Genetics of Cancer Predisposition

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Formal genetic analysis of cancer engenders several paradoxical situations: (a) although familial instances are well described, most disease occurs as sporadic isolated cases; (b) even in those cases arising subsequent to inheritance, tumors are focal in nature rather than affecting all cells of a particular tissue; (c) individuals in those families who exhibit high propensities to malignancy appear to be at risk for broad, but specific, subsets of cancer. Here we have consolidated evidence from familial, epidemiological, cytogenetic, and molecular genetic studies which support the notion that cancer is a profoundly genetic disease and which help explain the aforementioned seeming paradoxes. We review approaches and results addressing these questions afforded by molecular biological technologies and speculate on the nature of genetic lesions responsible for predisposition to cancer.

Familial Clustering

The observation of a family with a high proportion of members who developed breast or liver cancer led to the first proposal in 1866 (1) that familial aggregation of malignancy was due to some inherited aberration within the affected tissue that allowed tumor development. Similarly, studies of spontaneous mammary tumor formation among inbred strains of mice indicated that predisposition to tumorigenesis behaved in a formal sense as a Mendelian genetic trait (2) such that female mice whose recent female ancestors had developed breast tumors inured a much higher risk of developing similar tumors than females from strains with non-tumor-bearing ancestors. Several subsequent studies of human cancer have supported these indications of inheritance as a component of the etiology. Analysis of cancer patients from five pedigrees indicated that immediate family units tended towards an increased incidence of disease although the inheritance patterns of their extended families were not necessarily clear (3). These limited sets of tumor aggregation were termed “cancer fraternities” and were postulated to arise through the mating of two heterozygously susceptible but unaffected parents (3). Analyses of the pedigrees of all cancer patients admitted to the University of Michigan hospital between 1895 and 1913 indicated that 15% of 3,600 patients had some familial history of cancer (4). Accurate multigenerational data from four families were compiled and susceptibilities to several specific cancer types appeared to be transmitted as autosomal dominant Mendelian traits. The largest of these families was afflicted exclusively by cancers of the digestive tract and uterus that were predominantly of earlier onset than the general population. The term “cancer family” was coined to designate this aggregation of specific types of tumors. Subsequent studies of other cancer families suggested that many other types of tumors also have a genetically determined component in their etiology (5–9).

The proposal that neoplasia has an inherited sensitivity as part of the process has a corollary that such genetically marked individuals should often develop tumors at multiple distinct sites. In fact, as early as 1932 it was possible to document 1,259 patients with multiple primary cancers (10), a number that has been extended to well over 30,000 cases to date (11). Such tumors can be categorized as simply multifocal or multicentric or as different types of cancers in different tissues. One particularly striking example of multicentric neoplasia arose through careful gross and microscopic examination of tissues from patients treated for primary epithelial carcinoma (11); between 20 and 93% of all cases showed multiple primary epithelial cancer of shared epithelial surfaces [e.g., lips and skin; kidney, ureter, urethra, and stomach (12); colon (13); liver (11); and breast (14)]. Although these multiple tumors appeared to be independent primary events, the level of resolution of histopathological analysis was not, of course, fine enough to eliminate the possibility of multiple early metastases. Conversely, many patients who died of a first primary tumor would not have had the opportunity to develop others, thus making the incidence estimates cited above artificially low. Nonetheless, it is quite clear that the presence of one malignancy in these individuals indicates a great likelihood that a second malignancy of similar type has occurred. Thus, predisposition to these tumor types does not appear to simply reside in a single cell of a given histological type but rather in many, or even all, such cells.

A second clinical phenomenon suggesting a genetic diathesis of cancer is the occurrence of multiple primary malignancies arising in different tissues of the same patient. In adults, many tumor associations have been reported (11) to occur at greater frequency than would be expected by chance. For instance, breast cancer has been associated with cancers of the colon, larynx, bone, ovaries, salivary gland, and uterus while similar sorts of associations have been reported between other primary cancers. Although the numbers of cancers clinically associated one with the other appears large, clearly discernible groups can be ascertained; for example, leukemia appears to be associated with cancers of the lung, skin, thyroid, and melanocytes but not with those cancers associated with breast cancer. These specific associations may be defining the range of predisposition due to a single defect inherent in those individuals. Alternatively, these cancer-cancer associations may be merely an artifact of the relative thoroughness with which various cancer types are diagnosed and recorded, the age at which a second malignancy becomes apparent, differential geographic incidence of specific malignant neoplasias, or even of differential therapies eliciting subsequent second tumors.

The genetics of a familial inheritance of susceptibility to multiple primary malignancies is perhaps best documented in a clinical presentation known as “Cancer Family Syndrome” (15). These families are characterized by the occurrence of adenocarcinomas of the colon and endometrium in half the offspring of an affected parent. The risk for development of a second site primary cancer in an affected member of such a family is about 50% during the first 18 years after the first primary tumor. The predisposition to more than one tumor type in these families is inherited with a probability consistent
with a single highly penetrant autosomal dominant Mendelian trait. Similar families have been identified which appear to be segregating defective genes which predispose to specific groups of tumors, for example premenopausal breast cancer and osteosarcoma. Thus, it seems reasonable to postulate that these families are, in effect, defining the tissue specificities of the aberrant gene they are segregating.

The final example of tumor-tumor associations involves the examination of second primary tumors arising in children. Clearly, in adults some common tumor types may arise irrespective of a primary tumor and any aberration in geographic distribution which enhances the frequency of such tumor types would strengthen any apparent association. Additionally, many adult tumors are not readily treatable by current therapies and so such patients would not be expected to live long enough to develop a second tumor. Tumors of childhood, however, have recently proven more manageable. They also arise early in life so that, if successfully managed, the possibility for development of a second site tumor later in life is increased. Several such childhood tumor associations have been reported including: Wilms' tumor with hepatoblastoma, mesothelioma, adrenocortical carcinoma, and basal cell carcinoma; and Ewing's sarcoma with adenocarcinoma of the breast, rhabdomyosarcoma, and osteogenic sarcoma (16). The overall frequency of second malignant neoplasm development in survivors of first childhood tumors is about 15% (17), even though the average incidence of any of these tumors singly is about 1:10,000 live births. Thus, the specific types of second primary tumors arising in children with a first primary tumor may be indicative of the tissues which share a common predisposition to malignancy.

Two clear, and perhaps prototypic, examples of tumor clustering exist which may allow the combination of analyses of familial manifestation of multiple tumor types as well as those of second malignancies in children. The first of these examples, the association of bilateral retinoblastoma and subsequent osteosarcoma (18, 19), may represent the best in vivo evidence for cancer genes with less than absolute tissue specificity. The second example is the heritable predisposition to several tumor types classified as the multiple endocrine neoplasias, types I, II, and III (20–22).

Retinoblastoma is one of a group of childhood tumors to which predisposition can be inherited as an autosomal dominant trait (23, 24). The inherited form of the disease generally presents in both eyes whereas the sporadic form presents in only a single eye (25). Advances in surgical technique and radiotherapy have led to survival rates exceeding 90% for retinoblastoma patients. However, it has been observed (18, 19) that patients with bilateral but not unilateral retinoblastoma have a high incidence of the development of independent second-site tumors; of 693 bilateral retinoblastoma cases, 15% developed second primary tumors, about 45% of which were osteogenic sarcomas (19). Importantly, survivors of nonheritable unilateral retinoblastoma appear to have no increased risk for the development of these second site tumors. Additional support for the idea of a genetic association between these two disparate tumor types comes from a family in which two half-sibs with bilateral retinoblastoma were fathered by the same man who subsequently developed osteosarcoma himself (26).

The second example of familial clustering of multiple tumor types are the multiple endocrine neoplasias. MEN type I appears to be inherited as an autosomal dominant trait com-
"latency" period prior to its expression (30). However, analysis of tumors arising subsequent to X-irradiation suggested a latency period of approximately 9 years, a time span that could not account for the occurrence of tumors in young children, particularly those apparent prior to the first year of age. Subsequently, epidemiological analyses of stomach cancer incidence at different ages (31) led to the proposal that cancer arose not by one mutation in a single cell, but rather through single mutations in several adjacent cells. Subcritical masses of these mutant cells were postulated to remain dormant until sufficient adjacent cells had undergone mutation to achieve a critical mass which then cross-fed and subsequently developed into a tumor.

A mathematical treatment of these data suggested that the number of cancer cells present in any tissue would be proportional to the age of the tissue raised to the power of one less than the number of cells necessary to form a tumor island. Supportive evidence for this formulation was provided by statistical studies of the incidence of deaths due to stomach cancer in American females (31). This analysis appeared particularly appropriate due to the short interval between diagnosis of stomach cancer and death, which made the incidence of cancer deaths an accurate reflection of cancer onset. A graphical comparison of the logarithm of the age at diagnosis and the corresponding logarithm of the death rates indicated a linear relationship with a slope (which is equal to one less than the number of cells critical to form an island) of 5.7, indicating that the number of cells necessary to form a stomach cancer island was between six and seven.

There were, however, several problems that this hypothesis could not explain. First was the problem of early onset disease, such as childhood tumors, which showed a corresponding relationship requiring far fewer than the six or seven mutant cells necessary for an adult stomach cancer island. It was suggested that the rapid division of tissues during organismal development might lead to an enhanced susceptibility to mutation and that the number of cancer cells necessary to form an island might also be fewer in the developing fetus (31). Secondly, this hypothesis could not explain the incidence of carcinogen-induced cancers in experimental animals since one would predict that this cancer incidence should be proportional to the concentration of the carcinogen, raised to the fifth or sixth power. However, experimentally, the proportionality of tumor incidence and concentration of carcinogen vary in arithmetic rather than exponential order (32).

Alternative hypotheses to explain the age-dependent increase in cancer incidence were put forth which proposed that a single somatic mutation was insufficient to elicit a tumor and that tumors arose from a single cell which underwent multiple somatic mutations (29, 33). A comparison of the incidence of cancer deaths and age of diagnosis in 25- to 74-year-old males in the United States, Great Britain, France, and Norway produced the best fit with a model requiring six independent mutations to produce a tumor, assuming that the rate for each mutation was constant and equal throughout life (30). Similar analyses of Welsh cancer deaths corroborated this quantification (34–36). This hypothesis still did not explain the high incidence of childhood cancers, a discrepancy noted by Nordling (29) in his proposal that childhood cancers arise from altered fetal cells which are inherently more susceptible to mutation. The hypothesis was, however, in agreement with the evidence from experimental carcinogenesis in its prediction that the incidence of tumor formation would be directly proportional to the concentration of carcinogen (32).

This notion of cancer being elicited by multiple mutations in a single cell gained support through the examination of death rates for men and women of differing age groups from cancers of the esophagus, stomach, colon, rectum, pancreas, lungs, bladder, prostate, breast, ovary, cervix, and corpus uteri, which showed that the first four types clearly increased in incidence with age (32). Formally, the data were consistent with approximately six to seven intracellular events being required for tumor formation. However, the incidence of cancers of lung, bladder, and prostate in men and lung, breast, ovary, cervix, and corpus uteri in women showed a significant departure from predicted mortality that was not consistent with this number of events. It was suggested that this departure was due to the presence of factors which increased the likelihood of cancer and thereby biased the incidence rates. For example, the incidence of lung tumors in the older age groups was lower than in the middle age groups. Since cigarette smoking had been popular for less than 50 years, members of the older age groups were less likely to have indulged in this practice than members of the middle age groups. Thus, a reasonable explanation for the departure from expected incidence ratios was that the middle age group had been exposed to the mutating effect of cigarette smoke for a larger proportion of their lives than had the older age groups.

A refinement of the "one cell, multiple hits" theory proposed that one effect of a first mutation might be to increase the growth rate of the mutated cell (37). One prediction of this hypothesis is that as the tissue proliferates, the proportion of cells in the tissue which carry the first mutation will increase, thereby enlarging the population of cells at risk for a subsequent mutation. It was reasoned that if the probability of undergoing a first mutation was \( p_1 \) per unit time, if each cell that had undergone the first mutation gave rise to a clonal population of cells of \( e^{kt} \) at time \( t \) after the initial mutation where \( k \) is a constant, and if the probability of a second mutation in one of those cells in which a first mutation has already taken place was \( p_2 e^{kt} \) per unit time \( t \), then the incidence of a cell undergoing both mutations at any given time could be mathematically predicted by the expression

\[
I = N p_1 [1 - \exp((-p_2/k)(\exp(kt - 1)))]
\]

where \( N \) is the number of cells in the tissue at risk for the first mutation (38). As \( t \) becomes larger, the expression asymptotically approaches \( I = Np_1 \).

The aforementioned multiple-step carcinogenesis hypotheses of Muller (33) and Nordling (29) required only that the first mutation be time independent, and thus their predictions of the incidence of cancer simplified mathematically to this same asymptotic approach. This suggested that it was possible to require as few as two mutations to fit the incidence curves, if the first mutation led to growth of the mutated cells. In this way, it was possible to account for the perturbations in the previously proposed mathematical models. This formulation could also account for the latency period between exposure to a mutagenic agent and the appearance of the tumor by considering that a tumorigenic agent induces a first mutation leading to an increased growth rate and a consequently larger population of vulnerable cells, any one of which could produce neoplastic growth upon a second mutational event. It could also account for earlier findings that tumors in murine mammary glands were surrounded by "hyperplastic nodules" of rapidly growing cells (2) and for the clinical observation of hyperplasia in premalignant tissues such as intestinal polyps in humans (39). The model would predict that the nodules were composed of cells which had received the first (growth-stimulating) mu-
tation but had not undergone the second (tumor-forming) event.

The first model (40) to explain the incidence of childhood malignancies arose from a joint consideration of suggestions that tumors arose subsequent to two events (38) and that a premalignant mutation might be inherited but not expressed until the cell was exposed to an unfavorable environment (41). This model proposed that latency could be due to a prezygotic mutation, which was not itself oncogenic but which instead predisposed the cells to a second somatic alteration under conditions of stress which then gave rise to neoplastic growth (40). This idea did not require that the initial somatic mutation lead to favored growth of the cell but instead postulated that the initial mutation was present in all cells of the body by virtue of germline inheritance. Since the likelihood of a second event occurring in a previously mutated cell over a long period of time is essentially independent of the frequency of the second event (38), those individuals who inherited the first mutation would inevitably suffer the second alteration in at least one target cell and, consequently, develop cancer. The application of this hypothesis to childhood leukemia showed that early onset forms of leukemia occurred with kinetics consistent with a single event, suggesting that the first mutation occurred prezygotically, and led to the suggestion that childhood leukemia could be grouped into those that had a prezygotic mutation and thus required further somatic alterations and those that did not inherit such a mutation but rather required cumulative postzygotic events for oncogenesis. This hypothesis was extended to leukemias of known familial or environmental origin (42) and the data indicated that inherited mutations could substitute for somatic mutations in all cases where the etiology of a tumor required multiple postzygotic events. Furthermore, one stated constraint on this model was the requirement for one or more somatic mutations together with other epigenetic events for onset of the disease; otherwise such a mutational combination would probably be prenatally lethal.

Analysis of solid tumors of childhood has been most fruitful and has led to specific hypotheses about the number, nature, and relationship between pre- and postzygotic genomic alterations in the process of human cancer (43–47). The data have indicated the necessity of as few as two mutations in the same cell for the initiation of a tumor; that sporadic and heritable forms of disease were likely to arise by alterations of the same genetic loci; and that the kinetics of cell renewal in the stages of tumor formation plays a role in determining the frequency and age of onset of various tumor types (43). These proposals distill all of the common themes of the aforementioned approaches and, furthermore, overcome most of their weaknesses. The initial comparison of bilateral and unilateral retinoblastoma cases (44) indicated that the two forms of disease differed significantly in age of onset, number of tumor clones, and heritability. When the ages of diagnosis for the two forms of the diseases were graphically displayed, bilateral cases appeared to show kinetics requiring a single event, while unilateral cases appeared to require two events in their etiology. It seemed that these differences were unlikely to be due to mutations at different loci, since unilaterally affected parents could sometimes give rise to bilaterally affected progeny. It was proposed that both forms of retinoblastoma occur by sequential mutations, each of which arises at a rate of \(2 \times 10^{-7}\) per cell per year; the essential difference between the two forms of the disease was hypothesized to be the somatic or germlinal occurrence of the first mutation. This formulation was entirely consistent with the aforementioned models (38, 40), as well as the clinical evidence, and furthermore made specific predictions about the mechanism of tumorigenesis in this disease. Similar results were obtained with a variety of other solid tumors of childhood, particularly Wilms' tumor, neuroblastoma, and pheochromocytoma (45, 46). Importantly, the general model (47) could be extended to adult cancers such as breast cancer (48) and medullary thyroid carcinoma (49) with only minor modifications.

Thus, the analyses described in the preceding section have converged to provide a mechanistic model which specifies the involvement of prezygotic and postzygotic lesions in the genesis of human cancer. They do not address, except in a speculative way, the biological nature of these lesions.

Somatic Cell Genetics of Tumorigenesis

A great deal of important information concerning the genetic behavior of cellular transformation has been obtained through ascertained of the tumorigenicity of somatic fusions between normal and tumor cells in vitro (50–54). Early experiments using interspecies hybrids between mouse and hamster cells provided conflicting data indicating that fusion progeny could have either normal or tumorigenic phenotypes (55). Karyotypic analyses suggested that a major difficulty with this line of experimentation was chromosomal instability of the hybrid cell such that genetic material of the normal parent in such crosses was rapidly lost upon passage in culture (55).

More informative and reproducible results were obtained when both normal and tumorigenic parents were derived from the same species since these crosses produced relatively stable progeny. A particularly interesting set of experiments of this type involved the fusion of normal human fibroblasts with various human tumor cell lines (56, 57). In these studies, the progeny of normal × normal parents were nontumorigenic while those of the fusion of similar tumor type were tumorigenic. However, in each instance, progeny of normal × tumorigenic parents were completely nontumorigenic. Furthermore, rare revertants of this latter class were isolated (58) which had become tumorigenic. In these revertants, particular pairs of chromosomes had been lost. These general results have been complemented by an elegant series of studies using Chinese hamster embryo fibroblast cell lines (59). Two possible interpretations of these data are that normal cells express gene products which function in the suppression of tumorigenicity or that the normal cells were providing a function that had been lost by the tumor cells which was required for maintenance of the normal phenotype. The first, more activist, interpretation is consistent with a model proposed by Comings (60) in which cells carry genes which encode products which act as repressors of other tumor-causing genes. The second interpretation is consistent not only with this view but also with the broader notion that tumorigenicity arises subsequent to the total loss of some required cellular function, i.e., by the development of homozygosity for recessive mutant alleles at some unspecified loci. The major difficulties with this line of experimentation are the lack of objective criteria for transformation and tumorigenicity and the extension of information obtained in cultured cell lines to the human neoplastic process. The definition of suppression of tumorigenicity is necessarily separated from certain aspects of transformation such as anchorage independence, serum requirements, and contact inhibition. These in vitro criteria for transformation have been suggested (61, 62) to be unnecessary for in vivo tumorigenesis. Similarly, when individual chromosomes are introduced into tumor cells, the resulting
phenotype does not resemble normal cells but is, rather, different from either tumor or normal parental cells (63, 64).

**Retinoblastoma, a Prototypic Neoplasm**

**Cytogenetics.** Evidence supporting the notion of a genetic basis of human cancer has been provided through direct cytogenetic examination of the chromosome numbers and their arrangement in tumor cells. As the resolution of these analyses has increased and they have been extended to solid tumors, various chromosomal abnormalities have been described which appear to be consistent within a tumor type (65). These abnormalities most frequently take the form of deletion of particular portions of the genome or chromosomal interchanges between specific regions of two chromosomes.

Although these aberrations are best documented in lymphoid tumors, several instances have been reproducibly described in solid tumors as well (66, 67). Perhaps one of the clearest examples is the deletion of the interstitial band q14 of chromosome 13 in retinoblastoma. Beginning in the early 1960s and 1970s, cases have been reported (68–70) with karyotypes of constitutional cells showing deletions of the long arm of chromosome 13. Although these deletions were discernible in only about 5% of retinoblastoma patients and the extent of deletion varied considerably, the smallest overlapping region of aberration was the band 13q14. Furthermore, analysis of tumor cells from patients with normal constitutional karyotypes indicated that about 5% of cases had tumor-specific deletions of chromosome 13, each of which included the q14 band (71–74). Although this lineage, the significance of these observations was unclear for several reasons. First, only a small proportion of cases carried visible constitutional or somatic deletions. Second, in those cases in which a 13q14 deletion was apparent in all cells, not all retinal cells were neoplastic. Clearly these alterations were insufficient in and of themselves to elicit disease. Finally, tumor (but not constitutional) karyotypes have shown several other chromosomal aberrations in addition to deletion of 13q14 such as triplication of 1q23-1qter and an isochromosome 6p (74).

However, these deletion cases have been useful in defining the region of the genome likely to contain a locus involved in the genesis of retinoblastoma not only by inference from the physical map but also by genetic linkage mapping. Activity levels of esterase D, an enzyme of unknown physiological function, were shown to be reduced in patients with deletions of 13q14 as compared to their karyotypically normal family members (75). This enzymatic activity also displayed isozymic forms in the human population so that the cosegregation of a specific allelic variant with retinoblastoma could be determined in families (23). In fact, no meiotic recombination events could be detected, strongly suggesting that a genetic locus influencing tumor development resided in the 13q14 region. In this family, both affected and unaffected members were described that carried an interstitial 13q14 deletion. However, several of the unaffected members also carried a chromosome 3 which included an interstitial translocation of this 13q14 region. Thus, all family members who were constitutionally monosomic for the region developed disease whereas all who were either disomic or trisomic were spared. These data strongly suggested that the deletion event predisposed to retinoblastoma, that this region did indeed contain a locus or loci involved with tumor development, and that the initial mutation was unlikely to be acting in a dominant genetic fashion.

The limitations of these sorts of analyses in describing unifying genetic mechanisms are obvious. The first is the relatively small proportion of diseased individuals who display cytogenetically visible chromosomal aberrations. This could be due to the restricted precision of the analysis but could also be due to random rearrangements in the process of tumorogenesis. Second, the combination of low level analytical resolution with allelic data that relies on enzyme expression can be misleading as well. One particularly appropriate example of this was a reported case of a child with bilateral retinoblastoma (76). Analysis of esterase D activity in blood and fibroblasts from this patient indicated about one-half the level of that in the general population. However, cytogenetic analysis of these same cells revealed no observed deletion of chromosome 13. Tumor cells from this child showed an absence of esterase D activity and a single distinguishable chromosome 13. On this basis, it was concluded that the chromosome 13 in the tumor cells carried a submicroscopic deletion of 13q14 that entirely eliminated both the esterase D (ESD) and retinoblastoma (RBI) loci. This proposal would, of course, be consistent with the idea that the initial deletion mutation in this child was recessive at the cellular level and was unmasked by chromosome loss of the homologue carrying the wild type RBI allele. However, it is also possible (and, in light of recent molecular data discussed below, likely) that these results could be explained by simultaneous intralocus mutations in the ESD and RBI loci, in which case they shed little light on the genetic mechanisms involved in tumorogenesis. A final example of how this combined determination of esterase D activity or isozymic forms with cytogenetics can be misleading is illustrated in a study of six cases of retinoblastoma (77). Each of these patients was constitutionally heterozygous at the ESD locus (i.e., displayed both electrophoretic variants) and none had any obvious deletion of their chromosome 13. The corresponding tumor cells from four of these six patients showed only one isoform of esterase D and no deletion in either chromosome 13 homologue. These data are consistent with mechanisms involving either somatic mutation/deletion/inactivation of one allele of the ESD locus, mosaicism for a 13q14 deletion and clonal expansion of a cell containing such an aberration, or somatic loss of one chromosome 13 homologue with duplication of the other resulting in two chromosomes 13 identical at every locus. The second possibility was discounted by examining several individual fibroblast clones from each patient and establishing that each expressed both ESD isozymes. The third possibility was discounted on the basis of cytogenetic examination of fibroblast and tumor cells from one patient, both of which were heterozygous for a chromosome 13 fluorescent-staining satellite sequence. It was proposed therefore that retinoblastoma arose in these patients through a spread of somatic inactivation from the ESD to the RBI locus in a manner analogous to X-chromosome inactivation in females. However, molecular genetic analyses described below indicate that the third mechanism above, or variations thereof, was inextant in these and other cases.

**Molecular Genetics.** The foregoing discussion raises several salient points regarding mechanisms of development of human neoplasia. It seems likely that cancer is a disease with a genetic basis; specific chromosomal aberrations appear to be a route to its expression. Single genetic events, even in individuals prone to cancer development by virtue of inheritance, seem to be insufficient to elicit disease; epidemiological and theoretical considerations suggest that, in some cancer types, as few as two genetic alterations may be required. The behavior of the transformed phenotype in vitro indicates that tumorigenicity may...
arise through the elimination of tumor suppressor genes and analysis of the pathology of individuals with familial cancer suggests that those cells with only the initial prezygotic lesion have no heterozygote phenotype.

A model was proposed which could account for each of these points and which predicted several specific chromosomal mechanisms capable of unmasking predisposing mutations. Each of these chromosomal mechanisms would be expected to be mitotic and each would have as its net effect the somatic attainment of homozygosity for a mutant allele at a relevant locus. These chromosomal mechanisms included: mitotic nondisjunction with loss of the wild type chromosome, which would result in hemizygosity at all loci on the chromosome; mitotic nondisjunction with duplication of the mutant chromosome, which would result in homozygosity at all loci on the chromosome; mitotic recombination between the chromosomal homologues with a breakpoint between the tumor locus and the centromere, which would result in homozygosity at loci in the proximal region of the chromosome and homozygosity throughout the rest of the chromosome, including the tumor locus; or regional second events such as gene conversion, deletion, or point mutation. Sporadic disease could also arise through the appearance of homozygosity at the tumor locus, the difference being, in this instance, two somatic events as compared to one germinal and one somatic event in heritable cases (44).

The method that was used to examine these hypotheses (78) relies on the variability of DNA sequences among humans which results in inherited differences in restriction endonuclease recognition sites. In this approach, segments of the human genome are isolated in recombinant DNA form and the loci homologous to these probe segments are tested for their encompassing restriction endonuclease recognition sequences which may vary among unrelated individuals. Two types of such variation have been defined. The first, and most abundant, results from simple base pair changes within the recognition site sequence of a particular restriction endonuclease and yields alleles of longer (when the effect of the mutation is loss of a site) or shorter (when the effect of the mutation is the gain of a site) length. The second type results from the insertion or deletion of varying numbers of blocks of like DNA sequence into or out of the genomic locus. Practically, the net result is the observation of two alleles at the locus encompassing a site change (presence or absence of the site) in the first case or numerous alleles at a locus subject to insertion or deletion of larger segments of DNA. In either case, however, any given individual will reveal only two alleles at the locus, one from the paternally derived and one from the maternally derived homologue. In all cases examined to date these types of markers have been shown to behave in family studies as would be predicted for simple Mendelian codominant alleles. It is notable that these RFLP would be apparent regardless of the tissue origin of the DNA sample and also that this type of analysis does not rely on the expression or determination of different protein isoforms. It is also noteworthy that a RFLP locus is defined by the probe and the relevant restriction endonuclease; consequently the number of such loci is virtually limitless. Recombinant DNA segments were isolated from human chromosome 13, as well as other chromosomes, and used as probes to examine loci scattered along the chromosome in order to detect changes in the somatic genotypes of tumors, as compared to the germline genotypes of the individuals harboring the tumors (78).

A schematic illustration of the model and its specific experimental predictions is shown in Fig. 1. In this model the hereditary form of the disease (Fig. 1, left) arises subsequent to the inheritance of a germlinal mutation at the RBI locus from the mother and is, therefore, present in all somatic and germ cells of the daughter. The sporadic form (Fig. 1, right) arises subsequent to a somatic mutation at the RBI locus in a single retinal cell. Any subsequent event that results in hemizygosity or homozygosity for the mutant allele (i.e., mutant at the RBI locus on both chromosome 13 homologues) will result in a tumor clone. Furthermore, these chromosomes are differentially marked by restriction fragment length polymorphisms at the loci designated A and B that flank the RBI locus. Several chromosomal mechanisms which could accomplish the somatic attainment of homozygosity for a mutant allele at the RBI locus are illustrated at the bottom of the figure.

If the extant mechanism was mitotic nondisjunction with loss of the wild type chromosome, the net result would be hemizygosity for all loci on chromosome 13; a comparison of the normal and tumor DNA at the two RFLP loci would show a coordinate loss of constitutional heterozygosity. The intensity of hybridization of the remaining allele at each locus in the tumor DNA would be equal to the intensity of hybridization of either allele in the normal DNA. If the mechanism involved mitotic nondisjunction and loss of the wild type chromosome followed by duplication of the mutant chromosome, analysis would yield slightly different results. The prediction would be coordinate loss of constitutional heterozygosity at both loci with the intensity of hybridization of the remaining allele at each locus being twice that of either allele in the normal DNA. If the mechanism of loss of the wild-type allele was mitotic recombination with a breakpoint between the proximal RFLP locus and RBI, then genotypic determinations in normal and tumor DNA would be expected to show a loss of constitutional heterozygosity at the distal RFLP locus. The proximal RFLP locus, however, would be expected to remain heterozygous. The intensity of hybridization of the remaining allele at the distal locus would be twice the intensity of either allele in the normal DNA, suggesting that there was not simply a deletion of the proximal region of the chromosome. The identified RFLP loci are physically distant from the RBI locus, so other proposed mechanisms leading to loss of the wild-type allele, such as gene conversion, point mutation, and small deletion which result only in alteration at the RBI locus, would not be detected by this analysis.

Thirty-three cases of retinoblastoma have been analyzed in this manner, and the relative distribution of chromosomal events accomplishing loss of constitutional heterozygosity at the RBI locus in these tumors is shown in Fig. 1, bottom. The data indicated that 24 of these cases arose through mitotic nondisjunction or recombination. Interestingly, several of the tumors which had been reported to undergo somatic inactivation of the region encompassing the ESD and RBI loci (77), when examined at this level of resolution, had instead undergone aberrancies in mitosis. For example, the case which had a distinguishing satellite region (77) had, in fact, arisen through mitotic recombination (78). Furthermore, these chromosome rearrangements and losses appeared to be specific to chromosome 13 since tested loci on other chromosomes retained heterozygosity in the tumors. Corroboration of these inferences has been obtained by examining cases of inheritable retinoblastoma (79) and showing that the chromosome 13 homologue retained in the tumor was derived from the affected parent as would be predicted.

These observations are consistent with all of the lines of investigation discussed above and suggest that chromosomal
mechanisms are involved in multistage carcinogenesis and that their effects can be to unmask recessive mutations at specific loci. It is noteworthy that, although the unmasking of predisposing mutations at the RB1 locus occurs in mechanistically similar ways in sporadic and heritable retinoblastoma cases, only the latter carry the initial mutation in each of their cells. Heritable cases also seem to be at increased risk for the development of second primary tumors, particularly osteogenic sarcoma (18, 19, 26). A testable corollary of the model outlined above is that this high propensity is not merely fortuitous but is genetically determined by the predisposing RB1 mutation. This notion of a pathogenetic causality in the clinical association between these two rare tumor types was tested by determining the constitutional and osteosarcoma genotypes at RFLP loci on chromosome 13. The data indicated that osteosarcomas arising in retinoblastoma patients had become specifically homozygous around the chromosomal region carrying the RB1 locus (80). Furthermore, these same chromosomal mechanisms eliciting losses of constitutional heterozygosity were observed in sporadic osteosarcomas suggesting a genetic similarity in
pathogenetic causality. These findings are of obvious relevance to the interpretation of human mixed cancer families inasmuch as they suggest differential expression of a single pleiotropic mutation in the etiology of clinically associated cancer of different histological types.

This model of chromosomal mechanisms unmasking recessive predisposing mutations has been tested in a variety of tumor types and in each case has been confirmed. The loci for the associated neoplasms hepatoblastoma, rhabdomyosarcoma, and Wilms' tumor have been mapped to chromosome 11 (81-86) as have those for adenocortical carcinomas and transitional cell bladder carcinoma (87). The loci for small cell lung carcinoma (88) and renal cell carcinoma have been mapped to chromosome 3p. The low differentiation form of premenopausal ductal breast carcinoma appears to be raised by similar mechanisms on chromosome 13 (89) whereas meningioma and acoustic neuroma involve chromosome 22 (90, 91).

The importance of these mutations in the process of human tumorigenesis is underscored by three points. First, the examination of any given tumor type with the approaches described above indicates that the majority of cases of each have undergone similar chromosomal rearrangements. Secondly, several groupings of clinically associated tumors have been found to arise through similar shared mechanisms; these have not been observed in nonassociated cancers. Finally, the specificity of these chromosomal events is notable, particularly in light of the plethora of chromosomal rearrangements accompanying the neoplastic process (66, 67). Published data obtained from retinoblastoma (78, 79, 92), osteosarcoma (80, 93), Wilms' tumor (81-84), hepatoblastoma (85), and acoustic neuroma (90, 91) indicated no nonspecific loss of constitutional heterozygosity. In fact, in unpublished studies from this laboratory, over 300 cases of 27 different types of predominantly pediatric or adolescent tumors have been genotyped at some 45 loci and in only 3 instances has apparently random loss of heterozygosity been observed. However, it has been reported (94) that such random losses do occur in cell lines derived from metastatic adult melanoma, and some limited nonoverlapping sets of chromosomes do appear to be lost in breast carcinoma (89) and meningioma (90). The significance of these latter findings is at present unclear; in fact other studies of primary uveal melanomas have not shown random chromosomal losses (95).

Molecular Biology: Nature of the Gene Products of These Loci

Several points regarding recessive genes predisposing to tumorigenesis must be considered in any strategy concerned with the elucidation of their structure and function. First, what is the biological role of these genes? One possibility is that the products of these loci act as suppressors of other transforming genes (60). This idea would predict a requirement for the continuous presence of the gene product to maintain such suppression and that the chromosomal events described above would suffice to alter or eliminate the suppressing function. It might also be reasonable to postulate that the activity of such suppressors would be required in any tissue which can be transformed by deregulation of their targets.

Several groups have examined the possibility that the retinoblastoma gene product is responsible for controlling the expression of N-myc, a cellular gene that was originally found to be amplified in human neuroblastomas (96). The mouse homologue has been shown to be under stage-specific regulation during normal murine development and embryogenesis (97). One report suggested that N-myc was deregulated and amplified in retinoblastomas as compared to normal fetal or adult retina (98), data which would be consistent with the product of the RB1 locus suppressing N-myc expression in normal tissue. However, subsequent analyses (99) indicated that the N-myc gene product is normally expressed at high levels in fetal retina to a certain stage and not in adult retina; in retinoblastoma tumors, the level of N-myc expression is comparable to early fetal retina. Furthermore, Squire et al. (99) and we have been unable to consistently detect N-myc expression in osteosarcomas, a result which is at odds with a suppressing role of the RB1 locus. The most reasonable interpretation of these results is that expression of N-myc in retinoblastomas is merely a reflection of the embryonal nature of these tumors. It is, of course, possible that the retinoblastoma gene product functions as a suppressor gene but that its target gene(s) has yet to be identified.

An alternative hypothesis is that the recessive oncogene product plays a direct role in organismal development as a differentiation, cell recognition, or growth arrest factor the absence of which causes the omission of some signal in the normal developmental program resulting in the continuation of growth in an abnormal fashion. Recently, the isolations of two genes which fulfill many of the theoretical criteria for a recessive oncogene have been reported (100-102). The first of these is the 1(2)g1 gene of Drosophila melanogaster. Flies carrying homozygous recessive mutations of this locus appear to arrest developmentally; they do not pupate but continue larval growth (100). Additionally, in the resultant giant larvae there is autonomous proliferation of neuroectodermal neuroblasts that give rise to transplantable tumor-like growths resembling neuroblastomas. It has been convincingly shown that the excessive larval growth and the neuroblastoma-like tumors are the direct result of the absence of the 1(2)g1 gene product because reintroduction of a single copy of 1(2)g1 by P-element transformation into defective flies results in both normal development and suppression of the tumor formation (100). The molecular function of this gene remains unknown but is an area of intense investigation.

Recently, two groups (101, 102) have reported the isolation of a gene from human chromosome 13q14 that is expressed as a 4.7-kilobase mRNA. The genomic locus was shown to be altered from the germline configuration in 4 of 45 retinoblastoma tumors and cell lines as judged by aberrancies upon Southern analysis. Furthermore, many retinoblastoma and osteosarcoma cell lines express either no mRNA or altered size transcripts. These results are, of course, consistent with the genetic predictions of homozygous loss of function in tumors. Furthermore, this gene appears to be expressed in all normal tissues and all other tumor types examined and at levels suggesting constitutive, perhaps housekeeping, function. DNA sequence analysis reveals little homology with any other known gene and structural features that are undistinguished. Certainly, the determination of the biological function of this gene is likely to shed a great deal of light on the molecular mechanism of human carcinogenesis.

Significance

The discovery of genes with heritable recessive alleles that predispose to cancer has immediate medical relevance. Most notable is increased precision in the accuracy of premorbid prediction of tumor development in cases of familial retinoblastoma (103). Such predictions of predisposition to disease...
depend on the ability to detect the chromosomal origin of the predisposing mutation (79). Identification of the chromosome carrying the mutation in these linkage-based approaches is independent of the determination of the primary defect and depends instead upon flanking RFLP loci which are in informative allelic combinations in the parents and either the parental tumor or the first affected child (103). Routine prenatal or premorbid screening of children at risk for retinoblastoma as an adjunct to the earlier application of powerful therapeutic approaches (104) should dramatically reduce morbidity and mortality in these cases. Furthermore, these methodologies should serve as the conceptual basis for their application to other, more common, cancers.

At a more fundamental level, the discovery of loci with recessive alleles that predispose to cancer may provide an interface between tumor biology and developmental biology. The normal biological role of these genes and their role in organ/sal development remains to be uncovered. It is conceivable that the developmental stage at which an embryonal tumor initiates may be due to the loss of function of these genes thereby determining the histogenetic basis for tumor structure and behavior. Whether the groups of clinically and pathogenetically associated tumors described by these molecular genetic analyses arise through the inactivation of single loci or through multilocus aberration requires the physical and genetic mapping of these heretofore unsuspected genomic regions. In either case, the data which are being rapidly generated promise to provide fundamental information of relevance to a wide range of biological and medical specialties. Methodologies which must be developed to analyze the function of these loci and their mutant alleles promise to be inventive, novel, and applicable to other problems in cancer and developmental biology. Manipulations of these genes in vivo and in vitro should provide data with which to interpret their role in the initiation of human tumors, may allow for the experimental induction (and perhaps reversal) of tumors, and may ultimately allow the distinction between initiating and progression events (105) in the complex pathway whereby a normal cell becomes malignant.

References


Genetics of Cancer Predisposition
Marc F. Hansen and Webster K. Cavenee


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