Mutations and Cancer

The somatic mutational hypothesis for the origin of cancer carries several implications of importance: (a) spontaneous mutations should produce some irreducible "background" level of cancer; (b) agents that can change the host genome by mutagenesis or by addition or deletion of genetic material should increase this background incidence; (c) abnormalities that favor either spontaneous or induced mutations should impose an elevated risk of cancer; and (d) inheritance of an initiating mutation should strongly predispose to cancer. Consequently, there should be 4 groups of persons with respect to carcinogenesis (29). These groups may be designated as "oncodieses," because they are demographic units with different expectations of cancer, depending upon environmental and hereditary variables.

The first of these oncodieses should ensure that every cancer has some universal incidence below which we cannot go unless we devise methods for reducing spontaneous mutagenesis; i.e., we shall always have cancer. This group may be the 20% that would remain if the 80% of "environmentally induced" cancer of the world were prevented. Most investigators now believe that most of the world's cancer befalls the second group, whether the causative agent is a chemical mutagen, radiation, or a virus. The third group is exemplified by xeroderma pigmentosum but could be much larger than currently suspected if it turns out that there are significant genetically determined differences among individuals with respect to the activation or inactivation of mutagens. It is even possible that most of the persons thought to be in the second group actually belong to the third group. For both of these groups, the probability of the occurrence of one or more steps in oncogenesis is increased, although the number of steps is not decreased. The fourth group should be at very high risk of cancer, especially if the number of steps on the way to cancer is small. In this group, the number of steps in oncogenesis is reduced by one. It is this group whose cancers are designated "hereditary" and with which this commentary is concerned.

The somatic mutation hypothesis also implies that there are critical genetic loci with mutations that lead to cancer. Any attempt to understand the process of carcinogenesis would necessarily be concerned with discovering these genes and understanding their behavior in health and disease. We now seem to have identified 2 large classes of such genes. The first class, consisting of the oncogenes, was originally discovered through the study of critical genes in the acutely transforming retroviruses. The second class includes what I have called "antioncogenes" (31) and was identified through the study of the hereditary cancers of the fourth oncodiese.

Hereditary Cancers

The hereditary cancers of humans number about 50, but there is good reason to believe that there are more to be discovered. For every cancer, there seems to be at least one inherited form; i.e., all cancers exist in both hereditary and nonhereditary form. Well-known examples of hereditary cancer include polyposis of the colon and colon carcinoma, neurofibromatosis and various cancers associated with it, and the multiple endocrine neoplasia syndrome type 2, which predisposes to pheochromocytoma and medullary carcinoma of the thyroid. The term "hereditary cancer" is a misnomer in that what is inherited is a predisposition to cancer. In the typical case, the pattern of inheritance is mendelian dominant, a pattern that is particularly striking in very large pedigrees extending over several generations. Although 50% of the offspring of affected persons are affected in model cases, it happens for many of the hereditary cancers that penetrance is incomplete; therefore, an unaffected person can even have affected parent and child. In still other instances, the penetrance is of the order of magnitude of 50%, so that as many gene carriers do not develop the cancer in question as do.

The cancers of predisposed persons are generally restricted to one or a few; predisposition is not to cancer generally but to a specific cancer or cancers. At a given age, the risk that a particular cancer will occur is increased manyfold. Thus, the age-specific incidence of carcinoma of the colon is increased 1000 times or more in subjects with polyposis coli (2). A consequence of this is that persons carrying such genes often die at an earlier than usual age. If part of this mortality occurs before the end of the reproductive period, as happens for most, perhaps even all, of the hereditary cancers, there would be selection against the germlinal mutations, consistent with the fact that the heritable cancers are all uncommon. The frequency of such genes would be more or less constant from generation to generation, however, as new mutations occur, establishing mutational equilibrium for the population with respect to the particular gene in question; the rate of gene mutation would then equal the rate of gene loss. For the hereditary cancers of children, most cases are in fact new germlinal mutations; knowledge that they are heritable derives from the occurrence of the cancer in the offspring of survivors. When the cure rate for such a tumor improves, as with retinoblastoma, the mutational equilibrium can shift, and the disease can become more common.

For some cancers, there are 2 or more hereditary forms. One of the clearest examples is colon carcinoma. Mutation of one gene produces polyposis coli and a high risk of carcinoma, particularly in the descending sigmoid colon and rectum. Mutation of the other accounts for the cancer family syndrome first reported by Warthin (50), predisposing to both endometrial and colon carcinoma, the latter unassociated with polyps and located
exists for breast cancer. In some families, only breast cancer is
found at high incidence, while in others it is associated with
endometrial carcinoma and, in still others, with a diversity of
cancers, including soft tissue sarcomas and brain tumors (40).
This last form of breast cancer often strikes at an early age
(before 40 years) and bilaterally; it is one of the clearest excep-
tions to the generality that cancer genes usually predispose to
one or few cancers (38).

Most of the cancer genes fail to manifest features that permit
the identification of the gene carrier in the general population.
Exceptions include polyposis coli and neurofibromatosis. In some
instances, microscopic lesions can be detected in the target
tissue. For example, in hereditary medullary carcinoma of the
thyroid, C-cell hyperplasia is regularly found in the medulla of
the thyroid (25). Some studies on such precursor lesions found in
hereditary cancers involve the attempt to decide whether they
arise as a consequence of some rare event that affects one cell,
which then multiplies in clonal fashion, or whether they result
from a multicellular, even though local, phenomenon. This prob-
lem was attacked by Friedman et al. (20), using the elegant
technique of Linder and Gartler (39) to study uterine myomas.
This technique depends upon mosaicism in normal females with
respect to the activity of X-linked genes; in a given cell, one X-
chromosome is active and one is inactive. Once this dichotomous
decision is made in young embryos, all of the X-chromosomes
descended from an active chromosome are active; the action is
vice versa for inactive chromosomes. When a female is hetero-
zygous for an X-linked gene such as glucose-6-phosphate de-
hydrogenase, a study of clonality of tumors is possible. Myomas
were shown to be clonal in origin, some of them, even when
several are present in a particular female, being active for one
allele, some for the other, but none for both. Neurofibromas in
normal individuals are also clonal, but those of patients with
neurofibromatosis are multicellular in origin. On the other hand,
neurofibrosarcoma arising from a neurofibroma is clonal (20). A
multicellular origin has also been demonstrated for polyps in
polyposis coli (24), and clonality has been shown for malignant
medullary carcinoma of the thyroid (5); therefore, it seems that
the precancerous lesions in the hereditary cancers are multicel-
lar in origin and the malignant tumors arising from them are
clonal. We conclude that the precancerous lesions result from
local phenomena that affect numerous cells, whereas each ma-
lignant tumor results from a very rare second event that affects
a single cell.

Antioncogenes

Recessive Cancer Genes

The hereditary cancers have revealed a new class of gene
that is important in the pathogenesis of cancer. The genes of
this class, clearly different from oncogenes, have been called
antioncogenes (31), because they produce cancer in a recessive
mode, one normal allele being adequate to protect against a
particular cancer. Abnormally activated or mutant oncogenes,
on the other hand, can produce cancer in the heterozygous
state, and the continued presence of the oncogene is required.

It may be useful to discuss here the meaning of the words
"dominant" and "recessive." The terms were originally used in a
strict mendelian sense. If an allele of interest were expressed in
the heterozygous state with the normal allele, it was said to be
dominant. Such an allele would be transmitted to 50% of the
offspring. Familiar examples in medicine include Huntington’s
disease and polyposis coli. If an allele were expressed in the
homozygous, but not heterozygous, state, then it was said to be
recessive. Homozygous individuals constitute 25% of the
offspring of matings between 2 heterozygous persons. Now
consider a disease like sickle cell anemia. The heterozygous
person has a condition called sickle cell trait (AS) which is
dominantly inherited. On the other hand, sickle cell anemia (SS)
is recessive. Consider further that somatic mutation or recom-
bination could occur, producing a homozygous cell in a hetero-
yzygous person; for example, it must surely happen that persons
with sickle cell trait (AS) develop some recessive sickle cells
(SS). For nearly all diseases, such cells would be inconsequential,
because they would differentiate and die. Even if a stem cell
became homozygous, it would produce a small and harmless
d ended. However, if such a homozygous cell were a cancer cell, it
would continue to grow until, if untreated, the host would die. In
homozygotes, every cell in the target tissue should be homozy-
gous; this might produce cancer in all cells of the target tissue
or prevent development of the fetus. Such cancer genes could
also mutate and lead to cancer in normal persons. If somatic
mutation produced a heterozygous stem cell in a normal person,
no important effect should be produced; e.g., if such a mutation
occurred in the polyposis gene, the host would develop a polyp.
However, since these cells could persist, they could survive to
develop the homozygous state, by new mutation or by genetic
recombination and thus become malignant. All 3 host genotypes
(normal (+/+), heterozygote (+/-), abnormal homozygote (-/-)
for a particular cancer gene could develop recessive (---)
cancers. In the first (+/+), cancer would result from 2 somatic
events; in the second (+/-), cancer would result from one germ
line and one somatic event; and in the third (-/-), it would result
from 2 germ line events (Table 1). In practice, the third group
would not exist; therefore, a particular cancer would occur in
normal persons and in heterozygous persons described as hav-
ing dominantly inherited predisposition to that cancer. However,
the tumors in both would be homozygous for a recessive cancer
gene and lack the normal (antioncogenic) allele.

Physical evidence for the existence of 2 such genes (antion-
cogenes) has been presented for 2 cancers, retinoblastoma and
Wilms’ tumor. In both instances, the demonstration of their
recessiveness has depended upon the existence of constitutional
deletion cases that permitted chromosomal localization of the
genes and upon linked polymorphic genetic markers that permitted
a test of homozygosity.

Retinoblastoma

Carcinogenesis in 2 Steps. Antioncogenes were discovered
in retinoblastoma. This ocular tumor is usually recognized before

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Host</th>
<th>Tumor</th>
<th>No. of somatic events</th>
<th>Probability of tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>+/+</td>
<td>—/—</td>
<td>2</td>
<td>Rare</td>
</tr>
<tr>
<td>Heterozygote</td>
<td>+/-</td>
<td>—/—</td>
<td>1</td>
<td>Many? (lethal)</td>
</tr>
<tr>
<td>Abnormal</td>
<td>—/-</td>
<td>—/—</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 1

Tumors caused by recessive antioncogenes in hosts of different genotypes
the age of 5 years and has had a reasonably high cure rate for much of this century, both of which facts permitted the early recognition that some survivors produced affected offspring. When patients have had bilateral tumors, one-half of their children are affected and one-half are unaffected; i.e., such patients bear a “retinoblastoma mutation” and transmit it in dominant mendelian fashion. On the other hand, most unilaterally affected survivors produce unaffected offspring, although 10 to 15% produce affected offspring, most of whom are bilateral cases.

Knudson (28) observed that the numbers of tumors that heritable cases acquired fitted closely to a Poisson distribution, with a mean number of 3 to 4 tumors/gene carrier. Most carriers acquired bilateral disease, some carry unilateral disease, and a small minority have no tumors. It was estimated that 35 to 40% of retinoblastoma cases were of this heritable type, the remainder being nonheritable and unilateral. The disease has an incidence of about 5/100,000 children; therefore, the probability that a noncarrier will acquire the tumor is about 3/100,000. If the mean number of tumors per gene carrier is 3, then the risk for one tumor in gene carriers relative to noncarriers is 100,000 (30).

Since the retina contains more than 10^6 cells that are descended from normal retinoblasts, it is a very rare cell that becomes malignant even in the gene carrier. This strongly suggests that a second event is involved in hereditary cases, the inherited mutation not being sufficient; a Poisson distribution further suggests that this event is random. It was hypothesized that 2 events are necessary not only for the hereditary cases but also for the nonhereditary cases (28, 30). In the former, one event is in the germ line and one is somatic, while in the latter both are somatic. The 2 events were regarded as involving the same genes for both the hereditary and nonhereditary forms. The 2 events were further thought to involve the 2 copies of the same gene; i.e., the tumors were homozygous for a cancer mutation.

Mutation at the Same Locus in Hereditary and Nonhereditary Cases. About 3 to 5% of retinoblastoma cases are heterozygous for a chromosomal abnormality that has permitted localization of the retinoblastoma gene. Although the deletions are of various lengths and there is no single breakpoint at either end, they do all include one particular band on the long arm of chromosome 13, 13q14 (33, 51). These deletion cases also have a deficiency in a serum enzyme, esterase D, the deficiency being a quantitative reduction to 50% of normal, from which it has been concluded that the deletions include both the esterase D and retinoblastoma loci (49). With a few exceptions, the nondeletion cases show a normal level of esterase D, and it has been proposed that these exceptions are cases of deletions that are too small to be visualized microscopically. The 2 loci are evidently closely linked.

It was hypothesized that the 13q14 band contained the gene the mutation of which was responsible for the hereditary cases that do not show deletion (33). This hypothesis was verified by the demonstration of linkage between the retinoblastoma and esterase D genes (13, 48). This verification utilized a polymorphism for esterase D, consisting of 2 electrophoretic forms of enzyme, designated EsD1 and 2. In some retinoblastoma pedigrees, these alleles segregate too, and a test of linkage is possible. For example, if a retinoblastoma survivor is heterozygous, EsD1/EsD2, and marries an individual who is EsD1/EsD1, then the offspring will be either EsD1/EsD1 or EsD1/EsD2. If the retinoblastoma mutation in a particular pedigree is on the EsD2 chromosome, then the heterozygous offspring will have the retinoblastoma mutation and the EsD1 homozygotes will not. An exception would be due to genetic recombination. If recombinants are 50%, the 2 genes are on different chromosomes or far from each other on the same chromosome. In fact, no recombinants have yet been observed, indicating close linkage. Therefore, the 2 kinds of heritable cases, deletion and nondeletion, both involve the same genetic site, contrary to the conclusion of Matsunaga (41) that they involve 2 different genetic loci.

The first prediction was that the nonhereditary cases would be caused by mutation at the same genetic locus affected in the hereditary cases (27, 28, 30). This prediction has been tested by cytogenetic analysis of tumors of nonhereditary cases. In 20% or so, there is an abnormality, usually absence or deletion, of one of the 2 No. 13 chromosomes in tumors, and the common deletion site is the same as in the constitutional cases (4, 6, 21). The remainder cannot be evaluated by this method. However, the tumors of some patients who are heterozygous for EsD have been examined. In some tumors, only one allele is expressed, suggesting the possibility of a submicroscopic deletion (23), although this finding evokes another interpretation, as discussed below.

Homozygosity or Hemizygosity in Tumors. In approaching the problem of the nature of a second event, attention is called to 3 classes of events that could lead to loss of the normal allele at the retinoblastoma locus: submicroscopic mutation (which has been designated 13q-, the normal allele being 13q+); deletion (13q-); and chromosomal loss (13-). If the gene is recessive and the second event involves the development of homozygosity, then tumor genotypes can be imagined: 13q+/13q-, 13q+/13q+, 13q+/13q-/13-, 13q-/13q+, 13q-/13q-/13- (30). The last genotype would probably be lethal, as would the fourth and fifth if the deletions were large enough to include many other genes. The first 3 genotypes might well be compatible with tumor survival. If the first mutation were 13q-, then the second event would be a mutation in the first case, a deletion in the second, and chromosomal loss by nondisjunction in the third. The first genotype could also be caused by the mechanism of somatic recombination, whereby a heterozygous cell (13q+/13q-) could produce 2 homozygous cells, one normal (13q+/13q+) and one tumorigenic (13q-/13q-).

The first evidence for recessiveness came from the study of a case in which quantitation of esterase D in the parents and affected child showed a 50% level of the enzyme in the child, although no deletion could be detected in karyotyped leukocytes (7, 42). In the tumor, however, only one chromosome 13 was found. If that chromosome was the normal No. 13, there should have been esterase D activity in the tumor; if abnormal, there would be no activity. No enzyme activity was found, indicating that the genotype of the tumor was 13q-/13-, the deletion being so small that the genotype was not lethal.

Further evidence for recessiveness has been obtained by the comparison of RFLPs in bloods and tumors of patients. RFLPs are very common, and 7 or so have been localized to various regions of chromosome 13 by deletion mapping. Study of the patient noted above revealed polymorphism for 2 of these sites

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2 The abbreviations used are: RFLP, polymorphic restriction enzyme-digested DNA fragment lengths; BL, Burkitt's lymphoma.
in leukocytes (10). However, only one allele of each site was found in the tumor, as expected, since one chromosome 13 was lost. In some patients, there were no deletions in the tumors and no deficiencies in esterase D to suggest occult deletion. Presumably, the tumors were of the 13q10/13q14 genotype. These could have been produced by a new event or by recombination. Some showed heterozygosity for all of the loci that were heterozygous in leukocytes. If these tumors were homozygous at the retinoblastoma locus, they became so by acquiring a new submicroscopic mutation or deletion that did not include any of the RFLP sites. On the other hand, one tumor showed heterozygosity for a RFLP located between the centromere and the retinoblastoma (rb) locus but homozygosity for more distal RFLPs that were heterozygous in leukocytes (10). It was concluded that somatic recombination had occurred in the proximal long arm of chromosome 13 between the first RFLP locus and the rb locus, giving rise to a cell homozygous for loci beyond the cross-over site.

In some other patients, all of the sites heterozygous in leukocytes have been homozygous in the tumor (10, 15). Two possible explanations have been offered (10, 15, 42); one involved recombination between the centromere and the proximal marker, and the other involved the loss of one chromosome and the acquisition of a second copy of the other one by nondisjunction. Centromeric markers could distinguish these possibilities, but none have been noted in the cases reported. In the first possibility, the centromeres would be derived from both parents; in the latter, they derive from just one parent.

It appears then that the second event can arise, as predicted, as a new event (including mutation and chromosomal loss) or by somatic recombination. The second event is clearly genetic rather than epigenetic as suggested by Matsunaga (41). In approximately one-half of cases, there is evidence for recessiveness of the mutation in tumors, thereby distinguishing this kind of gene from the oncogene.

Wilms' Tumor

Similar findings have been reported for Wilms' tumor. Hereditary cases are not as numerous for this tumor, and about 50% of persons with the mutation do not develop the tumor. Deletion cases are also known. These were discovered because of the association of sporadic, not inherited, aniridia in 1 to 3% of tumor cases. Knudson and Strong (34) proposed that these cases might be caused by constitutional deletions of neighboring genes, one for aniridia and one for Wilms' tumor. The first report confirming this prediction concerned a case of translocation between chromosomes 8 and 11, but the authors mistakenly concluded that there was a small deletion in chromosome 8 (36). Subsequent analyses of this case and of pure deletion cases have localized the critically deleted segment to a specific band in the short arm of chromosome 11, 11p13 (45).

Localization of the gene responsible for the hereditary but not the deleted form of Wilms' tumor has not been accomplished directly, as with retinoblastoma, because familial cases are rare and because there is no marker analogous to esterase D. However, the nonhereditary form of tumor has been shown in some instances to contain a deletion of the 11p13 band (26, 47). Furthermore, studies with polymorphic loci on chromosome 11 have revealed only one allele in almost 50% of the tumors in persons showing heterozygosity in leukocytes (18, 35, 43, 44), apparently due in some cases to homozygosity and in some to hemizygosity.

These findings show that Wilms' tumor is very much like retinoblastoma. Both tumors seem to be caused by recessive mutations, at a single site for each tumor. Heterozygosity precedes the development of homozygosity, sometimes by germinal mutation or deletion (hereditary cases), sometimes by somatic mutation or deletion (nonhereditary cases). Homozygosity follows a second, somatic, event such as mutation, chromosomal loss (with or without duplication of the remaining chromosome), or genetic recombination.

Other Cancers

It is unlikely that retinoblastoma and Wilms' tumor are the only examples of tumors caused by the mechanism of mutations in oncogenes. Neuroblastoma is a good candidate. If oncogenes are important in the origin of common tumors, it is improbable that only one genetic locus is involved. For colon cancer, for example, the nonhereditary form should sometimes result from somatic mutation at the locus responsible for polyposis coli and sometimes at the locus responsible for the Warthin type of hereditary colon cancer.

Relationship between Oncogenes and Antioncogenes in Carcinogenesis

The retroviruses and the hereditary cancers have revealed 2 different kinds of genes, oncogenes and antioncogenes, respectively. The salient feature of viral oncogenes is that they exert a positive effect. They can do this in the heterogeneous state and, when associated with viral promoter sequences (long terminal repeats), they are sufficient for transformation (3). The cellular homologues of viral oncogenes have also been implicated in carcinogenesis, through \textit{in vitro} transformation by DNA transfected from tumors (14) and through their activity in association with tumor-specific translocations (1, 46). It is thought that the translocations activate the host oncogene in abnormal ways and thus lead to cancer. This would mean that a primary abnormality of either an antioncogene or an oncogene can lead to cancer. A comparison of the 2 classes of genes is shown in Table 2. It is seen that no constitutional abnormalities of oncogenes have yet been described, so that a test of the idea that either germinal or somatic mutation of oncogenes could lead to cancer has not yet been possible.

The 2 most thoroughly studied examples of tumors implicating these different classes of cancer genes are BL and retinoblastoma. In BL, specific translocations bring the c-myc oncogene on chromosome 8 into proximity with immunoglobulin loci on chromosomes 2, 14, or 22 (46). Let us assume that these translocations activate the host oncogene in abnormal ways and thereby cause BL, whereas the loss of both normal alleles at the rb locus

<table>
<thead>
<tr>
<th>Oncoenes</th>
<th>Antioncoenes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene active</td>
<td>Gene inactive</td>
</tr>
<tr>
<td>Specific translocations</td>
<td>Deletions or invisible mutations</td>
</tr>
<tr>
<td>Translocations not hereditary</td>
<td>Mutations hereditary and nonhereditary</td>
</tr>
<tr>
<td>Dominant</td>
<td>Recessive</td>
</tr>
<tr>
<td>Tissue specificity may be broad</td>
<td>Considerable tissue specificity</td>
</tr>
<tr>
<td>Especially leukemias and lymphomas</td>
<td>Solid tumors</td>
</tr>
</tbody>
</table>

Table 2

Comparison of oncogenes and antioncogenes in human cancer

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1440

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causes retinoblastoma. Chronic myelocytic leukemia seems to involve a mechanism similar to that of BL; that of Wilms' tumor is like that of retinoblastoma. Is one or both of these mechanisms operating in other cancers? Why do these cancers seem to use only one of the 2 mechanisms? It will be important to establish whether one (or both, or neither) is operating in cancer generally. Is it possible that the translocation mechanism is peculiar to tumors of the hematopoietic system, possibly because this tissue normally uses genetic rearrangements in its ontogeny?

Enhanced activity of an oncogene, N-myc, has also been reported for retinoblastoma, a tumor caused by recessive loss of normal antioncogenes (37). Is N-myc activation another event in oncogenesis, or is it a secondary effect of antioncogene loss? Comings (12) had proposed that what are here called antioncogenes are suppressors of what are now known as oncogenes, that some cancers are caused by loss of regulatory antioncogenes, and that others (e.g., some viral cancers) are caused by primary oncogene abnormality. If this were true, one might expect to transform cells with DNA transfected from tumors caused in the latter manner but not from those caused by antioncogene loss.

At least one kind of oncogene activation requires another event; i.e., it is associated with tumor progression. This is the N-myc amplification seen in neuroblastoma. This gene is amplified 100 times or more in some cases. A recent study has shown, however, that tumors assessed pathologically as Stage I or II do not show this amplification, whereas 50% or so of tumors of Stage III or IV show amplification (9). Clearly, this amplification is not an initiating phenomenon.

Caution should be exercised in assigning a primary role to the translocations found in BL and chronic myelocytic leukemia. Fialkow has studied chronic myelocytic leukemia for evidence of its clonal origin (19). As noted earlier, the glucose-6-phosphate dehydrogenase polymorphism permits the analysis of clonality in heterozygous females. In a carefully studied case, abnormal clones of B-lymphoid cells were negative for the Philadelphia chromosome, but the leukemic myeloid cells were positive. The authors concluded that formation of the Philadelphia chromosome was a secondary event, not the primary one.

The case for a primary role for antioncogenes in oncogenesis depended upon finding the same initiating defect in both hereditary and nonhereditary forms. The case for a primary role for oncogenes (not associated with viruses) in oncogenesis would similarly be strengthened if both hereditary and nonhereditary forms of abnormality were found. Thus far, no constitutional genetic aberrations have been shown to cause primary oncogene abnormality. One family with many cases of renal carcinoma has been reported in which affected persons have a 3/4 translocation in leukocytes (11). The probability that a carrier will develop this tumor by the age of 60 years is approximately 90%. As with other hereditary cancers, a second event seems to be necessary, however. It is not known whether an oncogene is near or in the translocated segments.

We probably should not expect to find either germlinal or somatic mutations of oncogenes that would be capable of causing cancer without the intervention of another step. Cancers arising in one step must be rare, because the age-specific incidences of virtually all cancers suggest at least 2 steps in their origin. An autonomously oncogenic mutation in the germ line would almost certainly be lethal, because every target cell would be malignant. Any nonlethal constitutional abnormalities of oncogenes would necessarily be less severe and probably require another event to produce cancer. The strongly oncogenic retroviruses are evidently capable of producing cancer in one step, but natural selection must operate strongly against them; indeed, such viruses occur rarely in nature.

It has now been demonstrated that, in the absence of such strongly oncogenic mutations, 2 steps are necessary for the in vitro transformation of normal cells by DNA transfected from human tumors. One step involves "immortalization," as by the c-myc oncogene, while the other involves transformation, as by the ras oncogenes. If this situation prevails in vivo as well, then there could be 2 classes of heritable predisposition to cancer, caused by germinal mutations in these 2 classes of oncogenes. We may therefore discover that not all "hereditary cancers" are caused by heterozygosity for recessive mutations of antioncogenes.

Cancer Genes as Developmental Genes

Finally, we turn to the normal physiological role of oncogenes and antioncogenes. Clearly, their purpose is not causation of cancer. Oncogenes obviously play an important role in cell proliferation. The tissue specificities of some of them, as judged by expression in tumors, can be very broad. Antioncogenes are apparently more numerous, show much narrower tissue specificity, and may play important roles in tissue differentiation. Both probably play major roles in histogenesis. Broadly speaking, histogenesis proceeds in 3 phases: (a) commitment of some cells to differentiate in restricted fashion; (b) proliferation of committed cells to generate tissue precursors; and (c) differentiation to a terminal postmitotic state (32). Is it possible that oncogenes play a major role in the proliferation phase and antioncogenes play one in the differentiation phase of histogenesis? The idea that antioncogenes may be tissue-specific tissue differentiation genes can be developed in connection with tumors of neural origin. The neuroectoderm, including the neural crest and its derivatives, is the source of a series of tumors of great histopathological diversity, each of which occurs in both hereditary and nonhereditary form. There are considerable restrictions upon the tumors expressed by most of the germinal mutations. The genetic conditions and their tumors are summarized in Table 3.

At least 13 different dominantly heritable predispositions affect this one system, many quite specifically. If the mutations are shown to be recessive for oncogenesis, as with retinoblastoma, then the genes may be classified as antioncogenes. Their specificity with respect to the nervous system suggests that the normal alleles of the various genes are important for differentiation within the nervous system.

If the normal alleles of cancer genes do influence histogenesis, then their mutant alleles should produce not only predisposition to cancer but also developmental anomalies. In the case of an oncogene activated by a somatic mechanism such as translocation, there is no opportunity to study the role of the gene during development. As noted above, however, there is reason to believe that germ line mutations of oncogenes may exist. These might also lead to developmental abnormalities, as a result of excessive proliferation of certain tissues. Therefore, some congenital defects that manifest hyperplasia may be caused by
germinal oncogene mutations, but thus far there are no reports of such. The idea that constitutional abnormality of an oncogene can predispose to neoplasia and cause hyperplastic lesions is supported by a recent experimental report in mice (8). The injection of SV40 early-region genes into the male pronucleus has been shown to lead to tumors of the choroid plexus and, in some cases, to thymic hypertrophy. Furthermore, predisposition to the choroid plexus tumor is transmitted to subsequent generations in dominant fashion. However, mutants of normal cellular oncogenes were not used in this experiment, so the question remains what the phenotype of such a heterozygote might be.

Although mutations of oncogenes are known (e.g., hereditary retinoblastoma), they are known only in the heterozygous state, and phenotypic abnormalities are minimal or absent. If we wish to learn about their developmental physiology from developmental pathology, we must discover, or produce, homozygotes for oncogene abnormality. Yet there are no reports of such homozygous individuals. However, there may be an animal model that will be useful in this regard. Eker and Mossige (16) reported dominantly inherited predisposition to renal carcinoma in rats. The tumors appear to be identical to the human ones, which are also known to exist in a heritable form. The Norwegian investigators reported that matings of heterozygous animals yielded smaller than usual litters, with live offspring in the ratio of 2 heterozygotes to one normal homozygote (17). Evidently, the abnormal homozygotic state was lethal, prenatally. Even though tumors appear in adult rats, the gene seems to be highly important in early development.

Further afield from human tumors are the tumors observed in Drosophila. The most thoroughly studied of these tumors are the neuroblastomas that arise in larvae in cells that are precursors of the adult’s optic center (22). These highly malignant tumors are caused by recessive mutations at any of several loci. One of these loci is near the terminus of the left arm of chromosome 2. Mutation causes inappropriate proliferation of ganglion precursor cells, leading to enlargement of the brain and, ultimately, of the larva. The locus is therefore called lethal (2) giant larvae, abbreviated l(2)gl. Mutations at this locus are common and variable in their effects. The locus has now been cloned and the mutants analyzed; most are deletions6. The normal transcriptional unit is disrupted by all of the mutant alleles studied, and the expression of the normal transcript coincides with the critical periods of cell growth and differentiation in the early embryo and in third-instar larvae. There is a difference in that heterozygotes do not develop tumors in Drosophila, but this may simply be due to the small number of target cells in the larva, so the probability of a second event, somatic mutation or recombination, is too low. It would be interesting to irradiate third-instar larvae in an attempt to induce mutation or somatic recombination that would lead to homozygous cells and tumors.

At this time, we cannot say whether the Drosophila tumors are homologous to human tumors. If conservation of oncogenes is as strong as that of oncogenes, it could be revealing to use the Drosophila gene as a probe for homologous DNA in humans. Of particular interest would be any homology with DNA on the short arm of chromosome 1, where the human neuroblastosma oncogene may be located.

### Conclusions

The burden of cancer falls unequally upon a population, which in effect consists of different oncodemes. One of these oncodemes includes persons with hereditary cancer. The mutations that cause these cancers have revealed a new class of “cancer genes,” different from the oncogenes originally discovered in tumor viruses, and which have been called oncogenes.

Cancer can be produced when both normal alleles of an oncogene are mutated or lost; i.e., the carcinogenic mutations are recessive. Remaining questions concern the general contribution of this class of gene to human cancers and their mechanism of action. Their normal alleles may be important development genes, and germ line homozygosity for defective mutants may lead to developmental abnormality and prenatal death. A beginning seems to have been made toward understanding genes that are important for both teratology and oncology.

### References

Hereditary Cancer, Oncogenes, and Antioncogenes

Alfred G. Knudson, Jr.


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