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OVERVIEW

Potential Utility of Differentiation Markers as Intermediate Biomarkers in Colon Carcinogenesis

Alterations in growth regulation and differentiation occur early in multistep carcinogenesis. Phenotypic and genotypic markers that reflect these changes in early stages of colon carcinogenesis will be very useful tools in identifying subjects at high risk for developing colon cancer, but may also be useful in assessing the efficacy of chemopreventive measures. The colonic mucosa is composed of many tubular glands which are lined by several types of epithelial cells. Some of the cell lineage-associated differentiation markers have been identified in both normal and cancerous colon. These include: 1) the phenotypic markers that are expressed by cells of a particular lineage in an advanced stage of differentiation or maturation; 2) phenotypic markers that are expressed in a stage-specific manner during fetal colon development; these are also expressed in premalignant and malignant colon but not in adult colon. In this session, altered expression of colonic cell-associated differentiation markers, such as carbohydrate-associated antigens, growth factors and their receptors, and of cytoskeletal elements in mature and immature colonocytes, preneoplastic and neoplastic colon are discussed.

The session entitled "Colon Marker III: Differentiation Markers" included presentations by five investigators.

An overview of glycoconjugate structure, which included details on the structure of glycolipids, glycoproteins, and the *N*-glycosidic and *O*-glycosidic carbohydrate linkages found on glycoproteins, was given by Y.S. Kim. Attention has been focused on mucin-type glycoproteins over the past 5-10 years because it is now appreciated that they are the locus of many neoplasm-associated structural changes.

Progress in this area has been enhanced by the recent cloning of the genes for four different mucin-apoproteins. Each of these shares a tandem repeat motif, but each shows unique sequences in the tandem repeat sequence and each gene is located on a different chromosome. Two of these genes, MUC2 and MUC3, are expressed in the normal colon, whereas MUC1 is not. MUC1, MUC2, and MUC3 are all expressed in some, but not all, colon cancers. A model of altered glycosylation of mucins and colon cancer was proposed in which the cancer-associated mucins may contain fewer oligosaccharide chains that might permit a greater exposure of the peptide core, and populations of shorter and modified oligosaccharide chains in the tandem repeat regions which may serve as tumor markers.

The role of the blood group antigen expression as an intermediate marker of neoplasia in the large intestine was discussed by S. Itzkowitz. The proximal colon normally retains its fetal blood group antigen expression into adulthood, and this tends to be lost in cancers. The distal colon is distinct from the proximal colon in that fetal blood group expression is normally lost in adult life, and certain of them are re-expressed in tumors. Both proximal and distal colon may show the appearance of incompatible blood group antigens in neoplastic tissues as well. These patterns of inappropriate blood group expression are seen in benign adenomatous polyps, with a pattern that is generally proportional to the size and degree of dysplasia in the polyp. Interestingly, hyperplastic polyps, which are not neoplastic, show some of these changes at reduced frequency. Immunohistological data derived from the Lewis antigens and modifications of the T-antigen family were presented. The most important of these was the sialyl Tn antigen, whose expression correlates well with tumor progression in preneoplastic lesions, and whose appearance in colon cancer correlates with a poorer prognosis. Patients with ulcerative colitis who have developed high grade dysplasia or cancer were likely to have expressed sialyl Tn-antigen in prior biopsies (when studied retrospectively). Sialyl Tn-antigen

tends not to be expressed in hyperplastic polyps.

The use of lectins as markers of differentiation and tumor progression in the human colon was discussed by C.R. Boland. The lectins DBA and RCA1 preferentially labeled goblet cells in the upper portion of the normal colonic crypt, whereas other lectins preferentially labeled lower in the crypt. Peanut agglutinin (PNA) binds to mucin in larger tubular adenomas, colon cancer, transitional epithelium, and in preneoplastic tissue in two animal models. PNA also binds to goblet cell mucin in hyperplastic polyps. A biometric system was devised to give lectin binding scores ranging from 0-400 in rectal biopsies taken from patients at increased risk for colon cancer. Flat, normal-appearing epithelium of patients with familial polyposis had significant changes in their lectin binding, including reductions in binding by DBA. A population of patients with hereditary nonpolyposis colon cancer (HNPCC) also showed a reduction in DBA binding, but none of these tissues show labeling by PNA. The lectin amaranthin (ACA) binds best to a modified T-antigen structure, and labels the proliferation region of the normal colonic crypt in the distal human colon. The lectin is not reliable as a marker in the proximal colon. Studies were performed in which proliferation was measured using BrdU incorporation and compared with ACA binding. Adenomatous and carcinomatous tissues showed significant increases in labeling and a loss of compartmentalization using either BrdU incorporation or ACA binding. ACA also bound in the superficial portion of the normal-appearing colonic epithelium in both familial adenomatous polyposis and HNPCC.

The role of tumor growth factors (TGF) in the colon was reviewed by R. Coffey. TGF-alpha binds specifically to the EGF receptor which is expressed throughout the colon. TGF-alpha expression is relatively low in the colon and its role in colorectal neoplasia remains uncertain. However, two transgenic animal models have been developed in which the TGF-alpha gene has been placed under the control of different promoters. TGF-alpha driven by a steroid-inducible promoter results in mice that develop mammary carcinomas. Transgenic expression begins at approximately five weeks and produces elevated circulating levels of the peptide. Tumor expression is localized to a small number of tissues. Curiously, TGF-alpha expression is greater in the villus than in the crypt, based upon immunohistochemistry and message production on serially eluted cells from villus to crypt. It appears that TGF-alpha may play an important role in regulating normal and neoplastic growth in the colon; however, the details remain to be elucidated.

Cytoskeletal markers in the GI tract were summarized by S.B. Ho. An overview of cytoskeletal constituents and assembly was provided, including the roles of villin, fodrin, intermediate filaments, cytokeratins, desmin, vimentin, and others. Cytoskeletal elements are dynamic proteins that require assembly and turnover repeatedly throughout the life cycle of an epithelial cell. Limited information is available at present on abnormalities that occur during disordered differentiation. Abnormalities of actin have been reported in sporadic adenomas, carcinomas, and in normal-appearing tissue of patients with familial polyposis. The impact of cytoskeletal abnormalities in the expression of microvillar enzymes has been not fully explored. Preliminary data suggest that normal cytokeratin expression may accompany certain premalignant conditions. Of interest, the recently cloned APC gene shares some structural similarity to intermediate filaments.

To summarize, besides their association with colon cancer as tumor markers, some of these changes in the expression of differentiation markers have also been observed in premalignant, immature and regenerating colonic epithelial cells as well as in the mucosa of patients with ulcerative colitis and in the flat mucosa of affected members of familial polyposis coli. Further detailed prospective and retrospective studies using a combination of these and other differentiation markers are needed to assess their usefulness as intermediate endpoint markers.

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