Experimental technique may have future applications for lung and other cancers.

**Antisense RNA technology successfully blocks K-ras–induced cancer**

Can anything stop the rapid and destructive proliferation of cancer cells while preserving normal tissue? The answer may be yes, according to recent findings by a team of researchers at The University of Texas M. D. Anderson Cancer Center. The team is headed by Jack A. Roth, M.D., chairman of the Department of Thoracic Surgery. They used a relatively new technology in which "antisense RNA" inactivates the K-ras oncogene, a gene known to play a role in the development of lung cancer. Roth and his team's studies are still limited to the laboratory, but using antisense RNA to inactivate oncogenes may eventually have clinical applications for lung and other cancers.

The team's work is based on oncogene theory, one of the most widely accepted explanations for the origin of tumors. The existence of oncogenes, genes that promote the development of cancer when they are activated by carcinogens, had been suspected by tumor biologists since early in this century; these suspicions were confirmed in 1975 when an oncogene was first characterized in normal human cells. Since then, about 60 different oncogenes have been identified. However, said Roth, "the name oncogene is really a misnomer. Oncogenes are not present in cells to cause cancer. They are normal genes present in all normal cells, and they perform very vital functions related to cell growth and cell division. They have in some way become activated by mutation, translocation, or deletion to perform functions that they don't normally perform."

Some oncogenes work in a dominant fashion; that is, change in only a single allele of the gene is sufficient to cause that cell to become cancerous. In other cases, these oncogenes have a negative function: in their normal state, they suppress the growth of the cell, and only when both alleles of the gene are lost is the cell likely to become cancerous. These "negative" genes actually protect against getting cancer; they are usually called tumor suppressor genes. (The classic example is the retinoblastoma gene: both copies of the gene must be deleted or inactivated in order for the retinoblastoma to develop.)

Roth's team selected the ras family of oncogenes for their studies of cell growth in lung cancer. The ras family includes the Kirsten rat sarcoma virus (K-ras) oncogene, the Harvey ras (H-ras) oncogene, and the N-ras oncogene. Originally identified in the rat sarcoma virus, K-ras was one of the first oncogenes to be implicated in human cancer. K-ras is one of the dominant, or what Roth calls positive, oncogenes. It produces a protein called p21 (because its molecular weight is 21,000) that binds to the inner surface of the cell membrane and acts, probably, as a transducer, modifying signals that reach the cell's surface in some way so that the cell can interpret the signal. Mutations in the oncogene alter the protein's transducing ability. The precise mechanism of signaling and function, however, is not clearly known.

**Normal function of K-ras is altered**

The researchers did know, however, that single nucleotide mutations in the ras genes in human cells caused these cells to take on the features of cancer cells: rapid growth, tissue invasion, and metastasis. In the mutated state, ras genes are constantly activated rather than being switched on and off by the signals at the cell surface. Ras investigators think this constant activation has something to do with the cell becoming malignant. Therefore, the M. D. Anderson Cancer Center team wanted to figure out a way to inactivate ras. Previous studies had found ways to do this, but these methods had also destroyed the cells needed for normal functioning. A new method was needed that would inactivate ras while preserving normal cells, targeting only the mutated genes so that the normal ras gene could help keep the cell alive and functioning.
"We're trying to alter the cancer cell directly by altering the expression of cancer genes."

"Antisense" technology counteracts the cancer-causing protein

The advent of antisense RNA technology made such an objective possible. In Roth's studies, an antisense RNA construct was injected into a commonly used experimental lung cancer cell line in which K-ras was known to spontaneously mutate. The construct caused the cells to express an artificial gene that produced messenger RNA that was exactly complementary ("antisense") to the abnormal RNA produced by the cancer cell. For example, for all cytosine nucleotides in the cancer cell's RNA, the antisense RNA contained complementary guanine nucleotides that bound to the cytosines. The same is true for the other pair of complementary nucleotides: uracil and adenine. The binding of these complementary nucleotides forms a K-ras-antisense RNA duplex that essentially inactivates the abnormal RNA's ability to produce the cancer-causing protein (Figure 1). In these experiments, the growth rate of the cells slowed to about one third of their malignant rate, closer to the normal rate of growth, and continued to function normally. When the antisense RNA was injected into cells that did not have mutated K-ras, the cells were unaffected. The team also tested these cells in animals. The cells with the cancer-causing mutated gene were injected into nude mice, which began to develop fatal tumors. The mice whose injected cells were first treated with antisense RNA did not develop these tumors. This inactivation of the K-ras protein had no effect on the other ras gene products.

Future research will focus on the clinical applications of this technique. Although others have heralded the development as a step toward gene therapy, Roth sees the technique as more promising in the area of what he calls molecular therapy. "We're trying to alter the cancer cell directly," he said, "by altering the expression of cancer genes." He doubts whether gene therapy, which has gotten much attention in recent years, will have many applications in cancer. He sees gene therapy, which he defines as inserting a normal gene into a cell to produce a protein that cannot be produced by the defective host gene, chiefly as a drug delivery system. Molecular therapy, on the other hand, involves changing how the gene behaves. Roth sees the major challenge in this technique as increasing the efficiency of uptake of the antisense constructs by the cancer cells; experiments now under way are looking at viral systems for delivering the antisense RNA. For example, a retrovirus carrying the antisense RNA would infect the proliferating cancer cells and leave the normal cells unaffected. Although Roth warns that such a method is far from perfected, he is optimistic that such an agent may be ready for clinical trials in a few years.

Roth also points out that cancer cells probably have about 15 to 20 different gene abnormalities; researchers are beginning to identify many of these in

continued on page 7
Renowned as a giant of pediatric oncology and beloved by patients, colleagues, and friends, Grant Taylor, M.D., is recognized internationally as a founding father of pediatric oncology in the nuclear age. After receiving his M.D. from Duke University School of Medicine, he entered the military and was decorated for valor as a battlefield doctor in World War II. In the late 1940s, he helped establish the Atomic Bomb Casualty Commission (ABCC) and directed its initial study of the effects of radiation bomb survivors in postwar Japan, where he recorded some of the first cases of lymphoid neoplasia resulting from radiation exposure. Taylor is remembered not only for his medical skill and leadership, but also for his compassion and deep sensitivity to the trauma of the bomb survivors. His tenure at the ABCC set the stage for his years at The University of Texas M. D. Anderson Cancer Center, which he joined in 1954 as head of pediatric oncology. At M. D. Anderson Cancer Center, he assembled one of the most effective teams to manage and develop therapies for childhood leukemia.

—James M. Bowen, Ph.D., Professor of Virology, Vice President for Academic Affairs

Hiroshima, 1949—Science in the midst of suffering.

The human face of data

On a small hill at the edge of Hiroshima in 1949, the Atomic Bomb Casualty Commission (ABCC) established its permanent quarters in eight buildings of corrugated iron. Grant Taylor, M.D., then director of the ABCC, hoped the location would attract bright American researchers to study the effects of the atomic bomb on Hiroshima’s survivors. The buildings were spare, but the view was beautiful. Amidst tall pine, one could see the seven small peninsulas of Hiroshima below, where the delta of the Ota river met the Inland Sea, and on the hill, far from the hypocenter of the blast, the atmosphere was serene.

And perhaps deadly. Taylor, now professor emeritus at The University of Texas M. D. Anderson Cancer Center and The University of Texas Health Science Center at Houston, recalls that in 1949 little was known about radiation. How long would the bomb’s effects last, and would situating the ABCC in Hiroshima endanger the researchers? As a precautionary measure, workers were housed in Kure, a small town outside Hiroshima, and were bussed into the ABCC each day.

The ABCC’s purview was strictly research. It was to retrieve as much medical data as possible about survivors, focusing on their location at the time of the blast and the symptoms they experienced since then. But it became obvious to Taylor that his endeavors could not remain dispassionately scientific. From his first visit to Japan, his experience there would be colored by powerful images of destruction and intense human pain. None of his research could be performed without in some way reminding him of some aspect of the horror of the bomb.

Awed by the devastation

“I made my first visit to Japan in 1946 during my military assignment in Seoul,” Taylor said. “I went first to Hiroshima, on to Nagasaki and Sasebo, and then back to Hiroshima. I’ll never forget the devastation. But the physical destruction was only the tip of the iceberg in terms of the total human impact. The extent of outright deaths, those that slowly died, and the crippling and the physical and mental anguish of untold thousands was yet to be evaluated.”

While in Nagasaki, Taylor explored the remains of the pathology building in the medical center. “Two walls of the main pathology unit remained standing. Against one of them, on a shelf, a stack of histologic slides had melted into a solid block of glass; some had melted so that the glass hung in long ‘icicles’ from the edge of the shelf. I could reconstruct the furnishings of the room by the way the ashes had fallen.”

Taylor made his way to the medical school, one of the few structures that remained standing. He found the dean sitting behind what Taylor calls an “improvised desk.”
"He was the only one who survived. He was completely submerged when the bomb exploded."

Taylor could find no words that seemed appropriate. "We just sat and looked at each other. Finally, he told me that he had remained in his office late the night before the bomb to reprimand one of his professors and then departed for his home in the country. Now he was obsessed with the thought that there was no way to unsay the words he had used."

The dean then offered to introduce Taylor to a medical student. "The student had gone swimming with friends from the pathology laboratory," Taylor said. "He had been reluctant to return and pled with his colleagues to wait for him while he took one more dive. He was the only one who survived. He was completely submerged when the bomb exploded."

After his military assignment, Taylor accepted a position at Duke University Medical School in Durham, North Carolina. "My research program progressed very nicely, and as associate dean I enjoyed my student and faculty responsibilities," Taylor said. But that was to change when he accompanied Wilbert C. Davison, M.D., dean of the school, to Washington, D.C., for a planning meeting of the ABCC.

Data analysis a daunting task

He had heard about the newly formed commission, but had some doubts about its feasibility. What kind of data should they gather? No one was quite sure. "Aside from some mouse studies by Ernest Goodpasture, little in the scientific literature was helpful to those interested in studying the medical effects of irradiation," Taylor said. But even if appropriate data were gathered, the amount would be massive. How could such data be analyzed? While Taylor was in Washington, D.C., his doubts were allayed by two encounters, one with an emphatic man, the other with an odd-looking contraption.

"Dr. John Lawrence of the UCLA Medical School had a big impact on me. He told me, 'When you don't know what to look for, comprehensively examine your patients and compare them with appropriate controls!'" Simple advice, but said with enough conviction to give Taylor confidence.

Later Taylor visited the Library of Congress, where he saw a strange mechanical device. "It was primarily a cataloging system for book titles and author names. Information was stored on 16-mm film. The film would stretch every time the braking system was applied, but I was very impressed, for here was a device that could be used to store large amounts of medical data. The availability of a machine like the one I saw (Taylor later arranged for one to be sent to Hiroshima) and Dr. Lawrence's comments were the reason for my going to the ABCC."

Courtesy a veil for sorrow

So it was that Taylor returned to Hiroshima in 1949. Surprisingly, he found the Japanese courteous and friendly. "Although food was scarce and they lived in dwellings largely made with debris from the blast, they generally did not react toward us as though we had been the cause of their tragic misfortune," he said.

But beneath the courtesy there was much sorrow. Taylor was invited by the chairman of the city council to attend a party, which would also be attended by the governor, the mayor, and other local officials. Taylor and other ABCC personnel typically refused such invitations as graciously as they could. The prospect of being entertained while people in the city were under such economic hardship was something Taylor wanted to avoid. But he accepted this invitation because of the arrival of Dr. Alan Gregg from the Rockefeller Foundation. "The Japanese were very excited about his arrival, so we accepted their invitation."

The party began with a cordial introductory welcome by the chairman of the city council. "He stood in the center of the room, facing me," Taylor said. "And then his manner abruptly changed. In a deeply emotional voice he said, 'Tonight I am going to tell it!' He told of the blast, first the blinding bright flash, the thundering crash, the demolition of his home, the darkness and the heat. Frantically he had moved through the wreckage of his home, which was beginning to burn to the area where his children slept. His home was a tangled mess of planks, tile, wooden frames, and sheets of zinc roofing. He could not reach where his children had been sleeping, so he tried to crawl under the wreckage. He was choking with smoke; it was warm and he couldn't see. His outstretched hand touched a foot. It was one of his sons; nearby he located the body of his other son. Both were dead.

"The chairman, who had been standing in front of
me, slowly turned and made his way across the room to his ozan, where he stood, sobbing, and his shoulders shaking. His sobs were the only sound in the room. I did not know what to do, but with an outstretched hand I rose and made my way slowly toward him. He shoved his hand into one of his pockets and brought out an engraved Zippo lighter I had previously given him. He raised his hand and shouted ‘Okay! Okay! Okay!’ The party was over.”

It was such events, the courtesy of the Japanese notwithstanding, that colored the experience of every American in Hiroshima. Every survivor of the blast probably had a similar tale to tell, which made conducting scientific studies all the more difficult. Using Japanese midwives as translators, ABCC personnel would go to the homes of survivors, many of whom were very ill, to interview them. In some cases, however, survivors were picked up and brought to the ABCC complex, which did not go over very well with some of the Japanese. “Some were very suspicious of us taking sick people, some of them on stretchers, to the facility,” Taylor said.

Three ABCC researchers go to M. D. Anderson

Ironically, the data that Taylor spent years gathering had no direct application to his subsequent career as a pediatric oncologist, but the ABCC had a tremendous impact on M. D. Anderson. Taylor had heard about a fledgling cancer hospital in Houston named The University of Texas M. D. Anderson Hospital and Tumor Institute. As his term with the ABCC came to an end, he spoke with the hospital’s director, R. Lee Clark, M.D., at a meeting in Washington. Clark convinced Taylor to direct pediatric cancer research at M. D. Anderson.

But Taylor did not go to M. D. Anderson alone. During his tenure at the ABCC, he met two bright, young physicians. One a Houston woman educated in biology and chemistry at Rice University and in medicine at Duke University, and the other a Japanese-American physician whose medical training had been temporarily interrupted by detainment in a WWII internment camp.

Margaret P. Sullivan, M.D., spent 18 months at the ABCC beginning in 1952. She and her group were responsible for performing health examinations of 5,000 children—2,500 who had been exposed to the bomb’s radiation and 2,500 who had not. Impressed by her keen intelligence and toughness, Taylor later asked her to join his staff at M. D. Anderson. Sullivan works there to this day.

Taylor was also accompanied by Wataru W. Sutow, M.D., who at the ABCC had been in charge of the pediatric research group. Sutow had spent one two-year term at the ABCC beginning in 1947, but in 1950 he was called into the U.S. Army Medical Corps. After officer training, he returned to the ABCC for an additional term. His ability to develop an instant rapport with the children amazed Taylor. Sutow was renowned for the “Sutow magic toy” (a tape measure, much loved by infants) and for his ability to captivate preschoolers by drawing a butterfly or a flower on the back of their hands. Sutow remained a physician at M. D. Anderson until 1981, when he died of lung cancer. Ever the scientist, Sutow demanded that his colleagues assure him that tissue methotrexate levels would be taken and studied after his death.

Taylor said that one thing that he, as well as Sutow and Sullivan, brought back with them was hope. “We had begun to see an increased incidence of leukemia at the ABCC, a late effect of the radiation. By the time we came to the States, chemotherapy was beginning to show promise for leukemia. After we had seen the death in Hiroshima, the possibility of life was very exciting.”

Portions of this article were adapted, with permission, from Remembrances and Reflections, written by Grant Taylor and edited by Don Macon and John G. McGovern, M.D., The University of Texas Health Science Center at Houston, Houston, 1991.

Physicians who desire additional information may write Grant Taylor, M.D., Department of Continuing Education, The University of Texas Health Science Center at Houston, P.O. Box 20367, Houston, Texas 77030, or call (713) 792-4671.
"When the breast mound is restored before the patient recovers from her anesthetic, much of the psychological pain... is avoided"

Breast reconstruction
continued from page 8

of the shorter incisions and limited exposure, but when properly executed allows the reconstructive surgeon to closely match the shape of the original breast (Figure 1). Moreover, if the scars are limited to the areolar area and to the upper lateral quadrant of the breast, low-cut dresses and bathing suits can be worn without fear of scars becoming exposed. If reconstruction is being accomplished with simple implant insertion or with tissue expansion, the additional preserved skin allows much better ptosis and a better chance of achieving a normal breast shape (Figure 2). Even when flaps are used to replace missing skin, however, the amount of exposed distant skin (which may have a different color or texture from that of the surrounding normal breast skin) and the extent of visible scarring are reduced. For these reasons, the cosmetic appearance of immediate breast reconstructions is almost always better than that of reconstructions performed at some later time.

Risk of tumor recurrence not increased

Many patients and physicians have asked if immediate reconstruction and preservation of uninvolved breast skin adversely affects the risk of tumor recurrence. Provided that patients are properly selected, we have found that such risks are not increased. Out of 140 patients with early breast cancer followed for over one year, only three have developed local recurrences. This number is actually lower than that expected from a comparable group after a more conventional mastectomy, supporting the concept that preservation of uninvolved skin is not harmful. Appropriate candidates for a skin preservation mastectomy include patients who have T2 and smaller tumors (less than 5 cm in diameter) without clinical skin involvement. Any skin within 1 cm of the tumor should be removed, along with the nipple, the areola, and any biopsy scars. Patients with advanced tumors, who will require postoperative radiotherapy, are not appropriate candidates. Although they still may be candidates for eventual breast reconstruction, it is usually better to do so later.
"The cosmetic appearance of immediate breast reconstructions is almost always better"

Antisense RNA technology
continued from page 2

a variety of cells. It appears that these gene abnormalities may be very dependent on one another and function in a cascade; that is, a certain sequence of mutations or abnormalities must occur to cause a cell to become malignant. In the K-ras experiments, the team was very surprised that interrupting just one event caused such a profound change in the character of the cells. They did not expect it to cause such a large decrease in tumor growth or growth rate. A follow-up experiment on another gene abnormality, p53, which is found in many different kinds of tumors, also showed a profound and dramatic decrease in malignant cell growth. The team concluded from this finding that it may not be necessary to reverse every one of the 15 or 20 malignant changes that are present in the cancer cells; it may be enough to interrupt only one or two of them.

Molecular therapy may have uses for cancer prevention
Roth foresees that this technique may be broadly applicable to a wide variety of tumors. The prevention of cancer may be another application; alteration of the genetic changes may be possible at the premalignant stage. Roth's team has, in fact, recently identified oncogene mutations in premalignant lesions. This gives researchers the opportunity to identify patients who may be at risk of developing cancer very early and to try to manipulate the genes before the cancer develops. For example, patients who smoke, have changes in their bronchial mucosa, or have had previous lung or throat cancers have a high risk of developing lung or esophageal cancer. These patients would be monitored very carefully for premalignant conditions, and molecular therapy could be used to inactivate the genes causing these conditions before they become malignant.

Physicians who desire additional information may write Jack A. Roth, M.D., Department of Thoracic Surgery, Box 109, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, Texas 77030, or call (713) 792-6932.

MD Anderson Oncolog
The University of Texas M. D. Anderson Cancer Center
President, Charles A. LaMaistre, M.D.
Vice President for Academic Affairs James M. Bowen, Ph.D.
Associate Vice President for Academic Affairs Robin R. Sanderfer, Ph.D.
Director, Department of Scientific Publications Walter J. Pagel
Editor, Kevin Flynn
Contributing Editor Kathryn L. Hale
Production Edith K. Wilson
Photographs Donald G. Kelley
Editorial Board
David M. Gershenson, M.D.
Frankie A. Holmes, M.D.
Raymond E. Meyn, Jr., Ph.D.
William Plunkett, Ph.D.
Tyvin A. Rich, M.D.
S. Eva Singletary, M.D.
Michael J. Wargovich, Ph.D.

Published quarterly by the Division of Academic Affairs, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, Texas 77030.

Made possible by a gift from the late Mrs. Harry C. Wiess and by grants from The University Cancer Foundation.
Immediate breast reconstruction reduces the trauma of mastectomy

By Stephen S. Kroll, M.D.

Since late in the past decade, The University of Texas M. D. Anderson Cancer Center has been a pioneer in the concept of breast reconstruction immediately following mastectomy. This early performance of reconstruction has become very popular among women who must undergo mastectomy. The first and most obvious reason for this is convenience for the patient. She can obtain her breast mound reconstruction without having to take time off for a separate hospital admission, and she leaves the hospital with her breast contour restored. This early rehabilitation not only preserves time and effort, but also provides a second advantage in that she is spared the psychological trauma of having to live with physical deformity. Although not all women are bothered by such deformity, often those who are young or single are considerably upset by it. When the breast mound is restored even before the patient recovers from her anesthetic, much of the psychological pain caused by loss of the breast is avoided.

A third advantage is that of reduced expenses. The patient, already obligated to remain in the hospital for at least a few days recovering from her mastectomy, can recover from her reconstruction at the same time for little or no additional cost. Anesthetic risks and costs are also reduced, since she is already anesthetized when the reconstruction begins and a separate anesthetic induction is not required.

Another significant benefit is the aesthetically improved outcome. When reconstruction is immediate, the reconstructive surgeon and the oncologic surgeon can work together to conceal the scars and preserve uninvolved breast skin. This teamwork adds to the technical difficulty of the mastectomy because

continued on page 6