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A look into centrosome abnormalities in colon cancer cells, how they arise and how they might be targeted therapeutically

Lauren E Harrison, Marina Bleiler, and Charles Giardina*

Department of Molecular and Cell Biology, 91 North Eagleville Road, U3125, University of Connecticut, Storrs, CT 06269

Abstract

Cancer cells have long been noted for alterations in centrosome structure, number, and function. Colorectal cancers are interesting in this regard since two frequently mutated genes, *APC* and *CTNNB1* (β -catenin), encode proteins that directly interact with the centrosome and affect its ability to direct microtubule growth and establish cell polarity. Colorectal cancers also frequently display centrosome over-duplication and clustering. Efforts have been directed towards understanding how supernumerary centrosomes cluster and whether disrupting this clustering may be a way to induce aberrant/lethal mitoses of cancer cells. Given the important role of the centrosome in establishing spindle polarity and regulating some apoptotic signaling pathways, other approaches to centrosome targeting may be fruitful as well. Basic information on the nature and extent of centrosome defects in colorectal cancer, including why they over-duplicate and whether this over-duplication compensates for their functional defects, could provide a framework for the development of novel approaches for the therapeutic targeting of colorectal cancer.

Graphical abstract

1. Colon cancer relevance and therapy

Colorectal cancer is the third most common cancer diagnosed in both men and women in the United States, afflicting approximately one in twenty individuals [1]. The death rate from

*Corresponding author. Phone: 860-486-0089, charles.giardina@uconn.edu.

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colorectal cancer has been dropping in recent decades, thanks largely to screening and early diagnosis [2]. Early stage lesions are removed by surgery, with more advanced lesions requiring both surgical and systemic treatments. Chemotherapy is the mainstay of most current treatment regimens for colon cancers that have progressed beyond stage 1, with targeted therapies also being increasingly utilized. The 5-year survival for early stage colon cancer is greater than 90%. However, as cancers progress and chemotherapy becomes necessary, the survival rate drops steadily and is around 10% for advanced metastatic disease [1]. Further reductions in the colon cancer death rates would therefore benefit greatly from improvements in treatment.

Standard chemotherapies for colorectal cancer include metabolic and DNA-targeting compounds. Most patients receive FOLFOX (5-FU, leucovorin, and oxaliplatin) or CapeOx (capecitabine and oxaliplatin), with or without an accompanying targeted therapy [3, 4]. Targeted therapies typically include agents like bevacizumab (Avastin) for VEGF signaling inhibition or cetuximab (Erbix) for EGFR signaling inhibition [5]. Response to therapy varies with cancer stage, but is also affected by specific molecular defects of the cancers. For example, approximately 20% of colon cancers are defective for DNA mismatch repair. These cancers are often not responsive to 5-FU-based (FOLFOX) therapies, which may be due to the fact that they don't recognize uracil misincorporation into DNA, attenuating the response to therapy [6]. Although present treatment approaches are beneficial to most patients, complementary or alternative approaches will likely be necessary to substantially improve current outcomes.

2. The centrosome

2.1 Normal centrosome functioning

The centrosome is the primary microtubule organizing center (MTOC) in most eukaryotic cells that determines the number, distribution, and organization of microtubules throughout all stages of the cell cycle. The centrosome consists of two centrioles surrounded by a mass of proteins that make up the pericentriolar material (PCM). During S phase, duplication of the centrioles occurs alongside chromosome duplication. The movement of the two resulting centrosomes to opposite poles of the cell helps establish cell polarity during division and allows for faithful separation of the chromosomes [7]. The centrosome is also involved in many signaling pathways, including Wnt, NF- κ B and integrin-regulated pathways [7]. The role of the centrosome in these signaling pathways appears to be that of a scaffold that organizes multiple interacting signaling proteins. For this reason, the centrosome changes found in cancer cells are likely to affect cell signaling and chromosomal stability [8–10].

2.2 Centrosome assembly

Centrosomes form as a result of centriole assembly and subsequent pericentrosomal material (PCM) recruitment around these centrioles (Figure 1). The daughter centriole is formed by budding from the mother centriole. The first step in assembling the daughter centriole is the recruitment of the kinase PLK4 to the side of the mother centriole. This recruitment is mediated by two proteins, CEP152 and CEP192 [11]. PLK4 then works to bring the proteins SAS-6 and STIL to the mother centriole [12]. Together these two proteins form a cartwheel-

like structure to create the classic 9×2 symmetry of the daughter centriole. SAS-6 and STIL then recruit CPAP to the outside of the cartwheel structure where it serves to assemble the surrounding centriolar microtubules [13]. Once the centrioles are formed, they can begin to recruit PCM. The kinases PLK1 and Aurora A contribute to the recruitment of multiple PCM components to the centrioles and are essential for centrosome maturation [14]. PLK1 phosphorylates pericentrin to induce the movement of other PCM proteins to the centrosome. PLK1 also stimulates γ -tubulin recruitment through phosphorylation of NEK9 and subsequent phosphorylation of NEDD1 [15]. The maturation of the centrosome, including the assembly of additional γ -tubulin ring complexes (γ -TuRC), increases the microtubule nucleating capacity of the centrosome required for efficient mitotic spindle assembly and function [9].

2.3 Centrosome abnormalities lead to cancer

The idea that centrosome abnormalities promote cancer development can be traced back to a 1902 paper written by Theodor Boveri in which he speculated that multipolar mitoses can lead to tumorigenesis. Interestingly, Boveri was not studying cancer cells, but was working on doubly fertilized sea urchin eggs and his theory about centrosomes and cancer was a footnote to his conclusions. These eggs had extra centrosomes and underwent multipolar mitoses to create aneuploid cells, which led him to connect his findings to cancer development [16]. Since then, the link between centrosome abnormalities and cancer have become well documented. Centrosome abnormalities have been identified in all major classes of human cancer including solid tumors of the breast, prostate, colon, ovaries, and pancreas, as well as hematological neoplasms, such as multiple myeloma, non-Hodgkin's lymphoma, Hodgkin's lymphoma, acute myeloid leukemia, and chronic myeloid leukemia. These abnormalities can be seen in both early and late stage tumors, further implicating their role in tumor progression [7]. However, the nature of these defects, and their underlying causes, are likely to differ between and within cancer types.

Centrosome amplification/overduplication has been identified in many cancers and is thought to play a critical role in tumorigenesis as a major cause of multipolar mitoses leading to chromosome instability (CIN) and aneuploidy [17]. Cells with amplified centrosomes have more than two normal or abnormally structured centrosomes prior to the onset of mitosis [18]. Amplification is associated with high-grade tumors (stages III and IV) and a poor prognosis [19]. Amplification can occur through a number of mechanisms, including cell-cell fusion, de novo centriole formation, centrosome fragmentation, dysregulation of the centriole duplication cycle, or cytokinesis failure. The amplified centrosomes are often found in unlikely locations within the cell and may also have functional abnormalities [17].

In cancer cells, the centrosome can be misshapen in the form of string-like linear arrays or ring-like arrays. They can have a corkscrew shape, or an amorphous structure with atypical filaments. These structural defects may contribute to their functional abnormalities. Defects in centrosome positioning (random cytoplasmic locations, scattered, or clustered) as well as altered protein composition (high protein levels, aberrant phosphorylation, missing centrioles) are other defects that can contribute to overall centrosome dysfunction [19].

Varying amounts of PCM or PCM fragmentation can itself cause loss of spindle pole integrity and the development of multipolar spindles. Pericentriolar satellite proteins such as PCM-1, centrin-2, and ninein aid in the formation of highly conserved PCM structures. These proteins may play a role in microtubule anchoring to the centrosome [20]. This subset of PCM proteins may therefore directly regulate the formation of the mitotic spindle as it extends outward from centrosomes. If any of these proteins dissociate from the centrosome, multipolar spindles can potentially form [20].

Centrosome changes, both in number and composition, have been widely-reported in colorectal cancer. Interestingly, many oncogene and tumor suppressor gene mutations appear to directly impact centrosome structure and activity in colorectal cancer. The near ubiquitous changes in the centrosome accompanying cell transformation suggests that some of these changes might be necessary for cancer cell survival and expansion. As such, targeting these aberrant centrosome assemblies may provide a novel route for the treatment of colorectal cancer. Finding the right target(s) in the centrosome complex that selectively target transformed cells is the present challenge.

3. Mutations contributing to centrosome abnormalities

3.1 APC – Adenomatous Polyposis Coli (APC)

The APC protein is a large multi-domain protein of 2843 amino acids in humans. The *APC* gene is mutated in the majority (>80%) of colorectal cancers, with mutations most frequently leading to the expression of a truncated protein [21]. Since *APC* mutation is the underlying genetic defect in familial adenomatous polyposis, and is frequently found in sporadic pre-cancerous adenomas, it is considered one of the earliest events in colon tumorigenesis [22–24]. The tumor suppressor function of *APC* includes the regulation of Wnt signaling by targeting the β -catenin transcription factor for degradation through the ubiquitin/proteasome pathway [25, 26]. Wnt signaling regulation by *APC* ensures the proper organization of proliferation, migration and differentiation of intestinal crypt cells [27]. However, APC has a distinct function as an MT binding protein. In conjunction with the EB1 plus-end binding protein, APC serves to stabilize the plus ends of growing MTs, to facilitate their growth and attachment to kinetochores and the cellular cortex [28–30].

The truncated APC protein found in most colon cancers lacks the C-terminal MT and EB1 binding domain, resulting in deficiencies in cytoskeleton assembly and mitosis [28, 31]. This has been demonstrated in *APC*-mutant intestinal epithelium cells (in *APC^{Min/+}* mice). While normal cells undergo mitosis with their spindles parallel with the basement membrane, *APC* mutant cells show large variations in this alignment [31]. Other mitotic abnormalities in *APC* mutant cells include the formation of multinucleated cells, unanchored mitotic spindles, and failure to form a cytokinetic furrow. Mouse embryonic stem cells (ESCs) homozygous for the *APC^{Min}* mutation likewise display extensive spindle aberrations including mono- and multipolar spindles that result in chromosomal aberrations [32]. The aberrant mitosis in *APC*-mutant tissue is often ascribed to defects in APC's role in stimulating MT growth, which results in the inhibition of cytokinesis due to weakened spindle anchoring at the kinetochores and cortex, and defective cytokinetic furrow

formation. However, the role of APC in MT regulation extends to the primary MT organizing center (MTOC) of the cell, the centrosome.

A portion of cellular APC associates with the centrosome throughout of cell cycle, where it promotes MT nucleation [33]. Detailed image analysis shows that APC preferentially wraps around the length of the mother centriole to form a U-shaped tube (Figure 2). Association with the daughter centrosome occurs as it matures. The association of APC and its partner EB1 with the centrosome does not depend on polymerized microtubules and can be observed in both control and nocodazole-treated cells (a drug that disrupts the microtubule polymerization). Centrosomes purified from nocodazole-treated cells maintain their association with APC, consistent with a stable mode of interaction [33]. APC interacts with the centrosome through its armadillo repeat domain extending from amino acids 334–625 of the N-terminus [34, 35]. Truncated APC lacks the EB1 binding domain, so interaction with EB1 is not essential for the centrosome localization of APC. Instead, γ -tubulin binding to N-terminal amino acids of APC appears to be an important feature of centrosome association [34]. A functional role for APC at the centrosome first came from the study of an in vitro MT nucleation system (employing *Xenopus* oocyte extracts). In these studies, Dikovskaya et al. showed that APC-depleted extracts were defective for the assembly of centrosome-nucleated MTs, but not kinetochore-nucleated MTs [36]. By studying knockdown and mutant cancer cell lines, Lui et al. also showed that APC associates with the centrosome, where it promoted aster formation in MT re-growth assays [35]. Based on present data, APC appears to be recruited to the centrosome through its N-terminus where it stimulates aster formation, and then in association with EB1, stimulates the outgrowth of newly nucleated MTs (Figure 2)[35].

Evidence has also been obtained that the *APC* mutation can lead to centrosome amplification (Figure 2). Mouse embryonic stem cells homozygous for the *APC*-Min mutation frequently form multipolar spindles resulting from the formation of additional centrosomes [32]. Aberrant cell divisions resulting from this increased centrosome number may underlie the chromosomal aberrations found in the *APC* mutant ESCs (and cancers). Interesting observations have also been made in studies of *APC* mutant liver tissue. Méniel et al. utilized AhCre mice to selectively induce *APC* mutations in hepatocytes [37]. *APC* mutation caused an increase in hepatocyte proliferation (likely due to increased Wnt signaling), but also increased apoptosis and triggered double strand DNA breakage. Interestingly, centrosome overduplication was observed, leading the authors to postulate that multipolar mitoses resulting from the additional centrosomes might cause DNA shearing. It is not clear how centrosome numbers increase in *APC* mutant cells. Possibilities include aberrant cell cycle signaling or failed cytokinesis. It is also possible that the additional centrosomes form as a response to the deficiencies in centrosome function associated with an *APC* mutation. These studies provide important insight into the early stages of chromosomal instability following *APC* mutation.

3.2. β -catenin

β -catenin functions in the Wnt signaling pathway as a transcriptional regulator and promoter of cell proliferation. When the Wnt pathway is activated, β -catenin enters the nucleus and

activates transcription of growth-promoting genes (Figure 3). In the absence of Wnt, axin and APC form a complex that promotes β -catenin phosphorylation by GSK3 β , which then targets β -catenin for degradation by the proteasome. *APC* mutations cause the accumulation of β -catenin, which then activates target genes in an unregulated manner [25, 26]. β -catenin itself can also become mutated into an oncogenic form that resists phosphorylation GSK3 β and subsequent degradation [38, 39]. In addition to its role in Wnt signaling, other important roles of β -catenin include cell adhesion through interaction with adherens junctions, microtubule maintenance, and the regulation of centrosome cohesion/separation [18, 40, 41]. Initial experiments employing β -catenin over-expression showed centrosome disruption, featuring debilitated MT elongation and cortical anchoring [42]. These findings suggest that β -catenin over-expression in colon cancers may have similar effects.

Some of the cellular β -catenin accumulates on mitotic centrosomes and where it helps regulate centrosome trafficking [43]. During S phase, the centrosome duplicates in preparation for mitosis. These two centrosomes, which are held together by a complex composed of c-Nap1 and rootletin, need to separate and move to opposite sides of the cell to establish polarity (Figure 3). The Nek2 kinase phosphorylates c-Nap1 and rootletin to stimulate centrosome separation [44, 45]. β -catenin is also phosphorylated by Nek2, which stimulates its association with the centrosomes during the separation process [40, 46]. Experiments support a role of β -catenin association in centrosome separation; specifically, reducing β -catenin levels using RNAi prevents centrosome separation and results in monopolar spindle formation [43]. These and other experiments indicate that β -catenin plays a role in centrosome organization. Its overexpression in colon cancers may serve to disrupt these processes.

Mutations in the N-terminal CK1/GSK3 β phosphorylation sites of β -catenin found in some colon cancers suppress its degradation and result in a highly expressed and constitutively active β -catenin [47]. These mutations also cause the appearance of structures in colon cancer cells that contain a subset of centrosome proteins, including γ -tubulin and centrin, that are not fully functional in microtubule nucleation (Figure 3)[48]. The formation of these structures appears to be independent of the transcription activation function of β -catenin. It is not clear how these unusual structures form, or what the consequence of their presence is, but they may interfere with the function the intact centrosomes. Deletion of the mutated β -catenin significantly reduced the appearance of these structures [48]. In addition, deletion of the mutated β -catenin has been found to eliminate over-replicated centrosomes in colon cancer cell lines [48]. These findings suggest that mutated β -catenin found in some colon cancers can induce the formation of aberrant assemblies of centrosomal proteins and also promote the over-replication of centrosomes.

3.3. BRAF

Early studies of BRAF pointed to a role in mitosis distinct from its functions in G1. Using siRNA, BRAF knockdown was found to cause widespread spindle abnormalities and misaligned chromosomes (whereas CRAF knockdown did not have this effect)[49]. A portion of BRAF in mitotic cells can be detected at spindle structures, including the spindle poles and kinetochores [49], consistent with a direct role in mitosis. The most common

mutation in the BRAF gene is the V600E point mutation, which occurs in 8–10% of colorectal cancers, and is associated with a CpG Island Methylation Phenotype (CIMP) and a poor prognosis [50, 51]. The V600E mutation results in unregulated BRAF activity and high levels of ERK activation through disruption of the RAF-MEK-ERK pathway. Expression of the constitutively active BRAFV600E oncogene also leads to the hyperphosphorylation and stabilization of Mps1, a protein that plays a pivotal role in regulating the SAC [52, 53]. As a result, cells progress slowly through mitosis, which may provide time for centrosome amplification [54]. Centrosome amplification under these conditions involves unregulated ERK activity, and may also be related to the ability of Mps1 to promote centrosome re-duplication [55]. The connection between oncogenic BRAF signaling and centrosome amplification has been uncovered primarily in melanoma cell lines, but these principles may extend to colorectal cancer. As discussed below, the prolonged SAC resulting from Mps1 stabilization may also promote survival of cells with over-replicated centrosomes by giving them additional time to cluster their supernumerary centrosomes.

3.4. p53

The tumor suppressor gene p53 is mutated in almost half of all colon cancers, usually through missense mutations in its DNA binding domain [56]. Mutations of p53 have long been associated with chromosomal abnormalities in cancers, pointing to a role for p53 in mitotic regulation. Part of this regulation appears to involve centrosome duplication, as centrosome over-duplication is generally not seen when wild type p53 is present [57]. p53 may control centrosome duplication through a number of mechanisms, including a transactivation-dependent mechanism. CDK2/cyclin E activation is sufficient to trigger centrosome re-duplication, even if the cell is not in S phase. As an activator of the CDK inhibitor p21Waf1/Cip1, p53 can suppress the aberrant CDK2/cyclin E kinase activity, and thus ensure that centrosome duplication is coordinated with the cell cycle [58]. The ability of p53 to recognize centrosome defects also appears to involve the interplay between p53, ATM, and the centrosome itself [59]. Specifically, ATM associated with the centrosome can induce p53 phosphorylation at Ser15 [60, 61]. This modification causes p53 to associate with the centrosome. This stabilized, centrosome-associated p53 is propagated to daughter cells where it can become active to safeguard against the expansion of cells that divided with a damaged centrosome [62]. The loss of p53 in colon cancers can therefore impact the cell's ability to both monitor centrosome integrity and to regulate its duplication.

4. Therapeutic implications

As discussed above, colon cancer cells often have defects in their ability to grow microtubule arrays from the centrosome, to regulate centrosome separation and trafficking, and to control centrosome duplication. These abnormalities arise in part from mutations in *APC*, β -catenin and other genes (Figure 4). Given the functional and numerical changes of the centrosomes in colon cancer cells, general approaches to therapeutic development include: 1) further interference with the centrosome function in a manner that is not tolerated by cancer cells, or 2) prevention of centrosome over-duplication and 3) disruption supernumerary centrosome clustering such that cancer cells form multipolar spindles.

4.1. Targeting centrosome function

Of the mutations common to colorectal cancer, mutation of the *APC* tumor suppressor gene appears to have the most direct effect on centrosome function. Among other roles, APC interacts with γ -tubulin to direct the growth of microtubules from the centrosome-associated γ -tubulin ring complex. As a result, the establishment of “K-fibers” that attach the centrosome to the kinetochore are formed more slowly in *APC* mutant cells. Cancer cells may adapt to this deficiency by increasing the expression of proteins that drive and stabilize K-fiber formation. Many proteins with this function are found to be up-regulated in colon cancers, including EB1, Aurora A, CHC, chTOG and TACC3 [63–67]. EB1 works together with APC to promote MT plus end growth, so its increased expression may directly compensate for the loss of APC function. Aurora A phosphorylates TACC3 to stimulate K-fiber elongation directed by the TACC3, CHC and chTOG complex, which may help compensate for the reduced efficiency of MT elongation from the centrosome in *APC* mutant cells [68, 69].

Compensation for *APC* mutation through increased activity of other proteins suggest potential vulnerabilities for *APC*-mutant cancer cells. With regard to the potential over-reliance on EB1, eribulin mesylate is known to bind microtubules at the plus-end to rapidly disrupt EB1-MT association [70]. Although the impact of *APC* mutation status on the sensitivity of cells to eribulin has not been extensively tested, it has been shown that *APC* mutant COLO 205 cells are sensitive to eribulin [71]. Stimulation of MT growth from the centrosome also requires Aurora A [72]. A number of specific Aurora A inhibitors have been developed, which tend to have broad anti-cancer activity and function by inducing G2/M arrest and apoptosis. However, cancer cell lines show a range of sensitivities to Aurora A inhibitors. Mutations in *APC* and/or other genes that affect centrosome function may underlie the sensitivity of cancer cells to Aurora A inhibitors. Another potential target is the MT-regulator TACC3. Recent data indicate that TACC3 over-expressed in cancer cells promotes colorectal tumor growth and metastasis [66, 67]. Knockdown of TACC3 reduced proliferation, migration, and invasion capabilities in HCT116 and SW480 cell lines, and inhibited tumor growth in xenograft tumor models and *APC*-mutant mice [67]. Inhibitors of TACC3 have been developed, and are presently being evaluated [73]. In general, compounds that disrupt the already debilitated centrosome function in *APC* mutant cells may kill cancer cells through mitotic catastrophe, or through activation of centrosome-based apoptotic pathways, such as ATM/p53.

4.2 Targeting centrosome overduplication

Centrosome overduplication is a common event in colon cancer, and has been linked to mutations in *APC*, *CTNNB1*/β-catenin, *BRAF*, and p53. The mechanism and consequences of this overduplication are not always clear. In some instances, such as cells with *BRAF* and p53 mutations, overduplication appears to arise from aberrant cell cycle signaling [53, 58]. Other potential mechanisms can include failed cytokinesis followed by centrosome re-duplication. As discussed above, the overduplication of the centrosomes may contribute to the viability of colon cancer cells, potentially compensating for defects in centrosome function resulting from *APC* mutations or other changes. If over-duplication of the centrosome is required for cancer cell viability, inhibiting the underlying pathway may be

sufficient to restore normal centrosome number and debilitate cancer cell proliferation and survival. Based on present cell-based studies, pharmacological BRAF, CDK, or PLK inhibitors may have this effect. Inhibition of centrosome overduplication may be particularly effective when combined with other mitotic challenges, such as spindle-targeting agents, which take advantage of centrosome defects in the cancer cells.

4.3 Targeting centrosome clustering

Supernumerary centrosomes occur almost exclusively in cancers cells, making centrosome clustering a problem exclusive to transformed cells. Interference with centrosome clustering can result in the formation of multipolar spindles, aberrant division, and subsequent cell death. Evidence has also been found that supernumerary centrosome de-clustering directly induces apoptosis, even in the absence of cell division. It is not entirely clear how de-clustering directly induces apoptosis (without cell division), but this finding highlights the presence of apoptotic signaling pathways that can sense centrosome aberrations.

A number of different experimental approaches have indicated that maintaining tension on the mitotic spindle is critical for keeping supernumerary centrosomes clustered. Using a broad-based RNA interference analysis, Leber et al. identified numerous cellular components necessary for supernumerary centrosome clustering [74]. They found that knockdown of the microtubule-kinetochore attachment complex, sister chromatid cohesion proteins, and the augmin complex induces multipolarity leading to cell death [74]. A common result of all these knockdowns is the disruption of spindle tension. These findings are consistent with work showing a role for dynein in centrosome clustering; the dynein motor is important for obtaining the proper spindle tension [75, 76]. Disrupting spindle tension is therefore one approach to centrosome de-clustering. Many MT-targeting compounds have this activity, which may account (in part) for their cancer cell selectivity. Other, more targeted approaches, are also possible [77], as there are many possible targets that could be disrupted to reduce the spindle tension. Although targeting some of these proteins may have deleterious effects on normal cells, some may have specificity for colon cancer cells with supernumerary centrosomes.

The RNA interference screens by Leber et al. also indicated that proteins within the chromosome passenger complex serve to stabilize supernumerary centrosome clustering. This complex includes Aurora B, a kinase that enforces the spindle assembly (SAC) checkpoint by phosphorylating histones and other chromosomal proteins [74]. RNAi knockdown or pharmacological inhibition of Aurora B causes centrosome de-clustering and ultimately apoptosis. This observation can be rationalized by considering that a strong SAC provides time for the supernumerary centrosomes to cluster. This model predicts that a debilitated SAC may selectively target cancer cells with supernumerary centrosomes, which has experimental support. In this regard, BRAF inhibitors (e.g., vemurafenib and sorafenib) may function as SAC inhibitors in BRAF-mutant colon cancers by virtue of BRAF's role in Mps1 stabilization [52]. Together with agents that disrupt spindle tension, inhibition of the SAC may be an effective approach for selectively targeting colon cancer cells. Development of this type of combinatorial approach would require careful target selection and agent

administration, since the proteins under analysis also play important roles in the division of normal cells.

In addition to inducing the de-clustering of supernumerary centrosomes to promote multipolar mitosis, evidence has also been obtained that cancer cells can be induced to form acentrosomal spindles that can result in aberrant mitoses. Colon cancer cells carrying a β -catenin mutation cells form multiple acentrosomal MT bundles that can potentially create acentrosomal spindles to disrupt bipolarity [48]. Knockdown of chTOG, HAUS3, and CEP164 have been shown to induce the formation of acentrosomal spindles in cancer cells and thus may be fruitful targets in colon cancer cells that readily form acentrosomal MT bundles. A mitotic disruptor with high apoptotic activity that we developed in our laboratory promotes the formation of multiple MT organizing centers in cells with a β -catenin mutation [78]. We are presently assessing the relationship between the β -catenin-induced MT bundle formation and these acentrosomal MTOCs.

5. Conclusions

Colon cancer cells carry many mutations that affect centrosome function, and frequently over-replicate and cluster supernumerary centrosomes. Since the centrosome plays a critical role in the distribution of genetic material following division, and can regulate cellular apoptotic pathways, we propose that effective colon cancer treatments may be designed through the rationale targeting of these centrosome changes and defects.

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Abbreviations used

PCM	pericentriolar material
MT	microtubules
MTOC	microtubule organizing center
APC	adenomatous polyposis coli
GSK3β	glycogen synthase kinase-3 beta
SAC	spindle assembly checkpoint

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Figure 1.

Centrosome assembly. Top panel shows the growth of a daughter centrosome, occurring during G1/S phase. Prior to M phase the pericentriolar matrix (PCM) expands to increase γ -tubulin ring complex (γ TuRC) association and the microtubule nucleation capacity of the centrosome (bottom panel)

A. **B.****Multipolar spindles** **Figure 2.**

A) Association of APC with the centrosome. Immunofluorescence and confocal microscopy show APC associated at the outer surface of the peri-centrosome material (PCM), partially wrapping around the γ -tubulin and centrin core. The N-terminus of APC associates with γ -tubulin, whereas the C-terminus associates with the MT plus-end and EB1 to promote elongation. APC mutations in colon cancer result in the expression of a truncated protein that still associates with γ -tubulin, but cannot interact with EB1. Cells expressing mutant APC are therefore defective for aster formation and spindle growth. **B)** APC mutations may also result in centrosome over-duplication, producing multipolar spindles with defective aster formation and spindle dynamics, due to the mutated APC.

Figure 3.

A) β -catenin expression is controlled by WNT signaling. APC, Axin and GSK3 β keep β -catenin expression low in normal, unstimulated cells. β -catenin expression increases with active Wnt signaling, or following APC or β -catenin mutation. Increased β -catenin expression leads to the activation of growth-promoting genes, and may affect centrosome separation and duplication. **B)** Nek2 phosphorylates β -catenin, in conjunction with c-NAP1 and rootletin, to promote centrosome separation during mitosis. **C)** Constitutively active mutated β -catenin promotes centrosome over-duplication, and produces aberrant centrosomal structures through poorly understood mechanism.

Figure 4. Centrosome regulation in normal cells, and the impact of colorectal cancer mutations on this process.