

The Biology Behind

E1A as a Tumor Suppressor Gene

Commentary re S. Madhusudan et al. A Multicenter Phase I Gene Therapy Clinical Trial Involving Intraperitoneal Administration of E1A-Lipid Complex in Patients with Recurrent Epithelial Ovarian Cancer Overexpressing HER-2/*neu* Oncogene. Clin Cancer Res 2004;10:2905–2907.

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Adenovirus E1a protein presents an interesting paradox. When expressed in primary rodent cells, E1a facilitates oncogenic transformation by activated ras or an activator of c-src signaling, polyoma middle T protein (1). However, adenovirus E1a is nononcogenic in human cells: adenovirus sequences have not been found in human tumors, despite widespread infection of the human population, and intensive efforts to transform human cells with E1a-oncogene mixtures have failed (2, 3). In some instances, small and infrequent tumors arose after a long latency period, but E1a retention was not verified in these tumors, suggesting that they arose from the loss of the E1a transgene (4).

In 1991, two groups published tumor-suppressive effects of E1a in human tumor cells. Our group reported that E1a suppressed tumorigenicity and anchorage-independent growth in three diverse human tumor cell types, carcinoma, fibrosarcoma, and melanoma (5). This was (pleasantly) surprising because the diversity of genetic alterations in these three tumor cell types suggested that E1a might override many different genetic abnormalities to perhaps universally suppress (human) transformation.

The other report (6), which was from the Hung laboratory, indicated that E1a suppressed tumor growth by repressing HER2/c-erbB2/c-neu expression.

To date, parallel lines of investigation have been pursued by these and other groups. Subsequent reports from the Hung laboratory indicated that the E1a effect was specific to HER2/c-erbB2/c-neu-overexpressing tumor cells (7), although reverse transformation of tumor cells independently of this oncogene was later confirmed in several reports (8–10).

HER2/*neu* is a member of the epidermal growth factor receptor family that heterodimerizes with other epidermal growth factor receptor proteins on ligand stimulation. It has tyrosine kinase activity that autophosphorylates and phosphorylates its dimerization partners, simultaneously activating several signal transduction pathways, leading to Akt and mitogen-activated protein kinase activation that induce mitogenesis, cell survival, and, by mechanisms that are not well-defined, genomic instability (11, 12). It is the target of the

breast cancer-suppressing monoclonal antibody Herceptin. The mechanism of repression of HER2/*neu* by E1a is straightforward because the Her2/*neu* promoter has several positive elements that require the p300/CREB-binding protein (CBP) coactivator proteins, which are inhibited by direct E1a interaction (13).

Some of the HER2/*neu*-independent tumor-suppressive effects of E1a are due to the E1a-p300 interaction; others may involve the interaction of E1a with the retinoblastoma protein (Rb), whereas yet others depend on the interaction of E1a with a transcriptional corepressor known as COOH-terminal binding protein (CtBP). These proteins and the effects of E1a on their functions are summarized as follows.

p300/CBP

These proteins are required cofactors for a large variety of tissue-specific transcription factors (reviewed in Ref. 14). E1a binds to a key domain of these related proteins (the transcriptional adaptor motif region), functionally inactivating the protein. The fact that cells are able to metabolize and proliferate well in the absence of functional p300 is consistent with the independence of the “housekeeping gene” promoters from this coactivator. P300 is also required for the induction of important tumor angiogenic genes such as vascular endothelial growth factor by the transcription factor hypoxia-inducible factor-1 (15). Consistent with this, E1a has been reported to inhibit tumor angiogenesis, a primary effect of its NH₂-terminal (p300-interacting) domain (16).

E1a causes p53 protein to accumulate (17), and p53 can induce either growth arrest or apoptosis, depending on the cellular context. One factor determining which will occur is the expression of p21^{Cip/Waf} (18): E1a inhibits the transactivation of this gene by p53, which tends to favor apoptosis *versus* growth arrest. This inhibition is caused by the interaction of E1a with the p300, which is required for transactivation by p53 (19). Also, transactivation by p53 requires its acetylation of p53 by p300 and/or p300/CREB-binding protein-related enzymes, enhancing the interaction of p53 with the p21 promoter, which is inhibited by E1a (20, 21). It should be remembered that E1a displays impressive tumor suppression activity in p53-null or p53-mutant tumor cell lines (8).

Rb and Related Proteins

The E1a-Rb interaction is oncogenic in rodent cells but has been reported to contribute to E1a tumor suppression in human cells (16), although this contribution has not been evident in

Received 4/2/04; accepted 4/2/04.

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other cell systems.¹ One possible mechanism for this is the activation of the transcription factor E2F, which is proapoptotic in certain contexts, by E1A-mediated release from sequestration on Rb. E2F in turn activates the p19^{Arf} gene (22), which, through relocalization of the p53-regulatory-protein mdm2, results in p53 accumulation and increased apoptotic potential. Although the tumor-suppressive effect of the E1A-Rb interaction is poorly documented and somewhat counterintuitive, it should be remembered that E2F, which is liberated from a repressive complex by E1A, behaves mostly as a tumor suppressor gene in knockout mouse models (23). Direct transactivation of various caspase genes by E2F has been proposed as a possible mechanism of the proapoptotic behavior of E2F (24).

CtBP

The expression of E1A in diverse human tumor cell lines that did not overexpress HER2/neu consistently caused tumor suppression that correlated with two phenotypic effects. First, E1A up-regulated the expression of epithelial cell adhesion molecule genes (E-cadherin, tight junction proteins, and desmosomal proteins) as well as epithelial cytokeratin genes (25). In fact, E1A essentially reversed the epithelial-to-mesenchymal transition that characterizes tumor cells, explaining at least phenomenologically the basis for the nonselectivity of E1A as a tumor suppressor gene (26). Second, E1A dramatically sensitized tumor cells to apoptosis induced by chemotherapeutic DNA-damaging agents, tumor necrosis factor, and cell matrix detachment (8, 27–29). Other groups showed that E1A sensitized tumor cells to the apoptotic actions of natural killer cells, which may play an additional role in tumor suppression *in vivo* (30). Whereas multiple mechanisms are quite possibly involved, we found that apoptosis sensitization in the absence of p53 function was attributed mainly to the interaction of E1A with CtBP (27).

CtBP is a corepressor protein, *i.e.*, it can be recruited to promoters by repressor proteins that contain the minimal CtBP-interaction motif (PXDLS) plus critical flanking sequences, where it causes chromatin remodeling that silences transcription (reviewed in Ref. 31). E1A disrupts the interaction of CtBP with repressors, one potential way for E1A to up-regulate cellular genes. The aforementioned effects of E1A on human tumor cells suggested that perhaps CtBP selectively targeted certain epithelial and proapoptotic genes for repression. By characterizing cells from CtBP knockout mice, we confirmed that CtBP down-regulates a battery of epithelial cell adhesion genes as well as certain proapoptotic genes, including PERP, Bax, Noxa, three forkhead family transcription factors, and three insulin-like growth factor-binding proteins (32). In fact, cells from the CtBP knockout mice were hypersensitive to a wide variety of apoptotic stimuli, emulating the effect of E1A.

Bystander Effects

The efficacy of cancer gene therapy at its current efficiency level is critically dependent on the induction of a bystander effect. Three effects of E1A suggest that it may induce a by-

stander effect, although these have yet to be investigated rigorously.

(a) The simple failure of E1A-expressing cells to support angiogenesis would not be expected to generate a bystander effect *in vivo* because the majority of cells in a tumor that fail to express E1A should be able to provide enough vascular endothelial growth factor to drive angiogenesis. It is conceivable that E1A could potentially induce the expression of angiogenesis-inhibitory proteins (*e.g.*, thrombospondin) that might be predicted to generate a bystander effect; the possible existence of such an activity has been suggested (33), but the factor itself has not yet been identified, nor is there convincing evidence as yet that E1A-expressing cells inhibit angiogenesis.

(b) The conferral of tumor necrosis factor (34) and natural killer cell (30) sensitivity on tumor cells could evoke a sufficiently non-cell-specific effect so as to eradicate most or all of the tumor.

(c) The mesenchymal-to-epithelial conversion effect might induce the formation of gap junctions. This could increase the efficacy of cell-to-cell communication of small molecules such as DNA-damaging drugs that are used in conjunction with E1A.

Several Phase I and Phase II clinical trials have now been completed using E1A in a gene therapy format in which it is delivered by liposome vectors (35–38). The article by Madhusudan *et al.* (39) in this issue of *Clinical Cancer Research* reports a new clinical trial in patients with advanced ovarian cancer, showing evidence of safety and gene transfer, but no evidence of efficacy. Based on the considerations discussed above, several improvements that might render E1A a useful gene for cancer therapy are suggested: (a) characterize and optimize the use of the bystander effect; (b) conduct trials on earlier-stage patients; (c) combine E1A gene therapy with conventional chemotherapy; and (d) use cell-based or protein-based assays to identify compounds that mimic the effect of E1A and then develop drugs that might circumvent the problem inherent in gene delivery to the target sites.

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¹ S. M. Frisch, unpublished observations.

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