Adenomatous polyposis coli in cancer and therapeutic implications

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Abstract

Inactivating mutations of the *adenomatous polyposis coli* (*APC*) gene and consequential upregulation of the Wnt signaling pathway are critical initiators in the development of colorectal cancer (CRC), the third most common cancer in the United States for both men and women. Emerging evidence suggests *APC* mutations are also found in gastric, breast and other cancers. The *APC* gene, located on chromosome 5q, is responsible for negatively regulating the β-catenin/Wnt pathway by creating a destruction complex with Axin/Axin2, GSK-3b, and CK1. In the event of an *APC* mutation, β-catenin accumulates, translocates to the cell nucleus and increases the transcription of Wnt target genes that have carcinogenic consequences in gastrointestinal epithelial stem cells. A literature review was conducted to highlight carcinogenesis related to *APC* mutations, as well as preclinical and clinical studies for potential therapies that target steps in inflammatory pathways, including IL-6 transduction, and Wnt pathway signaling regulation. Although a range of molecular targets have been explored in murine models, relatively few pharmacological agents have led to substantial increases in survival for patients with colorectal cancer clinically. This article reviews a range of molecular targets that may be efficacious targets for tumors with *APC* mutations.

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Introduction

Loss of a functional adenomatous polyposis coli (*APC)* tumor suppressor gene has long been implicated in the initiation of colorectal cancer, a leading cause of cancer mortality worldwide. Aside from CRC, the *APC* mutation has additionally been linked to gastric, breast, lung, and brain neoplasms.[1-4] The *APC* gene, located on chromosome 5q, negatively regulates the β-catenin/WNT pathway that facilitates cell growth and differentiation.[5-7] Wnt is a growth stimulatory protein that when activated facilitates an increase in β-catenin levels and transcriptional upregulation of Wnt target genes implicated in invasive pathologies.[5] Mutations affecting the *APC* gene prohibit the formation of a β-catenin destruction complex composed of the APC protein, Axin/Axin2, glycogen synthase kinase-3β (GSK-3β), and casein kinase 1 (CK1).[8-10] Without a functional destruction complex, β-catenin accumulates, leading to carcinogenic proliferation of gastrointestinal epithelial stem cells.[6,11] As the bulk of *APC* mutations are found in colorectal pathology, the majority of this paper will focus on the pathophysiology and associated molecular targets being investigated as they relate to CRC and associated syndromes, though other solid tumors will also be described.

Transition of epithelial cells to a state of chromosomal instability (CIN) occurs through a series of events. In colorectal cancer, the first pathogenic step that leads to loss of *APC* heterozygosity and CIN has been associated with both DNA hypomethylation, increasing genomic instability, and hypermethylation, which results in silencing of the *APC* promotor sequence.[12-14] Loss of *APC* gene function and subsequent accumulation of β-catenin is linked to decreased E-cadherin expression and, therefore, decreased intracellular adhesion.[15] A subsequent *KRAS* mutation leads to the formation of colorectal polyps or benign adenomas, and a final loss of either p53 or Deleted in Colorectal Carcinoma (*DCC*) tumor suppressor genes leads to malignancy.[16,17]

Several genetic and modifiable factors have been proposed to contribute to the etiology of dysfunctional *APC* mutations, including hereditary components, dietary components, age, obesity, and colon inflammation (*e.g*, history of inflammatory bowel disease). For sporadic cases of colorectal cancers with an aberrant *APC* gene, loss of *APC* tends to be an initiating event while in colitis-associated cancer (CAC) it tends to be a later event.[18] Patients with colitis have a 1.5-2 times greater likelihood of developing cancer, and it is hypothesized that inflammatory cytokine mediators such as IL-6 induce oxidative damage and influence dysplastic and neoplastic progression.[19-32] Similarly, obese patients are hypothesized to require fewer somatic mutations to progress to microsatellite instable colon cancer as elevated cytokines are linked to obesity. Women qualifying as obese appear to particularly be at risk for early onset colorectal cancer.[24] While more studies are needed to confirm the exact connection between obesity, inflammation, and cancer development, it is currently thought that obese patients have a lower threshold for carcinogenesis and the

chronic inflammatory state contributes to over expression of related signaling pathways (*e.g.* JAK/STAT) that mediate cancer development.[25,26] Familial adenomatous polyposis (FAP) is an autosomal dominant inherited syndrome affecting the *APC* gene. The most common disease progression without surgical intervention is to colorectal carcinoma, though some patients carry additional risk for other cancers, including of the duodenum, pancreas, thyroid, liver and central nervous system (CNS).[27] Phenotypically, patients will develop hundreds of premalignant polyps as early as their teenage years due to loss of heterozygosity of the *APC* allele. In addition to the original germline mutation most commonly found from codons 1061 to 1309, a second mutation renders both *APC* alleles nonfunctional.[28] Progression to colorectal cancer in these patients is predictable and typically occurs by age 40 to 50, therefore prophylactic colectomies are often required as a preventative measure.[29-31] However, most *APC* mutations are somatic, or non-inherited. Approximately 60% of these sporadic cases appear to have mutations localized at the exon 15 region of the *APC* gene, termed the mutation cluster region (MCR).[32] Interestingly, for unknown reasons patients with FAP who have germline mutations outside of codons 1194 and 1392, a region frequently associated with loss of heterozygosity (LOH) mutations, tend to also develop their second tumor-initiating mutation in the MCR.[33,34] It is hypothesized that despite the differences in sporadic and hereditary colorectal cancers, genomic sequencing may be beneficial in both cases to identify potential therapeutic targets and interventions.[35]

FAP is composed of several minor variants, including attenuated FAP (AFAP), Gardner Syndrome, and Turcot Syndrome, that are related to *APC* gene malfunction. AFAP is a category of *APC* mutation associated FAP typically denoted by fewer polyps, later onset of disease, and lower overall risk of development of colorectal cancer.[36] This disease in general is thought to be milder and it has been implied that a unique third hit, or mutation, of a germline allele may occur in some instances of polyp formation.[36] In turn, Gardner syndrome denotes a subset of FAP that is characterized by premalignant colonic polyps, but also extracolonic features including desmoid tumors, osteomas, fibromas, epidermal cysts, and ocular involvement.[37] Finally, Turcot syndrome is both associated with FAP and hereditary non-polyposis colorectal cancer (HNPCC) which is characterized by colonic involvement with the addition of a primary brain tumor, such as glioblastoma or medulloblastoma.[38,39] These syndromes have unique features though each have an underlying propensity to develop colonic adenocarcinoma.

A growing literature base addresses an array of therapeutic regimens that could prove helpful in patients with *APC* mutated colorectal cancer.[40] Typically, colorectal cancer patients receive a combined regimen of leucovorin, 5-fluorouracil, and oxaliplatin (FOLFOX), with survival rates for stage II or III cancer after 3 years reaching approximately 78.2%.[41] However, given the high mortality rates with approximately 50,000 deaths per year in the United States alone, current therapies are inadequate.[42-45] In cancers with *APC* inactivation, targeted treatment options are lacking and provide minimal increases in survival rates.

Molecular pathway

The *APC* gene codes for the APC protein, a multi-domain protein capable of binding to various cellular proteins. The APC protein is involved in many functions of the cell, with roles in development, tumor suppression, cell adhesion, cellular migration, and chromosome segregation through protein-protein interactions.[46]

APC is thought to play a vital role in fetal development. Its expression has been demonstrated in most fetal tissues.[47] Embryonic stem cells without the *APC* gene are unable to differentiate.[48] In murine studies, complete knock-out in embryos is lethal.[49-51] Heterozygous *APC* mutant mice are able to survive but develop many intestinal polyps.[52] These mutant mice, referred to as multiple intestinal neoplasia or "Min" mice, have been used as models for intestinal tumorigenesis as the polyps closely resemble those of FAP patients.[46]

Wnt signaling and β-catenin regulation

As a tumor suppressor, APC acts to suppress the Wnt signaling pathway, which plays a key role in cellular proliferation and differentiation in several organ systems.[46] With proper Wnt-receptor/ligand interaction, buildup of β-catenin occurs and translocates to the nucleus, where it complexes with and activates T-cell factor (TCF), a Wnt signaling mediator (Figure 1). This interaction with TCF results in activation of target genes such as cyclin D1 (*CCND1*), *MYC*, and *EphB*, the ultimate targets of the signaling pathway which promote oncogenic proliferation and differentiation.[46,53-55] Without proper Wnt signaling, APC inhibits β-catenin, preventing the downstream signaling of TCF and gene activation. APC prevents the accumulation and function of β-catenin by several mechanisms including, forming a complex with GSKb and axin which marks α-catenin for degradation; promoting β-catenin export from the nucleus; directly binding β-catenin, blocking its interaction with TCF; and forming a repressor complex which inhibits TCF-dependent transcription.[49-51,56]

In cell adhesion, APC binds with β-catenin to adhere E-cadherin to both a-catenin and the actin cytoskeleton in the lateral plasma membrane of epithelial cells.[57] Loss of this interaction between APC and β-catenin, and consequent loss of cell adhesion, is thought to contribute to tumorigenesis.[58]

Role in cytoskeleton stability, cell migration, and chromosome segregation

APC is important in the control and function of the actin cytoskeleton, particularly in cell polarity and migration. APC binds and activates Asef1, a guanine nucleotide exchange factor which in turn activates Cdc42, an important control protein in signaling pathways which alter the cytoskeleton and cause cell flattening, membrane ruffling, and cell migration.[59] APC also binds with IQ-motif-containing GTPase activation protein 1 (IQGAP1) to influence actin filaments and microtubules.[60] In migrating cells, IQGAP1 and APC complex with CLIP-170, along with either Rac1 or Cdc42, in order to stabilize microtubules and to aid in polarized migration of the cell.[60] Microtubule stabilization and polymerization are further regulated by APC forming a complex with the Rho downstream target EB1.[61] Together, APC and EB1 bind the formin protein mDia on the plus-end of microtubules, capping and stabilizing the microtubule.[61]

APC has been found to be important in chromosome segregation during mitosis. Though its exact mechanism is not known, many different mutations of the *APC* gene have led to mitotic malfunction and tumor formation.[46] During mitosis, APC is found associated with the kinetochores, mitotic spindles, and centrosomes.[62] It is thought that *APC*'s role in microtubule regulation is important in kinetochore function and chromosome segregation.[63]

Mutations which cause truncation of *APC* cause aneuploidy and chromosomal aberrations.[64]

Cell cycle regulation

The APC protein plays several roles in regulating the cell cycle, namely during the G1/S transition, the G2/M transition, and during base excision repair.[65-67] First, with respect to the G1/S transition, APC has been shown to perform regulatory functions upstream of the Rb/E2F complex.[66] β-catenin, which is inhibited by APC, activates cyclin D1.[66] This in turn phosphorylates Rb, separating it from the E2F transcription factor and allowing E2F to make protein regulators such as cyclin E and cyclin A. These cyclins progress the cell past the G1-S restriction point.[68] β-catenin also interacts with and activates c-MYC, which can enhance E2F and progress cells into the S phase as well.[66] In this manner, inhibition of APC, which is common in colorectal cancers, can lead to unregulated cell cycle progression through lack of upstream modulation of β-catenin, cyclin D1, and c-MYC. Inhibition of APC and its effects on the G1/S transition may occur through its interaction with hDLG.[69] hDLG is a member of the membrane-associated guanylate kinase protein family (MAGUK) and has been shown to form a complex with APC to inhibit further cell cycle progression.[69] When hDLG is mutated, APC-regulated cells pass the G1/S restriction point unhindered.[69] This response to a hDLG mutation implies that if the APC-hDLG complex cannot be made due to an hDLG mutation, then the G1/S restriction point may become unregulated and the cell cycle will proceed.

Furthermore, APC can regulate the cell cycle at the G2/M transition, though an exact mechanism has not been outlined.[65] The APC protein has been shown to contain consensus sequences for phosphorylation *via* the CDC2 kinase, which usually binds to cyclin A to form a complex that assists in the progression from the G2 to M phase of the cell cycle.[70,71] When APC is phosphorylated by CDC2, it is inhibited from binding to and stabilizing microtubules at their kinetochore sites.[70,72] Microtubules are formed from centrosomes during mitosis and help pull chromosomes to the opposite ends of the cell. As stabilization of microtubules would impede dynamic tubulin polymerization during mitosis, the cell would subsequently be arrested in the G2 phase.[72] In this way, unphosphorylated APC inhibits G2/M progression, whereas a phosphorylated APC allows mitosis to advance. Consequently, when CDC2 is upregulated in cancer settings, APC is phosphorylated and mitosis can proceed past the G2/M checkpoint.[73] APC also regulates the G2/M transition through interactions with topoisomerase IIa (topo IIa).[65] Topo IIa introduces double-stranded breaks into the DNA to facilitate strand passage and relegation, both of which are essential for DNA replication and chromosome condensation.[65] It has been proposed that APC interacts with topo IIa and regulates its function during chromosome condensation at the beginning of cell cycle during the M phase.[65] Consequently, when the APC protein is truncated due to a mutation, it ceases to modulate topo IIa's effects which allows for chromosome instability during the M phase, a hallmark of several cancers including colorectal cancer.[65] From these findings, it has been suggested that topo IIa inhibitors may be an effective chemotherapeutic target in *APC*-mutated colorectal cancer as this unregulated protein may be an essential component of cell cycle progression.[65,74]

Role in base excision repair

Lastly, APC may be involved with base excision repair.[67] In low levels of DNA damage, the process of base excision repair occurs to replace single mispaired nucleotides.[75] However, in high levels of DNA damage, such as significant exposure to alkylating agents, *APC* gene expression is stimulated.[67] APC proteins form complexes with base excision repair modulators such as Fen-1 and Pol-b, which together block the overall base excision repair process. Because of this unrepaired DNA damage, the cell subsequently undergoes apoptosis.[67] If APC is inhibited, base excision repair will not be blocked and damaged cells will proceed to survive and replicate. Overall, APC is involved in regulation of sev-

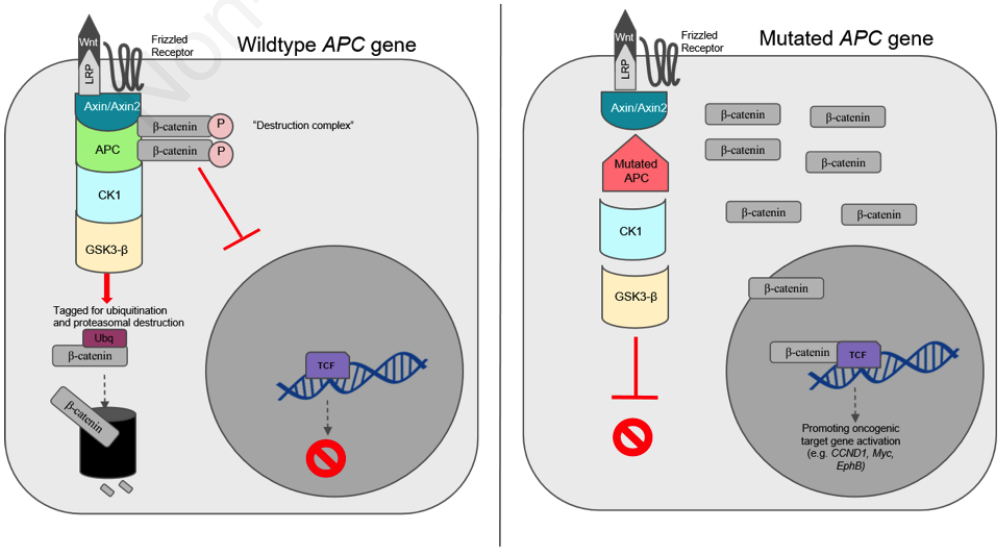

Figure 1. A mutated *APC* gene leads to an nonfunctional destruction complex and subsequent accumulation of β-catenin. After translocating to the nucleus, β-catenin complexes with T-cell factor (TCF) to enhance transcription of oncogenic promoting genes, including *CCND1*, *Myc*, and *EphB*.

eral areas of the cell cycle and DNA repair. Therefore, when APC is inhibited or downregulated, unfettered progression of cell replication can occur.

Path to tumorigenesis

As the *APC* gene functions as a tumor suppressor, a loss of heterozygosity via somatic mutation is needed for the protein to be rendered functionally inactive.[76] This mutation is often a result of a truncation or insertion in the portion of the gene that codes for a protein binding region, which prevents binding to either Large Tumor Kinase 1 (LATS1) or β-catenin.[76]

Malignant transformation in colorectal cancer is driven by the loss of heterozygosity of *APC* within the JAK-STAT pathway which regulates IL-6.[77] Within this pathway, the APC protein binds with LATS1 to form a complex, which inhibits the Yes-Associated Protein (YAP). YAP can function as an inducer to gp130, a protein that is part of the transmembrane tyrosine kinase receptor of IL-6.[78] When IL-6 is bound extracellularly to this receptor, the membrane protein undergoes a conformational change, which allows JAK2 to bind to the intracellular side of the receptor and phosphorylate STAT3.[78] This phosphorylation process occurs with the assistance of SRC Family Kinases (SFK's), which remain activated due to the induction of YAP.[79] When STAT3 is phosphorylated, it can act as a transcription factor and regulate expression of numerous genes including NF-kB (46). Subsequently, transcription for cytokines like IL-1 and TNF-a, results in pro-inflammatory effects within the tumor microenvironment.[78] Phosphorylated STAT3 can also activate genes that promote proliferation and evasion of apoptosis.[78] Therefore, the loss of function of *APC* renders the inhibitory complex with LATS1 inoperable and causes the constitutional activation of YAP and its downstream effects.

Neoplasms are promoted with DNA hypomethylation. DNA hypomethylation occurs when there is a loss of a maintenance methyltransferase named DNMT1.[80] Total loss of DNMT1 in $APC^{Min/+}$ mouse model with intestinal cancer showed an increase in progression of adenoma initiation due to hypomethylation.[81] Two months after the removal of DNMT1, the $APC^{Min/+}$ also showed an increase in macroadenoma load. However, there was no effect on tumor cell proliferation or apoptosis. Therefore, the loss of DNMT1 that leads to hypomethylation causes genomic instability, which accelerates loss of heterozygosity at the *APC* gene locus and ultimately causes an increase in tumor initiation.[81]

DNA hypermethylation in the promoter region of the *APC* gene leads to the inactivation of the *APC* gene. Since the *APC* gene is located on 5q (long arm of chromosome 5), a study investigating 137 sporadic colorectal cancer specimens with and without loss of heterozygosity at the 5q locus was performed.[82] The results showed that tumors with a 5q loss of heterozygosity with reduced *APC* gene expression were more often hypermethylated than tumors with a 5q loss of heterozygosity with normal *APC* gene expression. Therefore, hypermethylation is associated with loss of *APC* gene expression.[82]

Preclinical studies-targeting therapeutics related to APC gene mutations

Role of COX-2 inhibition

The vast majority of studies aimed at understanding the effectiveness of different pharmacological agents for colorectal cancer recognize the role of inflammation in tumorigenesis. Cyclooxygenase-2 (COX-2), an enzyme in the arachidonic acid pathway involved in pro-inflammatory signaling of cytokines such as IL-6, is overexpressed in subgroups of colonic tumors. Celecoxib is a selective COX-2 inhibitor of frequent discussion in the context of inflammation and carcinogenesis. One study explored the effects of celecoxib in combination with piperine, a chemopreventative agent and p-glycoprotein inhibitor thought to increase drug delivery of combined agents.[83,84] Evaluated in an HT-29 colon adenocarcinoma cell line, this combination was notably cytotoxic *via* induction of the caspase cell-death cascade and reactive oxygen species.[83]

In preclinical studies of APC^{Min} mice, COX-2 has been found to initiate the development of intestinal and colonic polyps. COX-2 (*Ptgs2*null mutation*)* knockouts in *APC Δ716* mice demonstrated significant decrease in the number of intestinal polyps.[85] Additionally, *APC Δ716* mice treated with MF Tricyclic, a selective COX-2 inhibitor, showed marked decrease in tumors compared to treatment with sulindac, a non-selective, non-steroidal anti-inflammatory drug (NSAID); thus, COX-2 inhibition has been implicated as a potential chemo-preventative strategy.[85] Furthermore, one study suggested that elevated prostaglandin E_2 (PGE_2), a pro-inflammatory mediator derived from COX-2 activity, in APC^{Min} mice promotes tumorigenesis.[86] The potency of NSAIDs directed at colonic tumors largely relies on the indirect ability to reduce PGE_2. In addition, the authors also recognized the involvement of the C-X-C motif chemokine receptor 2 in advancing chronic inflammation and colorectal cancer pathogenesis via recruitment of myeloid-derived suppressor cells (MDSCs). This is significant because it provides another pathway that can be targeted in order to inhibit inflammation and tumor progression.[86]

A study by Bi *et al*. (2013), suggested that synergistic effects of sulindac, which is thought to inhibit prostaglandin synthesis *via* COX-1 and COX-2 inhibition, and selenium, a dietary supplement, can negatively impact tumor growth.[87] In *APC* +/-/*p21*+/- mouse models given the sulindac/selenium combination for 24 weeks had meaningfully decreased tumorigenesis, with a 52% reduction in tumor incidence and approximately 80% reduction in tumor multiplicities (the number and variety of tumors). Mechanistically, this dual therapy increased expression of tumor suppressors p27 and p53, which are thought to play a role in β-catenin downregulation *via* E-cadherin/β-catenin adhesion mechanisms and ubiquitin/proteasome degradation systems, respectively.[88,89] Additionally, JNK1, a MAPK family protein kinase, is thought to promote functional intestinal development and, like p27 and p53, is thought to negatively regulate β-catenin. It was also found that JNK1 phosphorylation increased in mouse models treated with selenium and sulindac, thus highlighting a promising anti-cancer treatment option.[87]

Antibiotics: macrolides and aminoglycosides

Aminoglycoside and macrolide family antibiotics have been found to induce read through of nonsense mutations in plasmids and colorectal cancer cell lines.[90] Erythromycin, a macrolide antibiotic that inhibits the 50s subunit of bacterial ribosomes, has also been implicated in the treatment of colorectal cancer. Aside from the antimicrobial properties of this drug, erythromycin can also suppress development of intestinal tumors via inhibition of two transcriptional regulators: nuclear factor- κB (NFκB) and activator protein-1 (AP-1). In a sample of APC^{Min} mice who were administered erythromycin for 15 weeks, polyps in the proximal small intestine were reduced approximately 71% compared to untreated control ($P<0.01$).[91] Additionally, mRNA expression of IL-6 and COX-2, downstream targets of AP-1 and NFκB, were

reduced in the treatment population.[92] Another macrolide antibiotic, tylosin, had similar effects of tumor inhibition in *APC*^{Min} mice that specifically had a nonsense mutation at codon 850.[91]

Due to their ability to induce stop codon read through, aminoglycosides, such as gentamicin and negamycin, a dipeptide antibiotic, have been explored to target nonsense mutations of the *APC* gene in cell lines. Two nonsense mutations, R1114X and L360X, were particularly susceptible to read through mechanisms by gentamicin.[93] Overall, both therapies were able to reintroduce *APC* activity in cell cultures with *APC* nonsense mutations.[93]

Herpesvirus vehicles

A study by Macnab *et al.* (2011) reintroduced a complementary DNA strand of the *APC* gene into a virus-based Herpesvirus samiri (HVS) vector with successful translation of full APC protein in two separate colorectal cancer cell lines: SW480 and a metastatic SW620. In both cell lines, further cancer cell growth was inhibited. Specifically in the SW480 line, a monolayer wound assay effectively measured diminished in vitro cell migration for 2 months, thus demonstrating the potential for virally-delivered gene therapies.[94]

Renin-angiotensin downregulation

Renin-angiontensin system targeting agents, including angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin II receptor blockers (ARBs) are widely used in a number of conditions, namely hypertension; however, these agents have recently been implicated in colorectal cancer therapy and may decrease rates of liver metastasis.[95-97] One study examined the combination of losartan, an ARB, and/or vitamin D, which is thought to downregulated renin in Apc+/LoxP Cdx2P-Cre mouse models to assess effects on tumor burden.[98] Overall, it appeared that addition of vitamin D and losartan individually decreased tumor burden through upregulation of specific metabolites though the combination of vitamin D and losartan was not significant.[98]

mTOR inhibition

Sirolimus, or rapamycin, is a drug known to bind the binding protein FKBP-12 and inhibit the mechanistic target of rapamycin (mTOR), a serine/threonine protein kinase involved in cell proliferation that has become a topic of discussion for cancer therapies.[99] A mouse model for *APC*-deficient colonic polyposis, CDX2P-CreERT2 Apcfl/fl, demonstrated that treatment with rapamycin led to better median survival (P=0.003), fewer polyps (P=0.001), and decreased expression of β-catenin and Sox9 compared to the control cohort.[100] Similar to rapamycin, everlimous has mTOR blocking effects by specifically downregulating mTORC1.[101] In a ApcΔ716 mouse model, everolimus was shown to inhibit adenoma proliferation, tumor angiogenesis, and size and number of polyps.[102]

Mycophenolate mofetil and tacrolimus

Mycophenolate mofetil (MMF) and tacrolimus are agents that have complex mechanisms in immunosuppression, including inosine monophosphate dehydrogenase inhibition and calcineurin phosphatase inhibition, respectively. Classically, immunosuppressive therapies have been considered to increase risk for certain cancers, including lymphoma, following solid organ transplantation; however, MMF and tacrolimus have not specifically been shown to increase risk of colorectal cancer compared to other agents.[103] One study interestingly reported a case of a teenage patient with FAP who following therapy with MMF and tacrolimus for 6 months after a kidney transplant experienced halted proliferation and dysplasia in colonic adenomas and decreased Ki-67 expression following treatment (P<0.001).[104,105] In addition to pre- and post-treatment Ki-67 staining of a low grade dysplastic tubular adenoma from the patient, a human colonic adenocarcinoma cell line HT-29 containing a nonsense *APC* mutation was additionally treated with tacrolimus, MMF, or combination at varying doses. The combination of 10 μg/mL MMF and 0.1 μM tacrolimus demonstrated the greatest inhibition of the proliferating cell line at 24, 48, 72, and 96 hours compared to both solely MMF treatment (P<0.01) and the control (P<0.005). The cell-cycle phases of the HT-29 cells were also analyzed for percentage of cells in S phase, with increasing levels indicating cell arrest. After 48 hours, various treatments and dosing with 10 μg/mL MMF alone, combined 1 μM tacrolimus with 10 μg/mL MMF, combined 1 μM tacrolimus with 1 μg/mL MMF, combined 0.1 μM tacrolimus with 10 μg/mL MMF, and combined 0.1 μM tacrolimus with 1 μg/mL MMF were all significant compared to the control (respectively P<0.04; P<0.001; P<0.03; P<0.001; P<0.001). Overall, combined therapy with MMF and tacrolimus led to a regression in adenoma proliferation in an FAP patient and decreased cell cycle progression in a HT-29 cell line, thus indicating potential for future studies with these immunosuppressing therapies.

Src family kinases

As mentioned previously, *APC* loss of function mutations rather than β-catenin (CTNNB1) gain of function mutations are specifically linked to colorectal carcinogenesis.[78] *APC* mutations appear to increase IL-6 signal transducers and subsequent activation of phosphorylated Src, YAP, STAT3, and Notch.[78] Through antibody staining, 59% of human surgical colorectal cancer tumor samples were found to have overexpression of these signals, which was additionally replicated in *Villin-Cre* colonic mouse tumors.[78] *APC*$^{-/-}$ mouse small intestinal organoids created *via* Adeno-Cre virus vectors demonstrated upregulations in the following mediators in mucosal regeneration: STAT3, Src tyrosine (Y) phosphorylation, YAP, mRNA for YAP targets, *Jag1* (a Notch ligand), YAP targets cysteine-rich angiogenic inducer 61 (*Cyr61*) and connective tissue growth factor (*Ctgf*), leucine-rich repeat-containing G-protein couple receptor 5 (*Lgr5*) and polycomb complex protein *Bmi1*.[78] Each of these mediators has a variety of functions. STAT3 and YAP are transcription regulators that promotes cell cycle progression and oncogenesis.[106,107] *Jag1* is a ligand part of the Notch signaling pathway that through complex mechanisms promote cancer growth and angiogenesis.[108] *Lgr5* and *Bmi1* are act on stem cells and when elevated are thought to be pathologic due to their ability to enhance the Wnt/β-catenin signaling pathway and downregulate E-cadherin, respectively.[109,110]

The role of the IL-6 signal transducer was also explored, as IL-6 upregulation in *APC*-deficient intestinal cells is thought to direct activation of Src, YAP, STAT3, Notch and overall accelerate tumorigenesis. *APC*-null enteroids also demonstrated increased expression of cytokine markers: *Il6st* (gp130), *IL6r* mRNAs, *Il11r* mRNAs, *Lif* mRNAs, *IL17ra* mRNAs, and *Il17rc* through qRT-PCR, with *Il6st* (gp130) and *Il6r* mRNAs being the most substantial. *Il6st* (gp130) has a binding TEA domain transcription factor 4 (Tead4), which forms a complex with YAP in human colorectal cells via chromatin immunoprecipitation.[111] Downregulation of YAP and TAZ consequently reduces *IL6st* mRNA expression, which suggests that YAP is necessary for induction of the gp130 signal transducer.[78] Because IL-6, the IL-6 receptor, and IL-11 first activate Src family (SFK) kinases which in turn upregulate YAP, SFK inhibitors are thought to reduce YAP gene expression and may have a potential role to indirectly reduce the expression of these cytokine signal transducers.

PP2, a selective Src family kinase inhibitor, saracatinib (AZD0503), a selective Src and Bcr-Abl tyrosine kinase inhibitor, and dibenzazepine (DBZ), a Notch/γ-secretase inhibitor, were individually administered at 10 μM for 24 hours to both wildtype and *APC* $^{-/-}$ enteroids to assess targeted treatment efficacy.[78] AZD1480, a small JAK2 inhibitor, and ruxolitinib, a JAK1 and JAK2 inhibitor, were also individually administered at 3 μM for 24 hours. Notably, PP2 and saracatinib each inhibited YAP and Src tyrosine phosphorylation in *APC*-null human enteroids while PP2 additionally inhibited Notch activation.[78] No substantial β-catenin downregulation was noted with Src inhibitors and no other agents demonstrated substantial inhibition in their molecular targets.[78]

As previously described in Taniguchi *et al*. (2015), gp130 in turn also activates YAP and Notch and leads to epithelial proliferation and colorectal pathology.[112] However, intestinal tumor growth in $APC^{+/IEC\Delta}$; $Vil\text{-}gp130^{Act}$ mice utilizing the *Villin* promoter for STAT3, SFK, YAP, and Notch signaling was sustained when either YAP or STAT3 was inhibited.[112] More importantly, SFK and JAK inhibitors synergistically lead to tumor death. Combination PP2 and Ruxolitinib therapy in *Cdx2-Cre* x $APC^{F/+}$ mice for 1.5 months reduced tumor load ($P<0.05$) while PP2 and AZD1480 administration for 3 days lead to tumor death ($P<0.05$).[112]

Overall, the work by Taniguchi *et al.* (2015, 2017) in mouse models highlighted how constitutive expression of IL6st/gp130 in intestinal epithelial cells leads to progression of colorectal tumors whereas SFKs and JAK tyrosine kinase inhibition leads to downregulation of tumorigenesis and shrinkage of previously established colorectal pathologies.[78,112] Authors noted that specifically blocking YAP and STAT3 *via* SFK and JAK inhibitors presents a unique opportunity for targeting therapeutics through interaction with mediators of the aberrant Wnt/β-catenin signaling pathway in *APC* mutations.

Targeting the Wnt signaling pathway

The *APC* gene is inactivated through mutation in familial adenomatous polyposis associated and sporadic colorectal cancers.[113] However, mutation is not the only mechanism through which loss of expression occurs. Methylation of promoter regions is another common mechanism. Utilizing 22 different colorectal cancer cell lines, one study analyzed the methylation status of the *APC* promoter.[113] This study found that methylation of the CpG sites in the promoter region of the gene correlated with a loss of the *APC* gene expression.[113] In cell lines lacking *APC* mutations, 5-Aza-2-Deoxycytidine, a methyltransferase inhibitor, was successful in inducing expression of *APC*.[113]

Dysregulation and overactivation of the Wnt pathway have also been found to be associated with cancer. R-spondins, RSPO1-4, are proteins responsible for activation of the Wnt/β-catenin pathway. RSPO fusion proteins were found to be mutually exclusive with *APC* gene mutations, which shows that these proteins have a role in increasing Wnt signaling and tumorigenesis.[114] Chromosomal translocations that combine regulatory sequences with RSPO2 and RSPO3 are found in about 10% of colorectal cancers.[1] Wnt is activated through palmitoylation, a post translational modification which occurs through Porcupine (PORCN). The compound ETC-159 was found to inhibit palmitate incorporation into Wnt3a and inhibit growth of teratocarcinoma in human cell lines (115). Also, ETC-159 was found to inhibit the growth of colon cancers with RSPO translocations, making it a potentially effective treatment.[115]

CREB binding protein inhibition

Another drug that targets the Wnt pathway is PRI-724. PRI-724 inhibits the CREB binding protein from interacting with β-catenin. Treatment with PRI-724 has been found to promote stem cell differentiation by increasing p300/β-catenin binding.[116] Increased stem cell differentiation inhibits tumor initiating cells and increases sensitivity to targeted drugs.[116]

Casein kinase 1 inhibitors

The dysregulation of casein kinase 1 (CK1), a type of serine/threonine kinase, is another potential cause of colorectal cancer.[117,118] CK1α and CK1δ are isoforms of CK1 and play a regulatory role in the *APC*/Wnt/β-catenin pathway by a number of complex mechanisms including inducing β-catenin phosphorylation, influencing cell cycle progression, and co-activating mTOR.[119,120] When these two isoforms of CK1 are overexpressed, it leads to dysregulation of the *APC*/Wnt/β-catenin pathway and leads to colorectal cancer.[117,118] Comparison of normal and tumor tissue showed that CK1α was overexpressed in colorectal cancer and thus has the potential to serve as prognostic biomarker. The study also determined that higher CK1α expression leads to poorer prognosis and decreased survival, though this finding has not yet been extended to other cancers with CK1α overexpression. The drugs D4476 and lenalidomide have been shown to potentially inhibit and degrade CK1, respectively.[117,121,122]

Tankyrase inhibitors

Several therapeutic agents that modify axin to increase β-catenin destabilization have been tested. Tankyrase inhibitors small molecule inhibitor, XAV939, promotes destruction of β-catenin via inhibition of β-catenin transcription and Axin stabilization. XAV939 stabilizes Axin, which forms part of the β-catenin destruction complex, by blocking the activity of both tankyrase 1 and tankyrase 2 enzymes that promote axin degradation.[123] Another tankyrase inhibitor, G007-LK, has also promoted axin stabilization and decreased tumorigenesis in *APC* mutant mice, though clinical potential may be limited due to intestinal toxicity.[124] KYA1797K, which binds to axin's G-protein signaling domain, has noted anti-tumorigenic properties in mutant *APC* and *KRAS* mice through stabilization of the proteasome complex that degrades β-catenin.[125]

TNIK Inhibitors

After translocating to the nucleus, β-catenin accumulates and forms complexes with several different transcription factors that lead to gene expression. The T-cell factor/lymphoid enhancer factor (TCF/LEF) family includes T-cell factor 4 and β-catenin transcriptional complex (TCF4), which is frequently stained in colorectal cancer tissue.[126] Its abnormal overactivation is thought to be essential for halting differentiation of intestinal stem cells and promoting malignant proliferation.[127] Traf2- and Nck-interacting kinase (TNIK) are known to be vital regulators of Wnt pathway activity by binding β-catenin, phosphorylating, and activating TCF4.[128,129] Thus, its inhibition may provide potential clinical relevance for colorectal cancer patients. A small molecule TNIK inhibitor, NCB-0846, decreased tumor proliferation in both in vitro and *in vivo* APC^{Min} mice and decreased expression of CD44, CD133 and aldehyde dehydrogenase-1 (ALDH1) cell markers, which typically are markers of cancer stem cell functioning and tumorigenesis.[130]

Frizzled receptor targeting

Another targeted therapeutic approach of the Wnt/β-catenin signaling pathway involves the Frizzled (Fzd) Wnt receptors. Frizzled-7 (Fzd7) receptor expression was recently linked to decreased survival in gastric cancer patients.[131] Targeting Fzd7

with vantictumab, an IgG2 monoclonal antibody that binds Fzd receptors, notably mitigated proto-oncogenic Myc expression while additionally reducing chemoresistance.[132] In mouse models, Fzd7 deficient cell lines were unable to sustain cancer growth via Wnt signal induction, while Fzd7 deletion or vantictumab therapy were effective in deterring tumor proliferation.[132] Vantictumab has promising anti-oncogenic properties, and its effects were noticeable regardless of mutations in the *APC* gene.[132] Ipafricept, a fusion protein containing part of Fzd8 linked to IgG1, has additionally been evaluated in solid tumors and was suggested to have benefit in a patient with a germ cell cancer that overexpressed β-catenin and had stable disease after treatment.[133]

Dikkopf-1 targeting

Dickkopf-1 (Dkk1) is a glycoprotein involved in antagonizing the Wnt signaling pathway through competitive LRP5/6 inhibition.[134] Overexpression of Dkk1 confers a poor prognosis in hepatocellular, esophageal, non-small cell lung, and ovarian cancers and could be an optimal target for therapy.[135-140] The humanized monoclonal IgG4 antibody, DKN-01, is emerging as a potential immunomodulatory therapy for patients suffering from cancers with a high Dkk1 burden.[141,142] Conversely, Dkk1 has been demonstrated to have decreased expression in colon cancer and melanoma.[143,144] In sample of *APC*-mutated nude mice with D-1 deficiency, reintroduction of a functional DKK-1 gene led to decreased tumorigenesis.[145] At present, no targeted therapies specific to low Dkk-1 expression are in preclinical trials; however, the Dkk-3 protein, also in the Dkk family, is being investigated as a potential biomarker in colorectal cancer due to its typical underexpression compared to healthy controls.[146]

Chemotherapeutic drugs targeting the APC gene

Cisplatin is a common chemotherapeutic drug used to treat different cancers. Cisplatin works by crosslinking purine bases on DNA, which inhibits DNA repair mechanisms and causes cellular apoptosis.[147] Cisplatin has been tested in $APC^{Min/+}$ mice with mouse mammary tumor virus (MMTV) that were crossed to the polyoma middle T antigen (PyMT) transgenic model. Cells were extracted from MMTV-PyMT; $Apc^{Min/+}$ mice tumors and were treated with cisplatin as well as doxorubicin and paclitaxel, which are also chemotherapeutic drugs. The tumor cells expressed multidrug resistance protein 1 (MDR1) after treatment with the three drugs, meaning that drug resistance occurred. Also, MMTV-PyMT; $APC^{Min/+}$ cells became more resistant to cisplatin and doxorubicin-induced apoptosis.

The same drugs were then tested in human metaplastic breast cancer cell line MDA-MB-157 with an *APC* knockdown. The results showed that without the *APC* gene, there was greater resistance to paclitaxel and cisplatin. However, when cisplatin was combined with PP2, a Src inhibitor, or SP600125, a JNK inhibitor, there was less resistance to treatment. Src and JNK regulate increased tumor cell proliferation downstream of the *APC* mutation, so targeting them would inhibit proliferation. The combination of cisplatin with PP2 and SP6000125 could prove to be clinically beneficial for breast cancer resistance caused by *APC* gene mutation.[148]

Targeting *APC* in the clinical setting

While an *APC* mutation is an initiating factor in roughly 80% of all colorectal cancers and is increasingly being explored in other cancers, there have not been many therapeutic targets identified. Most of the research conducted has focused on downstream signaling pathways regulated by *APC*, including WNT/β-catenin. The majority of the clinical trials have been directed at treatment for patients with familial adenomatous polyposis, which as mentioned previously is predominated by *APC* germline mutations. Table 1 provides a brief outline of the mentioned studies.

Erythromycin

Since the strategy of inducting read through of nonsense mutations in the *APC* gene was successful in murine models, one study investigated administration of erythromycin to humans with nonsense *APC* gene mutations. In a clinical trial, ten patients with FAP and *APC* nonsense mutations were given erythromycin for 4 months and had endoscopies at baseline, 4 months, and 12 months after treatment.[149] Results indicated that the number of adenomas and cumulative and maximal polyp size decreased. Tissue samples were also collected for assessment and were then molecularly and genetically analyzed. Treatment not only reduced the number of somatic *APC* mutations, but also restored *APC* gene function and decreased cellular proliferation.[149]

Erlotinib and sulindac

The pro-inflammatory signaling role of COX-2 and the overexpression found in colorectal cancer has previously been described in this review. Aspirin, an irreversible and nonselective

Table 1. The above are clinical studies that target *APC* mutations, namely studied in familial adenomatous polyposis.

Pharmacologic agent	Trial	Phase	Mechanism of action	Therapeutic use	Outcome
Erythromycin	NCT02175914	IV	Induction of read through of nonsense mutations in the *APC* gene	Familial adenomatous polyposis (FAP)	i) Decreased number of adenomas ii) Decreased polyp size
Erlotinib + sulindac	NCT01187901	II	EGFR inhibition	FAP, attenuated FAP	i) Decreased quantity of polyps
Eicosapentaenoic acid	NCT00510692	II, III	Omega-3 polyunsaturated fatty acid that modulates cyclooxygenase metabolism	FAP	i) Decreased polyp size
Celecoxib + difluoromethylornithine	NCT00033371	II	Blocking upregulation of polyamine synthesis	FAP, colorectal cancer	i) No significant decrease in polyp burden compared to placebo

inhibitor of COX-1 and COX-2, has been shown to improve the survival for patients with non-metastatic colorectal cancer positive for COX-2 overexpression (HR, 0.39; 95% CI, 0.20-0.76).[150]

Prostaglandin E2 (PGE2) is produced by COX-2 and found to transactivate EGFR (epidermal growth factor receptor). EGFR has been increased in *APC* deficient murine models which indicate EGFR inhibition may be beneficial.[151] Patients were given the EGFR inhibitor, erlotinib at 75 mg twice a day and sulindac (non-steroidal anti-inflammatory) at 150 mg/day for 6 months.[152] The goal of this study was to show decreased duodenal polyp amounts after 6 months of treatment. Ninety-two patients with FAP participated in this study and half of them received the drug combination while the other half received a placebo. The total number of polyps found in the colorectum, ileal pouch anal anastomosis, or ileo-rectum were recorded at baseline and reassessed after six months. After six months, it was found that the group that received erlotinib and sulindac had 69% less polyps compared to the placebo group. Four main side effects of these drugs include acne like rash, diarrhea, nausea, and oral mucositis.

Eicosapentaenoic acid

Eicosapentaenoic acid (EPA) is an omega-3 polyunsaturated fatty acid (O3FA) that has been shown to decrease colorectal cancer in preclinical studies through multiple different mechanisms such as modulating cyclooxygenase metabolism, which is elevated in colorectal cancer, as well as reduction of prostaglandins.[153] Therefore, the free fatty acid version of EPA (EPA-FFA) was tested in patients who were diagnosed with FAP. These patients were monitored through endoscopies of their retained rectum postcolectomy and were given 2 grams of EPA-FFA for six months. The number and size of their polyps were demarcated with a tattoo so a comparison before and after treatment could be made. At the end of this study, it was found that 22.4% of the polyps decreased after 6 months of treatment with EPA-FFA.[154] Also, 29.8% of polyps decreased in the sum of the diameter compared to the placebo group. EPA-FFA was also well tolerated amongst the experimental group.[154]

Celecoxib and difluoromethylornithine

Another study combined celecoxib with difluoromethylornithine (DFMO) to see if polyp burden decreased in patients with FAP.[155] DFMO is an irreversible enzyme-activated inhibitor of ornithine transcarbamylase which assists in polyamine synthesis. Polyamines have been found to potentially contribute to cancer formation in the colon and rectum through upregulation of polyamine biosynthesis enzymes.[156] This makes the polyamine pathway a potential target for inhibiting tumor proliferation. Therefore, if DFMO can block the formation of polyamines, it might have an anticancer effect. After analyzing the polyp burden, there was no significant difference in the overall amount between placebo and DFMO treated patients.[155]

Ongoing clinical trials targeting Wnt signaling

A previous review highlighted emerging therapies for gastrointestinal tumors via different small molecule targets in the Wnt pathway.[40] Currently, there are several clinical trials underway that are directed at components that are part of the Wnt signaling cascade. Of note, four of the six trials mentioned in Table 2 are DKK1 inhibitors, which are soluble inhibitors previously mentioned in this review. These trials are mostly related to gastrointestinal tract malignancies, though the PORCN inhibitor ETC-1922159 may be applicable to a number of solid tumors.

The role of azithromycin in inducing readthrough of *APC* nonsense mutations and inducing functional APC protein has previously been discussed in this review. One proposed phase IV study (NCT04454151) will investigate the effect of azithromycin therapy for 4-6 months on number and size of colonic adenomas, duodenal adenomas, and desmoid tumors in FAP patients. Additionally, samples will be taken to determine APC protein levels following medication administration. This study is not yet recruiting.

A separate study (NCT02521844) is a four-part, open label study evaluating ETC -1922159, a PORCN inhibitor, in a variety of unresectable solid tumors. Overall, the study evaluates the safety and efficacy of this medication for solid malignancies that lack other known treatment options with dose escalation scales. In certain parts of the study, the medication will be combined with denosumab (Part A extension) and pembrolizumab (Phase B). PORCN inhibitors and their connection to the Wnt signaling pathway have been described previously.

Several studies have also begun to investigate the DKN-01, a Wnt signaling pathway effector, in combination with other medications. NCT04166721 is an actively recruiting phase II study evaluating safety, tolerable dosing, and efficacy of DKN-01 and atezolizumab combination therapy in both metastatic esophageal and gastric cancer. NCT0436801 is an additional actively recruiting phase II study evaluating use of IV DKN-01 and tislelizumab with or without CAPOX in patients with inoperable gastric adenocarcinoma or gastroesophageal cancer. In a group of patients with advanced liver cancer, a combination of DKN-01 and sorafenib

Table 2. The listed studies are currently actively recruiting participants for pharmacological trials linked to Wnt pathway inhibition.

Pharmacologic agent	Trial	Phase	Mechanism of action	Therapeutic use
Azithromycin	NCT04454151	IV	Ribosomal readthrough of *APC* nonsense mutations	Familial adenomatous polyposis
ETC-1922159	NCT02521844	I	PORCN inhibitor	Multiple solid tumors
DKN-01 plus atezolizumab	NCT04166721	II	DKK1 inhibitor plus anti-PD-L1 monoclonal antibody	Metastatic esophageal cancer Metastatic gastric cancer
DKN-01 plus tislelizumab	NCT04363801	II	DKK1 inhibitor plus IgG4 anti–PD-1 monoclonal antibody	Gastric cancer Gastric adenocarcinoma Gastroesophageal cancer
DKN-01 plus sorafenib	NCT03645980	I, II	DKK1 inhibitor plus protein kinase inhibitor	Hepatocellular cancer
DKN-01 plus nivolumab	NCT04057365	II	DKK1 inhibitor plus IgG4 monoclonal antibody and PD-L1/L2 inhibitor	Biliary tract cancer

will be evaluated in an actively recruiting phase 1/II study (NCT03645980). Finally, DKN-01 and nivolumab in being investigated in patients with biliary tract cancer patients who are refractory to other therapies in a recruiting phase II study (NCT04057365).

Conclusions

In the United States alone, it is projected that in 2021 there will be 149,500 new diagnoses and 52,980 deaths from colorectal cancer.[44,45] Recent predictions have estimated that by 2040 the incidence of colorectal cancer will be similar, approximated at 147,000 cases.[157] In CRC, the *APC* gene on chromosome 5q is the most frequent initiating site of sporadic and familial mutations that leads to polyp formation and inevitable carcinoma, if untreated. Of note, *APC* gene mutations may also be found in a number of malignancies aside from CRC, though CRC is the most hallmark association with these mutations. The progression from adenoma to carcinoma has a typical stepwise progression after the loss *APC* functionality. In many cases, a subsequent activating *KRAS* mutation followed by a tumor suppressor genes mutation (TP53, DCC) leads to eventual tumor burden.

Surgery remains a mainstay of treatment to prevent malignant progression of CRC, though at present pharmacological options that can effectively mitigate disease are needed. Several cytotoxic, chemotherapeutic agents, including the FOLFOX regimen, have been implicated in the treatment of colorectal cancer for the past couple decades and are associated with significant side effect profiles; however, CRC remains the third most deadly cancer despite efforts to increase surveillance and scientific advances in treatment.

While several preclinical and clinical studies have addressed targeting upregulated inflammatory factors in CRC, including COX-2 and IL-6, therapies more specific to specific cytokine mediated transcription factors and Wnt pathway signalers are increasingly being explored. Potential molecular targets in mouse model from preclinical studies included IL-6 signal transducers (YAP, Src, STAT3, Notch), tankyrase enzymes, Traf2- and Nck-interacting kinase, and Frizzled 7 Wnt receptors. This review highlights several avenues that may prove efficacious for patients suffering from *APC*-mutated colorectal cancers and warrant further exploration.

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