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A Genetic Model for Colorectal Tumorigenesis

Review

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Tumorigenesis has long been thought to be a multistep process (Foulds, 1958); however, only recently has it become possible to identify the molecular events that underlie the initiation and progression of human tumors (Weinberg, 1989; Bishop, 1987). Colorectal tumors provide an excellent system in which to search for and study the genetic alterations involved in the development of a common human neoplasm. Abundant clinical and histopathological data suggest that most, if not all, malignant colorectal tumors (carcinomas) arise from preexisting benign tumors (adenomas) (Sugarbaker et al., 1985). Tumors of various stages of development, from very small adenomas to large metastatic carcinomas, can be obtained for study, unlike the situation in most other common human tumor types. Furthermore, both hereditary and environmental factors contribute to the development of colorectal neoplasia, allowing for the study of both inherited and somatic genetic alterations.

In this review we present a model for the genetic basis of colorectal neoplasia that includes the following salient features. First, colorectal tumors appear to arise as a result of the mutational activation of oncogenes coupled with the mutational inactivation of tumor suppressor genes; the latter changes predominate. Second, mutations in at least four to five genes are required for the formation of a malignant tumor. Fewer changes suffice for benign tumorigenesis. Third, although the genetic alterations often occur according to a preferred sequence, the total accumulation of changes, rather than their order with respect to one another, is responsible for determining the tumor's biologic properties. Fourth, in some cases, mutant tumor suppressor genes appear to exert a phenotypic effect even when present in the heterozygous state; thus, some tumor suppressor genes may not be "recessive" at the cellular level. The general features of this model may be applicable to other common epithelial neoplasms, in which tumors of varying stage are more difficult to study.

The Clonal Nature of Colonic Neoplasia

Various hypotheses for the development of cancer have been proposed. Mutational theories predict that neoplasms will have a monoclonal composition, whereas aberrant differentiation processes or field effects might be predicted to give rise to neoplasms with a polyclonal composition. Although the earliest events in human colorectal tumor formation are not yet defined, study of the clonal composition of human colorectal tumors has demonstrated that all tumors examined, including very small adenomas, have a monoclonal composition (Fearon et al., 1987). In contrast, normal colonic epithelium is polyclonal, having arisen from numerous stem cells. Thus, adenomas arise from a single pocket of epithelial stem cells (Ponder and Wilkinson, 1986), consistent with the idea that one or a small number of cells from within this pocket initiate the process of neoplasia by clonal expansion.

In an attempt to gain an understanding of the molecular basis for this clonal expansion, investigators have sought to identify somatic alterations present at various stages of colorectal tumor formation. Of particular interest are alterations present in all or virtually all of the neoplastic cells studied. The presence of such an alteration suggests that the genetic alteration itself provided the cell with a growth advantage, allowing it to outgrow other neoplastic cells within the tumor to become the predominant cell type constituting the neoplasm (clonal expansion). Alternatively, the genetic alteration, even if present in every neoplastic cell of an individual tumor, could have arisen coincidentally with another change that actually provided the selective growth advantage. When the same genetic alteration is observed in many different tumors, the former explanation is more likely.

Genetic Alterations in Oncogenes

One important type of somatic alteration identified in colorectal tumors is *ras* gene mutation. When transfected into appropriate recipient cells, mutated *ras* genes confer neoplastic properties (reviewed in Barbacid, 1987; Weinberg, 1989). Approximately 50% of colorectal carcinomas (Bos et al., 1987; Forrester et al., 1987) and a similar percentage of adenomas greater than 1 cm in size have been found to have *ras* gene mutations (Vogelstein et al., 1988). In contrast, such mutations have been identified in fewer than 10% of adenomas less than 1 cm in size (Figure 1), regardless of whether the adenomas arose sporadically or in patients with an inherited predisposition for their formation (Vogelstein et al., 1988; Farr et al., 1988). *ras* gene mutations may be the initiating event in a subset of colorectal tumors, and adenomas with *ras* gene mutations may be more likely to progress than adenomas without *ras* gene mutations. Alternatively, *ras* gene mutations may not be the initiating event in most tumors, usually occurring only in one cell of a preexisting adenoma. Such mutations would then be responsible for the conversion of a small adenoma to a larger and more dysplastic one, through clonal expansion of the cell with the mutation.

In addition to somatic activation by point mutation, oncogenes may be activated by amplification or rearrangement. Although the existence of amplified genes in a significant percentage of colorectal tumors was suggested by the presence of double-minute chromosomes or homogeneously stained regions in karyotypic analyses (Reichmann et al., 1981), few cases of specific gene amplification have been reported. These cases include examples of *neu*, c-*myc*, or c-*myb* amplification in primary colorectal tumors or their derived cell lines (D'Emilia et al., 1989; Alitalo et al., 1983, 1984; Finley et al., 1989). In addition, oncogene rearrangements, other than a single case of

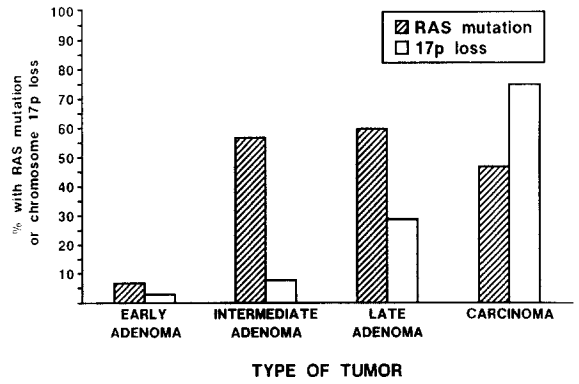

Figure 1. Frequency of *ras* Gene Mutations and Chromosome 17p Deletions in Colorectal Tumors

Early-stage adenomas were defined as adenomas that were 1.0 cm in size or less. Intermediate stage adenomas were greater than 1.0 cm in size and did not contain foci of carcinoma. Late-stage adenomas were greater than 1.0 cm in size and contained foci of carcinoma (the adenomatous elements were separated from the nonadenomatous elements before analysis). DNA was prepared from all specimens by a cyrostat sectioning technique to enrich for areas of tumor that comprised 70% or greater neoplastic cells. *ras* gene mutations were identified by oligonucleotide hybridization to DNA samples in which *ras* gene–specific sequences had been amplified by the polymerase chain reaction. Chromosome 17p losses were detected by study of DNA from normal colorectal mucosa and tumor tissue with probes detecting DNA polymorphisms on chromosome 17 (Vogelstein et al., 1988).

rearrangement of the *trk* oncogene (Martin-Zanca et al., 1986), have not been observed in colorectal tumors. Thus, the evidence to date does not support a major role for amplification or rearrangement of oncogenes in the genesis of colorectal neoplasms.

Allelic Losses and Tumor Suppressor Genes

A loss of specific chromosomal regions occurs frequently in colorectal neoplasia. Usually the losses involve only one of the two parental chromosomes present in normal cells. These allelic losses have been interpreted as evidence that the regions affected contain tumor suppressor genes, whose products normally regulate growth and differentiation in a negative fashion and thus indirectly suppress neoplastic development (Knudson, 1985). Chromosomal losses in colorectal tumors were first detected cytogenetically (Reichmann et al., 1981; Muleris et al., 1985), but have been studied more recently using probes that detect restriction fragment length polymorphisms to determine whether one of the two parental alleles is lost specifically in tumor DNA.

Previous studies of allelic losses in tumors from patients with inherited predisposition to particular tumor types, such as retinoblastoma and neurofibromatosis type II, focused on the chromosome known to carry the locus segregating with the disease (e.g., Cavenee et al., 1983; Seizinger et al., 1987). An inherited predisposition to colorectal tumor formation occurs in an autosomal dominant syndrome, familial adenomatous polyposis, in which hundreds of colorectal adenomas develop in affected individuals. The locus linked to familial adenomatous polyposis has been mapped to chromosome 5q (Bodmer et al., 1987; Leppert et al., 1987). In patients without polyposis, allelic losses of chromosome 5q have been observed in 20%–50% of colorectal carcinomas and in about 30% of colorectal adenomas (Vogelstein et al., 1988; Sasaki et al., 1989). However, in adenomas from patients with familial adenomatous polyposis, allelic losses of chromosome 5q are rare (Solomon et al., 1987; Vogelstein et al., 1988; Sasaki et al., 1989). Thus, although each adenoma in a patient with polyposis appears to arise by clonal expansion (Fearon et al., 1987), this expansion is not associated with allelic losses of the loci flanking the region linked to the disease. This finding contrasts with the pattern of allelic loss seen in other inherited tumor predisposition syndromes; the implications of this finding will be discussed below.

The loss of a large portion of chromosome 17p, through chromosome loss or mitotic recombination, has been seen in more than 75% of colorectal carcinomas (Vogelstein et al., 1988; Delattre et al., 1989), but such loss is relatively infrequent in adenomas of any stage (Figure 1). Moreover, in several patients the 17p allelic losses were found to be associated with the progression of individual tumors from adenoma to carcinoma (Fearon et al., 1987; Vogelstein et al., 1988). Other common adult tumors, including those of the breast (Mackay et al., 1988; Devilee et al., 1989), lung (Yokota et al., 1987; Weston et al., 1989), bladder (Tsai et al., 1990), and brain (James et al., 1989), also have been found to have frequent losses of chromosome 17p alleles. The common region of loss on chromosome 17p in colorectal tumors has been identified and contains the p53 gene (Baker et al., 1989). Furthermore, mutations resulting in amino acid substitutions in the p53 gene product have been observed in the remaining p53 alleles of several colorectal cancers that had concomitant allelic losses of chromosome 17p (Baker et al., 1989; Nigro et al., 1989). Thus, point mutation of one allele of the p53 gene coupled with loss of the remaining wild-type allele appears to occur frequently in colorectal tumors. Similar mutations have been observed in the remaining p53 alleles of lung, breast, and brain tumors with chromosome 17p losses (Nigro et al., 1989; Takahashi et al., 1989; Iggo et al., 1990). In addition, accumulating evidence from in vitro transformation systems suggests that the wild-type p53 gene can function as a tumor suppressor (Finlay et al., 1989; Eliyahu et al., 1989). The data are therefore consistent with the hypothesis that the wild-type p53 gene inhibits colorectal tumor growth, and removal of the wild-type p53 gene is the selective pressure underlying chromosome 17p allelic losses in these tumors. The remaining mutant p53 allele presumably cannot suppress further tumor progression.

The second most common region of allelic loss in colorectal tumors is chromosome 18q, which is lost in more than 70% of carcinomas (Vogelstein et al., 1988, 1989; Delattre et al., 1989) and in almost 50% of late adenomas (Vogelstein et al., 1988). The common region of loss has been mapped on chromosome 18q, and a candidate tumor suppressor gene from this region has been identified (Fearon et al., 1990). This gene, termed DCC, encodes a protein with significant homology to the cell

adhesion family of molecules (reviewed in Edelman, 1988). The DCC gene was found to be expressed in normal colonic mucosa, but its expression was reduced or absent in the majority of colorectal carcinomas. This loss of expression was associated in some cases with somatic mutations of the DCC gene. Thus, this gene might play a role in the development of colorectal tumors, perhaps though alterations in normal cell–cell and/or cell–extracellular matrix interactions.

Tumor Suppressor Genes and Recessive Models

Tumor suppressor genes have been hypothesized to act "recessively" at the cellular level, so that both maternal and paternal copies of the gene must be inactivated in order for the growth-suppressive function to be eliminated (Knudson, 1985). This model has been supported by numerous studies, most notably through the molecular cloning and analysis of the retinoblastoma gene (reviewed in Hansen and Cavenee, 1987; Weinberg, 1989). However, several observations suggest that this recessive model for tumor suppressor genes may need to be conceptually expanded.

The predisposition syndromes are generally thought to result from the germline inactivation of one copy of a tumor suppressor gene (a unique gene in each syndrome). If this premise and the "recessive" hypothesis are both correct, the tumors that develop in these patients would be predicted to have inactivated the remaining wild-type allele at the suppressor/predisposition locus. Indeed, in the inherited form of retinoblastoma and some other inherited tumor syndromes, the wild-type alleles at the predisposition locus are lost (Hansen and Cavenee, 1987). However, as reviewed above, study of adenomas from numerous patients with familial adenomatous polyposis has usually not revealed allelic losses of the chromosome region linked to this syndrome (Vogelstein et al., 1988; Solomon et al., 1987; Sasaki et al., 1989). Similar observations have been made for multiple endocrine neoplasia type 2 (Nelkin et al., 1989; Landsvater et al., 1989), in which wild-type alleles on chromosome 10 do not appear to be lost in the tumors from patients with the inherited form of the disease.

In sporadic tumors, the recessive model predicts that two genetic events are necessary to engender a phenotypic effect. The first event mutationally inactivates one tumor suppressor allele, and the second event inactivates the other (through localized mutation, mitotic recombination, or chromosomal loss). This scenario presents an inherent difficulty with recessive models for sporadic tumors. Clonal expansion of cells with a genetic alteration can occur only if a phenotypic effect, however small, results from the genetic alteration. If somatic mutation of one tumor suppressor allele did not result in a selective growth advantage, it might be difficult to amass enough cells to allow a reasonable probability for the second event, i.e., inactivation of the remaining allele, to occur.

These dilemmas could be resolved if some suppressor gene mutations affected growth even in the presence of a wild-type allele. Recent data suggest that p53 gene mutations may act in this fashion. Mutated p53 genes from mice (Jenkins et al., 1984; Eliyahu et al., 1984; Parada et al., 1984; Hinds et al., 1989) can cooperate with *ras* to transform primary rodent cells in vitro, even though the rat cells express wild-type p53. Thus, at the cellular level, p53 gene mutations may function as dominant negative (Herskowitz, 1987) rather than recessive mutations. This dominant negative effect may in part be explained by oligomerization of the p53 gene product (Eliyahu et al., 1988; Kraiss et al., 1988). A mutant p53 gene product may inactivate the wild-type gene product by binding to it and preventing its normal association with other cellular constituents.

On this basis, it seems likely that a mutated p53 gene in a colorectal tumor cell could provide a selective growth advantage leading to tumor progression, even in the presence of a wild-type p53 allele. The subsequent loss of the wild-type p53 allele is often associated with the progression from adenoma to carcinoma, and probably amplifies the growth advantage provided by the mutation. Further support for this model of p53 mutation and its phenotypic effect in the presence of a wild-type allele in human tumor cells is provided by the discovery of at least one colorectal tumor demonstrating the postulated intermediate step (Nigro et al., 1989). This tumor contained one wild-type p53 allele and one mutant allele, both expressed in RNA at approximately equal levels. The p53 mutation was present in all cells of the tumor population, presumably as a result of expansion of the cell in which the mutation initially occurred. We hypothesize that one of the cells of this tumor, had the tumor not been removed surgically, would eventually have lost the wild-type allele through an allelic loss and, by a process of clonal expansion, become the predominant cell type. We also hypothesize that the clonal expansion of the cell with the 17p loss would be accompanied by tumor progression. This hypothesis is supported by studies showing that colorectal carcinomas with 17p allelic losses are more aggressive than those without them (Kern et al., 1989).

It seems likely that other tumor suppressor genes also may not conform to recessive models. Mutation of one allele may be sufficient to provide an altered cellular phenotype, perhaps through a dominant negative effect. Alternatively, the level of expression of some tumor suppressor genes may be critically important; reduced expression of the corresponding protein (even in the absence of mutation) may alter some aspect of growth regulation. For example, loss of one allele of a cell surface receptor gene that normally negatively regulates growth might lead to defective control of colonic epithelial cell proliferation. Indeed, the DCC gene (described above) may provide a prototype for this scenario. The biologic function of some cell adhesion molecules is critically and nonlinearly related to the level of cell surface expression. A 2-fold decrease in the surface density of such molecules may result in a 30-fold reduction in cell adhesion (Hoffman and Edelman, 1983). Because the DCC gene is likely to represent a cell adhesion molecule, reduction in its expression in colorectal epithelial cells could lead to altered adhesion and to diminution of the growth-restraining signals associated with such adhesion. A resultant clonal expansion could

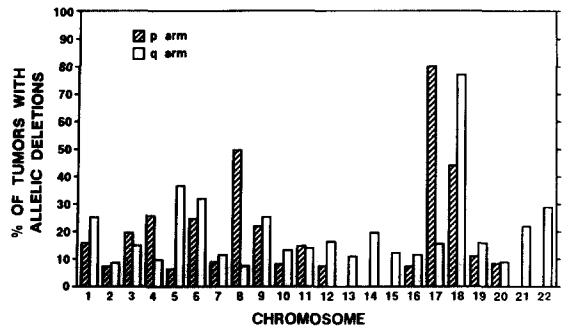

Figure 2. Frequency of Allelic Deletions in Individual Chromosomes in Colorectal Carcinomas

Allelic deletions were evaluated by studies of paired samples of DNA from normal colonic mucosa and cryostat sections of colorectal carcinoma tissue from 56 patients using probes that detected DNA polymorphisms on all nonacrocentic autosomal arms. The frequencies of allelic losses were determined only from informative cases, that is, those in which the normal tissue of the patient exhibited a heterozygous pattern for one or more allelic markers on the chromosomal arm. For each arm at least 40% of the cases were informative. An allelic loss was scored if one of the two alleles present in the normal DNA was lost in at least 80% of the neoplastic cells (modified from Vogelstein et al., 1989).

occur, even if the remaining allele was not mutated and was expressed in these cells. Although further studies are obviously necessary to determine which tumor suppressor genes have a phenotypic effect when only one allele is mutated or lost, the findings reviewed above suggest that dominant negative and dosage effects need to be integrated into the conceptualization of suppressor gene action.

Multiple Allelic Losses in Colorectal Tumors

In addition to the allelic losses noted on chromosomes 5q, 17p, and 18q, many other chromosome losses can be observed in colorectal carcinomas. In an attempt to examine the extent and variation of the losses, polymorphic markers from every nonacrocentric autosomal arm were studied in a large number of colorectal carcinoma specimens (the acrocentric arms carry predominantly ribosomal genes). Regions from chromosomes 1q, 4p, 6p, 6q, 8p, 9q, and 22q were lost in 25%–50% of the cases studied, and losses of each of the other chromosomal arms were identified in at least one case (Figure 2). The frequency of chromosomal losses when viewed from the perspective of individual tumors was less complex, with a median of four to five chromosomal arms suffering allelic losses per tumor. The group of patients with more than the median number of losses in their tumors had a considerably worse prognosis than the other patients, although the size and clinical stage of the primary tumors were very similar in the two groups (Vogelstein et al., 1989; Kern et al., 1989).

The allelic loss composite of Figure 2 may reflect two processes. First, some chromosomal regions, particularly those on chromosomes 17p and 18q, are lost in the majority of colorectal carcinomas, presumably because they contain suppressor genes that are the "targets" of the losses. Second, many other regions are lost in a more heterogeneous fashion, with each loss often found in only a minority of the tumors. There are at least two possible explanations for these additional allelic losses in individual tumors. One hypothesis is that some of the losses detected might have no specific effect on the phenotype of the cell, but may have arisen coincidentally with other genetic alterations upon which selection could act, perhaps in a complex mitotic event that segregated numerous chromosomes aberrantly. Alternatively, there may be many tumor suppressor genes present throughout the genome, and each of the chromosomal regions lost could contain such a gene. This latter hypothesis may not be as far-fetched as it seems, as studies of several tumor types in vivo as well as in vitro experiments with tumor cell lines suggest that tumor suppressor genes reside on many different chromosomes (reviewed in Ponder, 1988; Sager, 1989). The heterogeneity in allelic loss patterns of individual tumors might therefore be responsible for the observed heterogeneity in the biologic properties of colorectal tumors from different patients (Owens et al., 1982). Tumors with a specific subset of suppressor gene alterations would behave differently from those with an overlapping but distinct subset.

Other Somatic Alterations

A significant loss of methyl groups in DNA has been found to occur very early in colorectal tumorigenesis. Examination of DNA from even very small adenomas revealed that approximately one-third of the DNA regions studied had lost methyl groups present in the DNA of normal colonic mucosa, suggesting a loss of 10–20 million methyl groups per cell (Goelz et al., 1985; Feinberg et al., 1988). Although colorectal tumors are globally hypomethylated, a few specific regions of the genome may be hypermethylated (Silverman et al., 1989). The loss of DNA methylation has been shown to inhibit chromosome condensation and might lead to mitotic nondisjunction (Schmid et al., 1984), resulting in the loss or gain of chromosomes. Thus an epigenetic change, like hypomethylation, could contribute to instability in the tumor cell genome and alter the rate at which genetic alterations, such as allelic losses, occur.

In addition to the structural alterations of the oncogenes and tumor suppressor genes noted above, consistent differences in expression or activity of other genes or gene products have been noted in colorectal tumors. For example, *c-myc* is expressed at high levels in most colorectal carcinomas, especially those derived from the descending colon (Rothberg et al., 1985; Stewart et al., 1986). Tyrosine kinase activities of specific proteins are also elevated in many colorectal carcinomas (Bolen et al., 1987); such activities are noteworthy because similar activities are associated with several oncogenes (Bishop, 1987). In addition, the expression of several glycoconjugates has been found to be increased in colorectal carcinomas compared with normal colorectal epithelial cells (Kim, 1989). The significance of increased or decreased expression or activity of any gene product is, however, difficult to assess when the gene encoding the product is not demonstrably altered by mutation. For example, many

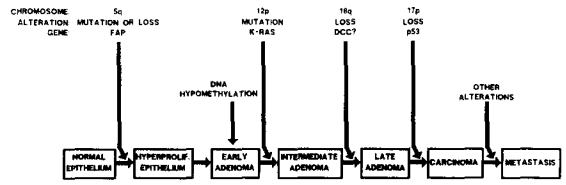

Figure 3. A Genetic Model for Colorectal Tumorigenesis

Tumorigenesis proceeds through a series of genetic alterations involving oncogenes (*ras*) and tumor suppressor genes (particularly those on chromosomes 5q, 17p, and 18q). The three stages of adenomas are defined in the legend to Figure 1 and, in general, represent tumors of increasing size, dysplasia, and villous content. In patients with familial adenomatous polyposis (FAP), a mutation on chromosome 5q is inherited. This alteration may be responsible for the hyperproliferative epithelium present in these patients. In tumors arising in patients without polyposis, the same region may also be lost and/or mutated at a relatively early stage of tumorigenesis. Hypomethylation is present in very small adenomas in patients with or without polyposis, and this alteration may lead to aneuploidy, resulting in the loss of suppressor gene alleles (see text). *ras* gene mutation (usually K-*ras*) appears to occur in one cell of a preexisting small adenoma and through clonal expansion produces a larger and more dysplastic tumor. The chromosomes most frequently deleted include 5q, 17p, and 18q; the putative target of the loss event (i.e., the tumor suppressor gene) on each chromosome is indicated as well as the relative timing of the chromosome loss event. Allelic deletions of chromosome 17p and 18q usually occur at a later stage of tumorigenesis than do deletions of chromosome 5q or *ras* gene mutations. However, the order of these changes is not invariant, and accumulation of these changes, rather than their order with respect to one another, seems most important (see text). Tumors continue to progress once carcinomas have formed, and the accumulated loss of suppressor genes on additional chromosomes correlates with the ability of the carcinomas to metastasize and cause death.

genes associated with cellular proliferation (even those devoid of any conceivable oncogenic property) are expressed at much higher levels in colon cancers than in normal colonic mucosa (Calabretta et al., 1985). Thus, further studies are necessary to determine whether the differences in expression or activity of c-*myc*, tyrosine kinases, and other gene products are a result or cause of the neoplastic state.

Accumulation, Rather Than Order, Is Most Important

A model for colorectal tumor development is depicted in Figure 3. Histopathological and clinical data (Sugarbaker et al., 1985) suggest that most colorectal carcinomas arise from adenomas, which gradually progress through increases in size, dysplasia, and the acquisition of villous (finger-like) morphology. Although the process of adenoma progression is actually a continuum, presumably resulting from successive waves of clonal expansion (Nowell, 1976), three discrete stages of adenoma formation are depicted in Figure 3. Two processes that undoubtedly are complex—the progression to carcinoma and then to metastasis—are each depicted as occurring in a single step in an attempt to keep the model as simple as possible. The process of colorectal neoplasia depicted occurs over a period measured in decades.

Data obtained from in situ DNA labeling of colonic epithelium from patients with or without polyposis suggest that tumorigenesis is preceded by widespread cellular hyperproliferation (Lipkin, 1988). In polyposis patients, and perhaps others, the proliferation may be induced by loss or inactivation of the familial adenomatous polyposis gene on chromosome 5q. One of these hyperproliferating cells may then give rise, by clonal expansion, to a small adenoma in which the genome is hypomethylated. The genetic event responsible for the transition from hyperplasia to neoplasia is not clear, but as noted above, it does not appear to involve loss of the remaining wild-type familial adenomatous polyposis gene, at least in polyposis patients. It is possible that the genetic events controlling this transition are heterogenous and involve *ras* gene mutations in some tumors, p53 mutations in others, and mutations on chromosome 18q or other chromosomes in the remainder.

The relative timing of *ras* gene mutations and chromosome deletions with respect to different stages of colorectal tumorigenesis is summarized in Figure 3. Although the alterations usually occur at characteristic phases of tumor progression, two observations argue that it is the progressive accumulation of changes, rather than their order of occurrence with respect to one another, that is likely to be most important in colorectal tumor progression. First, the occurrence of any given alteration was not found to be restricted to a particular stage of tumorigenesis. For example, chromosome 17p deletion events were usually observed only in carcinomas or late-stage adenomas, but a few very small adenomas were found to have already suffered a chromosome 17p loss (Figure 1). Second, in several cases it was possible to study different stages of neoplasia in the same tumor specimen by isolating DNA from both adenomatous and carcinomatous regions of individual tumors (Bos et al., 1987; Vogelstein et al., 1988). That the carcinomatous regions were derived from (and not simply adjacent to) the adenomatous regions was proven in several cases by the finding that the identical *ras* gene mutation was present in both regions. In all cases, however, the carcinomatous regions contained at least one alteration not found in the adenomatous region. The occurrence of the genetic alterations in these specimens was generally in accord with the preferred order shown in Figure 3; however, there were definite exceptions noted. For example, in one case a chromosome 17p deletion (identified in both the adenomatous and carcinomatous regions) preceded deletions of chromosomes 5q and 18q (found only in the carcinomatous region). In another case, a chromosome 18q deletion preceded a *ras* gene mutation. Therefore, although a preferred order for the genetic alterations in colorectal tumorigenesis exists, the data suggest that the progressive accumulation of these alterations is the most consistent feature of the clinical and histopathological progression of colorectal tumors.

Of note, the four genetic alterations discussed above (*ras* gene mutations and deletions of chromosomes 5q, 17p, and 18q) have been found to occur at similar frequencies in ethnically and geographically diverse populations with varying incidences of colon cancer (Vogelstein et al., 1988; Sasaki et al., 1989; Ashton-Rickardt et al., 1989; Delattre et al., 1989; Farr et al., 1988). In addition, these genetic alterations have been observed at roughly equal

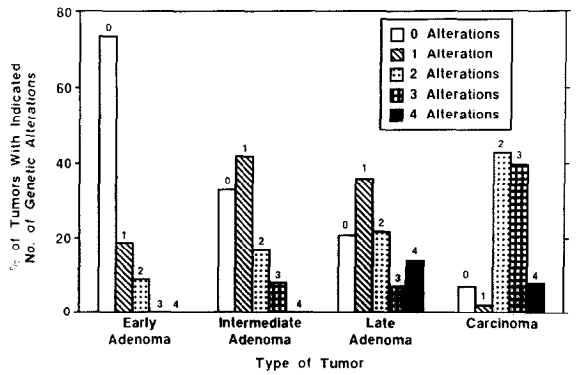

Figure 4. Multiple Genetic Alterations Are Present in Colorectal Tumors

Four genetic alterations (*ras* gene mutations and allelic deletions of chromosomes 5q, 17p, and 18q) were studied in each colorectal tumor; thus, each tumor could have had none, one, two, three, or all four of these alterations. The results are shown only for tumors that were informative for all four alterations (i.e., those in which DNA from the normal tissue of the patient was heterozygous for the restriction fragment length polymorphism markers used). These tumors included 53 carcinomas, 14 late-stage adenomas, 12 intermediate adenomas, and 27 early adenomas. The adenoma stages were defined as in the legend to Figure 1.

frequencies (with the exception of 5q loss in colorectal adenomas) in colorectal tumors from patients with or without polyposis (Vogelstein et al., 1988; Sasaki et al., 1989). Differences in the frequencies noted in the various studies can probably be explained by sample preparation; studies using enriched populations of neoplastic cells have generally found higher rates of alterations than those using unfractionated tumors (Vogelstein et al., 1988; Delattre et al., 1989). The data, in toto, suggest shared molecular mechanisms for the development of colorectal cancers, irrespective of varying hereditary factors and specific environmental exposures.

Multistep Basis of Tumorigenesis

Study of the process of tumorigenesis in experimental models has suggested that at least three steps can be defined: initiation, promotion, and progression. The process of tumorigenesis can be studied only indirectly in humans; measurements of age-dependent cancer incidence have shown that the rate of tumor development is proportional to the fourth to sixth power of elapsed time, suggesting that four to six independent steps are necessary (Peto et al., 1975; Dix, 1989).

Preliminary estimates of the number of genetic alterations necessary for different stages of colorectal tumorigenesis can be made. Four genetic alterations (*ras* gene mutations and deletions of chromosomes 5q, 17p, and 18q) were studied in colorectal tumors of various stages (Figure 4). More than 90% of the carcinomas had two or more of the alterations. In contrast, only 7% of early adenomas had more than one of the four genetic alterations, and this percentage gradually increased to 25% and then 49% as the adenomas progressed to intermediate and late stages, respectively. Interestingly, each of two late-stage adenomas contained all four genetic alterations. This observation supports the idea that alterations of the four genes were not sufficient for progression to malignancy. A further analysis showed that most colorectal carcinomas had at least one additional allelic loss (in addition to those on chromosomes 5q, 17p, and 18q), with a median of four or five allelic losses per tumor (Vogelstein et al., 1989).

As a subset of the allelic losses is likely to unmask mutations in the retained allele (discussed above), some of these losses should perhaps be counted as revealing two independent genetic events. Thus, most carcinomas probably arise from a minimum of five or more genetic alterations, while adenomas appear to require correspondingly fewer alterations. The number of genetic alterations necessary for cancer formation, as estimated from the study of genetic alterations in colorectal tumors, is therefore roughly consistent with the number of steps predicted by measurements of age-dependent cancer incidence.

Implications of the Colorectal Tumor Model and Unanswered Questions

The genetic model for colorectal tumor development depicted in Figure 3, although rudimentary, provides a framework for the study of the independent steps involved in a complex human disease. In addition, the discovery of genetic changes that are restricted to neoplastic cells suggests that the detection of early-stage colorectal tumors may be possible, perhaps through the identification of mutant gene products secreted into the blood or feces, or through the detection of antibodies made by the patient against the mutant gene products. The predisposition to form colorectal tumors can be inherited, and the identification of individuals who have inherited defective tumor suppressor genes on chromosome 5q, 18q, or other chromosomes should lead to more effective methods for prevention. Mortality from colorectal tumors is preventable when tumors can be identified at any stage prior to the last (metastatic) stage depicted in Figure 3. Moreover, the identification of the genetic alterations present in tumors may provide a molecular tool for improved estimation of prognosis in patients with colorectal cancer (Vogelstein et al., 1989; Kern et al., 1989). Finally, the findings suggest that multiple pathways exist in which new chemotherapeutic agents might achieve a therapeutic advantage. Some agents might be sought that would selectively inactivate mutated gene products (e.g., *ras*); others might be obtained that could mimic or restore the normal biologic action of suppressor genes (e.g., p53 or DCC).

Although the findings summarized in Figure 3 provide some insight into the pathogenesis of colorectal neoplasia, numerous questions remain unanswered. Little is known about the normal function of any of the genes implicated in colorectal tumorigenesis, a prerequisite for understanding the biochemical and physiologic effects of the mutations. The number of genetic alterations sufficient for tumorigenesis at the adenoma and carcinoma stages is not known, and this number may vary in different members of the population. Do most cases of colorectal tumors occur in individuals with an inherited predisposition (see Cannon-Albright et al., 1988)? Do some patients

inherit altered p53 or DCC alleles, predisposing them to malignancy? Do familial colon cancer syndromes without polyposis (Lynch et al., 1985) involve inherited mutations of the polyposis gene on chromosome 5q? In those tumors without identifiable *ras* (or p53 or DCC) gene mutations, do mutations in other genes confer an equivalent selective growth advantage? In addition to hereditary factors, environmental factors have been shown to be involved in determining colorectal tumor incidence (Willett, 1989). What is the relationship between these environmental factors and the genetic alterations depicted in Figure 3? Can the mutations identified or others yet to be identified influence either directly or indirectly the rate of subsequent alterations in an initiated cell? Is the progressive accumulation of mutations in colorectal tumors inexorable, or can it be arrested by nonsurgical means?

Although the work ahead is daunting, the relevance of the colorectal model to other tumor types, such as small cell carcinoma of the lung (Yokota et al., 1987; Minna et al., 1986; Weston et al., 1989), astrocytomas (James et al., 1988; Bigner et al., 1990), bladder cancers (Tsai et al., 1990), and breast tumors (Callahan and Campbell, 1989), is beginning to gather support. An optimistic outlook is that the colorectal model will prove relevant to other common human neoplasms in which tumor development is less well defined and tumors of varying stage are difficult to study. According to this optimistic outlook, the pathogenesis of human neoplasia is a puzzle that might prove solvable in the coming decades.

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