OXFORD

JNCI J Natl Cancer Inst (2017) 109(8): djw332

doi: 10.1093/jnci/djw332
First published online April 7, 2017
Review

REVIEW

Multiple Roles of APC and its Therapeutic Implications in Colorectal Cancer

Lu Zhang, Jerry W. Shay

Affiliations of authors: Department of Cell Biology, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390, USA.

Correspondence to: Jerry W. Shay, Ph.D., Department of Cell Biology, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX, 75390-9039, USA (e-mail: jerry.shay@utsouthwestern.edu).

Abstract

Adenomatous polyposis coli (*APC*) is widely accepted as a tumor suppressor gene highly mutated in colorectal cancers (CRC). Mutation and inactivation of this gene is a key and early event almost uniquely observed in colorectal tumorigenesis. Alterations in the *APC* gene generate truncated gene products, leading to activation of the Wnt signaling pathway and deregulation of multiple other cellular processes. It has been a mystery why most patients with CRC retain a truncated APC protein, but accumulating evidence suggest that these C terminally truncated APC proteins may have gain of function properties beyond the well-established loss of tumor suppressive function. Here, we will review the evidence for both the loss of function and the gain of function of APC truncations and how together they contribute to CRC initiation and progression.

Adenomatous polyposis coli (*APC*) has been regarded as a colorectal cancer (CRC) tumor suppressor gene and it is dysregulated at both the germline and somatic level (1). The *APC* gene is located on chromosome 5q21-q22, consists of 8535 nucleotides and spans 21 exons. The *APC* gene encodes a 310 kDa protein which is composed of 2843 amino acids. Approximately 75% of the coding sequence is located on exon 15, which appears to be the most common region for both germline and somatic mutations of *APC* (2). Germ-line mutations in the *APC* gene result in familial adenomatous polyposis (FAP), the major hereditary predisposition event leading to CRC development (3). Somatic *APC* mutations are found in more than 80% of sporadic colorectal tumors and loss of heterozygosity (LOH) of chromosome 5q is reported in 30%–40% of CRC cases (4, 5). APC is a multi-domain protein and it serves multiple functions through different binding partners. From the N terminus to the C terminus, there is an oligomerization domain, an armadillo repeat-domain, a 15- or 20-residue repeat domain, SAMP repeats domain, a basic domain and C-terminal domains (6). The oligomerization domain has been shown to be a binding site for APC mutants. *APC* mutant proteins that retain at least the first 171 amino acids are able to bind to this region and may produce a dominant negative effect (7, 8). The armadillo repeat domain is the most conserved domain and has been shown to bind to IQ-motif-containing GTPase activation protein 1 (IQGAP1), PP2A, Asef, and KAP3 (9–12). These interactions largely contribute to stimulation of cell migration and cell adhesion. The following 15-, 20-residue repeat domain, and SAMP repeats play central roles in negative regulation of the canonical Wnt signaling pathway by aiding in proteasomal degradation of β-catenin. The basic and C-terminal domains that can bind to microtubules, directly or indirectly, through interaction with EB1 are important for microtubule stabilization, kinetochore functions, and chromosomal segregation. Due to its interactions with a variety of proteins, APC is involved in cellular processes related to cell migration, cell adhesion, proliferation, differentiation, and chromosome segregation (13–17) (**Figure 1A and B**).

Alterations in the *APC* gene is an early, if not an initiating, event for 80%–85% of sporadic colorectal cancers, except for those that exhibit a CpG island methylator phenotype (CIMP) or a hypermutable micro-satellite instability (MSI) phenotype due to mismatch repair (MMR) deficiency (18–20). Exome sequencing

Received: September 21, 2016; **Revised:** November 29, 2016; **Accepted:** December 21, 2016

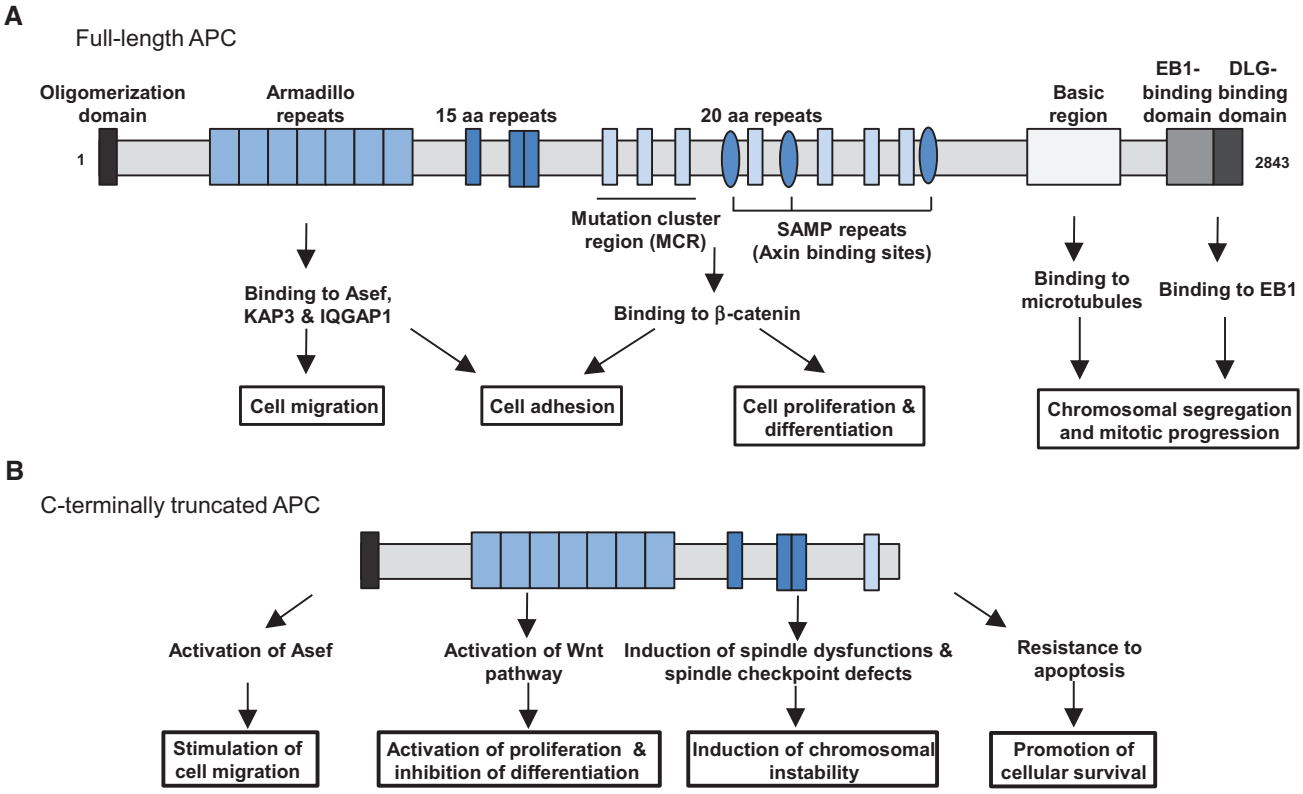

Figure 1. Structure and major functions of full length and truncated APC. **A**) Full length APC proteins contain multiple domains including oligomerization domain, an armadillo repeat-domain, a 15- or 20-residue repeat domain important for binding to β-catenin, SAMP repeats for axin binding, a basic domain for microtubule binding, and C-terminal domains that bind to EB1 protein. Due to its numerous interactions with a variety of proteins, APC is involved in cellular processes related to cell migration, adhesion, proliferation, differentiation, and chromosomal segregation. Most of APC mutations occur within mutation cluster region (MCR). **B**) The C-terminally truncated proteins present in CRC lack the domains that are required for binding to microtubule, EB1 and β-catenin, thus leading to the induction of chromosomal instability, activation of proliferation, and inhibition of differentiation. Truncated APC may have dominant properties that lead to stronger stimulation of cell migration and promotion of cellular survival.

of human colon adenomas and adjacent mucosa reveals that *APC* mutations are present in premalignant lesions, supporting its role in early growth of colon adenoma and the possibility of targeting *APC* loss as a chemopreventive strategy (21, 22). The new classification system has placed CRC into four consensus molecular subtypes (CMSs) among which CMS2, CMS3, and CMS4 have a higher frequency of *APC* mutations (over 50%) compared to CMS1 and are thus more often associated with *APC* mutated CRC (23). Genetic studies using a wide variety of *Apc* mutant mouse models demonstrated that mutations in the *Apc* gene are responsible for intestinal tumorigenesis (24). Although both alleles are altered in colorectal cancer cells mostly by mutations or LOH (1), *APC* mutations generally do not cause complete loss of APC protein. Instead, more than 90% of *APC* mutations generate premature stop codons, resulting in stable truncated gene products, most (~60%) of which occur within a region referred to as the mutation cluster region (MCR) (25). The mutational spectrum of *APC* in CRC is displayed in **Figure 2** and is based on datasets from The Cancer Genome Atlas. As indicated in the lollipop plot, 14.3% (131/913) of detected *APC* mutations are missense mutations whereas 85.7% (782/913) are truncating mutations. Stable expression of truncated APC proteins have been detected in FAP and CRC patient samples (26). The C-terminally truncated proteins present in CRC lack the domains that are required for binding to microtubules, EB1, and β-catenin, potentially leading to the induction of chromosomal instability, activation of proliferation, and inhibition of differentiation (**Figure 1B**) (6). While loss of tumor suppressing functions due to the mutational loss of the APC C-terminal sequence has been regarded as a critical event in the initiation of colon cancer, accumulating evidence suggest that APC truncations exert dominant functions that might contribute to colon tumorigenesis (**Figure 3**). Below, we discuss the loss of tumor suppressive function and the recent evidence for the gain of function of APC truncations, examining their potential roles in colon cancer initiation and progression.

Loss of Tumor Suppressive Function of APC in CRC

Colorectal tumors are known to arise from preexisting premalignant lesions through the acquisition of genetic alterations in a specific oncogene or tumor suppressor, the so-called "adenoma-carcinoma" sequence (3, 27). Mutational loss of the APC C-terminal sequence is regarded as essential to the initiation of colon cancer through loss of APC tumor suppressive functions. The following sections provide a brief review of the well-established tumor suppressive role of APC in CRC.

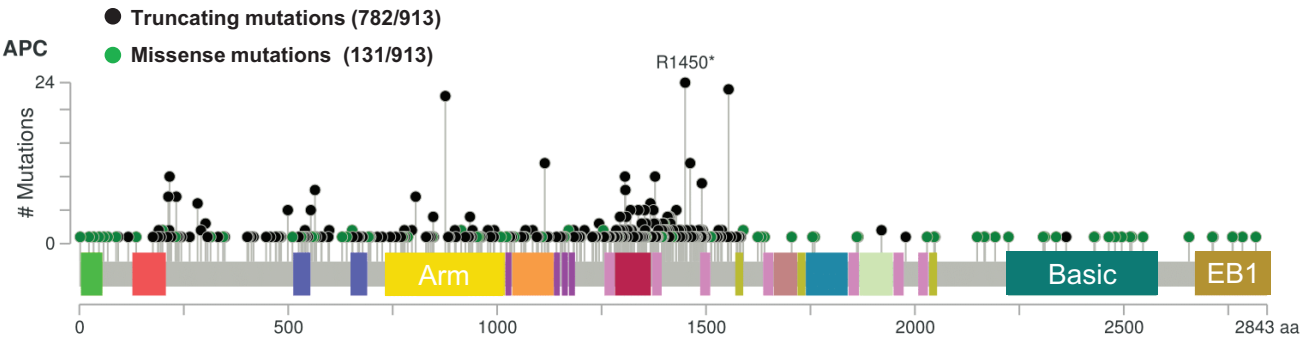

Figure 2. Lollipop plot showing the distribution and classes of mutations in APC across colorectal cancer datasets in The Cancer Genome Atlas. Plot originally generated using the cBioPortal (103). **Light circles** indicate missense mutations and **dark circles** indicate truncating mutations (including nonsense, nonstop (mutation occurring within a stop codon), frameshift deletion, frameshift insertion and splice site mutations). Arm=armadillo.

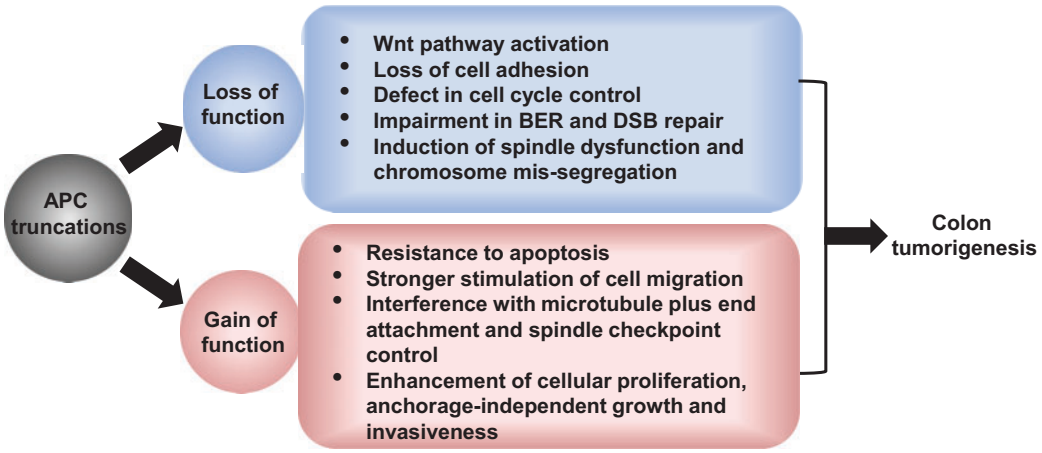

Figure 3. Multiple roles of APC truncations in colorectal tumorigenesis. Both the loss of tumor suppressive functions and gain of functions of truncated APC proteins contribute to the initiation, progression and maintenance of colorectal cancer. BER=base excision repair; DSB=double stranded break.

REVIEW

Wnt Pathway

APC is a key negative regulator of the canonical Wnt signaling pathway which controls the coordinated cell proliferation and differentiation in the gastrointestinal tract (28). The primary mechanism through which APC inhibits β-catenin/T-cell factor (TCF)-dependent transcription is by providing a scaffold for the destruction complex that stimulates phosphorylation and subsequent ubiquitin-dependent degradation of β-catenin (29). APC increases the efficiency of this destruction machinery by promoting Axin multimerization and stabilizing the Axin complex (30). Other regulatory mechanisms include reducing nuclear β-catenin levels by promoting export of β-catenin (31), blocking interactions with TCF by directly binding to β-catenin (31), and facilitating C terminal binding protein (CtBP)-mediated repression of Wnt-target genes through direct interaction with a repression complex (32, 33). Alterations in *APC* lead to activation of β-catenin/TCF transcriptional activity by both increasing nuclear β-catenin levels and attenuating CtBP-mediated inhibition of the repression complex. This activation leads to up-regulation of downstream targets, such as cyclin D1 and Myc, which are important drivers in tumor formation due to their well-established roles in cell proliferation, apoptosis, and cell-cycle progression (34, 35). Interestingly, there is a strong selection pressure favoring the retention of the first 20 APC amino acid repeat that can bind to β-catenin and regulate its transcriptional activity (36, 37). One explanation of this phenotype is the "just-right" signaling model which proposes that selection for *APC* genotypes is aimed at a specific degree of β-catenin that is optimal for tumor formation (38). According to this model, deletion of all β-catenin binding sites are less favorable because constitutive activation of β-catenin pathway leads to extensive changes in gene regulation that results in increased risk of cell death. In contrast, retaining some of the β-catenin binding sites may allow for partial down-regulation thus providing optimal Wnt signaling activity that is sufficient to confer proliferation advantage without inducing cell death. In agreement, the "three-hit" hypothesis was subsequently proposed which states that the optimal Wnt level for CRC tumor cells might change during tumor progression due to changes in the microenvironment or by acquisition of additional genetic alterations. Some CRCs can respond by modulating Wnt signaling through copy number changes or other types of "third hit" at *APC* (39). In addition to the "just-right" model, an alternative theory called "just-right nuclear-export activity" has been proposed (40). This theory hypothesizes that APC truncations, due to the loss of the central nuclear export signals which are 3' adjacent to the MCR, have reduced nuclear-export activity that compromises its maximum tumor-suppressing function (40, 41).

Cell Adhesion

Inactivation of *APC* has also been proposed to promote tumorigenesis through loss of cell-cell adhesion (42). APC interacts

with a subcellular pool of β-catenin which serves as a component of adherens junctions, linking E-cadherin to α-catenin and the actin cytoskeleton (43, 44). Enterocytes and tumor cells from $Apc^{min/+}$ mice which has one copy of mutant *Apc* have decreased levels of E-cadherin at the cell membrane and reduced associations between β-catenin and E-cadherin (45). Expression of full length APC in CRC cells harboring truncated APC results in increased levels of E-cadherin at the cell membrane and causes translocation of β-catenin from the nucleus and cytoplasm to the cell periphery, which enhances cell adhesion (46). Therefore, APC may regulate cell adhesion by controlling the distribution of β-catenin and E-cadherin between the cytoplasm and the cell membrane. APC mutants lacking the binding domains for β-catenin result in weakened cell adhesion.

Cell Cycle Control

Overexpression of full length APC is proposed to block serum-induced cell cycle progression from G0/G1 to the S phase in NIH3T3 fibroblasts whereas the inhibitory effects of mutant APC are less (17). Consistent with these data, transient overexpression of full length APC induces G1 phase arrest in colorectal cancer cell lines (47). This function is partially attributable to regulation of β-catenin/Tcf–mediated transcription of S-phase regulators such as *cyclin D1* and *c-myc* (47). It is also proposed that APC may affect proliferation in a β-catenin–independent manner. Thus, mutant APC defective in maintaining G1/S checkpoint control may contribute to aberrant cell proliferation.

DNA Repair

APC is primarily located in the cytoplasm, yet it has also been found to shuttle into the nucleus to regulate nuclear functions, including DNA repair (31, 48). Full length APC can directly bind to polymerase β (Pol-β), FEN1 endonuclease, and APE1 endonuclease, inhibiting the assembly of base excision repair (BER) proteins on damaged DNA and blocking long patch BER (LP-BER) (49, 50). The DNA repair inhibitory (DRI) domain of APC, which is the binding site for Pol-β and FEN1, is located in the N terminal region and is retained in APC mutants. Thus most truncated APC proteins are still capable of modulating BER, possibly to different extents (51). For example, it has been reported that LoVo, colon cancer cells expressing truncated APC protein, shows an accelerated assembly of BER proteins, as well as higher activity of APE1, FEN1 and Pol β (50). Re-expression of full length APC decreases FEN1 expression and sensitizes this cell line to 5-fluorouracil (52). Increased APE1 activity results in unbalancing of the BER pathway and may favor chromosome instability (CIN) and cancer progression (53). APC can also interact with replication protein A 32 (RPA32) to modulate the replication stress responses (54) and is directly involved in DNA double stranded break (DSB) repair as a part of the nuclear complex containing DNA-dependent protein kinase (DNAPK) (55). Collectively, mutated APC may have attenuated functions in BER and DSB repair, allowing for CRC cells to accumulate genetic alterations. With respect to therapeutic implications, CRC cells harboring mutant APC may be more vulnerable to DNA damaging chemotherapeutic agents, such as oxaliplatin and 5-fluorouracil.

Tumor Suppressor Role in Chromosome Instability (CIN)

APC can bind to and stabilize microtubules directly or through its association with EB1, a microtubule binding protein that is enriched at microtubule plus ends (14, 56, 57). APC is also found to localize to kinetochores in mitotic cells and form a complex with checkpoint proteins Bub1 and Bub3 (13). Removal of APC in U2OS and HCT116 cancer cells decreases association of checkpoint proteins Bub1 and BubR1 with kinetochores, alters mitotic progression, and increases mitotic slippage (58). Cells carrying a truncated *APC* gene (Min) are defective in chromosome segregation (13). In addition, small interfering RNA (siRNA)-mediated knockdown of APC and/or EB1 causes defects in mitotic spindle and chromosome alignment (59). Aberrant spindle structures and weakened kinetochore-microtubule attachments have been observed in CRC cells harboring APC truncations (60, 61). Moreover, Apc deficiency leads to an increase in anaphase bridge and chromosome numbers within hepatocytes in mice (62). Taken together, the C terminal truncated APC can lead to dysfunction in spindle formation and mitotic progression due to the lack of binding domains for microtubules, contributing to CIN and CRC progression.

Dominant-negative Effects of APC Truncation

On the basis of the correlation between FAP severity and specific truncating *APC* mutations, and the association of mutant APC proteins with wild type (WT) APC protein in vitro as well as in vivo, it has been proposed that APC truncations may inactivate WT APC in a dominant negative manner (8). Exogenous expression of a mutant APC that is truncated at codon 1309 antagonizes WT APC induced reduction in Tcf-mediated transcriptional activity (7). In another study, expression of a mutant APC increased β-catenin levels, decreased the proliferation rate, and decreased cell migration in histologically normal Min/+ mucosa compared with that of +/+ mice (63). However, evidence against the dominant negative effects comes from a study in mice carrying a transgene encoding Apc amino acid 1-716 ($Apc^{\Delta 716/+}$). Since no difference is seen in terms of tumor number, distribution, or morphology in $Apc^{\Delta 716/+}$ mice with or without an extra truncated *Apc* transgene, it is concluded from this study that Apc truncation does not act in a dominant negative manner (64). While these studies provide evidence against the dominant-negative effects of APC truncations, this conundrum has not yet been completely resolved.

APC Truncation: Switch from Tumor Suppressor to Oncogene?

Although both alleles are altered in *APC*-defective colorectal tumors, homozygous deletion of *APC* is absent or very rare. Analysis of tumors from FAP patients and colorectal tumors reveal that there appears to be an interdependence of the "two hits" at *APC*. Specifically, germline mutations within the *APC* MCR are associated with allelic loss of the remaining wild-type allele whereas mutations occurring outside of this region are associated with truncating mutations (5). A correlation between the presence of a germline mutation in the MCR and the severity of polyposis has also been noted (65, 66). In addition to the widely accepted argument that the strong selection for these truncated APC proteins is largely due to the requirement for the optimal level of β-catenin activity (38, 67, 68), emerging evidence supports that APC mutants may have gain of function properties that promote tumor progression beyond simple loss of function. The following sections will review the evidence for the reported dominant functions of APC mutants in terms of cell survival, cell migration, chromosomal stability, and beyond.

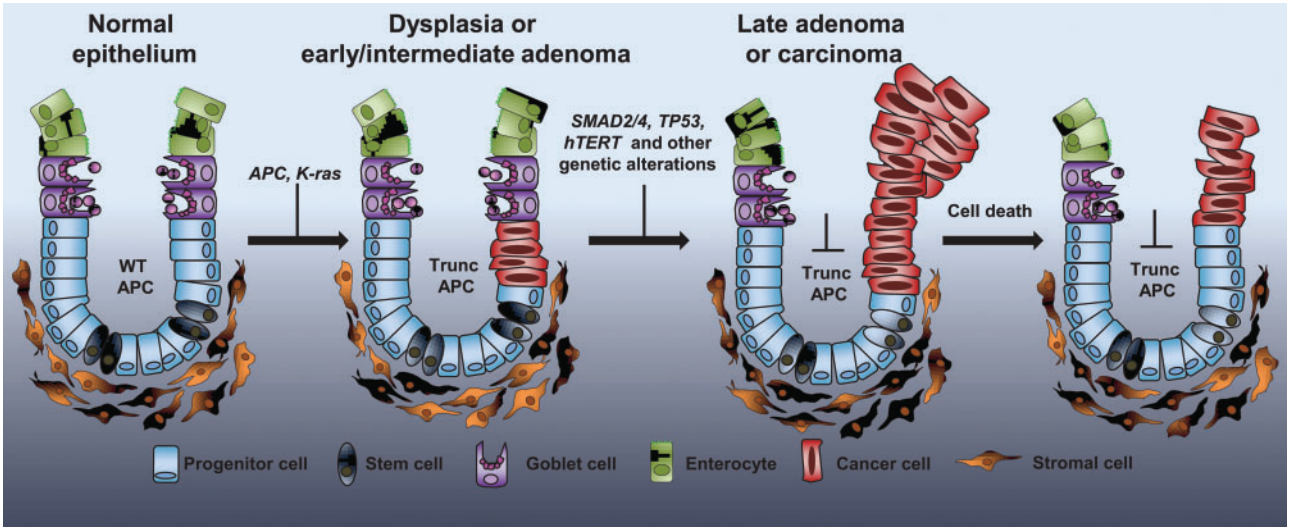

Figure 4. Dependency of colorectal cancer (CRC) cells on truncated APC for cell growth and survival. In early stage of CRC tumorigenesis, APC truncations can promote tumorigenic properties in the presence of other genetic alterations. Gradually, the late stage CRC cells with altered signaling network become "addicted" to truncated APC for cell survival and maintaining tumorigenic properties. Interfering with expression/functions of truncated APC can affect cellular proliferation and tumor cell survival. Trunc=truncated; WT=wild type.

Cell Survival

In contrast to full length APC, which promotes apoptosis when overexpressed in colon cancer cells (69), APC mutants may harbor anti-apoptotic functions through a transcription-independent mechanism. It has been shown that the addition of APC truncation 1309 into cell-free *Xenopus* egg extracts decelerates apoptosis-associated caspase cleavage through a mechanism that is independent of β-catenin-mediated effects on transcription (70). Further studies demonstrate that truncated APC proteins are resistant to cleavage by group II caspases and are unable to accelerate apoptosis in vitro or in vivo (71). In addition to aiding in evading apoptosis, APC truncations promote cell survival through other mechanisms as well. Truncated APC mutants preferentially localize to mitochondria and promote cell survival through regulation of Bcl-2 (72). In this study, exogenous expression of APC truncation, but not full-length APC, protects SW480 cells against sulindac-induced apoptosis whereas transient knockdown of APC truncation in SW480 cells leads to an increase of apoptotic cell death with concurrent down-regulation of Bcl-2 at mitochondria. Another study shows that transient knockdown of APC in SW480 cells compromises DNA replication and cell proliferation through down-regulation of cell cycle components (73). Altogether, these data can be interpreted to suggest a pro-survival function of truncated APC that CRC cells become addicted to for their survival and proliferation (**Figure 4**).

Cell Migration

APC regulates cell migration through different mechanisms, including control of the actin cytoskeleton (74), regulation of the microtubule network (75), and interaction with APC-stimulated guanine nucleotide exchange factor (Asef), a Rac-specific guanine-nucleotide-exchange factor (GEF) (11, 76). APC was initially identified as a binding partner with Asef1 through its armadillo repeat domain. This interaction enhances the GEF activity of Asef1 and stimulates Asef-mediated cell flattening, membrane ruffling, and lamellipodia formation in MDCK cells (11). Interestingly, exogenous expression of truncated APC but not full length APC stimulates Asef-mediated cell migration in MDCK cells. Additionally, short hairpin RNA (shRNA)-mediated knockdown of truncated APC proteins inhibits cell migration in SW480 and WiDr cells whereas expression of shRNA-APC in HCT116 and LS180 cells with WT APC does not affect cell migration (76, 77). These results demonstrate that truncated APC but not full length APC is an effective activator of Asef. Therefore, expression of truncated APC could lead to aberrant cell migration in CRC cells. More recently, N-terminal fragments of APC have been shown to cause dominant defects in directional cell migration in multiple model systems (78).

Oncogenic Role in Chromosome Instability (CIN)

Certain forms of APC truncations have been reported to have dominant effects on proliferation, spindle checkpoint control, survival, and chromosome stability (61, 79). Expression of mutant APC (1-1450) dominantly disrupts spindle microtubules and induces abnormal chromosome segregation in 293 cells (61). Cells expressing only the N-terminal 750 (N750) amino acids of APC show weakened kinetochore-microtubule interactions and exhibit a phenotype more typical of CIN lines (79). In an independent study, APC mutants dominantly inhibit cytokinesis by blocking initiation of the cytokinetic furrow (80). These results provide more direct evidence that the dominant functions of APC mutants may be important in the induction of aneuploidy in vivo (80). Although it remains to be answered whether CIN is a contributing factor or an indirect side effect of oncogenic processes, there is increasing evidence suggesting that CIN may play a role in tumor formation (81, 82).

Additional Support for Dominant Functions of APC Truncations

Until recently there has been limited, if any, cell-based studies on truncated APC's oncogenic properties, mostly due to a lack of optimum cellular reagents. The majority of previous studies

Box 1. Outstanding questions regarding truncated APC in CRC

- Why are APC truncations so common in colorectal cancer (CRC) and not other cancer types?
- Are there novel binding partners for truncated APC that differ from wild type (WT) APC protein?
- What are the molecular mechanisms underlying the dominant effects of truncated APC on tumorigenic properties?
- What is the functional link between truncated APC and cholesterol homeostasis? And what is the implication for CRC tumorigenesis?
- Are there additional synthetic lethal interactions with truncated APC that are druggable?

used cultured cell models of CRC of malignant origin containing multiple cytogenetic changes which imposes difficulties in assigning linear genetic relationships from gene function studies. As part of our efforts to elucidate the molecular underpinnings of colon cancer tumorigenesis, our lab has developed a series of human colonic epithelial cell (HCEC) lines immortalized with telomerase and cyclin-dependent kinase 4 (CDK4) (83). We showed that these cells are non-transformed, karyotypically diploid, and have multipotent stem-like characteristics. When placed in Matrigel in the absence of a mesenchymal feeder layer, individual cells divide and form self-organizing, crypt-like organoid structures with a subset of cells exhibiting markers associated with mature epithelium (83). Utilizing these immortalized HCECs, we demonstrated that truncated APC proteins are able to confer partially progressed HCECs more tumorigenic properties (84). Ectopic expression of APC1309 confers a moderate proliferative advantage and enhancement of soft agar growth as well as invasion through Matrigel compared to its matched parental cell lines. However, stable knockdown of greater than 90% WT APC does not have any of these effects, demonstrating that loss of APC function by itself does not drive colon cancer progression in this experimental cell culture model system. Consistent with our observations, Pineda et al. also show that ectopic expression of A1309 promotes tumorigenic properties in HCEC cells expressing oncogenic Kras (85). Additionally, knockdown of truncated APC proteins in DLD1 CRC cells slows down cell proliferation and leads to cell death in a subpopulation of the cells when cultured in more physiological low serum medium (84). In agreement with this observation, down-regulation of truncated APC reduces cellular proliferation in CRC cell lines in vitro and inhibits tumor formation in nude mice (86). These findings lend support to the notion that APC truncations can promote tumorigenic properties in the presence of other genetic alterations (in advanced lesions with additional genetic alterations). CRC cells harboring truncated APC may gradually become "addicted" to this truncated protein for cell survival and for maintaining tumorigenic phenotype (**Figure 4**), especially in nutrient starved conditions which is likely to exist in the emerging tumor microenvironment. This phenomenon of "oncogene addiction" for a mutant tumor suppressor is similar to the case of mutant p53 where malignant properties of cancer cells have been shown to be partially dependent on the presence of mutant p53 (87). The CRC tumors with LOH of 5q and large deletions of *APC*, however, must have evolved alternative mechanisms independent of APC's function to survive. Going forward, it would be important to investigate the downstream effectors/pathways that contribute to the progression of normal HCECs to tumor-cell-like HCECs (**Box1**). It may also be worthwhile to investigate the dominant effects of truncated APC using colon organoids culture system, which provides a potentially more physiologic context since colon organoids have recently been shown to successfully recapitulate human CRC progression (88, 89).

Lessons from Genetic Mouse Models

For the past two decades, extensive efforts have been made in generating and characterizing Apc mouse and rat models to investigate Apc functions in intestinal homeostasis and tumor suppression (90). The first hereditary mouse model of colon cancer, designated as multiple intestinal neoplasia (*Min*), was identified in an ethylnitrosourea (ENU) mutagenesis screen. The $Apc^{Min/+}$ mice harbor a nonsense mutation resulting in Apc truncation at codon 851 and have been used extensively to study Apc tumor suppressive functions since its first description in 1990. However, this model has many limitations in terms of human translation since these mice develop polyps and benign adenomas with most of them present in the small intestine (91). More recently, a wide variety of mouse models expressing truncated Apc protein longer or shorter than Apc^{Min} have been generated. Most, if not all, of these models develop adenomas (polyps) with varying numbers and varying intestinal distributions. However, there is no distinct correlation between the mutational spectrum and intestinal phenotypes (24, 90). Studies of these models have aided in discovery of important pathways involved in colon tumorigenesis but have also raised several unanswered questions. In particular, statistically significant phenotypic variation has been observed among the different models, even in the same model analyzed by different laboratories (24). Some possible explanations for these observed variations include different rates and mechanisms of WT Apc loss, differences in Wnt signaling, varying contributions of genetic modifiers or environmental factors, as well as differences in technologies used (24, 90).

Recently, a mouse model with a complete deletion of all 15 *Apc* exons ($Apc^{\Delta e1\text{-}15}$) was generated to test the requirement of truncated Apc for tumor formation. $Apc^{\Delta e1\text{-}15}$ mice develop intestinal polyps of the same distribution and histology as with $Apc^{Min/+}$ mice but at higher frequency in female mice. Colon tumors from these mice seem to have lower levels of Wnt pathway activation compared with $Apc^{Min/+}$ mice (92). This observation is consistent with the "just right" hypothesis that submaximal level of β catenin level is advantageous for tumor formation, yet the underlying mechanism remains unknown. This study superficially argues against the pro-tumorigenic functions of truncated Apc, however, several caveats need to be considered. First, since the more severe polyposis phenotype is only observed in female $Apc^{\Delta e1\text{-}15}$ mice on a C57BL/6 background but not in male $Apc^{\Delta e1\text{-}15}$ mice, it would be premature to draw any conclusions as to the tumorigenic functions of truncated Apc. In addition, there are no reports of this deletion in mice on a different background. As the phenotypes of CRC mouse models are greatly influenced by many factors, use of standardized conditions to compare the phenotype of different *Apc* mutant mouse models would be more appropriate for reliable genotype-phenotype analyses (24). Additionally, since these germline *Apc*

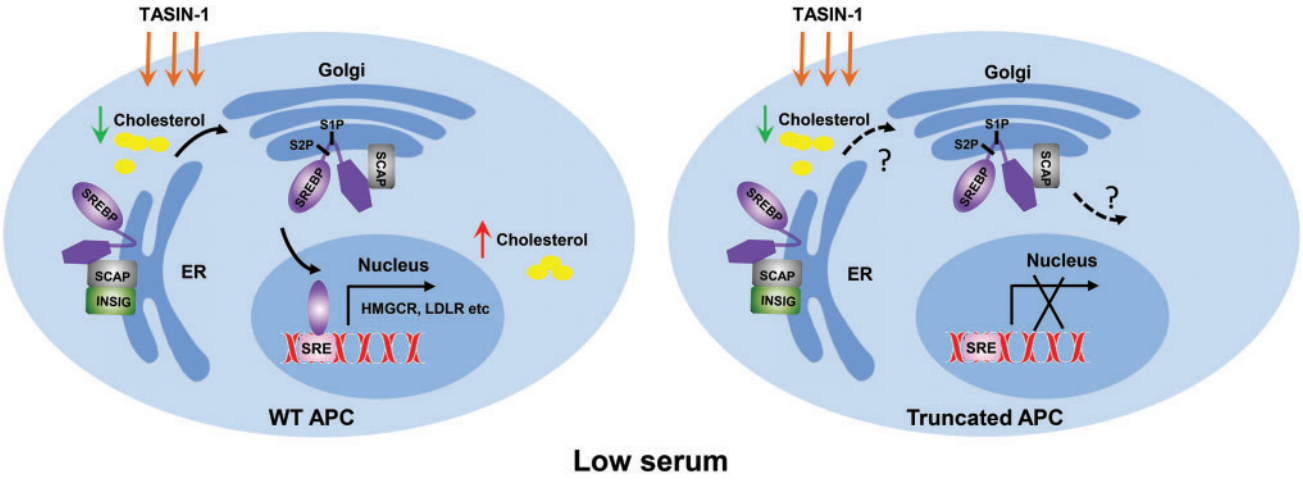

Figure 5. Therapeutics selectively targeting truncated APC cells. In wild type (WT) APC cells, TASIN-1 induced cholesterol depletion leads to conformational change of SCAP protein which is then released from INSIG and assists SREBPs transport from endoplasmic reticulum (ER) to Golgi. SREBP is cleaved by proteases S1P and S2P in the Golgi and the cleaved form of SREBP then translocates into the nucleus and activates expression of genes involved in cholesterol synthesis and uptake. This compensates for reduced cellular level of cholesterol, thus cells survive. In contrast, truncated APC compromises the normal feedback mechanism in response to TASIN-1 treatment when cultured in low serum conditions, therefore cells undergo apoptotic cell death due to cholesterol depletion. HMGCR=3-hydroxy-3-methylglutaryl-CoA reductase; LDLR=low density lipoprotein receptor; TASIN-1=truncated APC selective inhibitor.

mutant mice develop polyps mainly in the small intestine with few polyps in colon tissue, observations from these studies do not warrant definitive conclusions regarding human CRC tumorigenesis. Furthermore, these mouse models harbor *Apc* germline mutations whereas most CRC patients harbor somatic *APC* mutations which occur stochastically (93). Alternatively, it is possible that truncated APC becomes indispensable in later stages of tumor progression which cannot be reflected in these mice because the adenomas limit their life span. Finally, as is true with all mouse models of cancer, the CRC mouse models are different from humans in many aspects, such as size, life span, organ morphology, and physiology. In particular, in contrast to humans that have regulated telomerase, mice constitutively express active telomerase in many tissues and have very long telomeres which makes modeling of genomic instability in mice more problematic (93).

The Apc mouse models discussed above share the limitations that the majority of tumors appear in the small rather than large intestine and most of the mice develop adenomas with few, if any, occurrence of carcinoma (94, 95). Therefore, genetically engineered mouse models with colon specific disruption of Apc in the epithelial cells may represent a better tool for colon cancer research. For example, *CAC; Apc* and *CPC;Apc* mouse models are two such models harboring conditional inactivation of *Apc* with expression of *CAC-Cre* or *CDX2-Cre* transgene, both of which develop polyps mainly in the large intestine with low penetrance (96, 97). Although these models are much improved compared with previous ones, one limitation is that both models express Apc580 truncation which does not occur at a high frequency in human CRC. Future efforts are warranted to develop colon specific Apc mouse models with expression of Apc truncations within the MCR region, which will be more reflective of human CRC cases.

Therapeutic Opportunities

APC is clearly involved in many important signaling pathways and biological processes implicated in CRC carcinogenesis, with the Wnt signaling pathway as the most well-established one being affected by *APC* mutation. More recent work shows that Apc restoration drives tumor regression and reestablishes crypt homeostasis in CRC, validating the Wnt pathway as a therapeutic target for CRC treatment (98). In the past decade, intensive efforts have been focused on development of therapeutic strategies targeting the canonical Wnt signaling pathway in CRC and various small molecules have been shown to effectively inhibit Wnt signaling activity via targeting different components of this pathway (99–101). However, the potential toxicity of Wnt pathway inhibitors on normal intestinal epithelium as well as off-target effects may limit their clinical applications going forward (102).

Although it is well recognized that APC has diverse functions beyond regulation of the Wnt pathway, there are few therapeutic agents directly targeting the non-Wnt facet of APC truncation function. Utilizing the series of above mentioned isogenic HCEC cell lines, a small molecule named TASIN-1 (Truncated APC Selective INhibitor), that specifically kills cancer cells with APC truncations while sparing WT APC cells, has recently been identified (84). TASIN-1 can inhibit tumor growth of APC truncated CRC cells in both xenograft models and in a genetically engineered CRC mouse model with minimal toxicity. Mechanistic investigation of TASIN-1 reveals a synthetic lethal interaction between the endogenous cholesterol biosynthesis pathway and APC truncations in tissue physiologic low serum conditions, partially due to defective SREBP2 feedback activation in response to TASIN-1 induced cholesterol depletion in truncated APC cells (**Figure 5**). Importantly, this compound exerts its cytotoxicity independent of Wnt pathway inhibition and does not exhibit deleterious effects in general or specifically on normal human or mouse colonic epithelium. This discovery opens up the possibilities of employing more specific cholesterol biosynthesis inhibitors for the treatment of truncated APC CRCs, potentially in combinations with chemotherapeutic or other molecularly targeted agents, in combination with radiotherapy, or as a maintenance therapy in patients with minimal disease status. This compound and its analogs represent a potential paradigm shift in targeted therapy for CRC patients using

mutant APC as an enrollment biomarker for future personalized medicine clinical trials. Future investigations are warranted to elucidate the functional link between truncated APC and cholesterol homeostasis (Box 1). It can be envisioned that genome-wide loss-of-function screens will facilitate the identification of additional synthetic lethal interactions with truncated APC and novel drug targets (Box 1). It is worth emphasizing that TASIN-1 is only effective in 0.2% serum media and loses activity in typical culture media with 10% serum. The serum containing common culture conditions used in most, if not all, cancer biology studies are perhaps why the cholesterol connection to CRC has been missed in the past. In addition, since TASIN-1 inhibits tumorigenesis in a genetically engineered CRC mouse model, this result highlights that low serum/cholesterol culture conditions represent a more physiological relevant microenvironment. Therefore, it is important to rethink our current cell culture methodology and refine it in a way that is more reflective of the in vivo tumor microenvironment.

Conclusions and Future Perspectives

The tumor suppressive function of APC has long been a focus of research in the field of colorectal cancer, whereas emerging evidence has called attention to the oncogenic functions of mutated APC. There is accumulating support suggesting that both loss of tumor suppressive function and gain of function of APC mutants play critical roles in CRC tumorigenesis. As tumorigenesis progresses, cells undertake an altered pattern of signaling networks when they gradually become dependent on oncogenic function of the truncated APC proteins for survival or maintenance of their malignant phenotypes (oncogene addiction). Further studies of APC truncations' oncogenic properties are clearly warranted. The utilization of proteomics as well as advanced genome-wide high throughput screening approaches will aid in identifying the novel binding partners of truncated APC to obtain a more complete understanding of the signaling network in CRC cells. Understanding the comprehensive functions of these truncated APC mutants and downstream effector proteins will shed light on the molecular mechanisms of CRC tumorigenesis and reveal novel drug targets, allowing for the development of additional targeted therapeutics for the treatment of CRC.

Funding

This work was supported in part by CPRIT Grants #RP130189 and #RP160180 to J.W.S. This work was performed in laboratories constructed with support from NIH grant C06 RR30414. J.W.S. holds the Southland Financial Corporation Distinguished Chair in Geriatrics Research.

Notes

The funders had no role in the writing of the review or the decision to submit it for publication. Competing interests: JWS is a founding member of Elizabeth Therapeutics that is expanding TASIN analogues for clinical development.

References

1. Fodde R, Smits R, Clevers H. APC, signal transduction and genetic instability in colorectal cancer. *Nat Rev Cancer* 2001;1(1):55–67.
2. Beroud C, Soussi T. APC gene: database of germline and somatic mutations in human tumors and cell lines. *Nucleic Acids Res* 1996;24(1):121–124.
3. Kinzler KW, Vogelstein B. Lessons from hereditary colorectal cancer. *Cell* 1996;87(2):159–170.
4. Fearnhead NS, Britton MP, Bodmer WF. The ABC of APC. *Hum Mol Genet* 2001; 10(7):721–733.
5. Rowan AJ, Lamlum H, Ilyas M, et al. APC mutations in sporadic colorectal tumors: A mutational "hotspot" and interdependence of the "two hits". *Proc Natl Acad Sci U S A* 2000;97(7):3352–3357.
6. Aoki K, Taketo MM. Adenomatous polyposis coli (APC): a multi-functional tumor suppressor gene. *Journal of Cell Science* 2007;120(19):3327–3335.
7. Dihlmann S, Gebert J, Siermann A, et al. Dominant negative effect of the APC1309 mutation: a possible explanation for genotype-phenotype correlations in familial adenomatous polyposis. *Cancer Res* 1999;59(8): 1857–1860.
8. Su LK, Johnson KA, Smith KJ, et al. Association between wild type and mutant APC gene products. *Cancer Res* 1993;53(12):2728–2731.
9. Watanabe T, Wang S, Noritake J, et al. Interaction with IQGAP1 links APC to Rac1, Cdc42, and actin filaments during cell polarization and migration. *Dev Cell* 2004;7(6):871–883.
10. Jimbo T, Kawasaki Y, Koyama R, et al. Identification of a link between the tumour suppressor APC and the kinesin superfamily. *Nat Cell Biol* 2002;4(4): 323–327.
11. Kawasaki Y, Senda T, Ishidate T, et al. Asef, a link between the tumor suppressor APC and G-protein signaling. *Science* 2000;289(5482):1194–1197.
12. Seeling JM, Miller JR, Gil R, et al. Regulation of beta-catenin signaling by the B56 subunit of protein phosphatase 2A. *Science* 1999;283(5410):2089–2091.
13. Kaplan KB, Burds AA, Swedlow JR, et al. A role for the Adenomatous Polyposis Coli protein in chromosome segregation. *Nat Cell Biol* 2001;3(4): 429–432.
14. Nathke IS, Adams CL, Polakis P, et al. The adenomatous polyposis coli tumor suppressor protein localizes to plasma membrane sites involved in active cell migration. *J Cell Biol* 1996;134(1):165–179.
15. Browne SJ, MacFarlane M, Cohen GM, et al. The adenomatous polyposis coli protein and retinoblastoma protein are cleaved early in apoptosis and are potential substrates for caspases. *Cell Death Differ* 1998;5(3):206–213.
16. Sansom OJ, Reed KR, Hayes AJ, et al. Loss of Apc in vivo immediately perturbs Wnt signaling, differentiation, and migration. *Genes Dev* 2004;18(12): 1385–1390.
17. Baeg GH, Matsumine A, Kuroda T, et al. The tumour suppressor gene product APC blocks cell cycle progression from G0/G1 to S phase. *EMBO J* 1995; 14(22):5618–5625.
18. Powell SM, Zilz N, Beazer-Barclay Y, et al. APC mutations occur early during colorectal tumorigenesis. *Nature* 1992;359(6392):235–237.
19. Boland CR, Goel A. Microsatellite instability in colorectal cancer. *Gastroenterology* 2010;138(6):2073–2087 e3.
20. Worthley DL, Leggett BA. Colorectal cancer: molecular features and clinical opportunities. *Clin Biochem Rev* 2010;31(2):31–38.
21. Nikolaev SI, Sotiriou SK, Pateras IS, et al. A single-nucleotide substitution mutator phenotype revealed by exome sequencing of human colon adenomas. *Cancer Res* 2012;72(23):6279–6289.
22. Borras E, San Lucas FA, Chang K, et al. Genomic Landscape of Colorectal Mucosa and Adenomas. *Cancer Prev Res (Phila)* 2016;9(6):417–427.
23. Guinney J, Dienstmann R, Wang X, et al. The consensus molecular subtypes of colorectal cancer. *Nat Med* 2015;21(11):1350–1356.
24. Zeineldin M, Neufeld KL. Understanding phenotypic variation in rodent models with germline Apc mutations. *Cancer Res* 2013;73(8):2389–2399.
25. Miyoshi Y, Nagase H, Ando H, et al. Somatic mutations of the APC gene in colorectal tumors: mutation cluster region in the APC gene. *Hum Mol Genet* 1992;1(4):229–233.
26. Smith KJ, Johnson KA, Bryan TM, et al. The APC gene product in normal and tumor cells. *Proc Natl Acad Sci U S A* 1993;90(7):2846–2850.
27. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell* 1990;61(5):759–767.
28. Klaus A, Birchmeier W. Wnt signalling and its impact on development and cancer. *Nat Rev Cancer* 2008;8(5):387–398.
29. Rubinfeld B, Albert I, Porfiri E, et al. Binding of GSK3beta to the APC-beta-catenin complex and regulation of complex assembly. *Science* 1996; 272(5264):1023–1026.
30. Pronobis MI, Rusan NM, Peifer M. A novel GSK3-regulated APC:Axin interaction regulates Wnt signaling by driving a catalytic cycle of efficient betacatenin destruction. *Elife* 2015;4:e08022.
31. Neufeld KL, Zhang F, Cullen BR, et al. APC-mediated downregulation of beta-catenin activity involves nuclear sequestration and nuclear export. *EMBO Rep* 2000;1(6):519–523.
32. Hamada F, Bienz M. The APC tumor suppressor binds to C-terminal binding protein to divert nuclear beta-catenin from TCF. *Dev Cell* 2004;7(5): 677–685.
33. Sierra J, Yoshida T, Joazeiro CA, et al. The APC tumor suppressor counteracts beta-catenin activation and H3K4 methylation at Wnt target genes. *Genes Dev* 2006;20(5):586–600.
34. He TC, Sparks AB, Rago C, et al. Identification of c-MYC as a target of the APC pathway. *Science* 1998;281(5382):1509–1512.
35. Tetsu O, McCormick F. Beta-catenin regulates expression of cyclin D1 in colon carcinoma cells. *Nature* 1999;398(6726):422–426.

REVIEW

36. Schneikert J, Grohmann A, Behrens J. Truncated APC regulates the transcriptional activity of beta-catenin in a cell cycle dependent manner. *Hum Mol Genet* 2007;16(2):199–209.
37. Kunttas-Tatli E, Von Kleeck RA, Greaves BD, et al. The two SAMP repeats and their phosphorylation state in Drosophila Adenomatous polyposis coli-2 play mechanistically distinct roles in negatively regulating Wnt signaling. *Mol Biol Cell* 2015;26(24):4503–4518.
38. Albuquerque C, Breukel C, van der Luijt R, et al. The 'just-right' signaling model: APC somatic mutations are selected based on a specific level of activation of the beta-catenin signaling cascade. *Hum Mol Genet* 2002;11(13):1549–1560.
39. Segditsas S, Rowan AJ, Howarth K, et al. APC and the three-hit hypothesis. *Oncogene* 2009;28(1):146–155.
40. Henderson BR, Fagotto F. The ins and outs of APC and beta-catenin nuclear transport. *EMBO Rep* 2002;3(9):834–839.
41. Rosin-Arbesfeld R, Townsley F, Bienz M. The APC tumour suppressor has a nuclear export function. *Nature* 2000;406(6799):1009–1012.
42. Bienz M, Hamada F. Adenomatous polyposis coli proteins and cell adhesion. *Curr Opin Cell Biol* 2004;16(5):528–535.
43. Kemler R. From cadherins to catenins: cytoplasmic protein interactions and regulation of cell adhesion. *Trends Genet* 1993;9(9):317–321.
44. Su LK, Vogelstein B, Kinzler KW. Association of the APC tumor suppressor protein with catenins. *Science* 1993;262(5140):1734–1737.
45. Carothers AM, Melstrom KA, Jr., Mueller JD, et al. Progressive changes in adherens junction structure during intestinal adenoma formation in Apc mutant mice. *J Biol Chem* 2001;276(42):39094–39102.
46. Faux MC, Ross JL, Meeker C, et al. Restoration of full-length adenomatous polyposis coli (APC) protein in a colon cancer cell line enhances cell adhesion. *J Cell Sci* 2004;117(Pt 3):427–439.
47. Heinen CD, Goss KH, Cornelius JR, et al. The APC tumor suppressor controls entry into S-phase through its ability to regulate the cyclin D/RB pathway. *Gastroenterology* 2002;123(3):751–763.
48. Henderson BR. Nuclear-cytoplasmic shuttling of APC regulates beta-catenin subcellular localization and turnover. *Nat Cell Biol* 2000;2(9):653–660.
49. Jaiswal AS, Balusu R, Armas ML, et al. Mechanism of adenomatous polyposis coli (APC)-mediated blockage of long-patch base excision repair. *Biochemistry* 2006;45(51):15903–15914.
50. Jaiswal AS, Narayan S. Assembly of the base excision repair complex on abasic DNA and role of adenomatous polyposis coli on its functional activity. *Biochemistry* 2011;50(11):1901–1909.
51. Jaiswal AS, Narayan S. A novel function of adenomatous polyposis coli (APC) in regulating DNA repair. *Cancer Lett* 2008;271(2):272–280.
52. Das D, Preet R, Mohapatra P, et al. 5-Fluorouracil mediated anti-cancer activity in colon cancer cells is through the induction of Adenomatous Polyposis Coli: Implication of the long-patch base excision repair pathway. *DNA Repair (Amst)* 2014;24:15–25.
53. Tudek B, Speina E. Oxidatively damaged DNA and its repair in colon carcinogenesis. *Mutat Res* 2012;736(1-2):82–92.
54. Brocardo MG, Borowiec JA, Henderson BR. Adenomatous polyposis coli protein regulates the cellular response to DNA replication stress. *Int J Biochem Cell Biol* 2011;43(9):1354–1364.
55. Kouzmenko AP, Takeyama K, Kawasaki Y, et al. Truncation mutations abolish chromatin-associated activities of adenomatous polyposis coli. *Oncogene* 2008;27(36):4888–4899.
56. Su LK, Burrell M, Hill DE, et al. APC binds to the novel protein EB1. *Cancer Res* 1995;55(14):2972–2977.
57. Zumbrunn J, Kinoshita K, Hyman AA, et al. Binding of the adenomatous polyposis coli protein to microtubules increases microtubule stability and is regulated by GSK3 beta phosphorylation. *Curr Biol* 2001;11(1):44–49.
58. Dikovskaya D, Schiffmann D, Newton IP, et al. Loss of APC induces polyploidy as a result of a combination of defects in mitosis and apoptosis. *J Cell Biol* 2007;176(2):183–195.
59. Green RA, Wollman R, Kaplan KB. APC and EB1 function together in mitosis to regulate spindle dynamics and chromosome alignment. *Mol Biol Cell* 2005;16(10):4609–4622.
60. Fodde R, Kuipers J, Rosenberg C, et al. Mutations in the APC tumour suppressor gene cause chromosomal instability. *Nat Cell Biol* 2001;3(4):433–438.
61. Green RA, Kaplan KB. Chromosome instability in colorectal tumor cells is associated with defects in microtubule plus-end attachments caused by a dominant mutation in APC. *J Cell Biol* 2003;163(5):949–961.
62. Meniel V, Megges M, Young MA, et al. Apc and p53 interaction in DNA damage and genomic instability in hepatocytes. *Oncogene* 2015;34(31):4118–4129.
63. Mahmoud NN, Boolbol SK, Bilinski RT, et al. Apc gene mutation is associated with a dominant-negative effect upon intestinal cell migration. *Cancer Res* 1997;57(22):5045–5050.
64. Oshima M, Oshima H, Kobayashi M, et al. Evidence against dominant negative mechanisms of intestinal polyp formation by Apc gene mutations. *Cancer Res* 1995;55(13):2719–2722.
65. Nagase H, Miyoshi Y, Horii A, et al. Correlation between the location of germline mutations in the APC gene and the number of colorectal polyps in familial adenomatous polyposis patients. *Cancer Res* 1992;52(14):4055–4057.
66. Ficari F, Cama A, Valanzano R, et al. APC gene mutations and colorectal adenomatosis in familial adenomatous polyposis. *Br J Cancer* 2000;82(2):348–353.
67. Crabtree M, Sieber OM, Lipton L, et al. Refining the relation between 'first hits' and 'second hits' at the APC locus: the 'loose fit' model and evidence for differences in somatic mutation spectra among patients. *Oncogene* 2003;22(27):4257–4265.
68. Lamlum H, Ilyas M, Rowan A, et al. The type of somatic mutation at APC in familial adenomatous polyposis is determined by the site of the germline mutation: a new facet to Knudson's 'two-hit' hypothesis. *Nat Med* 1999;5(9):1071–1075.
69. Morin PJ, Vogelstein B, Kinzler KW. Apoptosis and APC in colorectal tumorigenesis. *Proc Natl Acad Sci U S A* 1996;93(15):7950–7954.
70. Steigerwald K, Behbehani GK, Combs KA, et al. The APC tumor suppressor promotes transcription-independent apoptosis in vitro. *Mol Cancer Res* 2005;3(2):78–89.
71. Qian J, Steigerwald K, Combs KA, et al. Caspase cleavage of the APC tumor suppressor and release of an amino-terminal domain is required for the transcription-independent function of APC in apoptosis. *Oncogene* 2007;26(33):4872–4876.
72. Brocardo M, Lei Y, Tighe A, et al. Mitochondrial targeting of adenomatous polyposis coli protein is stimulated by truncating cancer mutations: regulation of Bcl-2 and implications for cell survival. *J Biol Chem* 2008;283(9):5950–5959.
73. Schneikert J, Behrens J. Truncated APC is required for cell proliferation and DNA replication. *Int J Cancer* 2006;119(1):74–79.
74. Akiyama T, Kawasaki Y. Wnt signalling and the actin cytoskeleton. *Oncogene* 2006;25(57):7538–7544.
75. Nathke I. Cytoskeleton out of the cupboard: colon cancer and cytoskeletal changes induced by loss of APC. *Nat Rev Cancer* 2006;6(12):967–974.
76. Kawasaki Y, Sato R, Akiyama T. Mutated APC and Asef are involved in the migration of colorectal tumour cells. *Nat Cell Biol* 2003;5(3):211–215.
77. Kawasaki Y, Sagara M, Shibata Y, et al. Identification and characterization of Asef2, a guanine-nucleotide exchange factor specific for Rac1 and Cdc42. *Oncogene* 2007;26(55):7620-267.
78. Nelson SA, Li Z, Newton IP, et al. Tumorigenic fragments of APC cause dominant defects in directional cell migration in multiple model systems. *Dis Model Mech* 2012;5(6):940–947.
79. Tighe A, Johnson VL, Taylor SS. Truncating APC mutations have dominant effects on proliferation, spindle checkpoint control, survival and chromosome stability. *J Cell Sci* 2004;117(Pt 26):6339–6353.
80. Caldwell CM, Green RA, Kaplan KB. APC mutations lead to cytokinetic failures in vitro and tetraploid genotypes in Min mice. *J Cell Biol* 2007;178(7):1109–1120.
81. Fujiwara T, Bandi M, Nitta M, et al. Cytokinesis failure generating tetraploids promotes tumorigenesis in p53-null cells. *Nature* 2005;437(7061):1043–1047.
82. Dai W, Wang Q, Liu T, et al. Slippage of mitotic arrest and enhanced tumor development in mice with BubR1 haploinsufficiency. *Cancer Res* 2004;64(2):440–445.
83. Roig AI, Eskiocak U, Hight SK, et al. Immortalized epithelial cells derived from human colon biopsies express stem cell markers and differentiate in vitro. *Gastroenterology* 2010;138(3):1012–1021 e1-5.
84. Zhang L, Theodoropoulos PC, Eskiocak U, et al. Selective targeting of mutant adenomatous polyposis coli (APC) in colorectal cancer. *Sci Transl Med* 2016;8(361):361ra140.
85. Pineda CT, Ramanathan S, Fon Tacer K, et al. Degradation of AMPK by a cancer-specific ubiquitin ligase. *Cell* 2015;160(4):715–728.
86. Chandra SH, Wacker I, Appelt UK, et al. A common role for various human truncated adenomatous polyposis coli isoforms in the control of beta-catenin activity and cell proliferation. *PLoS One* 2012;7(4):e34479.
87. Alexandrova EM, Yallowitz AR, Li D, et al. Improving survival by exploiting tumour dependence on stabilized mutant p53 for treatment. *Nature* 2015;523(7560):352–356.
88. Fatehullah A, Tan SH, Barker N. Organoids as an in vitro model of human development and disease. *Nat Cell Biol* 2016;18(3):246–254.
89. Matano M, Date S, Shimokawa M, et al. Modeling colorectal cancer using CRISPR-Cas9-mediated engineering of human intestinal organoids. *Nat Med* 2015;21(3):256–262.
90. Zeineldin M, Neufeld KL. More than two decades of Apc modeling in rodents. *Biochim Biophys Acta* 2013;1836(1):80–89.
91. Su LK, Kinzler KW, Vogelstein B, et al. Multiple intestinal neoplasia caused by a mutation in the murine homolog of the APC gene. *Science* 1992;256(5057):668–670.
92. Cheung AF, Carter AM, Kostova KK, et al. Complete deletion of Apc results in severe polyposis in mice. *Oncogene* 2010;29(12):1857–1864.
93. Cheon DJ, Orsulic S. Mouse models of cancer. *Annu Rev Pathol* 2011;6:95–119.
94. Byun AJ, Hung KE, Fleet JC, et al. Colon-specific tumorigenesis in mice driven by Cre-mediated inactivation of Apc and activation of mutant Kras. *Cancer Lett* 2014;347(2):191–195.
95. Johnson RL, Fleet JC. Animal models of colorectal cancer. *Cancer Metastasis Rev* 2013;32(1-2):39–61.
96. Hinoi T, Akyol A, Theisen BK, et al. Mouse model of colonic adenoma-carcinoma progression based on somatic Apc inactivation. *Cancer Res* 2007;67(20):9721–9730.

97. Xue Y, Johnson R, Desmet M, et al. Generation of a transgenic mouse for colorectal cancer research with intestinal cre expression limited to the large intestine. *Mol Cancer Res* 2010;8(8):1095–1104.
98. Dow LE, O'Rourke KP, Simon J, et al. Apc Restoration Promotes Cellular Differentiation and Reestablishes Crypt Homeostasis in Colorectal Cancer. *Cell* 2015;161(7):1539–1552.
99. Anastas JN, Moon RT. WNT signalling pathways as therapeutic targets in cancer. *Nat Rev Cancer* 2013;13(1):11–26.
100. Huang SM, Mishina YM, Liu S, et al. Tankyrase inhibition stabilizes axin and antagonizes Wnt signalling. *Nature* 2009;461(7264):614–620.
101. Thorne CA, Hanson AJ, Schneider J, et al. Small-molecule inhibition of Wnt signaling through activation of casein kinase 1alpha. *Nat Chem Biol* 2010; 6(11):829–836.
102. Kahn M. Can we safely target the WNT pathway? *Nat Rev Drug Discov* 2014; 13(7):513–532.
103. Cerami E, Gao J, Dogrusoz U, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov* 2012;2(5):401–404.

REVIEW