Molecular Genetics of Small Bowel Cancer

Nadir Arber, Alfred I. Neugut, I. Bernard Weinstein, and Peter Holt

Department of Medicine [N. A., A. I. N., I. B. W., P. H.] and the School of Public Health [A. I. N., I. B. W.], College of Physicians and Surgeons, Columbia University, and the Department of Medicine, St. Luke’s-Roosevelt Hospital Center [P. H.], New York, New York

Abstract

Although the molecular genetic changes that take place during carcinogenesis in the large bowel have been well elucidated, very little work has been done on the carcinogenesis process in the small bowel where this phenomenon is much rarer. The few studies that have been done suggest that certain oncogenes, i.e., erbB2, K-ras, cyclin D1, and p53, are all altered in ways and in frequency similar to these phenomena in large bowel cancer. Some tumor markers have been noted to occur in frequency similar to these phenomena in large bowel. Although the molecular genetic changes that take place during carcinogenesis in the large bowel have been well elucidated, very little work has been done on the carcinogenesis process in the small bowel where this phenomenon is much rarer. The few studies that have been done suggest that certain oncogenes, i.e., erbB2, K-ras, cyclin D1, and p53, are all altered in ways and in frequency similar to these phenomena in large bowel cancer. Some tumor markers have been noted to occur in frequency similar to these phenomena in large bowel cancer.

Introduction

Although the molecular genetic changes that take place during carcinogenesis in the large bowel have been well elucidated, very little work has been done on the carcinogenesis process in the small bowel where this phenomenon is much rarer. The few studies that have been done suggest that certain oncogenes, i.e., erbB2, K-ras, cyclin D1, and p53, are all altered in ways and in frequency similar to these phenomena in large bowel cancer. Some tumor markers have been noted to occur in frequency similar to these phenomena in large bowel cancer. Although the small intestine or bowel contains almost 90% of the mucosal surface area of the gastrointestinal tract, cancer of the small intestine has an incidence rate which is one-fifteenth that of the large intestine (1). This is despite the fact that there are many similarities in the epidemiology of cancer in these two organs: they tend to covary in different countries (2); patients with small bowel adenocarcinoma are at elevated risk for large bowel adenocarcinoma and colorectal adenocarcinoma, it is highly likely that the same molecular genetic changes play a major role. Further work is needed to confirm this. If true, a potentially important area of future research would be to determine why these molecular genetic changes occur so much less frequently in the small bowel as compared to the large bowel.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 To whom requests for reprints should be addressed, at the Division of Oncology, Columbia-Presbyterian Medical Center, 630 West 168th Street, New York, NY 10032.
explored the molecular genetic changes associated with rapid transit or with the presence or absence of bacteria. This would be a fertile area for future research.

The small bowel contains certain enzymes that protect it from carcinogenesis. One example is benzopyrene hydrolase. This enzyme converts benzopyrene, a potent carcinogen, to a less active form. It is found in high concentrations in the small bowel. It is protecting the small bowel against carcinogenesis (29, 30).

One of the major advances in cancer research in the past 10–15 years has been the elucidation of the molecular genetics of colorectal cancer. Elegant studies by Vogelstein and others (31–33) have explored the somatic genetic changes that take place in the course of the adenoma-carcinoma sequence during colorectal carcinogenesis. The result is that we now have a clearer perception of the process of colorectal carcinogenesis on a molecular genetic level than that of any other solid tumor.

Clinical benefits have included the identification of germ-line mutations predisposing to colorectal cancer and the identification of new prognostic factors. Research efforts are currently underway to take advantage of these new findings in developing new screening tools for colon cancer (34, 35) and in developing pharmacologic agents that capitalize on the new genetic discoveries to treat colorectal cancer (36, 37). On the other hand, very little research has been carried out in the investigation of molecular markers in small bowel cancer; even those few studies that have been done have been limited in size.

Animal Studies

min Mouse System. Animal model and cell culture systems have shed some light on potential molecular biomarkers of small bowel tumors. Of particular importance is the min mouse model (38–43). min mice carry a dominant mutation in the homologue of the APC (adenomatous polyposis coli) gene. Genetic linkage analysis has localized the mutation to mouse chromosome 18, in a region known to contain the APC gene, the murine homologue of the human APC gene (40).

Mutations in the APC gene appear to be responsible not only for familial adenomatous polyposis but also for many sporadic cases of gastrointestinal cancer. It has been suggested that APC gene modification is a critical event in the initiation of small bowel tumor formation. Mendelian transmission of the mutated gene caused most homozygous mice to die in utero before day 8 of gestation. The heterozygous mice developed multiple polyps throughout the intestinal tract, mostly in the small intestine. All adenomas lost the wild-type APC allele, whereas the mutant allele remained unchanged. These results indicate that LOH2 is followed by formation of adenomas (42, 43).

mom-1. Further analysis of the min-1 mutation has identified, by quantitative trait loci studies, a locus designated mom-1 that dramatically modifies the min-induced tumor number. This locus maps to the distal region of chromosome 4, in a region syntenic to human chromosome 1p35–36. This region of human chromosome 1 is frequently rearranged or mutated in intestinal tumors. mom-1 is estimated to account for about 50% of the genetic variation in adenoma number in min mice (44). Further analysis of the mom-1 gene in tumors produced by min-1 mice should indicate if it is comutated during tumor progression. Recently, MacPhee et al. (38) have suggested that the gene for secretory phospholipase A2 is a candidate for the mom-1 locus, and that it modifies polyph number by altering the cellular microenvironment within the intestinal crypt.

Strain B10.A. Fijneman and Demant (41) reported that a high percentage of mice of strain B10.A that were treated prenatally with the carcinogen N-ethyl-N-nitrosourea developed macroscopically visible tumors of the small intestine. These tumors are described as adenocarcinomas containing cells of four histologically different types, each resembling one of the four main differentiated cell types in the mouse small intestine: mucous, enteroendocrine, Paneth, and columnar. Because these four cell types all originate from a common pluripotent stem cell, the crypt-base columnar cell, it is believed that tumors of the small intestine originate from neoplastically affected crypt base columnar cells.

LOH. LOH studies have presented a powerful tool for the study of the development and progression of cases. LOH in human tumors can be difficult to interpret due to the limitations of the number of tumors of a precise type, stage, genetic background, and environmental exposure (45). Transgenic mice would appear to offer an ideal situation to perform genomewide scans for LOH. The overall genomewide rate of LOH of carcinoid tumors in transgenic mice expressing the SV40 Tag was quite low. Chromosomes 9 and 16 showed high rates of allelic loss. The locus on chromosome 9 lies in a region with synteny to human chromosomes 3q, 6q12, 15q24, and 3p21, whereas the locus on chromosome 16 lies in a region corresponding to human chromosomes 3q and 22q. These regions do not encode the two tumor suppressors, pRB and p53, known to interact with SV40 Tag, suggesting the presence of new genes, the loss of function of which contributes to multistage tumorogenesis (45).

SV40 Tag, human K-ras and a dominant negative mutant of human p53 have been expressed singly and in all possible combinations in postmitotic enterocytes of transgenic mice to assess the role of these gene products in the pathogenesis of gut neoplasia. Transgenic mice that produce K-ras and/or p53 did not have any detectable phenotypic abnormalities. K-ras cooperates with SV40 Tag to generate marked proliferative and dysplastic changes. Yet, mice that carried one, two, or three of these transgenes did not form adenomas or adenocarcinomas. A modest increase in tumor number was noted in animals that express the min mutation and either SV40 Tag alone, SV40 Tag and K-ras, or SV40 Tag, K-ras, and p53. These results demonstrate the remarkable protective effect of a continuously and rapidly renewing epithelium in the small bowel (24).

Other Biomarkers. There is also evidence regarding the production of large quantities of epidermal growth factor and a polypeptide similar to it during experimental carcinogenesis in the small bowel mucosa of rats (46).

We showed that derivatives of the IEC-18 enterocyte cell line, originally isolated from normal rat ileum transformed by an activated human c-K-ras oncogene, display increased expression of both cyclin D1 and Rb genes, thus revealing novel effects of these oncogenes. The increased expression of these oncogenes in tumors may be relevant to small bowel carcinogenesis as well (47).

Oncogenes for Human Adenocarcinoma

Activation of the neu gene (also called erbB-2 and HER-2) encodes a transmembrane glycoprotein that has tyrosine-specific kinase activity. Cohen et al. (48) examined the expression of the p185neu protein in normal and malignant digestive tract tissues, including the small intestine. A point mutation in the neu gene leading to a single amino acid substitution (valine to glutamine at residue 664) is responsible for the transforming
phenotype (48). In the normal mucosa, there was prominent
p185neu expression in the villus, with little or no staining in the
crypts. Immunoreactivity was consistently greater in adenom-
atous polyps. These findings suggest that p185neu may play a
role in the transformation of these cells (48).

Our group examined the frequency of c-K-ras in small
bowel adenocarcinomas using a PCR-based method by RFLP.
c-K-ras mutations at codon 12 were observed in five of six
cases (49). Recently, we have identified another 6 k-ras muta-
tions in an additional 15 small bowel tumors3 for a total of 11
of 21 small bowel tumors.

We also evaluated the level of expression of cyclin D1
protein during the multistage process of human small bowel
carcinogenesis (50). Increased expression of cyclin D1 may
perturb cell cycle control early in the tumorigenesis process
and thereby enhance tumor progression. Cyclin D1 protein abun-
dance was determined by immunostaining samples of normal
mucosa (n = 34), adenomatous polyps (n = 24), and adeno-
carcinomas (n = 33). Cyclin D1 nuclear staining occurred in
33% of adenocarcinomas and 36% of adenomatous polyps but
not in normal appearing mucosa.

Genomic instability and replication errors play an impor-
tant role in the pathogenesis of tumor formation, especially in
intestinal tumors, including those of the small bowel (51). Hibi
et al. (52) observed replication errors in 5 of 11 cases (45%) of
small intestinal carcinomas, and Keller et al. (53) reported
errors in one case of five studied.

Spandidos et al. (54) found 46% (6 of 13) of the same
group of patients had increased expression of p53. These cases
included lymphoma, angiosarcoma, leiomyosarcoma, adeno-
carcinoma, and two metastases from adenocarcinomas of the
large bowel. Our own study of p533 found high levels of p53
expression in small bowel adenocarcinomas with significantly
lower expression in small bowel adenomas.

Markers for Carcinoid Tumors
Although most of this review has focused on small bowel aden-
ocarcinomas, small bowel malignant carcinoid tumors are also of
interest. They do not appear to have anything, aside from anatom-
ic location, in common with small bowel adenocarcinomas and
share no epidemiological characteristics with large bowel tumors.
Thus, on some level, they represent a "control" group for contrast-
ing small bowel adenocarcinomas. Biomarkers for the diagnosis
of carcinoid tumors are well established. Traditionally, they were
based on 5-hydroxy indole-3-acetic acid excretion in the urine and
serotonin measurement in blood platelets. Other specific markers
of carcinoid tumors include neuropeptide K, substance P, gastrin,
histamine, corticotropin-release factor, and growth hormone-re-
leasing factor (55).

The development of a RIA for the analysis of chro-
mosarin in plasma has improved the diagnostic possibilities of
early carcinoid. Moreover, changes in chromogranin A and
B levels correlate with changes in other markers, and can be
used to monitor treatment (55). Funa et al. (56) reported on an
in situ hybridization study of chromogranin A and B mRNA in
carcinoid tumors. They claimed that mRNA for chromogranin A
and B was a reliable marker for the carcinoid tumors, especially of
mid-gut origin. They also found that mRNA expression of chro-
mosarin A after IFN therapy indicated an inhibition at the pre-
translational level. Amplification and increased expression of the
neu gene was seen on both the mRNA and protein levels in
carcinoid tumors. Moreover, quantitation of actual copy number
may be an important prognostic determinant.

The tumor markers CA-19-9 and CA-50 are based on
monoclonal antibodies to colonic carcinoma cell lines. Immun-
ohistochemical studies have shown that both markers were
expressed in 50–60% of patients with small bowel tumors (55).
Carcinoembryonic antigen and proliferating cell nuclear anti-
gen production were noted in 8 of 10 cases from Japan (57).

Future Directions
Despite the difference in incidence rates, small bowel ade-
ocarcinomas and large bowel adenocarcinomas share a great
many characteristics. In particular, the adenoma-carcinoma se-
quence appears to operate in as significant a fashion in the small
bowel as in the large bowel.

Vogelstein and his coworkers (31–33) have identified
many of the molecular genetic changes that occur at various
stages in the adenoma-carcinoma sequence. It does not take a
great leap in imagination to hypothesize that a similar sequence
may be operating in the small bowel. To date, changes in ras,
p53, and the APC gene have been examined to a limited
degree. However, a systematic investigation of these and other
genetic changes in adenomas and adenocarcinomas needs to be
pursued. If these changes are not parallel in the two organs, it
would suggest that the resistance of the small bowel to carci-
nogenesis lies in a mechanism that prevents the occurrence of
certain molecular genetic changes. A mechanism for such re-
stance might be transferable to the large bowel.

Alternatively, if the multistage carcinogenesis process in
the small bowel were similar to that in the large bowel, at least at
the molecular genetic level, other mechanisms would need to be
sought that would explain the low-incidence rate in the small
bowel. The rapid turnover rate of the small bowel epithelium, for
example, could be used to explain why the molecular genetic
changes that appear to occur so commonly in the large bowel do
not have an equal opportunity to propagate in the small bowel.

This review article does not provide many answers regard-
ing the mechanisms or molecular genetic changes that play a
role in small bowel carcinogenesis. Instead, it raises these
issues to encourage others to pursue this line of inquiry. The
rarity of small bowel cancer makes its prevention or intensive
study unnecessary. Nonetheless, its similarity to the large bowel
suggests that lessons learned from its research might prove
helpful in our understanding and approach to large bowel
cancer and perhaps other cancers as well.

References
1. Ross, R. K., Hartnett, N. M., Bernstein, L., and Henderson, B. E. Epidemi-
3. Neugut, A. I., and Santos, J. The association between cancers of the small and
4. Sellner, F. Investigations on the significance of the adenoma-carcinoma se-
the jejunum occurring in a case of regional enteritis. Surgery (St. Louis), 39:

3 Unpublished data.
4 N. Arber, A. Neugut, I. B. Weinstein, and P. Holt, p53 in small bowel malign-
nancies, manuscript in preparation.

Downloaded from cebp.aacrjournals.org on March 30, 2021. © 1997 American Association for Cancer Research.
Molecular genetics of small bowel cancer.
Cancer Epidemiol Biomarkers Prev 1997;6:745-748.