The Battle Behind

The Battle between Tumor Suppressors: Is Gene Therapy Using $p16^{INK4a}$ More Efficacious Than $p53$ for Treatment of Ovarian Carcinoma?

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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 transcriptionally up-regulate the promoter of the cdk2 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 $p14^{ARF}$, 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 bc12 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 $p16^{INK4a}$ tumor suppressor protein negatively regulates cell cycle progression. This gene is encoded by the $p16$ locus on chromosome 9p21; this locus actually encodes for two different gene products that use separate promoters and distinct reading frames: $p14^{ARF}$ and $p16^{INK4a}$. The $p14^{ARF}$ protein negatively regulates p53 function, whereas the $p16^{INK4a}$ protein is a cdk inhibitor. $p16^{INK4a}$ 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 $p16^{INK4a}$ protein plays a normal role in the replicative senescence induced by cumulative cell

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The abbreviations used are: cdk, cyclin-dependent kinase; RB, retinoblastoma; pRB, RB tumor suppressor protein; Ad, adenoviral.
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 p16INK4a in vivo? Additionally, what is the possibility that induction of replicative senescence is not the only way that p16INK4a inhibits tumor cell growth.
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 p16INK4a 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 p16INK4a (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 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.

REFERENCES

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