NEWS

Debate Surges Over the Origins Of Genomic Defects in Cancer

Cancer cells are chock-full of mutations and chromosomal abnormalities, but researchers can't agree on when and how they come into play

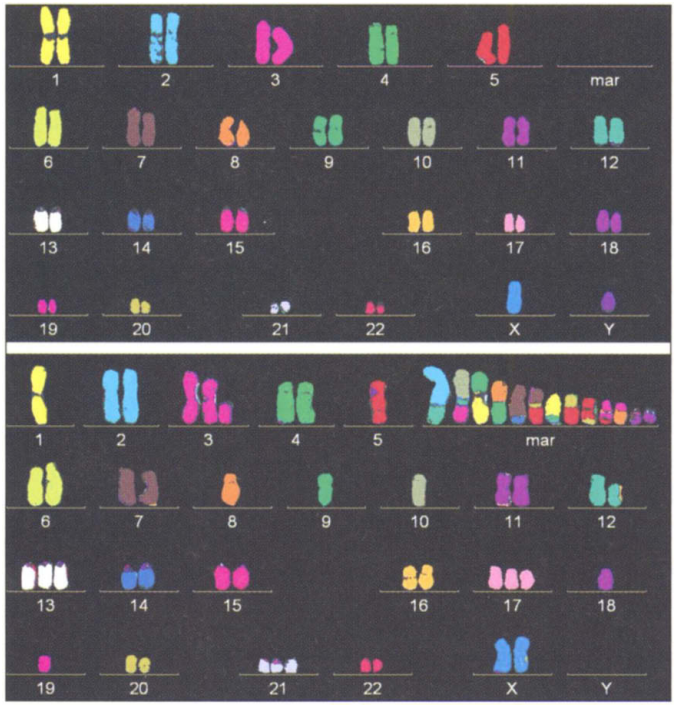
Inside a cancer cell is a veritable gallery of horrors: inactivated genes, extra or missing chromosomes, and a host of other genetic abnormalities, large and small. Most researchers agree that the great majority of cancers are triggered by accumulation of several such changes—some wiping out tumor suppressor genes and others activating growth-promoting oncogenes, for instance. But there's no agreement on how incipient cancer cells acquire so many mutations and chromosomal abnormalities. Increasingly, the debate is focusing on the role of genomic instability: some kind of inherent defect that makes the cancer cell genome more susceptible than that in normal cells to developing the various abnormalities.

Some researchers maintain that genomic instability is needed early on to set cells on the path to cancer. Even within this camp, however, the members disagree about what kind of genomic instability comes into play. Some think that cancer cells have what is sometimes called a "mutator phenotype" that makes them more prone to acquiring small mutations, simple base changes or insertions or deletions of small DNA segments that can cause trouble if they happen to strike oncogenes or tumor suppressors. Others within the genome-instability camp think that much bigger changes are needed, such as gains or losses of whole chromosomes, or shuffling of large segments either within or between chromosomes.

On the opposite side are researchers who assert that cancer cells start out no more prone to genomic instability than normal cells. The cells mutate at a normal rate, they say, but because they divide more often than normal cells do, they have more opportunities to accumulate mutations. At most, these researchers maintain, genomic instability might arise late in the development of a tumor and might contribute to its ability to spread in the body. But it's not necessary for a cancer to occur.

"The field, even though it's been going on for a while, isn't mature yet. There are a lot of divergent views," says Garth Anderson of Roswell Park Cancer Institute in Buffalo, New York. But that leaves a large gap in researchers' picture of carcinogenesis.

"If you want to understand cancer, you need to know the answers" to the many questions about the role genome instability plays in cancer, says Bert Vogelstein of Johns Hopkins University School of Medicine in Baltimore, whose own lab's work supports the view that cancer cells are especially susceptible to large chromosomal changes. Less clear is whether having those answers will aid in the development of better cancer therapies, although researchers favoring the different views can come up with various scenarios where it might. "That's speculative," Vogelstein says. "But so much of what has been learned about cancer has been so surprising that these ideas shouldn't be dismissed."



Scrambled. In contrast to the chromosome complement of a normal cell (*top*), that of a cancer cell (*bottom*) is highly abnormal, with extra copies of some chromosomes, lost copies of others, and chromosomes made of fused pieces of other chromosomes.

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Mutations great and small

Researchers have been speculating that genomic instability might be involved in cancer development for roughly a century. The earliest work on genomic aberrations in cancer cells focused on big changes easily detected by peering at cells through a microscope. In 1914, for example, German biologist Theodor Boveri postulated that cancer cells might be aneuploid: having an abnormal chromosome number. They might possess extra copies of some chromosomes while lacking others altogether. That suggestion has stood the test of time, particularly for the common tumors of the colon, lung, and breast. "It's extremely difficult to find a cancer cell with a normal karyotype [chromosome composition]," Vogelstein says. Conversely, he adds, "you can't find a normal cell with an abnormal karyotype."

The idea that increased genomic instability might lead to cancer by producing a much less extensive type of DNA damage—mutations of individual genes—got a big boost in the mid-1990s. Several teams, including Vogelstein's, showed that a hereditary form of colon cancer known as HNPCC (for hereditary nonpolyposis colon cancer) is caused by mutations in so-called mismatch-repair genes needed to repair a certain kind of DNA damage that occurs when the DNA is copied incorrectly prior to cell division. The inability to repair this damage leaves the cells vulnerable to other mutations that can, by hitting genes involved in growth control, lead to HNPCC and related cancers. Other hereditary cancers, including breast cancer and some skin cancers, have also been linked to defects in the cell's DNA repair machinery (see also the Report on p. 606 and the Perspective on p. 534).

But the particular mutations that cause HNPCC and breast cancer are relatively minor causes of their respective cancers, each accounting for approximately 5% of the total cases. A big question still remains about whether various types of genomic instability contribute to the much more common forms of these cancers, which are apparently not inherited, or to other forms of cancer. "Since tumor cells have these [genome] changes, it's reasonable to assume

that there is genetic instability behind it, but that's not necessarily so," says Felix Mitelman of University Hospital in Lund, Sweden, who has been cataloging the larger chromosomal abnormalities associated with human cancers.

To determine whether cancer cells are in fact genetically unstable, researchers have to know the rate at which DNA alterations accumulate—generally expressed as the number of mutations per cell division—in both normal and cancer cells. But as Mitelman points out, we "don't know how much instability goes on normally." As a result, researchers have a hard time establishing whether the mutation rate is elevated in cancer cells or not.

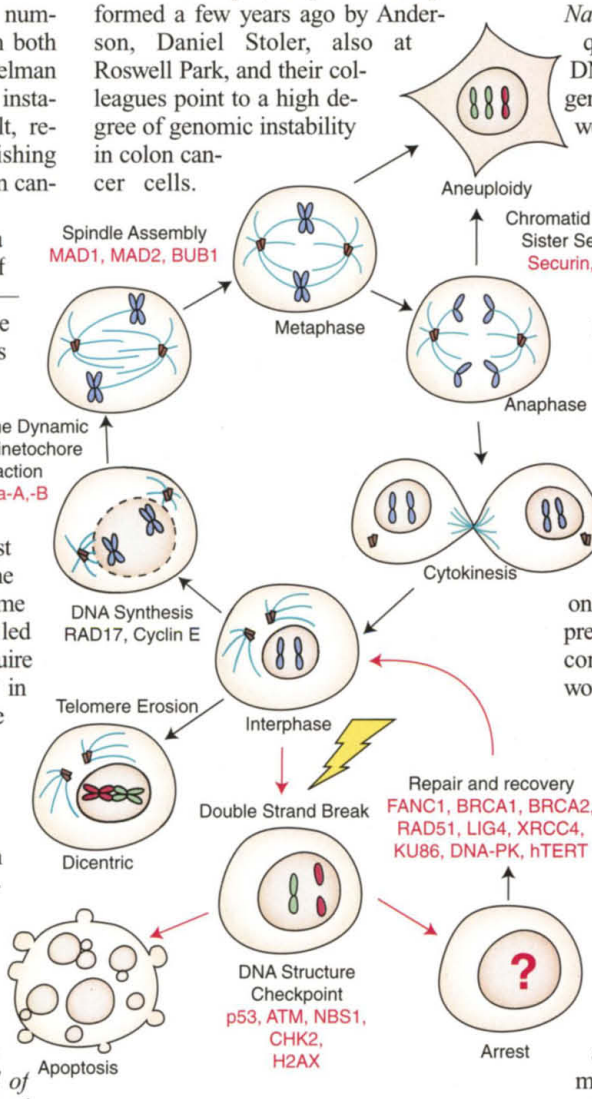
That leaves those who favor the idea that genomic instability is a cause of cancer—as well as those who don't—plenty of room to argue their cases. One long-time proponent of the idea is Lawrence Loeb of the University of Washington, Seattle. Some 25 years ago, he calculated that the normal mutation rate, estimated at 2×10^{-7} mutations per gene per cell division, isn't fast enough for cancer cells to acquire the mutations needed to make them become malignant and grow out of control. That led Loeb to suggest that cancer cells acquire some kind of early mutation, possibly in their DNA-repair machinery or in the polymerase enzymes that copy their DNA, that give the cells a mutator phenotype that predisposes them to the accumulation of additional mutations.

But those calculations have been questioned by other researchers, including Ian Tomlinson and Peter Sasieni of the Imperial Cancer Research Fund in London and Walter Bodmer of John Radcliffe Hospital in Oxford, both in the United Kingdom. In an analysis reported in the March issue of the *American Journal of Pathology*, they started with an assumption that it takes many more cell divisions to produce a cancerous tumor than Loeb and colleagues had estimated. Given that, the researchers concluded that even normal mutation rates could account for very high numbers of mutations in the cells. "My view is that there has been a bit too much emphasis on genomic instability as a driving force [in cancer development]," Tomlinson says.

Tallying the damage

To assess genomic instability experimentally as opposed to mathematically, researchers have often turned to colon cancers, because they have a known progression from benign polyps and adenomas to full-fledged invasive cancers, and tissue samples from all these stages are available. This work, focusing mainly on nonhereditary cancers, has produced equally discordant results. Complicating matters, the researchers who implicate early genomic instability in cancer and those who don't often look at different types of mutations.

For example, experiments performed a few years ago by Anderson, Daniel Stoler, also at Roswell Park, and their colleagues point to a high degree of genomic instability in colon cancer cells.



Many ways to go wrong. The cell cycle provides numerous opportunities for defects to arise that can lead to mistakes in chromosome sorting. DNA-repair machinery can also malfunction and lead to genome instability. Genes linked to such defects are indicated in red.

The researchers modified a standard technique for amplifying DNA that enables them to detect insertions and deletions in the genome. When they applied the method to human colon cancer cells, they found that the cells contain roughly 11,000 such mutations, most of them small. "When you actively look within the chromosomes, you can pick up a lot of [mutational] events," Anderson says. Cells from colon polyps had almost as many of the mutations. In contrast, Anderson says, "we see none of this variation" in normal colon cells. These results indicate, he concludes, that colon cancer cells acquire genetic instability very early in cancer development, well before they become malignant.

Vogelstein and his colleagues disagree, at least with regard to such small mutations. In the 5 March issue of the *Proceedings of the National Academy of Sciences,* they report sequencing more than 3 million bases of DNA, including coding sequences from 470 genes, from human colon cancer cells that were grown either in culture or in mice. After eliminating harmless, natural DNA variations, they found that the sequences carried only three true mutations, two involving base changes and the other a 14–base pair deletion. Based on this finding plus the estimated number of cell divisions needed to form a tumor, the Vogelstein team concluded that colon cancer cells have no more of these small mutations than would be produced by normal mutation rates.

Loeb counters that the Johns Hopkins workers seriously underestimated the actual number of mutations in cancer cells because their analytic technique only detects mutations that were "clonal," or present in all the cells analyzed. Vogelstein concedes that their sequencing methods wouldn't pick up mutations present in a small minority of cells, but he contends that the issue is not the total number of mutations in all the cells of a tumor but the rate at which they appeared, which the team took into account. Other researchers who used different methods have come to similar conclusions for colon and other cancers, Vogelstein adds.

Major chromosomal upheavals

Mutation rate aside, other researchers are trying to see whether the cells are more prone to higher order derangements—at the chromosome level. In the 25 years or so since researchers began discovering first oncogenes and then tumor-suppressor genes, aneuploidy has generally taken a back seat to the smaller mutations affecting these genes. Many researchers still think that aneuploidy develops late—an effect, not a cause, of cancer development. Virologist Peter Duesberg of the University of California, Berkeley, and the University of Heidelberg at Mannheim, Germany, is a notable exception.

Duesberg, who is perhaps best known for his contrarian views on AIDS—he maintains that it's not caused by HIV (*Science*, 9 December 1994, p. 1642)—is also something of an iconoclast about genes and cancer. "The prevailing gene mutation hypothesis is, to say the least, defective," he says. Among

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other criticisms, Duesberg points out that some known carcinogens, including asbestos and arsenic, do not cause mutations. In addition, he notes, researchers have had a tough time making human cells cancerous through the introduction of human oncogenes. Although others attribute this to the likelihood that several mutations are needed to produce human cancers, Duesberg concluded, he says, that "something else has to happen [to make cells cancerous], and there's a good chance that it's aneuploidy." Gain or loss of whole chromosomes can easily upset the checks and balances that maintain normal cell growth patterns, he points out.

In support of his idea, Duesberg cites experiments in which he and his colleagues exposed cells in culture to chemical carcinogens. The treated cells became aneuploid long before they began showing signs of becoming cancerous. Duesberg's model also gains support from recent results from Vogelstein and his colleagues. Although the researchers didn't see an increased rate of small mutations in colon cancer cells, Christoph Lengauer of the Johns Hopkins group found that the most common type of colon cancer cells is genomically unstable when it comes to gross chromosomal changes.

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>
> —Bert Vogelstein

In these experiments, Lengauer compared the accumulation of gross chromosomal changes in two types of colon cancer cells: those with a mismatch repair defect like that in HNPCC and those without. Unlike most colon cancer cells, HNPCC cells have normal or nearly normal chromosome numbers, and the mismatch-repair–defective cells didn't acquire many chromosome-level oddities over the course of many cell divisions.

In contrast, cells that had been spurred to become cancerous by some other means showed a high rate of chromosome loss or gain. Such cells acquired chromosomal changes 10 to 100 times faster than the mismatch-repair–defective cells. This shows that the usual type of colon cancer cell has an unstable genome, Lengauer says. Vogelstein adds that the experiments also show that aneuploidy is not just an after effect of cells becoming cancerous. If it were, colon cancer cells with the mismatch-repair defect "should also have a high rate of the chromosomal abnormalities," Vogelstein says.

In more recent work published in the February 2001 issue of *Cancer Research*, Lengauer, Vogelstein, and their colleagues detected major chromosome abnormalities even in very small, precancerous colon adenomas removed from patients not known to have an HNPCC defect. "You may need the instability to ever get to a cancer," Vogelstein concludes.

Evidence for the other argument—that chromosomal abnormalities are not needed for cancer development—comes from William Hahn of Harvard Medical School in Boston, Robert Weinberg of the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts, and their colleagues. In the past few years they have shown that, contrary to Duesberg's assertion, a variety of normal human cell types can be made cancerous by introducing the right combination of genes, including the *ras* oncogene. There's no need to summon up aneuploidy to explain carcinogenesis, they say.

But Duesberg responds that he and his colleagues have looked at cells transformed by the Weinberg team and found that they are highly aneuploid. That, rather than the specific gene changes, is what makes the cells cancerous, Duesberg maintains. Weinberg responds that when his team grows the cells in culture, they see no sign of aneuploidy or other widespread genomic instability, and he suggests that Duesberg's culture conditions might have been too harsh.

Other studies have cast further doubt on the centrality of chromosomal abnormalities, and even of the kind of repair defect seen in HNPCC, in early development of the common forms of colon cancers. William Dove of the University of Wisconsin, Madison, and his colleagues used a mutant mouse to track cells destined to become cancerous. The mice have a defect in the *APC* tumor-suppressor gene that causes them to develop numerous intestinal cancers. In humans, loss or inactivation of this gene causes a hereditary condition called familial adenomatous polyposis with similar symptoms. In work published online on 13 June by the *Proceedings of the National Academy of Sciences*, Dove and his colleagues looked for both the small mismatch-repair type of defect and larger chromosomal abnormalities in premalignant adenomas from the mutant mice and from humans. They found none, leading them to conclude that colon cancer can develop without either kind of defect, although that does not eliminate the role of mismatch-repair mutations in HNPCC.

Tomlinson, Bodmer, and their colleagues have also looked for signs of genomic instability in early human tumors and have come to the same conclusion as Dove. "When a tumor starts to grow, genomic instability is not a big factor," Tomlinson concludes, although it might come into play later.

Research into how chromosomal derangements might arise is also lending support for the idea of an early role for genomic instability in cancer. Some cancers are associated with defects in the centrosomes, small cellular structures that help form the mitotic spindle and are thus necessary for normal separation of the chromosomes during cell division (*Science*, 20 April 2001, p. 426). Malfunction of the telomeres could contribute as well. For example, if the telomeres are lost, two chromosomes might fuse end to end and be missorted during cell division (see Maser and DePinho Review on p. 565).

Indeed, researchers estimate that hundreds of genes, many of which control so-called "checkpoints" that keep cells from dividing if the DNA is damaged or there are other problems with the chromosomes, are potential targets for aneuploidy-causing mutations.

At the moment there is no end in sight to the numerous debates on the role of genomic instability in cancer development. But one thing is certain: Cancer researchers don't have to worry about running out of ideas—or work—as they try to get a better understanding of how cancer arises. **–JEAN MARX**

ADDITIONAL READINGS

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