

## The Biology Behind

# The Battle between Tumor Suppressors: Is Gene Therapy Using *p16<sup>INK4a</sup>* More Efficacious Than *p53* for Treatment of Ovarian Carcinoma?<sup>1</sup>

Commentary re: S. C. Modesitt *et al.*, *In Vitro* and *In Vivo* Adenovirus-mediated *p53* and *p16* Tumor Suppressor Therapy in Ovarian Cancer. *Clin. Cancer Res.*, 7: 1765–1772, 2001.

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Ovarian carcinoma is a malignancy with a notoriously poor prognosis, primarily because these tumors are rarely diagnosed early, and the majority of patients have disease that has spread outside of the ovary at the time of initial diagnosis (1). Even with the significantly increased survival rates afforded by the introduction of platinum-based chemotherapy regimens, the mortality rate associated with this tumor type remains high, and the overall 5-year survival rate is only approximately 37% (1). Because of the poor prognosis associated with this cancer, it has become imperative that new treatment modalities continue to be introduced and tested. One treatment avenue that has received a great deal of attention in recent years has been the use of gene therapy with adenoviral vectors (Ad) constructed to express the protein products of tumor suppressor genes. Because of its inherent death-inducing activity, and because it is subject to mutational inactivation in 40–80% of ovarian tumors (2), the majority of gene therapy research has focused on the *p53* tumor suppressor gene.

*p53* is an attractive molecule for use in gene therapy for several reasons. First, tumor development is clearly accompanied by a strong selection against this protein, as evidenced by the fact that the majority of human tumors of multiple histological types suffer inactivating mutations in this gene (3). Second, *p53* directs more than one growth suppressive pathway in the cell. Specifically, induction of *p53* can lead to growth arrest in certain tumor and cell types, or this protein can induce programmed cell death (apoptosis). Both of these pathways are largely mediated through the function of *p53* as a sequence-specific DNA-binding protein and transcription factor (Fig. 1). For the growth arrest pathway, it is the ability of *p53* to tran-

scriptionally up-regulate the promoter of the *cdk*<sup>3</sup> inhibitor *p21/waf1* that plays a primary role. The proapoptotic pathway of *p53* is more diverse: *p53* can transactivate the proapoptotic genes *bax*, *fas*, *KILLER/DR5*, *PUMA*, *NOXA*, and *p53AIP1*, and each can contribute to programmed cell death (reviewed in Ref. 4). Additionally, the transcriptional repression function of *p53* (5) and a proposed mitochondrial function (6) may also play a role in apoptosis induction by this protein.

In some senses, the potency of *p53* as a tumor suppressor may also be its downfall as a gene therapy vehicle. Specifically, it is typically the case that tumors that retain wild-type *p53* have mutated other key regulatory proteins in this pathway (such as *MDM2* or *p14<sup>ARF</sup>*, which control *p53* stability), thereby rendering this pathway inactive. Additionally, apoptosis induction is a major tumor suppressive function of *p53*, and the apoptosis pathway has multiple components that are subject to mutation in human cancer. For example, tumor cells have been reported to delete or inactivate caspases, which are the penultimate enzymatic mediators of programmed cell death, or to exhibit altered expression of the *bcl2* or inhibitors of apoptosis (IAP) family, which control caspase activity and function (reviewed in Ref. 7). Therefore it is likely that the majority of tumor cells also have inactivated the apoptosis pathway functionally, and reintroduction of *p53* might be postulated to have limited growth-suppressive effect.

Like *p53*, the *p16<sup>INK4a</sup>* tumor suppressor protein negatively regulates cell cycle progression. This gene is encoded by the *p16* locus at chromosome 9p21; this locus actually encodes for two different gene products that use separate promoters and distinct reading frames: *p14<sup>ARF</sup>* and *p16<sup>INK4a</sup>*. The *p14<sup>ARF</sup>* protein negatively regulates *p53* function, whereas the *p16<sup>INK4a</sup>* protein is a *cdk* inhibitor. *p16<sup>INK4a</sup>* binds to *cdk4* and *cdk6*, inhibits cyclin D binding, and thereby prevents phosphorylation of the RB gene product *pRB* (reviewed in Ref. 8). When *pRB* is hypophosphorylated, it interacts with and inhibits E2F transcription factors; conversely, when *pRB* is phosphorylated by cyclin D/*cdk4–6* complexes, it releases E2F from negative regulation, allowing it to activate genes required for S-phase progression (see Fig. 1). The *p16* locus is subject to mutational inactivation at different frequencies in diverse tumor types, and germ-line mutations in this gene are found in familial melanoma kindreds (9). Recent data suggest that the *p16<sup>INK4a</sup>* protein plays a normal role in the replicative senescence induced by cumulative cell

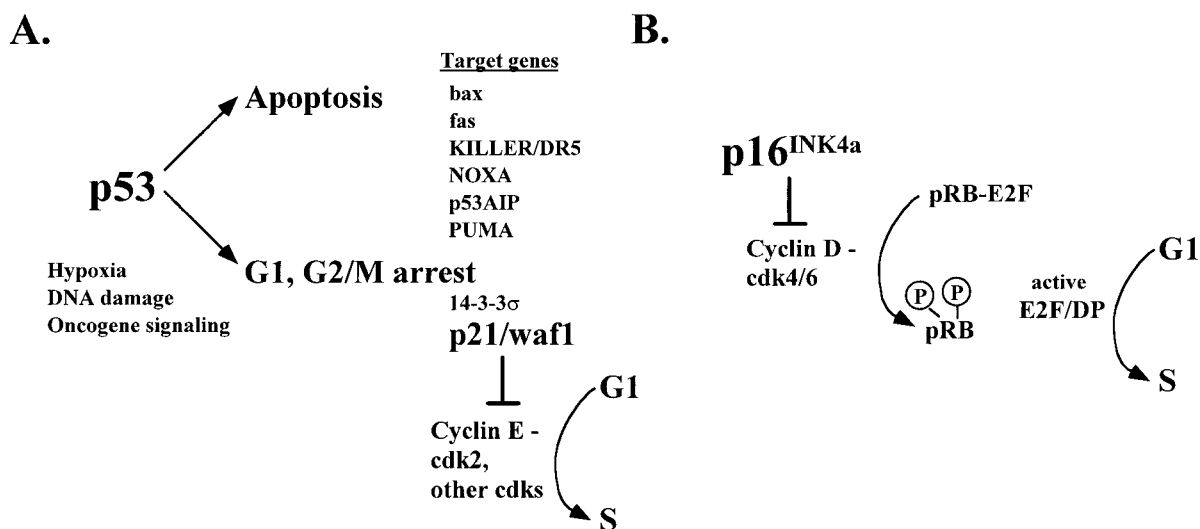
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<sup>3</sup> The abbreviations used are: *cdk*, cyclin-dependent kinase; *RB*, retinoblastoma; *pRB*, RB tumor suppressor protein; Ad, adenoviral.



*Fig. 1* A, p53 tumor suppressor pathway. p53 protein is latent in cells and can be activated by cellular stress signals such as hypoxia, DNA damage, and inappropriate oncogene signaling. In response to these stresses, p53 accumulates and transcriptionally up-regulates genes involved in G<sub>1</sub> and G<sub>2</sub>-M arrest, such as the cdk inhibitor p21/waf1 and 14-3-3 $\sigma$ , respectively. Alternatively, p53 can transactivate the proapoptotic genes *bax*, *NOXA*, *p53AIP*, and *PUMA*, which are involved in mitochondrial apoptotic signaling, or *fas* and *KILLER/DR5*, which play a role in death receptor signaling. B, p16<sup>INK4a</sup> growth suppressive pathway. p16<sup>INK4a</sup> protein interacts with cdk4 and/or cdk6 to inhibit cyclin D-binding and kinase activity. This allows transcriptional targets are necessary for the G<sub>1</sub> to S (synthesis) phase transition of the cell cycle.

divisions and constitutively active Ras signaling. Specifically, this gene is dramatically up-regulated in senescent fibroblasts (10), and enforced expression of p16<sup>INK4a</sup> induces a prolonged growth arrest with phenotypic features characteristic of senescent cells (10, 11). Notably, recent studies using adenoviral vectors to compare the growth-suppressive effects of a number of cdk inhibitors indicate that, although all cdk inhibitors tested induced a prolonged growth arrest, p16<sup>INK4a</sup> was the best to inhibit tumor progression (12). These data raise the interesting possibility that induction of replicative senescence is not the only way that p16<sup>INK4a</sup> inhibits tumor cell growth.

In the present report, Modesitt *et al.* (see this issue, pp. 1765–1772) follow up on previous studies on gene therapy for ovarian tumors (13–17) and compare the ability of adenoviral vectors expressing either the *p53* or the *p16<sup>INK4a</sup>* genes to inhibit the growth of ovarian carcinoma cell lines. Notably, these researchers have extended these studies to include an analysis of the ability of Ad-p53 and Ad-p16<sup>INK4a</sup> to inhibit the ability of ovarian carcinoma growth as xenografts in nude mice. Using downstream readouts such as cell proliferation, growth in soft agar, and metabolic assays (the MTT, or microtetrazolium conversion assay), these researchers found that adenoviral-mediated expression of p16<sup>INK4a</sup> was significantly better than p53 as a growth inhibitor for all of the cell lines tested. Interestingly, however, the ability of p16<sup>INK4a</sup> and p53 to induce apoptosis or to induce growth arrest did not differ significantly in these cell lines. Significantly, mice whose tumors were injected with the p16<sup>INK4a</sup>-virus showed the longest survival and, more frequently, showed no evidence of disease. The combined data argue that adenoviral expression of the *p16<sup>INK4a</sup>* gene may be more efficacious for ovarian and other tumor types.

These data are surprising in light of the fact that the *p16*

gene is rarely mutated in ovarian cancer (18), although decreased levels of p16<sup>INK4a</sup> protein have been detected in a low percentage of ovarian tumor samples (19). Additionally, Modesitt *et al.* report that the abilities of p16<sup>INK4a</sup> and p53 to induce growth arrest or apoptosis do not seem to be significantly different. Therefore, the combined data beg the question, why is gene therapy with *Ad-p16<sup>INK4a</sup>* so much more efficacious *in vivo*? The answer could lie in the above-mentioned possibility that components of the p53 downstream pathway are subject to mutational inactivation in the majority of tumor types. However, the same could be said for components of the RB pathway, which include p16<sup>INK4a</sup>. More likely then, the explanation lies in a growth suppressive activity of p16<sup>INK4a</sup> that is independent of pRB, which is an intriguing possibility. Along these lines, another group has reported that expression p16<sup>INK4a</sup> can cause down-regulation of the angiogenic factor vascular endothelial growth factor, and can inhibit angiogenesis in nude mouse xenografts (20). Additionally, the possibility exists that p16<sup>INK4a</sup> may be a more potent inhibitor of the growth of vascular endothelial and smooth muscle cells required for neovascularization of implanted tumors. Conversely, other mechanisms of tumor suppression by this molecule may yet be elucidated.

The present study offers a well-controlled and compelling comparison of the ability of different tumor suppressor proteins to work in gene therapeutic approaches. Future questions seem clear. Specifically, what is the requirement for pRB for tumor suppression by p16<sup>INK4a</sup> *in vivo*? Additionally, what is the contribution, if any, of the pRB family members p107 and p130 to growth suppression by this protein? Finally, the ability of the p16<sup>INK4a</sup> adenovirus to synergize with chemotherapeutic drugs needs to be determined. It seems unlikely that gene therapy

alone will be efficacious, so it becomes important to define drugs that will enhance the efficacy of this avenue. Finding such a drug may be more difficult than imagined; adenoviral-mediated delivery of p16<sup>INK4a</sup> has already been demonstrated to render tumor cells chemoresistant to agents commonly used in ovarian cancer, such as cisplatin and paclitaxel (21). Therefore, it will be important to identify drugs or therapies whose mechanism of action is not inhibited by p16<sup>INK4a</sup> (one possibility is radiotherapy; Ref. 22) and to compare these drugs in combination with gene therapy with the standard treatments for this tumor type. Additionally, the standard hurdles for gene therapy modalities, such as antiviral immune response and improved target cell specificity, need to be overcome. Finally, there is a compelling need for a mouse model of spontaneous ovarian carcinoma. Such a model, in which oncogenic transgenes are driven by promoters that are largely restricted to the ovarian surface epithelium, such as the ovarian specific 1 promoter (23), will greatly facilitate the discovery of novel mechanisms of detection and treatment for ovarian cancer.

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