Focus on colon cancer

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Epidemiology

The colon and rectum comprise the final portion of the human digestive tract, commencing at the ileocecal valve that marks the end of the small intestine, terminating at the anus, and measuring roughly one yard in length. Cancers of the colon and rectum are the second leading cause of cancer incidence and cancer death among adult Americans, with 135,000 new cases and 57,000 deaths in 2001, and with a 6% lifetime risk of developing the disease (Greenlee et al., 2001). Encouraging declines in the death rate from colorectal cancer in the last decade speak to the potential effectiveness of recent advances in prevention, screening, and therapy. Cancers of the colon arise from the colonic epithelial cells that line the lumen of the organ (Figure 1), which renew themselves every five days from a stem cell population located at the base of colonic epithelial cell crypts. Colon cancers are the end result of a multistep process of colon neoplasia that extends over several years (Figure 2). First, neoplastic tubular colon adenomas arise as pedunculated polypoid structures growing into the colon lumen. With time, they acquire increasingly disordered villous histology and dysplastic cellular cytology, and are recognized as frank cancers only when invasive cells breach the underlying epithelial basement membrane (Figure 1). Reproducible increases in incidence of the disease in populations that have migrated from low to high incidence regions of the world show the importance of environmental factors (Skibber et al., 2001). Cohort studies have rejected variations in intake of fiber, vegetables, and antioxidant vitamins as causative factors, but support that risk increases with red meat consumption, low folate intake, and sedentary lifestyle (Willett, 2001). The importance of genetic factors is shown by findings that germline mutations in key colon cancer genes give rise to familial hereditary colon cancer syndromes that account for 3%–7% of all cases annually (Kinzler and Vogelstein, 1996; Skibber et al., 2001). Genetic susceptibility factors also likely play a role in typical "sporadic" colon cancers, as indicated by the 2- to 3-fold increased risk of colon cancers in first degree relatives of persons affected by either colon cancer or by colon adenomas developing before age 60 (Skibber et al., 2001).

Screening

In concept, most colon cancers could be prevented by detection and removal of premalignant colon adenomas. Likewise, considerable benefit would be predicted by detecting colon cancers at early stages when the disease is amenable to cure by surgical excision. These considerations have lead to recommendations for mass screening starting at age 50 for the average risk adult population, and earlier for individuals at higher risk due to family history or other predisposing factors (Schoen, 2002; Smith et al., 2001). Available screening modalities include chemical testing for the presence of occult blood in the stool, endoscopic visualization of the lower portion of the colon by sigmoidoscopy, or full endoscopic visualization of the colon by colonoscopy, with sensitivities for detecting cancer of 15%–30%, 60%, and 90%, respectively (Schoen, 2002; Smith et al., 2001). The adoption of mass colonoscopic screening has been impeded by the expense of the procedure and the 24 hr required to undergo both a pretest laxative preparation and a posttest recovery from sedation. The recent successful detection of colon cancer-specific mutations in DNA from the feces of colon cancer patients has spurred considerable hope for the development of molecularly based screening (Ahlquist and Shuber, 2002; Traverso et al., 2002).

Staging, standard therapy, and outcomes

Colon and rectal cancers identified at early stages are highly treatable and often cured with standard therapies. Surgical resection of the involved colon is the initial treatment for colon and many rectal cancers. The likelihood of a recurrence is related to the degree of penetration of the tumor through the bowel wall and the presence or absence of nodal involvement (Skibber et al., 2001). These characteristics are the basis for the TNM clinical staging system shown in Figure 2 and guide recommendations for additional therapy.

Surgical resection is highly effective for early stage colon cancers, providing cure rates of over 90% in stage I and 75% in stage II disease. The presence of nodal involvement (stage III) predicts for a 60% likelihood for recurrence (Skibber et al., 2001). Treatment of these high-risk individuals with a postsurgical course of 5-Fluorouracil-based chemotherapy reduces the recurrence rate to 40%, increasing overall survival to 60%, and is now the standard of care for stage III patients (Skibber et al., 2001); however, newer adjuvant regimens are clearly needed to reduce the still substantial failure rate. The benefit of chemotherapy in stage II patients, who already have a good prognosis, has been harder to detect and remains controversial. There is certainly a need to identify better prognostic factors than TNM stage for guiding selection of individuals who will or will not benefit from adjuvant therapy. Initial reports suggest that good molecular prognostic markers (detailed below) include findings of microsatellite instability and TGF-β receptor II mutations (Watanabe et al., 2001), whereas adverse prognostic markers include allelic losses on chromosomes 8p and 18q (Zhou et al., 2002).

The management of rectal cancers is influenced by their having an increased risk of local recurrence and by the desire to maintain rectal sphincter function. The addition of radiation ther-

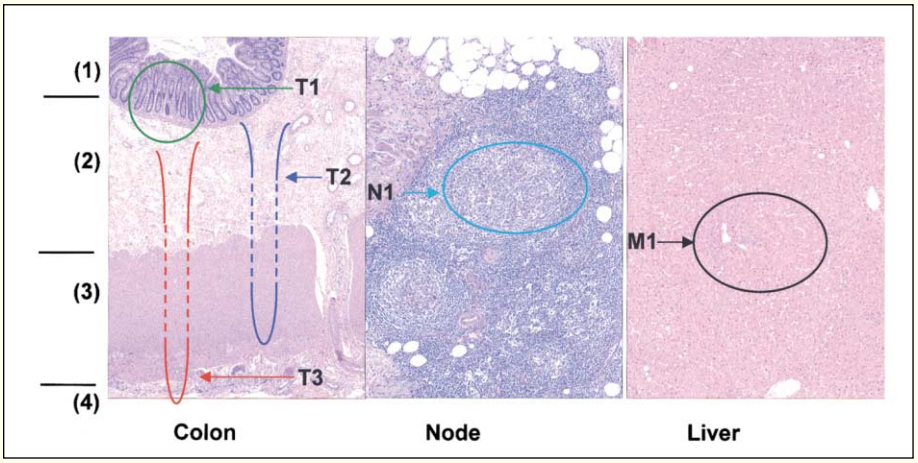

Figure 1. Clinical staging of colon cancer relative to the histology of the normal colon

Clinical staging of colon cancer relative to the histology of the normal colon. Shown are the principle layers of the colon including the mucosa (1), the submucosa (2), the muscle of the muscularis propria (3), and the subserosa (4). Also shown are micrographs of a lymph node in the colonic mesentery, and of the distant liver. The outlines show the extent of spread of different potential tumors and their stages as defined by the commonly employed TNM (tumor, nodes, metastasis) staging system. T1N0M0 (stage I) cancers are confined to the submucosa. T2N0M0 (also stage I) cancers invade the major muscular layer (muscularis propria). T3N0M0 (stage II) cancers breach the muscular layer. Cancers metastatic to mesenteric lymph nodes (N1–3) are designated stage III. Cancers metastatic to distant organ sites (M1) such as the liver are designated stage IV.

apy either before or after surgery decreases local recurrence, and the combination of chemotherapy and radiation therapy with surgical resection is the standard treatment for stage II and stage III rectal cancers (Skibber et al., 2001).

Metastatic colorectal cancer is present in 20% of individuals at the time of initial diagnosis and develops within five years in approximately 30% of those with initially localized disease. The liver and lung are the common sites for metastasis, along with peritoneal and local bowel recurrences. A small fraction of metastases are localized and can be surgically removed with a 30% cure rate (Skibber et al., 2001). When surgery is not feasible, treatment with 5-fluouracil-based systemic chemotherapy can have a palliative benefit with a modest increase in survival and quality of life, but most patients succumb to the metastatic disease within 2 years. Recently, several newer chemotherapies have been shown to provide an average improved survival of several months, including the topoisomerase I inhibitor irinotecan (Saltz et al., 2000) and the DNA adduct forming agent oxaliplatin. The major impact of these agents is likely to be as part of adjuvant therapies that are currently undergoing testing in clinical trials for high-risk patients following surgery.

Hereditary colon cancer

Germline mutations in the *APC* gene cause the inherited familial adenomatous polyposis (FAP) syndrome, in which hundreds to thousands of colonic adenomas develop during the third to fourth decade of life, and the lifetime risk of colon cancer approaches 100% (Goss and Groden, 2000; Kinzler and Vogelstein, 1996). *APC* is a classic tumor suppressor gene, with FAP tumors showing inactivation of the wild-type germline allele, most often by deletion (Goss and Groden, 2000; Kinzler and Vogelstein, 1996). APC protein functions as a suppressor of Wnt signaling by catalyzing the degradation of β-catenin, and thereby suppressing β-catenin mediated transcriptional activity (Goss and Groden, 2000; Kinzler and Vogelstein, 1996). The overwhelming majority of FAP associated *APC* mutations are nonsense mutations that truncate the APC protein amino terminal to the β-catenin interacting domain. Mutations at either the extreme 5′ or 3′ of the *APC* locus result in an attenuated polyposis phenotype. Intriguingly, adenomas in attenuated FAP often reveal somatic mutations targeting both the germline mutant as well as wild-type alleles (Goss and Groden, 2000; Kinzler and Vogelstein, 1996). Moreover, germline *APC* alleles with subtle 50% underexpression can also cause the full FAP phenotype (Yan et al., 2002). Lastly, an *APC* polymorphism (I1307K), recognized among the Ashkenazi Jewish population, creates a DNA sequence hotspot for subsequent somatic mutation, and this "premutation" increases colon cancer susceptibility approximately 2-fold (Fearon and Gruber, 2001).

Germline mutations in components of the DNA Mismatch Repair (MMR) complex are the genetic basis of hereditary nonpolyposis colon cancer (HNPCC) (Kinzler and Vogelstein, 1996; Kolodner, 1996; Markowitz, 2000). Carriers of these autosomal dominant mutations have an 80% lifetime risk of colon cancer, most often localized to the ascending colon, and an increased risk of gastric and endometrial cancers. Recent advances in "conversion" technology allow sequencing of individual germline alleles and show that over 90% of cases of classic HNPCC arise from mutations in *hMSH2* and *hMLH1*, which encode two required components of the mismatch repair complex (Yan et al., 2000). Moreover, germline mutations in *hMSH6*, which encodes a component present in a subset of repair complexes, have been associated with colon cancers with later age of onset and less striking familial aggregation than classic HNPCC (Kolodner et al., 1999). HNPCC tumors show somatic inactivation of the second germline MMR allele. MMR inactivation induces a nearly 1000-fold increased spontaneous gene mutation rates (Eshleman et al., 1995). This "mutator" phenotype accelerates the time for colon cancer development to less than 36 months. This reflects in part the creation of mutational hotspots within homopolymeric sequence repeats that are present in the coding regions of some tumor suppressor genes. The prototypical example is the biallelic mutational inactivation of the type II TGF-β receptor, discussed below (Markowitz, 2000; Markowitz et al., 1995). Moreover, MMR deficient tumors also accumulate frameshift mutations in over 40% of noncoding repetitive microsatellite alleles, and such "microsatellite instability" (MSI) is virtually diagnostic of MMR deficient cancers (Boland et al., 1998).

Somatic genetics of colon cancer

Genetic instability is a necessary molecular catalyst for colon carcinogenesis. Mismatch repair gene inactivation underlies genomic instability in the 13% of colon cancers with the MSI phenotype (Kinzler and Vogelstein, 1996; Markowitz, 2000). These sporadic MSI tumors are nearly always near diploid (Lengauer and Vogelstein, 1998). In contrast, chromosomal instability is demonstrated by microsatellite stable tumors that

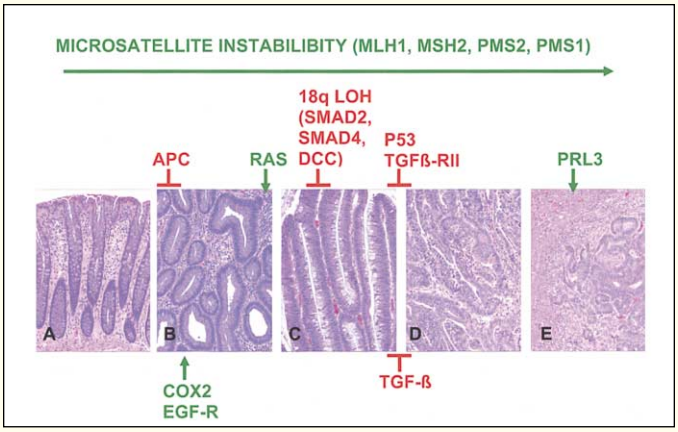

Figure 2. Colon cancer progression

Hematoxylin and eosin-stained sections show the progressive stages of colon neoplasia. **A:** normal colonic mucosa; **B:** tubular adenoma; **C:** villous adenoma; **D:** adenocarcinoma primary tumor; **E:** metastatic colon cancer to the liver. Shown above each stage are associated changes in tumor suppressor genes (designated by red lettering and red brakes) and oncogenes (designated by green lettering and green arrows). Mismatch repair gene inactivation is recognized by microsatellite instability and acts to accelerate the progression pathway through increased gene mutation rates. Shown below each stage are associated changes in growth factor activities. Activation of COX2 and EGF-R contributes to initiation of colon neoplasia, whereas TGF-β signaling suppresses neoplastic progression.

frequently show both chromosomal rearrangements and aneuploidies (Lengauer and Vogelstein, 1998). While rare mutations have been detected in BUB family genes that encode components of the mitotic spindle, the common basis of chromosomal instability remains unknown (Lengauer and Vogelstein, 1998).

MSI cancers largely arise in the right colon, most frequently in persons not belonging to HNPCC kindreds. The most common cause of these sporadic MSI tumors is epigenetic methylation affecting both *hMLH1* alleles (Markowitz, 2000). Such epigenetic methylation can serve as a tumor marker and has, for example, been detected preoperatively in blood from MSI colon cancer patients (Grady et al., 2001). Moreover, a propensity to aberrantly methylate multiple genomic loci may define a distinct class of colon cancers (dubbed CIMP) (Toyota et al., 1999). Confirming this model, and elucidating new genetic targets of aberrant methylation, are issues of great currency (Moinova et al., 2002).

Activation of the Wnt signaling pathway is an early event required for colon adenoma formation, and greater than 70% of nonfamilial colon cancers bear somatic *APC* mutations. Alternatively, some colon cancers activate Wnt signaling via activating mutations of β-catenin. Genes induced by Wnt activation include c-*myc*, *cyclin D1*, and *PPAR-δ* (Goss and Groden, 2000).

RAS mutations, mainly in K-*ras*, develop in 50% of large adenomas and 50% of colon cancers, and so are early steps in neoplastic progression (Fearon and Gruber, 2001).

Mutations inactivating the TGF-β receptor II (*TGFβ-RII*) gene arise in 30% of colon cancers and are temporally coincident with the further progression of colon adenomas to colon carcinomas (Markowitz, 2000; Markowitz et al., 1995). MSI cancers ubiquitously inactivate *TGFβ-RII* by frameshift mutations within a 10 base pair coding region poly-adenine repeat (Markowitz, 2000; Markowitz et al., 1995). In microsatellite stable colon cancers, TGF-β signaling is inactivated by somatic mutations in the *TGFβ-RII* kinase domain (15% of cases) or in *SMAD4* (15% of cases) or *SMAD2* (5% of cases) transcription factors that are phosphorylated by the TGF-β receptor complex (Markowitz, 2000).

Mutational inactivation of *p53* is also coincident with progression of colon adenomas to carcinomas. Greater than 50% of colon cancers bear *p53* mutations, and these mutations, first discovered in human colon cancers, are now recognized as the most common genetic event in human cancer (Fearon and Gruber, 2001).

Most recently, overexpression of the PRL-3 tyrosine phosphatase has been observed in metastatic colon cancers, and shown to be due to 25-fold gene amplification in 25% of cases, suggesting that PRL-3 is a direct genetic target contributing to metastatic progression (Saha et al., 2001).

Lastly, loss of Muc2 mucin expression, a frequent event in human colon adenomas and cancers, induces intestinal cancers in Muc2 knockout mice (Velcich et al., 2002).

Growth factor pathways are drug targets in colon cancer

Colon adenomas and colon cancers show increased levels of COX2 (Stack and DuBois, 2001), which with COX1 encodes enzymes that initiate the synthesis of prostaglandins and other eicosnoids. COX2 knockout mice demonstrate remarkable resistance to intestinal adenoma formation (Oshima et al., 1996), and nonsteroidal antiinflammatory drugs that inhibit COX2 induced shrinkage of some colon adenomas in human clinical trials (Stack and DuBois, 2001). Chronic use of COX2 inhibiting drugs, including aspirin, is associated with a decreased risk of human colon cancer, and the potential of these drugs for preventing colon adenomas and cancers is currently being evaluated in prospective clinical trials.

Studies in cell line and xenograft models suggest that autocrine activation of EGF-R and ErbB2, which are not amplified in colon cancer, is nonetheless requisite for growth of these cancer cells (Jiang et al., 1998), and is hence a target for anticancer therapeutics (Mendelsohn and Baselga, 2000). Pharmacologic or genetic inhibition of EGF-R activity impedes growth of human colon adenomas in culture and development of intestinal adenomas in murine models (Roberts et al., 2002; Torrance et al., 2000). Combining an anti-EGF-R monoclonal antibody, C225, with a cytotoxic agent, Irinotecan, gave significant antitumor effect in patients whose cancer was not responding to Irinotecan alone, implying that interruption of the EGFR signaling reversed resistance to Irinotecan (Saltz et al., 2001). While these initial studies need confirmation, they suggest that interruption of a growth factor signaling pathway overcomes resistance to a cytotoxic drug. Small molecules showing promise in preclinical models include inhibitors of EGF-R (Mendelsohn and Baselga, 2000), downstream inhibitors of the mitogen-activated protein (MAP) kinase pathway, and a rapamycin analog that targets TOR. However, disabling a single molecular target may not always give dramatic clinical effects, as shown by the lack of activity in the clinic of initially promising inhibitors of *RAS* oncogenes and of tumor angiogenesis.

Challenges

Colon cancer stands as a paradigm for our understanding of the molecular basis of human cancer. Much work remains to be done in discovering genes that contribute to such basic disease phenotypes as chromosomal instability and metastatic spread, and in translating the advances in understanding the molecular

biology of colon cancer into new clinical tools for prevention, early diagnosis, and better treatments of this disease.

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