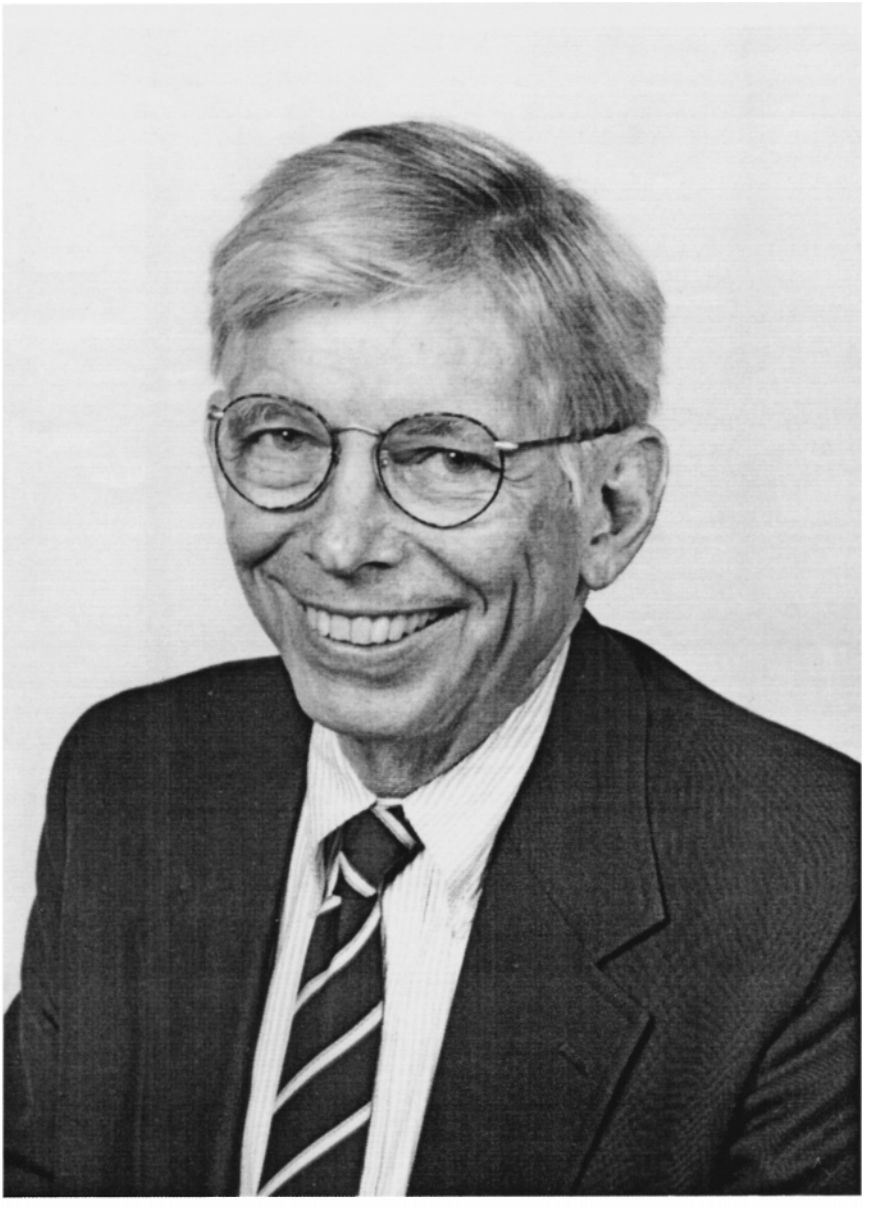

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Annu. Rev. Genet. 2000. 34:1–19

CHASING THE CANCER DEMON

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Key Words leukemia, retinoblastoma, phakomatoses, oncogenes, antioncogenes

■ **Abstract** Boveri's idea that somatic mutations are at the root of cancer found its first specific support with the investigation of leukemia and Burkitt's lymphoma, and the discovery of the mechanism of oncogene activation by balanced translocation. The study of retinoblastoma later led to the cloning of the first antioncogene, or tumor suppressor gene, and to understanding the mechanisms by which the wild-type genes lose activity. Only a small subset of cancer involves simple mechanisms. A category of hereditary disorders called the phakomatoses provide a perspective on the chain of oncogenic events in such cancers because of two-hit precursor lesions that have a low probability of malignant transformation. The common carcinomas are much more complex and are typically genetically unstable, owing either to mutational instability or chromosomal instability.

CONTENTS

INTRODUCTION

My interest in cancer dates back over 50 years, to 1949, when I was a pediatric resident at New York Hospital and spent one month in the pediatric unit across the street at Memorial Sloan-Kettering Cancer Center. There a world I knew only from books opened before me, with cases of leukemia, Wilms' tumor, neuroblastoma,

osteosarcoma, rhabdomyosarcoma, glioma, and teratoma, but, as I recall, not retinoblastoma. It was an exciting time as the first remissions were induced in acute lymphocytic leukemia by anti-folates, under the leadership of David Karnofsky and Joseph Burchenal, but even then I was more curious about the nature and origin of childhood cancer, and learned much about the natural history of these diseases from Harold Dargeon. Especially interesting were those tumors that could be found even in newborns, the so-called embryonal tumors, typified by retinoblastoma, Wilms' tumor, neuroblastoma, and teratoid tumors. My interest in embryology and in genetics was rooted in my college experience at Caltech with Albert Tyler for the former discipline, Alfred Sturtevant for the latter, and Thomas Hunt Morgan for both.

At Caltech again, after pediatric training and two years of Army service, new interests had developed because there was much excitement in the air, and I began to realize how special the times were. My first year at Columbia's College of Physicians and Surgeons was 1944, the year that Avery and his colleagues identified DNA as the genetic material. The first year of my return to Caltech was 1953, the year of Watson and Crick. In fact, I first met Jim Watson that summer when he came to Max Delbrück's laboratory to try to solve the structure of RNA. I was working in the laboratory of Henry Borsook on histidine metabolism, but he, Jacques Kruh from Paris, and Dick Schweet were investigating hemoglobin synthesis in reticulocytes, the big question being how the genes for this protein direct its synthesis in a cell that has no DNA. Two other projects at Caltech were also under way that had great implications for cancer research. One was the topic of immunological tolerance with Ray Owen, my genetics advisor, and the other the in vitro investigation of tumor viruses by Renato Dulbecco, whose course on that subject introduced me to the scientific study of cancer. One of Dulbecco's students, Marcel Baluda, was working with Harry Rubin in Dulbecco's laboratory on Rous sarcoma virus. He and I both moved to the City of Hope Medical Center in 1956.

LEUKEMIA, SOMATIC MUTATIONS, AND ONCOGENES

My greatest challenge as leader of a small pediatric cancer unit at the City of Hope Medical Center was leukemia, the major cancer treated in the children there. Remissions were regularly induced by steroid hormones, amethopterin and 6-mercaptopurine. The sudden disappearance of 10^{11} or more leukemic cells in a few days quite amazed me. So, too, did the fact that a normal adult produces about 10^{11} erythrocytes per day. How is such a production by a renewal tissue regulated, and why isn't cancer ubiquitous at an early age? It also seemed that there was a difference in the reasons for large numbers of leukemic cells in acute and chronic lymphocytic leukemia (CLL). My colleagues working with adult leukemias informed me that the lymphocytes in chronic lymphocytic leukemia survive much longer than is normal and are differentiated, whereas in children with

acute lymphocytic leukemia (ALL), the bone marrow is crowded with immature lymphoblasts; the former seemed to involve a decreased destruction of lymphocytes, the latter an increased production, with most of the cells being only partially differentiated.

In 1960, Nowell & Hungerford published their famous discovery of the Philadelphia chromosome (Ph^1) in chronic myeloid leukemia (CML) (72), which was subsequently shown by Janet Rowley to originate from a translocation between chromosomes 9 and 22 (76). This was an exciting time for the somatic mutation hypothesis because this was the first report of a specific chromosomal abnormality in a specific cancer. The new technology used to find it was directed at other leukemias and new findings were made in rapid succession. One of these findings was the 8;14 translocation in a majority of cases of Burkitt's leukemia, with others involving 2;8 or 8;22 translocations (14). It took little time to show that the break-point in chromosome 8 was at the site of the *MYC* protooncogene and that break-points at 2, 14, and 22 were at the immunoglobulin kappa, heavy, and lambda chains, respectively. In fact, the *MYC* oncogene was activated by immunoglobulin sequences, providing a wholly new way of regarding the meaning of these oncogenic translocations. When CML was investigated, a variation on the theme was found: Each break-point had interrupted a gene (*BCR* and *ABL*) and a new hybrid *BCR-ABL* "oncogene" was created. Since then many specific translocations have been investigated, especially for acute leukemia (64). Most of them operate as transcription factors. One of the most interesting genes has been *MLL*, which can participate in translocation with any of numerous different partners (77). The *MLL* gene is homologous to the *Trithorax* homeobox gene of *Drosophila melanogaster.* These translocations are typically found in cases of acute leukemia in infants, either myelocytic or lymphocytic in phenotype, hence the designation *MLL* for mixed lineage leukemia. Curiously, such balanced translocations are nearly all associated with a small part of the spectrum of cancer: leukemias, lymphomas, multiple myeloma, and a few sarcomas, including Ewing's sarcoma and alveolar rhabdomyosarcoma. An unusual feature of all of these conditions that are associated with the translocation mechanism is that their karyotypes show virtually no instability; other chromosomal aberrations are few or nonexistent. These are the simplest of cancers.

One of the hematologic malignancies that was clarified was CLL of the B cell form, which in a few instances showed a translocation that activated a gene, *BCL2*, named for its association with one form of B cell lymphoma. This gene is overexpressed in most cases of B cell CLL and thus interferes with apoptosis induced by the protein p53 (80). Another gene that may be inactivated or mutant in CLL is the ataxia telangiectasia mutated (*ATM*) gene (85), which operates upstream of p53 in the apoptotic signaling pathway. It is also of great interest that the recessively inherited disease, ataxia telangiectasia, predisposes to leukemia, including CLL of both B cell and T cell types. Most leukemias and lymphomas point to an oncogene that causes a loss of control of formation and differentiation of cells, whereas CLL discloses an impairment of the normal apoptotic control

of numbers of differentiated cells produced. The latter results in a disease that is usually not rapidly progressive, and is often compatible with life for another ten years or more. Obviously the excessive proliferation of a tissue stem cell, such as a lymphoblast or myeloblast, is more serious than is the failure of destruction of a differentiated cell.

Meanwhile, I had become interested in the possibility that ALL in children may be caused by an oncogenic virus, possibly integrated into the host genome (47) and, with Marcel Baluda, began to seek evidence for such in my patients. However, this effort led nowhere, and so have the efforts of other investigators since, even though there is still an interest in the possibility that at least some fraction of ALL cases may be of viral etiology. Meanwhile I turned to learning something about such viruses by working with Baluda on avian myeloblastosis virus (AMV), the first oncogenic virus to be discovered. We found that AMV resembled Rous sarcoma virus (RSV) in that its growth could also be impeded by inhibitors of DNA synthesis (52), as had been found previously for Rous sarcoma virus. Of course, it was later discovered that RSV was formed from a DNA template that was in turn synthesized from viral RNA, catalyzed by reverse transcriptase (3, 89); the viral genome thus becomes a part of the host genome.

The study of RSV later led to one of the great discoveries in the history of cancer research when it was found that the transforming gene, *SRC*, had strong homology with the mammalian (including human) *src* protooncogene (86). This proved to be true for other RNA viral oncogenes, including *myc*, the gene activated in Burkitt's lymphoma by translocation. In a curious way viruses could account for at least some leukemia, and unify the viral and somatic mutation hypotheses on the origin of cancer.

In a survey of 108 cases of leukemia in children for whom I had taken detailed histories, the only environmental factor that emerged was radiation, for which there was a significant history of exposure in 15 percent of the cases, most of these being acute lymphocytic leukemia (46). Of course this was not really news. Radiation was, however, important historically because it was discovered to increase not only the prospect of leukemia, but also, as Muller had discovered, the rate of new mutations, thus giving early support to the somatic mutation hypothesis that Boveri had formulated in his heuristic 1914 volume on the origin of cancer (7). Although Boveri had never investigated cancer himself, he had studied chromosome alterations connected with development in sea urchins, and was the first to suggest the functional individuality of different chromosomes. He cited the report of Hansemann in 1890 on mitotic abnormalities in cancer cells, and proposed that these were oncogenic. He even proposed that some chromosomes might stimulate cell division and that others might inhibit mitosis.

Among my patients were two who were first cousins; one child had acute lymphocytic leukemia and the other, juvenile chronic myeloid leukemia, a disorder that is not associated with the Philadelphia chromosome. Hereditary predisposition is quite uncommon for the malignancies that are associated with specific translocations. It may be that these translocations would be lethal to embryos

carrying them in the germline; in fact, this is true in transgenic mice in some instances. There are no examples of inherited strongly oncogenic translocations; however, there can be inherited mutations in genes, such as the ataxia telangiectasia gene, that predispose to translocations.

The age-specific incidence of ALL peaks in childhood and closely parallels the normal growth and regression of lymphoid tissues; leukemia incidence tracks with the target cell population, which could be expected for a "one event" cancer with a relatively short latent period between mutation and the appearance of disease. The population of target cells and the mutation rates per cell division are both important determinants of the incidence of a cancer; the product of the two should reflect the numbers of newly mutant cells per unit of time. This idea was reinforced strongly by a consideration of the age-specific incidences of the embryonal tumors, which arise from cells that are generators of a tissue but which disappear by differentiation.

TWO GENETIC SYNDROMES AND ANTIONCOGENES

Retinoblastoma and Two Hits

With an incidence in the United States, Europe, and Japan of approximately 1 per 20,000 (5×10^{-5}) births, retinoblastoma is an uncommon but well-known tumor through the age of 4–5 years, with some cases being detected at birth. Dominant transmission of predisposition to the tumor has been known since the last century. Some 25–30 percent of all patients are affected in both eyes, and a majority of hereditary cases are so affected. However, most bilateral cases do not have a positive family history, but 50 percent of their offspring are affected, usually bilaterally, thus offering convincing evidence that the bilateral cases nearly always carry a germline mutation; i.e. most of them represent new mutations. It now appears that 60 percent or so of all cases are unilateral and not heritable; about 10 percent are unilateral and heritable (with or without positive family history), 5 percent are bilateral, with family history, and 25 percent are bilateral new germline mutants. The germline mutation rate is about 0.8×10^{-5} per locus per generation. Family histories have occasionally included unaffected obligate carriers of the mutant gene, indicating that inheritance of the mutation is not sufficient for oncogenesis. I found that the numbers of tumors in bilateral cases were distributed among different cases in Poisson fashion, with a mean of three per case, thereby creating an expectation that five percent ($e^{-3} = 0.05$) of carriers should not develop any tumors, which was consistent with observation (48). Although the probability of developing at least one tumor is extremely high, the number of tumors is small. Since retinoblasts give rise to more than 10^8 differentiated descendants, the probability that one of them will develop into a tumor is of the order of magnitude of a somatic mutation rate. This suggested that hereditary retinoblastoma might depend upon one germline mutation and one somatic mutation, whereas nonhereditary retinoblastoma would result from a somatic mutation in a mitotic

retinoblast, its growth into a clone of mutant cells, and a second somatic mutation in one of them. Many, perhaps even most, persons have eyes with "one-hit" clones of cells that differentiated before sustaining a second hit. The frequencies of the two events in normal children are compatible with the rates of two mutations, and clonal growth, and with the observed incidence of the nonhereditary tumor, according to a mathematical model (36). Whereas the translocation-type leukemias and tumors may result from one somatic event and not be observed in hereditary form, a "two-hit" tumor can be observed in both nonhereditary and hereditary form.

A simple explanation of two mutational hits is that they occur in the two copies of the same gene; i.e. although predisposition is dominantly inherited, oncogenesis is recessive (12, 49). I suggested that the second event might be a new intragenic mutation, gene deletion, chromosomal loss, or somatic recombination (50). When Webster Cavenee, Ray White, and their colleagues subsequently utilized restriction-fragment-length DNA polymorphisms (RFLPs) to study retinoblastoma tumors, they indeed found that any of these mechanisms could account for second hits (9).

Retinoblastoma was thus the first example of a different kind of cancer gene, an antioncogene, or tumor suppressor gene, showing that cancer could be caused not only by activation of a protooncogene, but also by mutation or loss of a tumor suppressor gene. One childhood tumor, Burkitt's lymphoma, pointed to the first; another, retinoblastoma, to the second.

A few cases of heritable retinoblastoma are associated with deletion of chromosomal band 13q14, as Uta Francke's group and we demonstrated independently (25, 53). Using RFLPs located on this chromosome, Cavenee was able to prove not only the recessive oncogenetic hypothesis, but Friend, Dryja, Weinberg, and their colleagues were able, in 1986, to clone the *RB1* gene, the first tumor suppressor to be cloned (26). Cloning of the *RB1* gene permitted substantiation of the idea that both hereditary and nonhereditary tumors sustain mutation or loss of both alleles.

TP53 and the Li-Fraumeni Syndrome

The cloning of *RB1* aroused great interest in its mechanism of action. It was quickly discovered that it could interact with DNA, so a possible role in affecting gene transcription was considered. Here its history became entwined with that of an enigmatic protein, p53, that was found to be complexed with transforming proteins of certain DNA tumor viruses (56, 62). The transforming genes of these viruses did not have host counterparts as with the RNA viral oncogenes, but their protein products did interact with various host proteins. One such protein, in addition to p53, had a molecular weight identical to that of the protein encoded by *RB1*, so it was quickly shown that it was Rb protein (15, 100). Since it had been learned that *RB1* was acting recessively in oncogenesis, it was surmised for both *RB1* and *TP53* that their normal protein products could be inactivated by the viral transforming proteins, so *TP53*, which had at one point been considered as an oncogene, could

be categorized as a tumor suppressor gene. This was in agreement with other evidence that the wild-type p53 protein was a suppressor of transformation (23) and of colon cancer growth (2). Not long thereafter, it was shown that $p110^{RB}$ interacts with E2F transcription factors to stop progression from G1 to S phase of the cell cycle (1, 11, 34, 42, 83) and that p53 mediates G1 arrest, following irradiation (43), as well as restoring cell cycle control to cells mutant for it (102). The tumor suppressor genes were quickly connected with DNA replication as inhibitors, just as oncogenes had been connected with activators.

About the same time, *TP53* was discovered to be mutant in the germline of patients with a syndrome first described by Frederick Li & Joseph Fraumeni (59, 60, 65). These investigators were studying familial rhabdomyosarcoma in children and discovered that members of the pedigrees were affected by other tumors as well, notably osteosarcoma, brain tumors, leukemia, adrenocortical tumors, and, most notably, breast cancer among female carriers. The breast cancers were often diagnosed bilaterally, often in women in their twenties and thirties. The tumors, like retinoblastoma, typically had somatic mutation or loss of the second allele of the gene.

This phenomenon of predisposition to multiple tumors was also observed in survivors of retinoblastoma who carried a germline mutation in *RB1*(19). In fact, the two most frequently observed second tumors in the latter were osteosarcoma and soft tissue sarcomas. It has also been noted that nonhereditary osteosarcomas and embryonal rhabdomyosarcomas are often mutant for both *RB1* and *TP53* (17, 20, 67, 91, 92). However, *TP53* mutation or loss has not been found in retinoblastomas.

TP53 has also been connected with the process of apoptosis, and its loss, with failure of apoptosis of many kinds of tumor cells. Current thinking places *TP53* in the path of detection of DNA damage, whether produced by activated oncogenes or by radiation, to cell cycle arrest and DNA repair, or, lacking that, apoptosis, with the ataxia telangiectasia (*ATM*), and *hCHK*2 genes, and/or the p14ARF protein as important intermediates (44, 68). *TP53* is constitutively expressed at low levels but its expression is increased in response to induced DNA damage. *RB1* is regularly expressed constitutively, and regulates the cell cycle as its protein is phosphorylated and dephosphorylated. Put another way, loss of *RB1* function gives rise to an increased tumor cell birth rate, whereas loss of *TP53* function can produce a decreased tumor cell death rate. Incidentally, some cases of the Li-Fraumeni (LFS) that do not show mutations of *TP53* do show germline mutations of *hCHK*2 (4).

Germline mutations in *RB1* and *TP53* predispose to multiple tumors, but by no means all cancers. Especially curious is the fact that, although the tumors that occur in the syndromes are also often mutant for the same gene in their nonhereditary forms, as observed for retinoblastoma itself, the reverse is not always true. Thus, about 35 percent of nonhereditary breast cancers are mutant for *TP53*, whereas more than 80 percent of colon carcinomas are mutant for this gene, yet it is breast carcinoma, not colon carcinoma, that is featured in the **LFS**. The tumors of the

syndrome are particularly those of early life. Although the typical tumors are not embryonal, they do arise in organs that are growing in childhood, as with bone and breast during adolescence. One tumor, small cell carcinoma of the lung, is usually mutant in both *RB1* and *TP53*, yet again it is not featured in either hereditary predisposition. What we do know is that both of these tumors, lung and colon, also have mutations or loss of other specific genes at a high frequency, notably the *APC* gene for colon cancer and a gene at chromosome 3p14-21 in small cell carcinoma of the lung. Some tissues require more genetic changes to become malignant and this requirement apparently reduces the impact of the inherited mutation; these additional mutations are more likely to occur in growing tissues.

Osteosarcomas differ from the tumors with potently oncogenic translocations and from retinoblastoma in that their karyotypes are dramatically abnormal, both numerically and structurally (6). The obvious genetic difference between the two tumors in mutation of *TP53* compels consideration of the idea that *TP53* mutation is responsible. We also note that the vast majority of carcinomas, many of which contain mutated *TP53*, also display gross chromosomal abnormalities.

It may be that most cancers are mutant or inactive for *RB1* and *TP53*, or for genes functionally related to them (82). These two genes evidently play important roles in many organisms, as their orthologs have been found in *Drosophila melanogaster* (78).

HEREDITARY CANCER GENES

Incidence as a Function of Mutation and Selection

Hereditary retinoblastoma and the LFS are two instances of dominantly inherited predisposition to cancer from 50 or so known ones, for approximately half of which mutant genes have been cloned. Most of these are rare, with a birth incidence rate (I) of $1 - 10 \times 10^{-5}$, and show the typical features of mutational equilibrium: a significant fraction of new mutants and a reduction in survival value. Thus, as noted above for the hereditary form of retinoblastoma, approximately 80 percent of cases are new mutants, and for neurofibromatosis type 1 (NF1), 50 percent. For retinoblastoma the coefficient of selection (s) may be decreasing as the cure rate improves, so mutation and selection are probably not in equilibrium. For most of the remainder of the conditions that have been defined in molecular genetic terms the fraction of new mutants lies between 20 and 80 percent, and s would seem to be in the range 0.2–0.8, so $\mathrm{I} = 2\mathrm{pq}$, and $\mu\mathrm{p} = s\mathrm{q}$, where μ is the mutation rate, and p and q are the frequencies of the normal and mutant alleles, respectively, $\mathrm{p} + \mathrm{q} = 1$, and the fraction of newly mutant cases is $2\mu\mathrm{p} \cdot \mathrm{p}/2\mathrm{pq}$, or s. One condition, NF1, is exceptional in that it has an incidence of about 30×10^{-5}, which is in turn associated with a high rate of mutations ($\mu = 0.5 \times 2\mathrm{pq}\,/2 \approx 8 \times 10^{-5}$). For most of the conditions considered here, the mutation rate is of the order of 10^{-5} per generation and does not seem to vary much from one part of the world to another,

suggesting that germline mutations are occurring at a spontaneous background rate.

A few of the conditions under consideration are much more frequent, with their incidences being greater than 10^{-3}. These are hereditary breast cancer associated with *BRCA1* and *BRCA2*, and hereditary non-polyposis colon cancer (HNPCC) due to mutation of *MSH2* or *MLH1*. These incidences cannot be attributed to new mutation; in fact, it appears that new germline mutants for these genes are rare. It is also true that selection against these genes has not been important in past centuries, as revealed by the very extensive pedigrees of many generations, leading to well-known mutations associated with cohesive ethnic groups; however, animals made homozygous for mutations in these genes die in fetal or early postnatal life, so selection would seem to operate against the homozygote rather than the heterozygote. In other words, these genes are recessive for developmental lethal mutations, in which case mutational equilibrium occurs, but is independent of the heterozygote. Thus if a mutation rate of the order of 10^{-5} were operating, and $s = 1$ for the homozygote, equilibrium for the mutant gene frequency would occur at $\mu p = q^2$, and $q = 3 \times 10^{-3}$, giving a heterozygote frequency of 6×10^{-3}. The fraction of all newly mutant individuals would then be $2\mu p \cdot p/2pq = 2pq^2/2pq = q$, or approximately 3×10^{-3}, which is compatible with observation.

Classification of Hereditary Cancer Genes

On the basis of the two-hit hypothesis, I expected that the dominantly inherited cancer genes would all be tumor suppressors, and in fact that is true for most of these genes. Since activated oncogenes are very potent, as noted earlier, I had thought that mutations in them might be lethal to a fetus. It was therefore a surprise when multiple endocrine neoplasia type 2 (MEN2) was found to be due to a germline mutation in the *RET* oncogene, which codes for a tyrosine kinase receptor protein (18, 69). The two target tissues for tumors, the adrenal medulla and thyroid medulla, develop clonal tumors, thought to result from one or more mutations in other genes. Both organs show hyperplasia of target cells, however, which seems to be caused by the inherited mutation. The later events have not been identified. Two other diseases, hereditary papillary renal carcinoma and familial gastrointestinal stromal tumors, are also caused by mutations in tyrosine kinase receptor genes, *MET* and *KIT*, respectively (70, 81). The tyrosine kinase domains of each show considerable homology, and the mutations are in some instances in the same codons. The tumors are clonal, and in the case of HPRC they show a consistent cytogenetic abnormality, trisomy for chromosome 7, where the *MET* gene is located. Analysis of the tumors reveals that two of the chromosomes are mutant at the *MET* locus and one is not. The mutant gene is neither fully dominant nor fully recessive in oncogenesis, but the ratio of mutant and normal chromosomes appears to be critical. The trisomy apparently resulted from nondisjunction, and there is no suggestion of genomic instability in these tumors. A fourth oncogene mutation occurs in *CDK4* (103), causing a predisposition to melanoma, presumably

by increasing the phosphorylation rate of retinoblastoma protein and interfering with its sequestration of E2F transcription factor.

An even greater surprise was the discovery of the mutations responsible for the syndrome known as hereditary non-polyposis colon cancer (HNPCC), a condition that actually predisposes to several kinds of cancers. These genes, *MSH2* and *MLH1* being the most common, are indirectly cancer genes in that they regulate mutation rates through repair of DNA mismatches (8, 24, 58, 73). It had already been known that some cancers could occur excessively in a few recessively inherited DNA repair defects, such as xeroderma pigmentosum and ataxia telangiectasia. But in HNPCC it is the heterozygous carrier that develops cancers. Analysis of the tumors reveals that the second allele of the relevant gene is mutant or lost and that subsequent somatic mutation rates are elevated 10^2–10^3-fold, but only in the homozygously defective cells (5). The first phenotypic feature noted in the tumors was instability of microsatellite DNA sequences due to infidelity of replication of repeat sequences (39, 90). As a consequence, mutations at loci relevant to cancer occur at high rates and are selected by tumor growth; there is a *mutational instability.* The tumors usually arise from adenomatous polyps that contain *APC* mutations but the most common subsequent mutation is not in *TP53*, as in most colon carcinomas, but in the TGFβ R2 receptor gene (*TGFBR2*) (66), which contains a polyA tract that is regularly altered. The karyotypes of the tumors are often normal or nearly so; there is not a *chromosomal instability* with the remarkable aneuploidy that characterizes so many cancers. This kind of gene has been called a Caretaker gene in that it does not lead directly to cancer, but rather increases the rate at which other genes can mutate (45). On the other hand, oncogenes and tumor suppressor genes are referred to as Gatekeeper genes, because their mutations are directly on the path to cancer.

The *BRCA1* and *BRCA2* genes were discovered as the result of studies of dominantly inherited breast cancer. They are difficult to classify because they are connected to DNA repair, but also have some properties of tumor suppressor genes; i.e. they may be both Caretakers and Gatekeepers.

The Phakomatoses

In 1983, I wanted to study an animal model of dominantly inherited cancer, but I could find only one published example, inherited renal cancer in the rat, first described by Reidar Eker in Norway (21). He and his colleagues had noted that there was a ratio of two affected to one unaffected offspring of crosses between two affected animals, and genetic testing of the affected ones demonstrated heterozygosity (22). They therefore concluded that the homozygous state was lethal to the fetus. In 1983, my wife and I drove to Kennedy Airport in New York to pick up five rats that Eker sent me; all of the Eker rats now under study worldwide are descendants of the one mutation carrier of the five. We were able to show that the expected 25 percent of homozygous offspring had already died by fetal day 14 and that some of them had small heads. I sent some Eker rats to Cheryl Walker,

TABLE 1 The phakomatoses

Disease	Gene	Cloning reference
Neurofibromatosis 1	*NF1*	(10, 98, 99)
Neurofibromatosis 2	*NF2*	(75, 94)
Tuberous sclerosis 1	*TSC1*	(97)
Tuberous sclerosis 2	*TSC2*	(13)
von Hippel-Lindau syndrome	*VHL*	(57)
Gorlin syndrome	*PTCH*	(33, 41)
Cowden disease	*PTEN*	(61)
Familial adenomatous polyposis	*APC*	(31, 71)
Peutz-Jeghers syndrome	*STK11/LKB1*	(35, 40)
Juvenile polyposis	*SMAD4/DPC4*	(37, 38)

who demonstrated that the homozygotes frequently show neural tube defects (74). There was no evidence of neoplasm in the fetuses. Okio Hino from Tokyo's Cancer Institute and Raymond Yeung, a young surgeon at Fox Chase Cancer Center, joined me in an effort to find the responsible gene; the two of them, later, independently discovered that the responsible gene is the homologue of the human tuberous sclerosis 2 gene (54, 101).

Another animal model, the *min* mouse, discovered in the laboratory of William Dove (88), carries a mutation in the *APC* gene, and again the homozygous state is lethal to the fetus. This is also true for an increasing list of knock-out mice for tumor suppressor genes (29). Of interest is the fact that it is not true for *TP53*, which is reasonable since *TP53* is expressed at low levels in normal tissues, but at elevated levels in response to certain signals. Nearly all of the genes that require inactivation or loss of both copies for oncogenesis are in effect recessively inherited developmental lethals and would be known as such were it not for oncogenesis in heterozygotes.

I then became interested in the fact that tuberous sclerosis, familial adenomatous polyposis (FAP), and several other diseases whose genes were cloned belonged to a clinically recognized category of conditions known as the phakomatoses (95). This term was coined by Van der Hoeve in 1932 (96) using the Greek word "phakos", meaning "mother-spot" or birthmark. He applied it to three conditions, neurofibromatosis, tuberous sclerosis, and von Hippel-Lindau disease (VHL), because of their manifesting scattered benign lesions in one or more organs. The list has since grown and includes 10 conditions of the 25 or so for which the responsible mutant genes have been cloned (Table 1). Each of the genes is a tumor suppressor, and all appear to operate in signal transduction; thus *APC* operates in the Wingless/ Wnt pathway and *PTCH*, in the Hedgehog pathway. Orthologs of all of the 10 genes, except *VHL*, are known in *Drosophila* (78), suggesting their

importance in a wide range of organisms. The benign lesions typically show mutation or loss of the second allele of the inherited mutant gene; they are two-hit lesions.

The remarkable feature of these diseases is the large number of benign lesions, either hamartomas or small adenomas that are found in the target tissues. In FAP, there can be thousands of colonic polyps; in NF1, hundreds of cutaneous neurofibromas; in VHL, hundreds of renal adenomas or small carcinomas in the kidneys. In each condition the transition of such a lesion to a clinically malignant state occurs only rarely, but the large numbers of these precursors of malignancy lead to a common malignant outcome per patient. The malignant tumors are frequently carcinomas; e.g. colon or small intestinal carcinoma in FAP, juvenile polyposis, and Peutz-Jeghers disease, renal carcinomas in VHL and tuberous sclerosis (TS), basal cell carcinomas of the skin in the neroid basal cell carcinoma syndrome (NBCCS), or Gorlin's syndrome, and carcinoma of the breast in Cowden disease. The precursors in these diseases stand in contrast to their absence in hereditary retinoblastoma and the LFS, wherein the tumors that are seen are malignant from the outset. What is the reason for this difference?

The most obvious difference between hereditary retinoblastoma (Rb) and LFS on the one hand and the phakomatoses on the other is the target tissue (51). In the former, the usual target tissue is growing, frequently in embryonic life or during adolescence, while in the phakomatoses the targets are typical renewal tissues, as in the skin, gastrointestinal tract, or urinary tract. Yet, as noted previously, *RB1* and *TP53* are mutated somatically in many renewal tissues. The best-studied cancer is carcinoma of the colon, where the *APC* gene is responsible for FAP and is mutant in a large majority of nonhereditary colon carcinomas. But the Li-Fraumeni Syndrome does not feature colon carcinoma, yet 90 percent or so of sporadic colon carcinomas are somatically mutant for *TP53.* What is the difference between the two genes with respect to colon cancer? I think it is because *APC* mutations, whether germinal or somatic, lead to benign precursors in a renewal tissue, and *TP53* mutations do not.

The typical tumors seen in hereditary retinoblastoma and LFS arise in growing tissues, in which the tissue stem cells are presumably replicating. Under these conditions a first somatic mutation will result in a mutant clone of cells, and a second hit, whether in hereditary or nonhereditary cases, will lead to a tumor that will continue to grow. In a renewal tissue, these will not be the consequences, unless the mutation changes the renewal tissue into a growing tissue. This may be a key to understanding the lack of precursor lesions in hereditary Rb and LFS, and their presence in the phakomatoses. Furthermore, what is true for colon cancer may be true for all carcinomas; the latter may all begin with precursor lesions whose mutant genes in the germline can account for hereditary forms of the cancers.

Understanding the molecular actions of the phakomatoses genes does not explain their tumor biology. All of the genes appear to act in signal transduction that impinges on the cell cycle, but why do the benign lesions appear and why are they

not malignant until other events occur? Why do these lesions seem to be necessary for most, perhaps all, epithelial cancers? The answer to the latter question may simply be the resulting increase in probability that subsequent events necessary for transformation will actually occur. These could be events that decrease the probability of differentiation and/or apoptosis. This could be interpreted as a form of genetic instability in the target tissue, and indeed a recent report on colonic polyps concludes that new mutations occur in them in greatly increased numbers (87).

CHROMOSOMAL INSTABILITY IN CANCER

Hansemann's original observations of aberrant mitoses in cancer cells led Boveri to propose that somatic mutations were the cause of cancer. We now know that instability of the karyotype is a common feature in cancer, especially the carcinomas. Some carcinomas that demonstrate karyotypic stability are unstable with respect to mutations, as with mutations in DNA mismatch repair genes in HNPCC. It is of great interest that the tumors that are typically found in HNPCC are not the ones found in hereditary retinoblastoma or LFS, and vice versa. These observations invite a classification of cancers according to genetic mechanisms and to tissue site. First, we can take note that the cancers associated with specific translocations activate oncogenes or create new ones, they occur in hematopoietic and some other mesodermal tissues, and they do not show either kind of genomic instability. Second, retinoblastoma and possibly other cancers of embryonal origin result from mutations in tumor suppressor genes, may show some other somatic mutations, but do not manifest general genomic instability. The path to cancer in these tumors appears to involve small numbers [2–4] of genetic events. Third, some carcinomas show multiple mutations due to mutational instability but have normal, or nearly normal, karyotypes. Fourth, most carcinomas and some other cancers, e.g. osteosarcomas, show numerous karyotypic aberrations, both in chromosomal number and with respect to rearrangements. Tumor suppressor mutations are common, as are somatic oncogene mutations or amplification. Precursor lesions are common and produced by a two-hit mechanism, usually involving a tumor suppressor gene, and can be accounted for by usual somatic mutation rates. Other genetic changes effect malignant transformation and interfere with apoptosis; a limited number of these could occur at background mutation rates (84), but a large number would require a "mutator phenotype" (63). An intermediate view is that usual mutation rates and clonal expansion can be oncogenic, but a mutator phenotype may accompany the process, and even accelerate it (93).

Karyotypic instability of chromosomal number can result from mutation of *TP53*, in which case the mechanism is dysregulated replication of the centrosome, generating extra mitotic spindles and creating changes in ploidy but also with individual losses or gains (27). In addition there are frequent rearrangements in cancer cells, resulting from chromosomal breaks. These can be expected from

dysregulated centrosome replication too, so loss of *TP53* may be the direct cause of instability. These phenomena are not found in colon carcinomas that manifest a mismatch repair phenotype (28). It is also noteworthy that loss of normal *ATM* activity, which operates upstream of *TP53*, produces multiple chromosomal translocations in fibroblasts (55). Chromosomal breaks can be repaired by either homologous recombination, with Rad 52 protein playing a caretaker role, or by nonhomologous end-joining, with Ku70 or 80 playing such a role (16, 32). Recent work (30, 79) demonstrates that these breaks lead to the famous breakage-fusion-bridge cycle, first described in corn by Barbara McClintock. Here a break in both chromatids of a replicating chromosome leads to loss of a terminal fragment and to "sticky ends" that fuse together to form a dicentric chromosome, which then straddles the mitotic plate until it breaks, later fusing in each daughter cell and repeating the cycle at the next mitosis. Multiple chromosomal breaks can lead to heterologous chromosomal fusion and translocation. This sequence probably accounts for at least some of what Hansemann observed, and for what is now observed in karyotypes of many cancers, especially carcinomas. It is also interesting, even though expected, that the breakage-fusion-bridge cycle does not operate in tumors with strongly oncogenic balanced translocations (30).

PERSPECTIVE

All chasers of the cancer demon in the past century must surely have been fascinated by the object of their pursuit, while at the same time feeling the frustration of its elusiveness. As the new millennium begins, we have an optimism that the demon can at last be comprehended and that new approaches to conquering it can be formulated on the basis of this comprehension.

ACKNOWLEDGMENT

The author appreciates the constructive comments on the manuscript by Drs. Joseph Testa and Alfonso Bellacosa. He also regrets that many valuable publications were not cited, owing to limitations of space.

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