant to this discussion is that three of the five E4-ORF1-interacting PDZ proteins (PATJ, ZO-2 and Dlg1) represent key components of the cell machinery that controls polarity. For example, in mammalian epithelial cells, proper apical–basal polarity establishment depends on the two evolutionarily conserved polarity complexes, Crumbs-Pals1-PATJ and Par3-Par6-aPKC (Matter and Balda, 2003; Bilder, 2004; Shin *et al.*, 2006; Suzuki and Ohno, 2006; Siegrist and Doe, 2007), as well as the ZO-2 polarity protein (Hernandez *et al.*, 2007). In addition, recent evidence has revealed important functions of evolutionarily conserved Scribble and Dlg1, as well as PATJ, in determination of anterior–posterior polarity establishment in astrocytes, T cells and epithelial cells (Etienne-Manneville *et al.*, 2005; Humbert *et al.*, 2006; Krummel and Macara, 2006; Osmani *et al.*, 2006; Shin *et al.*, 2007). Providing a possible link to cancer, defects in anterior–posterior polarization are reported to increase the production of stem cells, from which cancers may originate (Wodarz and Gonzalez, 2006; Wodarz and Nathke, 2007).

The Dlg1 polarity protein

Dlg1/SAP97, a mammalian homolog of the *Drosophila* discs-large (dlg) protein (Woods and Bryant, 1991), was the first cellular PDZ-protein target identified for Ad9 E4-ORF1, as well as HTLV-1 Tax and high-risk HPV E6 (Lee *et al.*, 1997). Both Dlg1 and dlg are members of the membrane-associated guanylate kinase (MAGUK) family of proteins that contain, in addition to multiple PDZ domains, an Src homology region-3 or WW domain and a guanylate kinase-homology domain (Kim *et al.*, 1995) (Figure 3), all of which function as protein–protein interaction modules (Gonzalez-Mariscal *et al.*, 2000). In *Drosophila* imaginal disc epithelia, dlg localizes to the septate junction, which is analogous to the TJ of mammalian cells (Woods *et al.*, 1996). Homozygous *dlg* mutations in *Drosophila* cause embryonic lethality due to disruption of cell junctions, cell shape and apical–basal cell polarity, as well as neoplastic overgrowth of imaginal disc epithelia and hyperplastic growth within larval brains (Woods and Bryant, 1989). Furthermore, ectopic expression of mammalian Dlg1 reverses these *dlg* mutant phenotypes, including neoplastic cell

overgrowth (Thomas *et al.*, 1997). In addition to showing that *dlg* is a tumor suppressor, these findings are significant by linking the loss of cell polarity directly to neoplastic cellular transformation.

It has been postulated that mammalian Dlg1 likewise is a tumor suppressor and that the tumorigenic potential of Ad9 E4-ORF1, HTLV-1 Tax and high-risk HPV E6 depends in part on an ability to inactivate this cellular factor. In support of this idea, HPV E6 targets Dlg1 for proteosome-mediated degradation in cells (Gardiol *et al.*, 1999, 2002; Kuhne *et al.*, 2000; Pim *et al.*, 2000, 2002; Mantovani and Banks, 2001; Thomas *et al.*, 2001b; Massimi *et al.*, 2004, 2006; Matsumoto *et al.*, 2006; Kuballa *et al.*, 2007), and siRNA-mediated downregulation of Dlg1 augments HTLV-1 Tax-induced T-cell transformation (Ishioka *et al.*, 2006). Although $Dlg1^{+/-}$ mice are not reported to exhibit a heightened tumor incidence, increased proliferation of ocular lens cells is observed in $Dlg1^{-/-}$ mice (Nguyen *et al.*, 2003b), which die perinatally and display abnormal craniofacial, kidney and urogenital development (Caruana and Bernstein, 2001; Naim *et al.*, 2005; Mahoney *et al.*, 2006; Iizuka-Kogo *et al.*, 2007). Consistent with Dlg1 localization to the AJ of polarized epithelial cells (Muller *et al.*, 1995; Reuver and Garner, 1998; Wu *et al.*, 1998; Laprise *et al.*, 2004), cells treated with Dlg1 siRNAs show defects in AJ formation, as well as TJ function (Laprise *et al.*, 2004; Stucke *et al.*, 2007). Unlike *Drosophila* dlg, mammalian Dlg1 does not appear to determine establishment of apical–basal cell polarity. Instead, findings indicate that Dlg1 has an important function in the establishment of anterior–posterior cell polarization required for directed migration of astrocytes and epithelial cells (Etienne-Manneville *et al.*, 2005; Mimori-Kiyosue *et al.*, 2007) and for activation of T-cells (Xavier *et al.*, 2004; Round *et al.*, 2005, 2007; Krummel and Macara, 2006; Rebeaud *et al.*, 2007; Rincon and Davis, 2007). Thus, Dlg1 is an evolutionarily conserved polarity protein.

Notably, recent data indicate that Ad9 E4-ORF1 inhibits both directed migration and anterior–posterior polarity establishment in cells and that both E4-ORF1 D2 and PBM are required for these activities (L Waldron and RTJ, unpublished data). These observations suggest that Ad9 E4-ORF1 directly interferes with the cell polarity function of Dlg1. As this function depends on the interaction of Dlg1 with the adenomatous polyposis coli (APC) tumor suppressor (Etienne-Manneville and Hall, 2003a; Etienne-Manneville *et al.*, 2005; Gomes *et al.*, 2005), one interesting possibility is that Ad9 E4-ORF1 blocks anterior–posterior polarity establishment by disrupting the Dlg1–APC complex in cells. Consistent with this idea, the Dlg1 PDZ1+2 domain tandem mediates binding to the carboxyl-terminal PBM of both APC and E4-ORF1 (Matsumine *et al.*, 1996; Frese *et al.*, 2006; Chung *et al.*, 2008). A detailed discussion of anterior–posterior cell polarity and directed cell migration can be found in the accompanying review article by Etienne-Manneville in this issue.

Given evidence suggesting that HTLV-1 Tax and HPV E6 likewise bind and inactivate Dlg1, as well as Scribble that acts upstream of Dlg1 in the anterior–posterior polarity pathway (Nakagawa and Huibregtse, 2000; Etienne-Manneville and Hall, 2001, 2002, 2003b; Qin *et al.*, 2005; Osmani *et al.*, 2006; Arpin-Andre and Mesnard, 2007; Dow *et al.*, 2007), it is anticipated that these two viral oncoproteins also will be found to prevent anterior–posterior polarity establishment and directed migration of cells. Consistent with this prediction, unlike normal T cells, HTLV-1-infected T cells specifically fail to establish proper anterior–posterior polarity upon stimulation of the CD3 or CD28 receptor involved in T-cell receptor-mediated activation (Barnard *et al.*, 2005). Thus, considering that HTLV-1 infection activates human T cells (Gazzolo and Duc Dodon, 1987), Tax-mediated inactivation of the cell growth-inhibitory Dlg1–APC complex (Matsumine *et al.*, 1996; Ishidate *et al.*, 2000; Hirata *et al.*, 2004; Ishioka *et al.*, 2006) may cause a loss of polarity that, in conjunction with Tax1-mediated activation of the cyclic AMP, NFkB and PI3K pathways, provokes abnormal proliferation of human T cells. Because the Dlg1 and Scribble

polarity proteins also control immune synapse formation, migration and signaling in T cells (Xavier *et al.*, 2004; Ludford-Menting *et al.*, 2005; Round *et al.*, 2005, 2007; Krummel and Macara, 2006; Rebeaud *et al.*, 2007; Rincon and Davis, 2007), an interesting possibility is that the Tax oncoprotein additionally prevents host-mediated viral clearance by blocking these important T-cell functions, thereby aiding HTLV-1 establishment of persistent T-cell infections in people.

Also worth mentioning is the subversion of cell migration in cancer, where increased migration contributes to tumor invasion and metastasis. Consistent with this idea, loss or reduction of Scribble or Dlg1 expression is correlated with more invasive and aggressive human tumors (Humbert *et al.*, 2003; Nakagawa *et al.*, 2004; Navarro *et al.*, 2005; Gardiol *et al.*, 2006). At first glance, these observations seem at odds with compelling evidence showing that loss or reduction in Scribble or Dlg1 expression in normal mammalian cells instead inhibits migration (Etienne-Manneville *et al.*, 2005; Ludford-Menting *et al.*, 2005; Wada *et al.*, 2005; Osmani *et al.*, 2006; Dow *et al.*, 2007). In a recent review (Humbert *et al.*, 2006), this apparent discrepancy was proposed to reflect context-dependent functions of these tumor suppressors in migration (Goode and Perrimon, 1997; Abdelilah-Seyfried *et al.*, 2003; Pagliarini and Xu, 2003; Qin *et al.*, 2005). On the basis of this idea, it may be postulated that functional loss of Scribble or Dlg1 in normal cells contributes to the early stages of neoplastic transformation by preventing anterior–posterior polarity establishment (Wodarz and Nathke, 2007) and thereby directed migration, yet, in the context of additional oncogenic insults involved in late stage malignant progression, loss of Scribble or Dlg1 function instead acts to increase the migration of cancer cells.

E4-ORF1 binding to the Dlg1 polarity protein also promotes constitutive growth factor-independent PI3K activation

Phosphatidylinositol 3-kinase represents a key component of a signaling pathway triggered by activated tyrosine kinase and heterotrimeric G protein-coupled membrane receptors or Ras (Blume-Jensen and Hunter, 2001). These factors recruit PI3K to the plasma membrane, where this lipid kinase phosphorylates 4,5-phosphoinositides at the D3 position. The resulting 3,4,5-phosphoinositide products act as second messengers to recruit Akt/protein kinase B (PKB) to the membrane, where PDK1 and the TORC2 complex activate PKB by phosphorylating threonine residue 308 (T308) or serine residue 473 (S473), respectively (Bhaskar and Hay, 2007). Activated PKB promotes cell survival and proliferation through its ability to control the activities of multiple downstream effectors, including pro-apoptotic Forkhead transcription factors, translation and cell-cycle progression regulator p70S6-kinase (S6K) and cyclin-dependent kinase inhibitor p27Kip1 (Yu and Sato, 1999; Medema *et al.*, 2000). A critical antagonist of PI3K is the PTEN tumor suppressor protein, a lipid phosphatase that removes D3 phosphates from 3,4,5-phosphoinositides (Yamada and Araki, 2001). Significantly, human cancers are frequently associated with activating mutations in *PI3K* or *PKB* or loss-of-function mutations in *PTEN* (Engelman *et al.*, 2006), thereby widely implicating dysregulated PI3K–PKB signaling in the development of many human malignancies.

Findings have shown that Ad9 E4-ORF1 promotes constitutive growth factor-independent activation of PI3K, as well as its downstream effectors PKB and S6K, but not components of several other signaling pathways (ERK, β-catenin/TCF, JNK, NFκB, Notch, Stat3) (Frese *et al.*, 2003). In addition, the PI3K inhibitor LY294002 or mammalian target of rapamycin inhibitor, which blocks transformation by constitutively activated forms of PI3K and PKB but not 11 other oncoproteins (Aoki *et al.*, 2001), abrogates soft agar growth and focus formation by Ad9 E4-ORF1-expressing cells and reverses the transformed state of Ad9-induced mammary tumor cells (Frese *et al.*, 2003). Hence, cellular transformation induced by Ad9 E4-ORF1 depends on its capacity to dysregulate cellular PI3K signaling, an activity

shared by all Ad E4-ORF1 proteins (Frese *et al.*, 2003; O'Shea *et al.*, 2005a). As expression of a constitutively activated PI3K or PKB mutant fails to recapitulate Ad9 E4-ORF1-induced cellular transformation (Frese *et al.*, 2003), it also must be concluded that PI3K activation is necessary but not sufficient for the full oncogenic potential of Ad9 E4-ORF1. The capacity of E4-ORF1 to stimulate the PI3K pathway is abolished by an inactivating mutation in either the D2 or PBM (Frese *et al.*, 2003), identical to Ad9 E4-ORF1-induced disruption of anterior–posterior cell polarization. The fact that, unlike wild-type Ad9 virus, Ad9 viruses encoding PBM or D2 *E4-ORF1* mutants fail to activate PI3K during a viral infection and to elicit mammary tumors in rats (Frese *et al.*, 2003) additionally links PI3K activation, as well as disruption of anterior–posterior cell polarization, to Ad9-induced mammary tumorigenesis.

The finding that Ad9 E4-ORF1, high-risk HPV E6 and HTLV-1 Tax independently evolved to target the Dlg1 polarity protein have provided compelling evidence implicating this cellular factor in human cancer. Despite the general belief that these interactions solely function to inactivate this cell polarity protein, results showed that $Dlg1^{-/-}$ mouse embryo fibroblasts fail to support E4-ORF1-induced PI3K activation and cellular transformation (Frese *et al.*, 2006). This defect was specific to $Dlg1^{-/-}$ mouse embryo fibroblasts, as $MUPP1^{-/-}$ or $MAGI\text{-}1^{-/-}$ mouse embryo fibroblasts retain the capacity to support this E4-ORF1 activity. Moreover, growth factor-induced PI3K activation remained normal in $Dlg1^{-/-}$ mouse embryo fibroblasts, revealing a specific defect in E4-ORF1-induced PI3K activation. As expected, this Dlg1- and D2 element-dependent E4-ORF1 activity is mediated specifically by E4-ORF1 trimers (Chung *et al.*, 2007, 2008) and also depends on several different Dlg1 domains, including PDZ1+2, US3, Src homology region-3 and I3 (Frese *et al.*, 2006). These findings revealed the first known function for Dlg1 in virus-mediated cellular transformation and also exposed an unexpected oncogenic activity for this suspected cellular tumor suppressor protein.

The oncogenes encoded by DNA tumor viruses, such as Ad, evolved to promote quiescent cells to progress from G0 to S phase of the cell cycle to provide an optimal environment for viral DNA replication. As this aberrant proliferative signal often induces a cellular antiviral response that triggers apoptosis, DNA tumor virus oncoproteins also promote cell survival to ensure robust viral replication. Thus, it will be important to determine whether the perturbation of cell polarity proteins by Ad9 E4-ORF1 serves to promote both cell cycle progression and cell survival during the viral life cycle. This outcome seems likely given that the related Ad type 5 E4-ORF1 protein activates the cellular PI3K effector mammalian target of rapamycin and, in so doing, enhances S-phase entry and viral replication in quiescent primary cells under nutrient and growth factor-limiting conditions (O'Shea *et al.*, 2005a, b).

The PATJ and ZO-2 polarity proteins

The development of epithelial-derived cancers is commonly linked to a failure of tumor cells to form TJs and to establish proper apical–basal polarity (Cochand-Priollet *et al.*, 1998; Soler *et al.*, 1999). Evidence further suggests that such defects directly trigger neoplastic cellular transformation (Bilder, 2003, 2004; Humbert *et al.*, 2003; Matter and Balda, 2003; Aranda *et al.*, 2006). Given that MUPP1, PATJ, MAGI-1 and ZO-2 localize to the TJ (Beatch *et al.*, 1996; Hamazaki *et al.*, 2002; Hirabayashi *et al.*, 2003; Shin *et al.*, 2005) and become aberrantly sequestered by E4-ORF1 in the cytoplasm (Glaunsinger *et al.*, 2000, 2001; Lee *et al.*, 2000; Latorre *et al.*, 2005), a reasonable hypothesis would be that Ad9 E4-ORF1 binds and inactivates these PDZ proteins and, in so doing, blocks TJ formation and causes a loss of apical–basal polarity in epithelial cells. In support of this idea, both PATJ and ZO-2 are polarity proteins required for both TJ formation and proper apical–basal polarity establishment in epithelial cells (Shin *et al.*, 2005; Umeda *et al.*, 2006; Hernandez *et*

al., 2007) (Figure 3). Moreover, in Madin-Darby Canine Kidney (MDCK) epithelial cells, it was reported that Ad9 E4-ORF1 blocks proper TJ localization of PATJ and ZO-2, as well as their interacting partners, and also disrupts both the TJ barrier and apical–basal polarity (Latorre *et al.*, 2005). In contrast, E4-ORF1 fails to interfere with the proper AJ localization of Dlg1 or β-catenin in the same cells. In addition, E4-ORF1 D2 mutants, which cannot activate PI3K (Frese *et al.*, 2003; Chung *et al.*, 2007), retain a wild-type capacity to disrupt the TJ in MDCK cells, indicating that TJ disruption and PI3K activation are separable E4-ORF1 activities. The fact that siRNA-mediated downregulation of either PATJ or ZO-2 similarly blocks TJ formation and proper apical–basal polarity establishment in MDCK cells (Shin *et al.*, 2005; Hernandez *et al.*, 2007) argues strongly that analogous effects caused by E4-ORF1 are due to its direct inactivation of these two PDZ proteins. This work represented the first demonstration that PBM-mediated interactions of a viral oncoprotein with cell polarity proteins functionally disrupt the TJ and cause a loss of polarity.

Providing possible links for these observations to cancer, *ZO-2* is a candidate human tumor suppressor gene (Chlenski *et al.*, 1999a, b, 2000; Sato *et al.*, 2003; Fink *et al.*, 2006), and recent data suggest that *ZO-2*$^{+/-}$ mutant mice exhibit an elevated tumor incidence (I Latorre and RTJ, unpublished data). In addition, overexpressed ZO-2 interferes with focus formation induced by Ad9 E4-ORF1, as well as by the polyomavirus middle T (mT) and activated RasV12 oncoproteins (Glaunsinger *et al.*, 2001). Likewise, tumorigenic Ad9 E4-ORF1 and nontumorigenic Ad E4-ORF1 proteins interact with MUPP1, PATJ, MAGI-1 and Dlg1, whereas Ad9 E4-ORF1 uniquely binds ZO-2 (Glaunsinger *et al.*, 2001), thereby linking this particular interaction to the distinct Ad9 E4-ORF1 tumorigenic properties. Evidence further shows that high-risk HPV E6 binds PATJ (Latorre *et al.*, 2005) and targets it for degradation in cells (Storrs and Silverstein, 2007), suggesting that inactivation of this polarity protein may contribute to the development of cervical carcinoma in women.

Inactivation of TJ-associated polarity proteins likewise can be envisioned as contributing to Ad9-induced mammary tumorigenesis, as well as human breast cancer. Malignant Ad9-induced mammary tumor cells express myoepithelial cell markers (Javier *et al.*, 1991), implying an origin from polarized mammary stem cells of the lumenal epithelial lineage that form TJs (Gudjonsson *et al.*, 2002b). Notably, most breast carcinomas arise from this epithelial lineage, which functions to maintain normal mammary tissue polarity, a property lost during breast neoplasia (Pechoux *et al.*, 1999; Gudjonsson *et al.*, 2002a, 2005; Bissell and Bilder, 2003; Adriance *et al.*, 2005; Lakhani and Bissell, 2005; Polyak and Hu, 2005). Thus, the tumorigenic potential of Ad9 E4-ORF1 in mammary epithelial cells could conceivably stem in part from its capacity to prevent TJ formation and proper apical–basal polarity establishment by inactivating TJ-associated polarity proteins in polarized mammary stem cells.

Although TJ-associated MAGI-1 and MUPP1, a paralog of the PATJ polarity protein (Lemmers *et al.*, 2002; Michel *et al.*, 2005; Shin *et al.*, 2005), are involved in the assembly of membrane signaling complexes (Barritt *et al.*, 2000; Becamel *et al.*, 2001; Hamazaki *et al.*, 2002; Kimber *et al.*, 2002; Laura *et al.*, 2002; Patrie *et al.*, 2002; Hirabayashi *et al.*, 2003; Jeansonne *et al.*, 2003; Murata *et al.*, 2005; Heydecke *et al.*, 2006; Sakurai *et al.*, 2006; Balasubramanian *et al.*, 2007; Sugihara-Mizuno *et al.*, 2007), it is not yet known whether these two PDZ proteins likewise function in TJ assembly and apical–basal polarity establishment (Figure 3). In addition to their aberrant sequestration by Ad9 E4-ORF1 in insoluble cytoplasmic complexes, MAGI-1 and MUPP1 bind to and are targeted for proteasome-mediated degradation by high-risk HPV E6 (Glaunsinger *et al.*, 2000; Lee *et al.*, 2000). These observations, coupled with the requirement for MAGI-1 in localization and stabilization of the PTEN tumor suppressor at the plasma membrane (Kotelevets *et al.*, 2005; Valiente *et al.*, 2005) and the association of reduced MUPP1 levels with a poor prognosis in

breast cancer patients (Martin *et al.*, 2004), hint at possible functions of MAGI-1 and MUPP1 in cancer. On the basis of these observations, it seems conceivable that, similar to PATJ and ZO-2, MAGI-1 and MUPP1 are also cell polarity proteins targeted for functional inactivation by Ad9 E4-ORF1 and high-risk HPV E6 in cells.

Model for Ad9 E4-ORF1-induced cellular transformation through perturbation of cell polarity proteins

Figure 4 presents a model on the basis of available data indicating that the Ad9 E4-ORF1 oncoprotein promotes cellular transformation in large part through interactions with the cell polarity proteins Dlg1, PATJ and ZO-2. The model shows that E4-ORF1 exists in two oligomeric forms, which assemble different general types of protein complexes (complex A and complex B) and produce distinct functional consequences.

Complex A is formed through interactions of the E4-ORF1 trimer with Dlg1 and D2-interacting cellular proteins (Figure 4). TJ-associated PDZ proteins are excluded from complex A, as they cannot bind the E4-ORF1 trimer (Chung *et al.*, 2008). This specificity is determined in part by the unique capacity of E4-ORF1 trimers to bind cooperatively to the Dlg1 PDZ1+2 domain tandem and to form functionalD2 elements. Upon complex formation, Dlg1 mediates translocation of complex A to the plasma membrane (Frese *et al.*, 2006), in agreement with numerous studies implicating Dlg1 in receptor trafficking to the plasma membrane (Chetkovich *et al.*, 2002; Wu *et al.*, 2002; Lee *et al.*, 2003; Leonoudakis *et al.*, 2004; Cai *et al.*, 2006; Inoue *et al.*, 2006; Gardner *et al.*, 2007; Marcello *et al.*, 2007; Mauceri *et al.*, 2007). Complex A functions both to promote Dlg1-mediated PI3K activation by a Ras-dependent mechanism and to block Dlg1-mediated establishment of anterior–posterior cell polarity. This dual function of complex A interestingly suggests that subversion of Dlg1 for E4-ORF1-induced PI3K activation simultaneously blocks the Dlg1 cell polarity function, perhaps by disrupting the Dlg1–APC complex. Also worth mentioning is that proper anterior–posterior polarity establishment depends not only on Dlg1–APC complex formation at the leading edge membrane, but also on spatially restricted PI3K activation that stabilizes the polarized microtubule network at the same membrane site (Higuchi *et al.*, 2001; Procko and McColl, 2005; Onishi *et al.*, 2007; Primo *et al.*, 2007). Thus, an intriguing scenario would be that PI3K activation at the leading edge membrane is normally mediated by Dlg1 through an APC-independent mechanism and that E4-ORF1 usurps and dysregulates this additional Dlg1 polarity function to promote oncogenic PI3K activation in cells. Consistent with this hypothesis, Dlg1 has been shown to promote lamellipodia formation at the leading edge membrane by an APC-independent mechanism (Etienne-Manneville *et al.*, 2005; Humbert *et al.*, 2006).

Complex B, on the other hand, is formed by interaction of the E4-ORF1 monomer with individual TJ-associated PDZ protein targets (MUPP1, PATJ, MAGI-1 and ZO-2) (Figure 4) (Chung *et al.*, 2008). In contrast to soluble plasma membrane-associated complex A, complex B is insoluble and causes aberrant sequestration of TJ-associated PDZ proteins on cytoplasmic membrane vesicles. As PDZ proteins often link membrane proteins to the cortical cytoskeleton, the insolubility of these complexes may reflect an association with cytoskeletal proteins. Dlg1 and D2-interacting cellular proteins are excluded from complex B because they cannot bind the E4-ORF1 monomer (Chung *et al.*, 2007, 2008). Notably, complex B-mediated sequestration of PATJ and ZO-2 blocks their ability to promote TJ formation and proper apical–basal cell polarity (Latorre *et al.*, 2005). As the full oncogenic potential of E4-ORF1 depends not only on Dlg1-dependent PI3K activation, but also on other undetermined E4-ORF1 activities (Frese *et al.*, 2003), a reasonable hypothesis is that functional inactivation of the polarity proteins PATJ and ZO-2, as well as Dlg1, represents these additional crucial activities.

Conclusion

Findings suggest that key PDZ domain-containing polarity proteins are common cellular targets for inactivation by human virus oncoproteins and that such interactions contribute to virus-mediated tumorigenesis. In fact, available data support the notion that inhibition of proper cell polarity establishment may be the primary mechanism whereby the Ad9 E4-ORF1 oncoprotein induces mammary tumors in experimental animals. With respect to this idea, identification of Ad9 E4- ORF1 D2-interacting cellular proteins may reveal that they too are required for establishment of proper cell polarity and that the Ad9 E4-ORF1 oncoprotein likewise blocks their function. In addition, the essential function of Dlg1 in E4-ORF1-induced oncogenic PI3K activation predicts that Dlg1-mediated establishment of anterior–posterior cell polarity in part may depend on an ability of Dlg1 to promote spatially restricted PI3K activation at the plasma membrane of cells. Future experiments should explore this hypothesis.

Also worth mentioning is the suggestion that viral oncoproteins have evolved diverse mechanisms to perturb cell polarity pathways, as evidenced by the report of TJ disruption in polarized epithelial cells expressing the SV40 polyomavirus small t antigen oncoprotein (Nunbhakdi-Craig *et al.*, 2002, 2003), which due to the lack of a PBM is unable to interact with cellular PDZ proteins. This observation hints that cell polarity loss may have an even greater function in virus-mediated tumorigenesis than was thought previously. In this regard, it will be important to determine whether small t antigen expressed by the newly discovered Merkel cell polyomavirus associated with Merkel cell carcinoma (Feng *et al.*, 2008), a rare but aggressive human skin cancer of neuroendocrine origin, disrupts the tight junction and causes a loss of apical–basal polarity in epithelial cells. Additionally, although research to date has primarily focused on functions of polarity loss in the development of epithelial-derived carcinomas, the fact that Ad9, high-risk HPV and HTLV-1 promote sarcomas, carcinomas or leukemias, respectively, may indicate that loss of cell polarity is a contributing factor not only for carcinoma development, but also for the development of many other types of malignancies. This idea clearly warrants investigation.

It is important to mention that cell polarity loss is likewise implicated in the development of human cancers lacking an association with viral agents. For example, several different signaling pathways (for example, ErbB2, transforming growth factor-β, mammalian target of rapamycin) with involvement in human cancers coordinately regulate both apical–basal cell polarity and cellular proliferation, and evidence suggests that combined dysregulation of these processes cooperates to instigate malignant cellular transformation (Wodarz and Nathke, 2007). Moreover, the loss of apical–basal cell polarity and disruption of cell junctions is a hallmark of malignant human tumors. Thus, the development of drugs designed to inhibit cellular pathways that promote the loss of polarity represents a promising area for future cancer research.

It is presently unclear how cell polarity loss contributes to the development of cancer, although some reports have provided potentially important clues. For example, TJ disruption and loss of polarity in epithelial cells can activate a basolateral membrane-localized growth factor receptor by permitting inappropriate intermixing with its cognate apical membrane-localized growth factor (Vermeer *et al.*, 2003) and can also release growth-stimulatory transcription factors from the TJ and promote their translocation into the nucleus (Balda *et al.*, 2003; Betanzos *et al.*, 2004). Exciting results in *Drosophila* further show that perturbations in the establishment of anterior–posterior polarity required for asymmetric cell division increase the production of cancer stem cells (Wodarz and Nathke, 2007). Clearly, future research must explore the mechanisms by which polarity loss contributes to abnormal cellular proliferation that provokes the development of cancer. Studies of human tumor

viruses and their oncogenic determinants promise to serve as powerful tools to understand this important disease process.

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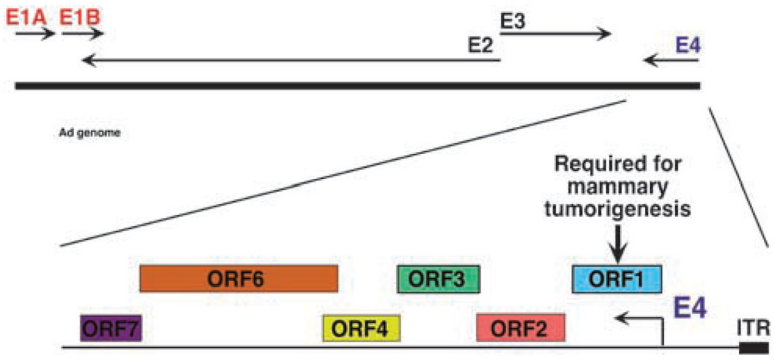

Figure 1.
The Ad genome and early transcription units with enlarged *E4 region* showing six open reading frames (ORFs). *E4 region*-encoded open reading frame (E4-ORF) 6/7 (not shown) consists of E4-ORF7 fused to the amino-terminal region of E4-ORF6.

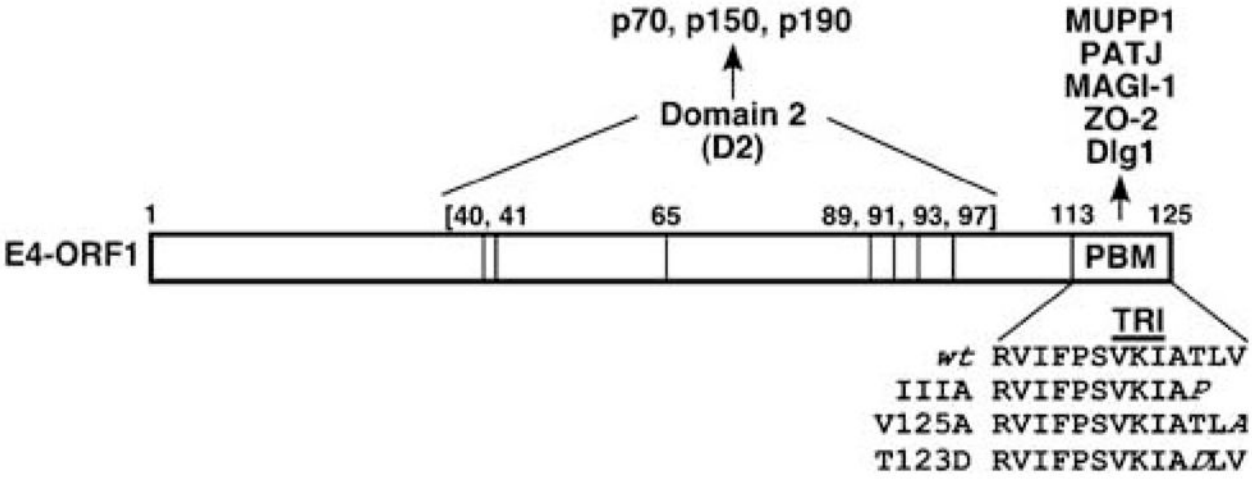

Figure 2.

Adenovirus type 9 (Ad9) *E4 region*-encoded open reading frame 1 (E4-ORF1) consists of two protein-interaction elements, designated domain 2 (D2) and PDZ domain-binding motif (PBM). Centrally located D2 and the carboxyl-terminal PBM are defined by mutants G40V, V41A, D65A, L89Q, F91S, H93A and F97A or mutants IIIA, T123D and V125A, respectively. CrucialD2 residues (bracketed numbers) and crucial PBM residues (italicized) are shown. Cellular targets that bind D2 or PBM are also indicated. The E4-ORF1 trimerization (TRI) element overlaps the PBM sequence.

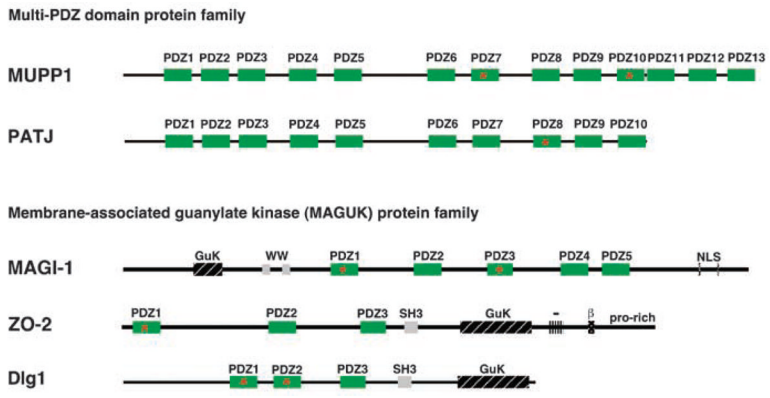

Figure 3.

Domain structures of E4-ORF1-associated cellular PDZ proteins. Red asterisks indicate specific PDZ domains that mediate binding to Ad9 E4-ORF1. SH3, Src homology 3 domain; GuK, guanylate kinase-homology domain; WW, WW domain; NLS, nuclear localization signal; –, acidic domain; prorich, proline-rich region.

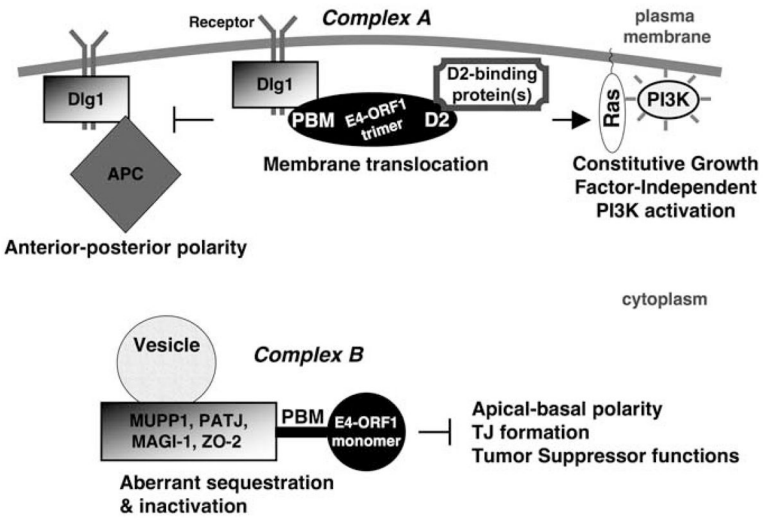

Figure 4.

Molecular model for polarity disruption and PI3K activation by the adenovirus type 9 *E4 region*-encoded open reading frame 1 (E4-ORF1) oncoprotein. See text for additional details about the model and E4-ORF1 complexes A and B.