that they would be receiving a 20% pay cut, to about $60 per month—endangering their ability to stay in science at all. They are reluctant to air their concerns publicly, fearing reprisals from the government of President Alyaksandr Lukashenka—so loathed by many researchers that rather than utter his name, they refer to him as "the top person in our government."

Researchers in Ukraine, meanwhile, have been more fortunate. With the country's economy booming, their salaries were doubled this year to $120 per month. And their government has aggressively courted Western support for Chornobyl research. In 1998, these efforts paid off with the creation of a radioecology laboratory in the exclusion zone and surrounding territories. Now Ukraine's Cabinet of Ministers is negotiating with China and Japan to launch a research center to study the population of Slavutych, a town built after the Chornobyl accident to accommodate the power plant's workers.

The center will probe the long-term health of residents, many of whom lived in Pripyat until the accident and received high radiation doses immediately after the explosion. Of particular interest to scientists are the 7000 or so Chornobyl engineers and scientists who work in and around the sarcophagus each day, and the 6000 to 7000 liquidators now living in Slavutych. "My dream is to have a research agreement ready by the end of this year," says Valeriy Glygalo, the one-time liquidator who is now director of the Cabinet of Ministers' International Chornobyl Center for Nuclear Safety, Radioactive Waste, and Radioecology in Kyiv. The residents of Pripyat are gone, but they are clearly not forgotten. **–RICHARD STONE**

CELL BIOLOGY

Do Centrosome Abnormalities Lead to Cancer?

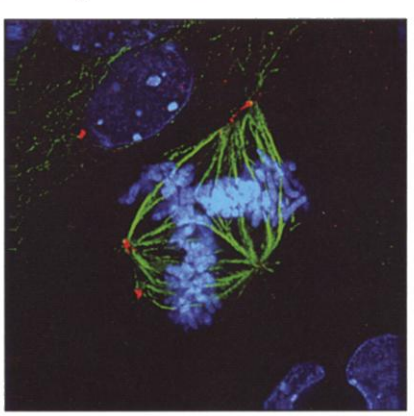
Evidence suggests that at least some cancers arise because centrosome malfunction causes chromosome damage and missorting

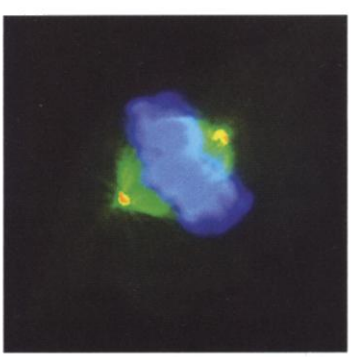
It may be small and inconspicuous, but the structure called the centrosome plays a big role in the cell. One key duty: helping to organize the mitotic spindle—the collection of protein filaments that pull the duplicated chromosomes apart during cell division, thereby ensuring that the two daughter cells each get a complete set. Without the centrosome, normal division of human cells could not occur. But accumulating evidence hints that this structure has a dark side as well. When the centrosome malfunctions, cancer may result.

Researchers have known for decades that cancer cells are rife with chromosomal abnormalities. Some cells lack one or more chromosomes, for example, while having extra copies of others. "Virtually every cancer cell has an abnormal chromosome complement, whereas virtually every normal cell has the [normal] diploid number," says cancer researcher Bert Vogelstein of Johns Hopkins University School of Medicine. The conventional wisdom has been that this aneuploidy, as it's called, is a late event in cancer development—the result of all the other disruptions in cancer cells. But now, "more and more it's coming out that [aneuploidy] is an early change and may be driving malignancy," says Vogelstein, whose own work has been pointing in that direction.

Contributing to this new view of aneuploidy is the realization that many types of cancer cells have abnormalities in their centrosomes—in particular, the cells often have extra copies. The supposition now is that the extra centrosomes lead to chromosome missorting and damage, thus causing the aneuploidy. Aneuploidy, in turn, may result in the loss of tumor suppressor genes or the gain or activation of cancer-causing oncogenes. "Once you have multiple centrosomes, that could increase the error rate [in chromosome replication and sorting], and those errors could be very dangerous," says centrosome researcher Greenfield Sluder of the University of Massachusetts Medical School in Worcester.

Researchers caution that the progression from centrosome derangements to aneuploidy to cancer isn't yet firmly established. Moreover, centrosome abnormalities likely aren't the only route to aneuploidy. For example, problems with the telomeres—the protective structures capping the ends of the chromosomes—have been implicated in the aneuploidy seen in some cancer cells. And two reports in the April issue of *Nature Cell Biology* suggest that mutations in a gene called *APC*, which are known to predispose to colon cancer, contribute to the chromosomal instability associated with that malignancy. But if centrosome abnormalities underlie at least some of the aneuploidy seen in cancer, they might be useful as diagnostic or prognostic indicators to help clinicians distinguish highly malignant cancers from those that are less dangerous. They might also point to possible new therapeutic strategies aimed at restoring normal centrosome function.





Centrosomal chaos. The mouse mammary cancer cell (left) has multiple centrosomes (red) and has generated four sets of spindle microtubules (green), which will lead to abnormal partition of the chromosomes (blue) in the daughter cells. At right is a normal dividing mammary epithelial cell.

CREDIT: W. BRINKLEY AND T. GOEPFERT

Hints of centrosome involvement

Early in the 20th century, a prescient microscopist named Theodor Boveri suggested that centrosome malfunction might lead to cancer. But the idea was more or less forgotten until about 5 years ago. At that time, Kenji Fukasawa, then in George Vande Woude's lab at the Frederick Cancer Research and Development Center in Frederick, Maryland, and colleagues found that cells lacking a critical tumor suppressor gene, known as *p53*, have multiple centrosomes instead of the normal one or two.

In work described in the 22 March 1996 issue of *Science* (p. 1744), the researchers reported that in cell culture, this centrosome amplification apparently disturbs mitotic fidelity, causing the cells to end up with abnormal chromosome complements. Because *p53*'s loss or inactivation is thought to contribute to the development of many human cancers, these findings suggested a new way that lack of a functional *p53* gene might lead to cancer: by disturbing centrosome function and thereby generating aneuploidy.

Two years later, a team led by Dennis Roop and William Brinkley of Baylor Col-

lege of Medicine in Houston, Texas, produced evidence that this can in fact happen, at least in an animal cancer model. When the researchers blocked *p53* activity in skin cells of living mice, those animals developed skin cancers when exposed to carcinogenic chemicals much more readily than controls did, and the centrosomes were amplified in the cells of 75% of the tumors. (The results appeared in the July 1998 issue of *Oncogene*.)

At about the same time, Jeffrey Salisbury of the Mayo Clinic Foundation in Rochester, Minnesota, and his colleagues found centrosome abnormalities in the cells of human breast cancers, and Stephen Doxsey and German Pihan of the University of Massachusetts Medical School detected them in most of the common human cancers, including breast, prostate, lung, colon, and brain. In addition to extra centrosomes, the researchers saw oversized centrosomes and some that contained more than the normal amounts of phosphate groups. "When we first looked at breast tumors, it was striking how unusual the centrosomes were. They stuck out like a sore thumb," Salisbury says.

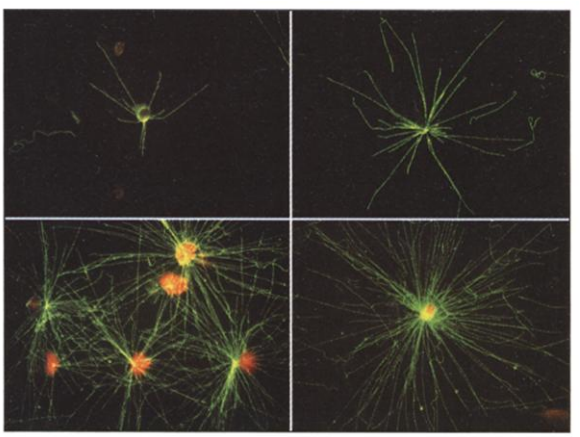
But could centrosomal defects such as these actually cause aneuploidy and thus possibly contribute to the development of the cancers? Some hints that they might came from Doxsey's work, which showed a strong correlation between centrosome abnormalities and chromosome instability, and from Thomas Ried and his colleagues at the National Cancer Institute in Bethesda, Maryland. As reported in the February 2000 issue of *Genes, Chromosomes and Cancer*, when the Ried team looked at cultured lines of human colorectal cancer cells, they detected aneuploidy only in those cell lines that also displayed centrosome abnormalities. Ried cautions, however, that this work simply shows a correlation between the centrosome and chromosome abnormalities: "Causality has not been established."

How centrosomes might go awry

Whatever their role in cancer, if any, researchers want to know what causes the centrosomal abnormalities. They have unearthed several intriguing possibilities. Fukasawa's earlier findings pointed to loss or inactivation of the *p53* tumor suppressor gene as one possibility. More recently, Fukasawa, who is now at the University of Cincinnati College of Medicine, has been working out just how that loss leads to centrosome amplification.

As shown 2 years ago by Sluder and Massachusetts colleague Edward Hinchcliffe, and independently by Tim Stearn's team at Stanford University, the activity of a kinase enzyme called CDK2 is needed for centrosome replication (*Science*, 5 February 1999, pp. 770 and 851). Because CDK2 activity is also needed to drive cells through the division cycle, this helps ensure that the centrosome replicates only once and at the right time. The Fukasawa team now has evidence that CDK2 controls centrosome replication by tacking a phosphate group onto a centrosome protein called nucleophosmin, causing it to leave the centrosome. Nucleophosmin's departure then initiates centrosome duplication, Fukasawa says. The *p53* gene comes into this picture because its protein product, working through another protein called Waf-1, inhibits CDK2. Thus, *p53*'s absence allows the centrosome to replicate when it shouldn't and accumulate extra copies.

Inappropriate activity of other kinases may also lead to the centrosome abnormalities seen in cancer cells. One of these is a so-called aurora kinase, discovered about 15 years ago in the fruit fly *Drosophila melanogaster* by David Glover of the University of Dundee, Scotland, and his colleagues. They found that when the gene encoding this kinase is mutated, mitosis is disrupted in fly cells, apparently because the kinase is needed to separate the duplicated centrosome before cell division.



Too much of a good thing? Centrosomes from cancer cells (bottom) nucleate the formation of many more microtubule fibers than do centrosomes from normal cells (top).

The first clue that an aurora kinase might be involved in human cancers came about 3 years ago. Two groups, one led by Brinkley and Subrata Sen of the University of Texas M. D. Anderson Cancer Center in Houston and the other by James Bischoff and Gregory Plowman of SUGEN Inc., in Redwood City, California, found an aurora kinase gene in a region of chromosome 20 that is amplified, or present in multiple copies, in many colon, breast, and other tumors. Presumably as a result of the amplification, the protein itself was present in the cancer cells in abnormally high concentrations.

To see whether the elevated aurora kinase levels actually cause the centrosome defects, and possibly the cancers, the Texas team genetically engineered noncancerous cells that had the normal one or two centrosomes to overproduce the enzyme. As a result, Brinkley says, the cells "produced multiple centrosomes, became aneuploid, and [displayed] other characteristics of transformed [cancerous] cells."

Abnormalities in the proteins that make up the centrosome structure have also been linked to cancer. For example, Doxsey and his team have found that concentrations of a centrosome protein called pericentrin are higher than normal in prostate and other cancers. And, similar to the situation with aurora kinase, when the researchers genetically engineered normal cells to overproduce pericentrin, the cells developed extra centrosomes. Says Doxsey: "We can create in vitro what's going on in tumor cells."

But questions remain ...

Many questions must still be answered to firm up the link between centrosome abnormalities and cancer development. For example, if the abnormalities are causative, one might expect to find mutations in the genes for centrosome structural or regulatory proteins in tumors. Aside from the amplification of the aurora kinase gene, none has been found so far, although Mark Winey, who studies centrosomes in yeast, thinks "it's just for want of looking."

Another question is how cells with centrosomal abnormalities manage to divide and survive at all. Normally, a variety of so-called checkpoints ensure that a cell doesn't divide if its DNA is damaged or abnormal. So "how does a cancer cell become a virtual dividing machine in the presence of all those centrosomes?" Brinkley asks.

One possibility is that multiple centrosomes coalesce at the poles of a dividing cancer cell. That way, instead of having multiple spindle poles, the cell would have just two functional poles that partition the chromosomes equally. Brinkley, Salisbury, and others have detected such structures in some cancer cells.

Perhaps the biggest question is when in cancer development the centrosomal abnormalities and aneuploidy arise. If they are driving cancer formation, as opposed to being a consequence of it, "they should be present not only in bad tumors, but in early ones," Doxsey says. Although the case isn't airtight, the researchers do have some evidence that the abnormalities are present in early tumors.

For example, in work that's not yet been published, Brinkley and his colleagues treated young mice with a carcinogen that induces breast cancer and then periodically

CREDIT: W. L. LINGLE AND J. L. SALISBURY

examined samples of mammary gland cells to see when extra centrosomes appeared. That turned out to be just 90 days after the carcinogen treatment began, when the tissue showed precancerous changes but had not formed full-fledged tumors, Brinkley says.

Salisbury has also detected centrosomal abnormalities in ductal carcinomas in situ—an early stage of human breast cancer. And Doxsey and his colleagues report in the March issue of *Cancer Research* that they have found the abnormalities in a variety of early cancers, including 15% to 20% of prostate cancers.

Doxsey and his colleagues are now exploring whether centrosome abnormalities, or concentrations of the centrosome protein pericentrin, can be used to help clinicians assess the aggressiveness of prostate tumors. Most of the tumors that are detected early will grow so slowly that they won't be a danger to the patient, but right now they can't be distinguished from the fast-growing ones. As a result, many men may have their prostate glands removed unnecessarily.

Doxsey notes that the percentage of early prostate tumors in which he found the centrosome abnormalities is about the same as the percentage of dangerous tumors. His team's work also shows a correlation between the degree of centrosome abnormality and cytological indicators of tumor seriousness. If those abnormalities can be used as a prognostic indicator, the tiny centrosome may prove a big help to patients with prostate and other cancers. **–JEAN MARX**

DEVELOPMENTAL BIOLOGY

The Hottest Stem Cells Are Also the Toughest

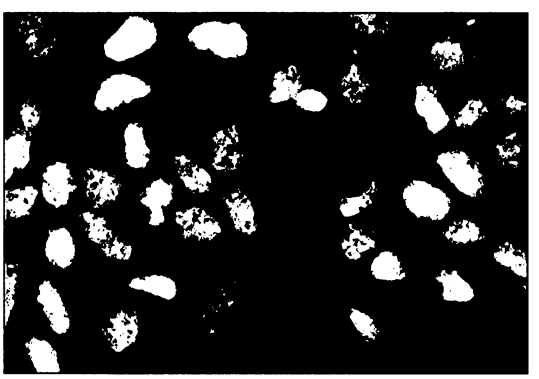
Although political uncertainty is rampant, researchers are making progress in the effort to tame human embryonic stem cells

DURANGO, COLORADO—In the United States, public funding for work with embryonic stem cells looks ever more uncertain, as the National Institutes of Health has put on hold its process for approving cell lines that government-funded scientists can use (see ScienceScope). That move comes as the handful of privately funded labs already using the cells are reporting progress—albeit limited—in manipulating these temperamental cells.

Although the cells were first derived more than 2 years ago, work has been frustratingly slow; indeed, only a few researchers have published *any* results with human embryonic stem (ES) cells. Not only are ES cells fussy about their growing conditions, but they also tend to differentiate spontaneously into a range of cell types other than the desired one, confounding research efforts. But at a recent Keystone meeting here,* researchers described new techniques to get around these obstacles.

Several teams are tackling the "very labor-intensive" process of growing human ES cells, as James Thomson of the University of Wisconsin, Madison, described it. Most researchers grow the cells on a feeder layer of mouse cells to keep them from differentiating. But before these cells can be used to treat disease in humans, researchers need to come up with a culture free of mouse cells. Melissa Carpenter and her colleagues at Geron Corp. in Menlo Park, California, reported that they have managed to grow cells on Matrigel, a commercially available gel commonly used to culture cells. They bathe the cells in a serum-free medium that is first "conditioned" by incubating it with irradiated mouse cells. The scientists do not yet know what the mouse cells add to the medium; they are working to characterize its active components. If they succeed, they might be able to produce a synthetic medium that could keep the cells dividing but not differentiating.

Stem cell researchers eagerly await the day when they can grow an unlimited supply of human liver cells. Not only would they be extremely valuable for tests of drug toxicity, but they might also be useful for treating some liver diseases. Geron scientists have taken a first step, reported Carpenter, coaxing their ES cell lines to produce "hepatocyte-like" cells. They first exposed the cells to sodium butyrate, a chemical known to promote cell differentiation. Many of the cells died, Carpenter said, but when the team cultured the survivors in media designed for growing hepatocytes, many of them began to store glycogen and express proteins typical of liver cells. Although the results are promising, Thomson cautions that liverlike cell markers appear on other cell types as well: "If they're really hepatocytes, it would be good. It's not yet clear to me that they are."



First step. Differentiating human embryonic stem cells express nestin (red), a marker for neural precursor cells. Cell nuclei are stained blue.

CREDIT: SU-CHUN ZHANG/UNIVERSITY OF WISCONSIN

Meanwhile, Thomson and his colleagues have found an efficient way to transform human ES cells into neural epithelial cells—precursors of more mature cell types in the brain. The team allowed the ES cells to cluster into balls of cells called embryoid bodies and then exposed them to media designed for neural cells. Next they isolated a partially differentiated cell type from the embryoid bodies and cultured those cells with basic fibroblast growth factor. Ninety-six percent of the resulting cells expressed neural markers, Su-Chun Zhang and Thomson reported. When transplanted into the brains of newborn mice, the cells migrated to several brain regions and showed signs of developing into mature neurons and glia, the neuronal supporting cells.

Stem cell researchers would like to create immune-neutral stem cells that wouldn't trigger rejection when transplanted into a patient. But that would require genetically altering ES cells. So far, that has proved far trickier in human cells than in their mouse counterparts. At the meeting, Joseph Gold of Geron said that he and his colleagues have genetically altered cell lines to express green fluorescent protein (GFP)—making them easier to track in animal transplantation experiments. And in a paper now in press, Nissim Benvenisty of the Hebrew University in Jerusalem, Joseph Itskovitz-Eldor of Rambam Medical Center in Haifa, Israel, and their colleagues report that they have created cells that express GFP only when they are undifferentiated. This would enable the scientists to weed out cells that have already chosen a developmental path.

Ultimately, researchers hope to devise a way to target genetic changes precisely. Several teams are working to adapt so-called homologous recombination—the technique that has made mouse ES cells such a valuable tool for geneticists—to human cells. So far none has reported success. **–GRETCHEN VOGEL**

* "Pluripotent Stem Cells: Biology and Applications," 6–11 February.