

Chromosomal Chaos and Cancer

Current wisdom on the role of genes in malignancy may not explain some features of cancer, but stepping back to look at the bigger picture inside cells reveals a view that just might

When I first began to study cancer as a young postdoctoral fellow in the early 1960s, it looked to leading scientists as though viruses could be the cause of most, if not all, malignancies. That idea was based on the discovery of several tumor- and leukemia-producing viruses that could infect a host cell and insert their own genetic material into its genome, sparking a cancerous transformation and proliferation of the cell. I was optimistic and naive enough to hope that if researchers could understand the exact molecular mechanisms by which such viruses caused cancer, we could develop vaccines to eliminate one of humanity's most dreaded diseases.

My own contribution to that pursuit came in 1970, when my colleagues, Michael Lai and Peter Vogt, and I managed to isolate a specific gene, *src*, which was suspected to be the tumor-initiating culprit in avian Rous sarcoma virus. Within a few years, more creative scientific minds than mine had followed this lead to a realization that a closely related gene was already present in the normal DNA of animals, including humans. And a new cancer model was born: it proposed that some triggering event, such as a mutation in a human cell's own version of *src*, could ignite tumorigenic powers like those possessed by its viral counterpart. The cancer-promoting potential of such a time bomb buried in our personal genomes earned it the title of "proto-oncogene." Once the mutation occurred, it would become a full-fledged oncogene.

The theory that mutations in certain key human genes are at the root of all cancers has dominated research for the past 30 years. Yet despite all the attempts of investigators, including myself, during that time to demonstrate that a handful of such oncogenes alone can transform normal cells into malignant ones, none have succeeded: The oncogene model also ignores what a casual observer might perceive as a rather large elephant in the room: in every known instance of cancer, individual genes may well contain mutations, but entire chromosomes, which carry thousands of genes, are also severely scrambled--duplicated, broken, structurally rearranged or missing entirely. Growing evidence suggests that this chaos on the chromosomal level is not just a side effect of malignancy, as the prevailing model holds, but the direct cause and driving force of cancer.

With several colleagues in the U.S. and Europe, I have been investigating this possibility for more than a decade, and the recent work of many other researchers is also pointing to the conclusion that changes to the number and structure of entire chromosomes, rather than single genes, are sufficient to initiate and sustain malignancy.

This view has important implications for cancer treatment and prevention, as well as for diagnosis of precancerous lesions when there may still be time to intervene. It also finally explains some characteristics of cancer cells and whole tumors that the gene mutation hypothesis leaves unresolved.

Invading Species

OUR RESEARCH GROUP arrived at a chromosomal theory of cancer in part by thinking about the basic biological features that make a normal human cell "normal," or even "human." Nature is extremely conservative with regard to chromosomes--the bound volumes of the encyclopedia of life--and the specific chromosome complements, or karyotypes, that define each species are final and stable for the duration of that species. Sexual reproduction also enforces conservation of a species-specific karyotype because embryonic development depends on absolute chromosomal conformity--cells containing altered or misallotted chromosomes are almost never viable. A rare exception, Down syndrome, illustrates the systemic damage that results from having just one extra copy of a relatively small chromosome, number 21, added to human cells.

Individual genes, in contrast, can be quite variable within a species. Single-nucleotide polymorphisms (SNPs), for example, which are mostly benign changes in the DNA sequence, are found scattered throughout every person's genome and passed from parent to child.

Cancer cells, and their less conspicuous precursors, are notorious violators of nature's karyotype stability laws, however. Whereas normal human cells are described as diploid, because they all have two copies of the chromosomes that define our species, the karyotypes of cells in solid tumors are always aneuploid--meaning that they have gained or lost whole chromosomes or segments of chromosomes. As a result of this aneuploidy, the total DNA content of a cancer cell can rise to more than twice or fall to nearly half that of a normal diploid cell. This situation would cause the cell to produce wildly skewed dosages of the proteins encoded by the thousands of genes that were multiplied or lost. Such gross imbalances would inevitably disrupt the work of critical teams of enzymes, including those involved in repair or disposal of damaged DNA, and would destabilize cellular structures and regulatory circuits. Indeed, the most complex coordinated squadron of proteins in a cell, and thus the most vulnerable, is the mitotic spindle apparatus that segregates chromosomes during cell division. Once aneuploidy is established, therefore, additional derangement of the chromosomes is likely.

This could explain why even cancer cells within the same tumor can exhibit different combinations and alterations of their chromosomes, making each cell a kind of new species unto itself. Their inherent instability also sets individual cancer cells free to evolve new traits and behaviors (phenotypes), unlike the normal cells in an organism,

which are destined to develop predetermined characteristics depending on the organ or tissue to which they belong and according to strict species-specific programming. With this license to change, an aneuploid cell can gradually drop more and more of its normal social responsibilities in a multicellular organism, multiplying ever more autonomously at the expense of other cells.

Collectively, cancerous cells tend to evolve from bad to worse. During this process, described as progression of carcinogenesis, the cells start developing their own exotic sizes, shapes, metabolisms and growth rates. Indeed, the very definition of malignancy includes the cells' acquisition of an unnatural ability to invade neighboring tissues and travel to distant organs, known as metastasis. The extreme variability of cancer cells and the enormous diversity of their phenotypes are primary reasons cancer has remained an intractable problem, scientifically and from the standpoint of treatment. Soon after a toxic drug is found to kill tumor cells, for instance, drug-resistant variant cells grow up in their place. Trying to tackle such entities with single drugs is like going up against an entire zoo of wild animals with a trap built only for crocodiles.

Yet scientists do know that in every case of cancer, the entire menagerie of malignant cells has arisen from a single unstable mother cell. This so-called clonal origin of a tumor is demonstrated by the presence in all or most of its cells of chromosomes whose rearrangements are so unique that they could not have arisen independently and so must all have been inherited from a common source. The challenge, therefore, is to find a theory that explains how one normal cell, out of the trillions that make up a human body, becomes chromosomally and phenotypically so unstable that it gives rise to a lethal cancer.

Until about 50 years ago, most cancer researchers saw the origins of cancer in aberrant chromosomes themselves. This idea had been advanced in the late 19th and early 20th centuries by two German scientists, David von Hansemann and Theodor Boveri. Von Hansemann, while studying cancer in Berlin, had discovered that all cancers contain abnormal chromosomes. Boveri, a biologist in Würzburg, had deduced that chromosomes are the vehicles for heritable information within cells by closely observing the steps of sea urchin embryo development. He reached his conclusion after witnessing the cellular degeneration that would follow if something went wrong during cell division and a single chromosome was broken or whole copies were unevenly distributed between two daughter cells. Boveri likened some of the resulting malformed embryos to tumors and, in 1914, predicted that gains or losses of specific chromosomes would initiate cancer.

That theory fell out of favor half a century later only because such patterns could not be detected in the chaotic and heterogeneous karyotypes of cancer cells, especially the most malignant ones, with the technology available at the time. In the absence of

consistent chromosomal changes, the bewildering cancer karyotypes were interpreted as mere consequences of malignancy, which must itself be caused by another unseen factor.

All along, however, some evidence that was largely ignored continued to support an important role for aneuploidy in the genesis and progression of cancer. In fact, degree of aneuploidy is a feature that is still sometimes used by pathologists and doctors to assess the cancerous potential of abnormal cells biopsied from the cervix, prostate, liver, stomach, throat, breast and other tissues. Once an abnormal growth is defined as a cancer, or neoplasm, high cellular aneuploidy is also considered by some to be a sign of aggressiveness.

Moreover, new technologies have enabled researchers to reopen the question of whether specific chromosomal changes can be detected in cancer cells, and many investigators are beginning to find those long-sought patterns. Even oncogene enthusiasts studying the effects of aneuploidy in existing tumors have generated evidence that points to chromosome-level alterations, rather than discrete gene mutations, as the driver of malignancy [see "Untangling the Roots of Cancer," by W. Wayt Gibbs; SCIENTIFIC AMERICAN, July 2003]. Intrigued by the emergence of these data over the past decade, as well as by the enormous potential of chromosomal alteration to generate new cellular phenotypes--much as it produces new species in nature--my colleagues and I set ourselves the challenge of explaining the instability inherent in cancer cells, which is the source of the chaos that became the nemesis of the original chromosomal cancer theory.

Effects and Causes

OUR STRATEGY was to collect and analyze the features of carcinogenesis that are most inconsistent with the prevailing gene mutation theory--by looking at the exceptions to the current rule, we hoped to find a better rule. In the end, we identified six features of cancer that are inexplicable by gene mutation alone but that can be explained by chromosomal changes, and a coherent theory did emerge.

Cancer risk grows with age. Lamentably common, cancer afflicts about one in three people at some point in their lives, but mostly after the age of 50, which is when chances for malignancy soar. Thus, cancer is, by and large, a disease of old age. The gene mutation theory of cancer's origins, however, predicts that the disease should be quite common in newborns. If, as that hypothesis holds, about half a dozen mutations to critical genes were necessary to ignite malignancy, certainly some of those mutations would accumulate like SNPs over the course of generations in the genomes of many individuals. A baby could thus inherit three of six hypothetical colon cancer mutations from her mother, for example, and two from her father and be at extremely high risk of

cancer from picking up the missing sixth mutation in any one of her billions of colon cells. Some babies might even be born with colon cancer from inheriting all six hypothetical colon cancer mutations from their parents. But colon cancer is never seen in children. Indeed, even laboratory mice intentionally engineered to carry an assortment of ostensibly carcinogenic mutations from birth can live and propagate happily, with no higher risk of developing tumors than normal lab mice.

Some proponents of the mutation theory maintain that except in rare cases of genetic predisposition to cancer--by which they mean the presence of inherited oncogenic mutations--the gene changes believed to cause malignancies must all be acquired after birth. That assumption implies a natural gene mutation rate over an individual's lifetime much higher than the norm, which is one change to a given gene in one out of every one million to 10 million cells (that is, once every 10^6 to 10^7 cell generations).

Interestingly, among the rare exceptions to cancer's age bias are children with congenital aneuploidy, as in Down syndrome, or with inherited chromosome instability syndromes, such as the disease known as mosaic variegated aneuploidy (MVA), which also causes severe mental retardation. Defects of mitotic spindle assembly in the cells of children with MVA produce random aneuploidies throughout their bodies, and nearly one third develop leukemia or unusual solid cancers.

Being born aneuploid, or prone to aneuploidy, clearly accelerates processes that lead to cancer. Indeed, the inherent instability of aneuploid cells would explain why most aneuploid embryos, as Boveri observed 100 years ago, would not be viable at all and thus why newborns are cancer-free and cancer is not heritable.

Carcinogens take a very long time to cause cancer. Numerous chemicals and forms of radiation have been shown to be carcinogenic in animals or established as the source of occupational or accidental cancers in humans. But even the strongest carcinogens at the highest survivable doses never cause cancer right away. Instead the disease emerges only after delays lasting years or even decades. In contrast, when substances known to cause gene mutations are administered to bacteria, the cells begin displaying new phenotypes within hours; in larger organisms such as flies, the effect is seen within days. A gene mutation scenario therefore does not explain why cells exposed to carcinogenic agents become cancer cells, much the way an undersea volcano becomes an island that appears only after many invisible eruptions.

Carcinogens, whether or not they cause gene mutations, induce aneuploidy.

Scientists have looked for the immediate genetic effects of carcinogens on cells, expecting to see mutations in many crucial genes, but instead have found that some of the most potent carcinogens known induce no mutations at all. Examples include asbestos, tar, aromatic hydrocarbons, nickel, arsenic, lead, plastic and metallic

prosthetic implants, certain dyes, urethane and dioxin. Moreover, the dose of carcinogen needed to initiate the process that forms malignant tumors years later was found to be less than one-thousandth the dose required to mutate any specific gene. In all cases, however, the chromosomes of cells treated with cancer-causing doses of carcinogens were unstable--that is, displaying higher than usual rates of breakage and disruption.

These findings suggest that carcinogens function as "aneuploidogens" rather than as mutagens. The gene mutation theory has never been able to explain how nonmutagenic carcinogens cause cancer. In fact, even the mutagenic carcinogens may actually work to trigger cancer by inducing aneuploidy through direct destruction or fragmentation of chromosomes. (Radiation, for example, induces mutations indirectly by first breaking the DNA strand. Cellular repair proteins attempt to fix the damage but may introduce errors or rearrangements in the nucleotide sequence.) Nonmutagenic carcinogens, such as aromatic hydrocarbons, can cause aneuploidy through a different mechanism. Those chemicals are known to destroy the microtubule polymers within a cell that normally pull duplicate chromosomes apart symmetrically during mitosis. Thus, the common denominator of all carcinogens seems to be their initiation of random aneuploidy.

Patterns of aneuploidy are seen in different tumors. If aneuploidy is only a side effect of cancer, then chromosomal changes in the cancers of different people should be random. But a pair of chromosome-painting technologies, known as comparative genomic hybridization and fluorescent in situ hybridization, have begun to reveal signature patterns amid the chaos in cancer cells. These techniques enable scientists to tag and track bits and pieces of chromosomes with colored DNA-specific probes to build a picture of all the chromosomal segments gained, lost or rearranged in a given cell.

Many researchers have begun to find evidence of "nonrandom" aneuploidies--specific chromosomal changes shared by most cancers of a certain kind, such as neoplasms of the breast or cervix, as predicted by the original chromosomal cancer theory. In just one example reported last year, scientists at Karolinska University Hospital in Sweden examined cells from 10 patients with Burkitt's lymphoma and found frequent translocations (swapping of chromosome fragments) involving chromosomes 3, 13 and 17, as well as specific losses or gains in segments of chromosomes 7 and 20.

Because such patterns in cancer cells of different individuals are specific to the type of tissue in which the cancer originates, they may well represent essential chromosome changes needed to overcome programmed constraints on the original mother cell's development. These changes would thus represent the minimum alterations needed for a viable aneuploid cell of that kind to start down the road toward becoming neoplastic.

In addition to such cancer-type-specific patterns, chromosome changes indicative of cancer stage, metastatic potential, and even drug resistance have also been identified by several research teams. For instance, the Karolinska group noted that translocations in one segment of chromosome 17 and gains on parts of 7 and 20 were associated with drug resistance.

As scientists have continued to work out the exact functional effects of such cancer-specific aneuploidies, many analyses of the amounts of different gene transcripts and proteins manufactured by cancer cells have also shown that the proteins encoded by specific purported oncogenes are actually often generated at the same levels as in normal cells. Among several interesting recent studies of this kind, one by a team of U.S. and Israeli researchers set out to assess the protein levels in colon cancer cells and did find that large numbers of proteins were being overproduced or underproduced--in amounts corresponding to the total DNA content changes within the cell. With greater aneuploidy, the protein imbalances increased, and so did the cells' cancerous progression. This example strongly supports the idea that by raising or reducing a cell's normal dosage of thousands of genes at once, aneuploidy produces malignant phenotypes.

Gratuitous traits do not contribute to the cancer's survival. Some of the most common and dreadful characteristics of cancer do not offer any competitive survival advantages to a tumor. Examples of these include intrinsic resistance to drugs the tumor has never encountered before and metastasis, which does not help tumor cells compete with normal cells at their site of origin. Individual gene mutations, which are rare to begin with, would only be selectively conserved in tumor cells if the mutation were advantageous, so the chances of an untreated tumor becoming drug-resistant through random gene mutations in its cells are practically zero. Because chromosomes are much larger and can harbor thousands of genes, however, they could be selectively retained for their contribution of some cancer-specific phenotype, and numerous unselected traits would be carried along as well. Indeed, the evidence of specific chromosomal changes associated with drug resistance or metastasis supports this possibility. And collectively, cancer cells can evolve all kinds of new traits very rapidly.

Cancer cells morph much faster than genes. Cancer cells generate new phenotypes and lose old ones exceedingly fast. Given a normal mutation rate--and many studies have demonstrated that in more than 90 percent of cancers, the cellular gene mutation rate is not accelerated--the odds are very long once again that a particular gene will mutate to generate a new phenotype as rapidly as is observed in cancer cells.

To test the hypothesis that whole-karyotype alterations could achieve such speedy change, our group recently examined the chromosomal variations present in highly aneuploid human breast and colon cancer cells, as well as the speed with which the

cells acquired resistance to toxic drugs. Karyotype changes were observed in these cells at rates of at least one in 100 generations, and drug-resistance-specific changes at a rate of one in 1,000 to one in 100,000 cell generations. In other words, aneuploid cells reshuffle their chromosomes and phenotypes much faster than mutation can alter their genes.

These and similar experiments also demonstrated that the more aneuploid the cancer cells were, the quicker new chromosomal alterations appeared. This pattern strongly supports a conclusion that the chromosomal instability seen in cancer cells is catalyzed by aneuploidy itself. Once this vicious cycle is under way, the fact that every cell would be randomly generating its own new phenotypes could explain an observation made decades ago by Leslie Foulds of the Royal Cancer Hospital in London that "no two tumors are exactly alike ... even when they originate from the same tissue ... and have been induced experimentally in the same way." Such individuality is yet another hallmark of cancer that cannot be explained by the activity or inactivity of specific genes, which would be expected to have consistent effects each time and in each cell.

Because every one of the extraordinary features of carcinogenesis that cannot be explained by the mutation theory is associated with chromosomal alterations, we have proposed a revised chromosomal theory of cancer that takes this inherent instability into account.

Deadly Dynamic

WHEN CANCER IS VIEWED as a chromosomal disease, carcinogens, rare genetic syndromes and accidental mitotic errors can initiate carcinogenesis by inducing random aneuploidies. Because aneuploidy unbalances thousands of genes and their protein output, it sets the stage for still more aneuploidy. This dynamic self-catalyzing condition becomes a steady source of variation from which, in classical Darwinian terms, selection of selfish chromosome combinations eventually gives rise to viable and competitive neoplastic cells. These are effectively new cell species, albeit parasitic ones, with unstable karyotypes.

Once cancer progression is under way, random chromosome reshuffling can rapidly generate gratuitous traits that include lethal properties such as drug resistance and metastasis. Thus, the prospects for success in treating tumors with individual drugs, particularly those targeted to single genes, are not good. Some investigators have recently proposed fighting fire with fire in the form of treatments that accelerate chromosomal DNA damage and aneuploidy, with the goal of making tumor cells so unstable that they are no longer viable. This might work in a very small, well-contained tumor, but it is a technique that could prove difficult to control.

The slow progression from early aneuploidy to aggressive neoplasm does, however, offer a wide window of opportunity for doctors to detect and surgically remove a potential cancer before it enters a neoplastic stage. Early cancers could also be distinguished from morphologically identical benign tumors by checking for aneuploidy. In more advanced tumors, chromosome patterns associated with drug resistance or metastatic potential could be used to guide treatment choices.

Finally, screening for chromosome-damaging substances in foods, drugs and the environment could significantly improve cancer prevention by identifying aneuploidy-inducing potential carcinogens. Today I am still optimistic enough to hope that by coming full circle, cancer scientists will eventually arrive at a basic understanding of this fearsome disease, yielding effective prevention, management and even cures.

HOW ANEUPLOIDY COULD CAUSE CANCER

Abnormal chromosome numbers in a cell create conditions that lead to further chromosome damage and disarray. With each new generation, resulting cells grow increasingly unstable and develop ever more malignant traits.

- 1.** Because of a random accident or damage by carcinogens to chromosomes or mitotic machinery, a dividing cell distributes its chromosomes unevenly between two daughter cells, leaving both aneuploid.
- 2.** Most resulting cells are not viable and die, but a surviving cell may continue proliferating. Low aneuploidy in its offspring begins to compromise their internal functioning, but they are not yet multiplying excessively.
- 3.** Skewed dosages of proteins generated by the cells' irregular chromosome complements cause instability that further disrupts regulatory and DNA-maintenance processes. Additional chromosome breakage, structural rearrangements and duplication errors arise.
- 4.** Cells begin exhibiting progressively more deviant traits as aneuploidy increases and their protein production grows more aberrant. These changes include atypical appearance and hyperproliferation, leading to formation of a tumor.
- 5.** Malignant features, such as the ability to invade neighboring tissue or metastasize to distant locations and intrinsic resistance to drugs, may also arise as random effects of the internal chaos caused by the cells' escalating aneuploidy.

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By Peter Duesberg

PETER DUESBERG is a professor of molecular and cell biology at the University of California, Berkeley, where he arrived from Germany in 1964 as a research virologist. Within six years he had isolated the first true oncogene, from within the Rous sarcoma virus, and mapped the genetic structure of the entire virus. He proceeded to do the same for 10 more mouse and avian sarcoma and leukemia viruses and was elected to the National Academy of Sciences in 1986. By 1987 his work with retroviruses led him to conclude that HIV is merely a bystander and AIDS results from chemical exposures and malnutrition. His ongoing work with cancer viruses also persuaded him that mutations in individual genes are insufficient to cause the malignant transformations seen in cancer.

Editors' note: The author, Peter Duesberg, a pioneering virologist, may be well known to readers for his assertion that HIV is not the cause of AIDS. The biomedical community has roundly rebutted that claim many times. Duesberg's ideas about chromosomal abnormality as a root cause for cancer, in contrast, are controversial but are being actively investigated by mainstream science. We have therefore asked Duesberg to explain that work here. This article is in no sense an endorsement by SCIENTIFIC AMERICAN of his AIDS theories.