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CL089

The Genetic Bases of Cancer

Lucy Wortham James Lecture

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• The past three decades have witnessed enormous progress made in our understanding of the pathogenesis of cancer. Research results of the previous decades had indicated that a variety of agents, the carcinogens, could induce cancer in experimental animals. By extension, similarly acting agents were presumed to intervene in human cancer. Beyond this, the precise nature of the pathogenetic mechanisms underlying human malignancies remained obscure. In these last decades, the origins of cancer have been uncovered: specific genes and biochemical mechanisms are now known to drive the process of neoplasia.
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An important advance came from observations made in the early 1960s that certain DNA tumor viruses, namely simian virus 40 and polyomavirus, could induce the malignant transformation of cells that they had infected.[1] Analogous work on Rous sarcoma virus, an RNA tumor virus, yielded the same result.[2] The importance of these observations was not in identifying possible causative agents of human disease; only a small proportion of the malignancies encountered in this country are triggered by viral agents. Instead, the salient conclusions of this work concerned the molecular mechanisms of malignant transformation. Stated simply, these experiments showed that a small amount of genetic information carried by these viruses could succeed in redirecting the complex metabolism of a cell, forcing it from its normal mode into a state characteristic of malignant cells. This established the principle that genes can act as central controllers orchestrating the malignant conversion of a cell. Moreover, the number of genes and the amount of associated genetic information was small compared with the complex genetic apparatus of the host cell. Indeed, the cancer-inducing genes of these viruses carry about one-millionth of the genetic information present in the host cell genome.

These virus-associated transforming genes came to be called "oncogenes." The relevance of these to clinical cancer remained suspect, however, since, as mentioned above, most tumors do not seem to be triggered by viral infections. Could similarly acting oncogenes also be involved in nonviral cancer? A breakthrough came in 1976 in the course of studies of Rous sarcoma virus and its associated oncogene termed *src*. Detailed analysis of the *src* gene revealed that it was not really a viral gene at all. It was, instead, of host cell origin and had been abstracted from the host genome by an ancestor of the Rous virus.[3] This kidnapping of a host cell gene by the virus through a process termed *transduction* enabled the virus to exploit a foreign gene for its own purposes, which, in this case, involved the transformation of chicken cells.

More important than these revelations about virus physiology were certain implications about the host cell genome that served as the source of this viral oncogene. This cell genome clearly contained a gene with latent transforming potential that could be unmasked through the genetic changes provoked by the viral genome. Extending this theme, some speculated that the cellular genome contains a series of genes (termed *proto-oncogenes*) that can become activated following transduction by one or another tumor virus. In the following years, other tumor viruses belonging to the retrovirus family were found to carry a number of distinct oncogenes, each of which could be traced to one or another normal cellular proto-oncogene.[4]

NONVIRAL ONCOGENES

Nonetheless, all this still seemed to show little about the pathogenesis of the nonviral cancers encountered in the clinic. Could these same proto-oncogenes, discovered by virtue of their alliances with retroviruses, also become activated by nonviral mechanisms, such as the genetic alterations of the cell genome induced by chemical carcinogens? Such evidence first came to light in 1979. Transfer of genes from chemically transformed mouse tumor cells into nontumorigenic recipient cells resulted in the malignant transformation of the latter.[5] Similar results were soon observed with DNAs of human tumor cells.[6] This showed that tumor DNAs indeed carry genes that somehow direct the cell to grow in a malignant fashion. The similarity of the genetic endowment of chemically induced animal tumors and spontaneously arising human tumors strengthened the analogy between the two types of tumors; moreover, it persuaded many that such experimentally induced tumors represent a good model of human disease.

Within several years, these hitherto mysterious cancer genes had been isolated by molecular cloning.[7] An important insight was gained from detailed molecular characterization of the initially isolated human oncogene derived from a bladder carcinoma cell line. It was found to be closely related to a gene present in all normal human cells. As expected, this bladder carcinoma oncogene gave no signs of having been activated or altered by a viral genome. Instead, detailed structural analysis showed that it was a slightly altered version of its normal cellular counterpart. The difference between the two genes, each 6000 DNA bases long, was very subtle—a single base change was responsible for converting

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the normal gene into a virulent oncogene.[8]

The difference between this oncogene and its normal antecedent, though slight, was critical in attempting to understand the molecular origins of this tumor and, by extension, the origins of countless other human tumors that have proceeded down similar paths in the course of their evolution from normalcy to full-blown malignancy. It appeared that an ancestor of the bladder tumor cells residing somewhere in the urothelium had sustained a critical alteration in one of its proto-oncogenes—a somatic mutation—that conferred on this cell and its descendants a substantial growth advantage. This advantage derived directly from the growth-stimulating effects of the mutant gene that pushes the cell into neoplastic growth.

None of this work directly addressed the biochemical mechanisms that enable this small gene to orchestrate a massive shift in cell phenotype. One clue came from the revelation that this bladder carcinoma oncogene was closely related to one of the oncogenes identified previously by virtue of its association with a retrovirus, namely, Harvey sarcoma virus.[9,10] The viral oncogene, termed Ha-*ras*, was known to encode a 21 000 d molecular weight protein that is responsible for redirecting the growth patterns of the cell. The mutation known to activate the oncogene in the bladder carcinoma cells was found to affect the structure of this protein.[8] It seemed that the normal version of this p21 protein found in cells mediates some aspect of the signal transduction regulating normal cellular proliferation. In contrast, the structurally altered protein seen in the tumor cell was apparently endowed with aberrant growth-inducing powers that elicit the malignant phenotypes of the tumor cell.

Parenthetically, I note that virtually identical p21 proteins and *ras* genes have been found in the cells of all metazoa and even in primitive organisms such as yeast. It would appear that these *ras* genes were developed early in the evolution of eukaryotic cells and have been retained in almost unaltered form for more than a billion years. The involvement of these genes in triggering cancer, now well-documented in hundreds of tumor samples, is a consequence of rare, randomly occurring mutations that subvert their normal functioning.

All this focuses attention on the mechanisms of action of the aberrant p21 *ras* oncogene protein and other similarly acting oncogene proteins. With rare exception, we do not understand how they are able to alter profoundly the biochemistry of the cell. It is presumed by many that these oncogene proteins act to deregulate the levels of certain intracellular hormones, so-called second messenger molecules, and that these low molecular weight compounds in turn elicit the wide variety of changes that accompany transformation of normal into malignant cells. Such oncoproteins represent tempting targets for those who would develop new types of chemotherapeutic compounds, designed using rational molecular criteria rather than the empirical, random searches that have led to existing anticancer drugs.

ONCOGENES AND MULTISTEP TUMORIGENESIS

In more recent years, it became apparent that the scheme of carcinogenesis suggested above is, at best, oversimplified. The formation of a tumor is known to be a complex multistep process, ostensibly involving a succession of genetic changes affecting the evolving tumor cell population. The above-mentioned "point-mutation" that created a *ras* oncogene can represent only one step in the process, and accordingly can only direct a partial conversion of a normal cell into one that is fully malignant. This notion has been verified directly by insertion of *ras* oncogenes into fully normal embryo cells. These cells undergo only partial conversion to malignancy in response to the *ras* oncogene. As later work showed, the *ras* oncogene requires the collaboration of other oncogenes to effect full malignant transformation. Thus, an oncogene like *myc* collaborates nicely with *ras* to achieve this end result.[11,12]

This collaboration between *ras* and *myc* oncogenes yields a number of useful insights.[13] The two oncogenes elicit a complementary set of changes in cell behavior. For example, the *ras* oncogene can induce the rounding-up of cells and loss of dependence on substrate adherence for growth. By contrast, the *myc* gene is effective as an immortalizing gene, conferring on cells an inability to replicate indefinitely in culture. When these changes occur together in the same cell, a tumor cell clone emerges from the cell's descendants. This suggests that a single oncogene is only able to deregulate a subset of the cell's growth regulatory circuits, and that two or more oncogenes must intervene for an incipient tumor cell to become truly autonomous in its growth.

In the last few years, we have begun to realize that oncogenes can represent only part of the genetic elements that intervene in tumorigenesis. This insight has come from two lines of logic. It is well known that certain types of cancer run in families. These familial cancers and the underlying genes are not explained by the existence of oncogenes. The latter become activated through somatic mutations that alter genes in one or another target organ during one's lifetime. Familial cancers, by contrast, must be triggered by genes that exist in mutant form in germ cells and are thus already present as mutant alleles in the conceptus. The oncogene model does not address the existence of these germline determinants of tumor susceptibility.

The oncogene paradigm proves inadequate in a second respect. Oncogenes all act as growth agonists as do many of the antecedent proto-oncogenes. However, cells may also carry an equally large repertoire of growth-regulatory genes that function in a countervailing fashion to constrain or antagonize growth. Such growth-suppressing genes might also participate in cancer when they are lost or inactivated by certain types of mutations. Inactivations of these genes could lead to cancer by removing barriers that normally act to prevent the cell from growing uncontrollably. Here again, oncogenes fall short of providing a complete explanation of cancer.

GROWTH-SUPPRESSING GENES

Several of these limitations in the oncogene model of cancer are addressed by a class of genes that has come to light in recent years through a series of indirect genetic analyses. This body of work provides compelling evidence that the process of tumorigenesis is often accompanied, indeed driven, by the loss of critical growth regulatory genes. These genes have been called "antioncogenes," "tumor suppressor genes," "emerogenes," and "growth-suppressing genes."[14] The last of these terms would seem most appropriate, as it most closely reflects the apparent role of these genes within the normal cell.

One revelation about these negatively acting genes came from a corpus of work, now almost two decades old, that describes the behavior of tumor cells after they become fused with normal cells. Such cell fusion, achieved in the laboratory through the processes termed *somatic cell hybridization*, results in hybrid cells whose genetic makeup derives from both parental cell types. These tumor/normal cell hybrids almost always lack an ability to form tumors.[15] This leads to the conclusion that genes initially present in the normal parent cell are able to impose normal growth control on the hitherto malignant partner. These growth normalizing genes would, one concludes, represent elements of the normal genetic repertoire that were lost from the malignant cell during its evolution from normalcy to neoplasia.

Careful study of the karyotype of these hybrid cells has shown that the tumor-suppressing gene(s) donated by the normal parent is often traceable to the presence of one or another chromosome present in the hybrid cell. Because of the karyotypic instability of the initially tetraploid hybrid cell, many chromosomes are shed during subsequent cell generations. Loss of specific chromosomes originating from the normal parent cell is often accompanied by reversion of the cell once again to a tumorigenic state. For example, the growth-normalizing effects imposed on Wilms' tumor cells and HeLa cells (a line of cervical carcinoma cells) are associated with the presence and retention of at least one copy of a normal chromosome 11.[16] By implication, this chromosome carries a gene or genes that were critical in constraining the growth of the progenitors of these tumor cells.

THE RETROBLASTOMA MODEL

Study of several unusual tumors has also added substantially to our insights on growth-suppressing genes as well as genes conferring inborn susceptibility to malignancy. Notable among these malignant neoplasms is retinoblastoma, a tumor that is exceedingly rare—being seen in only 1 in 20 000 children. However, this tumor has striking and unusual properties. Thus, retinoblastomas are often seen to run in families. The tumor occurs only in young children up to the age of 5 years; the retina would seem to present a fertile field for tumorigenesis only up to this age. Children cured of retinoblastoma through enucleation or radiation grow to adulthood and with substantial frequency have offspring, half of whom in turn contract this otherwise extremely rare malignant neoplasm.

In retinoblastomas, genetic loss would appear once again to play an important role in tumor formation. This is made most apparent by karyologic analysis of retinoblastoma tumor cells, which on occasion reveals specific interstitial deletions present on chromosome 13.[17] Although each detected deletion is distinct from those seen in other tumors, all would seem to converge on the q14 band of chromosome 13. This would suggest that loss of a specific chromosome 13–associated gene underlies formation of this particular type of tumor.

In reality, the process of retinoblastoma tumor formation is more complex than would be suggested by the above observations. As Alfred Knudson[18] first postulated, these tumors behave as if two distinct genetic alterations are required for a tumor cell clone to erupt into malignant growth. In making his arguments, Knudson distinguished between two types of retinoblastoma, termed *familial* and *sporadic*. He argued that children with familial tumors carry one of the required mutations from the moment of conception, and sustain the second mutation somatically in one of the cells of the target organ. In contrast, he suggested that in sporadic retinoblastoma, which is seen in children with no apparent familial predisposition, both of the essential mutations occur somatically within one or another target cells in the retina.

We now realize that both of these mutations involve chromosome 13 genes, specifically the two copies of 13q14–associated gene whose importance was first suspected on the basis of karyologic analysis. The two necessary mutations are required to knock out both copies of this gene present on the paired chromosomes 13 present in every cell. The apparent requirement for inactivation of both gene copies implies that either copy of this gene, termed *Rb*, can suffice to direct normal growth behavior in a cell. Thus, children who are born into the world with one intact and one defective copy of the Rb gene develop normally. However, when the surviving Rb gene copy is lost in one of their retinal cells, then this doubly defective cell begins the deregulated growth that soon spawns a tumor clone.

THE Rb GENE

Three years ago, the Rb gene was isolated by the process of molecular cloning.[19] Because of this advance, the arguments about its role in tumorigenesis need no longer be based exclusively on indirect types of genetic analysis. As predicted, the copies of this gene isolated from retinal tumors are frequently affected by alterations in structure. Rb is a massive gene, encompassing approximately 190 000 pairs of DNA on chromosome 13, and substantial portions of this gene are found to be missing in many retinoblastoma cells. However, subtle changes in Rb gene structure appear equally effective in knocking out its function; we have recently found point mutations affecting single base pairs that appear equally effective in Rb gene inactivation, in these cases, acting via their ability to affect the posttranscriptional processing (ie, splicing) of the Rb messenger RNA precursor.[20]

Like virtually all other genes, the Rb gene exerts its effects on cell behavior through the actions of an encoded protein. The Rb protein, of a mass of 105 kd, is found in the nucleus of the cell where it presumably acts as a regulator of the expression of other genes.[21] More precise insights into how it may act to regulate such genes and thus act as a governor of cell growth are still lacking. These questions can be addressed with great precision over the coming years.

One might speculate that the Rb-encoded protein normally acts to repress cell growth by shutting down the expression of certain critical cellular genes. When growth-stimulatory signals (eg, mitogenic growth factors) impinge on the cell, the growth-suppressing activities of this protein are reversed, allowing the cell to begin growth. In cells deprived of the Rb protein because of genetic inactivation of the Rb gene, the cell proliferation may proceed continuously, even in the absence of stimulation by mitogenic growth factors.

Within the past 2 years, the Rb gene and encoded protein have been shown to be involved in more than just retinoblastomas. Children who survive familial retinoblastomas early in life sustain sarcomas with high frequency later on.[22] In fact, the Rb gene has been seen in altered form not only in such sarcomas but also in a number of sarcomas arising in individuals having no history of eye tumors.[23] The rate of Rb alteration in such osteosarcomas and soft-tissue sarcomas is so high as to suggest that most of these tumors stem from somatic loss of Rb gene copies. A similar conclusion implicating somatic loss of Rb gene copies comes from studies of small cell lung carcinomas, almost all of which carry this lesion.[24] Moreover, about one third of bladder carcinomas have Rb alterations.[25] These results are puzzling, since children born with defective Rb alleles do not seem to be predisposed to small cell lung carcinomas or bladder tumors.

THE Rb PROTEIN AND DNA TUMOR VIRUSES

Yet another involvement of the Rb protein in tumorigenesis came to light last summer when it was revealed that the proteins encoded by three different viral oncogenes are complexed with the Rb protein in virus-transformed cells.[26-28] The initial observation concerned human adenovirus type 5, whose E1A oncogene contributes to the malignant transformation of rodent cells infected by this virus. The adenovirus E1A oncogene protein is seen to be tightly bound to the p105-Rb protein in these transformed cells.[26] Analogously, the monkey virus SV40 specifies a "large T" oncogene that also can induce transformation in infected rodent cells; it too is seen to be complexed with the Rb protein of the infected, transformed host cell.[27] Finally, the human papilloma virus type 16 uses an oncogene termed *E7* to transform cells; the encoded viral oncoprotein also has strong affinity for the Rb protein.[28] Significantly, this virus and its oncoproteins are

implicated as important agents causing human cervical carcinoma.

It would seem that these viruses have evolved an ability to transform infected cells by synthesizing oncoproteins that seek out and complex with the host cell's Rb protein. These viral oncoproteins may be able to inactivate or neutralize the functioning of the Rb protein, in effect removing it from cellular metabolism and thereby mimicking the effects of genetic inactivation of the Rb gene. Viewed from this perspective, the Rb protein would seem to be a centrally important regulator of cell growth whose activities may be perturbed by a number of distinct carcinogenic mechanisms.

Granting the rich insight provided by study of the Rb gene, it must be said that this particular gene stands as only one member of a potentially large class of analogously acting genes, each one of which is involved on a tissue-specific basis in the origin of one or another type of human malignant neoplasm.[14] Genetic analyses of a variety of tumors have already indicated that the homozygous inactivation of specific genes frequently accompanies the formation of acoustic neuromas, colon, lung, and mammary carcinomas, Wilms' tumors, and several unusual types of neoplasias. One anticipates that several of these will be isolated as molecular clones in the next several years. With such gene cloning will come abundant new insights into the molecular/genetic mechanisms underlying neoplasia.

Since the loss of genetic information through gene inactivation is achieved far more readily than hyperactivation of gene function, it would seem that the inactivation of growth suppressor genes may be as important in human cancer as the activation of oncogenes. It is likely that many tumor cell genomes will be found to contain both activated oncogenes and inactivated growth suppressor genes, and that these two types of genetic change conspire to create the tumor cell phenotypes encountered in the clinic. Within the next decade, we have the prospect of fully understanding the workings of these two types of genes and, with this, of gaining a complete understanding of the molecular mechanisms that trigger cancer.

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CORRECTION

Incorrect Legend.—In the article entitled "Improving Diagnosis of Appendicitis: Early Autologous Leukocyte Scanning," published in the October 1989 issue of the ARCHIVES (1989;124:1146-1152), images were incorrectly matched with diagnoses in the legend to Fig 2 on page 1149. The legend should have read as follows: Fig 2.—Early autologous leukocyte scanning images of other diagnoses. Top left, Colitis. Top right, Primary bacterial peritonitis. Bottom left, Pelvic inflammatory disease. Bottom right, Diverticulitis.