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Review

Antioncogenes and human cancer

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ABSTRACT **The antioncogenes, or tumor suppressor genes, as negative regulators of cell division, stand in contrast to oncogenes. For most human cancers, the more frequently mutated genes are the antioncogenes, the principal exception being the leukemias and lymphomas. Persons heterozygous for germ-line mutations in antioncogenes are strongly predisposed to one or more kinds of cancer, and most dominantly inherited cancer is attributable to such heterozygosity. Seven antioncogenes have been cloned through the study of these persons, and several others have been mapped. An eighth one was mapped and cloned through the investigation of tumors and is not yet known in hereditary form. Three dominantly inherited forms of cancer are not attributable to mutations in antioncogenes. The corresponding nonhereditary forms of most cancers generally reveal abnormalities of the same antioncogenes that are found in the hereditary forms but may also show additional ones. Some cancers, especially the embryonal tumors of children, have a small number of antioncogene mutations; some others, such as most sarcomas, have more, and the common carcinomas have the most, reflecting a hierarchy of controls over growth of stem cell populations. Still more members of this gene category remain to be mapped and cloned through the study of cancer families and of tumors. The genes that have been cloned act at diverse points in the signal transduction pathway in cells, from the outer cell membranes to sites of gene transcription, in some cases as negative regulators of oncogene expression.**

Virtually every human cancer can occur in genetically predisposed individuals. The most striking form of genetic susceptibility involves Mendelian dominant inheritance with high penetrance and appearance of cancer at earlier than usual age, as shown for colon cancer in persons with familial adenomatous polyposis. In this example, the heterozygous state of the germ-line mutation imparts a high risk for just one form of cancer, while in other examples, such as the Li–Fraumeni syndrome (LFS), it predisposes to several kinds of cancer, although never to all forms. The most frequent cancer in LFS is carcinoma of the breast, although it does not afflict all female carriers. This incomplete penetrance of a gene for a particular cancer typifies the heterozygous state for a familial cancer gene; its presence is not a *sufficient* condition for cancer.

A simple explanation for this incomplete penetrance is that oncogenesis requires a somatic mutation in some target cell, an event that may never occur in some heterozygous carriers; two mutations, one germinal and one somatic, would be needed (1). This hypothesis also relates the hereditary and nonhereditary forms of a cancer by a common mechanism; the same two mutations would operate in both, the first mutation being germinal in the former and somatic in the latter. In the hereditary case all of the somatic cells would carry a first "hit," whereas in the nonhereditary case only a clone of somatic cells would do so.

What might the targets of these two hits be? The simplest answer is the two copies of some autosomal gene; oncogenesis would be recessive at the cellular level in both the hereditary and nonhereditary cases (2, 3). The presence of one normal (wild type) copy of the gene would interfere with oncogenesis, and the normal allele could therefore be considered as antioncogenic. Such genes are known as antioncogenes, or tumor suppressor genes. If the 50 or so different hereditary cancers all arise by this mechanism, then there should be a corresponding number of antioncogenes.

The past several years have brought important discoveries regarding the genetic predisposition to cancer. Antioncogenes do exist and in fact play a major role in human carcinogenesis. Seven antioncogenes whose mutations in the germ line predispose their hosts to cancer have been cloned, and several putative genes of this class have been mapped to specific chromosomal bands (Table 1). These discoveries have illuminated our understanding of human cancer considerably and have revealed a group of genes that is important in cellular and developmental biology, making this a propitious time to review progress on the subject and to take note of some future prospects for extending this understanding.

Retinoblastoma: The Prototypic Hereditary Cancer

Although uncommon, occurring at a rate of approximately 5 cases per 100,000 children in most parts of the world, retinoblastoma has been a prototype in the study of hereditary cancer (1, 4). About 40% of cases are attributable to a germline mutation of the *RB1* gene, which is located in chromosomal band 13q14.2. Most of these affected persons have no previous family history of the tumor, a fact that is explained by new mutations occurring at a rate of 8×10^{-6} per locus per generation. However, ≈50% of the offspring of these newly mutant cases will develop the tumor. The remaining 60% of cases are nonhereditary, indicating that the incidence of tumor in children without the germ-line mutation is 3 per 100,000. These cases affect just one eye, whereas in the majority of germ-line cases both eyes are affected.

The number of tumors in germ-line cases is distributed in nearly Poisson fashion, with a mean of three tumors, and with the unaffected carrier class being approximately, as expected, e^{-3}, or 5%. Since the number of transformable cells derived from the normal target cells, the embryonic retinoblasts, is of the order of magnitude of 10^7, the probability that a second, tumor-forming event will occur can be estimated as no less than 3×10^{-7} per cell division (5). If this frequency applies to each of the two cell divisions necessary to form a nonhereditary tumor, and if we take into account the multiplication of once-hit retinoblasts, the expected incidence would be close to 3×10^{-5}, as observed. In addition, most persons in a population would have at least one once-hit cell that differentiates into a normal postmitotic cell before a second event can produce a tumor. The incidences of both the hereditary and nonhereditary forms of retinoblastoma can thus be accounted for by germ-line and somatic mutation rates that are similar to known "spontaneous" mutation rates, and there is no need to invoke induced mutations to account for the incidence of retinoblastoma in most countries.

About 5–10% of the hereditary cases show a constitutional deletion of part or all of chromosomal band 13q14, a phenomenon that revealed the site of the retinoblastoma gene (*RB1*) (6, 7). The use of syntenic polymorphic genetic markers (restriction fragment length polymorphisms, RFLPs) permitted demonstra-

Table 1. Characteristic neoplasms associated with germ-line mutations in selected antioncogenes

Antioncogene	Chromosomal location	Neoplasm		
		Sarcoma, Wilms tumor	Neurectodermal and endocrine	Carcinoma
Cloned				
RB1	13q14	Sarcoma	Retinoblastoma	
WT1	11p13	Wilms tumor		
TP53	17p13	Sarcoma	Glioma	Breast
NF1	17q11	Sarcoma	Glioma	
NF2	22q12		Schwannoma	
VHL	3p25		Pheochromocytoma	Kidney
APC	5q21			Colon
Uncloned				
NB1	1p36		Neuroblastoma	
MLM	9p21		Melanoma	
MEN1	11q13		Pituitary adenoma	
BCNS	9q31		Medulloblastoma	Skin
RCC	3p14			Kidney
BRCA1	17q21			Breast, Ovary

tion of the recessiveness of the gene in oncogenesis (8) and of the mechanisms predicted (4) for second events—namely, local mutation, deletion, chromosomal nondisjunction, and somatic recombination. Loss of heterozygosity of at least some syntenic markers is a feature of all of these mechanisms except the first. RFLPs were also used for the positional cloning of the *RB1* gene (9–11). The normal cDNA of *RB1* was then shown to cause reversion of the tumorigenic properties of cultivated tumor cells that were mutant for *RB1* (12). *RB1* thus became the first human antioncogene, or tumor suppressor gene, to be discovered.

The *RB1* gene itself has been the subject of intense investigation (reviewed in ref. 13). The retinoblastoma protein Rb is phosphorylated during the cell cycle. In its unphosphorylated form it blocks passage through G_1 to S, apparently by complexing with a transcription factor such as E2F, which normally can activate important cell cycle genes, perhaps including *MYCN*, which is overexpressed in fetal retina and retinoblastoma; an antioncogene may oppose the action of an oncogene. Phosphorylated Rb protein does not inhibit the factor and is therefore permissive for this passage. Since Rb exerts such an important effect and since it is expressed in all cells, its association primarily with a rare tumor of children is puzzling. Perhaps its action is circumvented by other regulatory mechanisms in tissues other than retina.

Survivors of retinoblastoma with germ-line mutations of *RB1* are also susceptible to other tumors, notably osteosarcoma and soft-tissue sarcomas (reviewed in ref. 13). The penetrance is much lower than the 95% or so observed for retinoblastoma itself, being of the magnitude of 10–15% for each of these classes of tumor by the age of 30 years. Some of these tumors were observed in irradiated orbital bone or soft tissues, but others were observed far from the eye, compelling the conclusion that genetic predisposition was not to a single tumor. The radiation-induced tumors do inform us that the frequency of second events can be increased by environmental agents. Furthermore, many nonhereditary tumors at these same sites, especially osteosarcomas, revealed mutations at the *RB1* locus. Even more surprisingly, several other tumors not typically observed in retinoblastoma survivors were mutated at *RB1*. Especially striking in this respect was small-cell carcinoma of the lung, where most, perhaps all, tumors are mutant or deleted for both copies of *RB1*; yet almost none show germ-line mutations of *RB1*. The relative risk that germ-line mutation of *RB1* imposes on its host is $\approx 10^5$ for retinoblastoma, 10^3 for osteosarcoma, and 10 or less for small-cell carcinoma of the lung (14). Why, then, would the penetrance be extremely high for one tumor, intermediate for others, and very low for still others? Small target-cell pools and very low tissue-specific mutation rates are not likely explanations, because osteosarcoma and small-cell carcinomas of the lung are much more common than retinoblastoma. As we shall see later, the apparent explanation is that for these other tumors, other controls on growth must be overcome; *RB1* is not the only gene that must mutate.

Mice that are constitutionally heterozygous for a nonfunctional allele of *RB1* do not develop retinoblastomas (15–17). One explanation is that the number of target cells—i.e., retinoblasts—is too small to lead to a second mutation in an observable number of animals. On the other hand, the mice do develop tumors of the pituitary, which are not seen in humans. These heterozygous mice can also be mated with each other to produce homozygously defective animals, which die before 16 days of fetal life, with abnormalities of the brain and hematopoietic system. Some cells in both tissues undergo too many mitoses and too little differentiation. From this experiment one can conclude that *RB1* is a developmentally critical gene, whose mutations are recessive in their developmental and oncogenic effects.

Wilms Tumor

Wilms tumor was the second cancer for which a relationship between hereditary and nonhereditary forms was proposed (18). This tumor occurs in ≈10 per 100,000 children. Here again the discovery of cases with constitutional deletions—at 11p13—pointed to the location of the putative *WT1* gene (19) and so to regional RFLPs that were used to find molecular mutations and deletions and to clone the gene (20, 21). Patients with constitutional mutations or deletions of *WT1* appear not to be susceptible to other tumors. The gene's specificity fits well with the limitation of its expression to the genitourinary tract, the mesothelial lining of the body cavities, the spleen, and parts of the central nervous system, in contrast to *RB1*'s ubiquitous expression (reviewed in ref. 22). Genitourinary anomalies are common in children who are heterozygous for mutation or deletion of *WT1*, and some mutations produce the Denys–Drash syndrome, which is also predisposing to Wilms tumor.

Mice made heterozygous for a mutation of *WT1* do not develop Wilms tumor, again probably because, as for *RB1* mice, the target-cell population is too small (23). Even in humans the penetrance of *WT1* mutations for Wilms tumor is lower than that of *RB1* for retinoblastoma. The homozygous mutant state is again lethal, at 13–15 days, with a failure of development of the metanephric kidney. Lethality is apparently attributable to widespread edema secondary to impaired development of the thoracic mesothelium, heart, and lungs. Like *RB1*, *WT1* is a developmentally lethal recessive gene that in the heterozygous mutant state predisposes its human host to cancer.

Wilms tumor differs from retinoblastoma in another important way. Whereas *RB1* appears to be the only gene whose mutations predispose to retinoblastoma, only about 10–20% of Wilms tumors can be attributed to mutation at *WT1*. A well-recognized but rare dominantly heritable disorder, the Beckwith–Wiedemann syndrome (BWS), also predisposes to this tumor. The *BWS* gene has not been cloned, but clinical features suggest that *BWS* may not be an antioncogene. Infants with the syndrome are large at birth and exhibit features that suggest the pos-

sibility of heterozygosity for an overexpressing mutation of an oncogene.

BWS has been mapped by linkage analysis to chromosomal region 11p15, a considerable distance from *WT1* (24). One gene in this region, that for insulin-like growth factor 2 (*IGF2*), is especially relevant because its product is a fetal growth factor, expressed strongly in fetal kidney and in Wilms tumor but not in the adult kidney. It is also an imprinted gene, the maternally derived allele being unexpressed. In BWS, and in some nonhereditary Wilms tumors, this imprinting is relaxed and both parental alleles are expressed, thereby presumably causing excessive fetal growth (25–27). In addition, the protein product of *WT1* can repress the *IGF2* gene *in vitro*, binding to a 5′ regulatory region of the latter. These findings suggest a kind of equivalence of the two genes that predispose to Wilms tumor, one mutation affecting an oncogene, and the other affecting an antioncogene that regulates it.

LFS and the p53 Gene

A role for the protein product of the *TP53* gene in carcinogenesis was originally deduced from the study of tumors induced by certain DNA viruses (reviewed in ref. 28). Following its mapping to human chromosomal band 17p13, loss of heterozygosity of 17p markers in many human tumors, especially carcinomas, implicated it as a member of the antioncogene category. Now it is known that a majority of mutations in the gene involve single base changes that produce a protein with an abnormally long half-life. When mutant, this protein, a DNA-binding protein as are the products of *RB1* and *WT1*, may interfere with the function of the product of the normal allele—i.e., it may have a "dominant negative" effect, which could explain its oncogene-like behavior. However, most tumors show abnormality of both alleles, and some tumors show complete deletion of both alleles.

Later it was found that *TP53* is constitutionally mutant in most cases of LFS (29), a dominantly inherited syndrome discovered through the study of familial rhabdomyosarcoma in children (30). The families of these children proved to have a high incidence of other cancers, including especially breast carcinoma, but also soft tissue sarcomas (including rhabdomyosarcoma), osteosarcoma, brain tumors, leukemia, adrenocortical carcinoma, and perhaps some other carcinomas. The breast cancers typically occur in subjects before the age of 50 years, even in their 20s. The incidence of LFS is probably 2–4 per 100,000 births, and the high mortality before the end of the age of reproduction assures that it is maintained in populations only by recurrent spontaneous germ-line mutations.

A remarkable feature of LFS is that some carcinomas are *not* typical. Thus, carcinoma of the colon and small-cell carcinoma of the lung, both with high incidence of somatic mutations of *TP53*, are not important tumors in LFS. Why does one carcinoma, that of the breast, but not two others, those of the colon and lung, develop frequently in LFS subjects? Perhaps there is a clue in a comparison of the tumors that typify the heterozygous carriers of *RB1* and *TP53*. In both instances sarcomas of soft tissues and bone are conspicuous among carriers in the first two decades of life. Mutations of both genes are common in nonhereditary tumors of these histologies; but they are also both common among cases of nonhereditary small-cell carcinoma of the lung, a tumor that is not typically found in constitutional heterozygotes for mutation at either gene. Interestingly, the sarcomas occur during a time of net growth in somatic tissues, which is accompanied by growth of tissue stem cells. In this respect these tissues partially resemble the growth of fetal retina and kidney, where clones of once-hit cells greatly magnify the number of target cells available for mutation or loss of the second allele of *RB1* or *WT1*, respectively. Breast is another tissue that undergoes net growth in adolescence. On the other hand, colon and lung are typical renewal tissues; development of once-hit clones in a renewal tissue is uncommon unless there is some stimulus to stem cell proliferation, as happens in the colon with chronic ulcerative colitis, and, possibly, in the lung in response to cigarette smoking.

The *TP53* gene, like *RB1*, can also influence passage through the G_1 phase of the cell cycle (31). Especially interesting is the observation that normal cells, but not those mutant at *TP53*, are arrested temporarily in G_1 by ionizing radiation (32). It is therefore something of a surprise that mice transgenic for expressing mutant p53 alleles (33) or homozygously defective for p53 (34) develop normally, although they have a high incidence of tumors, especially of lymphoid tissue. It seems that p53 regulation of the cell cycle is in some way conditional, and mutations are therefore permissive for normal development. This situation is compatible with the observation that overexpression of *TP53* in normal cells elicits a quiescent state *in vitro* in association with a reduction in available guanosine triphosphate (GTP), a key compound in the transduction of growth signals (35). One scenario (36) raises the possibility that tumors themselves, at some point in their histories, elicit a host defense in the form of increased production of p53, which is inhibitory to tumor growth. Only then would mutation or loss of *TP53* impart a selective advantage to a cell. Thus, mutation at *RB1* might initiate an osteosarcoma cell, but its malignancy would depend upon loss of normal p53 activity. In small-cell lung cancer, a tumor of a typical renewal tissue, yet another negative control might inhibit growth until it too is mutated. There seem to be three kinds of tissues: (*i*) embryonal tissues whose normal growth is unconditional once proliferation is initiated; (*ii*) "conditional tissues" like bone, whose growth can be induced as by hormones at puberty; and (*iii*) renewal tissues, which have a capacity for conditional response but in addition are programmed for daily replenishment (14). *TP53* plays an important role in these latter two tissue categories, but the second category is the principal target for oncogenesis in LFS.

Antioncogenes and Tumors of Neural and Neural Crest Origin

Neuroblastoma. In addition to *RB1* there are several antioncogenes that predispose to tumors of neural origin with some specificity. Along with retinoblastoma and Wilms tumor, neuroblastoma was one of three embryonal tumors treated as models of hereditary cancer involving constitutional heterozygosity for a mutant gene that is also important in the nonhereditary form of the same tumor (37). The tumors commonly show a deletion that includes all or part of chromosomal region 1p36, so that site has been suspected as the locus of a *NB1* gene (38, 39). However, familial neuroblastoma is rare and linkage analysis has not been conducted. Two case reports of neuroblastoma with constitutional aberrations of 1p36 enhance the candidacy of an antioncogene at this site (40, 41). The families that have been observed with this tumor typically do not develop other tumors, suggesting a considerable specificity for the neural crest-derived adrenal medulla and sympathetic nervous chain. This tissue can also be the site of pheochromocytoma, but families with neuroblastoma do not have pheochromocytoma, and vice versa. Thus, there is specificity not only for an organ but also for the degree of differentiation within that organ.

Neurofibromatosis. The term neurofibromatosis is applied to two entities, types 1 and 2 (NF1 and NF2). NF1 features the benign tumor, neurofibroma, and distinctive café-au-lait pigmented lesions of the skin. One of the most frequent dominantly inherited human diseases, NF1 affects 30 per 100,000 children, half of whom are new germ-line mutants. The *NF1* gene predisposes to several tumors: neurofibrosarcoma, malignant schwannoma, glioma, and pheochromocytoma, and, at low frequency,

leukemia, rhabdomyosarcoma, and Wilms tumor. The *NF1* gene was mapped by linkage analysis to chromosomal band 17q11 and subsequently cloned (42, 43). The gene codes for a large ubiquitously expressed protein, neurofibromin, one region of which is homologous to the two inhibitor genes of *RAS* found in yeast. The gene's function is similar to that of the *GAP* (GTPase-activating protein) gene product; loss of its activity results in failure of hydrolysis of GTP to GDP by the RAS protein. This loss of neurofibromin function is thought to result in elevated levels of the GTP-bound RAS protein that transduces signals for cell division (44–46). The high germinal mutation rate of *NF1* may be attributed to its heavy methylation (47). The mutations observed in the gene frequently produce truncated, functionless proteins. In a significant fraction of the characteristic malignant tumors of *NF1*, the second (normal) copy of the gene appears to be mutated or absent (48–50). Mutations of *TP53* are also observed in some cases of neurofibrosarcomas (51), as often observed in other sarcomas. *NF1* evidently qualifies as an antioncogene, with relatively narrow specificity for tumors of neural and neural crest origin, although mutations are found in some tumors that are not observed in NF1 patients. Wild-type *NF1* again exemplifies the inhibition of an oncogene by an antioncogene. It will be very interesting to discover why *NF1* has the tissue specificity that it does. The existence of both *GAP* and *NF1* suggests redundancy in the regulation of RAS protein. It is possible that the tissues that are susceptible to tumorigenesis have little *GAP* function, leaving *NF1* to be the sole regulator.

NF2, a much less common disorder than NF1, shows a high penetrance and great specificity for acoustic nerve tumors (vestibular schwannomas) and meningiomas. Sporadic, nonhereditary forms of these tumors are often monosomic for chromosome 22, implicating an antioncogene on this autosome. *NF2* has been mapped to chromosomal arm 22q12 and has recently been cloned (52, 53). Its protein product is unusual for tumor suppressor genes in that it is homologous with proteins found at the interface between the plasma membrane and the cytoskeleton. Mutations found in the tumors typically cause truncation of the protein product; *NF2* clearly qualifies as an antioncogene, although its precise mechanism of action is not yet known.

Melanoma. Malignant melanoma, another tumor of neural crest origin, exists in at least one, possibly two or more, hereditary forms. The gene for one of these, *MLM*, has been mapped by linkage studies and in a deletion case near the α- and β-interferon genes, on chromosomal arm 9p21, although it has not yet been cloned (54, 55). A claim has been made for another familial melanoma gene on chromosome arm 1p (56). Preneoplastic benign nevi usually precede the appearance of melanomas, suggesting one somatic event producing the nevus, and still further events leading to malignancy. Nonhereditary melanomas often show loss of heterozygosity for 9p markers and even homozygous deletions at band 9p21 (57), indicating the importance of the *MLM* antioncogene for both the hereditary and nonhereditary forms of melanoma. The 9p21 site has been implicated in other tumors, including brain tumors (58), some leukemias (59), and some lung cancers (60), but these tumors have not been associated with constitutional mutation of *MLM*. An apparently homologous site on rat chromosome 5q nearly always shows loss of heterozygosity in cell lines derived from renal carcinomas in the Eker rat (61), clearly in association with tumor progression. The possibility must be considered that not all of the tumors mentioned involve one and the same gene.

Multiple Endocrine Neoplasia. Two well-known syndromes, multiple endocrine neoplasia types 1 and 2, that predispose to endocrine tumors, some of neurectodermal and neural crest origin, have been mapped by linkage analysis to sites at 11q13 (MEN1) and 10q11 (MEN2) (62, 63). Heterozygotes for *MEN1* mutations are strongly predisposed to parathyroid hyperplasia and to tumors of the anterior pituitary and pancreatic islets. Furthermore, these tumors often show loss of heterozygosity for 11q markers, so *MEN1* appears to qualify as an antioncogene. Such is not the case for *MEN2*, whose mutations predispose heterozygotes to two neural crest-derived tumors, pheochromocytoma and medullary carcinoma of the thyroid. Loss of heterozygosity (LOH) for markers on 10q11 has been found rarely in these tumors, even in those persons with the syndrome; on the other hand, LOH for 1p markers is very common (64). Chromosome arm 1p has been implicated also in neuroblastomas and melanomas, other tumors of neural crest origin. The responsible gene on chromosome 10q11 has recently been identified as the *RET* protooncogene (65), a receptor tyrosine kinase gene, making it the only example so far of an oncogene whose mutations in the germ line place the host at risk of cancer. A plausible scenario for the development of these tumors is that the *RET* mutation produces the preneoplastic hyperplasia of the adrenal and thyroid medullae that characterizes the disease, increasing the number of target cells available for transformation to neoplasia by mutation at an antioncogene locus on 1p.

Brain Tumors. The principal tumors that arise in the brain are gliomas and primitive neurectodermal tumors (PNETs). Two hereditary conditions, NF1 and LSF, have already been observed to predispose to gliomas, although neither does so at high penetrance. Mutations at *TP53* are frequently seen in gliomas of high-grade malignancy, and clonal expansion of p53 mutant cells has been observed in association with progression in glioma (66). Deletions of chromosome 9p have been noted previously as common in glial tumors, and it remains to be determined whether the affected gene is identical with the one that is mutant in hereditary melanoma. The 9p site is apparently important in tumor progression, as is one on chromosome 10q, an almost universally affected chromosome in the very malignant glioblastoma (67). Still, there is as yet no explanation for pedigrees that show highly penetrant dominant inheritance of gliomas, even over three generations (for example, see ref. 68), which may point to initiating events in glioma formation and to a new antioncogene.

PNETs, which usually occur in children, are occasionally found in patients with a predisposing germ-line mutation in an antioncogene, as with *TP53* or *NF1*. However, one condition has regularly been associated with PNETs—namely, Gorlin syndrome, or basal cell nevus syndrome (BCNS), whose gene has been mapped by linkage analysis to 9q31 (69). Carriers of mutation at the *BCNS* locus almost invariably develop basal-cell carcinomas of the skin, and approximately half of these tumors reveal loss of heterozygosity for 9q markers, suggesting the presence of an antioncogene on that chromosomal arm (70). A few percentage of persons with the syndrome develop PNETs, and ≈25% of nonhereditary medulloblastomas also reveal loss of heterozygosity for 9q markers (70). It is interesting that syndrome subjects with PNET have in some cases received craniospinal irradiation, which in turn induced large numbers of cutaneous basal cell carcinomas, with a short latent period, suggesting the induction of numerous "second hits" that led to cancer (71).

Carcinomas

Carcinomas constitute the largest category of human cancer and take the largest toll of life. They are also the most complicated from the vantage point of genetics. Still, there has been considerable progress in recent years, with a promise of more to come in the near future.

The von Hippel–Lindau (VHL) Syndrome and Renal Cell Carcinoma. This dominantly inherited syndrome is characterized by nonneoplastic phenotypic features that facilitate its identification. Virtually every carrier of the *VHL* mutation develops one or more of three types of cancer: hemangioblastoma of the brain, pheochromocytoma, or renal

cell carcinoma (RCC). Curiously, the last two tumors are uncommon in the same family, although bilateral tumors are frequent at each site when they do occur (72). The gene was localized by linkage analysis to chromosomal band 3p25 and recently cloned (73). Its protein product appears to be a cell surface molecule involved in cell adhesion and signal transduction. It will be of interest to discover whether the protein interferes with the function of an oncogene that is involved in signal transduction. Examination of nonhereditary renal carcinomas reveals abnormality of *VHL* in a high percentage of cases, making it the principal RCC gene. Both of these nonhereditary tumors and the tumors found in the syndrome typically reveal mutation or loss of the second copy of the gene, as expected for an antioncogene. However, one family with high penetrance for RCC carried a constitutional translocation with one breakpoint at 3p14-21 far from the *VHL* gene (74). It seems likely that 3p harbors a second RCC gene, although it is possible that the tumors in translocation patients have sustained 3p deletions that cause loss of the *VHL* gene. Phenotypically, the tumors in both inherited forms are of the common clear cell histology. In addition, there are nonclear cell tumors that do not show LOH for chromosome 3p markers, suggesting the existence of yet another renal cancer gene. Nonclear cell RCC is known in hereditary form in the Eker rat, and the gene has been localized to rat chromosome 10q, in a region that seems to be homologous in part with 16p in humans (75). In neither humans nor rats do predisposed gene carriers develop Wilms tumor, nor do carriers of a Wilms tumor mutation develop RCC. Here again there is specificity for a developmental state in an organ, as with the adrenal medulla for neuroblastoma and pheochromocytoma.

Familial Adenomatous Polyposis and Colon Cancer. One of the best known hereditary conditions that predispose to a major cancer of adults is familial adenomatous polyposis (FAP), which occurs in persons heterozygous for a mutation in the adenomatous polyposis coli (*APC*) gene. *APC* is located at chromosomal band 5q21 and has been cloned (76, 77). Its protein product is located in the cytoplasm and has features suggesting potential for interaction with other proteins, perhaps the product of some oncogene. In typical FAP cases, the colonic mucosa is studded with hundreds or even thousands of benign adenomatous polyps. These polyps, which begin to appear even in the first decade of life, are clonal in origin. Polyps of this same type occur sporadically in normal individuals; cells of even very small polyps are mutated somatically at the *APC* locus (78). It has been reported that many, conceivably even all, polyps have sustained mutations or loss of *both* copies of the *APC* gene (79, 80).

The crypts of the colonic mucosa in FAP patients show a larger than normal proliferative compartment (81); in effect the *APC* mutation causes an expansion of the target cell compartment. This phenomenon is not clonal and appears to be a dominant effect of the constitutional mutation, even though some of the mutations involve total deletions of the gene. However, the mutations most often introduce termination codons that cause extreme truncation of the protein product, which can interact with the normal allele's product, interfering with the latter's function and causing the mutation to behave in a "dominant negative" manner, as with some mutations in *TP53* (82). It will be interesting to compare the phenotypic effects of null and truncating mutations.

Mutations at the *APC* gene in the mouse produce multiple intestinal neoplasia (*Min*)—i.e., polyps and carcinomas (83–85). Multiple cell lineages in the tumors suggest that the mutation exerts its effect upon a pluripotent stem cell. Homozygotes for the mutation die during fetal life (85), indicating that the gene has an important role in normal development, as is true also for *RB1* and *WT1*.

Even if mutation at the *APC* locus is a necessary condition for carcinoma of the colon, it does not suffice. Other genetic events are the rule for this tumor, the targets being the *KRAS* oncogene, the *TP53* antioncogene (86), and the *DCC* (Deleted in Colon Cancer) antioncogene (87). *KRAS* mutations are featured in large polyps, where their incidence is ≈40%. They are rare in very small polyps, and their incidence in carcinomas is about the same as in large polyps. They seem not to be directly involved in malignant transformation of the latter. On the other hand, *TP53* and *DCC* mutations occur uncommonly in polyps but in the majority of carcinomas. It has not been established whether there is a necessary sequence for mutation in these two genes.

The *DCC* gene was identified following LOH studies that showed frequent losses for markers on chromosome 18q (87). The gene encodes a cell surface molecule with considerable homology to the cell adhesion molecule of neural cells (N-CAM) and probably plays a role in self-recognition in colonic epithelium. The characterized mutations usually produce termination codons that in turn lead to truncated, presumably nonfunctional, proteins. No normal copies of *DCC* remain in the tumor cells, as expected for an antioncogene. This is the only antioncogene that has been cloned without the availability of constitutional mutations. So far there is no hereditary condition in which *DCC* is mutant. *DCC*, for example, is expressed in neural tissue as well as in the gastrointestinal tract (88), and one patient with a constitutional deletion of 18q, the arm that contains the *DCC* gene, developed a brain tumor (89). Could it be that germ-line mutations of *DCC* would be associated with brain tumors rather than with colon cancer?

Another hereditary condition, known as the Lynch cancer family syndrome type 2, imparts susceptibility to numerous types of carcinoma, including that of the colon. A gene for it (there may be more than one), *LCFS2*, has recently been mapped to chromosome 2, a chromosome not previously implicated in colon carcinogenesis (90). Although the gene has not yet been cloned, one remarkable property of it has been described. Tumors in heterozygous carriers show widespread rearrangement of microsatellite sequences (91–93) but not loss of heterozygosity for chromosome 2 markers, as expected for an antioncogene. However, they do show incidences of mutation at *KRAS*, *TP53*, and *APC* that are comparable with those in colon cancer in general. If the *LCFS2* mutation accelerates the rates of mutation at *APC*, *TP53*, and *DCC*, formation of polyps and their transformation to malignant carcinomas should be expedited.

Familial Breast Cancer. The clustering of cases of breast cancer in families has long been known. Disentangling the cause of this phenomenon as due to heredity, environment, or chance has been difficult. Particularly striking are families in which female descendants of an affected female are affected through two or more later generations, often at an earlier than usual age. Two genes are now known to contribute to this phenomenon—namely, *TP53*, in the LFS, and the breast cancer 1 (*BRCA1*) gene. Constitutional mutations of this latter gene predispose heterozygous carriers to carcinomas of the breast and ovary. The gene has been mapped to chromosomal band 17q21, and vigorous attempts are underway to clone it (94, 95). Breast and ovarian cancers from affected women in these families reveal a high incidence of loss of the wild-type allele, as expected for an antioncogene (96). The gene probably also plays a major role in nonhereditary cancer of these tissues. The frequency of heterozygous carriers of the gene can be estimated to be as high as 1 per 1000 (94). Such a high frequency for a serious single gene condition is extraordinary and, if true, would imply virtually no mortality before the end of the reproductive period. This gene is apparently the most common among those that predispose to breast cancer and perhaps the most common that predisposes to any cancer.

Lung Cancer. There are no pedigrees that unequivocally demonstrate segrega-

tion of a dominantly inherited predisposition to lung cancer, possibly because such a very large fraction of all cases is attributable to cigarette smoking. Studies of cytogenetic and molecular changes in tumors point to certain genes or chromosomal regions. Mutations at *RB1* and *TP53* are common (97, 98), especially in small-cell lung cancer (SCLC), although, as noted previously, this cancer is not a characteristic feature of hereditary retinoblastoma or of the LFS. A third change, found almost universally in SCLC, and in a majority of cases of non-SCLC, is deletion of chromosome 3p14-p21 (99, 100). There is at least one lung cancer antioncogene (*LC1*) on chromosome 3p. SCLC is similar to colon cancer in that three different antioncogenes appear to be critical to both, with p53 being common to them. A difference is that none of the three lung cancer genes (*RB1*, *TP53*, or *LC1*) resembles *APC* by providing strongly predisposing germ-line mutations.

Prostate Cancer. The genetics of prostate cancer is poorly understood, but the analysis of familial cases has led to the conclusion that there is a dominantly inherited predisposing gene (101). A possible clue to the location of such a gene has been provided by cytogenetic and loss of heterozygosity studies in tumors, which have revealed a few recurrent abnormalities. Deletion commonly occurs at 8p22, and was even homozygous in one case (102). Deletion at 10q24 has been reported as the sole anomaly in some tumors and displayed clonal evolution in one patient (103). Perhaps one of the candidate antioncogenes at these chromosomal sites is mutant in the hereditary form of this cancer. Other somatic mutations will probably prove to be important too, as happens with other carcinomas.

General Comments on Antioncogenes and the Carcinomas. The common and very important carcinomas, especially of breast, colon, and lung, have been discussed in part, especially in connection with the *TP53*, *APC*, *LCFS2*, and *BRCA1* genes. The phenomenon, noted for *RB1* and *TP53*, that carcinomas can show somatic mutations at these loci, without belonging to the constellation of tumors to which germ-line mutations predispose their hosts, apparently relates to the fact that carcinomas often show mutations at multiple loci, and no one of them would have a great impact in the germ line. *APC* mutations have the unusual property of causing an expansion of the renewal proliferative compartment and, judging from studies on the *Min* mouse, thereby expanding the number of stem cells that are available to sustain other oncogenic events. The colon cells assume properties that are similar to those of proliferating embryonal stem cells, such as retinoblasts and nephroblasts, and the stem cells that are stimulated by puberty and adolescence. The stem cells of normal renewal tissues do not expand in number, so some environmental or genetic stimulus must be provided. Evidently, germinal mutations in *TP53* do not provide such an impetus, which may explain why breast cancer, but not colon or lung cancer, is a distinctive feature of the LFS. Embryonal tissues require the fewest oncogenic events; conditional growth tissues, an intermediate number; and renewal tissues, the most.

Table 2. Cloned human tumor suppressor genes: protein products and homozygous mutant effects in mice

Gene	Protein	Mutant mouse
RB1	110-kDa transcription modulator, regulator of $G_1 \rightarrow S$ transition	Fetal lethal
WT1	45-kDa transcription factor, zinc finger protein	Fetal lethal
TP53	53-kDa transcription factor, conditional regulator $G_1 \rightarrow S$	Not lethal; predisposition to tumors
NF1	327-kDa activator of Ras GTPase activity	Not reported
NF2	66-kDa protein at membrane–cytoskeleton interface	Not reported
VHL	34-kDa cell membrane protein	Not reported
APC	310-kDa cytoplasmic protein	Fetal lethal
DCC	153-kDa cell adhesion molcule	Not reported

There are other genes whose mutations are highly penetrant for carcinomas, but none of them is so obvious as *APC* or *BRCA1*. Thus, rare pedigrees with carcinomas of the stomach, pancreas, or bladder have been reported, but there has been no progress in mapping the relevant genes.

Putative Leukemia and Lymphoma Antioncogenes. Many leukemias and lymphomas reveal somatic chromosomal translocations that activate or rearrange cellular protooncogenes. This mechanism for initiation of cancer is evidently limited to hematopoietic and a few uncommon neoplasms, such as Ewing sarcoma and alveolar rhabdomyosarcoma. The reason for such a limitation of target sites is not apparent. However, some leukemias seem to involve the antioncogene mechanism. For example, leukemia is prominent in LFS. Furthermore, *TP53* mutations are found in some leukemias and lymphomas, although it is uncertain whether they are changes associated with initiation or with progression. There are some pedigrees with many cases of leukemia or lymphoma, usually rather homogeneous for acute leukemia, chronic lymphocytic leukemia, or non-Hodgkin lymphoma. None of these genes has been mapped, and there is no evidence bearing on their identity as oncogenes or antioncogenes.

The Search for New Antioncogenes

So far eight antioncogenes (*RB1*, *WT1*, *VHL*, *APC*, *DCC*, *TP53*, *NF1*, and *NF2*) have been cloned. All except *DCC* have been found to be mutated in the germ lines of persons predisposed to one or more tumor types. Another seven putative antioncogenes (*BRCA1*, *RCC*, *NB1*, *MLM*, *MEN1*, *BCNS*, and *LC1*) have been mapped but not cloned. Germinal mutations in all except *LC1* are known to predispose to tumors. Obviously the analysis of hereditary cancer provides excellent entry to the world of antioncogenes.

The numerous reports of familial cancers at virtually all sites indicate that many more antioncogenes are to be discovered. The list of antioncogenes could reach 50 or so from the source of hereditary cancer alone. There is a problem, however. The most common conditions are those that are mapped first, and it already appears that the remaining hereditary cancers will be so rare as to render linkage analysis difficult. Of course, rare cases with constitutional deletions have pointed to genetic sites in the past and will continue to do so. Similarly, LOH studies can be used, as was done with *DCC* in colon cancers, although this is a very difficult process. However, there may be numerous genes whose mutations are rare or lethal in the germ line that could be important in the origin of tumors. Some of these genes may even occur as germ-line mutations but produce tumors very different from those found in the nonhereditary form. Thus, the discovery of somatic mutations in *RB1* in small-cell carcinoma of the lung would hardly have prepared one for its production of retinoblastoma in carriers of the germ-line mutation.

Another approach to the discovery of new members of this class of cancer-predisposing gene could involve mechanistic analysis of oncogenes and of the signal transduction pathway. The early proposals that antioncogenes might encode cell membrane molecules (2) or negative transcription factors (3) were both correct in a way. The antioncogenes that have been cloned include three genes (*RB1*, *WT1*, and *TP53*) whose protein products interact with DNA or with tran-

scription factors to regulate negatively the expression of other genes, which are in effect oncogenes (Table 2). The *APC* and *NF1* gene products appear to function in the cytoplasm, whereas the products of *NF2, VHL*, and *DCC* seem to be located at the cell membrane. If one assumes that positive and negative regulation occurs at all points in the signal transduction pathway, then it may be that the study of the control of oncogene activity could lead to identification of new inhibitory factors that would be candidates for antioncogenes.

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