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Centrosome Function in Normal and Tumor Cells

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Abstract Centrosomes nucleate microtubules that form the mitotic spindle and regulate the equal division of chromosomes during cell division. In cancer, centrosomes are often found amplified to greater than two per cell, and these tumor cells frequently have aneuploid genomes. In this review, we will discuss the cellular factors that regulate the proper duplication of the centrosome and how these regulatory steps can lead to abnormal centrosome numbers and abnormal mitoses. In particular, we highlight the newly emerging role of the Breast Cancer 1 (BRCA1) ubiquitin ligase in this process. J. Cell. Biochem. 99: 1240–1250, 2006. © 2006 Wiley-Liss, Inc.

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The centrosome is a centrally positioned organelle that functions as the major microtubule (MT) nucleating center in animal cells. Structurally, it is highly conserved among higher eukaryotes and consists of two cylindrical centrioles embedded in an electron dense mesh called the pericentriolar matrix (PCM). The centrioles are each composed of characteristic nine parallel triplets of microtubules. The PCM provides a scaffold for proteins that are important for regulating centrosome duplication and function, containing the basic unit for MT nucleation, the γ-tubulin ring complex (γ-TuRC) [Moritz et al., 1995a,b], a complex of six proteins in humans that includes γ-tubulin and GCP2-6 [Fava et al., 1999; Murphy et al., 2001]. In Drosophila, this γ-TuRC complex has been visualized by electron microscopy to form rings, and these rings position γ-tubulin to initiate the polymerization of α- and β-tubulin protomers into microtubules [Moritz et al., 2000].

The centrosome is a complex and dynamic organelle. At 1–2 μm in diameter, it dwarfs ribosomes (25 nm) and mitochondria (0.1–0.2 μm), and centrosomes contain at least 150 proteins [Andersen et al., 2003] that change their abundance as the centrosome and the cell proceed through the cell cycle. In dividing cells, the two centrosomes establish the poles of the bipolar mitotic spindle that controls the equal division of chromosomes. Clearly, it is vital to the health of the cell that there be exactly two centrosomes during cell division.

The function of the centrosome is equally important in the interphase cell as well as the mitotic cell. During interphase, centrosomes nucleate MTs that form functional arrays to maintain cell morphology and polarity. Centrosome repositioning has been shown to be essential for migration in many neural cells. As an example, in neural migration, the centrosome is required to pull the nucleus along with the moving cell body [Bellion et al., 2005]. Disruption of centrosome function can thus have dire consequences for non-mitotic cells.

THE PROBLEM OF CENTROSOME DUPLICATION

Similar to DNA, centrosomes divide once per cell cycle, but unlike DNA replication, there are no cell cycle checkpoint arrests if centrosome duplication is faulty [Uetake and Sluder, 2004; Wong and Stearns, 2005]. The interphase cell has one centrosome. The centrosome division cycle begins at the G1-S phase and by end of G2, each cell has two centrosomes. The centrosome

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duplication process (diagrammed in Fig. 1A) involves five defined steps.

Disorientation

The single post-mitotic centrosome contains two centrioles, termed the mother and daughter centrioles. The daughter centriole is attached at a right angle to the lateral base of the mother centriole. During late G1 phase, the mother and daughter centrioles separate, losing their orthogonal orientation with respect to each other but still attached via a fiber. These separated centrioles are the new mother centrioles that nucleate the synthesis of daughter centrioles. It has been suggested that this disorientation step licenses the subsequent duplication steps [Tsou and Stearns, 2006].

Duplication

At the beginning of S-phase, pro-centrioles, or daughter centrioles, appear close to the proximal end of each centriole, perpendicular to each mother centriole. New centriole formation can occur anywhere in the cytoplasm, but a poorly understood regulatory mechanism limits the sites of synthesis to the base of the mother centriole (reviewed in [Hagan and Palazzo, 2006]). It is likely that this limitation to centriole synthesis ensures that a single duplication event occurs.

Elongation

The nascent procentrioles elongate through S and G2 phase of the cell cycle.

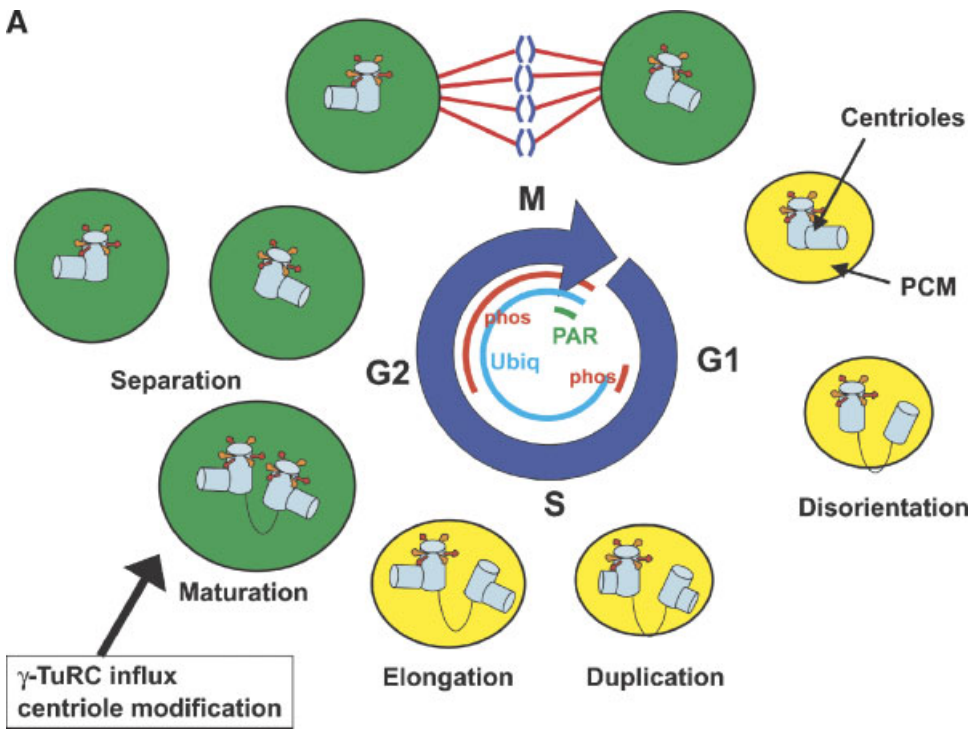

Fig. 1. **A**: The centrosome cycle. Centrosomes contain paired centrioles (blue cylinders) embedded in PCM (depicted as yellow in G1 and S phases). At the G1-S transition, the centrioles lose their orthogonal orientation (disorientation), duplicate, and the daughter centrioles elongate. In G2 phase of the cell cycle, the centrosomes mature by decoration of the mother centriole with appendages (depicted as red and orange knobs) and by increasing the γ-TuRC content of the PCM. The increase in γ-TuRC is associated with higher MT nucleation activity of the centrosome. The two pairs of centrioles split off the centrosome, migrate to opposite poles of the nucleus, and after nuclear membrane disintegration in mitosis, the centrosomes set up the mitotic spindle (depicted with red microtubules and blue chromosomes; not drawn to scale). After mitosis, the daughter cell has a single centrosome. The times at which kinases are known to affect centrosome duplication and maturation are indicated with red arcs, ubiquitin ligases with a blue arc, and poly-(ADP)-ribosylation (PAR) in green. **B**: Centrosome function. The main role of the centrosome is as the microtubule organizing center of the cell (MTOC). The γ-TuRC (depicted as a green ring) initiates the polymerization of α- and β-tubulin protomers into microtubules (MT, depicted as red rods). The γ-TuRC complex directly controls MT nucleation function of the centrosome. Thus, maturation of the centrosome in G2 controls the γ-TuRC content in the PCM. Likewise, BRCA1 function in S phase limits γ-TuRC influx into the centrosome, causing a reduction in MT nucleation activity. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

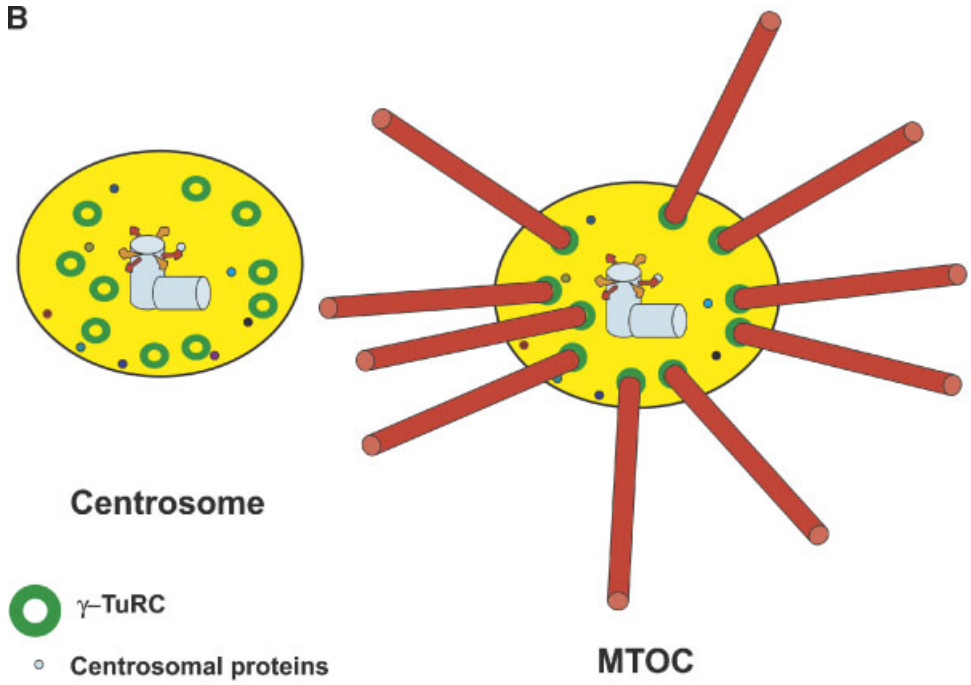

Fig. 1. (*Continued*)

Maturation

At the G2/M phase, the centrosomes undergo a dramatic increase in size due to accumulation of several proteins including γ-TuRC [Khodjakov and Rieder, 1999]. The influx of γ-TuRC into the PCM is associated with a higher level of centrosomal MT nucleation activity. The new mother centriole also matures via the binding of specific proteins.

Separation

The two centrosomes move to the opposite poles of the nucleus to form the mitotic spindle.

These steps are all regulated by a slew of kinases, and it has become increasingly clear that other protein modifications, including ubiquitination and ADP-ribosylation, regulate this process. This review will discuss the various regulatory modifications that occur at the centrosome and how disruption of these modifications can result in abnormal centrosome amplification associated with cancer. Special emphasis will be given to the Breast Cancer 1 (BRCA1) E3 ubiquitin ligase since significant elements of the mechanism by which this tumor suppressor protein regulates centrosomes have recently been determined.

CENTROSOME ABNORMALITIES IN CANCER

Abnormal centrosomes have been observed in a wide range of tumor types (reviewed by [Nigg, 2002]). The key finding is of amplification in centrosome number in tumor cells. This centrosomal amplification accompanies aneuploidy that typifies many cancers, and this correlation suggests a chicken-and-egg problem: is centrosome amplification the cause of aneuploidy, or vice versa? In our opinion, the data best support the concept that supernumerary centrosomes create a mutator phenotype that promotes cancerous transformation.

The first argument in favor of centrosome amplification driving the aneuploidy is from the investigation of early tumor lesions and the observation of extra centrosomes at the earliest stages of cancer [Lingle et al., 2002; Pihan et al., 2003]. If centrosomes re-duplicate to a total of four centrosomes in a cell that has completed DNA replication, then the extra centrosomes may group so that a bipolar spindle and normal division of DNA occur. However, often re-duplication of centrosomes results in multipolar spindles, and completion of mitosis would be catastrophic for the cell. This outcome is fine for the organism since the abnormal cell would

die, but in many cases, the cells would fail to divide resulting in a G1 cell with two centrosomes and a tetraploid genome. It had been originally suggested that such a cell cannot enter the cell cycle due to a p53-dependent cell cycle block [Andreassen et al., 2001], but subsequent experiments suggest that the observed cell cycle block was dependent on the techniques used to block cytokinesis. If other methods were used to create cells with supernumerary centrosomes and tetraploid genomes, then the cells could continue to cycle [Uetake and Sluder, 2004; Wong and Stearns, 2005]. It is easy to imagine that subsequent cell divisions with unequal distributions of the re-replicated chromosomes will result in cells that survive despite genomic amplifications and deletions. Studies of cells that are tetraploid reveal that such cells are more likely to be tumorigenic when compared to genetically identical diploid cells [Fujiwara et al., 2005]. The weight of the evidence thus suggests that overduplication of centrosomes can cause cancer.

In the tumor cell, a number of apparent abnormalities of the centrosome are observed. Structural changes in centrosomes include increase in number and volume, supernumerary centrioles, accumulation of excess pericentriolar material, and aberrant phosphorylation of centrosomal proteins [Lingle et al., 1998; Pihan et al., 1998]. There are several ways by which cells could accumulate supernumerary centrosomes (reviewed in [Nigg, 2002]): (1) re-duplication of centrosomes in a single cell cycle; (2) cytokinesis failure and re-duplication in the next cell cycle; (3) fragmentation; or (4) cell fusion. Further, centrosomes from breast cancer cells and tissues are hyperactive in terms of their microtubule nucleation capacity [Lingle et al., 1998, 2002; Salisbury et al., 1999; D'Assoro et al., 2002]. The consequences of hyperactive MT nucleation are currently unclear but likely contribute significantly to the tumorigenicity of the cell.

PROTEIN MODIFICATIONS AFFECT CENTROSOMAL DUPLICATION AND FUNCTION

Kinases

Cyclin/cyclin-dependent kinases (Cdk) activate the duplication process and a number of kinases regulate the changes in centrosome function preceding and during mitosis. The level of protein phosphorylation at the centrosomes significantly increases during mitosis [Vandre and Borisy, 1989]. Among the important kinases that have been established to affect centrosomal processes are cdk2, Aurora kinase, Polo-like kinase, and the NIMA-related kinase 2 (Nek2). Discussed below are some of the centrosome regulatory activities of these kinases. Table I includes a summary of the known kinases, ubiquitin ligases, and a poly-ADP-ribosyl polymerase that modify centrosomal proteins with known substrates indicated and functional consequences.

Cdk2. Cyclin/cdk enzymes control the progression through each stage of the cell cycle. Cdk2 bound to cyclin E is essential in initiating DNA replication in higher eukaryotic cells [Sherr, 1996]. Since centrosomes, like DNA, must divide once per cell cycle, it is not surprising that both processes require cycE/cdk2. In some cell types, cycA/cdk2 is the critical activity for initiating centrosome duplication [Meraldi et al., 1999]. The mechanics of how cyc/cdk2 initiates the centrosome duplication are unclear, but apparently one critical step for centrosomes to begin duplication is the displacement of nucleophosmin/B23 (Npm) from the centrosomes. This is accomplished by the phosphorylation of Npm by cycE/cdk2 at Npm residue thr-199 [Okuda et al., 2000; Tokuyama et al., 2001]. A T199A mutant of Npm has a dominant negative effect and arrests duplication at a single centrosome [Tokuyama et al., 2001]. CycE/cdk2-mediated phosphorylation of CP110 has also been shown to be important for centrosome duplication at G1/S transition [Chen et al., 2002].

Aurora kinases. The Aurora kinases are a family (Aur-A, Aur-B, and Aur-C) of serine/threonine kinases distinguished by a highly conserved catalytic domain. Aur-A and -C are localized to centrosomes whereas, Aur-B is localized to the mid-body and all three are key regulators of cytokinesis (reviewed in [Katayama et al., 2003]). Among the important substrates of the Aurora Kinases are: Eg-5 [Giet et al., 1999]; CENP-A by Aur-A and -B [Kunitoku et al., 2003; Zimmerman et al., 2004]; a kinesin-like protein MCAK by Aur-B [Andrews et al., 2004; Lan et al., 2004]; Tpx2 by Aur-A [Kufer et al., 2002]; and a component of the mitotic spindle, the microtubule de-stabilizing oncoprotein Op18/Stathmin by Aur-A [Gadea and Ruderman, 2006]. Inhibition of Aur-A

TABLE I. Centrosome Modifiers and Effects

Enzyme	Stage	Substrate	Effect of inhibition/overexpression
Kinases			
CycE/Cdk2	G1/S	Npm	Inhibition: arrest at single centrosome Tokuyama et al. [2001]
CycA/Cdk2	S	—	Inhibition: inhibits centrosome duplication Meraldi et al. [1999]
Aur-A	G2/M	CenpA, Tpx2, Op18	Inhibition: monopolar spindle Glover et al. [1995]; Roghi et al. [1998]
		—	Overexpression: Centrosome amplification Meraldi et al. [2002]; loss of mitotic checkpoint Anand et al. [2003]
Aur-B	G2/M	—	Inhibition: multinucleate cells Tatsuka et al. [1998]
Aur-C	G2/M	—	Overexpression: centrosome amplification Dutertre et al. [2005]
Plk1	M	Nlp1	Inhibition: smaller centrosomes with less γ-tubulin Tavares et al. [1996]
Plk2	G1/S	[β-tubulin, MAPs]	Inhibition: failure of centriole duplication Warnke et al. [2004]
Plk3	S/G2	—	Expression of mutants: altered cell morphology due to effects on microtubule dynamics Wang et al. [2002]
Plk4	S, G2, M	—	Overexpression: amplified centrioles Habedanck et al. [2005]
			Inhibition: blocked centriole overduplication during S-phase Habedanck et al. [2005]
Nek2A	G2/M	C-Nap1	Overexpression: split centrosomes and gradual disappearance of centrosomes Fry et al. [1998a]
Nek2B	M	—	Depletion from Xenopus egg extract: delayed MT aster formation from sperm basal body Twomey et al. [2004]
Ubiquitin ligases			
SCF	G1-S	CycE	Inhibition: centrosome overduplication and polyploidy Nakayama et al. [2000]
APC/C	M	CycA, Aur-A	Inhibition: arrest in metaphase Wasch and Engelbert [2005]
BRCA1	S-G2	γ-Tubulin	Inhibition: centrosome re-duplication Starita et al. [2004] and hyperactive MT nucleation Sankaran et al. [2005, 2006]
Poly-ADP-ribosyl-polymerase			
Tankyrase-1	M	—	Inhibition: loss of bipolarity of spindle Chang et al. [2005]

The kinases, ubiquitin ligases, and PAR polymerase that regulate centrosome function are indicated, along with stages of the cell cycle, identified substrates, and effects of perturbing the protein function. The actual substrate is not known for many of these enzymes, and in the cases of β-tubulin and MAPs, the specific Plk responsible for the phosphorylation was not determined.

causes formation of monopolar spindles [Glover et al., 1995; Roghi et al., 1998] and overexpression leads to amplified centrosomes [Meraldi et al., 2002] and cells override checkpoint controls to enter anaphase despite defects in the mitotic apparatus [Anand et al., 2003]. Based on these observations, we infer that Aur-A is required for centrosome duplication and separation. Inhibition of Aur-B leads to formation of multi-nucleated cells hinting at its role in regulating cytokinesis [Tatsuka et al., 1998]. Overexpression of Aur-C results in polyploid cells containing more than two centrosomes, and this phenotype is aggravated in the absence of a functional p53 [Dutertre et al., 2005].

The levels of all three Aurora kinases are elevated in many human cancers, directly correlating with chromosomal instability and aggressiveness of tumors. The activity of these kinases peaks at G2-M phase of the cell cycle, right before the time when there is a rise in the levels of phosphorylated epitopes at the centrosomes [Vandre and Borisy, 1989].

Polo-like kinases. Polo-like kinases (Plk 1-4) are a family of serine/threonine kinases that are highly conserved from yeast to human [Hamanaka et al., 1994]. In addition to the catalytic domain, Plks have a 30 amino acid polo box domain (PBD) that is essential for localization and substrate binding. Plks, in general, have peak activity at mitosis and their overexpression positively correlates with aggressiveness and prognosis in many cancers [Takai et al., 2005]. Some of the protein targets of Plk include β-tubulin [Tavares et al., 1996], two microtubule-associated proteins (MAPs), and a kinesin-like motor protein [Glover et al., 1998], and these proteins control centrosome separation. Centrosome separation proceeds in two steps. In the first step, independent of MTs, cohesion between the two mother centrioles is lost [Fry et al., 1998a; Mayor et al., 2002]. This is followed by a step involving MT-dependent motor proteins that recruit proteins required for the formation of the mitotic spindle. Disruption of Plk affects centrosome duplication. Using RNA interference and overexpression studies with a dominant negative mutant of Plk2, centrosome duplication was blocked in cells arrested in S-phase of the cell cycle [Warnke et al., 2004]. Plk2, unlike other Plks, is active at G1/S. Plk4 is localized to centrosomes through all phases of the cell cycle and increased activity of this kinase led to centriole amplification [Habedanck et al., 2005]. Cells

injected with antibodies against Plk1 had centrosomes drastically reduced in size and decreased γ-tubulin localization [Lane and Nigg, 1996], indicating the importance of this kinase in centrosome maturation. Prior to mitosis, the centrosomes displace several proteins including Ninein-like protein 1 (Nlp1). Nlp1 localizes to centrosomes during G1 and S phases of the cell cycle and recruits γ-TuRC to centrosomes [Casenghi et al., 2003]. Plk1 phosphorylation of Nlp1 during G2/M results in Nlp1 displacement from centrosomes [Casenghi et al., 2003], a critical step for proper centrosome maturation and spindle assembly.

NIMA-related kinase (Nek2). Nek2 is a coiled-coil domain containing serine/threonine kinase localized to centrosomes. It exists in two forms Nek2A and B, in mammalian cells. Nek2A has peak activity during S-phase and downregulated after onset of mitosis. Nek2B levels are high throughout mitosis. Nek2 phosphorylates C-Nap1 and displaces it from the centrioles, an essential step for subsequent splitting of centrioles [Fry et al., 1998a,b]. Nek2 also phosphorylates protein phosphatase 1 (PP1) to activate it, which in turn dephosphorylates C-Nap1 [Fry et al., 1998a,b; Helps et al., 2000; Eto et al., 2002].

Ubiquination of Centrosomal Proteins

Ubiquitination is a multi-enzyme cascade that appends the 76 amino acid ubiquitin protein onto substrate proteins. Beginning with the E1 ubiquitin activating enzyme, which charges the carboxy-terminal glycine on ubiquitin, the charged ubiquitin is then transferred to one of several E2 ubiquitin conjugating enzymes via a thioester linkage. The E2 then associates with an E3 ubiquitin ligase that transfers either single or multiple ubiquitin molecules to specific targets. There are many E3 ubiquitin ligases that provide the specificity for substrate protein modification. The E3 ubiquitin ligase is an unusual enzyme in that it acts as an adaptor protein that brings together the charged E2 with the specific substrate.

The G1–S transition of the cell cycle is regulated by one E3 ubiquitin ligase complex named SCF for its prototypical subunits (Skp1, Cullin, F-box). Entry into mitosis is regulated by a second multisubunit E3 ubiquitin ligase called anaphase promoting complex or cyclosome (APC/C). The activities of these ubiquitin ligases regulate centrosomes as well as the cell cycle. The heterodimeric BRCA1/BARD1 (in this review, the heterodimer will simply be referred to as BRCA1) is a third E3 ubiquitin ligase that regulates centrosome number and function, but surprisingly, the effects of BRCA1 appear to be specific for cell lines derived from breast tissue [Starita et al., 2004]. Experiments suggest that in non-breast cells, the same modifications executed by BRCA1 also occur, but are catalyzed by other, as yet unknown E3 ubiquitin ligases [Starita et al., 2004].

Skp1/Cullin/F-box complex. SCF (reviewed in [Cardozo and Pagano, 2004]) is a multisubunit ubiquitin ligase containing a catalytic RING-domain subunit, called Roc1/Rbx1. The F-box protein is the specificity determinant of the complex that binds targets. Proteins ubiquitinated by SCF, such as cyclin E, are targeted for degradation by the proteasome. Skp1 and Cullin1 localize to centrosomes and antibodies against either of these two proteins inhibited centriole separation in Xenopus extracts [Freed et al., 1999]. Addition of the proteasome inhibitor, MG132, to the extract also inhibited centriole disorientation, indicating a requirement for proteosomal degradation of the SCF target for this process. Mice with a targeted disruption of F-box protein Skp2 were viable, but many animals showed marked centrosome overduplication and extensive polyploidy [Nakayama et al., 2000].

Anaphase promoting complex and cyclosome (APC/C) ubiquitin ligase. The first multi-subunit E3 ligase described, APC/C is required for the transition from metaphase-to-anaphase and to exit mitosis. APC/C controls the cleavage of the cohesin complex, which holds sister chromatids together, by ubiquitinating securin, a negative regulator of separase. Separase is the protease that cleaves cohesin, allowing anaphase to begin [Nasmyth et al., 2000]. Among the key substrates of APC/C in the centrosome are the kinases that regulate centrosome function. The cullin homolog, Apc2 and a RING-H2 finger protein similar to Rbx1, called Apc11, form the catalytic core for APC and can polymerize ubiquitin chains in vitro [Gmachl et al., 2000; Tang et al., 2001]. APC/C associates with two WD repeat-containing adapter proteins, Cdc20/Fizzy and Cdh1/Hct1/Fizzy-related, which are important for substrate binding [Hansen et al., 2002]. Ubiquitination of targets by APC/C is regulated either by the phosphorylation of the core components as

well as the Cdh1 adapter [Kramer et al., 2000], or by inhibitory proteins that bind to the adapter proteins. These inhibitor proteins, for example, Emi1 [Reimann et al., 2001a,b; Hsu et al., 2002], maintain the inactive state of the ligase during interphase. The human Emi1 inhibits Cdh1 complex, thus stabilizing cyclin A [Hsu et al., 2002], an important kinase for initiating centrosome duplication in somatic cells [Meraldi et al., 1999]. Aur-A is an important target for APC/C [Castro et al., 2002; Littlepage and Ruderman, 2002; Taguchi et al., 2002].

BRCA1/BARD1. BRCA1 is a breast and ovary-specific tumor suppressor that in association with BRCA1-associated RING Domain 1 (BARD1), is a powerful E3 ubiquitin ligase. The paradox of BRCA1 is that its function is required in all cells, but loss of BRCA1 is only associated with breast and ovarian tumors. The E3 ubiquitin ligase activity of BRCA1 targets multiple proteins (reviewed in [Starita and Parvin, 2006]) for either mono-ubiquitination, for example, γ-tubulin [Starita et al., 2004], or poly-ubiquitination, for example RNA polymerase II [Starita et al., 2005] and Npm1 [Sato et al., 2004]. We [Sankaran et al., 2005] and others [Hsu and White, 1998] have shown BRCA1 to localize to centrosomes.

BRCA1 inhibition, in tissue culture cells, causes centrosome amplification, and this phenotype was specific to mammary cell lines [Starita et al., 2004]. BRCA1 inhibition in cells also stimulated centrosome MT nucleation function [Sankaran et al., 2005], suggesting that BRCA1 inhibits centrosome MT nucleation. Expression in cells of a mutant BRCA1, that was inert for ubiquitin ligase activity, resulted in a dominant-negative phenotype of hyperactive and supernumerary centrosomes, proving that the centrosome is a critical target for this enzymatic activity of BRCA1 [Sankaran et al., 2006].

REGULATION OF CENTROSOME NUMBER AND FUNCTION BY BRCA1-MEDIATED UBIQUITINATION

As described earlier, centrosomes start duplicating at the G1/S transition of the cell cycle. The centrosome duplication cycle must be coordinated with the DNA replication so that the chromosomes will be divided appropriately between the daughter cells. What controls whether a centrosome duplicates? Is it a cytoplasmic factor or intrinsic to the centrosome? Cell fusion experiments reveal that a centrosome from a G2 cell (that has already duplicated) will not re-duplicate in a G1 cytoplasm. Conversely, G1 centrosomes continued to duplicate in G2 cytoplasm [Wong and Stearns, 2003]. This implies that the centrosomes are marked as post-duplicated. We suggest that ubiquitination of centrosome proteins by BRCA1 may provide this covalent tag, indicating that the centrosome should not re-duplicate.

Arresting cells in early S phase, by including hydroxyurea or aphidicolin in the tissue culture medium, arrested centrosome development at two centrosomes per cell. Inhibition of BRCA1 in this setting did not result in re-duplication of centrosomes, indicating that the effect of BRCA1 was downstream of the hydroxyurea S phase block. Release of the S-phase block in these BRCA1-inhibited cells resulted in the re-duplication of centrosomes before the cell could pass through mitosis [Ko et al., 2006]. We model that BRCA1-mediated ubiquitination provides the covalent tags for centrosomal proteins, marking them as already duplicated. The loss of the BRCA1 ubiquitination would thus result in centrosome re-duplication before the cells reach mitosis.

BRCA1 ubiquitinates several centrosomal proteins, in vitro. One of the protein targets identified was γ-tubulin [Starita et al., 2004], an important centrosomal protein that was shown previously to bind BRCA1 [Hsu et al., 2001]. γ-tubulin is monoubiquitinated by BRCA1/BARD1; thus, this ubiquitination is not targeting the protein for degradation but rather altering its activity. It should be noted that this was the first report of a post-translational modification of γ-tubulin. Subsequently, others have observed modifications of γ-tubulin in the mammary glands of estrogen-treated rats [Li et al., 2004], and the phosphorylation of γ-tubulin in the amoeba *N. gruberi* was found to regulate basal body (centriole) duplication [Kim et al., 2005].

Two lysine residues were mapped on human γ-tubulin to be acceptor sites for BRCA1 ubiquitination. Mutation of one of these lysines to arginine (K48R) and expression in cells resulted in the dominant phenotype that was identical to that of BRCA1 inhibition. Expression of the mutant γ-tubulin caused both centrosome amplification and hyperactive MT

nucleation, implicating γ-tubulin as one of the important substrates in regulation of centrosome duplication and function by BRCA1 E3 ligase activity. Interestingly, expression of the other mutant of γ-tubulin (K344R), caused cells to accumulate hyperactive centrosomes without causing centrosome amplification [Sankaran et al., 2005]. Apparently there are specific effects of ubiquitinating lysine-48 versus lysine-344.

Inhibition of BRCA1 in breast-derived cell lines resulted in centrosome amplification, and expression of the substrate with mutated lysine acceptor sites also caused centrosome amplification. Since both manipulations produce the same phenotype, it is suggested that γ-tubulin is a critical substrate for the BRCA1 ubiquitin ligase. Surprisingly, expression of the mutant γ-tubulin in the non-breast cell line, U2OS, also caused centrosome amplification, although inhibition of BRCA1 had no effect in that cell line [Starita et al., 2004]. This result suggests that this pathway exists in non-breast cell types, but another E3 ligase modifies γ-tubulin in order to inhibit the function of the centrosome.

MODEL FOR REGULATION OF CENTROSOME FUNCTION AND DUPLICATION BY BRCA1-MEDIATED UBIQUITINATION

BRCA1 ubiquitination activity causes γ-tubulin to dissociate from the centrosome [Sankaran et al., 2005]. γ-tubulin content of centrosomes is regulated, and the influx of γ-tubulin to the centrosome associated with G2 phase of the cell cycle is associated with increased MT nucleation activity of the centrosome (Fig. 1A). This increased activity is probably due to the high γ-TuRC concentration in the centrosome since γ-TuRC functions as an initiation factor for MT formation (Fig. 1B). Inhibition of BRCA1 in cells resulted in an increase in γ-tubulin content of the centrosomes but not pericentrin or centrin. Conversely, in vitro ubiquitination of centrosomes by BRCA1 resulted in centrosomes with decreased γ-tubulin content and diminished MT nucleation activity [Sankaran et al., 2005]. Similar to this observation, centrosomes from breast tumors, which generally have lost BRCA1 function by either mutation or low expression [Wilson et al., 1999], have high γ-tubulin content, are hyperactive in nucleating MTs, and have an increase in number [Lingle et al., 2002].

How are the three centrosome phenotypes of BRCA1 inhibition linked? Does high γ-tubulin content make the centrosomes hyperactive? Is hyperactive MT nucleation causing the increase in centrosome number? Early breast tumor lesions have both hyperactive and high number of centrosomes [Lingle et al., 2002; Pihan et al., 2003]. Results showing that post-translational modification of γ-tubulin regulates both centrosomal function and number [Kim et al., 2005; Sankaran et al., 2005] suggest a possible link. A protein that controls γ-TuRC recruitment to the centrosome called NEDD1 (or GCP-WD) also controls both centrosome activity and number [Haren et al., 2006]. Haren et al., suggest that γ-tubulin is localized to the centriole as well as the PCM, and γ-TuRC in the centriole catalyzes the synthesis of the triplet MTs that comprise the bulk of the protein in the centriole. Thus, excessive γ-TuRC in the centrosome causes the high MT nucleation activity (Fig. 1B), which in turn causes high centriole synthesis and amplification of centrosome number.

We model that in early breast cancer, BRCA1 is lost by either mutation or epigenetic down-regulation, resulting in diminished ubiquitination of γ-tubulin. The ubiquitination of γ-tubulin causes γ-tubulin content of centrosomes to drop, thus decreasing the activity of the centrosome. It is probable that the high kinase activity in G2-M renders the centrosome refractory to the ubiquitination by BRCA1. The γ-tubulin content of the centrosome climbs in G2, making the centrosome highly active in nucleating the mitotic spindle. Thus, loss of BRCA1 function in breast cancer cells results in the inappropriate accumulation of γ-tubulin in S phase centrosomes, making these centrosomes hyperactive and more likely to re-duplicate. These effects are likely to result in genomic instability.

CONCLUSIONS

The centrosome is a dynamic organelle with various proteins associating, dissociating, and being modified very specifically through different phases of the cell cycle. Under disease conditions, these modifiers are either absent, decreased, or are present in excess shifting the delicate balance from the normally functioning and duplicating centrosome to one that loses its restraints preventing overduplication and hyperactivity. In this review, we focused on the example of the BRCA1 E3 ubiquitin ligase and

developed a model whereby loss of a critical ubiquitination event results in centrosome amplification, which we believe drives chromosomal instability.

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