

APC and chromosome instability in colorectal cancer

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ABSTRACT

Colon cancer is a common disease that can be sporadic or familial. An inactivated adenomatous polyposis coli (APC) suppressor gene is found in over 80% of colorectal tumors, this being an early alteration in the development of adenomatous polyps. APC function is not only critical for tumor initiation and progression, and chromosome instability (CIN) is another characteristic dependent at least partly on APC mutations.

Key words: Colorectal cancer. APC. Chromosome instability.

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INTRODUCTION

The colon is organized into cell compartments called crypts. It is widely believed that adenomas develop from normal stem cells located at the bases of normal crypts (1). The progeny of stem cells migrate up the crypt and continue to divide until they reach its mid portion. Subsequently, migrating epithelial cells stop dividing and differentiate to mature cells instead. When differentiated cells reach the top of the crypt, they undergo apoptosis and are engulfed by stromal cells or shed into the lumen. This journey from the crypt's base to the apex lasts 3-6 days (2,3). Usually, the birth rate of colonic epithelial

cells precisely equals the loss rate from the crypt apex. When the birth/loss ratio increases a tumor results.

Colorectal tumors progress through a series of clinical and histopathological stages ranging from dysplastic crypts through small benign tumors to malignant cancers. This progression is the result of a series of genetic changes that involve activation of oncogenes and inactivation of tumor suppressor genes (4). In colorectal cancer, chromosomal instability (CIN) is the major form of genetic instability (5). Mutation of the APC gene is the earliest event yet identified in sporadic colorectal tumorigenesis, and it is estimated that > 85% of colorectal tumors have somatic mutations of the APC gene (5).

APC STRUCTURE AND FUNCTION

The APC gene encodes a large multidomain protein that has many different sites for interaction with other proteins. It is present in a variety of epithelial tissues, usually in cells that are post-mitotic (6). Immunohistochemical studies show that APC is often diffusely distributed in the cytoplasm, although it can sometimes be found in the apical or lateral regions of epithelial cells (6). Studies indicate that APC participates in a variety of cellular functions including proliferation, differentiation, apoptosis, adhesion, migration, and chromosomal segregation (7).

Figure 1 shows the various domains within APC that interact with other proteins. The armadillo repeat at the N-terminal portion binds to the B56 regulatory subunit of protein phosphatase 2A and APC-stimulated guanine exchange factor (8). These two proteins may be involved in the Wntless (Wnt) signaling pathway, of which APC is a component (8,9). Another important domain includes three 15-amino acid repeats that bind β -catenin, and seven 20-amino acid repeats that are required for the down-regulation of β -catenin (10,11). Sites in APC have also

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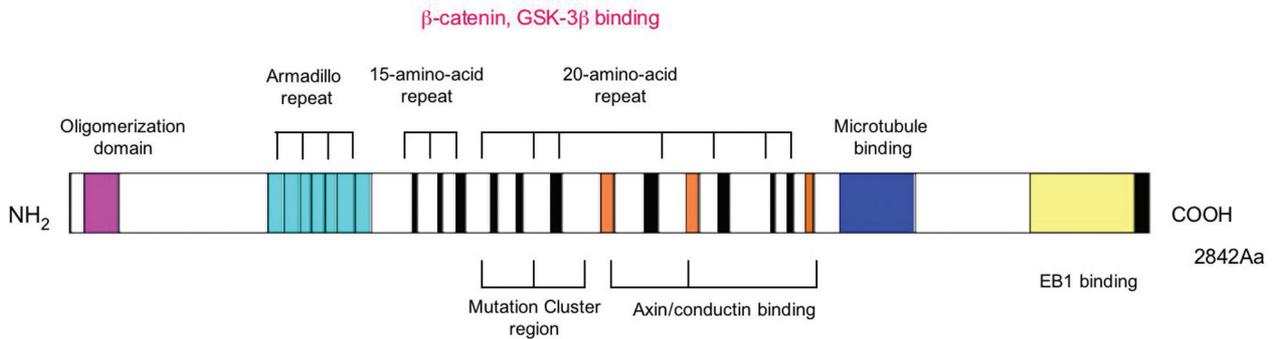


Fig. 1.- The different domains of the APC protein. Conserved regions, such as Armadillo repeats, and regions that interact with other proteins, including tubulin, the microtubule-associated protein EB1, β -catenin, and axin/conductin, are shown.

Dominios de la proteína APC. Presenta regiones conservadas como las repeticiones Armadillo; y regiones que interaccionan con otras proteínas, incluyendo tubulina, la proteína EB1 asociada a microtúbulos, β -catenina, y axina/conductina.

been identified that interact with axin and conductin (12,13), two inhibitory proteins of the Wnt signaling pathway. The C terminal portion of the protein is involved in binding to microtubules and tubulin-binding protein EB1 (14).

Mutational analysis of the APC gene indicates that the majority of germline mutations found in patients with familial adenomatous polyposis (FAP) are nonsense mutations, leading to the formation of a truncated protein. More than 60% of APC mutations are found in the central region (between codons 1284 and 1580) of the protein, which is called the mutation cluster region (MCR)

(15). The MCR region coincides with a region in APC that is important for the down-regulation of β -catenin, which suggests that this function is important for the pathogenesis of colorectal cancer. Subsequent studies demonstrated that APC and β -catenin are important parts of the Wnt signaling pathway (Fig. 2). The greatest progress in understanding the function of APC has been made in studying its interaction with glycogen synthase kinase (GSK)-3 β and β -catenin, both being essential components of the Wnt signaling pathway (5). GSK-3 β makes up complexes with APC, β -catenin, and axin, and then phosphorylates β -catenin. Phosphorylation targets β -catenin for degradation via an ubiquitin-mediated proteasomal pathway (16). Truncation of APC results in the disruption of complex formation and ultimately increased cytoplasmic levels of β -catenin. Free β -catenin is translocated to the nucleus, where it interacts with T-cell factors (TCFs) (Fig. 2). TCF-4 is the predominant member of this family of transcription factors in colonic epithelial cells, and activation of this pathway upregulates oncogenes c-Myc and cyclin D1 (17,18). These findings suggest that β -catenin upregulates TCF-responsive genes critical for the proliferation and transformation of colonic epithelial cells. In this context, it is noteworthy that a gain-of-function mutation in the β -catenin gene has been identified in as many as 50% of colon tumors with an intact APC (19).

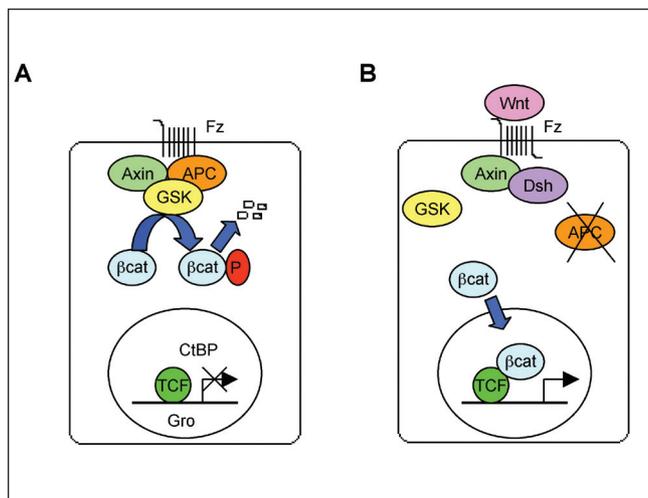


Fig. 2.- The signaling pathways in the absence (A) and the presence (B) of the Wnt ligand. Fz, frizzled receptor; Dsh, dishevelled protein. Upon the binding of the Wnt ligand to its receptor Fz, the dishevelled protein is activated.

Rutas de señalización en presencia (A) y ausencia (B) de un ligando Wnt. Fz, receptor frizzled; Dsh, proteína dishevelled. La unión del ligando Wnt a su receptor Fz, activa a la proteína dishevelled.

APC AND CHROMOSOME INSTABILITY (CIN)

Recent studies have shown that the C terminus of the APC protein is involved in maintaining chromosome stability during mitosis (20,21). APC is localized in the kinetochore of metaphase chromosomes, and this localization is likely dependent on the interaction between

APC and EB1. Accordingly, APC-mutant cells have an abundance of spindle microtubules that fail to connect to the kinetochore and are characterized by CIN (22). In the mouse model that involves a mutation at codon 1628 of the APC gene (APC¹⁶³⁸), this mutation truncates the C-terminal portion of APC responsible for CIN-related functions but retains the β -catenin regulatory domain (20). Consequently, embryonic stem (ES) cells isolated from homozygous APC^{1638T} animals were CIN (20). However, the corresponding mice were viable and tumor-free. In contrast, the classic mouse model APC^{Min} that carries a nonsense mutation at codon 850 truncated the region required to regulate β -catenin. The heterozygous APC^{+Min} animals develop numerous adenomas in their intestinal tract (23). These observations underscore the importance of the selective advantage provided by the loss of β -catenin control in tumor formation, and argue against the ability of chromosomal instability to initiate the oncogenic process.

CHROMOSOME INSTABILITY IN TUMOR INITIATION

Colorectal cancer is one of the best understood systems for the study of the genetics of cancer progression. Two types of genetic instability have been identified, with chromosomal instability predominating (24,25).

The molecular basis for CIN is just beginning to be explored (26). A large number of gene alterations can give rise to CIN in *Saccharomyces cerevisiae* (27,28). These genes include those involved in chromosome condensation, sister-chromatid cohesion, kinetochore structure and function, and microtubule formation and dynamics as well as checkpoints that monitor the progress of the cell cycle. To date, the only genes implicated in aneuploidy in human tumor cells are those of the latter class. Heterozygous mutations in the mitotic spindle checkpoint gene hBUB1 were detected in a small portion of colorectal tumors with the CIN phenotype (29). Mutations in hBUB1 can function in a dominant-negative manner in both mouse and human cells, conferring an abnormal spindle checkpoint when expressed exogenously (29,30). These results also confirmed cell-fusion studies that indicate that the CIN phenotype has a dominant quality and it might only require a single mutational "hit" to produce CIN (31).

Most APC mutations observed in patients lead to the truncation of the encoded protein, with loss of the carboxyl-terminal sequences that interact with microtubules (20). However, some well characterized human colon cancer cell lines with APC mutations have chromosome complements that have remained perfectly stable and invariable over thousands of cell divisions *in vitro* (31,32). Therefore, it is unlikely that APC inactivation itself triggers CIN in human colorectal cancer.

CONCLUSION

The fact that genomic defects in so many genes can lead to CIN, at least in yeast, suggests a heterogeneous basis for CIN in tumors, with many genes each playing a role in a small proportion of cases. Accordingly, CIN may be so common in tumors precisely because there are so many genes that, when mutated, can lead to this phenotype. Therefore, in colorectal tumors, chromosomal instability as observed is not only originated by APC mutations, and probably other mitotic checkpoint genes can be involved in this process.

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