Adenoma-specific Alterations of Protein Kinase C Isozyme Expression in ApcMIN Mice

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Abstract

Members of the protein kinase C (PKC) family appear to play important roles in colorectal carcinogenesis. To investigate the potential involvement of PKC isozymes in adenomatous transformation induced by inactivation of the adenomatous polyposis coli (APC) gene product, we examined protein levels and localization of ten PKC isozymes by immunohistochemistry in normal and adenomatous ileal epithelium of ApcMIN mice. Compared with surrounding normal epithelium, adenomas showed dramatically reduced staining for PKCs α, β1, and ζ, as well as dysplasia-specific punctate nuclear staining of PKC μ. We conclude that reduced protein expression of PKC α, β1, and ζ, and nuclear localization of PKC μ are markers of, and are perhaps involved in, adenomatous transformation induced by APC inactivation in ApcMIN mice.

Introduction

PKC3α is a family of 11 serine-threonine kinases that participate in a variety of cellular processes including mitogenesis, differentiation, and apoptosis (1). Several lines of evidence suggest involvement of PKC isozymes in colorectal carcinogenesis. Expression levels of PKC α, PKC β1, PKC δ, and PKC ζ are frequently lower in colorectal cancers of humans and carcinogen-treated rodents than in surrounding normal mucosa (2–6). In colorectal cancer cell lines, forced expression of PKC α or PKC β1 inhibits growth and tumorigenicity (7, 8), whereas activation of PKC δ induces apoptosis (9). In contrast, activation or overexpression of PKC ε stimulates proliferation in colon cancer cells (9) and induces transformation of rat colonic epithelial cells and other cell types (10, 11). Similarly, expression of a PKC β2 transgene in murine intestine enhances formation of carcinogen-induced preneoplastic lesions and appears to activate the Apc/β-catenin pathway in vivo (12). Thus, PKC isozymes exhibit features of tumor suppressor genes (PKCα, β1, and δ) and proto-oncogenes (PKC β2 and ε) in intestinal epithelium. However, the potential links between these activities and the molecular events involved in triggering progression of colorectal carcinogenesis remain unclear.

Among the earliest and most common molecular changes during colorectal carcinogenesis is inactivation of the APC tumor suppressor gene product (13). To determine whether adenomatous transformation induced by APC loss is associated with altered expression and/or localization of PKC isozymes, we examined the levels and distribution of PKC isozymes by immunohistochemistry of fresh frozen normal ileal mucosa and adenomas from ApcMIN mice.

Materials and Methods

Antibodies and Peptides. All primary antibodies and epitope competitive peptides used in this study were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Each are reported to recognize their respective murine PKC isoform with minimal or no cross-reactivity with other PKC isozymes. Optimal dilutions of antibodies used for the study were as follows: sc-208 (PKC α), 1:500; sc-210 (PKC β1), 1:100; sc-210-G (PKC β2), 1:100; sc-211 (PKC γ), 1:100; sc-213 (PKC δ), 1:200; sc-214-G (PKC ε), 1:50; sc-215-G (PKC η), 1:50; sc-935 (PKC μ), 1:200; sc-212-G (PKC ζ), 1:300; and sc-7262 (PKC θ), 1:500.

Immunohistochemistry. All animal procedures were approved by the Mayo Clinic (Scottsdale, AZ) Institutional Animal Care and Use Committee. Immediately after CO2 asphyxiation, ileal tissues from eight 60- to 80-day-old ApcMIN and eight 60- to 80-day-old wild-type C57BL/6J mice were removed and photographed. Representative 1-cm pieces of normal and adenoma-containing tissues were immediately embedded in Tissue-Tek II cryogenic embedding medium (Scientific Products, McGaw Park, IL) and snap frozen.

Results

PKC Expression in Normal Ileum. Table 1 summarizes the results of immunoreactivity scoring for each PKC isoform within the epithelial layer of the crypt villus axis, lamina propria, smooth muscle, myenteric plexus, and adenomas of ApcMIN mouse ileum. Normal ileal compartments of wild-type (data not shown) and ApcMIN mice
showed no differences in intensities or localizations of PKC isozyme immunoreactive staining. All PKC isozymes were expressed in the myenteric plexus with strongest staining observed for PKCs α, γ, and ε (Figs. 1 to 3). Staining of cells within the lamina propria was also present for most isozymes, including PKC η (Fig. 3), which was notably absent in epithelial compartments. PKC μ was the most strongly expressed isozyme in the circular and longitudinal smooth muscle layers (Fig. 2).

Within the epithelial layer of normal ileal mucosa, PKCs α, β1, β2, δ, and μ each showed higher staining intensity in villi compared with crypts. Staining was diffusely cytoplasmic for PKCs α, β1, and δ but predominantly localized to the apical cytoplasm or brush border for PKCs β2 and μ (Figs. 1 and 2). Specific staining for PKC γ was primarily nuclear within the proliferative zone of crypts and predominantly cytoplasmic toward the villus tips (Figs. 1 and 4). PKC ζ immunostaining was moderately strong along the apical cytoplasm of villus enterocytes (Figs. 2 and 3). In addition, isolated cryptal cells stained strongly for PKC ζ (Figs. 2 and 3). Levels of specific staining for PKCs ε, η, or θ were low or undetectable in the epithelial layer of the ileum (not shown).

**PKC Expression in Adenomas.** Compared with adjacent normal epithelium, epithelial cells of adenomas showed similar levels of staining for PKCs β2, γ, and μ; slightly reduced staining for PKC δ; and markedly reduced staining for PKCs α, β1, and ζ (Table 1; Figs. 1 and 2). As in normal enterocytes, PKC β2 immunoreactivity in adenomas was strongest in the apical cytoplasm at the luminal surface (brush border) of glandular structures (Fig. 1). Immunoreactivity for PKC γ in adenomas was primarily localized within nuclei as seen in normal cryptal cells (Fig. 4). PKC μ immunoreactivity showed strong punctate nuclear staining in adenomas. This pattern was unique to, and universally expressed in, dysplastic epithelial cells of adenomas (Figs. 2 and 4).

**Discussion**

We examined expression levels of 10 PKC isozymes in normal and adenomatous ileal epithelium of wild-type and ApcMIN mice and

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Eight regions examined include the crypt (Crypt); lower (L1/3), middle (M1/3), and upper (U1/3) third of the crypt villus axis; adenoma (Aden); lamina propria (LP); myenteric plexus (MP); and smooth muscle layers (SM). Notable staining characteristics included cytoplasmic (c), brush border (b), isolated cells (i), and nuclear (n).

Fig. 1. Immunohistochemical localization of classical PKC isozymes α, β1, β2, and γ in ApcMIN mouse ileum. Left panels, normal ileum at low power (left column; ×4–10), at higher power (middle column; ×16–40), and controls in the presence of competitive epitope peptide (+ pep) (right column). Right panel, ileal adenomas at low power (left column), at high power (middle column), and in the presence of competitive epitope peptide (right column). High power views of crypts are labeled as such. A, adenomas; N, normal epithelium.
found invariable reductions in the immunohistochemical staining of PKCs α, β1, and ζ, as well as dysplasia-specific nuclear localization of PKC μ, among adenomas compared with normal mucosa. Given that the common denominator of adenomas in Apc<sup>MIN</sup> mice is absent expression of the remaining wild-type APC allele (14), these adenoma-specific alterations should reflect downstream effects of abrogated APC function.

Transgenic overexpression of PKC β2 in murine colon increases colonic proliferation, accelerates formation of carcinogen-induced colon tumors, and appears to activate the Apc/β-catenin pathway (12). We found similar levels and patterns of expression of PKC β2 in adenomas and normal ileal mucosa of Apc<sup>MIN</sup> mice, suggesting that although PKC β2 may be an upstream activator of the Apc/β-catenin pathway, it appears not to be a downstream target of this pathway and may not play a role in adenoma formation induced by Apc loss.

Reduced protein levels of PKCs α, β1, and ζ are reported in colorectal cancers of humans and carcinogen-treated rodents (2–5, 15). Our data indicate that reduced expression of these isoforms occurs early during premalignant adenomatous stages after APC loss. All three of these isozymes exhibit properties of tumor suppressor genes when overexpressed in transformed cells (7, 8, 16), suggesting that the tumor suppressor functions of APC and PKCs α, β1, and/or ζ could be mechanistically linked. Future studies are needed to determine whether the suppressed levels of these isoforms are caused by...
transcriptional, translational, or posttranslational events and how they may be linked to APC loss. If abrogated expression of one or more of these isoforms proves to be important for adenomatous transformation induced by APC loss, then pharmacological induction of their expression or activity may prevent adenomas in ApcMIN mice, as well as adenomas associated with familial adenomatous polyposis and most sporadic adenomas in humans.

Among our most interesting and novel observations was the dysplasia-specific localization of PKC μ to discrete sites within nuclei (Figs. 2 and 4). Among PKC isozymes, PKC μ is the least well characterized. It is calcium independent and is activated by diacylglycerol, so it shares features of the “novel” PKC subgroup (δ, η, θ) (17). However, several features are unique to PKC μ, including the presence of a pleckstrin homology domain, lack of a pseudosubstrate domain, activation by heparin sulfate, and inhibition by basic proteins that ordinarily serve as substrates for PKCs including p53, myelin basic protein, and histone H1 (18, 19). PKC μ is overexpressed in many malignancies (18). The apparent translocation of PKC μ from the cytoplasm in normal ileal epithelial cells to the nucleus in adenomas suggests that this isoform is activated during adenomatous transformation. Further studies are required to determine whether dysplasia-specific nuclear localization of PKC μ occurs in human adenomas, whether this isoform plays a role in adenomatous transformation, and how it may be regulated by APC loss.

PKC γ, which is generally regarded to be neuron specific, was detected in nuclei of cells predominantly within the proliferative zone of normal crypts and in all nuclei of adenomas (Figs. 1 and 4). PKC γ mRNA is expressed in some human colorectal cancers (20) and is activated by insulin-like growth factor 1 in HT-29 colorectal cancer cells (21). Together, these observations support the hypothesis that PKC γ plays a role in growth factor-mediated signaling in normal and adenomatous intestinal epithelium.

Our observation of increased expression of PKCs α, β2, δ, and ζ toward the villus tips in normal ileal mucosa of ApcMIN mice is consistent with previous studies in normal rat ileum and supports the notion that these isoforms play roles in postmitotic processes (22). We additionally observed strong specific staining for PKC ζ in isolated cells within the crypts. The cell type and significance of PKCζ expression in this distinct epithelial compartment remain unknown.

Our analysis of PKC isozymes in ApcMIN mice revealed several novel observations related to their distributions of expression in normal ileum and adenomas in ApcMIN mice. We speculate that the adenoma-specific changes we observed are downstream effects of APC loss that may participate in adenomatous transformation. Future work will help determine the importance of these alterations as early events of colorectal carcinogenesis and as potential targets of preventative and therapeutic interventions.

References
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