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Suppressor Gene Alterations in the Colorectal Adenoma-Carcinoma Sequence

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Abstract Tumorigenesis is thought to be a multistep process in which genetic alterations accumulate to bring about the neoplastic phenotype. Colorectal tumors appear to arise as a result of the mutational activation of oncogenes coupled with the inactivation of several tumor suppressor genes. We have found frequent allelic deletions of specific portions of chromosomes 5, 17, and 18 which presumably harbor suppressor genes. The target of allelic loss events on chromosome 17 has been shown to be the p53 gene, which is frequently mutated not only in colon cancer but in several other tumor types as well. Candidate suppressor genes have also recently been identified on chromosomes 18 and 5. The DCC gene on chromosome 18q encodes a protein with significant sequence similarity to neural cell adhesion molecules and other related cell surface glycoproteins. Alterations of this gene may interfere with normal cell growth and differentiation by disrupting cell-cell or cell-substrate interactions. Two genes (MCC and APC) on chromosome 5q have also recently been identified and partially cloned. These genes are located in a region tightly linked to familial adenomatous polyposis (FAP). While MCC mutations have been found only in sporadic colon tumors, APC mutations have been identified in sporadic tumors as well as the germline of patients with FAP. Studies are currently in progress to increase our understanding of how alterations of these genes affect colorectal tumor cell growth.

Key words: APC, chemoprevention, colorectal tumors, DCC, familial adenomatous polyposis (FAP), intermediate biomarker, MCC, p53, tumor suppressor genes

Tumorigenesis is a complex process which appears to be largely based on the accumulation of genetic alterations within the tumor cells. The critical genetic lesions are not distributed randomly throughout the genome, but target specific genes, including oncogenes and tumor suppressor genes. These genes can be activated (oncogenes) or inactivated (suppressor genes) through several mechanisms including gene amplification, point mutation, rearrangement, and deletion.

Epithelial tumors of the colorectum provide an excellent system in which to study these genetic alterations and the manner in which they affect tumor progression. These tumors progress along a continuum through different clinicopathologic stages which can be sampled with relative ease. Recent studies have provided the basis for a model of tumorigenesis in which activational mutation of the ras oncogene, coupled with inactivation of several suppressor genes, combine to bring about the malignant phenotype in colonic epithelial cells (1).

Several suppressor gene candidates have recently been identified. Identification of the genes believed to be involved in colorectal tumorigenesis was aided by "allelotyping" DNA from a large group of paired samples of colorectal carcinomas and adjacent normal mucosa (2). This analysis used polymorphic DNA probes to determine the nature and extent of allelic loss events in colorectal cancers. Allelic deletions were found to be extremely common, with eight chromosomal regions affected in more than 25% of the carcinomas studied. A median of four to six allelic losses were observed in individual carcinomas. While some of the losses may be "passengers" coincidentally occurring in the same mitosis as another genetic alteration providing a selective growth

advantage, we believe that many of these changes reflect the presence of suppressor genes in the deleted regions. This is in keeping with the model of tumorigenesis proposed by Knudson, in which defective genes involved in inherited neoplastic syndromes normally function as tumor suppressor genes. At the cellular level, these genes function in a recessive manner such that both alleles must be inactivated to promote tumor growth (3). Inactivating lesions such as deletions, point mutations, or translocations, can be inherited through the germline or acquired somatically.

Deletions on the short arm of 17 (17p) occur most commonly, affecting over 75% of colorectal carcinomas. These deletions appear to be relatively late events in colorectal tumor progression and are associated with the transition from the benign adenoma to malignant carcinoma. Using 20 polymorphic chromosome 17p markers, a common region of deletion was localized to a region centered at 17p13 (4) which was known to contain the gene encoding the transformation associated protein p53 (5). The p53 gene has subsequently been identified as a suppressor gene which is routinely inactivated by mutation and allelic deletion in colorectal carcinomas (4,6), as well as in several other tumor types (reviewed in 7) including tumors of the lung, breast, colon, esophagus, liver, bladder, ovary, soft tissues, brain, and hematopoietic cells. Recently, germline p53 gene mutations were identified in DNA from patients with Li-Fraumeni syndrome, a rare syndrome in which affected patients are at high risk for developing malignancy of several tissues at early age (8). The p53 gene mutational spectrum differs among different tumor types, probably reflecting heterogeneity in the etiology and functional consequences of the various p53 gene mutations. For instance, transitions predominate in tumors of the colon, brain, and lymphoid cells, while transversions are most frequent in cancers of the lung and liver. All of the mutation types tend to occur in the evolutionarily conserved domains of the gene.

Allelic deletions of chromosome 18q are also very common, occurring in approximately 75% of colorectal carcinomas. Deletions are also seen in 47% of large adenomas, but in less than 10% of small and intermediate stage adenomas, suggesting that 18q loss events generally precede p53 gene alterations during colorectal tumorigenesis. Recent studies have found that fusion of normal chromosome 18 with colorectal carcinoma cell lines at least partially inhibits their tumorigenicity (9,10), providing further evidence that chromosome 18 is likely to harbor a suppressor gene. We have recently identified a candidate gene on chromosome 18q which appears to be altered in colorectal cancer (11). Using a panel of several polymorphic DNA probes to study tumors which had lost some, but not all of 18q, we identified a common region of deletion centered at 18q21.3. One anonymous probe from within this region detected homozygous loss of sequences in a primary colorectal carcinoma. Homozygous loss events are quite rare, and are thought to be a hallmark of suppressor genes. Using this probe as a starting point, we used a bidirectional chromosome walking strategy to clone 370 kilobases of contiguous genomic DNA from within the deleted region thought to harbor the suppressor gene target. Potential exons in the cloned region were identified by cross-species hybridization at reduced stringency followed by comparison of human-rodent sequence identities in which candidate exons were found to contain long open reading frames flanked by appropriate splice donor and acceptor sites and lariat sequences. Expression of these potential exons was identified using an "exon-connection" strategy based on the polymerase chain reaction (PCR). Using standard cloning techniques, we obtained a cDNA from this gene (DCC for deleted in colorectal carcinomas). The DCC cDNA encodes a putative translation start site, signal peptide, and hydrophobic transmembrane region dividing the predicted protein into extracellular (1100 amino acid) and intracellular (324 amino acid) domains. The coding region is composed of 28 exons and appears to be flanked by lengthy 5' and/or 3' untranslated regions, accounting for a significant portion of the 10-12 kb DCC mRNA. YAC cloning of the DCC locus suggests that the gene spans over 1.5 megabases (12). Although the sequence of the intracytoplasmic domain demonstrates no significant homology to any sequence in the Genbank data base, the extracytoplasmic portion of the DCC protein has significant sequence similarity to neural cell adhesion molecules and other related cell-surface glycoproteins. By mediating cell-cell and cell-substrate interactions, this class of molecules is thought to be important in mediating cell growth and differentiation. Alterations of the DCC gene may interfere with maintenance of these critical controls and thus may play a role in the pathogenesis of human colorectal neoplasia.

Numerous abnormalities of DCC have been identified in colorectal tumors. Using the PCR based

expression assay, DCC expression was identified in almost all of the normal tissues studied, including normal colonic mucosa. In contrast, expression of the same portion of the DCC mRNA was absent or reduced in 15 of 17 colorectal carcinoma cell lines. Somatic alterations of DCC have also been identified in several primary tumors, colorectal carcinoma xenografts, and cell lines. Demonstration of a functional tumor suppressive effect should provide further support for DCC as a suppressor gene. We have recently transfected DCC expression constructs into colorectal carcinoma cell lines and obtained several clones of cells expressing high levels of exogenous DCC (13). We are currently in the process of evaluating the effects of DCC expression in assays of tumor cell "invasiveness", anchorage independent growth, and tumorigenicity in nude mice.

Lynch et al. have described a syndrome with autosomal dominant inheritance in which affected individuals are predisposed to carcinomas of the colon and other organs (14). Candidate genes on chromosome 18q are of particular interest, since the gene causing this syndrome has been linked to the Kidd blood group antigen on this chromosomal arm (15). Unfortunately, recent linkage analyses support exclusion of the DCC gene as the locus for susceptibility to hereditary nonpolyposis colorectal carcinoma (HNPCC) in at least 5 of 7 kindreds studied (16). However, the interpretation of such linkage studies is complicated by the possible genetic heterogeneity of HNPCC kindreds and the potential occurrence of "sporadic" colon cancers in some family members arising independent of the putative HNPCC susceptibility gene.

Allelic losses on chromosome 5q are also quite common, occurring in nearly 40% of carcinomas and sporadic adenomas studied (17,18). These deletions are of special interest since the gene responsible for familial adenomatous polyposis (FAP) has been mapped to the deleted region (19,20). Two candidate suppressor genes located at chromosome 5q21 have recently been identified (21,22,23,24). The first is called MCC (for mutated in colorectal carcinomas). Using a strategy similar to that described for DCC, the MCC gene was identified and a partial cDNA cloned (21). The cDNA encodes an 829 amino acid protein with a short region of sequence similarity to the G protein-coupled m3 muscarinic acetylcholine receptor (mAChR). The region of sequence similarity coincides with that region of the mACHR which has recently been shown to be critical for G protein activation (25). Members of the G protein family are believed to be important in transducing signals within the cell. The connection between MCC and the G protein-activating region of mACHR is intriguing in light of previous demonstrations of the importance of G-proteins in neoplasia; however, it remains to be shown whether MCC actually interacts with G proteins *in vivo*. MCC alterations identified to date include one tumor with somatic rearrangement of one MCC allele, and six tumors with somatically acquired point mutations within the MCC coding region or at intron-exon boundaries.

A second interesting gene (APC) mapping to 5q21 has also been recently identified (22,23). The 8.5 kb APC gene open reading frame encodes an unusually large protein which, like MCC, contains local sequence similarity to the same region of mACHR as well as alpha helical coiled-coil regions which may function to mediate homo- or hetero-oligomerization. APC gene mutations have been identified in sporadic colorectal carcinomas as well as in the germline of patients with FAP and Gardner's syndrome. Studies are currently in progress to evaluate the relationship (if any) between these two proteins in colorectal tumorigenesis, to understand the normal functions of these proteins, and to determine how their alteration contributes to colorectal tumor formation in both inherited and sporadic cases.

In studying the genetic alterations occurring in colorectal tumorigenesis, several pertinent points have become evident. First, it appears that while specific alterations tend to occur at specific stages during tumor progression, exceptions certainly exist, and it is probably the total accumulation rather than order of changes that is most important. Second, genetic alterations probably continue to accumulate once carcinomas have developed and this accumulation may parallel clinical progression. The multiplicity of genetic changes cannot be over-emphasized and should prompt caution in overinterpreting the significance of any single alteration. Finally, carcinogenesis is complex in molecular terms. Although deletions of certain chromosomes occur very frequently, deletions of other chromosomes often occur as well, which may contain suppressor genes in addition to the ones discussed above. The complexity of genetic changes in human neoplasia is most certainly reflected in the biologic heterogeneity of these tumors.

The identification of specific genes involved in colorectal tumorigenesis will hopefully contribute to better understanding of the molecular pathogenesis of human neoplasia and provide molecular targets at which to aim screening, early diagnosis, and ultimately treatment of patients with this common and often lethal cancer.

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