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Centrosomal Clustering Contributes to Chromosomal Instability and Cancer

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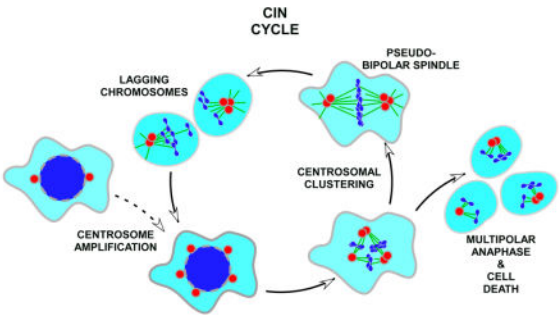
Abstract

Cells assemble mitotic spindles during each round of division to insure accurate segregation of their duplicated genome. In animal cells, stereotypical spindles have two poles, each containing one centrosome, from which microtubules are nucleated. In contrast, many cancer cells often contain more than two centrosomes and form transient multipolar spindle structures with more than two poles. In order to divide and produce viable progeny, the multipolar spindle intermediate must be reshaped into a pseudo-bipolar structure via a process called centrosomal clustering. Pseudo-bipolar spindles appear to function normally during mitosis, but they occasionally give rise to aneuploid and transformed daughter cells. Agents that inhibit centrosomal clustering might therefore work as a potential cancer therapy, specifically targeting mitosis in supernumerary centrosome-containing cells.

Graphical Abstract



Introduction

Transformed cells no longer respond to cues that normally suppress cell growth and division. This change, from a normal to a cancerous cell, can arise from mutations in specific genes or be caused by more dramatic alterations in genomic composition in the form of whole or

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partial chromosome loss or addition [1]. Indeed, chromosomal instability (CIN), defined in a population of cells as a systematic deviation from the normal genomic content, is a hallmark of most solid tumor cancers and likely contributes to the maintenance of transformed cell populations, drug resistance, and disease progression [2, 3]. Though there are several different paths to CIN, in this review we focus on how centrosomal amplification (CA), another hallmark of many cancers, affects the chromosome segregation machinery in cells and leads to CIN. Specifically, we introduce the centrosome and provide a brief overview of how centrosome number is controlled in normal cells. We then describe a mechanistic link between supernumerary centrosomes and CIN. Finally, we discuss how cancers maintain the CIN cycle, and potential therapeutic strategies designed to force cells to exit the cycle.

Centrosome Structure and Function

Overview of Centrosome Structure

Centrosomes are non-membranous organelles that contain a structurally-defined core consisting of a pair of longitudinally orientated mother and daughter centrioles and a surrounding region of electron-dense pericentriolar material (PCM). The centrioles themselves are cylindrical structures with a radially symmetrical arrangement of nine microtubule triplets circumscribing a central cartwheel-like structure ([4]; for a more detailed review of centriole structure see[5]). It is important to note that centriolar microtubules differ from their spindle counterparts. Evidence suggests that they are heavily modified by post-translational modifications [6, 7] and thus are behaviorally distinct from spindle microtubules in terms of both inherent dynamics as well as resistance to commonly used cytotoxic drugs [8]. In contrast to centrioles, the surrounding PCM is structurally ill-defined and until recently has been described as an amorphous, proteinaceous matrix ([9]; for a more detailed review of PCM organization see [10]). The PCM serves as a recruiting center for several proteins [11] involved in the nucleation of microtubules, including the yTURC ring complex [12], and provides a structural scaffold to which newly nucleated microtubules can be anchored [13].

Overview of Centrosome Function in Mitotic Spindle Assembly: A Numbers Game?

At the onset of mitosis, duplicated centrosomes occupy a singular site on the surface of the nucleus. Signaling events in the cell cause the pair to split, and each centrosome migrates along the surface of the nucleus until directly opposite the other. This geometry establishes the eventual pole-to-pole axis of the mitotic spindle, with each centrosome forming an important component of each individual pole [14, 15]. The spindle takes shape when the nuclear envelope breaks down and the centrosome-nucleated microtubules invade the now accessible nucleoplasmic space, eventually making connections with kinetochores as well as with chromosome-derived microtubules [14]. The formation of the bipolar spindle is very important because the spindle is responsible for accurately segregating chromosomes during cellular division, and because defects can lead to chromosomal instability [16].

The spindle is typically maintained until all chromosomes are aligned on the metaphase plate, satisfying the spindle assembly checkpoint (SAC; [17]) and allowing anaphase to ensue. Due to the dynamic nature of spindle microtubules ($t_{1/2}$ ~ 10–100secs [18]), this

maintenance requires continuous microtubule nucleation and spatial organization via microtubule-based motors such as dynein, kinesin-5, and kinesin-14 (HSET in human cells; [19, 20]), whose integrated activities shape the spindle by cross-linking and sliding microtubules [21]). Despite the seemingly prominent role of centrosomes during this process, spindle assembly (and even chromosome segregation) occurs unabated in mammalian tissue culture cells following laser ablation of one or both centrosomes [22]. Centrosome-independent spindle assembly is akin to the behavior of several well-characterized meiotic systems, which are capable of assembling bipolar spindles in the absence of centrosomes entirely [23], and similar mechanisms are likely responsible for the assembly of pseudo-bipolar spindles in cells that contain more than two centrosomes. In the presence of supernumerary centrosomes (i.e. >2), spindle assembly typically proceeds through a multipolar intermediate with centrosomes located at >2 spatially distinct foci of microtubule ends. This transient morphology is often resolved prior to anaphase onset by clustering extra centrosomes together into two independent spindle poles (see graphical abstract). Emerging spindles appear deceivingly normal (i.e. are the right size and shape), but are prone to contain erroneous kinetochore attachments that can increase the likelihood of chromosome missegregation [24], the prevalence of aneuploid daughter cells [16, 25], and malignant transformation (see discussion below [26–28]). It makes sense then that the control of centrosome numbers in cells is tightly regulated.

Control of Centrosome Number in Cells

A detailed discussion about the centrosome duplication cycle, beyond the scope of this review, is provided in excellent and recent reviews on the topic [29, 30]. In broad strokes, the centrosome replication cycle can be divided into six major events that occur in concert with the cell division cycle: duplication, elongation, linker dissolution, separation, maturation, and disengagement [29, 30]. Though each of these processes is distinct, involving a specific set of structural and regulatory proteins, there are critically important proteins and overarching control mechanisms that merit special consideration.

Coordination between centrosome duplication and other events of the cell cycle is achieved in part through the activity of cyclins and cyclin-dependent kinases (CDKs; [31, 32]). Cyclins are a family of proteins whose levels rise and fall during the cell cycle [32, 33], and when their intracellular levels are sufficiently high, they bind to and activate cyclin-dependent kinases (CDKs). At the G1/S transition for example, both mother and daughter centrioles serve as a template for procentriole formation [34], and this event is tightly controlled by levels of cyclin E/CDK2 [35], the same cyclin-CDK pair responsible for establishing the pre-initiation complexes required for DNA replication [36]. Similarly, centrosome linker dissolution, whereby the proteinaceous linkages between duplicated centrosome pairs are degraded, is regulated by cyclin B/CDK1 at the G2/M transition [37]. Many additional kinases are involved in the centrosome duplication cycle [38], including Polo-like Kinase 4 (Plk4). Often described as the master regulator of centrosome duplication [39], Plk4 activity is essential for the initiation of procentriole formation and its levels/activity have been shown to correlate with centrosome amplification in several model systems [40, 41]. The levels of Plk4 are strictly regulated as well, in part, through an auto-phosphorylation event that marks itself both for ubiquitination and destruction [42].

Dysregulation of the centrosome duplication cycle can lead to aberrant centrosome numbers. Indeed, experimental perturbation of the levels and/or activities of several of the proteins involved results in CA, and many cancers exhibit aberrant expression levels of these same proteins [43–45]. Though targeting of the centrosome duplication cycle might ultimately prove to be of therapeutic benefit, recent evidence has shown that transformed cells are actually refractory to centrosome loss – for example, transformed cells treated with the Plk4 inhibitor centrinone were able to divide even after drug-induced centrosome loss [46]. Thus, once cells are transformed, complete loss of centrosomes may do little to stop their proliferation.

Mechanistic Links between Centrosome Number and Cancer

In animal cells, the mitotic spindle is comprised of dynamic microtubules that are nucleated around several spatially distinct sources including chromosomes [23] and centrosomes [47]. A normal cell contains exactly two centrosomes at the onset of mitosis: one for each spindle pole. How might dysregulation of centrosome number result in malignant transformation? During mitosis, cells with supernumerary centrosomes often assemble mitotic spindles with supernumerary (i.e. >2) poles [44, 48]. In the late 19th century, Theodore Boveri hypothesized that these multipolar spindles could undergo a multipolar anaphase, resulting in transformed, aneuploid daughter cells with a malignant potential [49, 50]. Recent experimental evidence, however, has shown that multipolar anaphases are relatively uncommon and almost always produce inviable daughter cells [2, 26, 51, 52]. Instead, cells with CA tend to reshape transient multipolar spindles into pseudo-bipolar structures prior to anaphase onset via a dynamic process called centrosomal clustering [27, 51, 53]. This remodeling of spindle morphology requires many of the same proteins that are involved in maintaining and positioning the spindle pole, including dynein, kinesin-14, microtubules, and the actin cytoskeleton [27, 51]. Furthermore, activation of the spindle assembly checkpoint (SAC) appears to facilitate centrosomal clustering by delaying the onset of anaphase [28, 54], and proteins that are closely monitored by the SAC, such as NDC80 and its binding partners, are important for centrosomal clustering processes as well [55]. In summary, centrosomal clustering is a conserved mechanism that allows cells with CA to continue to proliferate.

Resolution of multipolar intermediates through centrosomal clustering results in a higher incidence of mis-attached kinetochores, especially merotelic attachments [24, 26]. These erroneous attachments, defined as an attachment of a single kinetochore to multiple spindle poles, can lead to lagging chromosomes during anaphase, which then are frequently damaged or mis-segregated [24, 52]. As such, merotelic attachments are a major driver of CIN [2, 26]. Because only a small percentage of spindles with supernumerary centrosomes contain merotelic attachments, random proliferative advantages are not quickly lost due to continued mis-segregation or cell death. This type of infrequent, low-level CIN can contribute to, and may possibility initiate, the development of cancer [16, 56], likely by altering the copy number of oncogenes [1]. The relationship between centrosomal clustering, chromosomal instability, and cancer is further described in Figure 1.

While it is clear that CA can contribute to the development of cancer, there are contrasting data in the literature. For example, two recent studies have demonstrated that supernumerary centrosomes are insufficient to initiate tumorigenesis in mice [40, 57]. We interpret these results to suggest that the promotion of cancer requires levels of centrosomal amplification within an acceptable range that confers sustainable genetic instability [16, 56]. It has been shown, for example, that increasing levels of genetic instability in cells already displaying CIN results in a higher incidence of apoptosis and therefore lower proliferation rates [56]. Instead, levels of CIN must be at a tolerably low level in order to promote the stable genetic changes that are necessary for malignant transformations and tumorigenesis.

Preventing Centrosomal Clustering and Promoting Multipolar Anaphase as an Anticancer Strategy

Definitive evidence that establishes a causal link between CA and tumorigenesis remains elusive, undermining confidence in the potential efficacy of novel anti-cancer therapies designed to reduce the incidence of CA. Regardless, most cancers exhibit CA and therefore require centrosomal clustering as a means to divide and proliferate [45]. As such, CA can serve as a “pan” marker of cancerous cells, and centrosomal clustering can serve as a target for anti-proliferative agents. Several screens have recently been used to identify proteins required for centrosomal clustering [58–60]. Candidates with mitosis-specific functions are of particular interest as they may be used to specifically target dividing cells with CA. However, many candidate targets identified so far also function to insure proper spindle assembly and chromosome segregation fidelity in normal cells and likely will suffer from the same issues that plague other commonly used anti-mitotic cytotoxic agents. Furthermore, centrosomes and their associated microtubule asters are involved in diverse non-mitotic functions such as nuclear expansion [61], chromosomal organization [62, 63], pronuclear migration [64], nuclear/spindle positioning [14, 65, 66] and ciliogenesis [67]. What, if any, contribution that these functions might make to the transformed cell state is now only poorly understood. As such, the challenge to the field is to identify and characterize biomolecules that are critical for centrosomal clustering, but have negligible roles in other critical processes in normal cells.

Currently, several centrosomal clustering inhibitors have been shown to be antiproliferative and partially selective for cells with CA. These include inhibitors of kinesin-14 family members [68], as well as compounds with unknown targets, such as the microtubule binding, anti-fungal griseofulvins [69–71] and nitrofuramides [60]. Inhibition of kinesin-14s is a particularly promising approach because these motors have been shown to be essential for clustering centrosomes in cancer cells, but are not required for division normal cells [28, 60, 72]. Results from experiments using derivatives of griseofulvin and/or nitrofuramide are also encouraging, as these drugs inhibit mitosis selectively in cancer cells [60, 70, 71]. The griseofulvin derivative, GF-15, was also found to inhibit tumorigenesis in mice [71]. Small molecule therapeutics that target centrosomal clustering therefore represent a promising strategy to selectively target populations of cells that require centrosomal clustering for division, but currently, only a disparagingly small number have been identified.

Recent advances in high-throughput data acquisition and data analysis have the potential to significantly improve upon existing screening technologies, enabling more efficient, and perhaps less costly, discovery of new centrosomal clustering inhibitors. For example, small molecule screens designed to examine the effects of compounds on tissue culture cell division have proven powerful, but are inherently limited in that they require the use of cell-permeable effectors. To expand the utility of these approaches, several labs have recently begun to explore the potential of combining cell-free systems with microfluidic-based encapsulation [73–76]. This experimental platform allows for the recapitulation of cellular processes such as spindle assembly and chromosome segregation in discrete volumes of defined size and composition. However, since these “open” systems lack membranes, they can be used to study the effects of a broader range of therapeutic compounds, including those not cell-permeable. Other microfluidic-based approaches, such as flow cytometry [77], will permit more efficient characterization of drug effects on cancer cells with CA and will likely continue to evolve as a proven platform for drug discovery [78]. When combined with tissue-on-a-chip [79] and/or 3D cell-culture technologies [80, 81], these high-throughput methods might one day streamline the drug development/approval process by accurately predicting *in vivo* results.

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Highlights

1. Cancer cells often have supernumerary centrosomes and rely on centrosomal clustering to divide.
2. Centrosomal clustering leads to chromosomal instability (CIN) through the formation of merotelic kinetochore attachments.
3. CIN results in changes to the sequence and copy number of oncogenes, providing a mechanism for cancer cells to adapt.
4. Inhibiting centrosomal clustering forces cells to undergo multipolar anaphase and often results in cell death.

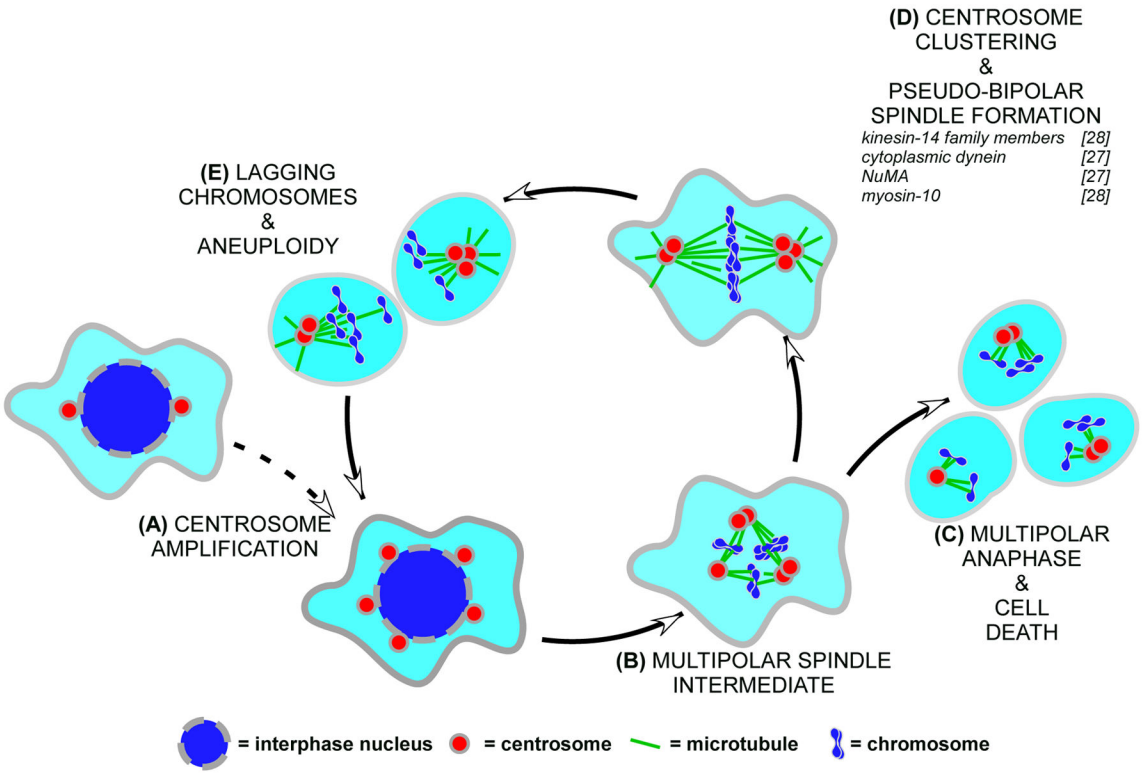

Figure 1.

Cartoon schematic of the CIN cycle. Cancer cells often possess supernumerary centrosomes due to dysregulation of the centrosome duplication cycle or failure of cytokinesis (**A**). Spindles that assemble in these cells typically form transient intermediates with more than two poles (**B**) leading to multipolar anaphase and apoptosis (**C**) or centrosomal clustering and the formation of pseudo-bipolar spindles (**D**). The latter path causes merotelic chromosome attachments and lagging chromosomes (**E**) sometimes resulting in aneuploidy and CIN. Several well-characterized targets for selective inhibition of centrosomal clustering and forced exit from the CIN cycle are listed in **D**.