

Targeted therapies for killing tumor cells

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In this issue of PNAS, Su *et al.* (1) present evidence that transfection and expression of the *mda-7* gene in human pancreatic cancer cells combined with antisense inhibition of expression of a mutant *K-ras* gene results in tumor cell growth suppression and induction of apoptosis. They show that both of these treatments are required for this effect and also indicate that these treatments should not affect normal cells having wild-type *K-ras*. These results suggest translating the laboratory findings into the clinic as a new rational approach for this deadly disease. How can this translation take place, what are the potential problems, and can this approach be applied to other types of cancer?

At the heart of these questions is a test of the central hypothesis underlying molecular oncology research for the past decade. In its simplest form this hypothesis states that when we understand the “wiring diagram” of a particular human cancer (that is, all of the genetic and epigenetic changes that are required for the malignant phenotype) we will have a rational approach for early diagnosis, prevention, and development of new curative treatments. Pancreatic cancer is an excellent candidate for the therapeutic aspects of this molecular oncology approach. It is a deadly disease and more than 90% of patients will die within 1 year of diagnosis (often a painful and debilitating process), and it is relatively uniform in its molecular abnormalities ($\approx 90\%$ of tumors have dominant oncogene *K-ras* mutations and disruption of the p16/RB tumor suppressor pathway, about 75% have p53 mutations, and more than 50% have SMAD4/DPC4 disruptions). Thus, many pancreatic cancers have the same lesions, and although we may not know the final biochemical mechanisms there have been hundreds of publications dealing with the biochemical function of these proteins. Current treatment success is so limited that oncologists will welcome novel therapeutics.

During the past 30 years our knowledge about the molecular pathogenesis of cancers has increased dramatically, generated in part by new molecular technologies and sequence information from the Human Genome Project and from public data-

bases with associated informatics tools for interrogating this information. An example of this is the National Center for Biotechnology Information database and the subset provided by the Cancer Genome Anatomy Program established by the National Cancer Institute and used daily by cancer investigators around the world. Also of importance are tissue resources with relevant clinical information and tumor cell lines that can be manipulated in the laboratory by using the ever expanding toolkit of molecular biologic techniques. This information provides us with strategies to find genetic abnormalities in tumors and to design and tailor therapy to specific tumor targets.

Invasive cancers in the adult develop after a lengthy multistep process (involving multiple different genetic and epigenetic events activating dominant oncogenes and inactivating tumor suppressor genes, as well as genes involved in mechanisms such as DNA repair) during which progressive molecular and pathologic changes can be detected. Study of cells during the preneoplastic process may permit the identification of individuals at increased risk (by detecting tissues with a few of these changes) and provides opportunities for cancer prevention (by blocking their progression which can be monitored by following these molecular changes). Hanahan and Weinberg (2) have suggested that these molecular alterations can be divided into six essential categories that collectively dictate cancer growth: self-sufficiency in growth signals, resistance to antigrowth signals, evasion of cell death (apoptosis), limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis. Malignant tumors usually have alterations, often multiple, in all of these categories. While multiple changes are present in tumors, in many past studies correcting one or a few of these changes (e.g., replacing a mutant with a wild-type *p53* gene) may reverse the malignant pheno-

type, resulting in growth cessation of tumor cells or increased sensitivity to more conventional therapies.

In previous studies of melanoma, Fisher and associates (3) identified a gene, *mda-7*, which selectively suppressed the growth and induced apoptosis in several types of human tumor cells when delivered via an adenovirus vector. So far it is not clear from sequence analysis or other studies what is the normal function(s) of *mda-7*, or if *mda-7* is a tumor suppressor gene undergoing biallelic inactivation in tumor cells. However, pancreatic carcinoma cells proved refractory to induction of *mda-7* expression. One obvious question raised by these results is whether normal pancreatic epithelial cells (or pancreatic stem cells) giving rise to these cancers normally express *mda-7*. If they do, then the strategy for detecting and treating very early or preneoplastic lesions could focus on detecting loss of *mda-7* expression, *K-ras* mutations, and interdicting the Ras pathway. If *mda-7* is not expressed normally, then both *mda-7* and *K-ras* treatments would have to be given.

Mutations of the *ras* gene family are the most frequent dominant oncogene abnormality discovered to date, being present in about 25% of all human cancers. Activating *ras* mutations continuously trigger a signaling pathway, resulting in increased cell proliferation. Apparently *mda-7* expression alone was insufficient to counteract the mutant *K-ras* signaling switch in pancreatic cancer cells. It was of interest that *mda-7* treatment alone or with antisense *K-ras* had no effect on the uncommon pancreatic cancers with wild-type *K-ras* or in normal prostate epithelial cultures. Thus, correction of two apparently very

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different molecular changes were necessary to kill pancreatic cells harboring *K-ras* mutations and this effect had specificity for the tumor's molecular ontogeny. These intriguing findings also indicate that successful molecular targeting of cancer cell treatment may, in some situations, require correction of more than one of the cancer hallmarks.

What is *mda-7* and how does it function? It was isolated by Fisher and his coworkers (4) by subtractive hybridization analysis of melanoma cells forced to undergo terminal differentiation. Progression from melanocyte to metastatic melanoma was associated with progressive reduction in *mda-7* expression. Induced expression in diverse human cancers caused growth suppression. Although induction is associated with an increase in apoptosis, it may function via a novel therapeutic action, differentiation therapy, whereby cancer cells are reprogrammed to revert to a more differentiated state and lose their tumorigenic potential (3).

Molecular targeting offers great promises for future cancer therapy. Recent striking examples of clinically beneficial rational therapy are: retinoid acid-induced remissions of acute promyelocytic leukemia carrying a molecularly rearranged retinoic acid receptor; selective tyrosine kinase inhibition-induced tumor responses in chronic myelogenous leukemias carrying *BCR-Abl* translocations, or gastrointestinal stromal tumors with an overexpressed and mutated *c-kit* receptor gene; locally administered *p53* gene therapy (5); and humanized anti-*Her2/neu* receptor antibody in the therapy of *Her2/neu* overexpressing breast cancers (6). Other new approaches involve targeting of angiogenesis/tumor vasculature, telomerase, apoptosis system, and invasiveness, all of which are implicated in pancreatic cancer pathogenesis. Antiangiogenic therapy prevents neovascularization by inhibiting proliferation, migration, and/or differentiation of endothelial cells, whereas vascular targeting is directed at the existing tumor vasculature with both approaches being tested in clinical cancer trials (7). To avoid senescence, cells on the way to malignancy have to stabilize the length of their telomeres. They achieve this by activating telomerase, a unique RNA-containing enzyme that complexes with other proteins and represents a target for development of inhibitors (8). Activation of a family of caspase proteins is necessary to initiate and perform the apoptosis death cascade. Regulated, caspase-based suicide gene therapy can inhibit the growth of cancer cells through induction of apoptosis, providing a rationale for further development (9). Members of the integrin family of cell adhesion recep-

tors influence cancer cell motility, invasiveness, cell growth, and cell survival (10). Thus, integrins offer excellent targets for the development of therapeutic agents (11).

Activating point mutations of the *ras* family of oncogenes (H, N, and, in particular *K-ras*) are frequent in many types of human cancer. Direct inactivation of the *ras* signal itself can be achieved by several different approaches (12). Antisense therapy, as used by Su *et al.* (1), may be performed with the use of antisense nucleotides or by gene therapy with ribozymes, which break down specific RNA sequences. An antisense-*H-ras* oligonucleotide (ISIS-2503) demonstrated antitumor activity in human tumor xenografts and has gone into clinical trials. To function, Ras proteins undergo post-translational modifications such as farnesylation. Inhibition of some Ras family members (but probably not *K-ras*) can be accomplished through inhibition of farnesyl transferase, and several farnesyl transferase inhibitors are undergoing clinical trials currently (13). Appropriate membrane localization also requires Ras methylation and the enzyme responsible for this is another potential drug target (14). Alternatively, downstream events in the Ras signal cascade involving protein kinases represent other attractive drug targets. These include Raf, mitogen-activated protein (MAP)/ERK kinase (MEK) and MAP kinase. These enzymes are required for normal cell signaling throughout the body. Nevertheless, a variety of agents are being developed and moved into clinical trials based on antitumor activity in preclinical models including antisense Raf, Raf kinase inhibitors, and MEK inhibitors (15). It should be possible to combine *mda-7* treatment with treatment with drugs that block downstream events in the Ras-activated pathway to achieve a similar effect to *K-ras* inhibition. Ras also activates phosphatidylinositol 3-kinase (PI3-kinase) which in turn activates the protein kinase Akt (a suppressor of apoptosis). Thus, inactivation of PI3-kinase or Akt should activate apoptosis in human tumors. Such agents (such as the kinase inhibitor LY294002) have shown activity in preclinical tumor models when combined with a farnesyl transferase inhibitor (16). Thus, it may be necessary to combine not only therapy with *mda-7* and a Ras inhibitor but also to block several steps of the Ras pathway.

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In the preclinical development of these approaches surrogate pharmacodynamic endpoints are being used for the development of such signal transduction inhibitors (15). They will also be essential for the introduction of these agents into the clinic. Thus, it will be of value to show in pancreatic tumor tissues after treatment that *mda-7* protein is expressed, the Ras signaling pathway has been inactivated, Bax protein is expressed, and apoptosis is induced by the combined therapy. These surrogate endpoints will be important where there may not be clearly defined dose-limiting toxicities. Monitoring these pharmacodynamic endpoints will indicate that a therapeutic dose has been given, and if this does not result in a beneficial antitumor effect, the particular approach needs to be reconsidered or discarded.

The complications in the Su *et al.* study (1) are potentially of use as well. Tumor formation was suppressed when *mda-7* adenovirus-infected cells (presumably nearly 100% of cells) were combined with transfection with an antisense *K-ras* plasmid (probably transducing only 10–30% of cells). Nevertheless, the population of tumor cells as a whole was suppressed. This must mean some “bystander” effect occurred whereby the tumor cells receiving both *mda-7* and antisense *K-ras* induced changes that led to cell death in tumor cells with none or only one of these two genetic manipulations. Understanding this bystander mechanism could provide new therapeutic insights as well. Although Su *et al.* state that *mda-7* was effective alone against other human tumors it will be important to see the effect of *mda-7* alone on colon cancer or nonsmall cell lung cancer cells containing *K-ras* mutations. Both of these tumor types are often resistant to our current chemotherapies and when metastatic have a 100% mortality and often short survival. If the combined *mda-7* replacement and *K-ras* inhibition approach was successful and required in preclinical models of these tumor cells, it would add great impetus to the further clinical development of this approach. Another unexpected result of the Su *et al.* study was the effect of *K-ras* inhibition on *mda-7* protein expression. Adenoviral *mda-7* vectors efficiently transfected cells, leading to the expression of large mRNA levels; however, *mda-7* protein expression was not seen until there was cotreatment with anti-

sense *K-ras*. In addition, under no conditions was *mda-7* peptide expression seen in cells (BxPC-3) with wild-type *ras*, so we don't know whether the effect was indeed specific for mutant *K-ras* containing tumor cells. Although these results are puzzling, they suggest there may be a more complex interaction of *mda-7* and *K-ras* pathways and raise the possibility that in tumors with *K-ras* mutations where *mda-7* protein expression can be detected endogenously that *K-ras* inhibition by itself may be therapeutic.

If this combined therapy indeed works how can it be delivered to patients, in proof-of-principle initial clinical tests? There is precedent for giving gene therapies by local injection, and surgical-laparoscopic skills readily exist to test such locally delivered gene therapy. One technical improvement suggested by Su *et al.* (1) is that a combined *mda-7* and antisense *K-ras* delivery system could be constructed into one virus. Other approaches could involve the combination of local *mda-7* adenovirus treatment and systemic

delivery of a *K-ras* inhibitor, or systemic liposomal delivery of the *mda-7* vector as well. Finally, if we knew more about *mda-7* function and targets it is conceivable that a small molecule could be designed as a drug for *mda-7* replacement therapy. We have entered one of the most exciting phases of medical science. While this approach may have unforeseen problems (6), the future holds enormous promise.

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