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Drug Wrecks the Power Plants of Cancer Cells

Researchers have identified a compound that selectively kills tumor cells by destroying their metabolic power plants. The researchers believe that the compound, code-named F16, could serve as a model for a targeted chemotherapy with low toxicity.

In an article published in the July 2002 issue of the journal *Cancer Cell*, researchers led by Howard Hughes Medical Institute senior investigator Philip Leder at Harvard Medical School reported that they screened 16,000 small molecules to look for compounds that would have a favorable effect on transgenic mouse cells engineered to overexpress the cancer-causing gene *neu*. The human counterpart of *neu*, which is called *HER-2*, has been implicated in 20-30 percent of human breast cancers, and is linked with a poor prognosis for breast cancer treatment.

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— Philip Leder

Because *neu* or its human analog are such important elements in breast cancer, we decided to carry out these experiments to identify metabolic pathways that might collaborate with *HER-2* or *neu* in the development of malignancy, said Leder.

In the experiments, the papers lead author, HHMI associate Valeria R. Fantin, introduced the *neu* gene into mouse mammary epithelial cells. Mouse mammary epithelial cells that overexpress *neu* bear some of the characteristics of human breast tumors. Fantin then tested each of the 16,000 molecules to see how it affected the growth of the transgenic and normal mouse cells. These studies showed that F16 selectively inhibited the growth of the *neu*-overexpressing cells, but not the normal cells.

Additional studies indicated that F16 also inhibited the proliferation of a number of mouse cancer-cell lines that were derived in Leder's laboratory and a panel of human breast cancer cell lines. The researchers found that F16 prevented the formation of tumors that would normally occur when *neu*-overexpressing cells are injected into otherwise healthy mice.

In investigating how F16 selectively killed the cells in which *neu* was overexpressed, the physical properties of the molecule yielded an approach to solving the mystery, Leder said. It turned out that when one of our co-authors, Marcelo Berardi, looked at the structure of the molecule, he recognized the possibility that it might be fluorescent. So, when we looked at the pattern of fluorescence within a cell when it took up F16, it resembled the pattern that would be given by using dyes that selectively stain mitochondria, he said.

Mitochondria are organelles that supply cells with energy. It has long been known that cancer cells undergo complex metabolic changes, which affect the mitochondria. In particular, damaged mitochondria can trigger programmed cell death, called apoptosis, by releasing the chemical cytochrome c.

Leder and Fantin collaborated with HHMI investigator Stanley J. Korsmeyer and Luca Scorrano at the Dana-Farber Cancer Institute to characterize how F16 interacted with the mitochondria. According to Fantin, the F16 molecule possesses a widely distributed positive charge on a lipophilic core that attracts it to the negatively charged membranes of mitochondria of cancer cells and allows the molecule to traverse them. The higher negative charge is a property that seems to be characteristic of mitochondria in many cancer cells. Because these mitochondria have a higher negative transmembrane potential, we believe that this compound is selectively concentrated by them, said Fantin.

The effects of high F16 concentrations on cancer cells' mitochondria are dramatic, Fantin said. Electron microscopy studies showed that when the mitochondria take up F16, they become swollen, and eventually the outer mitochondrial membrane ruptures, she said. And when we looked at markers of apoptosis like cytochrome c release, we could see clear evidence of such release in F16-affected cells.

According to Leder, F16 and perhaps other related compounds have properties that may make them promising anti-cancer drugs. First, F16 inhibits growth and induces cell death of tumor cells while apparently sparing normal cells, said Leder. And it does so by virtue of a property of tumor cells that can be exploited by this drug namely, the high charge of the tumor mitochondria, as compared to that in normal cells.

Secondly, this compound seems to be active at relatively low concentrations, which will be important in reducing any toxicity that this class of compounds may have as an anti-tumor agent, said Leder.

Leder and his colleagues are now exploring the metabolic and genetic basis for the difference in membrane potential between normal cells and tumor cells. They are also beginning studies to understand the effectiveness of F16 and related compounds on tumors.

We believe that this study also illustrates how basic investigations designed to answer fundamental questions about the mechanism of cancer can, at the same time, provide very interesting and practical leads such as this one, which may turn out to be of value in the future, said Leder.