Gene Therapy

Virus-Mediated Oncolysis

Of all the available gene therapy vector systems, recombinant adenoviral vectors are unparalleled in their capacity to achieve effective gene delivery in vivo. A recent report in *Science* extends the utility of this vector system to employ adenoviruses as specific oncolytic agents without adding therapeutic genes. Effective antitumor therapy was achieved with a mutant adenovirus able to replicate in neoplastic cells and thereby to lyse those cells in a selective manner.

The recent study extends a longstanding concept in cancer therapy. Early in this century, it was noticed that viral infections could have an inhibitory effect on tumor cell growth in certain contexts. Furthermore, certain viruses could be adapted in tissue culture to replicate in neoplastic cells but not in their normal tissue counterparts. Tumor cells would be killed when the virus completed a lytic cycle within those cells.

Viral agents that have been adapted to replicate selectively in tumor cells include adenovirus, mumps virus, vaccinia virus, myxovirus, West Nile virus, and Newcastle disease virus. Recent studies have employed the H-1 parvovirus and herpes simplex virus type 1 (HSV-1), which have achieved an antitumor effect with prolonged survival in rodent systems.

Despite these promising findings, virus-mediated oncolysis has had limited use because of safety concerns and because tumor responses are incomplete. With respect to the safety issue, a complete understanding of the basis for tumor-specific viral replication has been lacking. Even for the best-developed system, HSV-1, the biologic basis of selective replication in tumor cells has been only partially characterized.

The basis for the limited efficacy of viral agents in accomplishing tumor eradication is probably complex, reflecting viral lability in vivo and the host immune response. Less than optimal stability in vivo limits the ability of viral agents to infect neighboring tumor cells after replication within neoplastic targets. An ideal agent for virus-mediated oncolysis would possess a characterized basis of tumor selectivity as well as in vivo stability to achieve effective virus propagation.

Frank McCormick and his colleagues at ONYX Pharmaceuticals, Richmond, Calif., found that an adenovirus with a partial deletion of the *E1B* immediate early gene region can specifically replicate in tumor cells lacking functional p53, a tumor suppressor protein. After the virus infects a quiescent cell, its E1A protein induces cell division so that viral replication can proceed. In the process, cellular levels of p53 would also increase and p53-dependent cell death would ensue, except that the viral *E1B* gene region encodes proteins that inactivate p53. By this means, infected cells are made to survive until the late phase of the viral replication cycle.

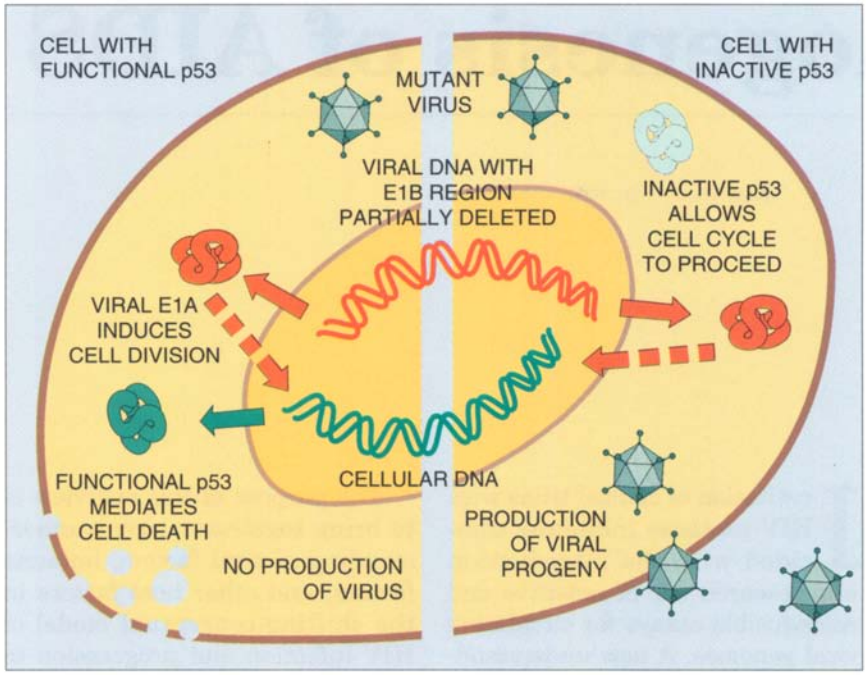
McCormick's group reasoned that because p53 is inactive in many tumor cells, there would be no p53-dependent apoptosis induced by adenoviral E1A. Therefore the cells would not need to be protected with E1B proteins. The hypothesis was that a deletion mutant adenovirus that did not express the E1B 55-kD protein would be capable of replicating in tumor cells, but not in normal cells with wild-type p53, and that infection would proceed to the late phase.

The mutant adenovirus, designated dl1520, was found to replicate as efficiently as wild-type virus in a cervical carcinoma cell line in which the p53 gene has an inactivating mutation. In an osteosarcoma cell line that expresses wild-type p53, the mutant virus replicated poorly, producing about 100 times less infectious virus than did wild-type virus. A cytopathic effect assay was done on a variety of tumor cell lines of known p53 status and showed that dl1520 lysed p53-null cells but not p53-positive tumor cells or normal cells. These results suggested a correlation between the status of p53 in the tumor cells and the cells' susceptibility to dl1520 infection.

Next, the ONYX investigators examined the in vivo growth of dl1520 in human cervical carcinoma cells (p53-deficient) and human glioblastoma multiforme cells (wild-type p53) as tumor xenografts in athymic mice. Tumor cells were injected subcutaneously, and once tumors were palpable, dl1520 was injected into the tumors every other day for a total of three doses. Tumor volume was measured after six weeks.

Cervical carcinoma tumors were reduced by 84% on average, with one complete regression, whereas treated glioblastoma tumors were comparable in size to control-injected tumors, with a little reduction in some cases. In another experiment, cervical carcinoma tumors were injected with dl1520 daily for five days, resulting in complete tumor regression in three of five mice and partial regression in one. Tumors responding completely were followed for three months and did not regrow during that period.

"Gene Therapy" is edited by Joanne T. Douglas and David T. Curiel of the Gene Therapy Program, University of Alabama at Birmingham.



Selective replication of the mutant adenovirus in neoplastic cells with defective p53 but not in cells with wild-type p53 is based on the observation that binding of the viral E1B 55-kD protein to cellular p53 is essential for viral replication in normal cells. Deletion of the part of the *E1B* gene region that encodes the 55-kD protein renders the virus incapable of replicating in cells with wild-type p53 but does not interfere with replication in p53-deficient tumor cells.

The innovative approach presented by McCormick's group deals with the problems of in vivo stability and a characterized basis for selective replication in an animal model of human cancer. Oncolysis is based on a cellular defect that is specific to many tumor cells, namely p53 inactivation. This provides a twofold advantage, because loss of p53 function is responsible for the poor response of some tumors to radiation and chemotherapy.

Some issues remain to be addressed. The ideal tumor-specific replication-competent virus should not replicate at all in normal cells, but the attenuated ONYX virus does still replicate and at a fairly high level in p53-positive cells. The authors could not report on viral replication in human cells in vivo because they used a mouse model, and human adenovirus type 5, from which dl1520 is derived, does not efficiently infect mouse cells.

Finally, in humans though not in immunodeficient mice, an immune response will be generated against the adenovirus infection and may limit the spread of newly made virions to neighboring tumor cells.

Even with caveats, the ONYX study is an important landmark, offering a possible means to solve practical problems that have limited the use of virus-mediated oncolysis. Further refinements in the approach can be anticipated; for example, my colleagues and I have shown that the cytokine signaling molecule IL-6 can cause defective adenoviral vectors to replicate selectively in tumor cells.

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James R. Bischoff et al., *Science* 274:373-376, October 18, 1996.

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