CANCER RESEARCH

Novel Anticancer Agents Move Closer to Reality

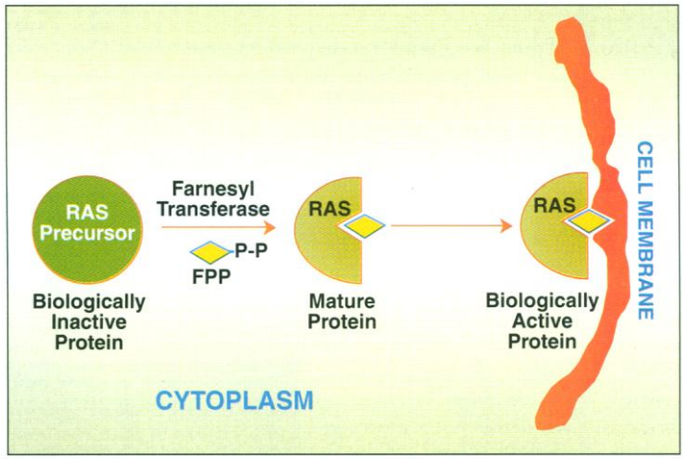
During the past decade, the marriage of cancer research and molecular biology has produced a much better understanding of the many biochemical changes underlying cancer development. Only recently, however, have researchers begun to apply that growing body of knowledge to the search for better therapies for cancer patients. Now, two reports in this issue of *Science* may have brought one potential treatment aimed at correcting a biochemical alteration of cancer cells a step closer to reality.

On pages 1934 and 1937, two independent teams, one from Merck Research Laboratories in West Point, Pennsylvania, and another including researchers from the biotech firm Genentech Inc., in South San Francisco and the University of Texas (UT) Southwestern Medical Center in Dallas, describe new compounds that apparently block the ability of mutated *ras* genes to make cells cancerous. (A third group from the Japanese-owned Eisai Research Institute in Andover, Massachusetts, has similar work submitted to the *Journal of Biochemistry* but was reluctant to discuss unpublished results.) A so-called oncogene, *ras* may contribute to as many as one-fifth of all human cancers, including more than half of such common ones as colon cancer.

While tests of the new drugs' effectiveness and safety are just beginning in lab animals, the hope is that they, or more likely their improved successors, will one day prove effective for treating human tumors in which *ras* activity plays a role. "We're finally targeting one of the fundamental mechanisms responsible for malignant transformation," comments Allen Oliff, Merck's director of cancer research.

A great deal of work has shown that the proteins encoded by *ras* genes occupy a central position in the signaling pathways used by cells to respond to growth factors. What apparently happens in cancerous tumors is that as a result of mutations in the genes, Ras proteins are always "turned on" so that they constantly promote cell division, even in the absence of stimulation by growth factors. But before either the normal or mutated Ras proteins can do anything they have to undergo a series of modifications that enable them to settle in their final home, a spot on the cell membrane from which they relay growth signals to the cell interior (*Science*, 1 November 1991, p. 650).

As details of how a newly formed Ras protein becomes biologically active emerged in recent years, intriguing possibilities began to stir in the minds of cancer researchers: Perhaps by interfering with the modifications of mutated Ras proteins, they might be able to keep the proteins from attaching to the membrane—and thus might be able to keep the growth switch off and prevent uncontrolled cell division. In theory, drugs designed on this principle might prove more powerful and have fewer side effects than conventional chemotherapeutic drugs, which attack rapidly dividing cells, whether they are cancerous or not.



MERCK RESEARCH LABORATORIES

Hooked up. Potential anticancer compounds block Ras attachment to the membrane by inhibiting the farnesyl group transfer.

Both teams now describe compounds that home in on the earliest step in Ras maturation, the transfer of the farnesyl group from farnesyl diphosphate—a cellular chemical used to synthesize cholesterol—to the protein by the enzyme farnesyltransferase. After a few other modifications, the protein moves to the cell's inner edge and the farnesyl group acts as a molecular hook that allows Ras to attach to the cell membrane. Since that's the essential first step in the production of a functioning Ras protein, this process, known as farnesylation, is a prime target for drugs aimed at preventing Ras activity.

In the past few years, those shooting for this target have often set their sights on the so-called CAAX box, a sequence of four amino acids—a cysteine followed by two aliphatic amino acids followed by any one of several different amino acids—that form the attachment site for the farnesyl group. If cancerous cells could be flooded with harmless CAAX-containing peptides or even nonpeptide chemicals that have the same overall shape, they might outcompete mutant Ras for the attention of farnesyltransferase and prevent the enzyme from attaching the farnesyl group to Ras itself.

That supposition was quickly born out when researchers tested CAAX box peptide mimics in cell extracts. But they encountered a road block when they tried to use the same compounds with whole cells. "The problem with peptides is they don't enter cells very well," explains Michael Brown, who with his longtime colleague Joseph Goldstein led the University of Texas team. (Brown and Goldstein also shared the 1985 Nobel Prize for their contributions to understanding cholesterol metabolism.) Peptides have trouble infiltrating cells because they carry charges that prevent them from crossing the lipids of the cell membrane, and even if they do cross that barrier they are readily broken down by the many proteases in cells. So the trick was to find a compound more lipid-loving in nature and more resistant to degradation while preserving the CAAX box's essential structure.

With some computer-aided structural analysis pointing the way, the Genentech-UT collaboration did just that by eliminating the two interior amino acids of a CAAX box peptide and replacing them with the organic molecule benzodiazepine. Benzodiazepine apparently has the same three-dimensional structure as the two amino acids it replaces but allows the altered compound to penetrate cells and protects it from proteases. The Merck group took a different, but equally successful, tack by modifying a CAAX-based peptide inhibitor that had worked well in test tubes but couldn't permeate cells. In addition to slightly altering the peptide to ward off degradation, they added a lactone to mask charges temporarily and help sneak the compound through the cell membrane, explains Merck's Jackson Gibbs.

And once inside, these inhibitors of farnesylation proved their worth. The Merck researchers, for example, took cells that had been made cancerous by introducing a mutated *ras* oncogene into them. They then incubated these "transformed" cells with micromolar concentrations of their compound. The result: The farnesyl hooks apparently weren't attached to the mutant Ras protein, which simply floated free in the cell as a result. In another experiment, the Merck group showed that cells transformed by a *ras* oncogene did not form their usual multiple,

large colonies in the presence of Merck's inhibitor, instead growing in a manner similar to that of more normal, untransformed cells. The Genentech-UT collaboration also showed they could reverse cancerous transformation by *ras*, restoring essentially normal growth patterns to cells that express a *ras* oncogene.

Considering the effectiveness of the enzyme inhibitors in test tubes, these results were not wholly unexpected. The crucial question now is whether inhibiting farnesyltransferase action might have too dramatic an impact on cells. To function normally, a cell must keep some level of Ras activity. Moreover, other vital proteins such as nuclear lamins and certain others found in the visual system must also be farnesylated before they become functional.

One encouraging sign, however, is that both groups' compounds do not seem toxic, at least to cells in lab cultures. Normal cells and those transformed with oncogenes other than *ras* seemed to grow as usual when exposed to the farnesyltransferase inhibitors. "In these particular cases, I think we lucked out. It appears we can limit farnesylation without seriously affecting normal cell growth," says Merck's Oliff. This result, as welcome as it may be, creates a lot of future work for puzzled scientists. "How do [these inhibitors] selectively target *ras*-transformed cells without affecting the viability of normal cells?" asks Channing Der of the University of North Carolina at Chapel Hill.

The simple answer is that nobody knows, but there are a number of possible explanations that researchers are exploring. As with many drugs, for example, the effect may simply be a matter of dosage, says Oliff, suggesting that there is a "therapeutic window" in which the compounds inhibit farnesylation just enough to prevent uncontrolled cell division while allowing the enzyme to keep the cell functioning. Still, simply documenting that certain cell lines appear to grow normally does not mean that the farnesyltransferase inhibitors can be used without dangerous side effects in living animals, cautions Genentech peptide chemist James Marsters: "Growth in cell culture and effects on animals are often two very different things."

Whether or not new chemotherapeutic drugs emerge from this work, researchers are also excited at the prospect of using these new inhibitors to understand better the process of farnesylation in general, and the function of Ras in particular. "For us it's going to be extremely useful as an experimental tool," says UT's Goldstein. For those dreaming of cancer treatments and profitable drugs, however, the hope is that farnesyltransferase inhibitors will prove to be much, much more than that.

–John Travis

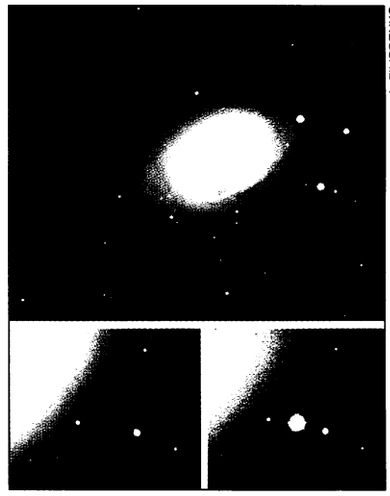
MEETING BRIEFS

Astronomers Watch the Stars Come Out in Berkeley

New and strange sightings caught the attention of astronomers at this June's American Astronomical Society (AAS) meeting in Berkeley: a supernova that has changed its identity, a clutch of mysterious blue stars, and objects at the edge of the universe, shining brilliantly at the far end of the ultraviolet spectrum. Meanwhile, a more familiar object–one species of supernova–is raising hopes of predicting the ultimate fate of this cosmic zoo.

A Split-Personality Supernova

Three months after its explosion, supernova 1993J has dwindled to nothing more than a wispy smudge, even in the 30-inch telescope at the University of California, Berkeley's Leuschner Observatory. But the great supernova of 1993 is far from forgotten. For one thing, the nearness of its host galaxy M81 made this supernova the brightest to burst into the northern sky since 1937. For another, Berkeley astronomer Alex Filippenko told the AAS meeting, SN1993J just isn't staying true to type. Over the past 2 months, Filippenko and other researchers studying its spectral lines —the fingerprints of its elements—have watched it shift from one established type to another, defying the known supernova categories. In the end, this metamorphosing explosion may force astronomers to redraw their supernova taxonomy.



A. FILIPPENKO

Shape-shifting supernova. Before *(left)* and after images show the explosion.

At first, lines of hydrogen in the supernova's spectrum had marked it as a classic type II, an event scientists believe originates when a massive star, eight or more times the mass of the sun, suddenly runs out of nuclear fuel. The core of the star then collapses into a dense ball of solid nuclear particles, sending out a shock wave that blows away the bulk of the star's mass. Some of the progenitor star's hydrogen, however, lingers around the core.

But, over the past 2 months nearly all of the hydrogen has vanished from the spectrum of SN1993J, while the signature of helium has strengthened. Helium is the trademark of another kind of supernova, type Ib. Along with another subspecies, type Ia, these explosions are thought to originate in white dwarf stars when they siphon enough material from a companion star to trigger a vast thermonuclear explosion. White dwarfs lack hydrogen but may harbor helium.

This seeming transformation may actually reflect a problem with the classification scheme, says Filippenko. After all, it has happened before. A 1987 supernova also showed hints of hydrogen at the beginning but later was dominated by helium, Filippenko points out. At the time, he suggested that the 1987 supernova was really "a type II without its clothes on." Like a conventional type II, he proposed, the explosion took place in a collapsing massive star, but a companion star had stripped away most of its hydrogen atmosphere before the explosion, leaving only small traces that could later be swamped by emission from the deeper layers of helium. If all type Ibs originate this way, he suggested, they are really a special case of type II.

Supernova 1993J provides confirmation, says Filippenko, because he and his colleagues had spotted the progenitor star on earlier photographs of the galaxy, and it was a giant star, not a white dwarf. Says Filippenko, "I'm overjoyed. It confirms the idea that I advanced 6 years ago." Agrees astronomer Brian Marsden of Harvard University, "This proves that type IIs and Ibs are related."

The finding may do more than clarify supernova taxonomy, says theorist Stan Woosley of the University of California, Santa Cruz. Studying exceptions like this one may help astrophysicists understand the mechanism of these mega-explosions. Says Woosley, "Because it's an unusual beast, there's a chance to learn a lot from it."

Tough Company in M15

The center of the globular cluster M15 is a rough-and-tumble neighborhood. This dense knot of stars, 30,000 light-years away near