Journal of Cellular Biochemistry, Supplement 16G:119-128 (1992)

Cytoskeleton and Other Differentiation Markers in the Colon

Samuel B. Ho

Department of Medicine, University of Minnesota and VA Medical Center, Minneapolis, Minnesota 55417

Abstract Differentiation of intestinal epithelial cells involves a complex process of establishment of cell polarity, commitment to cell lineage, and inhibition of cell division. Polarized epithelial cells are characterized by specific junctional complexes and cytoskeletal proteins which produce specific membrane domains. Intestinal cytoskeletal proteins are often preserved in neoplastic colonic tissues, and can be used to identify the cell of origin of poorly differentiated cancers. In this context, these proteins are markers of organ-specific differentiation. In addition, since loss of cytoskeletal polarity commonly occurs in transformed cells, aberrant expression of these proteins may be used as a marker of neoplasia in the colon. Normal polarization of basolateral proteins (secretory component) and apical proteins such as brush border hydrolases, cytoskeletal proteins (villin, fodrin), and carcinoembryonic antigen can become disrupted in adenomas and cancers. Cytoskeletal intermediate filaments (cytokeratins) demonstrate increased immunoreactivity in villous adenomas and cancers compared with normal colonic crypts. Altered actin bundles are found in preneoplastic mucosa such as colon from patients with familial polyposis coli. Molecular mechanisms responsible for altered cytoskeletal structures remain unclear; however, altered protein phosphorylation most likely plays a role. For example, the phosphorylation status of cytoskeletal and junctional complex proteins appears to influence their solubility and interactive properties, which may result in altered cell polarity. Markers of altered cytoskeletal structure and polarity can identify neoplastic colonocytes; however, the extent to which they can be used as intermediate markers of colonic neoplasia remains to be determined. © 1992 Wiley-Liss, Inc.

Key words: actin, brush border enzymes, cell polarity, chemoprevention, colorectal neoplasms, cytokeratins, cytoskeleton, cytoskeletal proteins, fodrin, integrins, intermediate biomarker, junctional complex, protein phosphorylation, villin

Cytoskeletal proteins are responsible for maintenance of cell polarity, motility, adhesion, differentiation and mitosis [1]. Loss of cytostructural integrity is a well known component of cellular transformation, and several cytostructural alterations, such as the loss of polarity and pseudostratification, are hallmarks of the morphologic diagnosis of dysplasia [2,3,4]. In the last several years numerous advances have been made in the characterization of the structure and function of cytoskeletal proteins in epithelial cells. This review will focus on intestinal cytoskeletal and related proteins and their alterations in colonic neoplasia and preneoplasia. The studies to date suggest that specific cytoskeletal markers have the potential to serve as markers of early cellular transformation in the colon.

Address reprint requests to Samuel B. Ho, VA Medical Center, Dept. of Gastroenterology (111-D), 1 Veterans Drive, Minneapolis MN 55417

© 1992 Wiley-Liss, Inc.

CYTOSTRUCTURAL ORGANIZATION OF INTESTINAL EPITHELIAL CELLS

In the normal intestine immature cells are produced by dividing stem cells in the crypt base. Differentiation of intestinal and colonic cells begins in the lower one third of the crypt and continues as these cells migrate to the luminal surface. This process of differentiation involves: 1) establishment of spatial organization by junctional complexes, 2) targeting enzymes, carrier proteins, and specific phospholipids to apical and basolateral membrane domains, 3) synthesis of specific cell lineage related proteins, and 4) loss of proliferative ability [5]. Ultrastructural studies

reveal that terminally differentiated colonic absorptive cells are characterized by numerous well formed microvilli, well developed apical tight junctions with a single apical desmosome, apical mitochondria, and scattered multivesicular bodies, lipid droplets, and lysosomes. Mature goblet cells have a distended cup-like collection of mucin granules in the apical portion of the cell, a sparse microvillar apical membrane, and a narrow, dense basal cytoplasm [6,7].

The structural components of a differentiated intestinal columnar cell are illustrated in Figure 1. Junctional complex proteins are responsible for the division of the cell into two distinct membrane domains: the luminal microvillus membrane and the basolateral membrane [5]. Proteins specific for the basolateral membrane include Na/K ATPase, histocompatibility antigens, adenylate cyclase, secretory component, and transferrin receptor. The microvillus "brush border" membrane is characterized by hydrolytic enzymes and transport systems designed for oligopeptide and carbohydrate digestion and solute or water resorption. Hydrolytic enzymes such as sucrase-isomaltase, dipeptidyl peptidase IV, aminopeptidase A/N, and alkaline phos-

Cytoskeletal Organization of Intestinal Epithelium

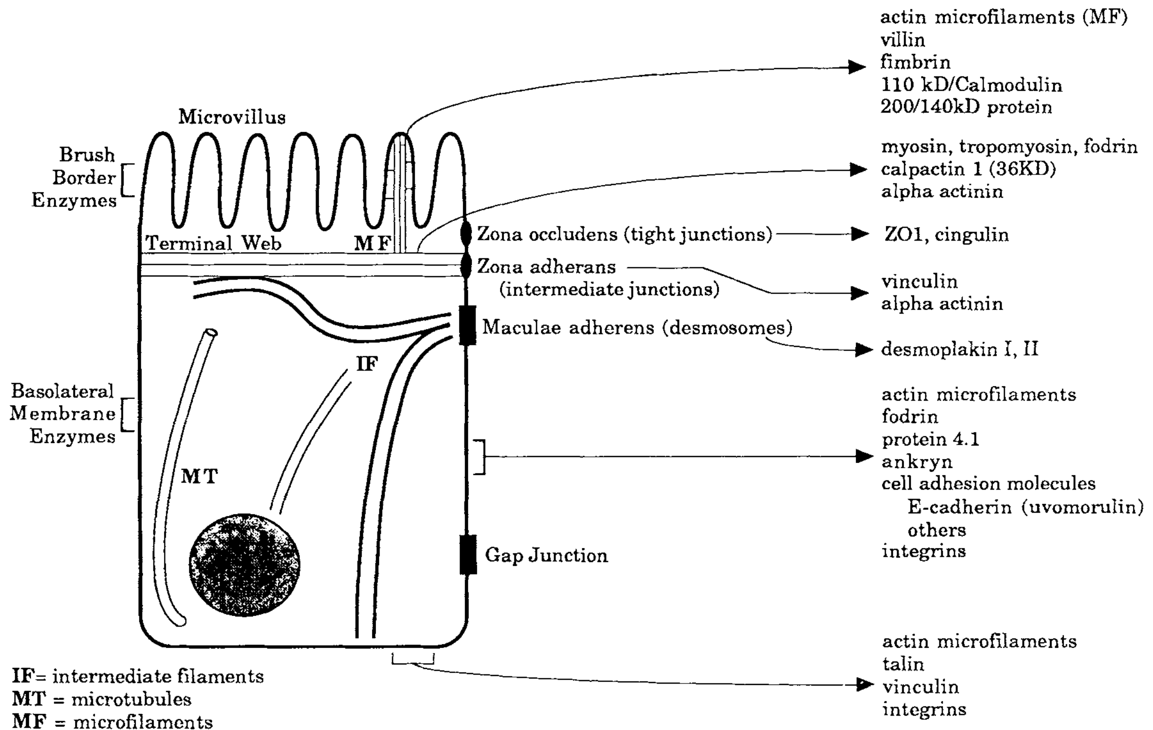

Fig. 1. The cytoskeletal framework of intestinal epithelial cells consists of actin microfilaments, intermediate filaments and microtubules. The junctional complex located on the lateral membrane has four components: 1) The zona occludens or tight junction is the "fence" that maintains the protein and lipid differences between the apical and basolateral membranes. The zona occludens also regulates the permeability of the paracellular pathway. 2) The zona adherens or intermediate junction and desmosomes maintain the structural integrity of the monolayer. 3) The gap junctions are protein channels that allow for intercellular communication. 4) The cell adhesion proteins along the basolateral membrane regulate cell/cell recognition and cell substratum adhesion [see reference 5]. The structural proteins associated with the intestinal brush border, junctional complexes, and basolateral membranes are listed.

phatase are present in colonocytes, albeit in lesser quantities compared with small bowel enterocytes.

The framework of the cell consists of actin microfilaments, intermediate filaments and microtubules. Actin filaments are responsible for both structural and mechanical properties of the cell matrix [1]. Actin exists as bundles (F-actin) or as monomers (G-actin), and is controlled by a diverse group of actin-binding proteins. Actin filaments are attached to membrane proteins (such as Na/K ATPase) and sites of cell-substratum adhesion via direct or indirect linkages to fodrin, ankyrin, protein 4.1, vinculin, 110 kD protein, or talin. This linkage may serve to anchor and segregate membrane proteins [5]. Intermediate filaments in epithelial cells consist of the multigene family of cytokeratins. Cytokeratin filaments are composed of bundles and form the skeletal framework of the cytoplasm, and are bound between terminal webb, desmosomes, and hemidesmosomes. They also may serve to bind nuclear lamins and integral membrane proteins such as ankyrin, forming a link between the nucleus and the peripheral membrane [8]. Microtubules are aligned with the apical/basal axis of the cell and determine the orientation of various organelles (endoplasmic reticulum, golgi apparatus, lysosomes) and the distribution of actin microfilaments [4,5].

The cytoskeleton of intestinal brush borders can be divided into two distinct areas--the microvilli and the terminal web [9]. Each microvillus contains a bundle of 20-30 actin microfilaments extending from the tip of the microvillus to the terminal web. The microfilaments are connected laterally by short microfilaments spaced regularly along the longitudinal axis. Two polypeptides of 68 kD (fimbrin) and 95 kD (villin) bind the actin filaments along its entire length. This microvillus core is connected to the plasma membrane by cross bridges. These connections are thought to contain a 110 kD protein which binds tightly to calmodulin. This protein complex is anchored by a 140 kD transmembrane glycoprotein. The terminal web has three filament networks: 1) the microfilament rootlets which are cross-linked by filamentous proteins fodrin, myosin, and calpactin 1; 2) the zona adherens bundle of actin microfilaments bound by myosin, tropomyosin, and actinin; and 3) the intermediate filaments. The zona adherens bundle and intermediate filaments form circumferential rings perpendicular to the microvilli [10,11].

The apical brush border of intestinal goblet cells is characterized by a sparse population of microvilli. They are structurally similar to absorptive cell microvilli; however, less is known about their associated actin binding proteins. Mucin granules are surrounded by a filament rich cytoplasm (theca) that separates the granules from the plasma membrane. The theca contains vertically oriented microtubules and circumferential intermediate filaments [12].

ULTRASTRUCTURAL ALTERATIONS OF NEOPLASTIC AND PRENEOPLASTIC COLONIC CELLS

Numerous ultrastructural studies have described the structural alterations of malignant colonic cells. In addition, several studies have examined the cytostructural alterations of colonic cells in premalignant and preneoplastic colonic mucosa. Cells with immature ultrastructural features are limited to the crypt base in normal colon [7]. Adenomas are characterized by the appearance of numerous structurally immature cells distributed along the full length of the adenomatous crypts. These immature cells are characterized by irregular microvilli, multiple desmosomes, many free ribosomes, and occasional cells with concentrations of secretory droplets at the apical pole. Both the apical and lateral membrane domains are poorly formed in immature cells. Some adenomatous cells can be identified by the complete lack of normal apical surface structures [7]. Similarly, preneoplastic colonic mucosa is characterized by the presence of increased numbers of cytostructurally immature cells. The histologically normal mucosa of familial polyposis patients was compared with rectal mucosa from patients with no known gastrointestinal disease. Ultrastructural alterations were present in 56% of the glandular structures of the familial polyposis patients examined and were not present in the controls. These alterations consisted of: 1) the presence of immature and undifferentiated cells in both the crypt base and mid crypt regions, 2) cells with increased vesiculation and small electron-dense granules, and 3) variation in goblet cells with increased numbers of atypical secretory cells [13]. Similar ultrastructural changes were demonstrated in transitional colonic

mucosa adjacent to cancer [14]. However, the specificity of these ultrastructural findings for preneoplastic colon has not been determined in rigorous studies using age and site-matched controls.

MEMBRANE DOMAIN MARKERS AND CELL POLARITY

If microvillar structures are altered in neoplasia, are microvillar enzymes also altered? Several studies have examined expression of brush border hydrolases, such as sucrase isomaltase, aminopeptidase, dipeptidylpeptidase IV, maltase-glucoamylase, and lactase. These enzymes are present in high amounts on apical membranes of fetal colon and small intestine, but they are present in small amounts, if at all, in normal colon, detection of which depends on the type of method and antibody used.

Most studies indicate that these enzymes act as oncofetal markers in the colon, and are more commonly expressed in adenomas, carcinomas, and possibly "pre-neoplastic" transitional mucosa (Table 1). Bealieu *et al.* [15] demonstrated that colonic sucrase-isomaltase was immunologically and biochemically distinct from small intestinal sucrase-isomaltase. These differences are thought to occur because of changes in degradation or cotranslational processing of sucrase-isomaltase. Sucrase-isomaltase immunofluorescence (using monoclonal antibodies which only recognize sucrase-isomaltase present in jejunal crypt cells) was present in the cells of the crypt base in "normal" colon; however, staining also extended to the surface epithelium in some specimens of transitional mucosa adjacent to cancers. Positive staining was present on the surface epithelium of 62% of adenomas; however, colon cancers were rarely positive in this study.

Using highly sensitive PCR methods for detection of sucrase-isomaltase mRNA, Wiltz *et al.* found 1.5-100-fold higher sucrase-isomaltase mRNA in cancers compared with normal colon, and immunoreactive sucrase-isomaltase (using a polyclonal antibody) was present in 80% of all cancers and adenomas [16,17]. Distribution was mainly cytoplasmic rather than localized to the apical membrane. Normal or transitional colonic mucosa was rarely positive with this antisera. Other preneoplastic colonic tissues have not been examined.

These data suggest that sucrase-isomaltase is altered in neoplastic cells in quantity, quality, and perhaps distribution. The mechanism involves translational and posttranslational changes and may reflect altered cell polarity or maturity. Few studies have examined high risk or preneoplastic colonic mucosa. Since transitional mucosa adjacent to carcinomas have been shown to differ from "normal" colon, further studies of sucrase-

TABLE I. Brush border hydrolases in colonic epithelium detected by immunocytochemistry
(number positive/ total number specimens)

	S-I*	S-I	S-I	S-I	APN	DPP-IV	MGA	L
Normal colon	55/58 (crypt cells only)	5/6 (focal)	0	0/15	0/15	0/15	0/15	0/15
Distant TM**		29/42						
Adjacent TM		31/41	rare	9/11	0/11	0/11		
Adenomas	10/16	18/21	23/32					
Carcinomas	4/61	22/57	33/39	7/27	7/27	7/27	0/27	0/27
Antibody***	Mab HSI-3, HSI-9,HSI-12	Mab HBB 2/614/88	Poly-clonal	Mab HBB 2/614/88	Mab HBB 3/153/63	Mab HBB 3/775/42	Mab HBB 2/143/17	Mab HBB 1/909/34/74
Detection method	IF****	IF	IP	IF	IF	IF	IF	IF
Reference	15	54	16,17	55	55	55	55	55

* S-I = sucrase isomaltase; APN = aminopeptidase N; DPP-IV = dipeptidylpeptidase IV; MGA = maltase-glucoamylase; L = lipase
** TM = transitional mucosa ***Mab = monoclonal antibody
**** IF = immunofluorescence; IP = immunoperoxidase

isomaltase in other preneoplastic tissues using a variety of antibodies and detection methods are warranted.

Other membrane specific markers, such as CEA and secretory component, lose their polarized expression in colon adenomas and carcinomas [18,19]. However, loss of CEA polarization has not been observed in specimens of preneoplastic colon. For example, CEA localization to the apical membrane was similar in specimens of colonic mucosa from non-neoplastic controls and specimens of histologically normal mucosa adjacent to colon cancers [20]. Secretory component is normally expressed on the basolateral membranes of colonocytes. In premalignant mucosa (adenomas and ulcerative colitis) secretory component is often expressed as in normal mucosa; however, expression is decreased or lost in dysplastic areas [19]. Secretory component expression in gastric intestinal metaplasia was localized to basolateral membranes; however, this polarized expression was lost in gastric cancers, resulting in secretory component localization over the entire cell surface [21]. Loss of polarity of membrane specific markers may be due to the disruption of the tight junction "fence" between apical and basolateral domains, as has been demonstrated to occur in carcinomas. The data to date indicate that loss of CEA or secretory component polarization is a relatively late phenomenon in the hyperplasia-dysplasia-carcinoma sequence. More data are needed before it can be determined how sensitive these markers are for preneoplastic changes such as might occur in aberrant or hyperproliferative crypts.

ACTIN MICROFILAMENTS

Actin disorganization is a characteristic of a variety of transformed cell lines. Friedman *et al.* studied actin organization in epithelial patches grown from explants of human colon in short term tissue cultures [22]. Actin was visualized by staining with rhodamine-conjugated phalloidin, which binds to actin. Specimens included biopsies from normal individuals with no family history of colon cancer; the histologically normal tissue from patients with familial polyposis (FP); the normal tissue from children of polyposis patients (who are at risk for FP); adenomas from FP and sporadic polyp patients; and explants from carcinomas.

These tissues can be considered a model of stepwise carcinogenesis in the colon, and a two-point modification of actin organization was observed. Actin cable organization present in normal colon was not present in preneoplastic FP tissue. Actin cables were then observed in the benign adenoma cell and lost again with the benign tumor to malignant tumor transition. The cause of this two point modification of the actin cytoskeleton is unknown. The modification of actin cytoskeleton may be due to actin binding proteins, cell attachment factors, or other regulatory molecules. It should be noted that dermal fibroblasts from FP individuals have been shown to contain fewer organized microfilaments compared to skin cells from normal individuals [23]. These data indicate that actin alteration can occur in preneoplastic tissue, and further studies of familial polyposis and other preneoplastic colonic mucosa are needed.

ACTIN BINDING PROTEINS

Several recent studies using actin binding protein-specific reagents have found that expression of these proteins also becomes altered in colonic neoplasia. One of the most intensively studied actin binding proteins is villin. Villin is found only in large amounts within brush borders, such as in differentiated intestine and kidney proximal tubule cells. Lesser amounts of villin are found in tissues embryologically related to intestine, such as pancreatic duct cells and biliary duct cells. In contrast, the actin binding protein fimbrin is ubiquitous. Villin has three calcium binding sites per molecule and acts to either bundle or sever actin molecules at low and high calcium concentrations, respectively. Severing activity is also regulated by phosphoinositides [10,24].

Microvillus actin binding proteins are assembled in a precise chronological order in the embryo. In undifferentiated cells of the intestinal tube of the chicken, Shibayama *et al.* [25] found villin, fimbrin, and the 110 kD complex diffusely distributed in the cytoplasm. As terminal differentiation of enterocytes occurred in late embryogenesis, first villin localized to the apex, then fimbrin two days later, then 110 kD complex last when the chicken hatched. Fibroblast cells transfected with full length villin cDNA developed microvilli along the dorsal cell surface, indicating that villin

may play a key role in the morphogenesis of microvilli [26].

Since villin is an important protein of the differentiated microvillus, how is villin altered in precancer and cancer? First, villin expression detected by monoclonal antibodies in adenocarcinomas reflects the distribution of villin in embryonic tissue [27,28,29]. In this respect it is an embryonic type differentiation marker, and may be of some use in determining the cell of origin of a particular cancer.

Immunohistochemical studies of colonic tissues indicate that villin is localized to the brush border of normal ileum and luminal border of normal colon, and is expressed with increasing staining intensity as cells migrate from crypt to surface [30]. West *et al.* observed villin immunoreactivity on luminal borders in most adenomas and cancers, but the normal polarized distribution of villin was lost in approximately 50% of these specimens [30]. In these cases cytoplasmic or basement membrane and lateral membrane staining was observed. However, inasmuch as transitional mucosa is preneoplastic, no abnormalities of villin polarization were seen, indicating that this type of alteration may only occur later in the carcinogenesis sequence.

In a similar study, Younes *et al.* examined the immunohistochemical expression of fodrin, or nonerythoid spectrin, an actin binding protein of the terminal web [31]. In normal crypt cells fodrin was found in the basolateral domain, with only sparse apical membrane staining. This distribution persisted except in cells of the luminal surface, where intense apical staining of fodrin abruptly became apparent, and corresponded with intense villin staining, presumably reflecting maturation of the terminal web and brush border cytoskeleton. In neoplastic lesions, the pattern of fodrin staining was always altered, which was more frequent than observed for villin. In adenomas, increased cytoplasmic and apical staining was seen. Carcinomas demonstrated similar localization and increased staining intensity compared with adenomas. The quantity of fodrin determined by western blotting was also increased in cancer compared to normal. These data indicate that redistribution and increased levels of fodrin may be sensitive markers of neoplastic changes in the colon. Again, further studies are needed to determine if alterations of fodrin occur in preneoplastic colonic mucosa.

CYTOKERATIN INTERMEDIATE FILAMENTS

Intermediate filaments are a multigene family of major structural proteins [32]. The expression of these is tightly linked to embryonic differentiation. Six groups have been identified on the basis of amino acid sequences. The largest group is the keratins or cytokeratins. Type 1 includes keratins 9-19 and Type 2 includes keratins 1-8. These are expressed in epithelial tissues as Type 1 plus Type II pairs. Type III filaments include mesenchymal proteins vimentin, desmin, glial fibrillary acidic protein, and peripherin. Type IV are neurofilaments, Type V are nuclear lamin proteins, and Type VI are restins found in CNS stem cells [33]. All intermediate filaments have long alpha helical rod domains, which dimerize to form a coiled coil 50 nm long, and they are flanked by nonhelical amino and carboxyl terminal domains. Phosphorylation sites lie in nonhelical terminal domains, and phosphorylation can interfere with polymerization of intermediate filaments [32].

Specific cytokeratin proteins are generally preserved in epithelial tissues following malignant transformation [34]. The cytokeratins have been used as a tissue-specific differentiation marker in diagnostic studies of poorly differentiated tumors. In addition, aberrant cytokeratin distribution has been reported to occur in precancer and cancerous colon [35]. Using monoclonal antibodies which recognize both Type I and Type II cytokeratins, one immunohistochemical study demonstrated that the cells of the upper crypt and surface of normal colon stain quite strongly, compared with minimal lower crypt staining. In contrast, a more patchy distribution and loss of this gradient was found in tubular adenomas and hyperplastic polyps. Tubulovillous adenomas were uniformly positive. In addition, all 12 colon carcinomas were strongly positive in this study. Interestingly, in FP patients the normal colon showed a uniform pattern with no gradient, similar to villous adenoma cells. However, this finding was not confirmed in another study using specimens of non-neoplastic tissue from FP patients compared with age and site-matched specimens of normal mucosa from patients with nonmalignant disease, colon cancer, and inflammatory bowel disease (mostly quiescent disease) [36].

In this study cytokeratin expression in the lower crypts was found to be similar in these four groups, with the exception that lower crypt staining was more frequently found in specimens with inflammatory bowel disease, and usually correlated with shortened or distorted crypts.

These data indicate that cytokeratin staining may not be a sensitive marker for early preneoplastic changes in the normal colonic mucosa. Altered cytokeratin distribution appears to occur most frequently in reparative or regenerative changes such as ulcerative colitis and in mildly dysplastic adenomas. The cause of altered cytokeratin immunoreactivity within colonic crypts is unknown. Other investigators have suggested that the phosphorylation status of intermediate filaments may influence their immunoreactivity [37].

CELLULAR ADHESION MOLECULES

Cell adhesion molecules include several families of molecules that mediate cell-cell and cell-substratum interactions and play important roles in the process of embryogenesis, differentiation, cell migration and polarity [38]. These include integrins, immunoglobulin superfamily molecules (e.g., N-CAM and cadherins), and white blood cell adhesion molecules that mediate adhesion with endothelial (LEC-CAM) and lymphoid tissue (lymphocyte homing receptors). Integrins are heterodimers of noncovalently bound α and β subunits and serve as receptors for extracellular matrix glycoproteins. Various members of the integrin family are found in all tissue types. Immunohistochemical studies demonstrate that integrins VLA-1,2,3 and 6 are expressed in all epithelial cells of the small and large intestine. However, integrin expression along the crypt axis may be heterogeneous, with decreased VLA-1 and possibly VLA-2 expression in villous cells of the small intestine and upper crypt and surface cells of the colon [39,40].

Alterations of several types of integrins and other cell adhesion molecules have been related to the process of tumor development and metastasis [38]. An immunohistochemical study comparing expression of VLA-2 and VLA-6 in adenomas and cancers of the colon found partial or complete loss of VLA-2 in 3/20 adenomas and 37/96 cancers. Loss of VLA-2 was associated with stage C or D cancers more frequently than with early stage cancers. In contrast, VLA-6 was expressed equally in all colonic tumors. The DCC tumor suppressor gene product is thought to play a role in cellular adhesion, due to sequence similarity with neural cell adhesion molecules [41]. The DCC gene is located on chromosome 18q and is absent or altered in 71% of colorectal carcinomas and almost 50% of late stage adenomas [42]. These data suggest that integrins and possibly other cellular adhesion molecules play a role in the normal cellular migration process and in determining invasive and metastatic ability of malignant cells. Further research is required to determine if alterations of cellular adhesion molecules exist in preneoplastic colonic tissues.

BIOCHEMICAL MECHANISMS OF CYTOSKELETAL PROTEIN CONTROL

The biochemical mechanisms controlling cytoskeletal protein polymerization, cell polarity, and differentiation are complex and poorly understood; however, recent reports indicate that phosphorylation of cytoskeletal proteins may play an important role in normal and transformed colonic cells.

Protein tyrosine kinase and their substrates differ in immature crypt cells compared with villous cells. Several studies have demonstrated that protein kinases display a defined topological segregation in the crypt. Protein tyrosine kinase and protein kinase C activity is greatest in the crypt compared with the surface colon in rat and human specimens [43,44,45,46]. These studies also indicate that tyrosine protein kinase activity and phosphotyrosine were primarily associated with the cytoskeleton fraction of these cells. These data suggest that cytoskeletal-associated phosphorylation may be linked to the proliferation/differentiation process in colonic crypts.

Numerous studies indicate that protein phosphorylation may regulate cytoskeletal function. For example: tyrosine phosphorylation of ezerin, a protein associated with the microvillus core bundle, correlates with major actin reorganization [47]. Phosphorylation of vimentin by cAMP dependent protein kinase and protein kinase C modulates its assembly into filaments [48,49]. Microinjection of the p34cdc2 kinase alters the cytoskeleton, which occurs during mitosis [50]. In addition, the actin binding protein tensin binds phosphoty-

TABLE II. Potential cytoskeletal-related markers of preneoplasia in the colon

A.	Ultrastructural morphology
	immature cells in mid-crypt region
	poorly-formed microvilli
	vesiculation and electron dense granules
	atypical secretory (goblet) cells
B.	Altered actin organization
C.	Increased expression or altered polarization of actin-binding proteins
	villin
	fodrin
D.	Increased expression or altered polarization of microvillar-associated brush border enzymes
	sucrase-isomaltase
E.	Altered expression (? phosphorylation) of cytokeratin intermediate filaments
F.	Altered expression of cell adhesion molecules
	integrins
	cadherins
	DCC
G.	Altered distribution of protein kinase activities/substrates
	cAMP-dependent and independent protein kinases
	tyrosine protein kinases (pp60c-*src*)

rosine and is a target for tyrosine kinase, representing a possible link between signal transduction and the cytoskeleton [51].

Altered cytoskeletal protein phosphorylation may play an important role in cellular transformation. The *src* tyrosine kinase oncogene has been extensively studied [52]. Following transformation of fibroblasts, the *src* oncogene is concentrated at adhesion plaques (the structures at which microfilament bundles terminate on the membranes). The adhesion plaque proteins vinculin, talin, and integrin are phosphorylated and some β1 integrins are lost. This results in disruption of adhesion plaques, loss of spatial organization of integrin and fibronectin, and the transformation of the cell to a rounded phenotype. Since activation of the pp60c-*src* oncogene appears to occur during early stages of adenoma formation [53], further investigations of altered pp60c-*src* and its cytoskeletal substrates in neoplastic and preneoplastic colon are warranted. In addition, several other tyrosine kinase oncogene products have been found to localize to cytoskeletal structures. These include *yes*, *abl* (localized to adhesion plaques), and *fms* (localized to intermediate filaments) [4].

CONCLUSION

In conclusion, what role do cytoskeletal proteins play as preneoplastic or "intermediate" biomarkers? Electron microscopic studies have indicated that the crypts of both neoplastic and preneoplastic colon are characterized by abnormal numbers of structurally immature cells. The data presented above indicate that several cytoskeletal and membrane domain-specific proteins are also altered in neoplastic and possibly preneoplastic colon, and may be related to the structural immaturity and/or altered proliferation known to occur in these tissues (Table 2). Numerous other cytoskeletal and associated proteins have been characterized, however the extent to which they are altered in preneoplastic and neoplastic intestine remains to be determined. Further research is needed to determine if immunohistochemical or molecular assays for these markers are useful for the detection of colonic mucosa at increased risk for neoplasia. These studies will require carefully collected specimens of colon from patients at risk for colon cancer and appropriate age and site-matched controls.

REFERENCES

1. Carraway KL, Carraway CAC: Membrane-cytoskeleton interactions in animal cells. Biochim Biophys Acta 988: 147-171, 1989.
2. Brown DT, Anderton BH, Wylie CC: The organization of intermediate filaments in normal human colonic epithelium and colonic carcinoma cells. Int J Cancer 32: 163-169, 1983.

3. Brinkley B, Chafouleas J: Cytoskeleton and the transformed phenotype of malignant cells. Prog Cancer Res Therap 29: 67-90, 1984.
4. Ben-Zeev A: The cytoskeleton in cancer cells. Biochim Biophys Acta 780: 197-212, 1985.
5. Molitoris BA, Nelson JW: Alterations in the establishment and maintenance of epithelial cell polarity as a basis for disease processes. J Clin Invest 85: 3-9, 1990.
6. Pitman FE, Pittman JC: An electron microscopic study of the epithelium of normal human sigmoid colonic mucosa. Gut 7: 644-661, 1966.
7. Kaye GI, Fenoglio CM, Pascal RR, Lane N: Comparative electron microscopic features of normal, hyperplastic, and adenomatous human colonic epithelium. Gastroenterology 64: 926-945, 1973.
8. Geiger B: Intermediate filaments, looking for a function. Nature 329: 392-393, 1987.
9. Coudrier E, Kerjaschki D, Louvard D: Cytoskeleton organization and submembranous interactions in intestinal and renal brush borders. Kidney Inter 34: 309-320, 1988.
10. Louvard D: The function of the major cytoskeletal components of the brush border. Curr Opin Cell Biol 1: 51-57, 1989.
11. Mooseker MS: Organization, chemistry, and assembly of the cytoskeletal apparatus of the intestinal brush border. Ann Rev Cell Biol 1: 209-241, 1985.
12. Specian RD, Oliver MG: Functional biology of intestinal goblet cells. Am J Physiol 260: C183-C193, 1991.
13. Mughal S, Filipe MI: Ultrastructural study of the normal mucosa-adenoma-cancer sequence in the development of familial polyposis coli. J Natl Cancer Inst 60: 753-768, 1978.
14. Dawson PA, Filipe MI: An ultrastructural and histochemical study of the mucous membrane adjacent to and remote from carcinoma of the colon. Cancer 37: 2388-2398, 1976.
15. Beaulieu J-F, Weiser MM, Herrera L, Quaroni A: Detection and characterization of sucrase-isomaltase in adult human colon and in colonic polyps. Gastroenterology 98: 1467-1477, 1990.
16. Wiltz O, O'Hara CJ, Steele GD, Mercurio AM: Sucrase-isomaltase: a marker associated with the progression of adenomatous polyps to adenocarcinomas. Surgery 108: 269-276, 1990.
17. Wiltz O, O'Hara CJ, Steele GD, Mercurio AM: Expression of enzymatically active sucrase-isomaltase is a ubiquitous property of colon adenocarcinomas. Gastroenterology 100: 1266-1278, 1991.
18. Ahnen DJ, Nakane PK, Brown WR: Ultrastructural localization of carcinoembryonic antigen in normal intestine and colon cancer. Cancer 49: 2077-2090, 1982.
19. Ahnen DJ, Brown WR, Kloppel TM: Secretory component: the polymeric immunoglobulin receptor. Gastroenterology 89: 667-682, 1985.
20. O'Brien MJ, Zamcheck N, Burke B, Kirkham SE, Saravis CA, Gottlieb LS: Immunocytochemical localization of carcinoembryonic antigen in benign and malignant colorectal tissues. Am J Clin Pathol 75: 283-290, 1981.
21. Nagura H, Tsutsumi Y, Shioda Y: Immunohistochemistry of gastric carcinomas and associated disease. J Histochem Cytochem 31: 193-198, 1983.
22. Friedman E, Verderame M, Lipkin M, Pollack R: Altered actin cytoskeletal patterns in two premalignant stages in human colon carcinoma development. Cancer Res 45: 3236-3242, 1985.
23. Kopelovich L, Lipkin M, Blattner WA, Fraumeni JF, Lynch HT, Pollack RE: Organization of actin-containing cables in cultured fibroblasts from individuals at high risk of colon cancer. Int J Cancer 26: 301-307, 1980.
24. Friederich E, Pringault E, Arpin M, Louvard D: From the structure to the function of villin, an actin-binding protein of the brush border. Bioessays 12: 403-408, 1990.
25. Shibayama T, Carboni JM, Mooseker MS: Assembly of the intestinal brush border: appearance and redistribution of microvillar core proteins in developing chick enterocytes. J Cell Biol 105: 335-344, 1987.
26. Friederich E, Huet C, Arpin M, Louvard D: Villin induces microvilli growth and actin redistribution in transfected fibroblasts. Cell 58: 461-475, 1989.
27. Bacchi CE, Gown AM: Distribution and pattern of expression of villin, a gastrointestinal-associated cytoskeletal protein, in human carcinomas. Lab Invest 64: 418-424, 1991.
28. Moll R, Robine S, Dudouet B, Louvard D: Villin: a cytoskeletal protein and a differentiation marker expressed in some human adenocarcinomas. Virchows Arch B 54: 155-169, 1987.
29. Robine S, Huet C, Moll R, Sahuquillo-Merino C, Coudrier E, Zweibaum A, Louvard D: Can villin be used to identify malignant and undifferentiated normal digestive epithelial cells? Proc Natl Acad Sci USA 82: 8488-8492, 1985.
30. West AB, Isaac CA, Carboni JM, Morrow JS, Mooseker MS, Barwick KW: Localization of villin, a cytoskeletal protein specific to microvilli, in human ileum and colon and in colonic neoplasms. Gastroenterology 94: 343-352, 1988.
31. Younes M, Harris AS, Morrow JS: Fodrin as a differentiation marker. Redistribution in colonic neoplasia. Am J Pathol 135: 1197-1212, 1989.
32. Steinert PM, Roop DR: Molecular and cellular biology of intermediate filaments. Ann Rev Biochem 57: 593-625, 1988.
33. Lu X, Lane E: Retrovirus-mediated transgenic keratin expression in cultured fibroblasts: specific domain functions in keratin stabilization and filament formation. Cell 62: 681-696, 1990.
34. Rafiee P, Ho SB, Bresalier RS, Bloom EJ, Kim JH, Kim YS: Characterization of the cytokeratins of human colonic, pancreatic, and gastric adenocarcinoma cell lines. Pancreas 7: 123-131, 1992.
35. Chesa PG, Rettig WJ, Melamed MR: Expression of cytokeratins in normal and neoplastic colonic epithelial cells. Am J Surg Pathol 10: 829-835, 1986.

36. Ho SB, Yan PS, Kern SE, Hamilton SR, Kim YS: Expression of glycoconjugate and cytokeratin antigens in non-adenomatous colonic epithelium of familial adenomatous polyposis. Gastroenterology 98: A286, 1990.
37. Leoncini P, Bellizzi De Marco E, Bugnoli M, Mencarelli C, Vindigni C, Cintorino M: Expression of phosphorylated and nonphosphorylated neurofilament subunits and cytokeratins in neuroendocrine lung tumors. Path Res Pract 185: 848-855, 1989.
38. Albelda SM, Buck CA: Integrins and other cell adhesion molecules. FASEB J 4: 2868-2880, 1990.
39. Choy M-Y, Richman PI, Horton MA, MacDonald TT: Expression of the VLA family of integrins in human intestine. J Pathol 160: 35-40, 1990.
40. Koretz K, Schlag P, Boumsell L, Moller P: Expression of VLA-a2, VLA-a6, and VLA-b1 chains in normal mucosa and adenomas of the colon, and in colon carcinomas and their liver metastases. Am J Pathol 138: 741-750, 1991.
41. Fearon ER, Cho KR, Nigro JM, Kern SE, Simons JW, Ruppert JM, Hamilton SR, Preisinger AC, Thomas G, Kinzler KW, Vogelstein B: Identification of a chromosome 18q gene that is altered in colorectal cancers. Science 247: 49-56, 1990.
42. Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakumura Y, White R, Smits AMM, Bos JL: Genetic alterations during colorectal-tumor development. N Engl J Med 319: 525-532, 1988.
43. Craven PA, DeRubertis FR: Subcellular distribution of protein kinase C in rat colonic epithelial cells with different proliferative activities. Cancer Res 47: 3434-3438, 1987.
44. Schwartz B, Fraser GM, Levy J, Sharoni Y, Guberman R, Krawiec J, Lamprecht SA: Differential distribution of protein kinases along the crypt-to-lumen regions of rat colonic epithelium. Gut 29: 1213-1221, 1988.
45. Burgess DR, Jiang W, Mamajiwalla S, Kinsey W: Intestinal crypt stem cells possess high levels of cytoskeletal-associated phosphotyrosine-containing proteins and tyrosine kinase activity relative to differentiated enterocytes. J Cell Biol 109: 2139-2144, 1989.
46. Schwartz B, Cagnano E, Braun S, Lamprecht SA: Characterization of tyrosine protein kinase associated with subcellular components of human colonic epithelium. Anticancer Res 10: 1747-1754, 1990.
47. Bretscher A: Rapid phosphorylation and reorganization of ezrin and spectrin accompany morphological changes induced in A-431 cells by epidermal growth factor. J Cell Biol 108: 921-930, 1989.
48. Inagaki M, Nishi Y, Nishizawa K, Matsuyama M, Sato C: Site-specific phosphorylation induces disassembly of vimentin filaments *in vitro*. Nature 328: 649-652, 1987.
49. Steinert PM, Liem RKH: Intermediate filament dynamics. Cell 60: 521-523, 1990.
50. Lamb NJC, Fernandez A, Watrin A, Labbe J-C, Cavadore J-C: Microinjection of p34cdc2 kinase induces marked changes in cell shape, cytoskeletal organization, and chromatin structure in mammalian fibroblasts. Cell 60: 151-165, 1990.
51. Davis S, Lu ML, Lo SH, Lin S, Butler JA, Druker BJ, Roberts TM, An Q, Chen LB: Presence of an SH2 domain in the actin-binding protein tensin. Science 252: 712-715, 1991.
52. Kellie S: Cellular transformation, tyrosine kinase oncogenes, and the cellular adhesion plaque. Bioessays 8: 25-30, 1988.
53. Cartwright CA, Meisler AJ, Eckhart W: Activation of the pp60c-*src* protein kinase is an early event in colonic carcinogenesis. Proc Natl Acad Sci USA 87: 558-562, 1990.
54. Czernichow B, Simon-Assmann P, Kedinger M, Arnold C, Parache M, Marescaux J, Zweibaum A, Haffen K: Sucrase-isomaltase expression and enterocytic ultrastructure of human colorectal tumors. Int J Cancer 44:238-244, 1989.
55. Zweibaum A, Hauri HP, Sterchi E, Chantret I, Haffen K, Barmat J, Sordat B: Immunohistological evidence, obtained with monoclonal antibodies, of small intestinal brush border hydrolases in human colon cancers and foetal colons. Int J Cancer 34:591-598, 1984.