The Genetic Basis of Cancer

An accumulation of genetic defects can apparently cause normal cells to become cancerous and cancerous cells to become increasingly dangerous

by Webster K. Cavenee and Raymond L. White

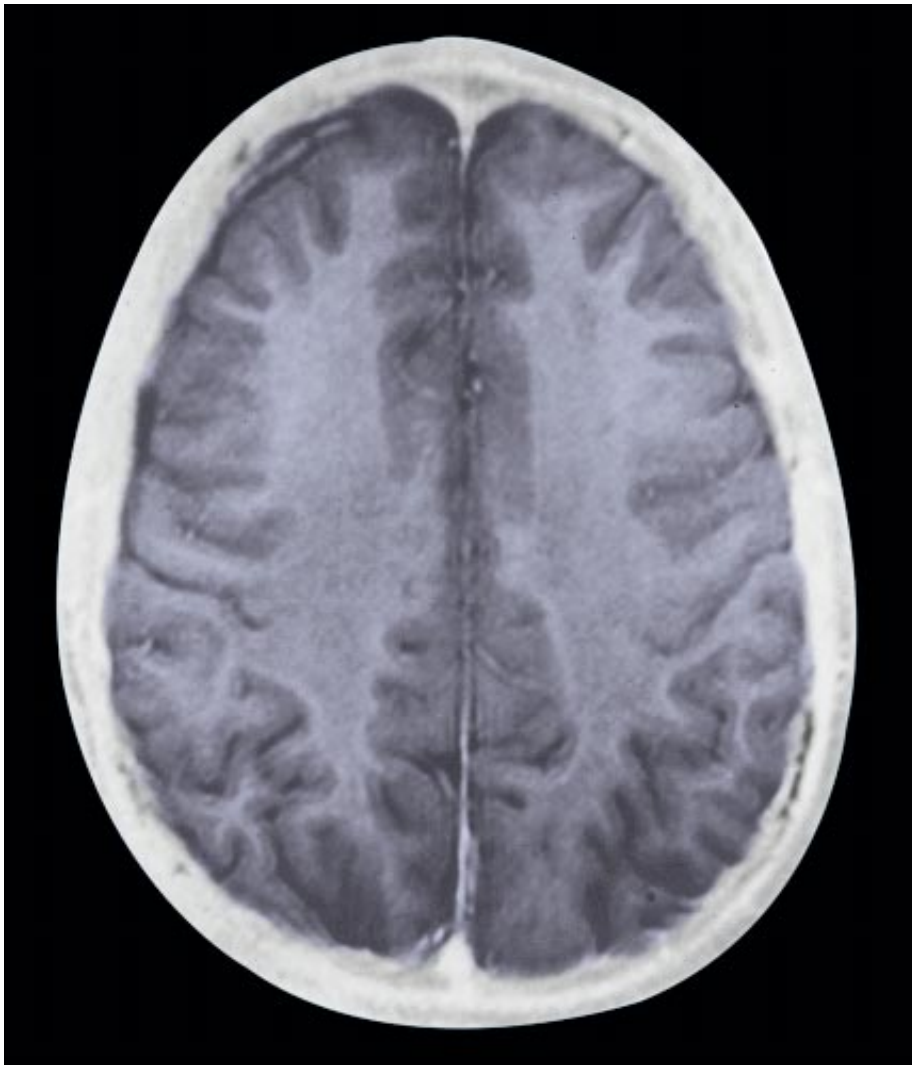
Patients stricken with cancer feel as if they have been invaded by an alien force. Yet malignancies arise from our own tissue. In fact, the weight of evidence today indicates that cancers generally derive from a single cell that is changed dramatically by a series of genetic alterations.

A healthy cell has a well-defined shape and fits neatly within the ordered array of cells surrounding it. It responds to the dictates of its environment, giving rise to daughter cells solely when the balance of stimulatory and inhibitory signals from the outside favors cell division. But the process of replication, or growth, carries the constant hazard of genetic mutations: random changes that can impair the regulatory circuits of a cell. If a single mutation occurs, the newly damaged cell, which may look normal and be slightly less responsive to external messages, may occasionally undergo unscheduled cell division.

Eventually, an accumulation of genetic damage can cause a daughter cell to become quite deaf to external messages and to display the signs of malignancy. In particular, it loses its distinctive shape and boundaries, ceases to respond to growth-inhibiting signals and gains the ability to replicate uncontrollably. The resulting mass, in turn, can compress and damage healthy tissue in its vicinity. What is worse, it can invade the barriers that separate one organ from another and can metastasize, establishing new colonies at distant sites.

Studies carried out over the past 20 years have begun to identify many of the genes that take part in this progression from normalcy to cancer. The ongoing research is confirming and extending early proposals that cancer develops primarily because cells suffer irreversible damage to particular classes of genes. It is also creating opportunities for improved diagnosis and therapy.

The emerging view of tumor progression reflects a convergence of several lines of research, the oldest of which still involves painstakingly looking at cells through a microscope. By 1914, for instance, the German cytologist Theodor Boveri had concluded from such



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observations that malignant cells had abnormal chromosomes and that any event leading to such aberrancy would cause cancer.

Microscopic observations became considerably more specific after 1970, when new staining techniques, together with improved equipment, made it possible to distinguish each of the 23 pairs of chromosomes that collectively contain all the genes forming the blueprint for a human being. (All human cells, except for sperm and eggs, carry two sets of chromosomes—one inherited from the mother and one from the father.) Each chromosome takes up the stain in specific regions and thus becomes marked by a characteristic series of light and dark bands, a kind of bar code identifying the individual chromosome.

By comparing stained chromosomes from normal cells with those from tumors, investigators noted many different signs of genetic disarray in cancers. The chromosomes of tumors were often broken, with some of the pieces joined to other chromosomes. Individual chromosomes were present in multiple copies rather than the normal two. Whole chromosomes, or sometimes internal segments, seemed to have disappeared entirely. Unfortunately, until the 1980s researchers generally lacked the tools they needed to determine whether the chromosomal rearrangements were among the causes of cancer or were a by-product of its development.

Two Hits

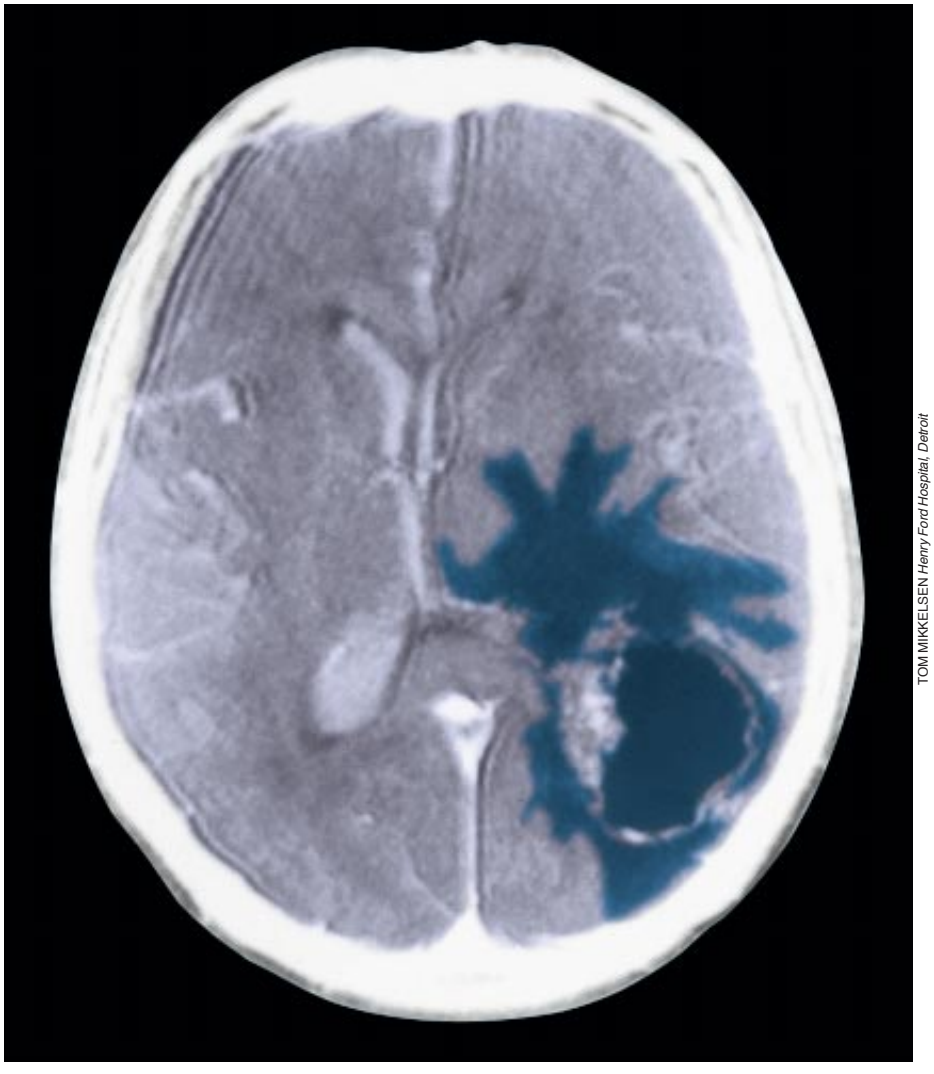
Quite different evidence that genes had a role to play came from observations that some extended families suffered an unusually high incidence of certain cancers. When particular diseases "run" in families in predictable patterns, an inherited defect is usually at fault.

Yet the discovery that some cancers could apparently be inherited also raised perplexing questions. A genetic defect passed to a child through the sperm or egg should appear in every cell of the body. Why, then, did people with inherited disease typically acquire only one or a few cancers and only at discrete sites? Further, did the existence of familial cancers necessarily mean that sporadic (nonfamilial) disease, which is much more common, also had a genetic basis? Or did sporadic cancers arise by completely different processes than inherited ones?

A proposal put forward in 1971 by Alfred G. Knudson, Jr., now at the Fox Chase Cancer Center in Philadelphia, seemed to offer an answer to both questions, although it took about a decade for his ideas to gain broad acceptance. Knudson had been puzzling over the cause of retinoblastoma, a rare childhood disorder in which malignant tumors develop in the retina before the age of six. He noted that sometimes the disease occurred in both eyes, but most of the time it affected only one eye. Moreover, children who were affected bilaterally often had close relatives afflicted with retinoblastoma.

A statistical analysis comparing the age at onset for each form of the disease showed that the bilateral type was usually diagnosed at an earlier age than was the unilateral type. Also, the shape of the age distribution curves suggested to Knudson that retinoblastoma resulted from two cellular defects arising at separate times. In bilateral disease the first defect was probably inherited and present in all cells of the body from the moment of conception. In unilateral disease the first defect probably arose during development or later and perhaps exclusively in retinal cells. In both cases, however, a tumor formed only if the first defect in a retinal cell was later accompanied by a second, independent one. Knudson's two-hit theory, as it is frequently called, turns out to be essentially correct for all cancers, not just retinoblastoma, although more than just two hits are often required.

The need for two hits—now known to constitute damage to genes—explains why patients in cancer-prone families are not riddled with tumors throughout their bodies: inheritance of just one genetic defect predisposes a person to



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CANCER OF THE BRAIN progressed in just three months from being invisible on a scan (*left*) to covering a large area of one hemisphere (*blue area, right*). The patient, whose initial complaint was an uncontrollable twitching in one eye, died two months after the second image was made. Recent evidence indicates that brain tumors and other malignancies arise when multiple genetic mutations combine to free a single cell from normal restraints on proliferation, invasiveness and movement.

cancer but does not cause it directly; a second event is required. Knudson's intuition that the causes of sporadic and familial cases can involve the same biochemical abnormalities has also been confirmed. But even back in the 1970s his insights provided justification for thinking that research aimed at discovering genetic and other cellular aberrations in rare familial cancers could shed light on the processes leading to sporadic malignancies.

Oncogenes Take Center Stage

As various researchers focused on the genetics of familial malignancies, other workers convinced that genes were at the root of cancer were taking a rather different approach to finding cancer-related genes. It had been known for many years that viruses can cause tumors in animals. That link had spurred a great deal of research aimed at identifying the cancer-causing genes carried by the viruses and at finding the host genes that were affected. Those efforts revealed, surprisingly, that the genes implicated in malignant diseases were often altered forms of human genes that the viruses had picked up during their travels. Other times the viruses activated host genes that were usually quiescent.

The normal versions of the pirated and activated genes—now called proto-oncogenes—carry codes specifying the composition of proteins that encourage cells to replicate. These growth-promoting genes come in many varieties. Some specify the amino acid sequences of receptors that protrude from the cell surface and bind to molecules known as growth factors. When bound by such factors, receptors issue an intracellular signal that ultimately causes cells to replicate. Others of the genes code for proteins that lie inside the cell and govern the propagation of the intracellular growth signal. Still others encode proteins that control cell division.

Discovery that the viral genes had human counterparts introduced the intriguing possibility that human cancers—including the majority not caused by viruses—might stem from mutations that convert useful proto-oncogenes into carcinogenic forms, or oncogenes. Consistent with this notion, studies indicated that alteration of just one copy, or allele, of these proto-oncogenes was enough to transform—render cancerous—some types of cells growing in culture. Such dominant mutations cause cells to overproduce a normal protein or to make an aberrant form that is overactive. In either case, the result is that stimulatory signals increase within the cell even when no such signals come from the outside.

Later studies supported a role for oncogenes—and also complicated matters. Notably, in 1982 and 1983, investigators in France and the U.S. conducted studies similar to the original cell-culture experiments, but with an important difference. Because normal cells would not grow indefinitely in a culture dish, those earlier studies had relied on rodent cells that were unusual in their ability to proliferate for a long time in culture. To eliminate this possibly confounding influence, François Cuzin of the University of Nice, Robert A. Weinberg of the Massachusetts Institute of Technology and H. Earl Ruley, then at Cold Spring Harbor Laboratory in New York State, asked whether single oncogenes could also transform normal rodent cells.

They found that mutations in at least two proto-oncogenes had to be present and that only certain combinations of mutations led to malignancy. These results suggested that individual oncogenes, though potentially quite powerful, were not able to cause tumors by themselves. A major effort was then launched to see whether human tumors carried oncogenic alterations of the types and combinations that were able to transform cells in culture.

For a while it seemed that oncogenes might explain most cases of cancer. This view was strengthened by discovery of more than a dozen of them in human tumors. The results were ultimately disappointing, however; a mere 20 percent of human tumors turned out to carry the expected alterations singly, and none of them had the pairs of cooperative alterations found in cultured cells. At the time, it also appeared that the inherited mutations responsible for predisposing people to familial cancers were not oncogenes. These were all strong hints that the full story was yet to be told.

Enter Tumor Suppressor Genes

Even before those hints attracted much attention, the two of us were beginning to suspect that damage to a different kind of gene might play a part in cancers. Such genes came to be known as tumor suppressors because many of them code for proteins that inhibit cell replication. In contrast to the mutations that activate oncogenes, mutations of these genes, we believed, would be recessive: they would affect cell function only when both alleles were damaged or lost. In testing this idea, we relied on new technology we had developed for the more general purpose of following the inheritance of genes and chromosomes through extended families [see "Chromosome Mapping with DNA Markers," by Ray White and Jean-Marc Lalouel; SCIENTIFIC AMERICAN, February 1988].

In the early 1980s, while collaborating at the University of Utah, we realized that our technique—which involved tracking genetic markers (identifiable segments of DNA) in tissues—could be used to determine whether segments of chromosomes carried by normal cells were missing in a tumor. For instance, if a selected region of a chromosome was deleted in a tumor, we could spot that loss by observing that a marker known to travel with that region was also missing.

Our experiments were focused by earlier studies of Jorge J. Yunis of the University of Minnesota and Uta Francke of Yale University. That research indicated a gene on chromosome 13 might be involved in retinoblastoma. With our DNA-marker technology, we were able to demonstrate in 1983 that large segments of chromosome 13 were missing in cells taken from sporadic as well as inherited retinoblastomas. This new evidence strongly supported the idea that the two hits hypothesized by Knudson could consist of the physical or functional loss of one allele of a gene followed by elimination of or damage to the normal copy. The missing DNA on chromosome 13, now known as the *RB* (retinoblastoma) gene, was isolated by Stephen H. Friend of Weinberg's laboratory in 1986 [see "Finding the Anti-Oncogene," by Robert A. Weinberg; SCIENTIFIC AMERICAN, September 1988].

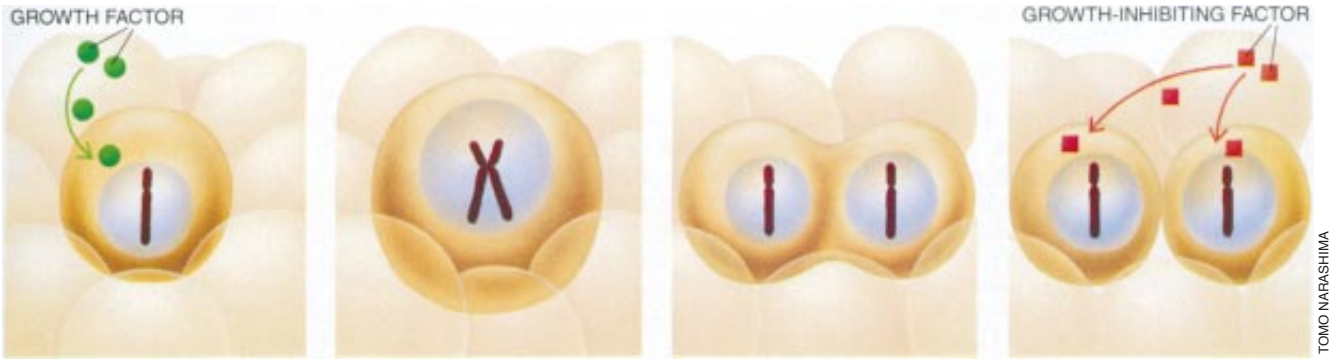
Subsequent studies have shown that recessive loss of the *RB* gene occurs in other cancers as well. What is more, inactivation or loss of DNA has now been shown to be a major feature in the genesis of every solid cancer examined so far. Breast cancer, prostate cancer, lung cancer, bladder cancer, pancreatic cancer and many others are marked by the disruption or elimination of multiple tumor suppressor genes.

By the late 1980s, then, there was good evidence that mutations in both proto-oncogenes and tumor suppressors could participate in causing cancer. It seemed reasonable to guess that some kinds of cancer resulted from a combination of such mutations. But did the mutations collect in the same cell or did some affect one cell, and others, different cells? A model of tumor progression proposed in the 1950s by Leslie Foulds of the Chester Beatty Research Institute in London and expanded in the 1970s by Peter C. Nowell of the University of Pennsylvania suggested that if both kinds of mutations were in-

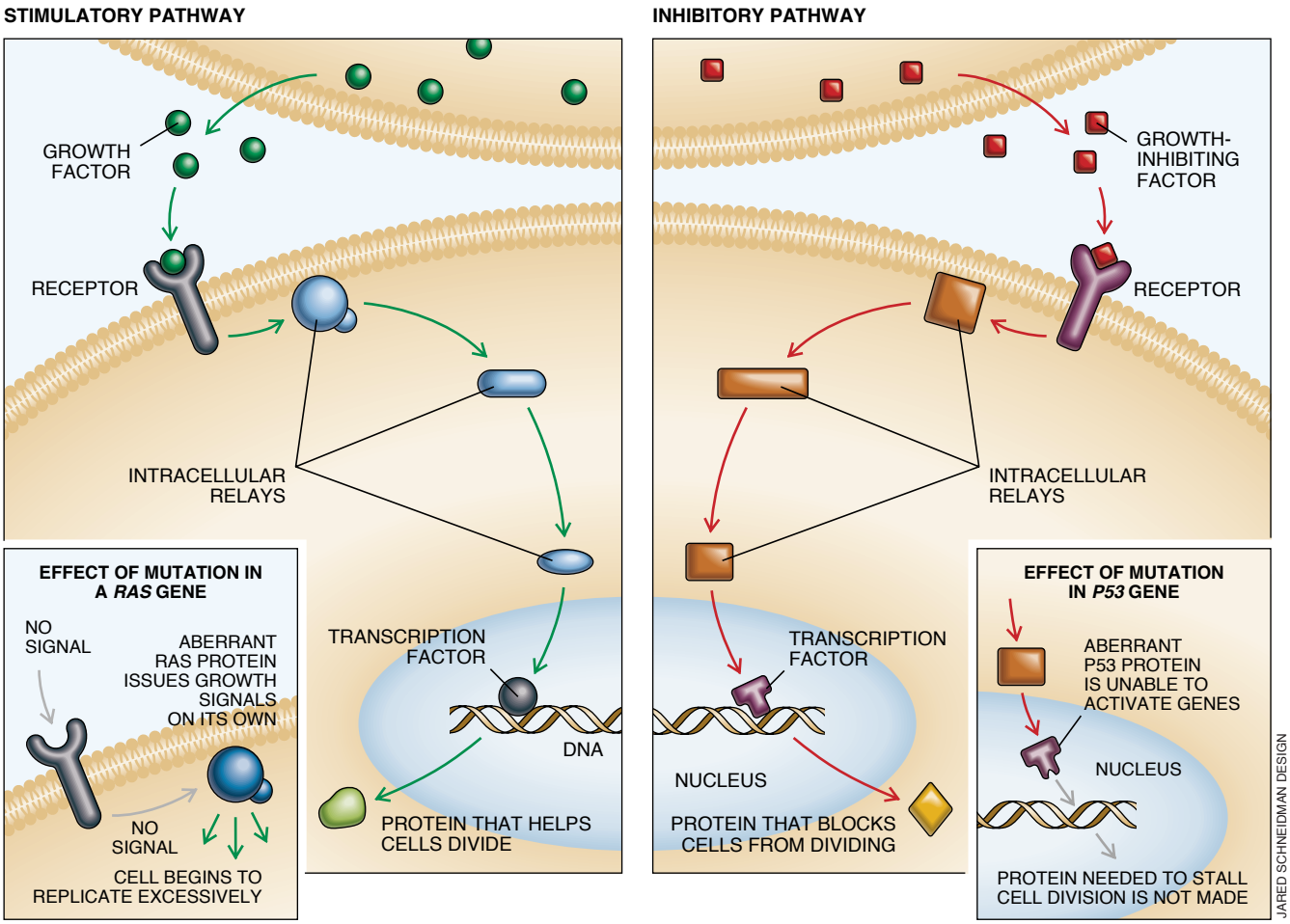
volved, they would accumulate in one cell and its direct descendants.

In this scheme, cancers are thought to arise and become more dangerous through a process known as clonal evolution. First, a single cell undergoes a genetic mutation that enables it to divide under conditions that cause normal cells to stop replicating. Because the inappropriately dividing cells copy their DNA and give identical sets to their offspring, the next generation of cells carries the same changes and shows the same inappropriate growth. Later, one of these cells or their descendants undergoes a mutation that further enhances its ability to escape normal regulation, perhaps allowing it to pass through surrounding tissue and enter the bloodstream. This mutation, too, is passed to daughter cells. Repetition of the process enables one cell to accumulate the mutations it needs to metastasize and colonize other organs.

If the theory were correct, it would mean the majority of cells in a tumor would carry the same defects. That being the case, therapy capable of counteracting one or more of those defects would be effective against all, or a great



TOMO NARASHIMA



JARED SCHNEIDMAN DESIGN

NORMAL CELL REPRODUCES ITSELF (*sequence at top*) in response to stimulation by external growth factors (*green*); it stops dividing in response to inhibitory factors (*red, far right*). For either reaction to occur, messages from the factors must be relayed deep into the target cell (*large panels*). Many cancer-causing genes are abnormal versions of ones that code for proteins in stimulatory pathways (*left panel*). The altered genes, called oncogenes, cause stimulatory proteins to be overproduced or overactive. In one example, mutation of a particular *ras* gene can lead to synthesis of a hyperactive ras protein (*inset at left*). Many other cancer-related genes code for proteins in inhibitory pathways (*right panel*) and are often called tumor suppressors. Damage to these genes can promote cancer if the defects prevent inhibitory proteins from being made or functioning properly—as often occurs when the *p53* gene is mutated (*inset at right*).

majority, of the cancer cells—a feature that is essential for eradicating any malignancy. For this reason and others, we set out to see if we could find evidence for the clonal evolution of tumors. One of us (White) focused primarily on colon cancer, and the other of us (Cavenee) on brain tumors. As part of this work, we had to identify many of the genes involved in these cancers.

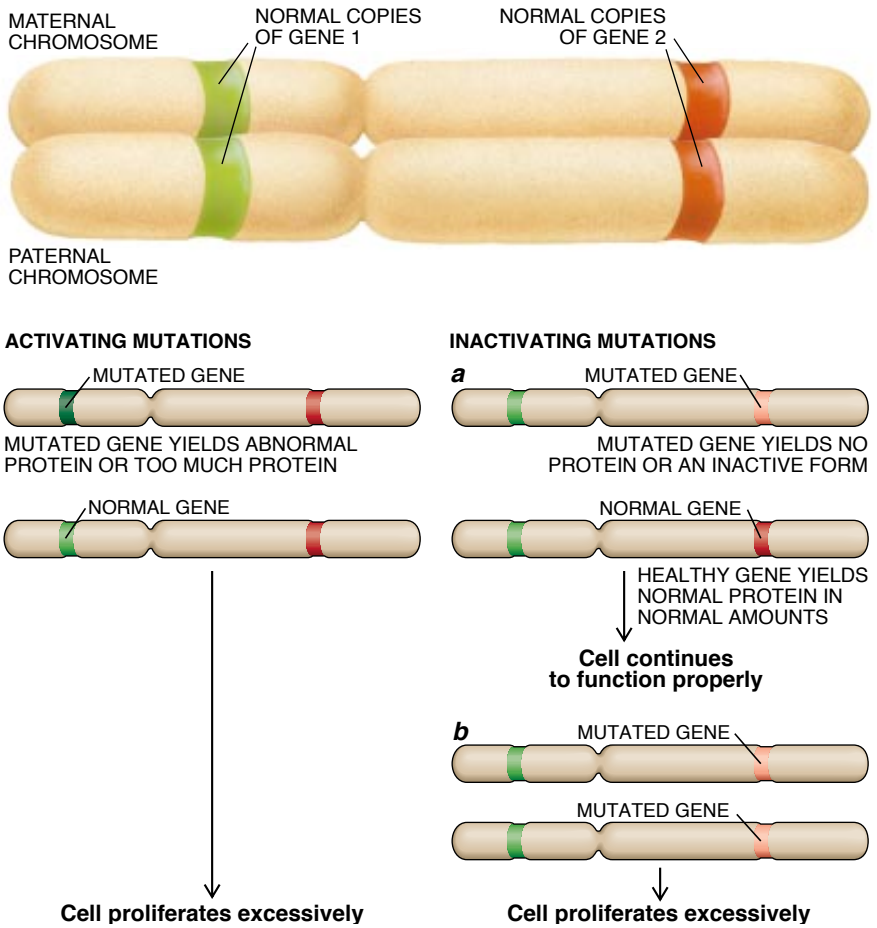
The Genetics of Colon Cancer

White turned to colon cancer in part because it usually emerges from a well-defined precursor—the colon polyp. If a cancer developed in a clonal fashion, mutations arising in an early stage of tumor development would be expected to be present in later stages, and each successive stage would be marked by additional mutations. To test this expectation experimentally, it is necessary to collect samples from the successive stages and compare their genes. In colon disease, samples are fairly easy to obtain. As a polyp, which is initially microscopic, becomes larger and more irregular, it becomes readily accessible to the gastroenterologist (who removes it for therapeutic purposes) and thus to the experimentalist.

Colon cancer also held appeal for our purpose because families that were genetically prone to a rare disease called familial adenomatous polyposis had been identified and were available for study. In affected individuals the colon becomes carpeted with hundreds or thousands of polyps, one or more of which is likely to become cancerous in midlife. Clearly, an inherited defect in some gene—called *APC* (for adenomatous polyposis coli)—was necessary for polyp formation and, in turn, for the development of colon cancer in such patients. It also seemed possible that appearance of a defect in the *APC* gene was one of the earliest steps, if not the first step, leading to many cases of sporadic colon cancer. If that gene could be isolated, these ideas could be tested, and investigators would have at least one of the genes needed for evaluating whether colon cancer developed in a clonal manner.

In 1987 Mark Leppert in White's laboratory at Utah and Walter F. Bodmer and his colleagues at the Imperial Cancer Research Fund in London separately demonstrated, through use of the marker technology described earlier, that the *APC* gene resided near the middle of the long arm of chromosome 5. Intensive work, often collaborative, by White's laboratory and those of two other investigators—Yusuke Nakamura of the Cancer Institute in Tokyo and Bert Vogelstein of Johns Hopkins University—eventually revealed the precise location of the gene. The research also identified several inherited *APC* mutations that appeared in sporadic as well as familial colon tumors. This work thus defined a first step in the evolution of colon cancer. It also provided additional confirmation of the speculation that the same genes are often mutated in both inherited and sporadic tumors.

The groups found, too, that all the cancer-related mutations in the *APC* gene led to production of an incomplete protein. Evidently, cells could operate relatively normally if they retained one normal *APC* allele and thus made some amount of the full APC protein. But if both alleles became damaged, a needed brake on replication disappeared. The precise function of the *APC* gene is unclear, but now that the gene is in hand, its normal responsibilities and its role in cancer should soon be defined.

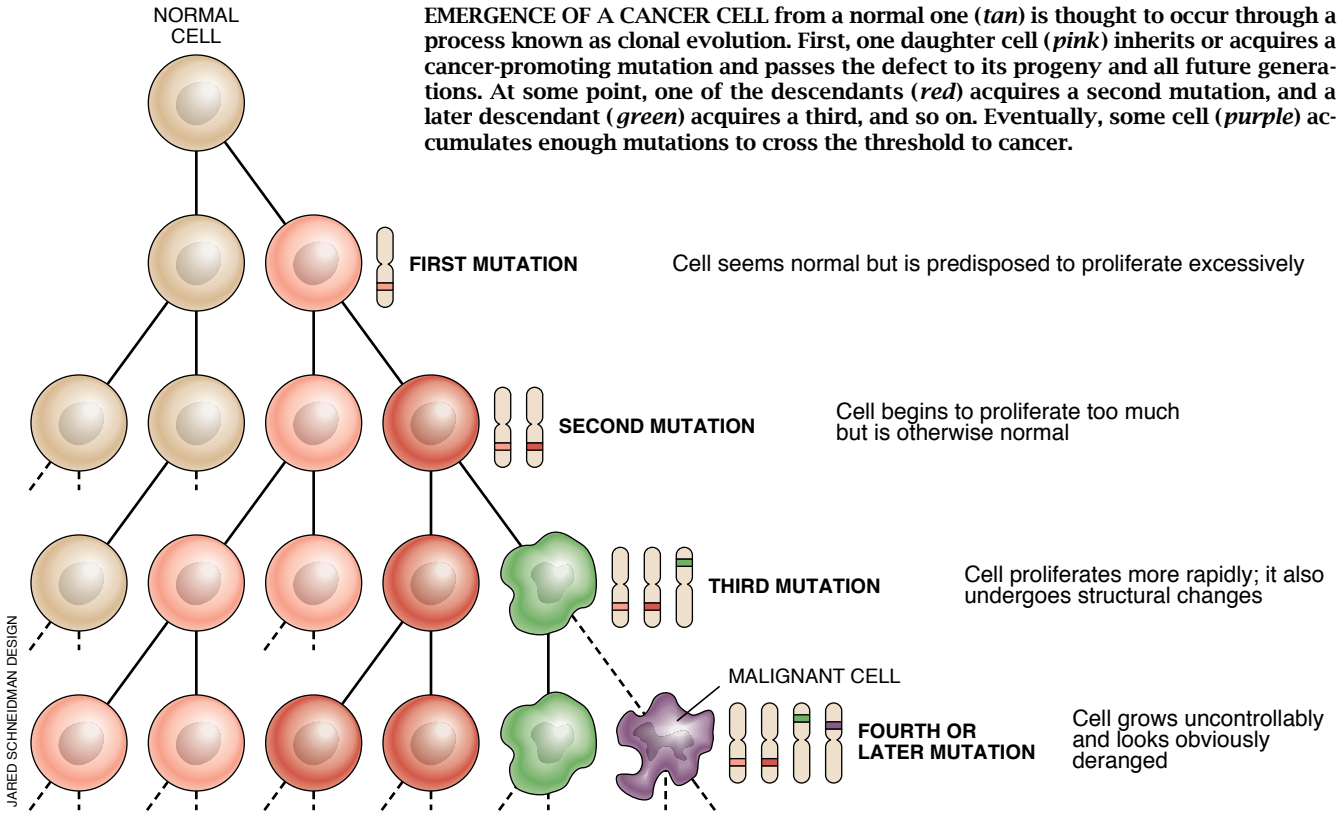


GENES ARE INHERITED IN MATCHING PAIRS—one from the mother and one from the father (*top*). Sometimes mutation of a single copy pushes a cell toward cancer (*left*)—such as when it leads to production of a protein that activates excessive cell division. (Oncogenic mutations fall into that category.) Other times both copies must be altered—such as when a gene coding for a protein that stalls cell division is inactivated (*right*). If only one copy of such a gene is affected (*a*), the other copy can still generate the needed protein. But if both copies are hobbled (*b*), an important brake on tumor development is lost.

Multiple Defects

The steps that follow immediately after the *APC* gene is inactivated are still obscure. In many cases, however, later mutation in a single allele of a particular proto-oncogene seems to push a polyp toward malignancy. This gene, as Manuel Perucho observed when he was at Cold Spring Harbor Laboratory, is one of several *ras* genes. The protein normally made under the direction of this gene sits under the cell membrane and relays stimulatory messages from growth factor receptors to other molecules in the cytoplasm. The mutant version does not wait for signals from the outside but issues its own autonomous growth signals.

Vogelstein and his group have shown that large polyps and colon cancers often carry only mutated copies of two additional tumor suppressor genes. One



EMERGENCE OF A CANCER CELL from a normal one (*tan*) is thought to occur through a process known as clonal evolution. First, one daughter cell (*pink*) inherits or acquires a cancer-promoting mutation and passes the defect to its progeny and all future generations. At some point, one of the descendants (*red*) acquires a second mutation, and a later descendant (*green*) acquires a third, and so on. Eventually, some cell (*purple*) accumulates enough mutations to cross the threshold to cancer.

is *p53,* which resides on chromosome 17 and is now known to be involved in many different cancers. The normal protein product of this gene functions in several biochemical pathways, including those enabling a cell to repair damage to DNA. The other is a gene—probably *DCC* (for deleted in colorectal cancer)—that resides on chromosome 18. *DCC* codes for a protein that appears on the cell surface and helps colon cells stick to one another.

The discovery that genetic changes in the *APC* gene occur early and persist, whereas other changes appear only in later stages, fits well with the theory of clonal evolution. But that conclusion was initially statistical and based on examining tissues removed from many different patients. That approach could not demonstrate conclusively that mutations appearing in one generation of cells are passed to later generations of those same cells. Another strategy, however, provided more convincing results.

Sometimes the polyp from which a cancer has emerged can be identified at the edge of a cancer. By comparing the DNA in a polyp with that in its adjacent cancer, Vogelstein showed that every mutational hit found in a polyp also appeared in the corresponding cancer, as would be expected if the tumor formed by clonal evolution. Further, the cancer invariably included mutations that were not found in the polyp, as would also be expected if the added mutations accounted for the increased aggressiveness of a cancer. For instance, some polyps carried a *ras* mutation without a *p53* defect, but the cancers growing from the polyps had both mutations. As yet, there is no strong evidence that mutation of *ras, p53* and *DCC* genes must happen in any particular order for a polyp to become cancerous, although the *ras* mutation seems to come first fairly often.

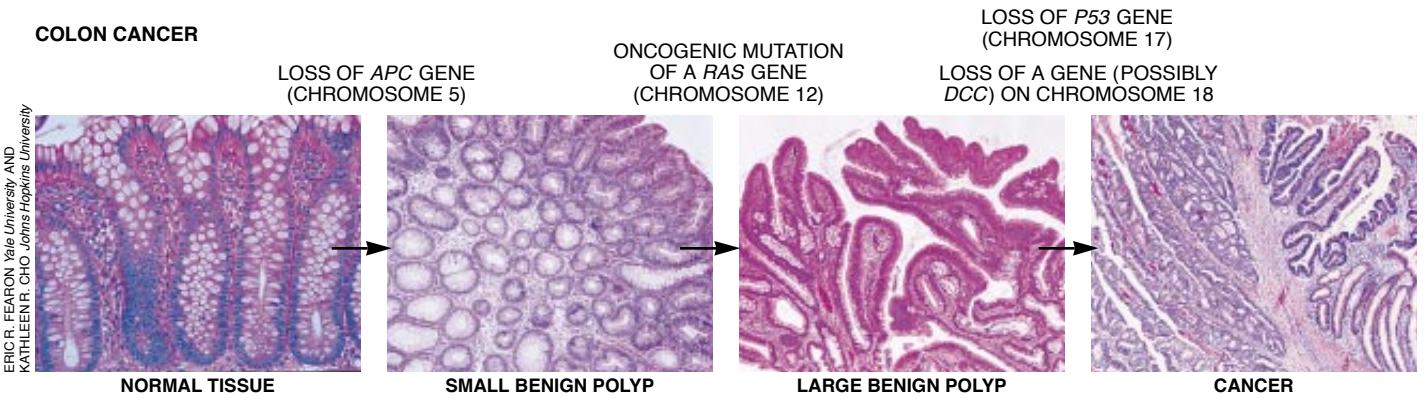
Brain Tumors Reveal Their Secrets

In spite of these encouraging findings, study of colon cancer has a major analytical limitation. To truly demonstrate that a given clone of cells is undergoing progressive changes in its genes, one needs to examine the same tumor over time. In the case of colon cancer, tumors are almost always removed at the earliest stage of detection. Such practice makes good clinical sense, but it prevents sequential observations. This consideration led Cavenee to seek out a disease in which removal of a tumor is sometimes followed by the reappearance of the tumor in a more aggressive form at the same site. In 1987, while he was at the Ludwig Institute for Cancer Research at McGill University, he and his co-workers settled on cancers known as astrocytomas—the most common tumors that originate in the brain.

Cancer of the brain is defined somewhat differently than it is in other tissues. In that organ, cells do not need to invade connective tissue or metastasize in order to be lethal; sadly, proliferation at a site critical to survival can sometimes be enough to kill a patient. Hence, most masses in the brain are called cancers. Cavenee's group examined progression of astrocytomas from their less malignant to more malignant stages, as determined by the size and shape of the tumors and by the structure of their constituent cells.

When the investigators began this work in 1987, they did not have the blueprint of genetic change that was emerging for colon cancer. They therefore began by laying the groundwork for future studies of individual patients. They obtained tumors from many different patients, grouped them according to stages, or grades, of advancing disease, and compared the genetic derangements found in each stage.

Over the next four years they made good headway. They learned, for instance, that tumors of every grade had inactivating alterations in chromosome 17, in a gene they had not yet identified. Moreover, the proportion of tumors displaying the mutation in the lowest stage was equal to that in all other stages; this pattern is a sign that the mutation came early and was retained. If a mutation generally occurred later in disease,

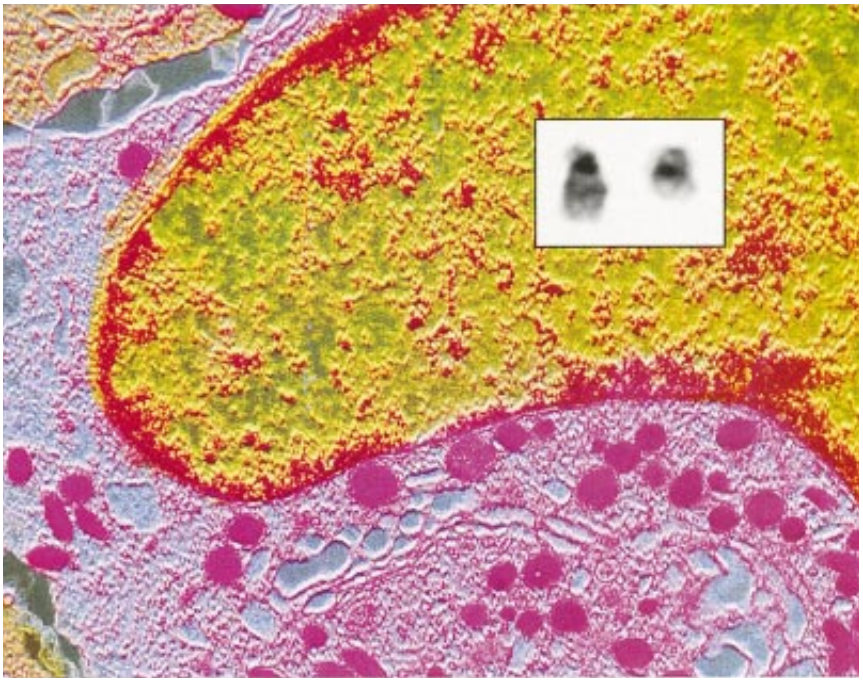


ERIC R. FEARON *Yale University* AND KATHLEEN R. CHO *Johns Hopkins University*

GENETIC CHANGES indicated at the top are among those thought to participate frequently in the development of colon cancer (*left*) or in the progression of a common brain cancer (astrocytoma) from its mildest to its most aggressive

the frequency would rise in the later stages. By the end of the 1980s Vogelstein's laboratory established that mutations in the *p53* gene, on chromosome 17, were among the most common alterations in human cancer. Subsequent analysis of Cavenee's tissue samples confirmed his growing suspicion that the chromosome 17 mutation was actually a defect in the *p53* gene.

Aware that a particular region of chromosome 9 was deleted in other kinds of brain tumors, C. David James on Cavenee's team, in conjunction with V. Peter Collins of the Ludwig Institute in Stockholm, examined this chromosome as well. Middle- and late-stage astrocytomas, but not early ones, often showed a loss in both copies of this chromosome. Thus, the deletion probably encouraged progression to middle-stage tumors from a lesser stage. The lost region contains a cluster of genes that code for proteins known as interferons. Such proteins can draw the attention of the immune system to diseased cells, and so elimination of their genes presumably helps cancer cells evade immune destruction. The missing region may additionally include two newly discovered genes, called *multiple tumor suppressors 1* and *2,* whose protein products are involved in regulating cell division. Disappearance of any of these genes could potentially contribute to a variety of cancers.



PETER C. NOWELL *University of Pennsylvania*

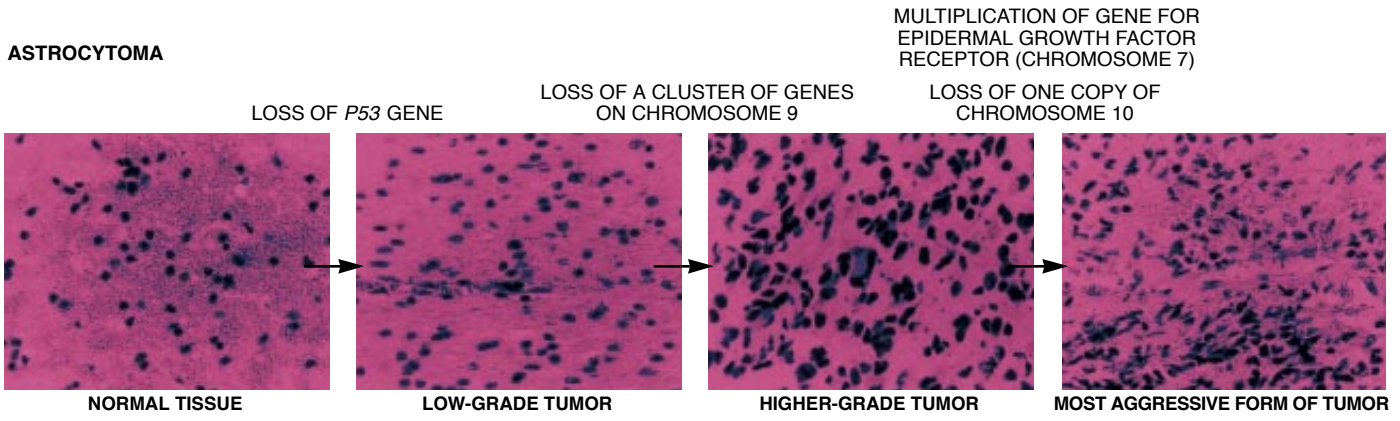
PHILADELPHIA CHROMOSOME (*at right in inset*) was the first chromosomal abnormality ever linked to a specific cancer. In the 1960s Peter C. Nowell of the University of Pennsylvania observed that the appearance of an unusually small chromosome in white blood cells was a hallmark of leukemia. It is now known that the aberrant structure forms when a normal version of chromosome 22 (*at left in inset*) swaps genetic material with another chromosome, in the process giving up more than it receives. Unfortunately, the DNA gained by chromosome 22 combines with a preexisting gene to form a hybrid oncogene.

The tissue studies also extended reports by Axel Ullrich of Genentech, Michael D. Waterfield of the Ludwig Institute in London and Joseph Schlessinger of the Weizmann Institute of Science in Israel that chromosomes in astrocytomas often carry more than one copy of the gene specifying the receptor for epidermal growth factor. Because each copy can be used to make the protein, cells will carry extra receptors on their surface. That abundance, in turn, can cause cells to overreact to the presence of the growth factor. This alteration seems to participate in bringing tumors from a middle to a late stage of disease.

Finally, Cavenee's group found that virtually all the end-stage tumors examined were missing one copy of chromosome 10 and that the loss was rare in earlier stages. This pattern says the loss is probably involved in advancement to the most virulent stage. Regrettably, though, we do not yet know which gene or genes on the lost chromosome are most important to the progression.

These results suggested by 1991 that formation of brain tumors involves, at a minimum, inactivation of the *p53* gene, loss of a gene on chromosome 9, oncogenic amplification of the gene for the epidermal growth factor receptor and, at a very late stage, loss of at least one copy of chromosome 10. But stronger proof that astrocytomas are caused by the accumulation of these, and possibly other, defects in cells required examining genetic changes in the cancer of single individuals over time.

At about that time Tom Mikkelsen joined Cavenee's laboratory and took on the challenge of comparing the genetic makeup of original astrocytomas with that of later recurrences arising at the same sites. This task was impossible earlier not only because the genes involved were not known but also be-



form (*right*). Other genes not listed here play roles as well. Unless otherwise indicated, the term "gene loss" indicates that both copies of a tumor suppressor gene have been damaged or deleted. The images show magnified slices of tissue.

cause matched pairs of tumors are hard to obtain. A patient seen initially at one institution may be cared for elsewhere when the cancer returns. Also, physicians do not remove tumors that reappear if it is thought that surgery is unlikely to extend survival. Luckily, however, two distinguished clinicians—Mark L. Rosenblum of the University of California at San Francisco and Karl Schwechheimer of Albert Ludwigs University in Freiberg, Germany—had come forward with collections of frozen tissue that included a few matched sets.

To Cavenee's satisfaction and delight, the genetic analysis of these tissues—done in collaboration with David Sidransky in Vogelstein's group—fulfilled the predictions of the theory of clonal evolution. The initial tumors possessed fewer mutations than did the recurrences. These alterations included one or more of the genetic hits (such as damage to chromosome 17) that had been identified in the low-grade tumors analyzed previously. And, most significant, the corresponding high-grade versions possessed each alteration found in the primary tumor as well as additional defects (of the kinds identified in the earlier studies). For reasons that are not obvious, progression of astrocytomas seems to follow a more defined sequence of genetic changes than is apparent in colon cancer.

Next on the Agenda

The collected results we have described offer strong support for the idea that cancer develops and becomes more dangerous primarily because cells in a single lineage accumulate defects in genes that normally regulate cell proliferation. Changes in other kinds of genes, many of which have not yet been identified, presumably facilitate the ability of tumors to grow, invade local tissue and establish distant metastases. Hormones and other factors in the environment around the genetically altered cells almost certainly enhance their genetically defined deregulation.

Questions remain. Why do cell types differ in the mix of mutations they require in order to become cancerous? And how is it possible for five or more mutations to accumulate in cells? After all, the probability is actually quite small that any given cell bearing a permanent mutation in a cancer-related gene will independently gain another mutation in such a gene.

Newly discovered genetic aberrations found in a second form of inherited colon tumors (hereditary nonpolyposis colon cancer) may offer a partial answer to the last question. The affected genes specify proteins responsible for identifying and repairing mistakes made when DNA in a replicating cell is copied. If these repair genes themselves are damaged, the number of mutations passed to daughter cells will go up dramatically. The daughter cells may then deliver DNA carrying still more mutations to their progeny. Defects in repair genes may thus play a role in making late-stage tumors highly aggressive. They may even account for the astonishingly fast rate at which some tumors arise and become killers.

Mutations in certain genes can also be especially devastating if the mutations have multiple effects. As a case in point, damage to the *p53* gene can apparently do more than release a brake on proliferation. Certain mutations seem to reduce the ability of cells to limit blood vessel formation. As extra vessels grow in a tumor, they help to nourish the mass and to serve as conduits through which malignant cells can spread to distant sites. In parallel, the abnormal proteins yielded by the altered gene may aid tumor cells in resisting the destructive effects of radiation.

As investigators gain clarity on the specific groups of genetic changes that lead to and exacerbate particular forms of cancer, their insights should point the way to practical benefits for patients. When the mutations follow in a fairly set sequence, their identification in a patient's tumor should be of value for clarifying the stage of disease and thus for tailoring therapy to the individual's needs. In addition, knowledge of the genes that are mutated in a primary tumor may make it possible to detect recurrences of some cancers earlier than is now possible—by spotting mutations that have occurred in tissues not yet displaying detectable masses.

Expanded understanding of the genetic bases of cancer can also be expected to lead to the introduction of drugs that will counteract the effects of selected mutations and thereby slow tumor development or halt it altogether. Some evidence suggests it may not be necessary to correct the effects of every mutation; doing so for one or two genes may well prove to be sufficient for taming renegade cells.

The process by which normal cells become cancerous and grow ever more dangerous is undoubtedly even more complicated than has been discovered so far. But continued investigation of the genetic changes underlying specific cancers seems a rational way to tease apart many of those complexities—and to gain new leads for treatment.

FURTHER READING

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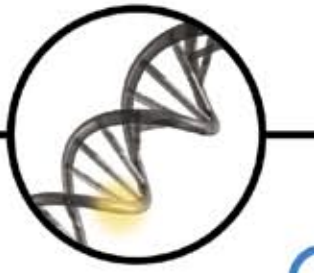
MAPPING THE CANCER GENOME



Pinpointing the genes involved in cancer will help chart a new course across the complex landscape of human malignancies

By Francis S. Collins and Anna D. Barker

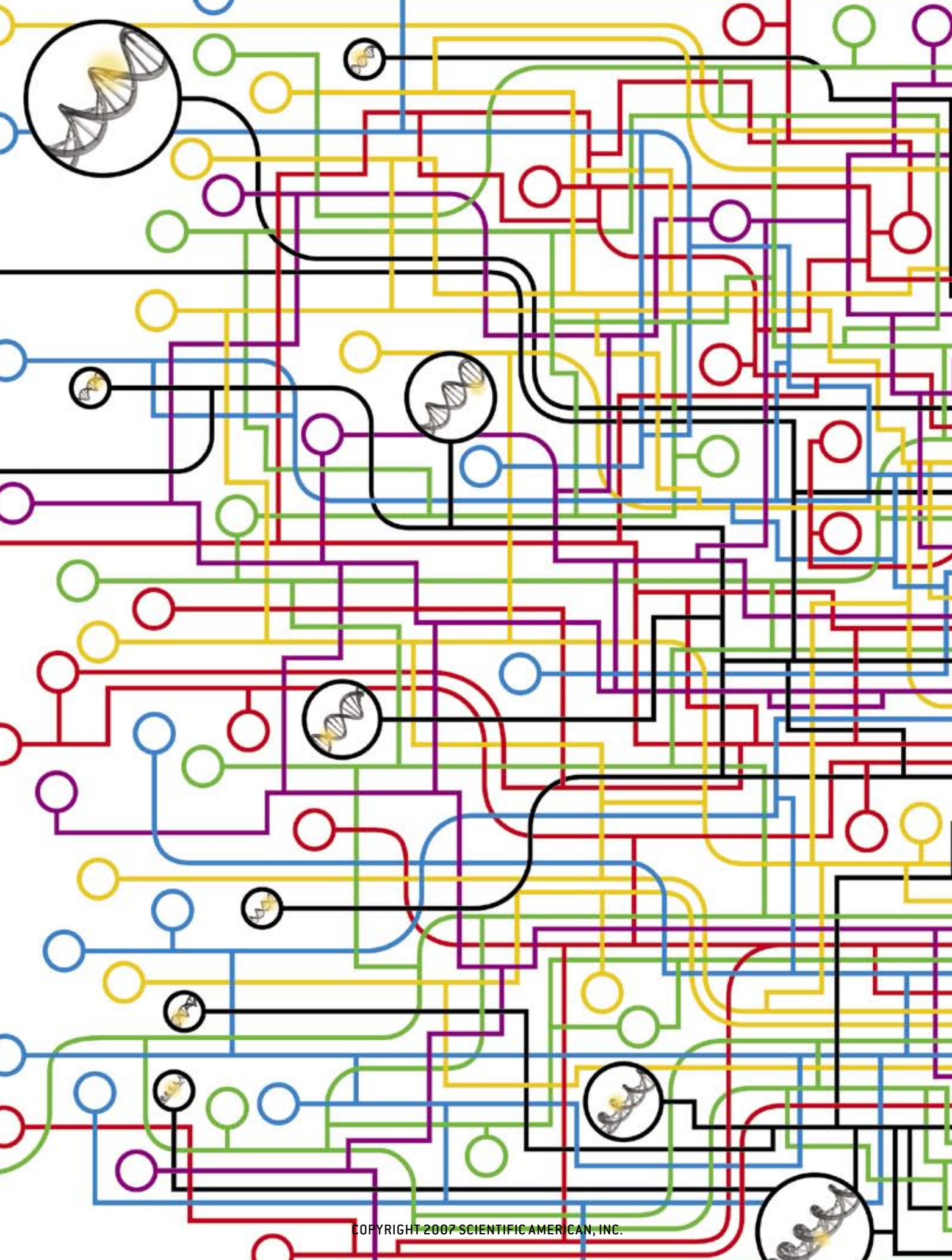
"If we wish to learn more about cancer, we must now concentrate on the cellular genome." Nobel laureate Renato Dulbecco penned those words more than 20 years ago in one of the earliest public calls for what would become the Human Genome Project. "We are at a turning point," Dulbecco, a pioneering cancer researcher, declared in 1986 in the journal *Science*. Discoveries in preceding years had made clear that much of the deranged behavior of cancer cells stemmed from damage to their genes and alterations in their functioning. "We have two options," he wrote. "Either try to discover the genes important in malignancy by a piecemeal approach, or … sequence the whole genome."

Dulbecco and others in the scientific community grasped that sequencing the human genome, though a monumental achievement itself, would mark just the first step of the quest to fully understand the biology of cancer. With the complete sequence of nucleotide bases in normal human DNA in hand, scientists would then need to classify the wide array of human genes according to their function—which in turn could reveal their roles in cancer. Over the span of two decades Dulbecco's vision has moved from pipe dream to reality. Less than three years after the Human Genome Project's completion, the National Institutes of Health has officially launched the pilot stage of an effort to create a comprehensive catalogue of the genomic changes involved in cancer: The Cancer Genome Atlas (TCGA).

The main reason to pursue this next ambitious venture in large-scale biology with great urgency is cancer's terrible toll on humankind. Every day more than 1,500 Americans die from cancer—about one person every minute. As the U.S. population ages, this rate is expected to rise significantly in the years ahead unless investigators find ways to accelerate the identification of new vulnerabilities within cancerous cells and develop novel strategies for attacking those targets.

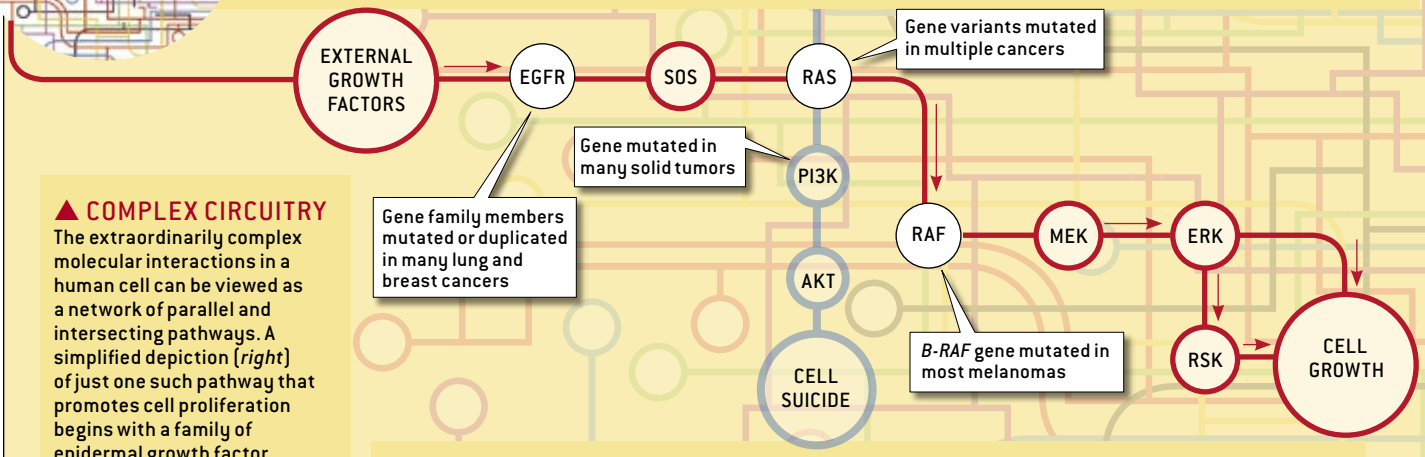
Still, however noble the intent, it takes more than a desire to ease human suffering to justify a research enterprise of this magnitude. When applied to the 50 most common types of cancer, this effort could ultimately prove to be the equivalent of more than 10,000 Human Genome Projects in terms of the sheer volume of DNA to be sequenced. The dream must therefore be matched with an ambitious but realistic assessment of the emerging scientific opportunities for waging a smarter war against cancer.

SLIM FILMS



MANY PATHWAYS TO MALIGNANCY

Gene malfunctions underlie the ability of cancer cells to escape normal constraints on a cell's behavior. Because genes give rise to proteins that serve as cellular building blocks, signals and regulators of other genes, a mutation that disables one gene, or causes it to be overactive, can have multiple deranging effects on the cell (*below*). Nevertheless, cells usually need to accrete several cancer-promoting, or oncogenic, mutations in separate genes to acquire the hallmark properties of malignancy (*box at right*). Identifying all the genes whose alteration can produce those traits should one day reveal which mutations are the true drivers of specific types of cancer—even of a specific patient's malignancy—and therefore the most effective ways to intervene in the disease.



▲ COMPLEX CIRCUITRY

The extraordinarily complex molecular interactions in a human cell can be viewed as a network of parallel and intersecting pathways. A simplified depiction (*right*) of just one such pathway that promotes cell proliferation begins with a family of epidermal growth factor receptors (EGFR) in the cell membrane. Their stimulation by growth factors outside the cell transmits signals to additional proteins and genes, ultimately prompting the cell to "grow" by dividing.

▲ ONCOGENIC MUTATIONS

In a significant portion of lung and breast tumors, members of the *EGFR* gene family are mutated or duplicated, which boosts the number or function of the receptors they encode, overstimulating this growth pathway. Damage to downstream genes can have similar results. Changes in the *B-RAF* gene, seen in some 70 percent of melanomas, also promote hyperactive cell proliferation. Versions of the *RAS* gene are mutated in many cancer types, which can affect cell growth as well as intersecting pathways—for example, interfering with a suicide program that normally destroys damaged cells.

A Disease of Genes

THE IDEA THAT ALTERATIONS to the cellular genome lie at the heart of all forms of cancer is not new. Since the first identification in 1981 of a cancer-promoting version of a human gene, known as an oncogene, scientists have increasingly come to understand that cancer is caused primarily by mutations in specific genes. The damage can be incurred through exposure to toxins or radiation, by faulty DNA repair processes or by errors that occur when DNA is copied prior to cell division. In relatively rare cases, a cancer-predisposing mutation is carried within a gene variant inherited from one's ancestors.

Whatever their origin, these mutations disrupt biological pathways in ways that result in the uncontrolled cell replication, or growth, that is characteristic of cancer as well as other hallmarks of malignancy, such as the ability to invade neighboring tissues and to spread to sites throughout the body. Some mutations may disable genes that normally protect against abnormal cell behavior, whereas others increase the activity of disruptive genes. Most cells must acquire at least several of these alterations before they become transformed into cancer cells—a process that can take years.

Over the past two decades many individual research groups have used groundbreaking molecular biology techniques to search for mutations in genes that are likely candidates for wreaking havoc on normal patterns of cell growth and behavior. This approach has identified about 350 cancer-related genes and yielded many significant insights into this diabolical disease. A database of these changes, called the catalogue of somatic mutations in cancer, or COSMIC, is maintained by Michael Stratton's group at the Wellcome Trust Sanger Institute in Cambridge, England. But no one imagines that it is the complete list.

So does it make sense to continue exploring the genomic basis of cancer at cottage-industry scale when we now possess the means to vastly increase the scope and speed of discovery? In recent years a number of ideas, tools and technologies have emerged and, more important, converged in a manner that

Overview/*Cancer Connections*

- Changes in the structure or activity of genes underlie the malignant behavior of cancer cells.
- Identification of genes involved in certain cancers is already advancing diagnosis and treatment.
- The Cancer Genome Atlas is a monumental initiative to eventually identify all the genetic alterations in different forms of cancer so that gene changes driving the disease can be targeted directly.

LUCY READING-IKKANDA; ACKNOWLEDGMENT: SPECIAL THANKS TO JEFFREY SETTLEMAN OF THE CENTER FOR CANCER RESEARCH AT MASSACHUSETTS GENERAL HOSPITAL AND DAPHNE W. BELL OF THE NHGRI CANCER GENETICS BRANCH FOR THEIR ADVICE ON THE PATHWAY ILLUSTRATION

Hallmarks of Cancer

The six abnormal capabilities listed below together give tumors their lethal power to overrun their native tissue and spread through the body.

Self-sufficiency in growth signaling
Cancer cells amplify external growth cues or generate their own.

Insensitivity to antigrowth signals
Cancer cells become deaf to quiescence cues from surrounding tissue.

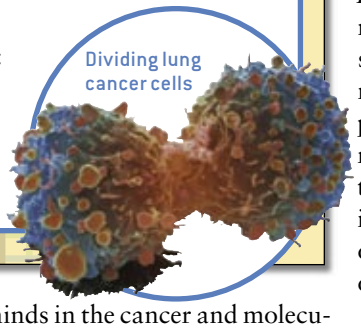
Evasion of cell suicide
Mechanisms that should trigger or carry out a self-destruct program in damaged cells are disabled or overridden.

Limitless replicative potential
Cancer cells evade intrinsic limits on the number of times a normal cell can divide.

Sustained blood vessel growth
Tumors emit signals promoting the development of new blood vessels to deliver oxygen and nutrients.

Invasiveness and motility
Cancer cells defy multiple signals and forces that hold a cell in place and prevent it from traveling to—and thriving in—other tissues.

Adapted from "The Hallmarks of Cancer," by Douglas Hanahan and Robert A. Weinberg, in Cell, *Vol. 100; January 7, 2000.*


Dividing lung cancer cells

STEVE GSCHMEISSNER *Photo Researchers, Inc.*

has convinced many leading minds in the cancer and molecular biology communities that it is time for a systematic, collaborative and comprehensive exploration of the genomics of cancer.

The Human Genome Project laid a solid foundation for TCGA by creating a standardized reference sequence of the three billion DNA base pairs in the genome of normal human tissues. Now another initiative is needed to compare the DNA sequences and other physical characteristics of the genomes of normal cells with those of cancerous cells, to identify the major genetic changes that drive the hallmark features of cancer [*see box above*]. The importance of international partnerships in large-scale biology to pool resources and speed scientific discovery was also demonstrated by the Human Genome Project, and TCGA is exploring similar collaborations.

Finally, the Human Genome Project spurred significant advances in the technologies used to sequence and analyze genomes. At the start of that project in 1990, for example, the cost of DNA sequencing was more than $10 per "finished" nucleotide base. Today the cost is less than a penny per base and is expected to drop still further with the emergence of innovative sequencing methods [see "Genomes for All," by George M. Church; Scientific American, January 2006]. Because of these and other technological developments, the large-scale approach embodied in TCGA—unthinkable even a few years ago—has emerged as perhaps the most efficient and cost-effective way to identify the wide array of genomic factors involved in cancer.

Proofs of Concept

PILES OF DATA are, of course, not worth much without evidence that comprehensive knowledge of cancer's molecular origins can actually make a difference in the care of people. Several recent developments have provided proofs of concept that identifying specific genetic changes in cancer cells can indeed point to better ways to diagnose, treat and prevent the disease. They offer encouraging glimpses of what is to come and also demonstrate why the steps toward those rewards are complex, time-consuming and expensive.

In 2001, when the Wellcome Trust Sanger Institute began its own effort to use genomic technologies to explore cancer, the project's immediate goal was to optimize robotics and information management systems in test runs that involved sequencing 20 genes in 378 cancer samples. But the group hit pay dirt a year later when they found that a gene called *B-RAF* was mutated in about 70 percent of the malignant melanoma cases they examined. A variety of researchers swiftly set their sights on this potential new therapeutic target in the most deadly form of skin cancer. They tested multiple approaches—from classic chemical drugs to small interfering ribonucleic acids—in cell lines and in mice, to see if these interventions could block or reduce the activity of *B-RAF* or inhibit a protein called MEK that is overproduced as a result of *B-RAF* mutations. Just five years later the most promising of these therapies are being tested in clinical trials.

Other research groups have already zeroed in on genetic mutations linked to certain types of breast cancer, colon cancer, leukemia, lymphoma and additional cancers to develop molecular diagnostics, as well as prognostic tests that can point to an agent in the current arsenal of chemotherapies to which a particular patient is most likely to respond. Cancer genomics has also helped to directly shape the development and use of some of the newest treatments.

The drug Gleevec, for example, was designed to inhibit an enzyme produced by a mutant fused version of two genes, called *BCR-ABL*, that causes chronic myelogenous leukemia. Gleevec is proving dramatically effective against that disease and showing value in the treatment of more genetically complex malignancies, such as gastrointestinal stromal tumor and several other relatively rare cancers that involve similar

THE AUTHORS

FRANCIS S. COLLINS and *ANNA D. BARKER* are leaders of The Cancer Genome Atlas initiative in their positions as, respectively, director of the National Human Genome Research Institute and deputy director for Advanced Technologies and Strategic Partnerships of the National Cancer Institute. Collins led the Human Genome Project to its completion of the human DNA sequence, and Barker has headed drug development and biotechnology research efforts in the public and private sectors, with a particular focus on fighting cancer.

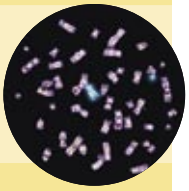
Genes and Cancer

A connection between genetic abnormalities and the aberrant features of cancer cells was first suggested more than 100 years ago by German biologist Theodor Boveri and others. But over the past few decades evidence that gene alterations directly cause the deranged behavior of cancer cells began accumulating. Calls arose by 1986 to sequence the normal human genome to study malignant gene changes comprehensively. The Human Genome Project was completed in 2003. The Cancer Genome Atlas project will start cataloguing the gene mutations found in three types of human cancer this year.

Theodor Boveri ▶

1890–1914
Studies of abnormal chromosome distribution during cell division suggest a role in malignancy.

1950s–1960s
Multiple discoveries reveal that tumor viruses can cause cancer by injecting their genes into cells.



1960
First genetic defect associated with a specific cancer—an abnormality known as the Philadelphia chromosome—is discovered in chronic myelogenous leukemia (CML) cells.

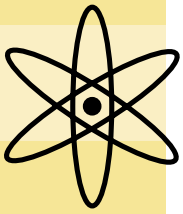
1976
Scientists discover that *src,* a nonviral gene found in animal cells, can cause cancer.

1979
P53, later found to be the most frequently mutated gene in human cancer, is discovered.

1981
H-RAS is the first human oncogene (a gene whose alteration is cancer-promoting) to be discovered.

1983
Altered methylation of DNA, suspected to affect gene activation, found in cancer cells.

1986
Renato Dulbecco, writing in *Science,* calls for sequencing the human genome to advance cancer research.



1986
U.S. Department of Energy considers sequencing the human genome to further study of radiation effects.

1986
First tumor suppressor gene, *RB1,* is identified.

1987
Fused gene *BCR-ABL* in the Philadelphia chromosome is found to cause CML.

1990
Model of multistep tumor genesis clarifies the role of accumulated gene changes in cellular transformation to malignancy.

1990
Human Genome Project begins.

enzymes. Herceptin, an agent that targets a cellular signal–receiving protein called *HER2,* is successful against breast cancers with an abnormal multiplication of the *HER2* gene that causes overproduction of the receptor protein.

Strategies for selecting treatments based on specific gene mutations in a patient's cancer are also being tested in studies of the drugs Iressa and Tarceva for lung cancer, as well as Avastin for lung, colon and other cancers. The performance of these new gene-based diagnostics, prognostics and therapeutics is certainly good news, although the list of such interventions remains far shorter than it would be if researchers in academia and the private sector had ready access to the entire atlas of genomic changes that occur in cancer.

A recent study led by investigators at Johns Hopkins University illustrates both the power of large-scale genomics applied to the discovery of cancer genes and the tremendous undertaking a comprehensive cancer genome atlas will be. The group sequenced about 13,000 genes in tumor tissues taken from 11 colorectal cancer patients and 11 breast cancer patients and reported finding potentially significant mutations in nearly 200 different genes. Interestingly, only about a dozen genes had previously been linked to these two types of cancer, and most scientists had generally expected to find just a few more.

Among the major challenges encountered by researchers sequencing cancer cell genomes is the difficulty of distinguishing meaningless mutations in the tumor samples from those that are cancer-related. Somewhat surprisingly, early sequencing studies have also found very little overlap among the genetic mutations present in different types of cancer and even substantial variation in the pattern of genetic mutations among tumor samples from patients with the same type of cancer. Such findings underscore the idea that many different possible combinations of mutations can transform a normal cell into a cancer cell. Therefore, even among patients with cancers of the same body organ or tissue, the genetic profile of each individual's tumor can differ greatly.

To grasp the full scope of what TCGA hopes to achieve, one must consider the complexities identified in such early efforts and imagine extending the work to more than 100 types of cancer. It is enough to give even veterans of the Human Genome Project and seasoned cancer biologists pause. Yet TCGA participants and other scientific pioneers from around the world are forging ahead, because we are convinced that amid the intricacies of the cancer genome may lie the greatest promise for saving the lives of patients.

Although researchers will probably take many years to complete a comprehensive catalogue of all the genomic mutations that cause normal cells to become malignant, findings with the potential to revolutionize cancer treatment are likely to appear well before this compendium is finished, as the proofs of concept have shown. As each new type of cancer is studied and added to TCGA, investigators will gain another rich new set of genomic targets and profiles that can be used to develop more tailored therapies.

PHOTO RESEARCHERS, INC. (*Boveri*); DEPARTMENT OF CLINICAL CYTOGENETICS, ADDENBROOKE'S HOSPITAL *Photo Researchers, Inc.* (*Philadelphia chromosome*); STEVE GSCHMEISSNER *Photo Researchers, Inc.* (*tumor cell*); MARK J. WINTER *Photo Researchers, Inc.* (*Gleevec molecule*); CECIL H. FOX *Photo Researchers, Inc.* (*Biospecimen Core Resource*); AFFYMETRIX (*Cancer Genome Characterization Centers*); NATIONAL HUMAN GENOME RESEARCH INSTITUTE (*Genome Sequencing Centers*); © CDC/PHIL *Corbis* (*Data Coordinating Center*)

Compiling a Colossal Atlas

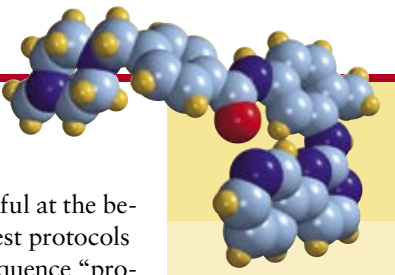
A PHASED-IN STRATEGY that proved successful at the beginning of the Human Genome Project was to test protocols and technology before scaling up to full DNA sequence "production." Similarly, TCGA is beginning with a pilot project to develop and test the scientific framework needed to ultimately map all the genomic abnormalities involved in cancer.

In 2006 the National Cancer Institute and National Human Genome Research Institute selected the scientific teams and facilities that will participate in this pilot project, along with the cancer types they will begin examining. Over the next three years these two institutes will devote $100 million to compiling an atlas of genomic changes in three tumor types: glioblastomas of the brain, lung cancer and ovarian cancer. These particular cancers were chosen for several reasons, including their value in gauging the feasibility of scaling up this project to a much larger number of cancer types. Indeed, only if this pilot phase achieves its goals will the NIH move forward with a full-fledged project to develop a complete cancer atlas.

The three malignancies that we selected for the pilot collectively account for more than 210,000 cancer cases in the U.S. every year and caused an estimated 191,000 deaths in this country in 2006 alone. Moreover, tumor specimen collections meeting the project's strict scientific, technical and ethical requirements exist for these cancer types. Last September our institutes announced the selection of three biorepositories to provide such specimens, along with new tumor samples as needed, and normal tissue from the same patients for comparison. Those facilities will deliver materials to a central Biospecimen Core Resource, one of four major structural components in TCGA's pilot project.

Cancer Genome Characterization Centers, Genome Sequencing Centers and a Data Coordinating Center constitute the project's other three main elements [*see illustration at right*], and all these groups will collaborate and exchange data openly. Specifically, the seven Cancer Genome Characterization Centers will use a variety of technologies to examine the activity levels of genes within tumor samples and to uncover and catalogue so-called large-scale genomic changes that contribute to the development and progression of cancer. Such alterations include chromosome rearrangements, changes in gene copy numbers and epigenetic changes, which are chemical modifications of the DNA strand that can turn gene activity on or off without actually altering the DNA sequence.

Genes and other chromosomal areas of interest identified by the Cancer Genome Characterization Centers will become targets for sequencing by the three Genome Sequencing Centers. In addition, families of genes suspected to be important in cancer, such as those encoding enzymes involved in cell-cycle control known as tyrosine kinases and phosphatases, will be sequenced to identify genetic mutations or other small-scale changes in their DNA code. At present, we estimate that some 2,000 genes—in each of perhaps 1,500 tumor samples—will be sequenced during this pilot project. The exact numbers will, of course, depend on the samples obtained and what is discovered



◀ Gleevec model

1993
Preclinical testing starts on drug that would become Gleevec, the first therapy developed to target a known gene-based cause of a cancer.

1999
Gene-activity profiles are first shown to distinguish between cancer types and to predict chemotherapy response.

2001
Gleevec earns FDA approval.

2002
Wellcome Trust Sanger Institute tumor genome survey discovers a mutation in *B-RAF* gene common to 70 percent of melanomas.

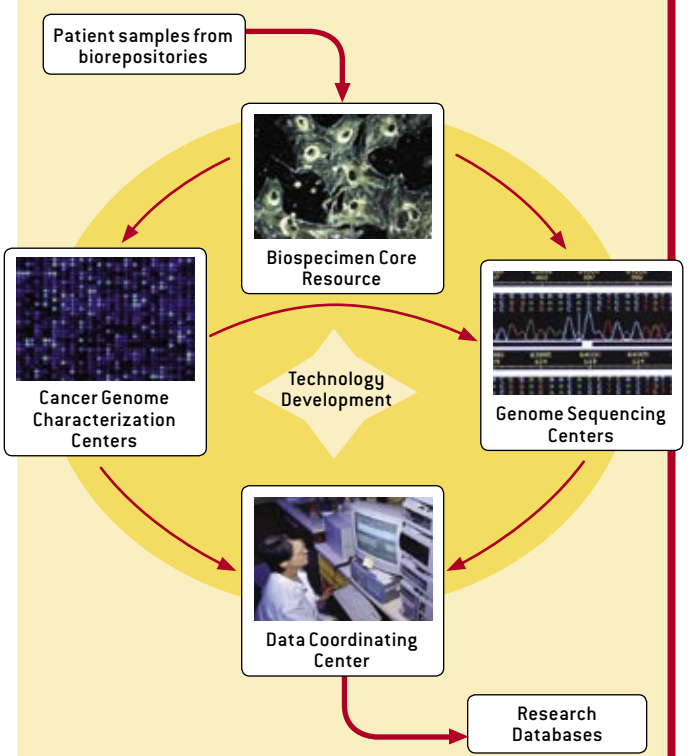
2003
Human Genome Project is completed.

2005
The Cancer Genome Atlas (TCGA) pilot project is announced by the National Institutes of Health.

2006
TCGA names pilot project participants and three cancer types for sequencing and genetic analysis.

2007–2010
TCGA will collect and analyze tumor samples obtained from designated biorepository institutions treating patients with cancer. The project's four primary components—a Biospecimen Core Resource, seven Cancer Genome Characterization Centers, three Genome Sequencing Centers and a Data Coordinating Center—will cooperate to test methods and technologies and to generate and manage data that will be made available to the wider research community.

How Will It Work?



about them by the Cancer Genome Characterization Centers.

Both the sequencing and genome characterization groups, many of which were participants in the Human Genome Project, can expect to encounter a far greater level of complexity than that in the DNA of normal cells. Once cells become cancerous, they are prone to an even greater rate of mutation as their self-control and repair mechanisms fail. The genomic makeup of individual cells can therefore vary dramatically within a single tumor, and the integrated teams will need to develop robust methods for efficiently distinguishing the "signal" of a potentially biologically significant mutation from the "noise" of the high background rate of mutations seen in many tumors. Furthermore, tumors almost always harbor some nonmalignant cells, which can dilute the sample. If the tumor DNA to be sequenced is too heterogeneous, some important mutations may be missed.

Following the lead of the Human Genome Project and other recent medical genomics efforts, all these data will be made swiftly and freely available to the worldwide research community. To further enhance its usefulness to both basic and clinical researchers and, ultimately, health care professionals, TCGA will link its sequence data and genome analyses with

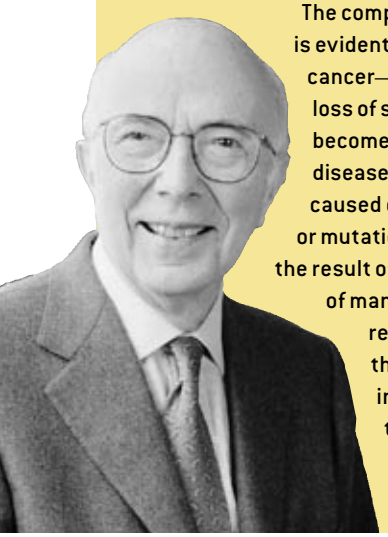
From Genome to Cancer—Why the Time Is Right

By Renato Dulbecco

When in 1986 I suggested a new project directed at identifying all human genes, one of my overriding goals was to find those genes involved in cancer development—a feat I hoped would lead to new tools for cancer research and, ultimately, to new therapies. That original human genome project has now been carried out and has demonstrated its usefulness for the discovery of genes involved in many diseases, including cancer. Moreover, the genome sequencing effort has been extended to other organisms—from bacteria to chimpanzees—and is showing the unity of life by revealing how many genes distant species share in common.

In the course of this work, new technologies have also provided a much more detailed understanding of the complicated processes by which genes give rise to a variety of functional molecules. An important outcome of this research is the realization that genes do not act alone but are participants in extensive networks of activity within cells. Any change in the functioning of one gene can therefore be accompanied by changes in the workings of multiple genes and proteins involved in the cells' self-maintenance.

Cancer results from the stepwise loss of cellular self-control.



The complexity of this system in normal cells is evident in what we already know about cancer—that it results from the stepwise loss of such cellular self-control, which becomes more and more complete as the disease progresses. That progression is caused only in part by physical alterations, or mutations, in specific genes; mostly it is the result of consequent changes in the activity of many other genes involved in cell regulation. Single genes may therefore be responsible in the initiation of cancer and so potential therapeutic targets. To reach the more advanced stages of these cancers (such as the acute phase of myeloid leukemia or the metastatic phase of other cancers), however, the participation of many other genes is required. Most of them are still unknown.

An exception is the recently observed phenomenon of oncogene addiction in certain tumor cells: despite the presence of numerous mutations to the cellular genome, turning off the activity of one so-called oncogene causes the cells to commit suicide via a mechanism known as apoptosis. But how generally this phenomenon occurs is also unknown. To approach these questions, it will be necessary to have a complete catalogue of the structural and functional alterations of genes and other cellular components that cause the loss of regulation in cancer cells. This process, in turn, will require a complete determination of their connections into networks by computational means—a task for the future.

On the way to this goal, however, many other unanswered questions can be explored by the research community. A possible role for stem cells in cancer, for example, is supported by similarities in the behavior of stem cells and cancer cells: both have an unlimited ability to divide; both are very sensitive to the cellular environment, or niche, in which they grow; and many of the genes known to be active in stem cells are also activated in cancer cells.

The advent of genomics has provided welcome insight into the mechanisms by which normal cells become cancerous, but our picture is still incomplete. The time has come to obtain a truly comprehensive catalogue of the genes involved in cancer, bringing to bear all the power of the new tools of genomics and molecular biology to the problem. The Cancer Genome Atlas project aims to do just that.

Renato Dulbecco is president emeritus of the Salk Institute for Biological Studies and co-recipient of the 1975 Nobel Prize in Physiology or Medicine for discoveries related to the interaction of tumor viruses and the genetic material of the cell.

SALK INSTITUTE FOR BIOLOGICAL STUDIES

information about observable characteristics of the original tumors and the clinical outcomes of the sample donors. Developing the bioinformatic tools to gather, integrate and analyze those massive amounts of data, while safeguarding the confidentiality of patient information, is therefore another hurdle that must be cleared to turn our vision into reality.

Uncharted Territory

THE ROAD AHEAD is fraught with scientific, technological and policy challenges—some of which are known and others as yet unknown. Among the uncertainties to be resolved: Will new sequencing technologies deliver on their early promise in time to make this effort economically feasible? How quickly can we improve and expand our toolbox for systematically detecting epigenetic changes and other large-scale genomic alterations involved in cancer, especially those associated with metastasis? How can we harness the power of computational biology to create data portals that prove useful to basic biologists, clinical researchers and, eventually, health care professionals on the front lines? How can we balance intellectual-property rights in a way that promotes both basic research and the development of therapies? When will Congress finally pass genetic nondiscrimination legislation so that knowledge gained through TCGA will have the maximum positive influence on Americans' health? The list goes on.

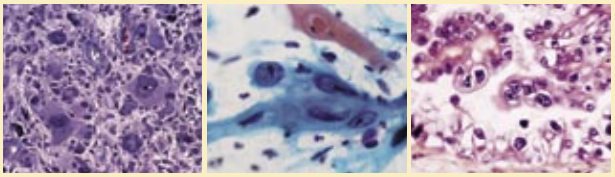
To avoid raising false expectations, we also must be clear about the questions this project will not attempt to answer. Although it will serve as a resource for a broad range of biological exploration, TCGA is only a foundation for the future of cancer research and certainly not the entire house. And we face the sobering issue of time—something that is in short supply for many cancer patients and their families. As we survey the considerable empty spaces that exist in our current map of genomic knowledge about cancer, the prospect of filling those gaps is both exhilarating and daunting. Scientists and the public need to know up front that this unprecedented foray into molecular cartography is going to take years of hard work and creative problem solving by thousands of researchers from many different disciplines.

Where all this work will lead can only be dimly glimpsed today. In this sense, our position is similar to that of the early 19th-century explorers Meriwether Lewis and William Clark. As they ventured up the Missouri River into the largely uncharted Northwest Territory in 1804, their orders from President Thomas Jefferson were to "take observations of latitude and longitude at all remarkable points.... Your observations are to be taken with great pains and accuracy; to be entered distinctly and intelligibly for others, as well as yourself."

Although Lewis and Clark did not find the much-longed-for water route across the continent, their detailed maps proved valuable to their fledgling nation in myriad ways that Jefferson could never have imagined. For the sake of all those whose lives have and will be touched by cancer, we can only hope our 21st-century expedition into cancer biology exceeds even Renato Dulbecco's grandest dreams. SA

Targeting Gene Changes in Cancer

TCGA pilot project teams will examine the DNA of some 1,500 tumor samples from patients with cancers of the lung, ovaries or brain (glioblastoma), looking for genetic changes. Approximately 2,000 suspect genes in each sample will be sequenced to identify specific mutations. The list of target genes will be tailored to each cancer type and largely determined by what the Cancer Genome Characterization Centers find in the samples, although candidates will also be drawn from categories of genes already associated with cancer.



From left to right: Glioblastoma, lung cancer, ovarian cancer

GENE CATEGORIES	EXAMPLES
Genes identified by TCGA Cancer Genome Characterization Centers as having aberrant structure or activity in a significant number of tumor samples	In some brain tumor cell lines, a gene encoding the intracellular protein NF-KAPPA B is much more active than in normal brain tissue
Well-known oncogenes (genes whose overactivity or alteration is cancer-promoting)	• Growth factor receptor genes: *HER2* (breast and lung cancers), *EGFR* (lung and colon cancers) • Signaling protein genes: *BCR-ABL* (chronic myelogenous leukemia), *RAS* (many cancers), *B-RAF* (skin cancers) • Regulators of cell death: *BCL-3* (lymphoma)
Well-known tumor suppressors (genes that protect cells from malignant transformation, unless disabled by mutation)	• Controllers of cell division: *RB1* (retinoblastoma) • DNA repairers: *HNPCC* (colon cancer, endometrial cancer) • Promoters of programmed cell suicide: *P53* (lung, colon, breast and brain tumors)
Genes related to known oncogenes and tumor suppressor genes by similarity or pathway membership	The oncogenes *HER2* and *EGFR* are part of the epidermal growth factor receptor signaling pathway, which contains at least half a dozen other genes suspected of playing key roles in cancer development and progression

MORE TO EXPLORE

The New Era in Cancer Research. Harold Varmus in *Science,* Vol. 312, pages 1162–1165; May 26, 2006.

The Consensus Coding Sequences of Human Breast and Colorectal Cancers. Tobias Sjöblom et al. in *Science,* Vol. 314, pages 268–274; October 13, 2006. (Published online September 7, 2006.)

The Cancer Genome Atlas: **http://cancergenome.nih.gov**

CNRI *Photo Researchers, Inc.* (*glioblastoma* and *ovarian cancer*); JONATHAN ASHTON *Photo Researchers, Inc.* (*lung cancer*)

The New York Times

December 9, 2012

In Girl's Last Hope, Altered Immune Cells Beat Leukemia

By DENISE GRADY

PHILIPSBURG, Pa. — Emma Whitehead has been bounding around the house lately, practicing somersaults and rugby-style tumbles that make her parents wince.

It is hard to believe, but last spring Emma, then 6, was near death from leukemia. She had relapsed twice after chemotherapy, and doctors had run out of options.

Desperate to save her, her parents sought an experimental treatment at the Children's Hospital of Philadelphia, one that had never before been tried in a child, or in anyone with the type of leukemia Emma had. The experiment, in April, used a disabled form of the virus that causes AIDS to reprogram Emma's immune system genetically to kill cancer cells.

The treatment very nearly killed her. But she emerged from it cancer-free, and about seven months later is still in complete remission. She is the first child and one of the first humans ever in whom new techniques have achieved a long-sought goal — giving a patient's own immune system the lasting ability to fight cancer.

Emma had been ill with acute lymphoblastic leukemia since 2010, when she was 5, said her parents, Kari and Tom. She is their only child.

She is among just a dozen patients with advanced leukemia to have received the experimental treatment, which was developed at the University of Pennsylvania. Similar approaches are also being tried at other centers, including the National Cancer Institute and Memorial Sloan-Kettering Cancer Center in New York.

"Our goal is to have a cure, but we can't say that word," said Dr. Carl June, who leads the research team at the University of Pennsylvania. He hopes the new treatment will eventually replace bone-marrow transplantation, an even more arduous, risky and expensive procedure that is now the last hope when other treatments fail in leukemia and related diseases.

Three adults with chronic leukemia treated at the University of Pennsylvania have also had

complete remissions, with no signs of disease; two of them have been well for more than two years, said Dr. David Porter. Four adults improved but did not have full remissions, and one was treated too recently to evaluate. A child improved and then relapsed. In two adults, the treatment did not work at all. The Pennsylvania researchers were presenting their results on Sunday and Monday in Atlanta at a meeting of the American Society of Hematology.

Despite the mixed results, cancer experts not involved with the research say it has tremendous promise, because even in this early phase of testing it has worked in seemingly hopeless cases. “I think this is a major breakthrough,” said Dr. Ivan Borrello, a cancer expert and associate professor of medicine at the Johns Hopkins University School of Medicine.

Dr. John Wagner, the director of pediatric blood and marrow transplantation at the University of Minnesota, called the Pennsylvania results “phenomenal” and said they were “what we’ve all been working and hoping for but not seeing to this extent.”

A major drug company, Novartis, is betting on the Pennsylvania team and has committed $20 million to building a research center on the university’s campus to bring the treatment to market.

Hervé Hoppenot, the president of Novartis Oncology, called the research “fantastic” and said it had the potential — if the early results held up — to revolutionize the treatment of leukemia and related blood cancers. Researchers say the same approach, reprogramming the patient’s immune system, may also eventually be used against tumors like breast and prostate cancer.

To perform the treatment, doctors remove millions of the patient’s T-cells — a type of white blood cell — and insert new genes that enable the T-cells to kill cancer cells. The technique employs a disabled form of H.I.V. because it is very good at carrying genetic material into T-cells. The new genes program the T-cells to attack B-cells, a normal part of the immune system that turn malignant in leukemia.

The altered T-cells — called chimeric antigen receptor cells — are then dripped back into the patient’s veins, and if all goes well they multiply and start destroying the cancer.

The T-cells home in on a protein called CD-19 that is found on the surface of most B-cells, whether they are healthy or malignant.

A sign that the treatment is working is that the patient becomes terribly ill, with raging fevers and chills — a reaction that oncologists call “shake and bake,” Dr. June said. Its medical name is cytokine-release syndrome, or cytokine storm, referring to the natural chemicals that pour out of

cells in the immune system as they are being activated, causing fevers and other symptoms. The storm can also flood the lungs and cause perilous drops in blood pressure — effects that nearly killed Emma.

Steroids sometimes ease the reaction, but they did not help Emma. Her temperature hit 105. She wound up on a ventilator, unconscious and swollen almost beyond recognition, surrounded by friends and family who had come to say goodbye.

But at the 11th hour, a battery of blood tests gave the researchers a clue as to what might help save Emma: her level of one of the cytokines, interleukin-6 or IL-6, had shot up a thousandfold. Doctors had never seen such a spike before and thought it might be what was making her so sick.

Dr. June knew that a drug could lower IL-6 — his daughter takes it for rheumatoid arthritis. It had never been used for a crisis like Emma's, but there was little to lose. Her oncologist, Dr. Stephan A. Grupp, ordered the drug. The response, he said, was "amazing."

Within hours, Emma began to stabilize. She woke up a week later, on May 2, the day she turned 7; the intensive-care staff sang "Happy Birthday."

Since then, the research team has used the same drug, tocilizumab, in several other patients.

In patients with lasting remissions after the treatment, the altered T-cells persist in the bloodstream, though in smaller numbers than when they were fighting the disease. Some patients have had the cells for years.

Dr. Michel Sadelain, who conducts similar studies at the Sloan-Kettering Institute, said: "These T-cells are living drugs. With a pill, you take it, it's eliminated from your body and you have to take it again." But T-cells, he said, "could potentially be given only once, maybe only once or twice or three times."

The Pennsylvania researchers said they were surprised to find any big drug company interested in their work, because a new batch of T-cells must be created for each patient — a far cry from the familiar commercial strategy of developing products like Viagra or cholesterol medicines, in which millions of people take the same drug.

But Mr. Hoppenot said Novartis was taking a different path with cancer drugs, looking for treatments that would have a big, unmistakable impact on a small number of patients. Such home-run drugs can be approved more quickly and efficiently, he said, with smaller studies than are

needed for drugs with less obvious benefits.

“The economic model is totally acceptable,” Mr. Hoppenot said.

But such drugs tend to be extremely expensive. A prime example is the Novartis drug Gleevec, which won rapid approval in 2001 for use against certain types of leukemia and gastrointestinal tumors. It can cost more than $5,000 a month, depending on the dosage.

Dr. June said that producing engineered T-cells costs about $20,000 per patient — far less than the cost of a bone-marrow transplant. Scaling up the procedure should make it even less expensive, he said, but he added, “Our costs do not include any profit margin, facility depreciation costs or other clinical care costs, and other research costs.”

The research is still in its early stages, and many questions remain. The researchers are not entirely sure why the treatment works, or why it sometimes fails. One patient had a remission after being treated only twice, and even then the reaction was so delayed that it took the researchers by surprise. For the patients who had no response whatsoever, the team suspects a flawed batch of T-cells. The child who had a temporary remission apparently relapsed because not all of her leukemic cells had the marker that was targeted by the altered T-cells.

It is not clear whether a patient’s body needs the altered T-cells forever. The cells do have a drawback: they destroy healthy B-cells as well as cancerous ones, leaving patients vulnerable to certain types of infections, so Emma and the other patients need regular treatments with immune globulins to prevent illness.

So far, her parents say, Emma seems to have taken it all in stride. She went back to school this year with her second-grade classmates, and though her grades are high and she reads about 50 books a month, she insists impishly that her favorite subjects are lunch and recess.

“It’s time for her to be a kid again and get her childhood back,” Mr. Whitehead said.

The New York Times

February 22, 2013

F.D.A. Approves a New Drug for Advanced Breast Cancer

By ANDREW POLLACK

The Food and Drug Administration on Friday approved a new type of drug that combines the widely used breast cancer medicine Herceptin with a powerful toxin to more effectively kill cancer cells while potentially reducing side effects.

The drug, which will be called Kadcyla but was known as T-DM1 during its development, extended the median survival of women with advanced breast cancer by nearly half a year in a clinical trial.

Genentech, which developed the drug, said it would cost about $9,800 a month, or $94,000 for a typical course of treatment. That is about twice the price of Herceptin itself, which is also made by Genentech, but it is similar to the price of some other new cancer drugs.

Kadcyla, which the company said could be available within days, is one of the first successful examples of a new class of drugs that link toxins to proteins known as monoclonal antibodies. The antibodies latch onto tumors and deliver the toxic payload. Because the toxin is not activated until it reaches the tumor, healthy cells are spared and some side effects are avoided.

Such medicines, known as antibody-drug conjugates, are a hot area for cancer drug developers, with around two dozen such drugs in clinical trials. Another antibody-drug conjugate, Adcetris, developed by Seattle Genetics, was approved in 2011 as a treatment for two rare types of lymphoma.

The linker and toxin used in Kadcyla were developed by ImmunoGen, based in Waltham, Mass., which will receive royalties on sales of the drug. This is the first approved product for ImmunoGen, which has been working on antibody-drug conjugates for three decades.

The main clinical trial leading to approval of Kadcyla involved 991 patients with metastatic breast cancer that was worsening despite treatment with Herceptin and a taxane chemotherapy drug, like paclitaxel. Half the women were given infusions of Kadcyla and the other half took two pills now commonly used for such patients: Tykerb, also known as lapatinib, and Xeloda, also known as capecitabine.

The patients getting Kadcyla lived a median of 30.9 months, compared with 25.1 months for those getting the two pills. The median time before the disease worsened was 9.6 months for those getting Kadcyla, compared with 6.4 months for those getting the other drugs.

While having greater efficacy, Kadcyla also had fewer side effects. About 43 percent of patients on Kadcyla had serious side effects compared with 59 percent of those getting the two pills.

Still, the label of Kadcyla has a warning saying the drug can cause liver toxicity, heart toxicity and death. It also can cause serious birth defects or fetal death, so women of childbearing age taking the drug are urged to use contraception.

Herceptin, also known as trastuzumab, binds to a protein on the surface of breast cancer cells called HER2. Since Kadcyla, known generically as ado-trastuzumab emtansine, incorporates Herceptin, it, too, is approved only for the roughly 20 percent of breast cancer cases with an overabundance of HER2.

Kadcyla's approval is for use after a patient has already failed to respond to Herceptin and a taxane. But Roche, the Swiss company that owns Genentech, is already testing it for use as an initial treatment for metastatic cancer. It is also testing it in combination with Perjeta, another of its drugs for HER2-positive breast cancer, which was approved in June.

Roche executives say they hope that Kadcyla, along with Perjeta, will make Herceptin somewhat obsolete by the time it could face competition from cheaper biosimilars, which are similar to generics. Roche says the United States patent on Herceptin expires in 2019.

Herceptin had global sales of 5.9 billion Swiss francs ($6.3 billion at current exchange rates) in 2012. It was the world's best-selling drug used only for cancer in 2012.

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ANALYSIS AIR DATE: Sept. 24, 2012

First Comprehensive Genetic Analysis of Breast Cancer Could Change Treatment

SUMMARY

Research published by Nature shows there are four distinct types of breast cancer and that genetic changes occurring as cancer cells spread are vastly different for each type. Judy Woodruff talks to National Cancer Institute's Dr. Harold Varmus for more on what the research could mean for treatment in the future.

Transcript

JUDY WOODRUFF: Next, new research that's changing our understanding of cancer.

Scientists say they have found new insights into four genetically distinct types of breast cancer, potentially altering the way doctors one day treat the disease.

The findings were published yesterday in the journal "Nature" as part of a comprehensive genetic analysis of breast cancer.

Among other discoveries, researchers say that a rare but deadly form of breast cancer bears a genetic resemblance to the kinds of tumors found in lung and ovarian cancers.

Doctors also learned that the two most common forms of breast cancer, both of which rely on estrogen to fuel their growth and have been treated similarly in the past, are actually genetically distinct from one another.

Well, for more on this, I'm joined by Dr. Harold Varmus. He's director of the National Cancer Institute. The institute helped to lead the work as part of a larger project to map genetic changes in cancer.

Dr. Varmus, thank you for being here.

DR. HAROLD VARMUS, National Cancer Institute: My pleasure.

JUDY WOODRUFF: So, tell us what is significant about what you found about these four types of breast cancer.

HAROLD VARMUS: Well, these four types have actually been known for some time based on work done nearly a decade ago that was intent on characterizing which genes were off and on in breast cancer types.

And to the surprise of many, it was possible to form four large groups that most breast cancers could fit into.

What these studies show -- and they are part of a much larger effort that the Cancer Institute and the Human Genome Institute are carrying out on many different types of cancer -- is that by using a variety of new techniques to sequence the genome, to count the number of copies of genes, to look at which genes are being read out and which proteins are being made, that we can begin to look at the heterogeneity of these four groups and define certain commonalities within the groups that give us -- will give us some insight into which therapies are most appropriate and what kind of new therapies might be envisioned.

JUDY WOODRUFF: So, is this telling you that the genetic markings are more important than just about any other distinction to these breast cancers?

I mean, we mentioned...

HAROLD VARMUS: Well, in general, all cancers have been traditionally characterized by the way they appear under the microscope and the organs in which they arise.

But as we learn more and more about cancer of every type, including breast, what we learn is that the drivers of cancer are mainly mutations and changes in chromosome organization or numbers of copies of genes, and that those are the instruments that drive a cancer and therefore become ways of categorizing cancer, ways of designing new therapies that specifically target those changes, and markers for knowing whether or not these cancers

will respond to conventional existing therapies.

JUDY WOODRUFF: So, was this a shocking piece of information?

HAROLD VARMUS: It wasn't shocking, no.

We have been going through many kinds of cancers, and many more are to come within this large study.

And what we're trying to do is to create a warehouse, a compendium of information. The project is called the Cancer Genome Atlas.

It's an atlas, a warehouse, a storehouse, a database which everyone is free to look at, because all this information is being made publicly available.

If you go to our website and look at the Cancer Genome Atlas, you will see the information. You can -- all these papers are freely accessible to everyone.

And the point is that we know that every time we approach a cancer with these technologies and look at many hundreds of individual cancers of a certain traditional grouping, like pancreatic cancer or liver cancer or gastric cancer or breast and other cancers that have been published, that we're going to see interesting patterns.

Every cancer looks different. Every cancer has similarities to other cancers. And we're trying to milk those differences and similarities to do a better job of predicting how things are going to work out and making new drugs.

JUDY WOODRUFF: And how will that affect the treatment of these cancers? I mean, do you already know how that might happen, or is that just...

HAROLD VARMUS: Well, we have an idea.

First of all, there is the long-range view that, as we understand exactly what's wrong, we will make targeted therapies that are specific for cancers that have certain kinds of genetic aberrations.

But even in the more immediate future, it's going to be possible to put together our understanding, our description of the genetic changes in a cancer and the responses to existing therapies. And that's the piece that we still miss.

And one way in which I believe that patients who have cancer now and are being treated now can make a major contribution to the development of more effective and more accurate treatment, using existing therapies.

JUDY WOODRUFF: So, this -- you're saying this could make a difference in the very near future?

HAROLD VARMUS: In the next few years. It is not going to change practice overnight.

Some of the ideas that are in this paper, the connection you mentioned between some of the genetic changes seen in a certain particularly severe from a breast cancer and ovarian cancer, for example, suggest that those cancers have an instability in their genome that can be addressed with some existing therapies. And those therapies are being tested now in those breast cancer patients.

But what remains to be figured out is how we get the clinical information together with the genetic information in the kind of database that we can all use to begin to predict who is going to respond to which drugs.

JUDY WOODRUFF: And why is that as hard as it is? What would make that easier?

HAROLD VARMUS: Well, in part because it's hard to get the clinical information into a form that can be put into a database that is interpretable.

Some of this is a matter of learning how to massage the data so we make the correlations that are truly helpful.

The second is that we need to overcome a reluctance to provide personal clinical information and genetic information to a database that will help others, to provide the right kinds of consent forms and privacy protections that allow this all to happen.

And I would urge patients who have cancer now to think of themselves as information donors who can benefit not just others who will have cancer later, but themselves over the next few years.

Because cancer patients are living longer and better lives, thanks to better symptom control, more effective therapies, and a deeper understanding of cancer that has come about through research over the last decade.

JUDY WOODRUFF: So, finally, just to broaden this out, what are your hopes, Dr. Varmus, for this larger genetic study of all kinds of cancer?

HAROLD VARMUS: Well, I believe that we are going to have a much deeper appreciation of what kinds of abnormalities in cancer cells and in the surrounding cells that feed and respond to cancers are vulnerabilities that will allow us to make better predictions of which kinds of drugs will work to treat these cancers.

They also become markers that allow or enable early detection. They become signposts for thinking about what the environmental causes of cancer might be and for thinking about how we can prevent cancers more effectively.

But this is not just all about treatment. And we need to think imaginatively about how we prevent cancers, which is the ultimate goal.

JUDY WOODRUFF: It must be very exciting for you.

HAROLD VARMUS: Well, it's a difficult problem that we think we're making great progress against these days. And it is an affirmation of the importance of medical research to the nation.

JUDY WOODRUFF: Dr. Harold Varmus, we thank you very much for being here.

HAROLD VARMUS: Pleasure. Thanks.