Cell Biology Yields Clues to Lung Cancer

Researchers are growing lung cancer cells in culture and are learning how these cells multiply—and how to stop them

Small cell cancer of the lung (SCCL), often called oat cell cancer, is one of the most common and also one of the most deadly cancers, killing nearly all patients within 2 years. It also is something of a medical curiosity because the cancer cells secrete a variety of hormones including calcitonin, bombesin, arginine vasopressin, and ACTH. In culture, the cells secrete up to 18 different hormones.

Because of this hormone secretion, many patients get paraneoplastic syndromes, which are adverse side effects of hormone secretion. For example, some patients develop Cushing's syndrome from excess production of ACTH by the SCCL cells—they get high blood pressure, wasted muscles, florid faces, and hirsutism. Many of the symptoms of the cancers—such as depression, muscle weakness, and loss of appetite—may be caused by the hormones that the tumors produce.

This hormone production was, for years, called ectopic secretion, meaning that the tumor cells were metabolically deranged. But now researchers believe that the hormone production reflects what happens normally in certain nonmalignant lung cells. And they are using this hormone secretion as a tool to study the cell biology of SCCL and the normal lung. As a result, they are nearing new diagnostic tests for SCCL, improved treatments, and a better understanding of normal lung cells.

George Sorenson and Olive Pettengill of Dartmouth University Medical School and, independently, Adi Gazdar and his associates at the National Cancer Institute (NCI) have established numerous cell lines of SCCL cells. "The reason there has been some progress with small cell cancer is that we were able to establish the cells in culture," says Sorenson. "These cells produce hormones so they are somewhat different than other types of tumor cells. Because of that, one has handles."

Desmond Carney and his associates at the NCI recently worked out a way to grow the cells in a defined medium, without serum, and thereby substantially increased their success rate in culturing the cells. In conventional tissue culture systems, which include serum, only 30 to 40 percent of cells from fresh tumors grow. Carney, however, can grow 80 to 90 percent of cells with his system. One of the keys to his success is to give the lung cancer cells some of the same hormones as the cells produce, such as bombesin and arginine vasopressin. If the tumors require their own hormones to grow, as these results suggest, it may be possible to treat SCCL by preventing the cells from getting those hormones, for example, by blocking cell surface receptors for them.

Using their cell culture system, Carney and his colleagues can selectively grow SCCL cells from clinical samples containing both normal and malignant cells. They then can test the cells for drug or radiation sensitivity. Small cell cancers, unlike other lung cancers, initially are exquisitely sensitive to chemotherapy and radiation, although patients eventually relapse and their cancers become resistant to treatment.

"We are able to do drug testing and correlate it with a retrospective analysis," says Carney. "For example, if a patient failed to respond to six drugs we can test his tumor in our assay and it will be resistant to those drugs. But the assay is not as good for telling sensitivity." Thirty to 40 percent of the time, the assay will predict incorrectly that a patient's tumor will respond to a particular drug. "The assay is good mainly for identifying drugs the patient should not receive," says Carney.

Using Gazdar's cell lines, Frank Cuttitta and John Minna of the NCI have made a collection of monoclonal antibodies specific for several different markers on the surfaces of SCCL cells. These antibodies could be used to diagnose and type lung cancers and, eventually, to deliver drugs or radiation directly and specifically to the cancer cells. Cuttitta and Carney find that some of these antibodies block the growth of cultured SCCL cells and so these cancers may respond to the antibodies alone. Cuttitta also has made antibodies against bombesin which binds to the hormone's active site and thus could be used to prevent the hormone from stimulating the growth of SCCL cells.

Since the SCCL cells produce hormones, a number of investigators hope that the concentrations of the hormones in patients' blood may be a measure of response to therapy or that they may be able to use hormone production by isolated cells to diagnose SCCL. Kenneth Becker of the Veterans Administration Hospital and George Washington University Medical Center in Washington, D.C., for example, measures serum and urine calcitonin and finds that calcitonin levels remain high in those patients who do not respond to therapy. But there is a problem with this approach—it is not sufficiently specific. Becker explains, "We realize that although small cell cancer has a tremendous propensity to release hormones, other cancers can also release them." What is needed, then, is a more specific marker—perhaps a spectrum of hormones that would characterize SCCL.

The NCI group, in collaboration with Terry Moody of George Washington University, Paul Marangos of the National Institute of Mental Health, and Mark Zweig of the National Institutes of Health Clinical Center examined many SCCL lines and cultures and found that they all consistently express very high concentrations of three markers—bombesin, neuron-specific enolase, and the brain form of creatine kinase. The concentrations of these markers are much lower or absent in other kinds of lung cancer. "Serum measurements of these substances can be used as clinical markers for disease extent and response to therapy," Gazdar says.

Sorenson together with Pettengill and their colleagues at Dartmouth find that when they grow small cell cancers in culture, the cells produce a vast array of hormones, including peptide hormones, glycoproteins, and estrogen. Their particular interest is to learn how this hormone production is controlled and to relate it to hormone production by normal lung cells.

One thing the Dartmouth group finds is that hormone production by SCCL cells is regulated the same way as it is in nonmalignant cells. The cells also seem to have hormone receptors on their surfaces. All this argues against the hypothesis that SCCL cells make hormones

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because a few genes are turned on when the cells become cancerous. The estrogen production also contradicts this hypothesis. Sorenson explains, "The finding of estrogen was quite unexpected. It goes against the dogma. The thinking is that the formation of steroids is a multistep process with several enzymes necessary for each step." Therefore, it is hard to see how a single transformation event could allow cells to produce estrogens and all the other hormones—unless the normal lung cell precursors already produce these hormones.

Says Sorenson, "I suspect that these small cells are derived from cells in the normal lung that have a commitment to being endocrine cells. This is not a transformation of an undifferentiated cell."

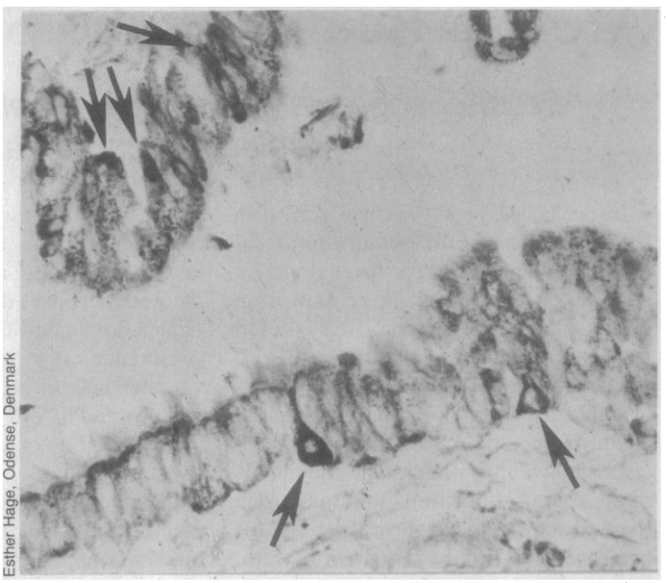
Until fairly recently, investigators did not suspect that normal lung cells might produce hormones. The lung was simply a respiratory organ, they thought. But now a number of researchers have demonstrated that lung cells do, in fact, produce hormones, although, Becker remarks, "To tell the truth, we don't know what these substances are doing in the lung."

Researchers have identified one type of lung cell, which they call the pulmonary endocrine cell, that may be a precursor of SCCL. Pulmonary endocrine cells are classic hormone-producing cells and they secrete calcitonin, bombesin, and Leu-enkephalin, all of which also are secreted by SCCL cells.

Becker finds that patients with lung diseases such as emphysema, acute pneumonia, or cystic fibrosis, have large amounts of calcitonin in their blood, which may mean that their pulmonary endocrine cells are proliferating and producing hormones in response to injury.

In support of this hypothesis, Hildegard Reznik-Schüller of the Frederick Cancer Research Center showed that hamsters get lung cancer when they are given nitrosamines. One of the first and most striking effects of these carcinogens is a proliferation of pulmonary endocrine cells. Then Ilona Linnoila of the NCI showed that when hamsters are given nitrosamines in sufficient quantities to injure their lungs but not to cause cancer, their pulmonary endocrine cells proliferate and their blood calcitonin concentrations increase.

But do small cell cancers and only small cell cancers come from these pulmonary endocrine cells? Stephen Baylin of Johns Hopkins University Medical School is not so sure. "We've been very interested in where lung cancer cells with endocrine functions come from and where normal endocrine cells come from," he says. "Evidence is building that rather than small cell cancers being the only lung cancers with endocrine properties, endocrine properties are spread out among the spectrum of lung cancers. Quantitatively, the small cell cancers have more endocrine properties but other types of lung cancers also have these properties."



Esther Hage, Odense, Denmark

Pulmonary endocrine cells of the normal bronchiolar epithelium of a spontaneously aborted human fetus. This hormone-containing cell is the putative cell of origin of small cell carcinoma of the lung.

The SCCL tumors often change to other kinds of lung cancers and frequently there is a mixture of histologically different lung cancer cells in one lesion. Baylin and Gazdar have noted a similar effect in vitro. Although small cell cancers are sensitive to chemotherapy and radiation, the other cancers are not. "The changes from one cell type to another could be important clinically," Baylin says.

So the approach Baylin and his colleagues, in collaboration with the NCI group, have taken is to look for cell surface markers and to use these markers to establish the lineages of lung cancer cells. Baylin, working with Joel Shaper of Johns Hopkins, finds that 12 cell surface proteins are present on small cell cancer but not on other lung cancers and that these markers are similar to the cell surface proteins on neuroblastoma cells, which also are hormone-producing tumor cells.

Cultured small cell cancers spontaneously can turn into other lung cancer types after a number of months, but the cells retain their surface proteins. Baylin and Shaper find these proteins are retained even though the cells lose their endocrine properties.

Jacqueline Whang-Peng and her associates at the NCI came to the same conclusion by doing cytogenetic analyses of cultured cells. Small cell tumor cells, they found, are missing part of the short arm of chromosome 3. When these cultured cells change into other types of lung cancer cells, they retain this cytogenetic marker of their origin.

Baylin and his associates next tested to see if the SCCL cells retain their cell surface proteins in vivo when they change to other kinds of lung cancer cells. He and Gregory Goodwin took large undifferentiated cancer cells—which are a histologically distinct lung cancer—from a patient whose tumor consisted mostly of small cells but which had nests of large cells in it. They cloned the large cells and found that the cloned cells had the SCCL proteins on their surfaces but also had several key non–small cell proteins. What this means, Baylin believes, is that small cell and non–small cell tumors can arise from the same normal lung cells. "This probably is a single cell which is pluripotent—it can go in different paths. Our working hypothesis is that stem cells in normal lung mucosa can make several types of cells. When the lung is injured, the activity of these stem cells is revved up. The final transformation could be along any direction that the cell could take," Baylin says.

It is Baylin's hope that by studying how lung cancer cells convert from one type to another, he may learn how to prevent SCCL cells from changing to other kinds of lung cancer cells which are treatment-resistant. But his work and that of the other researchers in this field has an obvious slant toward basic cell biology. "It is our basic prejudice that we need to understand the cell biology," he says, "We are using the neoplasm to glean information about the normal cell and vice versa."—GINA KOLATA