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The Adenoma-Adenocarcinoma Sequence in the Large Bowel: Variations on a Theme

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Abstract Most adenocarcinomas of the colorectum arise in a visible benign precursor lesion, the adenoma, which is a monoclonal proliferation of dysplastic nonmalignant epithelial cells. The resultant adenoma-adenocarcinoma sequence represents the predominant pathogenetic pathway, in contrast to *de novo* carcinoma. Therefore, the adenoma is a tempting endpoint for chemoprevention trials.

The adenoma-adenocarcinoma sequence occurs in diverse clinical settings. In familial adenomatous polyposis (FAP) syndrome, autosomal dominant inheritance of the mutated APC (adenomatous polyposis coli) gene on chromosome 5q21 typically results in thousands of adenomas in the colorectum and in lesser numbers in the proximal small bowel. Adenocarcinoma usually develops in only a few of these adenomas, typically in the left colon and duodenum. In hereditary nonpolyposis colorectal cancer (HNPCC) syndrome, autosomal dominant inheritance of an unidentified gene appears to result in small numbers of adenomas which progress frequently to adenocarcinoma, predominantly in the right or transverse colon. In familial aggregation of colorectal cancer without a recognizable syndrome, cancer and/or adenomas occur in pedigree members. In "sporadic" cancers and adenomas, family history is absent and the tumors are mainly in the left colon.

Colorectal adenomas have variable characteristics including size, shape (polypoid vs. flat), villous architecture, and dysplasia. A variety of oncogenes and tumor suppressor genes are altered during progression. Epigenetic factors are important as evidenced by the disappearance of adenomas in FAP patients after ileorectal anastomosis or treatment with the nonsteroidal antiinflammatory drug sulindac.

Several variations on the theme of the adenoma-carcinoma sequence are evident. Identification of the inherited and acquired genetic alterations as well as the interacting environmental factors will provide a rational basis for chemoprevention. Conversely, effective chemopreventive agents will contribute to understanding of the critical pathogenetic mechanisms.

Key words: chemoprevention, colorectal adenoma, colorectal neoplasms, familial adenomatous polyposis (FAP), hereditary nonpolyposis colorectal cancer (HNPCC), intermediate biomarker

The pathogenesis of colorectal carcinoma is unique among human tumors. This common malignant tumor usually develops in a benign precursor lesion, the adenoma, which is visible on the mucosal surface of the large bowel, constituting an adenoma-adenocarcinoma sequence [1]. In some instances, the precursor adenoma remains detectable at the periphery of an infiltrating adenocarcinoma which arose in it. In addition, adenomas which contain no cancer are very frequent findings in the large bowel since most adenomas do not develop adenocarcinoma. Consequently, benign and malignant tumors at various stages of evolution can be identified and obtained from surgical resection specimens and colonoscopic polypectomy specimens. These tumors, including banked specimens, provide a valuable resource for study of the molecular and cellular biology of the stages of the adenoma-adenocarcinoma sequence in the large bowel. Furthermore, the grossly normal colorectal mucosa in patients with colorectal neoplasia has widespread abnormalities of epithelial proliferation and differentiation which may be antecedents to neoplasia [2]. By contrast, the precursor lesions of most other common human cancers are not as evident or as accessible.

MORPHOLOGIC CHARACTERISTICS OF THE ADENOMA-ADENOCARCINOMA SEQUENCE

Clinically significant adenocarcinoma in the large bowel is characterized by infiltration through the muscularis mucosae [3]. The histopathologic features of adenocarcinoma include abnormalities of glandular architecture, epithelial morphology, and cytologic characteristics. Rarely, lesions with the morphology of adenocarcinoma are seen invading the lamina propria but not beyond the muscularis mucosae. These intramucosal lesions do not constitute clinically significant adenocarcinoma because invasion across the muscularis mucosae has not occurred, and metastasis from carcinoma confined to the mucosa of the large bowel is extremely rare. Therefore, the term "adenocarcinoma" is best reserved for tumors which invade into the submucosa. From the investigative viewpoint, however, the cells of intramucosal adenocarcinoma do have the characteristics of cancer cells, e.g. invasion.

Adenocarcinoma is more common in adenomas which are large, have extensive villous architecture, and/or have severe epithelial dysplasia [1]. The adenomas which give rise to adenocarcinoma are heterogeneous, however, and rare examples of infiltrating carcinoma arising in small tubular adenomas with low-grade dysplasia do occur. Adenomas are characterized by dysplastic epithelium representing unequivocally neoplastic epithelial proliferation [4]. Adenomatous epithelium typically has increased numbers of spindle-shaped nuclei, often with stratification and loss of polarity as compared to non-neoplastic colorectal epithelium. The severity of the histopathologic abnormalities forms the basis for grading of dysplasia in adenomas."High-grade dysplasia" in adenomas includes "carcinoma in situ" (morphological adenocarcinoma confined within the basement membrane) and "intra-mucosal adenocarcinoma" (confined by the muscularis mucosae), neither of which have metastatic propensity.

Colorectal "polyp" is not synonymous with adenoma. Hamartomatous polyps including juvenile polyps and those of Peutz-Jegher's syndrome, hyperplastic (metaplastic) polyps with characteristic serrated epithelium and distended mucus vacules, and inflammatory polyps also occur in the large bowel [5]. Dysplasia in the form of an adenoma evolves areas of cancer in the usual pathogenesis of colorectal cancer. Dysplasia complicating chronic inflammatory bowel disease, including idiopathic inflammatory bowel disease and schistosomiasis, and dysplasia in hamarto-matous polyps can also lead to colorectal carcinoma but with different clinical and pathological circumstances than the adenoma-adenocarcinoma sequence [4,6,7]. "De novo" carcinoma arising in previously normal mucosa without antecedent dysplasia is extremely unusual, if it occurs at all. On the other hand, dysplastic lesions termed "flat adenomas" can lead to adenocarcinoma with only subtle evidence of the precursor lesion on the luminal surface of the large bowel [8], leading to confusion with "de novo" cancer.

EVIDENCE FOR THE ADENOMA-ADENOCARCINOMA SEQUENCE

The vast majority of colorectal carcinomas appear to arise in precursor adenomas, and a variety of lines of evidence supports the occurrence of the adenoma-adenocarcinoma sequence. As discussed above, histopathologic examination of adenomas sometimes shows small areas of infiltrating adenocarcinoma within the larger benign lesion. Thus, the adenoma appears morphologically to be the precursor lesion. In addition, the distribution of adenomas of the large bowel parallels the distribution of colorectal carcinomas, with both adenomas and carcinomas being most frequent in the sigmoid colon [9]. Epidemiologic studies have demonstrated increased rates of colorectal carcinoma in patients with adenomas as compared to the general population [10]. Adenomatous polyposis syndrome provides evidence for a temporal relationship. This rare syndrome is an autosomal dominantly inherited condition characterized by the development of hundreds to thousands of adenomas in the large bowel during adolescence or young adulthood, considerably younger than in the general population. Patients who are not treated by prophylactic colectomy develop colorectal adenocarcinoma at a mean age approximately three decades younger than in the general population [11]. Additional temporal evidence is provided by colono-scopic follow-up studies which show that the mean age of patients with adenomas is approximately five years younger than patients with invasive carcinomas [12]. This finding suggests a lag time between the occurrence of adenomas and carcinomas. Finally, in the era before colonoscopy was

available for relatively easy removal of colonic polyps without laparotomy, radiographically detected polyps were sometimes left untreated; in one study the cumulative occurrence of invasive carcinoma during follow-up was 35% at 20 years [13]. These various lines of evidence support a temporal as well as morphological adenoma-adenocarcinoma sequence in the large bowel.

MOLECULAR GENETICS OF THE ADENOMA-ADENOCARCINOMA SEQUENCE

Specific molecular genetic alterations in tumors representing the continuum of the adenoma-adenocarcinoma sequence have been evaluated. Both adenomas and adenocarcinomas are clonal as determined by use of X chromosome-linked polymorphic probes [14]. Aberrant DNA methylation is evident even in small tubular adenomas [15, 16]. Activating mutation of ras proto-oncogenes and abnormal expression of c-src gene are relatively early events as evidenced by high frequency of occurrence in adenomas [17,18]. By contrast, deletion of the DCC gene on chromosome 18q as well as mutation and deletion of the p53 gene on chromosome 17p are relatively later events, with high prevalence only in adenomas at the periphery of infiltrating carcinomas and in the carcinomas themselves [19,20]. Of particular note, the prevalence of p53 alteration increases remarkably from adenomas to carcinomas [21]. The increasing prevalence of these various abnormalities suggests a preferred temporal sequence of molecular genetic alterations in the adenoma-adenocarcinoma sequence. The events, however, are complex. First, not all of the characteristic molecular genetic alterations are usually evident in a given carcinoma. Furthermore, allelotyping demonstrates allelic deletions throughout the genome of some carcinomas, suggesting that there are additional specific molecular genetic alterations of interest [22]. Also, comparison of the alterations in a group of adenomas to those in the carcinomas which arose in them demonstrates many exceptions to the preferred sequence. For example, ras gene mutation is sometimes found in a carcinoma but not in the adenoma at its periphery, whereas ras gene mutation is usually considered to be an early event based on the prevalence data. Thus, the molecular genetics of the adenoma-adenocarcinoma sequence appear to involve accumulation of several alterations in both oncogenes and tumor suppressor genes.

INHERITED SYNDROMES WITH THE ADENOMA-ADENOCARCINOMA SEQUENCE

Inherited predisposition plays an important role in the development of colorectal adenocarcinoma. Two autosomal dominant syndromes have been identified. As discussed earlier, the adenomatous polyposis syndrome is characterized by the occurrence of hundreds to thousands of colorectal adenomas, and adenocarcinoma of the large bowel is inevitable in patients who do not undergo prophylactic removal of the large bowel. This syndrome is due to germline abnormality of the APC gene (adenomatous polyposis coli) on the long arm of chromosome 5, (most frequently missense mutation or deletion resulting in a downstream stop codon which lead to truncated gene product), and inheritance of the abnormality accounts for familial adenomatous polyposis [23,24]. Altered epithelial proliferation and differentiation is evident in the large bowel mucosa of affected patients even before the appearance of adenomas [25]. Thus, the normal function of the APC gene product appears to be related to control of cellular kinetics and differentiation. Extracolonic manifestations occur in some patients and kindreds with adenomatous polyposis. Adenomas of the duodenum, especially the periampullary region, are frequent. Pigmented ocular fundic lesions characterized by hypertrophy and hyperplasia of the retinal pigment epithelium are common [26,27]. The eponym "Gardner syndrome" is applied to the occurrence of adenomatous polyposis along with epidermal inclusion cysts, osteomas, odontomas, and cutaneous fibromas [11,28]. The eponym "Turcot syndrome" is applied to brain tumors, especially medulloblastoma and glioblastoma multiforme, in association with adenomatous polyposis [29]. Abnormality of the APC gene appears to account for adenomatous polyposis without extracolonic manifestations as well as with the additional findings. Of note, colorectal carcinomas in patients with adenomatous polyposis are usually on the left side, similar to "sporadic" colorectal carcinoma. Also of note, the number of colorectal cancers which occur in an individual patient with adenomatous polyposis syndrome is tiny compared to the very large number of adenomas. Thus, the complete adenoma-adenocarcinoma sequence occurs in only a very few of the precursor adenomas in this syndrome and is probably influenced by environmental factors.

Specific factors modulating the phenotype of adenomatous polyposis remain to be defined. Some pedigrees have relatively sparse adenomas whereas the mucosal surface is completely carpeted in other families [30]. Sometimes members of a family, who have inherited the same abnormal APC gene, have strikingly different numbers of colorectal adenomas. Likewise, the heterogeneity of extracolonic manifestations within and between families remains to be explained.

The second autosomal dominant syndrome which follows the adenoma-adenocarcinoma sequence is hereditary nonpolyposis colorectal cancer syndrome (HNPCC or Lynch syndrome). This syndrome is characterized by the occurrence of colorectal cancer at young age in families with an autosomal dominant pattern of inheritance, a predominance of proximal involvement of the colon, increased multiplicity, and possibly improved stage-specific survival [31]. Patients in pedigrees with HNPCC have few adenomas, hence the term "nonpolyposis". Recognition of HNPCC is often difficult because the individual patients lack the distinctive phenotype of adenomatous polyposis syndrome. A current working definition of HNPCC is the presence of 3 members of a family with colorectal carcinoma, with one of the affected members being a first degree relative of another, and the occurrence of colorectal carcinoma at less than 50 years of age in at least one member [32]. In addition to colorectal carcinoma, other cancers, especially endometrial and ovarian carcinoma, occur in some families. Additional sites at increased risk include the ureter and renal pelvis for transitional cell carcinoma, and the pancreas, biliary tree, small bowel and stomach for adenocarcinoma [33]. The precursor colorectal adenoma in HNPCC is sometimes difficult to identify and has been termed "flat adenoma". In contrast to adenomatous polyposis, the adenoma-adenocarcinoma sequence is frequent in the small number of adenomas in HNPCC and usually occurs in the right or transverse colon. The molecular genetic abnormality responsible for HNPCC has not yet been identified. The DCC gene on chromosome 18q has been excluded [34], despite previous reports of linkage of HNPCC to Kidd blood group which is also located on chromosome 18.

In addition to these two syndromes associated with colorectal carcinoma, familial aggregation without an apparent syndrome is frequent. First-degree relatives of patients with colorectal carcinoma or adenoma have approximately a two- to four-fold increased risk of colorectal carcinoma as compared to the general population [35]. Studies in large Mormon pedigrees have suggested that predisposition to colorectal adenoma is inherited as an autosomal dominant trait with high frequency but low penetrance [36]. The identification of a susceptibility gene would have a dramatic impact on screening strategies by allowing genetic testing which can currently be done only for adenomatous polyposis syndrome. The contribution of inheritance to the occurrence of "sporadic" colorectal neoplasia remains to be determined.

CHEMOPROTECTION AND THE ADENOMA-ADENOCARCINOMA SEQUENCE

As discussed above, colorectal adenomas are premalignant neoplastic lesions which indicate individuals at increased risk for colorectal carcinoma. These precursors, therefore, are tempting target lesions for chemoprotection studies. Patients with adenomatous polyposis due to abnormal APC gene have been studied in chemoprotection trials. High dietary fiber and vitamins C and E [37] have shown evidence of reducing the numbers of adenomas in these patients who represent the most obvious example of genetic susceptibility to colorectal neoplasia. More dramatic resolutions of adenomas in polyposis patients have occurred with administration of the nonsteroidal anti-inflammatory drug sulindac, a prostaglandin synthesis inhibitor via its effects on cyclooxygenase [38-40]. Of note, in one study epithelial cell proliferation as evaluated by Ki-67 immunohistochemistry for an antigen expressed in cycling cells was not altered in either adenomas or nonpolypoid mucosa, despite the disappearance of adenomas [39]. This disparity between the effects of the drug on neoplasia and on intermediate biomarkers may have implications for selection of endpoints for chemoprotection trials.

The potential impact of genetic predisposition to colorectal neoplasia has not been considered in most chemoprotection trials of patients with "sporadic" adenomas due to the lack of identifiable molecular genetic susceptibility other than the APC gene. Identification of the inherited and acquired genetic alterations as well as the interacting environmental factors

(especially dietary factors) important in the genesis of colorectal carcinoma may provide a specific basis for chemoprotection. On the other hand, numerous molecular biological, cell biological, and phenotypic abnormalities have been identified in the adenoma-adenocarcinoma sequence. Pharmacologic agents which mimic the effect of tumor suppressor genes inactivated in the adenoma-adenocarcinoma sequence, or oppose the actions of oncogenes, may be developed. In turn, the identification of effective chemoprotective agents will contribute to understanding of the critical pathogenetic mechanisms via their efficacious interaction with specific pathways.

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