Double Trouble

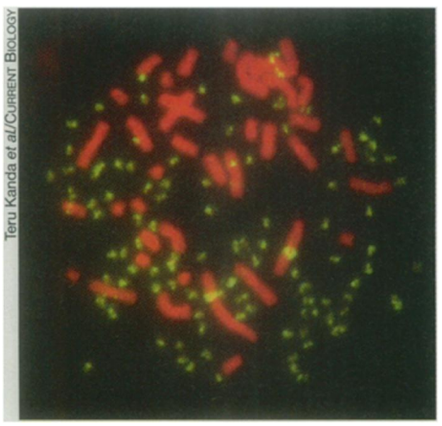
Tiny genetic loops aid cancer cells, offer target for therapy

By JOHN TRAVIS

The compelling tale of Dr. Jekyll and Mr. Hyde endures because it appeals to a reader's fascination with the idea that a murderous monster lurks inside an outwardly normal person. A similar theme—the normal transformed into the deadly—drives the story of cancer.

Mr. Hyde proved difficult to catch in large part because he reassumed the guise of Dr. Jekyll. As for cancer, the body's immune system often fails to recognize tumor cells as dangerous because they retain so many aspects of normal cells. Moreover, those similarities make it difficult to kill tumor cells without damaging healthy tissues in the process.

Consequently, scientists search vigorously for features—surface proteins, gene mutations, enzymes, and so on—that differentiate tumor cells from normal cells and that may eventually be used in the design of therapies that target only cancer cells. Small rings of DNA known as double minute chromosomes may represent one such distinguishing feature.



Teru Kanda et al/Current Biology

Double minute chromosomes containing a growth-promoting cancer gene (green) reside in the nucleus alongside normal chromosomes (red).

More than half of all tumors contain these genetic loops, whereas normal cells do not. The minichromosomes usually bear extra copies of genes that are useful to a cancer cell, such as those that override a cell's growth controls or make a cell resistant to drugs used in chemotherapy. Thus, double minute chromosomes seem to increase a tumor cell's chances of surviving and proliferating.

Although scientists have known about these minichromosomes for several decades, they remain relatively obscure. "I think double minute chromosomes have escaped attention because people haven't realized how interesting they are as molecular entities and unique targets for therapy," asserts Geoffrey M. Wahl of the Salk Institute for Biological Studies in La Jolla, Calif.

Wahl hopes to shine a spotlight on these DNA loops. He and his colleagues have recently found a way to track double minute chromosomes in living cells. Other studies by this group help explain how cells shed the chromosomes, a process that may suggest novel forms of cancer therapy. Indeed, tumor cells that get rid of their double minute chromosomes often have greater difficulty proliferating or become more vulnerable to cancer drugs.

The earliest report Wahl has seen that mentions double minute chromosomes was published in the 1960s. "Cytogeneticists have observed these things in cancer cells for years," he says.

The minichromosomes are thought to result from the general genetic instability of dividing cancer cells. As chromosomes fragment, the pieces either reintegrate into other chromosomes or form independent rings.

While scientists have long observed such isolated chromosomal fragments, it wasn't until 1979 that they realized the pieces contain copies of genes, not just meaningless DNA. That year, a research group reported that tumor cells which resist the chemotherapeutic drug methotrexate harbor double minute chromosomes composed of many copies of the gene that encodes dihydrofolate reductase, an enzyme that deactivates the drug (SN: 1/3/87, p. 12).

Since then, investigators have identified many other genes that can appear in the circular DNA fragments. Several of these genes endow tumor cells with greater resistance to chemotherapeutic drugs, while others are oncogenes, mutated genes that encourage a cell's proliferation or growth.

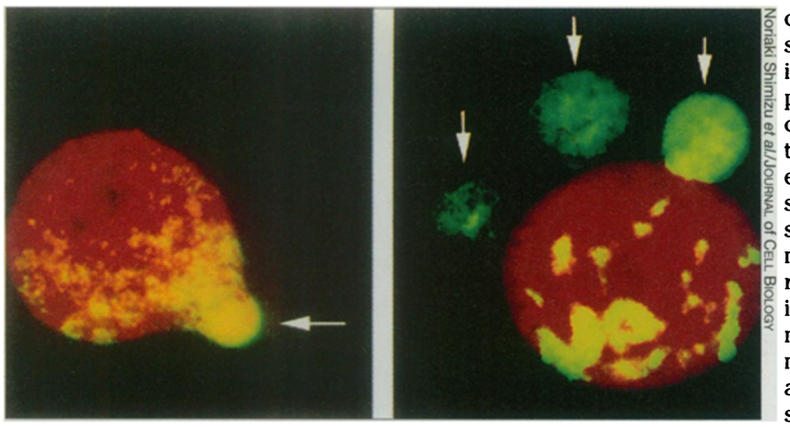
Although double minute chromosomes probably can form with all sorts of genes, those that do not offer the cell some advantage would disappear in time. Unlike normal chromosomes, double minute chromosomes do not have centromeres, the DNA sequences that a dividing cell uses to ensure that its progeny receive an equal number of chromosomes. Consequently, the dozens to hundreds of double minute chromosomes split unevenly between a dividing cell's two progeny.

"One cell is going to get more than the other does," says cytogeneticist Jerome L. McCombs of the University of Texas Medical Branch in Galveston. If the minichromosomes offer a growth advantage, cells with more of them will outpace cells with fewer. Such a selection process will gradually increase the overall number of double minute chromosomes in a cell population.

The finding that these DNA loops contain extra copies of genes useful to cancer cells wasn't totally unexpected. Frequently, full-size chromosomes in tumor cells had been observed to contain additional copies of such genes. What is unusual, and of potential medical interest, says Wahl, is that tumor cells sometimes discard double minute chromosomes and that certain compounds encourage this process.

Evidence that tumor cells could be "cured" of double minute chromosomes first emerged in 1983, when Robert M. Snapka and Alexander Varshavsky, both then at the Massachusetts Institute of Technology, reported that the chemotherapy drug hydroxyurea eliminates these circular fragments from methotrexate-resistant tumor cells grown in test tubes. As might be expected, the treatment restored the cells' sensitivity to methotrexate. Hydroxyurea affected only cells with double minute chromosomes—it didn't restore sensitivity when the genes for methotrexate resistance were integrated stably into normal chromosomes.

Since that 1983 report, investigators have recognized that hydroxyurea's curative powers extend to minichromosomes that contain genes countering other chemotherapeutic drugs or genes, such as *c-myc*, that promote the growth of cancer cells. In the latter case, Wahl and his colleagues have shown that treated cancer cells, bereft of their helpful *c-myc*-containing DNA circles, grow poorly in soft



Noriaki Shimizu et al/JOURNAL of CELL BIOLOGY

Micronuclei (arrows) budding out of these normal nuclei contain double minute chromosomes.

agar and don't form tumors as readily when implanted into mice. Several other drugs, adds Wahl, have a similar impact on laboratory-grown cells that contain double minute chromosomes.

How does hydroxyurea, at concentrations much lower than those given to cancer patients, eliminate double minute chromosomes from laboratory-grown cells?

In the absence of a chemotherapy drug, double minute chromosomes containing resistance genes are a burden to cancer cells grown in a test tube, notes Snapka, now at Ohio State University in Columbus. "Cells with fewer double minute chromosomes have a growth advantage," he says.

Consequently, laboratory-grown cells with few of the minichromosomes outpace the other cells, which become a smaller and smaller fraction of the overall population. Hydroxyurea, Snapka contends, somehow exaggerates the growth disadvantage of cells with double minute chromosomes.

Wahl has been pursuing a different explanation for hydroxyurea's skill at eliminating double minute chromosomes. His research indicates that cells can actively discard these fragments and that hydroxyurea, as well as related drugs, encourages that shedding of DNA. Exactly how the drugs speed this process is unclear, Wahl acknowledges, although they seem to lengthen the time a cell spends synthesizing DNA.

Investigators have recently made some progress in understanding how cells naturally cast off these abnormal chromosomes. In the March 23 JOURNAL OF CELL BIOLOGY, Wahl, Noriaki Shimizu of Hiroshima University in Japan, and their colleagues report that the removal of double minute chromosomes occurs through a novel variation of a process called micronucleation.

The nucleus is the membrane-surrounded structure that holds a cell's DNA. When a cell divides, the nuclear membrane breaks down, allowing identical sets of chromosomes to segregate into the cell's two progeny. A new nuclear membrane then forms around each cell's chromosomes. At this time, so-called micronuclei may surround any free-floating DNA that remains outside the nuclei—at least, according to the standard theory of micronucleation.

Wahl's group has found that double minute chromosomes are incorporated into micronuclei but that the process occurs inside the cell's nucleus. At some point after minichromosomes form, perhaps because they don't have centromeres, some of these chromosome fragments may move to the nuclear membrane. Then, when the cell is synthesizing new DNA in preparation for division, the double minute chromosomes replicate and may be enveloped by micronuclear membranes that bud out of the nuclear membrane. Ultimately, cells discard the micronuclei, according to unpublished work by Wahl's group.

This unexpected evidence that micronuclei can also originate inside a full-fledged nucleus is compelling to one of the scientists who developed the original model of micronucleation. "It is new, and it is right," says John A. Heddle of York University in Toronto.

Wahl speculates that this cellular process originally evolved as a way to remove any potentially dangerous DNA, such as that of a virus or bacterium. "Any DNA that doesn't have a centromere, you don't want, even if it came from you," he says.

Wahl and his colleagues have recently developed a colorful new tool that allows them to look at double minute chromosomes in living cancer cells. They took the gene for a histone, one of the cellular proteins around which DNA wraps itself, and joined it to the gene for green fluorescent protein (GFP), a jellyfish molecule that lights up when excited by a laser. When this engineered gene is added to cells, the resulting protein labels the DNA, seemingly without damaging the cells.

"It decorates chromosomes. The fluorescence is so bright you can see chromosomes with very high sensitivity and resolution. You can even see little chromosome fragments, such as double minute chromosomes," says Wahl.

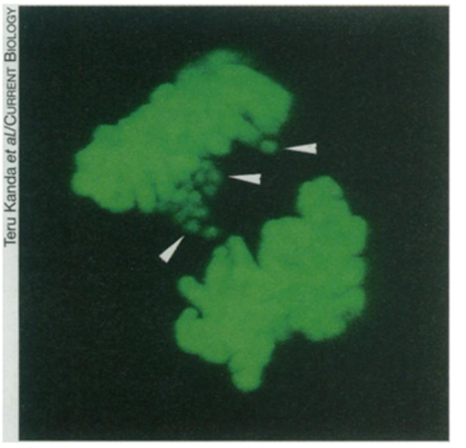
There are other methods of labeling chromosomes in living cells, but they have various disadvantages, such as ultimately damaging the cells being studied. "While other agents are useful for short-term analysis, this can be used for studying cells over, perhaps, days," says Wahl.

Although the investigators believe that the histone-GFP protein offers an excellent way to study overall chromosomal dynamics, including how DNA moves in a dividing cell, they have concentrated their initial efforts on double minute chromosomes. In a study reported in the March 26 CURRENT BIOLOGY, the histone-GFP method revealed that some of the minichromosomes hitchhike on full-sized chromosomes as they segregate in dividing cells. The fragments also associate with one another. "They look like grapes in a cluster," says Wahl.

The investigators hope eventually to use the histone-GFP protein, and their new understanding of how cells oust double minute chromosomes, to design a test of how effectively various compounds spur the elimination of the DNA loops. Any drug discovered in such a test should affect cancer cells regardless of whether their double minute chromosomes contain oncogenes, drug resistance genes, or some other type of genes.

"Here's an approach for therapy where you don't care what the gene is, you don't care what is encoded by the double minute chromosomes, you just know that whatever is encoded is important for cancer progression," explains Wahl.

While tumor cells that have lost their double minute chromosomes may retain the original gene mutations that made them cancerous, the experiments with hydroxyurea suggest that the forced shedding of these genetic loops can reduce the deadliness of cancer cells or their invulnerability to treatment.



Teru Kanda et al/CURRENT BIOLOGY

Fluorescently tagged histone proteins, around which DNA wraps itself, help light up chromosomes in living cells. The arrows point to double minute chromosomes, which sometimes attach to full-sized chromosomes.

Snapka says, "If you could selectively act against cells carrying the [extra] genes, even without correcting whatever initial mutations they had, you could still possibly lengthen someone's life." □