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Colorectal cancer: the APC-IncRNA link

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The adenomatous polyposis coli (APC) gene plays, among other things, a crucial role in the regulation of cell proliferation and survival through its ability to regulate canonical Wnt signaling. In this issue of the *JCI*, Wang et al. provide an intriguing new mechanism for APC function involving the regulation of a novel long noncoding RNA (lncRNA), leading to changes in exosome production. APC signaling via this novel pathway can regulate cell proliferation and invasion as well as angiogenesis. In addition to enhancing our understanding of APC function, this new mechanism is of particular clinical significance, as it may provide additional targets for the treatment of APC-mutated cancers.

The APC tumor suppressor gene

The adenomatous polyposis coli (APC) tumor suppressor gene was first discovered over 25 years ago as the gene that is mutated in familial adenomatous polyposis (FAP), a hereditary cancer syndrome characterized by the development of a large number of adenomas, some of which eventually progress to cancer (1). The APC gene is also mutated in the vast majority of sporadic colorectal cancers (CRCs), and APC mutations are observed in the earliest premalignant lesions (2). For these reasons, APC is believed to play crucial roles in the normal homeostasis of the colonic epithelium, and mutation of the APC gene is thought to be the first step in the series of genetic changes required in the progression from normal colorectal epithelium to the fully malignant state (3).

Functions of APC

The APC gene encodes a large 312-kDa protein that has been shown to have multiple cellular functions and act through several molecular pathways (summarized in Figure 1). One of the first and best-known functions of APC is its role in the negative regulation of the canonical Wnt signaling pathway, which it achieves by downregulating the transcriptional activator β-catenin (4). This is accomplished through the formation of a large protein complex, whose core components include APC, the scaffold protein axin, GSK3β, casein kinase 1α, β-catenin, and the E3-ubiquitin ligase β-TrCP. This complex can phosphorylate β-catenin at specific sites, leading to its proteasome-mediated degradation. β-Catenin has a central role in the canonical Wnt pathway, as it binds the T cell factor (TCF) family of transcription factors and mediates the transcriptional activation of target genes important in tumorigenesis (4). Inactivating mutations in the APC gene (or, in some cases, activating mutations in the β-catenin gene [CTNNB1]) lead to the stabilization of β-catenin and overexpression of β-catenin-regulated genes (5), such as those encoding cMYC (6), cyclin D1 (7, 8), and other proteins important in CRC formation (4). Activation of the canonical Wnt pathway through APC mutations has been shown to lead to increased cell proliferation, survival, and differentiation, as well as to changes in the cell cycle (9). Moreover, APC may exert negative regulatory effects on the canonical Wnt pathway by means of additional mechanisms. For example, APC can be imported into the nucleus, where it can remove β-catenin from specific genomic loci and facilitate its export back to the cytoplasm (9). Moreover, APC may have additional nuclear functions, such as DNA repair and cell-cycle control, unrelated to the Wnt pathway. For example, APC has been found to be bound to DNA, where it may block cell-cycle progression (10). The exact effects of oncogenic APC mutations on these nuclear functions are still being elucidated.

Another important role for APC is its involvement in the regulation of mitotic chromosome separation and stability (11). APC has been shown to bind to the kinetochore and promote kinetochore-microtubule attachment (12, 13). Mechanistically, it appears that APC can affect mitotic spindle dynamics through its ability to bind to and regulate the function of the microtubule-binding protein EB1 (13, 14). Regardless of the exact mechanisms, several studies have shown that loss of the APC-mediated mitotic function can lead to chromosomal instability (15). Interestingly, APC has also been implicated in the mediation of apoptosis (16), which may be crucial for regulating the homeostasis of normal colonic mucosa, where epithelial cells undergo apoptosis and shed off as they reach the top of the colonic crypts. The mechanism of apoptosis may involve the canonical Wnt pathway through the transcriptional activation of survivin, also known as baculoviral IAP repeat–containing 5 (*BIRC5*), and other proapoptotic genes (17). However, the proapoptotic effects of APC may also involve noncanonical pathways as, following APC cleavage by caspases, the N-terminus of APC can localize to the mitochondria and increase cell sensitivity to apoptosis through interaction with multiple proteins, including Bcl2 (10, 11). Finally, APC has also been suggested to have roles in cell migration, adhesion, and polarity through its ability to bind a number of cytoskeletal and junction proteins at the cell border (1, 18).

Many of the APC functions described above are typically disrupted in cancer cells through various mutations clustered...
in a region of APC called the mutation cluster region (MCR). It is important to note that the vast majority of these mutations lead to a truncated APC protein, which, in addition to having lost C-terminal functionality, may also have dominant negative effects (19).

A new pathway for APC function
In this issue of the JCI, Wang et al. describe an entirely new pathway for APC function (20). First, they identified a novel lncRNA that they named lncRNA-APC1, which is upregulated by WT APC expression and appears to be independent of the canonical Wnt pathway. Consistent with this finding, they showed that lncRNA-APC1 is frequently downregulated in CRC tissues and that low expression of lncRNA-APC1 is correlated with shorter survival. Analysis of the lncRNA-APC1 promoter and further experiments identified PPARα as a mediator of this effect through the ability of APC to downregulate PPARα DNA binding in the lncRNA-APC1 promoter. The mechanisms by which APC can reduce the DNA binding activity of PPARα were not investigated in the present report but may be of great interest in future studies. The authors showed that lncRNA-APC1 expression can reprogram cancer cells and affect multiple cancer behaviors such as cell proliferation and migration, metastasis, and angiogenesis. Interestingly, one mechanism by which lncRNA-APC1 appears to mediate its biological effects is through its ability to bind directly to and decrease the stability of Rab5b mRNA, thereby decreasing overall exosome production. While not investigated in the study by Wang et al. (20), it appears likely that lncRNA-APC1 can modulate the expression of multiple mRNAs and possibly miRNAs, and future studies will likely be aimed at identifying and studying these targets. In any case, the exact mechanisms by which the modulation of exosome production exerts its biological effects is probably quite complex, but the authors show that the exosomes may help activate the MAPK pathway in endothelial cells.

Are all APC pathways created equal?
As the number of pathways and roles attributed to APC continues to expand (Figure 1), it will be important to gain a better understanding of the relative contribution of each of these pathways to colorectal tumorigenesis, as it is unlikely that all of the pathways play equivalent roles in the development of cancer. Are some pathways only important under certain situations? Or are some pathways only relevant in combination with other genetic or epigenetic alterations? The complexity is, of course, immense, but we do have some clues. Through a large amount of mechanistic as well as genetic evidence, it is clear that the canonical Wnt pathway plays a major role in the initiation and development of CRC (4). But what is the relative importance of the other pathways? Could they have roles at different stages, or under different conditions (survival during chemotherapy, for example)? While the identification of new signaling pathways has brought to light the complexity and versatility of the APC protein, there has been little integration of these different mechanisms and functions as they relate to cancer development. It will be important to determine which of these pathways are true cancer drivers, and under what conditions.

Clinical implications
As described above, the importance of the canonical Wnt pathway in CRC is well established, and, because of its numer-
ous components and signaling nodes, this pathway has been an area of intense focus for drug development (1, 18). For example, glycogen synthase kinase 3β (GSK3β), casein kinase 1α, CREB-binding protein, and many other proteins of this pathway have been identified as possible targets for the development of small-molecule CRC drugs. An important aspect of the current study is the identification of potential new targets for the treatment of APC-deficient cancers. In particular, PPARα is a druggable molecule with existing antagonists and therefore provides a testable hypothesis for rapid translation to the clinic.

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