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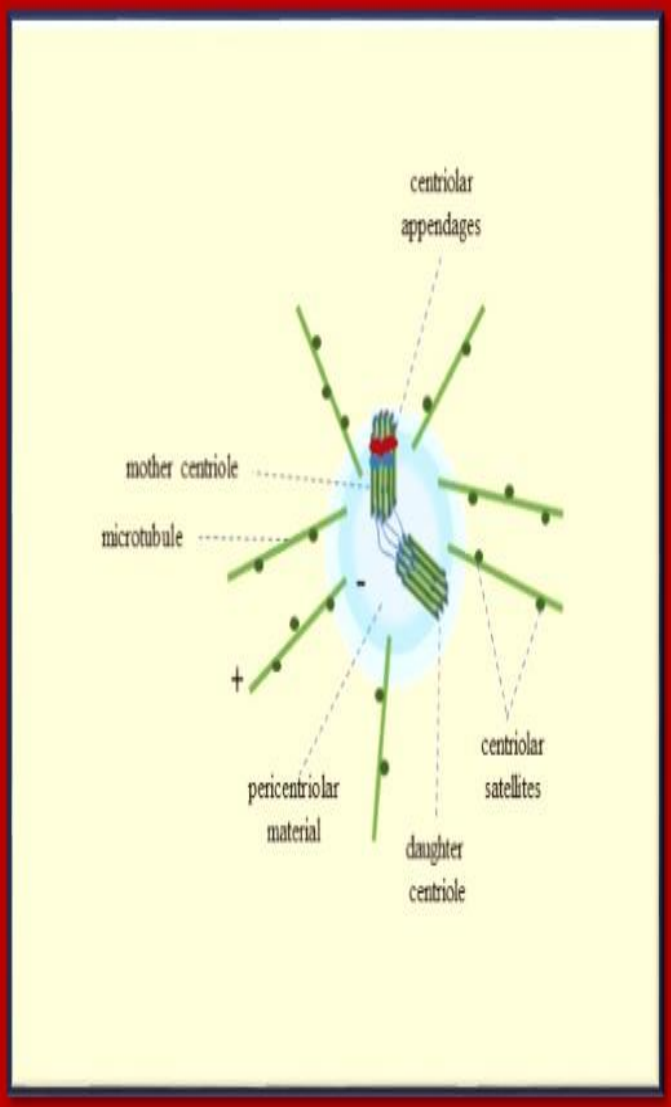
Insights into Centrosomes and The Implications of Their Aberration in Human Diseases



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Insights into Centrosomes and The Implications of Their Aberration in Human Diseases

Atef Sobhy Iskander

Centrosome is a complex organelle that serves as a microtubule organizing centre, and is involved in several cellular process including mitotic spindle assembly, cell cycle progression, neurogenesis, cell polarity and migration. Thus, centrosomal abnormalities lead to a variety of diseases and disorders ranging from neurodegenerative diseases, developmental disorder to cancer. The objective of this study is to identify new hypothetical therapeutic strategies, which can be considered as a platform for exploring new ideas to overcome centrosome instability and its implication in human diseases. To realize such a goal, a brief general survey is introduced to gain a better understanding of how the roles of centrosome play in cell cycle and checkpoint signaling; how the structure and function of centrosomal-associated proteins orchestrate cell division and what are the consequences of their overexpression that impact centrosome stability, which can lead to centrosome amplification and tumor malignancy. Finally, some new proposed therapeutic interventions are introduced, which may be useful to screen novel strategies to overcome the consequences of centrosome instability.

Keywords: Centrosome, Cell cycle, Checkpoint proteins, Centrosome-associated proteins, p53 signaling, Integrin adhesion, Nucleophosmin, p38 MAPK, Cancer.

1. Introduction

The centrosome is a multifunctional organelle, that plays a pivotal role in cell cycle regulation, coordination of signal transduction molecules, and cellular metabolism. Centrosome dysfunctions have been implicated in a wide range of diseases and disorders ranging from neurodegenerative diseases, developmental disorder to cancer. The centrosome is a non-membrane bound organelle that serves as the principal microtubule organizer in animal cells. In eukaryotic cells, it consists of a 9-fold symmetric centriole pair, a mother and a daughter centriole, made of microtubule-based cylinders in an orthogonal configuration, which are surrounded by an electron-dense matrix of proteins, the pericentriolar matrix (PCM) (Figure 1). The centriole determines most properties of the centrosome, such as stability, capacity to reproduce, dynamicity and polarity. The PCM harbors molecules that anchor and nucleate cytoplasmic microtubules in interphase and mitosis.[1,2] The mother centriole functions as the basal body that assembles the primary cilium, which, among other roles, can function as a centre for cellular signaling.

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The centrosome is participated in many cellular processes including mitotic spindle assembly, cell cycle progression, neurogenesis, cell polarity and migration.[3] Moreover, this complex organelle operates beyond its canonical role as a microtubule organizing centre, that a multitude of centrosomal interactions with diverse biological functions have been revealed including metabolism, protein synthesis, autophagy and inflammation.[4,5] Thus, centrosomal abnormalities can lead to a wide range of human diseases and commonly found in cancer.[6-8] For instance, the chromosomal instability may alter the chromosomal content of a cell (aneuploidy).[9-11] The tumor cells experience two main forms of aneuploidy, numerical and segmental. The first, is caused by errors in chromosome partitioning during mitosis, while the second is defined by copy number variations of sub-chromosomal regions.[9,12,13] In fact, centrosome amplification itself, can promote invasive phenotypes,[14] induced by high oxidative stress levels downstream of supernumerary centrosomes within the cells, which mediates secretion of invasive factors, such as interleukin-8 (IL-8) that alters the cellular microenvironment *via* extracellular vesicles promoting cell invation.[15,16] In addition to these phenotypes, there is tumor genetic diversity, a process known as intra-tumor heterogeneity (ITH) that can be observed at the genetic, proteomic, morphological, and environmental levels.[9,17] Furthermore, mutations on several centrosome components in animal models have been recognized as tumor drivers due to impairing centrosome structure.[18,19] Such centrosome abnormalities are caused by defects in centrosome structure, *i.e.* alterations in centriole length; defects in the amount of PCM or numerical aberrations.

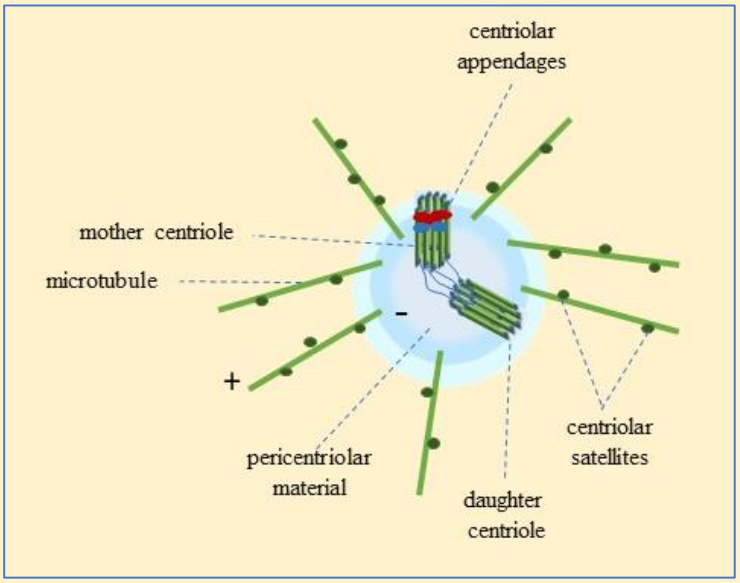

Figure 1. Schematic representation of the mammalian centrosome. The structure constituent of centrosome is the centriole. The centrosome is composed of a pair of orthogonally localized centrioles, a mother and daughter centriole, surrounded by a dense protein matrix, the pericentriolar material that acts as the primary microtubule organizing center. The mother centriole shows subdistal appendages, where the microtubules are docked, and distal appendages, which are crucial for docking to the plasma membrane. Satellites are granular structures surrounding the centrosome that are implicated in trafficking of material involved in centriole assembly.

As a consequence of centrosome abnormalities, lagging chromosomes could occur during mitosis, which could be a source of DNA damage through encapsulation into micronuclei. Such DNA damage in micronuclei is associated, at least in part, with their fragile nuclear envelopes, which can undergo abrupt loss of integrity, leading to mitotic errors and mutations.[20,21,22]
The objective of this study is to provide novel hypothetical therapeutic strategies, which can be considered as a platform for exploring new ideas to overcome centrosome instability and its implication in human diseases. To realize such a goal, a brief general survey is discussed to get a better understanding of how the roles of centrosome play in cell cycle and checkpoint signaling; how the structure and function of centrosomal-associated proteins orchestrate cell division and what are the consequences of their overexpression that affect centrosome stability, which can lead to centrosome amplification and tumor malignancy. Finally, some new proposed therapeutic interventions are introduced, which may be useful to screen novel strategies to overcome the consequences of centrosome instability.

2. The role of centrosome in cell cycle transitions

The biogenesis of centrosome is closely linked to cell cycle, which is in part controlled by tight regulation of many of its protein components that have been conserved during evolution (Figure 2).[20] In the cell-cycle transitions, a series of events takes place in the cell resulting in replication all of its material and the division into two daughter cells. These events include four steps: Gap phase-1 (G1) [the cell prepares for the DNA synthesis], DNA synthesis (S), Gap phase-2 (G2) [the cell prepares for mitosis], and mitosis (M). Next, new cells enter the quiescent G_o stage. During the cell cycle progression, centrosomes duplicate only once during S phase to ensure that at mitotic onset a cell carries two centrosomes that anchor the poles of the mitotic spindle.

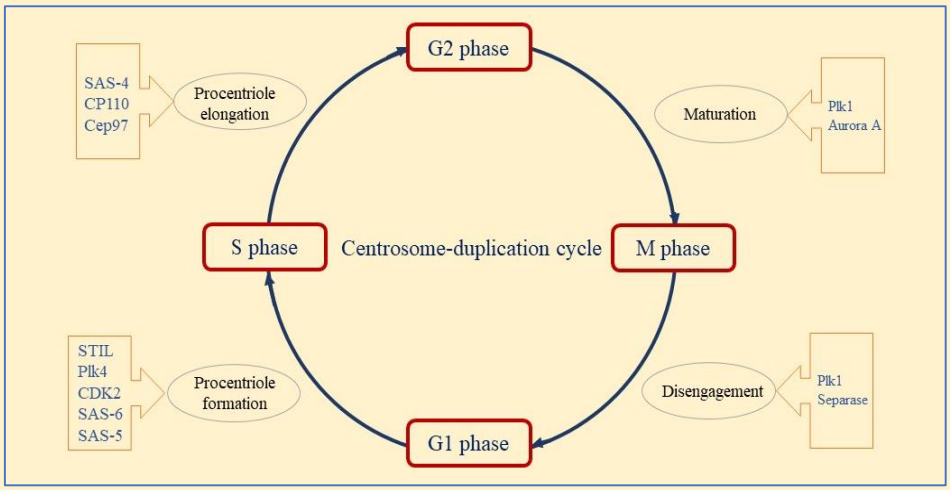

Figure 2. The centrosome duplication cycle (G1 ⟶ S ⟶ G2 ⟶ M) and the main proteins involved in the regulation and maintenance of cell-cycle transitions eukaryotic cells. The principal protein regulator of centrosome duplication is Plk4. Another essential core protein is SAS-6. After initial formation, centrioles are stabilized and elongated until G2 phase, in which CPAP/SAS-4 is involved and centriole length is controlled by CP110 and Cep97. After centriole duplication, the maturation of centrosome takes

place during G2/M phases, in which the process dependents upon Plk1 and Aurora A kinases. At the end of M phase, the disengagement process occurs through the activities of Plk1 and separase.[20] G_1 and G_2 are gaps (cell growth), S is the period of nuclear DNA synthesis, and M is the period of mitosis.

Cell cycle transitions are driven by the activation of the cyclin-dependent kinases (Cdks). In the G1 phase, entry into S-phase depends on the activation of Cdk4/6 complexes by mitogens, initiating a signaling cascade that leads to the inactivation of the Retinoblastoma (Rb) protein and the activation of Cdk2 complexes (Figure 3 **A**). Cdk2-Cyclin E is associated with the centrosome during interphase, whereas the domain centrosome localization signal (CLS) is responsible for targeting Cyclin E to the centrosome to allow S-phase entry.[18]
In the G2 phase, the activation of Cdk1-Cyclin B and its translocation from the cytoplasm into the nucleus drives entry into mitosis. The presence of the cell-cycle regulator Cdk1 is required for the activation of Cdk1-Cyclin B and mitotic entry. Cdk1-Cyclin B activity is restrained in the interphase through inhibitory phosphorylation in the active site of Cdk1 by Wee1 kinases (Figure 3 **B**). These phosphates are removed by Cdc25 phosphatase to drive cells into division.[18]
The activation of Cdk1-Cyclin B is facilitated by mitotic kinases, Aurora A and Polo-like kinase 1 (Plk1), which the activation of these two kinases plays a crucial role in promoting timely mitotic entry. The recruitment of Aurora A in G2 to the centrosome is mediated by the PCM component Cep192, leading to its self-activation, which in turn activates Plk1. Subsequently, Plk1 simultaneously activates Cdc25 (Cell division cycle 25) and inhibits Wee1, triggering the transition into mitosis.[18]

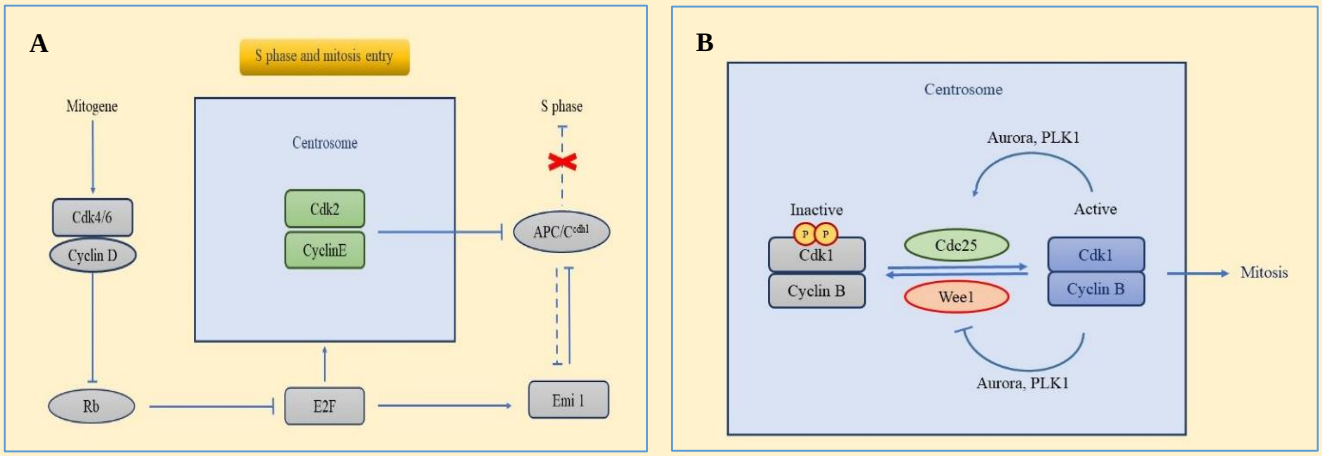

Figure 3. Regulation of cell cycle progression from the centrosome.[18]

3. Regulation of centrosome duplication cycle

Abnormalities in centriole duplication can result in the production of extra copies of centrosomes (centrosome amplification), which can lead to errors in mitotic spindle formation and chromosome missegregation. Thus,

accurate control of centrosome number is essential for the maintenance of genomic integrity. In normal cells, many of positive and negative protein regulators prevent centrosome amplification. Among these proteins is the Polo-like kinase 4 (Plk4), a conserved upstream regulator of centriole assembly, which is considered as the cardinal regulator of centriole duplication. Another core protein is SAS-6, a structural component of the centrosome, which is essential for the formation of the cartwheel structure that ensures the centriole's nine-fold symmetry.[20] Thus, it is important to highlight those two proteins in brief.

3.1. Polo-like kinase 4 (Plk4)

In a general sense, Polo-like kinases (Plks) are substantial regulators of the cell cycle, centriole duplication, mitosis and cytokinesis. They are transiently associated with many mitotic structures such as centrosomes and spindle poles, kinetochores and the central spindle. There are five members of polo-like kinase family: Plk1, Plk2, Plk3, Plk4 and Plk5. They have a similar structure, expect for Plk5, with a canonical serine/threonine kinase domain at the amino terminus (KD), and a carboxy-terminal regulatory domain that contains signature motifs, known as polo boxes (PBD) (Figure 4). The polo-box domain (PBD) binds to phosphopeptides and is required for Plk localization and activation.[23] The KD determines the kinase activity, and PBD is crucial to binding substrates and regulates their kinase activity. The architecture of a PB domain consists of an anti-parallel six-stranded β sheet that lies across a C-terminal helix. The Plks are activated by direct phosphorylation within the kinase domain by upstream kinases and *via* the binding of the PBD to phosphorylated docking proteins. Plk4 is a major regulator of centriole number, as any aberration of Plk4 activity leads to a variation of centriole numbers, and hence to chromosomal mis-segregation and cytokinesis failure, which is closely related to tumorigenesis and progression.[20,24] Plk4 contains only a single carboxy-terminal PB, which allows homodimerization and moderate centriole localization by binding an unidentified target. Plk4 also contains a conserved central domain, known as ''cryptic polo box'' (CPB), which bridges the kinase domain and the carboxy-terminal PB. Thus, Plk4 has a triple polo box architecture (PB1, PB2 and PB3) that facilitates oligomerization, targeting, and promotes trans-autophosphorylation, limiting centriole duplication to once per cell cycle.[25] The expression level of Plk4 increases at the G1/S phase, remains at the later M phase, and finally decreases at the early G1 phase. In prophase, Plk4 is positioned in the centrosomes; when the cell cycle progresses to the anaphase, Plk4 is localized to the whole cell, and then to the cleavage furrow at the telophase.[24]

The Plk4 transcription could be activated or repressed by a number of transcription factors through modulating Plk4 promoter activity. Evidences revealed that the E2F transcription factor, a key regulator of cell cycle progression, could increase Plk4 promoter activity, leading to Plk4 overexpression and centrosome amplification, which ultimately results in genomic instability and tumorigenesis.[24,26] Furthermore, nuclear factor kappa B (NF-κB) is also served as transcriptional activator of the Plk4.[24] It was also demonstrated that the ATPase family AAA domain-containing protein (ATAD2) activated the transcription of Plk4 and their overexpression resulted in Plk4 upregulation.[24] On the other hand, the Krüppel-like factor 14 (KLF14) gene is transcriptionally repressed Plk4 and played a role in preventing centriole duplication in human cancer cells. In addition, p53 was involved in the transcriptional repression of Plk4 *via* various pathways.[24]

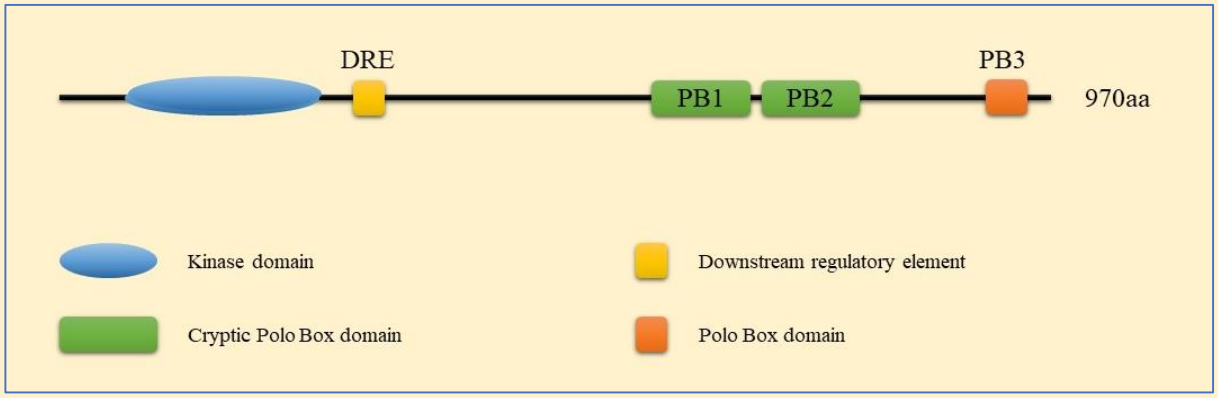

Figure 4. Schematic representation of Plk4 domain structure.

The levels of Plk4 are regulated by SCFβ^{TrCP} (ubiquitin-dependent proteolysis) that recognizes Plk4 after homodimer-dependent *trans*-autophosphorylation of the phosphodegron known as the downstream regulatory element (DRE).[27,28] The Plk4 phosphorylates itself to promote its own destruction through the ubiquitin–proteasome pathway. The SCF (Skp/Cullin/F-box) E3 ligase associates with phosphorylated Plk4 through the F-box protein β-TrCP (known as Slimb in *Drosophila*). Phosphorylation of two residues within the β-TrCP-binding motif of Plk4 promotes the binding of β-TrCP and subsequent ubiquitylation and destruction of the kinase. In *Drosophila*, the phosphatase PP2A in complex with its targeting subunit, Twins (known as B/PR55 in humans), counteracts Plk4-mediated autophosphorylation to stabilize Plk4 during mitosis.[29] Evidence has shown that self-catalyzed destruction of Plk4 plays a crucial role in both controlling endogenous protein levels, and in restricting centriole duplication to allow the production of a single new centrosome per cell cycle.[29] Therefore, autoregulated control of endogenous Plk4 levels is essential for long-term cell growth. In addition, increased Plk4 levels resulting from loss of Plk4 autoregulation causing a p53-dependent cell cycle arrest.[29] In other word, Plk4 autoregulation protects against genome instability by limiting centrosome duplication to once per cell cycle.

3.2. Centriolar protein SAS-6

The centriolar protein SAS-6 is a conserved structural component, which forms rod-shaped homodimers that interact through their N-terminal domains to form oligomers. Such oligomerization is essential for centriole formation in human cells. Their C-terminal contains KEN box that required to target the protein for destruction at the end of mitosis. The Plk4 activity is upstream of the SAS proteins and primes the mother centriole for S-phase duplication. Daughter centriole assembly begins at the proximal end of the mother centriole with the formation of a 9-fold symmetric cartwheel structure composed of SAS-6 homodimers.[29]
The overexpression of SAS-6 proteins with a mutated KEN box induces the formation of multiple new centrioles adjacent to the existing one in human cells, resulting in decreased cell proliferation through the stabilization of p53 and the induction of p21, and ultimately promoting centrosome amplification.[6,30]

4. The correlation between the centrosome and checkpoint signaling

Cell cycle checkpoints are essential for maintaining genomic integrity in proliferation cells. In response to DNA damage, the cell must detect the sites of this damage and either transiently block cell cycle progression, or exit the cell cycle. The response of this cell depends on its exact cell-cycle position and that checkpoints are phase-dependent, stringent or relaxed, and graded or all-or-none.[31] Centrosome is linked to the checkpoint signaling through regulating vital cell cycle proteins, anchoring the DNA damage checkpoint proteins and activating p53 signaling *via* the PIDDosome (Figure 5). In response to cellular stress (centrosome amplification and DNA damage), the centrosome stimulates the checkpoint responses, whether directly or indirectly inhibiting the Cdk-Cyclin complexes and arresting the cell cycle. In the next section, a discussion is introduced to understand such a role of centrosome in checkpoint signaling.

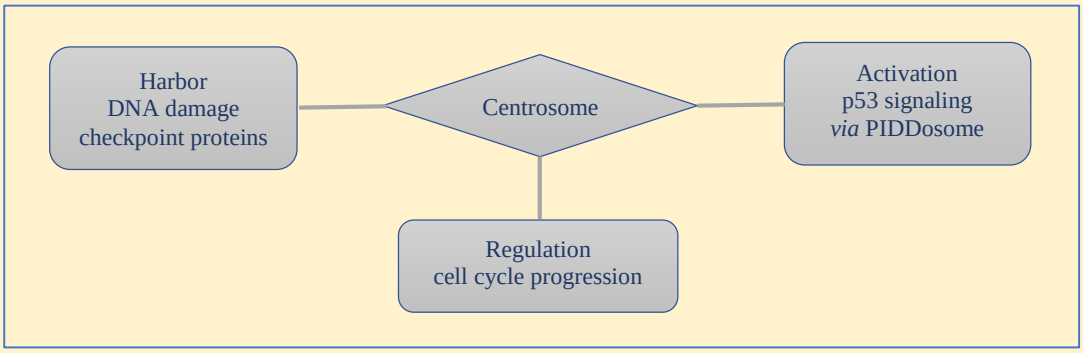

Figure 5. Schematic diagram of participation of centrosome in checkpoint signaling.

4.1. Checkpoint signaling

DNA damage response (DDR) is a complex interconnected signaling network that is essential to defend human genome integrity against a variety of exogenous and endogenous genotoxic insults. In G2, both the cell cycle checkpoint proteins ataxia-telangiectasia-mutated (ATM) and ataxia-telangiectasia-and-Rad3-related (ATR) signaling pathways are activated upon exposure to DNA damage (Figure 6). Consequently, their major downstream effectors checkpoint kinases Chk2 and Chk1, respectively, play a pivotal role in preventing the entry of cells with damaged or incompletely replicated DNA into mitosis through the inhibition of Cdk1-Cyclin B.[18] Checkpoint kinase 1 (Chk1) is a serine/threonine-specific protein kinase and its structure consists of a N-terminal kinase domain, a linker region, a regulatory serine-glutamine/ threonine-glutamine (SQ/TQ) domain and a C-terminal domain.[31] Chk1 has four SQ residues. Its activation takes places mostly through the phosphorylation of the conserved sites, Ser-317, Ser-345 and less often at Ser-366.[32,33] Whereas checkpoint kinase 2 (Chk2) is a serine-threonine kinase contained 543 amino acids that consists of an N-terminal SQ/TQ cluster domain (SCD), a central forkhead-associated (FHA) domain, and C-terminal serine/threonine kinase (KD).[34] The SCD domain contains multiple SQ/TQ motifs that serves as ATM phosphorylation sites, with Thr

68 being the most active phosphorylated site in response to DNA Damage.[34] Chk2 is a monomer existed in inactive state. However, in the event of DNA damage, SCD phosphorylation causes Chk2 dimerization. The phosphorylated Thr-68, located on the SCD segment of one Chk2 molecule, interacts with the FHA domain of a second molecule to form the dimer, followed by the KD activation through autophosphorylation. Once the KD activated the Chk2 dimer dissociates.[34]

The centrosome is linked to the DNA damage checkpoint response as mutations on several PCM components, including PCNT, MAPH1 and DK5RAP2. These PCM components have been shown to impair checkpoint mediated cell cycle arrest.[18] Cell defective in these centrosomal components are unable to respond to ATR signaling, failing to arrest its cell cycle at the G2/M boundary upon exposure to DNA damage.[18] This event is related to the failure to recruit Chk1 to centrosomes. Where the recruitment of checkpoint protein Chk1, a key component of DNA-damage-activated checkpoint signaling response, to centrosome is vital to protect centrosomal Cdk1 from unscheduled activation by cytoplasmic Cdc25B, and is needed to regulate the activation of Cdk1-Cyclin B at the centrosome.[35]

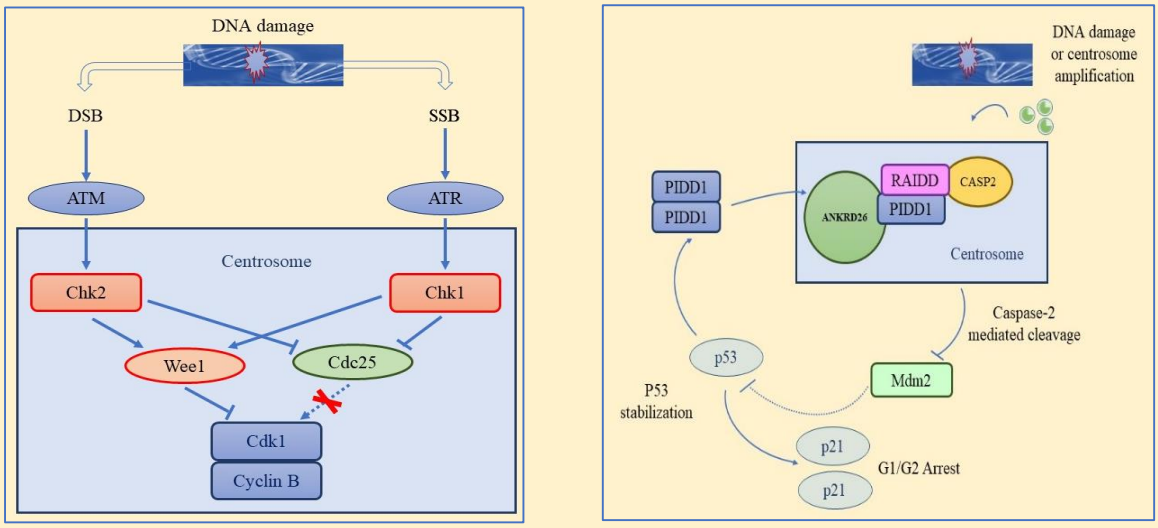

Figure 6. The centrosome is linked to the checkpoint signaling by anchoring the DNA damage checkpoint proteins and the PIDDosome. In the presence of cellular stress such as centrosome amplification and DNA damage, the centrosome promotes the checkpoint responses, which directly or indirectly inhibit the Cdk-Cyclin complexes and arrest the cell cycle.[18]

4.2. Activation of p53 signaling

The p53 protein is a transcription factor regarded as the “guardian of the genome” due to its vital function in preserving genomic integrity. The mutation of p53 protein leads to the unrestricted cell cycle and replication of the damaged DNA, resulting in uncontrolled cell proliferation and cancer. Thus, p53 deficiency modulates the ability of cancer cells to proliferate, escape apoptosis, invade and metastasize.

The activation of the DNA-damage response pathway triggers p53 (tumor suppressor protein) signaling. The canonical pathway that regulates p53 signaling is through Mdm2, a p53-specific E3 ubiquitin ligase. In the

presence of DNA damage and other cellular stress, Mdm2 is cleaved, leading to the accumulation of p53 and the promotion of cell cycle arrest or cellular death.[18] The centrosome can engage in the activation of p53 signaling through the PIDDosome, which is anchored to the centriolar distal appendages (Figure 6). The PIDDosome is a protein complex, that includes p53-induced death domain protein 1 (PIDD1), Caspase-2 and RAIDD, serving as an inducer of apoptosis. Such linkage of PIDDosome to p53 is to arrest the proliferation of cells bearing more than one mature centrosome to maintain genome integrity.[36] In response to centrosome amplification generated by disrupting cytokinesis or forcing centrosome overproduction, vital events are triggered through the activation of the PIDDosome multi-protein complex as a protective response to inhibit cell proliferation, leading to Caspase-2-mediated Mdm2 cleavage, p53 stabilization, and the induction of p21-dependent cell cycle arrest.[36] In cells with extra centrosomes, the centriole distal appendages is crucial for PIDDosome activation. The distal appendage protein ANKRD26 interacts with and recruits the PIDDosome component PIDD1 to centriole distal appendages, which such interaction is needed for PIDDosome activation in response to centrosome amplification.[37] Moreover, DNA damage-induced cleavage of Mdm2 seems to require ANKRD26, suggesting that centrosome may be contributed in other PIDDosome dependent p53 stabilization response.[18]

4.3. Centriole depletion

Abnormalities in centriole duplication can result in the production of extra copies of centrosomes, which can lead to errors in mitotic spindle formation and chromosome missegregation. Evidence has shown that the loss of centrioles impaired regular centrosome assembly leading to the accumulation of cells that lack a detectable Cep192 or γ-tubulin foci in interphase cells.[18,38] These results, coupled with other observations, have revealed that cell cycle progression is not inherently linked to the presence of an intact centrosome.[18] As a result of the loss of centrioles, endogenously tagged Cep192 will form a discrete foci to facilitate bipolar spindle assembly, as the cell progression from G2 to early prophase.[18] This reveals that in the absence of centrioles, some of the PCM components are dispersed within the cell in interphase, leading to the relocalization of several PCM components (AKAP9, CDK5Rap2 and PCNT) to the Golgi Apparatus (GA).[18,39] Such relocalization results in the association of γ-tubulin to the GA, enhancing the microtubule nucleating capacity of the Golgi.[39] Taken together, the loss of centrioles results in the relocalization of centrosomal proteins to other cellular compartments, transferring the microtubule organizing function of the centrosome to the compartments they associated with.[18] Such disruption could lead to the proximity of the cell cycle regulatory proteins being impaired. Consequentially, this would result in the failure of the checkpoint signaling within the cell to arrest cell cycle progression, as they are unable to efficiently influence the activation of Cdks.[18] Disruption of such localization by centriole depletion blocks PIDDsome, which is vital for the triggering of Caspase-2 mediated stabilization of p53.[18]

It is worth noting that centriole length is controlled by proteins such as CP110 and Cep97, which function as capping proteins. CP110 levels are also regulated by $SCF^{cyclinF}$/ubiquitin-dependent proteolysis, which is counteracted by deubiquitinating enzyme USP33.[6] Overexpression of USP33 leads to increased CP110 levels and centrosome amplification, which may arise from centriole overduplication as the case in some tumers.[6] Overexpression of PCM components such as pericentrin can also induce centrosome amplification. Moreover, increasing the levels of the PCM component γ-tubulin through the loss of the tumor suppressor BRAC1 leads

to centrosome amplification.[6] Cells have different mechanisms to restrict the detrimental consequences of a multipolar mitosis including: i) centrosome inactivation *via* a decreasing of PCM levels; ii) centrosome elimination during fertilization; and iii) centrosome clustering through allowing cells to undergo a pseudo-bipolar mitosis. Several microtubule-associated proteins (MAPs) play a crucial role in centrosome clustering, such as minus-end-directed motor HSET/KIFC1. Normally, inhibition of spindle assembly checkpoint (SAC) leads to decreased time in mitosis, that causes defects in centrosome clustering, promoting multipolar mitosis.

5. The convergence between centrosome and other centrosome-associated proteins

The biological functions of centrosomes are mainly dependent on the structure and a series of centrosome-associated proteins to maintain their normal functions. For example, cyclins family proteins are located in the centrosome and mediated the normal functions of centrosome, including mitosis, and directed cell motility. This section focuses on the key centrosome-associated proteins.

5.1. The Cyclin-Dependent Kinases (CDKs)

The kinases are activated by binding to regulatory proteins, commonly cyclins, but also kinases or phosphatases for regulation of various processes. Cyclins form a complex with kinases to activate them in the presence of phosphorylated proteins. Cyclins have no enzymatic activity, but have binding sites for some substrates targeting the kinases to specific subcellular locations. Chromosomal instability arises often from mitotic errors in the cell cycle, due to defects in the checkpoint loyalty. Cyclin-dependent kinases (CDKs) are the principal regulators of mitosis. They assign the mitotic proteins to other downstream transducers that influence various components of mitotic chromosomes, the spindle apparatus and the cytoskeleton.[40] CDKs are members of the serine/threonine kinase family existed in all eukaryotic cells. CDKs play an essential role in regulating cell cycle, transcription, differentiation of nerve cells, apoptosis, mRNA processing, DNA repair, and organizing cytoskeleton dynamics.[41] The human genome encodes twenty members of CDK family and are divided into two main subfamilies based on their regulation of different cellular processes: cell cycle CDKs (CDK1-6, -11, -14-18) and transcriptional CDKs (CDK7-13, -19, -20).[42] The cell-cycle CDKs regulate cell-cycle transitions and cell division. Whereas the key function of transcriptional CDKs is to allow cells to copy their genomic DNA into messenger RNA (mRNA), which subsequently is translated into proteins. This process is catalyzed by RNA polymerases: I, II, and III (RNAP-I, RNAP-II, RNAP-III). It is worth noting that the CDK7 and CDK8, can have dual functions, participating in both transcriptional processes and cell cycle regulation. They are integrated into large functional complexes, which require the participation of additional subunits for full activation. The conversion of an inactive kinase protein into an active state was studied in the light of the activation mechanism of CDK2 along with its complex with the cyclin partner CycA. The CDK protein structure consists of a β-sheet-rich N-terminal lobe and a compact C-terminal lobe, which encloses a catalytic cleft responsible for ATP binding and substrate recognition. The T-loop monomeric (the activation segment of CDK) adopts an inactive conformation, which obstructs the catalytic cleft and renders substrates inaccessible. To activate the kinase function of CDK, the cyclin forms a dimer complex with associated CDK, by interacting with the catalytic cleft of CDK, inducing conformational changes in its αC-helix (the PSTAIRE helix) and T-loop. This event alleviates the steric blockade of the catalytic cleft, which partially activates the kinase function of CDK as well as adjusting the positions of the active site residues. The αC-helix is shifted closer to the ATP-

binding site, which, subsequently, directs the inactive T-loop away from the entrance of the catalytic cleft, resulting in the phosphorylation of Thr160, a highly conserved threonine residue on the T-loop throughout the entire CDK family.[43-45] This conformational change is selective for the cyclin partner. The phosphate group of Thr160 acts as a re-organization center, reshaping the T-loop through interactions with three conserved arginine sidechains within the CDK. In addition, a conserved tyrosine residue Y180 in Y-loop, a short loop between two conserved helices adjacent to the T-loop, undergoes repositioning to participate in the interaction network mediated by phosphorylated Thr160. All these conformational changes upon cyclin binding and T-loop phosphorylation result in the correct disposition of substrate binding and relief of steric blocking to allow access of substrates to the catalytic site and, hence, fully active CDK. Some cyclin-dependent kinases, that are involved in cell cycle, would be further briefly discussed.

5.1.1. Cyclin-Dependent Kinases 1 (CDK1)

Cyclin-dependent kinase 1 (CDK1) binds with cyclin B1 to allow the transition from G2 phase into mitosis. This enzyme is controlled by Wee1-like protein kinase (Wee1) and checkpoint kinase 1 (CHK1), to ensure appropriate regulation.[46] The activity of cyclin B1–CDK1 increases gradually once cells enter prophase, and active cyclin B1–CDK1 translocates to the nucleus, triggering several mitotic events such as cell rounding, nuclear envelope breakdown, chromosome condensation, and spindle formation.

5.1.2. Cyclin-Dependent Kinases 2 (CDK2)

Cyclin-dependent kinase 2 (CDK2) is a key cell cycle component that regulates the G1/S and S/G2 transitions. CDK2 regulates the phosphorylation of several transcription factors, which cooperate to drive the cell cycle through different transition phases. Cyclin E binds CDK2 complex mostly controls cell cycle progression and DNA replication through phosphorylation of specific substrates. Hyperactivation of Cyclin E/CDK2 induces genomic instability in human cancers, characterized by the increased frequency of chromosomal gains and/or losses and rearrangements. Oncogenic activation of Cyclin E/CDK2 complex has been shown to impair DNA replication, causing replication stress and DNA damage through several different mechanisms.[47]

5.1.3. Cyclin-Dependent Kinases 4 and 6 (CDK4/6)

The cyclinD-CDK4/6 dimer activity is crucial for cell cycle progression from the G1 to the S phase of the cell cycle. Activated CDK4/6 complexes are responsible for the phosphorylation of retinoblastoma gene product (Rb) through functionally inactivating it. Phosphorylation of Rb allows dissociation of the transcription factor E2F from the Rb/E2F complexes, thus facilitating the subsequent transcription of E2F target genes, such as those for the E-type cyclins (cyclins E1 and E2).[46]

5.1.4. Cyclin-Dependent Kinase 5 (CDK5)

CDK5 plays a vital role in the central nervous system, where it activates several functions, by binding to p35 and p39 neuronal proteins, as well as their proteolytic cleavage products, p25 and p29, respectively (central nervous system cells). In contrast to other CDKs, CDK5 is not activated upon binding with a cyclin and does not require T-loop phosphorylation for activation. CDK5 is responsible for neuronal migration, synaptic

plasticity, neurite growth, and maintaining the entire neurogenesis process in adult life.[46] Furthermore, it plays a key role in cell division, cell differentiation, gene expression, angiogenesis. CDK5 activity also inhibits secretion of insulin from pancreatic β-cells in response to an increase of the plasma glucose concentration. Evidence identifies that the Rb is considered as a vital CDK5 downstream target. It has been shown that CDK5 could regulate the activation state of the tumor suppressor Rb, implicating CDK5 in the regulation of cell cycle progression.[46]

5.1.5. Cyclin-Dependent Kinase 7 (CDK7)

In the cell cycle, the activation of CDK7 leads to the phosphorylation of CDK2/cyclin E complex, allowing the cell to transfer from G_1 state into S phase. Thereafter, it activates CDK1/ cyclin B complex, allowing mitotic entry. It plays multiple key roles in cell-cycle control, the DNA repair process and gene transcription.[46,48] Although the ATP-binding site of CDK7 is accessible, its substrate-binding site remains hindered by the inactive T-loop, leading to the lack of kinase activity. Thus, the binding of CDK7 with its cyclin partner CycH requires an additional subunit, the RING finger protein MAT1 (ménage-a-trois 1), to form an active ternary CDK-activating kinase (CAK) complex. The CAK plays a crucial role in activating and phosphorylating cell cycle-related CDKs. As a dual functional kinase, the CAK also forms part of the general transcription factor TFIIH, a 10- subunit complex composed of a large Core and a CDK7/CycH pair connected through the MAT1. CDK7 requires both the binding of CycH and the C-terminus of MAT1 for kinase activation, in order to phosphorylate Ser5 and Ser7 of the RNAPII CTD during transcription process.
In the human CAK structure, the αC-helix of the CDK7 is shifted toward the catalytic site, and the T-loop adopts an active conformation. The tip of the activated T-loop is in extensive contacts with a C-terminal α-helix from MAT1. This helix extends along the groove between the C-lobe of CDK7 and the C-CBF of CycH.
The CDK7 harbors an additional phosphorylation residue S164, located within its T-loop. The phosphorylated S164 is in proximity to a positively charged pocket formed by three arginine residues from each subunit of the CAK complex. This interaction network contributes to the assembly of the complex and potentially stabilizes the T-loop of human CDK7.[45] Taken together, the CDK7 activation, along with CycH binding and T-loop phosphorylation, MAT1 establishes extensive contacts with both T- and Y-loops, resulting in a stabilized T-loop that releases the hindrance in the catalytic cleft (Figure 7).[45]

5.1.6. Cyclin-Dependent Kinases 8 and 19 (CDK8, CDK19)

The CDK8 or its paralog CDK19, together with the regulatory subunits cyclin C, MED12, and MED13 constitute a 600 kDa protein complex, called the kinase-module. They are components of the Mediator complex, which they serve as a negative regulator of transcription by influencing the biochemical activity of RNAP-II and general transcription factors GTFs. CDK8 has the ability to phosphorylate the CTD of RNAPII at Ser2 and Ser5 in vitro. In addition, CDK8 phosphorylates cyclin H within the transcription initiation factor TFIIH, thereby obstructing transcription initiation. However, the CDK8-Mediator complex acts as a positive regulator of transcriptional elongation, facilitating release of RNAPII from the paused state and promoting transcription elongation during serum and hypoxia responses. Moreover, CDK8 activates IFN-γ pathway by phosphorylating the STAT1 transcription factor.[45]

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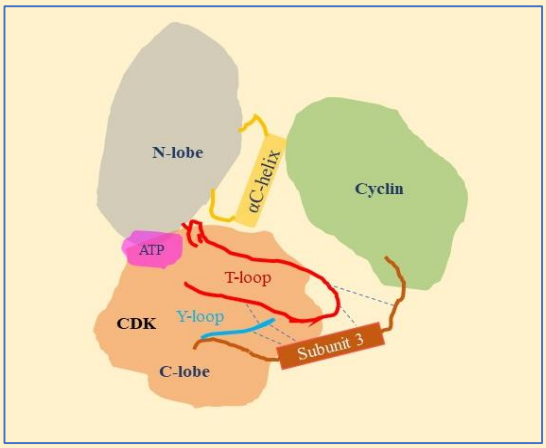


Figure 7. Schematic diagram illustrating the activation mechanism of CDK proteins. Cyclin binding pushes the αC-helix of CDK towards the ATP-binding site, causing the T-loop to move away from the entrance of the catalytic pocket. Then, the phosphorylation of the activation segment (T-loop), that stabilizes by the third subunit *via* contacting with its tip and binding to the Y-loop leading to the interaction with a conserved arginine residue from the C-lobe, enhances the kinase activity to full activation.[45]

Deviation of a number of Mediator subunit have been associated with the pathogenesis of a range of disorders including cancer. Such divergence can be either chromosomal or gene mutations.[46] Accumulated evidence has demonstrated that CDK8 acts as major oncogenic driver in different signaling pathways including Wnt/Catenin signaling and TGFβ/SMAD-driven metastases, and has been shown to be implicated in many tumor types.[46] On the other hand, CDK19 is also involved in a wide spectrum of cancers.

The structure of CDK8 is similar to that of CDK2 with an additional N-terminal αB-helix, which is responsible for recognizing cyclin C and binding with it. Such binding stimulates CDK8 to adopt an "αC-helix pushed-in" conformation.

The CDK8 is inactive state even when bound by cyclin C as shown by the partially absence of the T-loop. Moreover, the conserved threonine, the phosphorylation residue, is absent within T-loop, which distinguish CDK8 from other CDKs. In the absence of phosphorylated residue, the sidechain of the conserved tyrosine Y211 in human CDK8 Y-loop adopts an orientation that might potentially clash with the T-loop, resulting in destabilization of T-loop.[45] To replace the function of the conserved phosphorylation threonine residue, the MED12N, the N-terminal segment of MED12, contains an "activation helix" in proximity to the T-Loop of CDK8, which contacts with both T- and Y-loops, enhancing their stabilization and overall complex assembly.

5.2. Integrin-dependent regulation of the cell cycle

For most cells, cell cycle progression depends on cells engaging extracellular matrices (ECMs) *via* integrin transmembrane receptor and the formation of actin-associated adhesion complexes. Integrin adhesion receptors sense the composition and mechanical properties of the ECM and transmit the information via several signaling pathways to modify the cell behavior, including proliferation, differentiation, and migration. Before entry into mitosis, adhesion complexes are rapidly disassembled, and cells retract from their surroundings and round up

to divide. This disassembly requires elevated cyclin B1 levels and is mediated by inhibitory phosphorylation of CDK1–cyclin complexes. In fact, cell rounding is required for accurate spindle formation and chromosome capture. Integrin-mediated adhesion is also required to determine the orientation of cell division and to allow efficient cytokinesis to occur. The inactivation of CDK1 is therefore the trigger that initiates remodeling of adhesion complexes and the actin cytoskeleton in preparation for rapid entry into mitosis.[49]

Polo Like Kinase 1 (Plk1) plays a major role in the execution of diverse mitotic events in a coordinated manner. PLK1 is required for the activation of the cyclin B-CDK1 complex, whose kinase activity is needed for mitotic entry and the cell cycle progression through the early mitotic stages. Plk1 is also participated in the maturation of centrosomes. The critical mitotic regulator Plk1 induces phosphorylation of the mitotic Eg5, a kinesin-family motor protein driving the centrosome separation, whereby the activation of Eg5 generates the main force resulting in the centrosome translation to form a bipolar mitotic spindle.[50]

In addition to Plk1 and Eg5, the focal adhesion kinase (FAK) is implicated as signaling intermediates linking integrin to the formation of a bipolar mitotic spindle and error-free chromosome distribution. FAK is a cytoplasmic protein tyrosine kinase that distinctly co-localizes with integrins at sites of attachment to their ligands. Attachment of integrins to their extracellular matrix ligands is a major regulatory stimulus for FAK, leading to its tyrosine phosphorylation and enzymatic activation. FAK associates with a large number of enzymes, adaptor and scaffold proteins and serves both enzymatic and scaffolding roles in the transduction of signals. Aberrant FAK signaling results in altered cellular phenotypes, including increased invasion, growth in soft agar, tumorigenesis and metastasis.[50] Taken together, the lack of integrin adhesion, PLK1 or FAK activity destabilized the structural integrity of centrosomes and often caused detachment of pericentriolar material from the centrioles.

5.3. The Never in mitosis gene A (NIMA) family of kinases

The NIMA family of serine/threonine kinases is a diverse group of protein kinases implicated in a wide variety of cellular processes, including cilia regulation, microtubule dynamics, mitotic processes, cell growth, and DNA damage response. The NIMA is required for the initiation of mitosis, whereas its inactivation is necessary for mitotic exit. In mammalian cells, the overexpression of NIMA leads to the early onset of mitotic events including chromatin condensation and depolymerization of microtubules. Furthermore, the appropriate degradation of NIMA is required for mitotic exit.

The human family comprises 11 members (Neks for NIMA rElated Kinase), named Nek1 to Nek11, which their functions are poorly understood and are quit diverse. The Neks have been implicated in many human diseases including developmental disorders, amyotrophic lateral sclerosis, polycystic kidney disease, lethal skeletal dysplasia, primary ciliary dyskinesia, nevus comedonicus, multiple cancers, and multiple organ dysplasia.[51] They are conserved kinase domain, and contain a tyrosine inhibitory residue that protrudes into the catalytic site (with the exception of Nek3, Nek5, and Nek11). They are classified into three distinct clades: clade 1 consisting of Nek6, 7, and 10; clade 2 consisting of Nek1, 2, 3, and11; and clade 3 consisting of Nek8, and 9, although most of these are relatively similar, there are some outliers. Their predicted structures show great diversity in how these proteins are shaped, reflective of their diverse functions within cells.[51]

The activity of NIMA is tightly controlled by several mechanisms that ensure the protein is only active during G2/M transition, including phosphorylation at multiple sites by Cdc2/Cyclin B complex, dephosphorylation by Cdc25, and loss of protein stability *via* proteolytic degradation. Therefore, the activation of NIMA is dependent upon the activity of Cdc2, which is probably directly phosphorylated and activated by Cdc2. It is worthy to

mention that Cdc2 activation and its nuclear localization is a prerequisite for entry into mitosis and, in most cases, once Cdc2 has been activated, mitosis will occur. In fact, the NIMA protein is nuclear and is associated with chromosomes, spindles, and spindle pole bodies. Evidence has revealed that the NIMA promotes nuclear localization of the Cyclin B/Cdc2 complex.[51,52]

6. Consequences of centrosome abnormalities

To understand how centrosome amplification could contribute to human diseases, especially tumorigenesis, it is important to shed the light on the mechanisms behind such a correlation, at least in part, in both of mitosis and interphase cell (Figure 8).

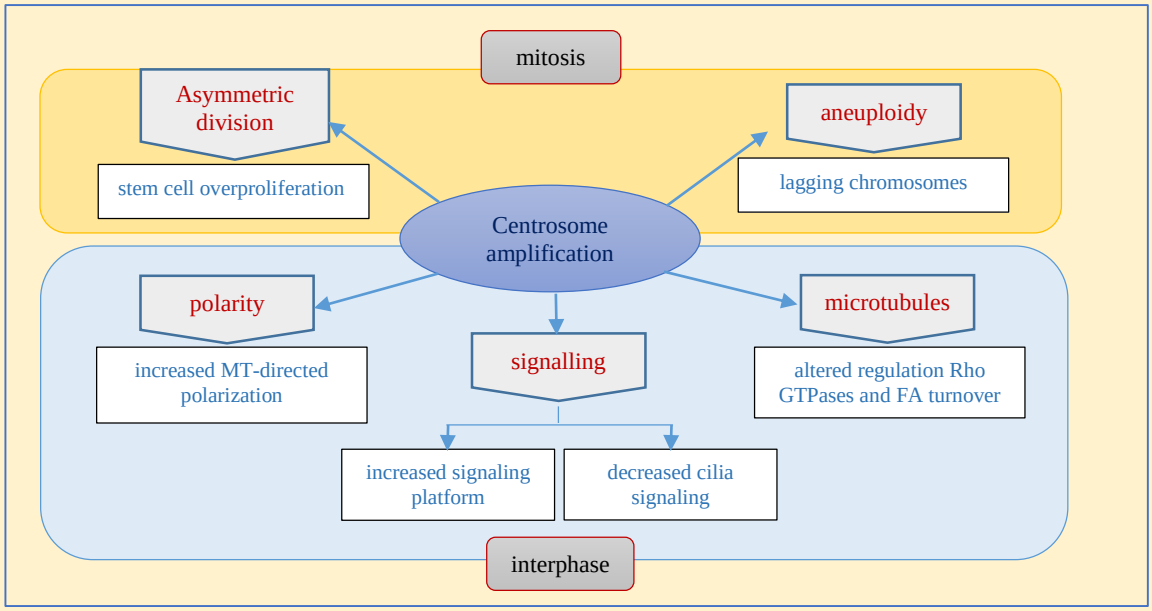


Figure 8. A Model of consequences of centrosome amplification in cancer.

6.1. Generation of aneuploidy in mitosis

During the process of clustering supernumerary centrosomes in mitosis, multipolar spindles are transiently formed, which tends to form defective attachments of chromosomes to the spindle microtubules (MTs), known as merotelic kinetochore-MT attachments.[20] Because of these merotelic attachments, which can escape the robust control of the spindle assembly checkpoint (SAC), they are a critical source of aneuploidy owing to the cells undergoing the missegregation event without control and, hence, not being culled from the population. Developing aneuploidy could provide a platform for evolving tumours.[20] Notably, chromosome segregation errors or DNA replication stress can lead to fuel aneuploidy through polyploidy, in which a cell contains more

than two genome copies, e.g. tetraploidy.[9] Tetraploid cells can be emerged from mitotic errors, including cytokinesis failure, mitotic slippage, endoreduplication or cell-cell fusion, hence, causing centrosome abnormalities and tumorigenesis.[6] Additionally, lagging chromosome can cause extensive DNA damage in micronuclei, which can result in fragile nuclear envelopes and thus undergo an abrupt loss integrity.[20]

6.2. Asymmetric cell division in mitosis

During mitosis, extra centrosomes can induce tumorigenesis independent of chromosome segregation.[20] Evidence has shown that in *Drosophila* neuroblasts containing extra centrosomes exhibit defects in asymmetric cell division, as a result of formation of multipolar spindles and delaying in mitosis, which can cause the expansion of the neuronal stem cell compartments, leading to tumours.[20,6,53]

6.3. Cell polarity and microtubules in interphase

Centrosome amplification could influence the biological architecture of tumor tissue by altering cell shape, polarity or motility. In interphase, extra centrosomes are clustered, that recruit extra PCM, resulting in an enlarged centrosome with increased MT nucleation capacity, which affected the cell shape and motility. The role of centrosome positioning, by directing MT nucleation, influences many aspects of cell signaling. For instance, it can determine the site of axon outgrowth in neurons, the proper secretion of lytic granules during formation of the immunological synapse, or directional migration by positioning the Golgi towards the leading edge.[20,54] In fact, increased MT nucleation capacity could influence cellular physiology in many ways. For example, for cells to migrate directionally, they need to coordinate signaling pathways to control polarity and cytoskeleton rearrangements to generate forces required for directional movement. Force generation relies on the ability of cells to dynamically remodel adhesion sites that connect them to the underlying extracellular matrices, known as focal adhesions (FAs). MTs are essential for cells to migrate directionally, and that microtubules are associated with FAs in migrating cells. The focal adhesion disassembly is controlled by MTs, which includes components of the endocytic machinery, inhibition of actomyosin-mediated contractile forces, or proteolytic cleavage of the link between FA and the actin cytoskeleton.[20,55] Furthermore, MTs influence the activity of Rho GTPases (Ras homologous family), which correlates with tumor progression, metastasis, and poor prognosis.[56] Indeed, centrosome amplification, leads to increased MT nucleation, resulting in Rac1 activation, increased actin polymerization and disruption of cell-cell contacts, promoting invasive behavior (invadopodia).[56,57]

6.4. Signaling in interphase

Component of numerous signaling pathways can associate with the centrosomes, even if only transiently, as revealed by proteomic analysis of purified centrosomes.[20] Members of the Wnt, NF-κB and integrin signaling can localize to the centrosomes, which they contribute to tumorigenesis. The centrosome is the cellular location at which proteasomes normally accumulate in many cell types.[58] In response to Bone Morphogenic Proteins (BMP) signaling, phosphorylated and polyubiquitinated Smad1 is associated to the centrosome. Inhibition of the proteasome results in accumulation of phospho-Smad1 at the centrosome, indicating that centrosome might act as a place for proteasomal degradation.[20]

7. Rho GTPases signaling

Proteins controlling microtubule dynamics and processes that require changes in cell shape and motility are important for tumor dissemination. The Rho (Ras homolog) family is an integral part of the Ras superfamily guanine nucleotide-binding proteins. Rho switches between being in a GDP-bound, inactive state and a GTP-bound, active state. Rho GTPases are mainly activated by guanine nucleotide exchange factors (GEFs), that catalyze GTP loading. The active state of Rho family proteins is vital for various cellular processes. Rho GTPases play a role in the establishment of cell-cell contacts and cell-matrix interactions. They regulate local dynamics of microtubules, centrosome activity, and the function of the Golgi/centriole comples.[9] By locally modulating Rho GTPases signaling pathways that control actomyosin-mediated contractility, microtubules could influence integrin-based focal adhesions dynamics. Centrosome disruption induces excessive Rac1 activation around the cell periphery, causing rapid focal adhesion turnover, a disorganized actin network, randomly protruding lamellipodia, and the loss of cell polarity. In cells with extra centrosomes, increased centrosomal microtubule nucleation leads to Rac1 activation, disruption of cell-cell contacts, and invasive behavior. In human non-transformed cells, these responses referred to as mitotic surveillance pathways depend on p53-activating signaling *via* the PIDDosome (a multiprotein complex that includes p53-induced death domain protein 1, PIDD1). Many studies reveal that activated Rho GTPases have tumor-promoting effects, but also tumor-suppressive functions. Studies on cancer and rare genetic disorders point out that centrosome dysfunction alters the cellular microenvironment through Rho GTPases, p38, and JNK (c-Jun N-terminal Kinase)-dependent signaling in a way that is favorable for pro-invasive secretory phenotypes and aneuploidy tolerance. These contrasting effects of Rho GTPases in cancers may be due to cell-type-specific functions or insufficient available cancer models.[9]

8. Centrosome stability and inflammatory response mechanisms

Genomic instability emerged from centrosome aberrations and DNA damage leads to DNA and cGAS-STING (cyclic guanosine monophosphate-adenosine monophosphate (cGAMP) synthase (cGAG)-stimulator of interferon genes (STING)), an important DNA-sensing machinery in innate immunity and viral defense, to induce inflammation signaling, which affects cellular antigen presentation.[9,59] These pathways are mediated by non-canonical Nuclear factor-κB (NF-κB), and also coopting of myeloid cell mobility programs. To maintain cell genomic integrity, cells have evolved mechanisms to generate various inflammatory response systems to address DNA and centrosome instability.[9] This section briefly discusses examples of these mechanisms.

8.1. Nucleophosmin

Nucleophosmin (NPM1) is an abundant nucleolar protein found primarily in the nuclei of proliferating cells, but a proportion of the protein continuously shuttles between the nucleus and cytoplasm. NPM1 is a ubiquitously expressed phosphoprotein belonging to a histone chaperones family, the Nucleophosmin/ nucleoplasmin (NPM) family of chaperones, a group that comprises multiple major functional members (NPM1, NPM2, NPM3 and invertebrate NPM-like), and can be found amongst all Metazoan. All members of the NPM family exhibit conserved structural motifs. The structural architecture of NPM1 is described by the

N-terminal core domain that mediates NPM1 oligomerization and interactions with other proteins (Figure 9). This region is marked by extremely asymmetric with negatively charge residues. Two pentamers of NPM1 are arranged so that a monomer of the pentameric ring only contacts a single monomer of the other pentamer, allowing structural plasticity at the pentamer–pentamer interface. This multimeric state is modulated by numerous post-translational modifications, especially phosphorylation events that regulate the monomer-pentamer equilibrium by promoting the disassembly of the pentamer into unstable, unfolded monomers. This structural polymorphism participates in the regulation of NPM1 localization and function. As such, oligomerization of NPM1 has been linked to its nucleolar localization and role in cellular proliferation, while the monomeric form of NPM1 is associated with its role in the DNA damage response and induction of apoptosis.[60]

The central region of NPM1 is characterized by the presence of highly acidic regions composed of strings of aspartic and glutamic acids (A1, A2 and A3). They provide long tracks of negatively charged residues. This region contains a nuclear localization signal.[60]

The C-terminus of NPM1 is characterized by the presence of a basic, positively charged cluster of amino acids, immediately followed by a stretch of aromatic residues, providing an adequate platform allowing the binding to nucleic acids and ATP. These aromatic residues constitute an atypical nucleolar localization signal (NoLS), and their mutation are responsible for the unfolding and the aberrant NPM1 localization typical in acute myeloid leukemia (AML) cases.[60]

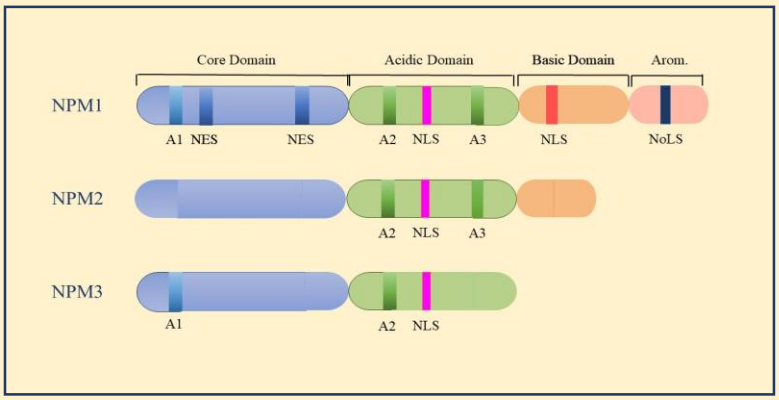


Figure 9. Schematic structure of NPM proteins. All proteins share a core hydrophobic domain (blue) responsible for oligomerization and chaperone activity, followed by an acidic domain required for ribonuclease activity. A basic domain implicated in nucleic acid binding is common to NPM1 and NPM2, but absent in NPM3. Finally, only NPM1 exhibits a C-terminal aromatic stretch required for its nucleolar localization. NPM members harbor nuclear-localization signals (NLS), nucleolar-localization signal (NoLS), nuclear export signal (NES) and acidic clusters.[60]

NPM plays multiple roles within human cells including ribosome biogenesis, mRNA processing, chromatin remodeling, embryogenesis, regulation of apoptosis and maintenance of genome stability. The critical role of NPM in the maintenance of genome stability takes place through its interaction with unduplicated centrosomes. The phosphorylation of NPM by Cdk2/Cyclin E promotes the release of NPM from the centrosome during

duplication, which represents an essential step for duplication to occur.[60] However, during mitosis NPM re-associates mitosis NPM re-associates with the centrosomes at the spindle bodies and appears to be controlling centrosome duplication.[60] The mechanism of this event is described by that pro-inflammatory cytokines activate the IKKα (inhibitor of NF-κB kinase α), that induces nucleophosmin hexamer formation, which in turn, leads to the interaction of NPM with centrosomes in the M phase. This pathway allows the maintenance of normal centrosome number and genome integrity. Consistently, loss of IKKα or NPM, reduces the levels of NPM and its association with centrosomes, thereby promoting centrosome amplification and genome changes that predispose to cancer (Figure 10).[9] Given the critical role NPM1 plays in genome stability and apoptosis it is obviously that NPM1 dysfunction is a frequent feature in cancers, as evidence of NPM1 function in DNA repair pathways increases, leading at least in part to the genetic instability associated with cancers such as AML. In addition, NPM1 deregulation or mutation will suppress the ability of that cell to respond to apoptotic stimuli, allowing for the tolerance of the genetic instability.[60]

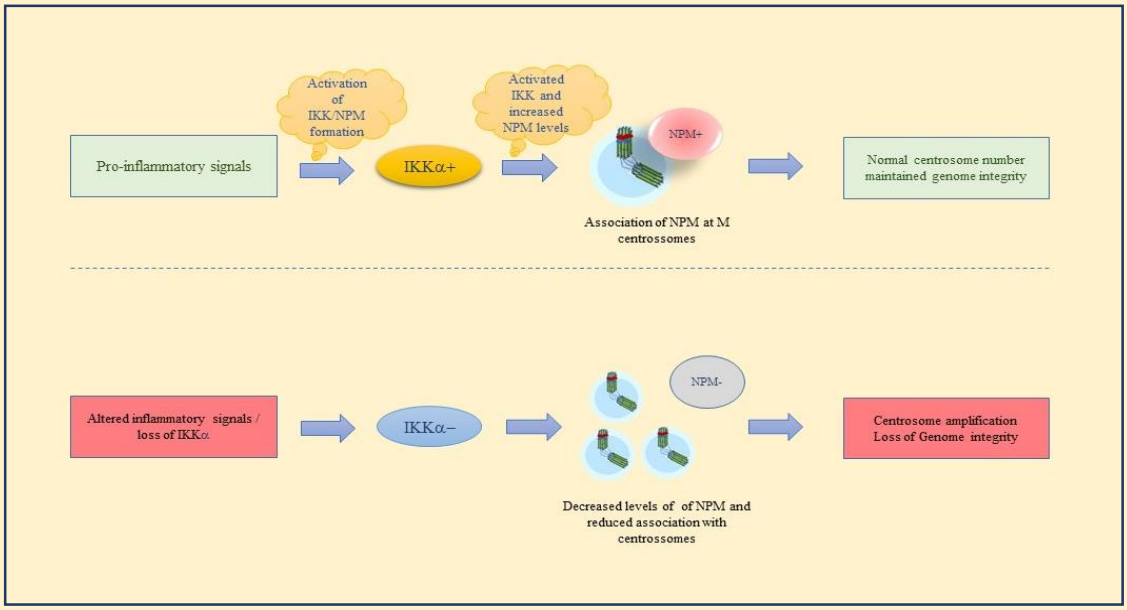


Figure 10. Pro-inflammatory cytokines activate the IKKα/Nucleophosmin (NPM) hexamer formation. IKKα activation and increased NPM levels allow NPM-Centrosome interaction, which allows the maintenance of normal centrosome number and genome integrity. Whereas IKK or NPM loss decreases the levels of NPM hexamers and the association of NPM with centrosomes, thereby promoting centrosome amplification and genome changes that predispose to cancer.[9]

8.2. p38 MAPK kinase

Mitogen-activated protein kinases (MAPKs) are serine/threonine kinases, which belong to conserved signaling components involved in transducing extracellular stimuli into cellular responses through phosphorylation of various substrates, thereby controlling a wide range of cellular processes including cell division, differentiation,

proliferation, survival and death. In mammals, p38 represents one subgroup of MAPKs, that is activated following a series of phosphorylation events. The p38 family consists of 4 related kinases, p38α (MAPK 14), p38β (MAPK 11), p38γ (MAPK 12), and p38δ (MAPK 13). The p38α is ubiquitously expressed in human tissues, while the expression of p38β and p38γ is largely restricted to brain and muscle, respectively. The p38δ isoform plays a role in the regulation of cellular processes. Variable levels of p38δ expression were detected in endocrine tissues.[61]

Mutations in key G2/M-phase checkpoint proteins that involved in mitotic regulation lead to genomic instability. The p38 MAPK kinase plays a crucial role in orchestrating the regulation of centrosome dynamics with cell cycle as well as normal immune and inflammatory responses, such as controlling mitotic entry timing.[62] Accumulated evidence has demonstrated the correlation between centrosome amplification and p38 kinases. For example, loss of centrosome integrity activates p38 MAPK, resulting in a p38-p53-p21-dependent cell-cycle (G1-S) arrest or induction of cellular senescence or apoptosis, revealing its important role in maintaining chromosome stability and a mitigated inflammatory response.[9] Furthermore, loss of p38 allows aneuploidy tolerance by enhancing HIF-1α and glycolysis, limiting metabolic collapse, as the case in tumor cells. p38 MAPK acts as a negative regulator of interferon signaling downstream of STING (Stimulator of interferon genes), therefor, in the cases of advanced viral infection, p38-mediated phosphorylation of USP21 (Ubiquitin Specific Peptidase 21), a deubiquitinating enzyme, inhibits STING.[9]

Chromosomal instability, a driving force of tumorigenesis, is mainly emerged by the deregulation of the cell cycle.[40] p38MAPK regulates cell cycle and controlling genomic instability through different pathways. For example, p38MAPK controls cell cycle transition stages to ensure their genetic integrity and stability. It also regulates actin cytoskeleton organization for cytokinesis and mitosis needs.[9] It was revealed that p38MAPK activation promotes p53 stability dependent-apoptosis by suppressing HIF-1α (Hypoxia-inducible factor-1α) among other mechanisms.[9] Moreover, p38MAPK activity effectively contributes to proper spindle assembly and controls mitotic entry through phosphorylation of Cdc25B in normal cells. Its localization at the kinetochores and centrosome is also substantial for proper chromosomal segregation, which its activity is required for the timely stable attachment of all kinetochores to spindle microtubules.[63]

Notably, p53 regulates centrosomal instability surveillance and chromosome segregation. Therefore, chromosomal mis-segregation, due to mitotic errors, leads to activation of p53, which in turn arrests cell cycle, inducing senescence or apoptosis.[9,63] In addition to the unique role of p38 in inflammation and immunity, cell cycle arrest is also regulated by p38, which directly or indirectly influences motor proteins, microtubule dynamics, and centrosome activity. Collective evidence reveals that p83MAPK plays a substantial role in detecting and transforming chromosome alteration into robust G1 arrest, often dependent on p53.[9]

The p38MAPKs have a key role in the signaling pathways that coordinate various types of cellular stresses, but also regulate the activity and expression of essential inflammatory mediators, including cytokines and proteases, which may act as potent tumor promoters.[64] The innate and immune systems have the ability to recognize and eliminate the tumor cells. However, if tumor cells are not completely removed, they might enter a dormant state that is reversible and can last many years. In that state, they can evade immune surveillance, and proliferate in an unrestricted way, using different strategies.[9,65] The p38 MAPK can regulate the immunosuppressive properties of some immune cells, such as Myeloid-derived suppressor cells (MDSCs), natural killer cells, or dendritic cells.[9] p38 MAPKs also regulates dendritic cells (DC) functions, promoting immune tolerance. The combined inhibition of STAT3 (Signal Transducer and Activator of Transcription 3) and p38 pathways promote DC differentiation in the tumor microenvironment and increased allogeneic T-cell reactivity against glioma,

melanoma cells, or multiple myeloma. Furthermore, p38 MAPK inhibition during DC differentiation decreased PPARγ (Peroxisome proliferator-activated receptor γ) expression, which activates p50 transcriptional activities, leading to overexpression of OX40L on DC membrane. This increases DCs ability to activate tumor-specific effector T cells (Teff), blocks Treg conversion and function, and inhibits tumor growth.[9,66]

9. The current therapeutic strategies targeting centrosome aberration

Centrosome instability, such as abnormal centrosome number, excess PCM, defects in centrosome maturation and microtubule-nucleating capacity, and centrosome misorientation, is a hallmark of cancer and a driver of metastatic dissemination, therapeutic resistance, and immune evasion.[67,68] In view of the complex cell-cycle of centrosomes, it is difficult to develop inhibitor drugs that allow to identify which patients will respond well to these inhibitors. The current therapeutic strategies targeting centrosome aberrations are mainly depended on inhibitors targeting centrosome-related proteins, which will ultimately promote normal mitosis and orientated cell migration and prevent cancer progression. Several treatment approaches targeting inhibitors of Aurora kinase, inhibitors of kinesin, p53-reactivating agents and PLK4 inhibitors, are currently being tested in clinical trials. Although several centrosome-related inhibitors have been identified and used in preclinical studies on tumor cells, no drug that targets the centrosome has been successfully used in clinical trials.[69] In the next section, I will provide with some hypothetical scenarios, which may serve as a potential promising therapeutic targets for centrosome aberration, and may overcome some of the challenges in the development of therapeutic interventions for patients with chromosomally unstable cancers.

10. Hypothetical scenarios targeting centrosome instability

In the view of the above discussion, one can conclude among the main causes that lead to centrosome amplification abnormalities are: i) hyperactivation of cyclin-dependent kinases; ii) overexpression of PMC components; iii) failure of DNA damage checkpoint proteins response ATM/Chk2 and ATR/Chk1; iv) overexpression of centrosome-associated proteins which their overexpression arises mostly from the over-phosphorylation of their conserved sites, namely serine and threonine; v) the lack of integrin adhesion, Plk1 or the focal adhesion kinase activity, leading to destabilized the structural integrity of centrosome and detachment of pericentriolar materials from centrioles; vi) overexpression of NIMA; vii) reduction of the nucleophosmin levels, leading to distortion of nucleolar structures and unexpected dramatic changes in nuclear morphology with multiple micronuclei formation; viii) p53 mutations, leading to the unrestricted cell cycle and replication of the damaged DNA; ix) contrasting roles of p38 MAPK.

10.1. Hypothetical strategies targeting centrosome instability

In view of the aforementioned discussion, one can envisage that besides the various approaches targeting centrosome-associated proteins, also the strengthening of the checkpoint protein kinases may play a vital role. Defects in the checkpoints that normally maintain the fidelity of mitosis event in the cell cycle process can lead to chromosomal instability, a driving force of tumorigenesis, as sister chromatids are irreversibly segregated to daughter cells. The motivation of accurate activity of checkpoint protein kinases, ATR, and its downstream the

serine/threonine kinase Chk1 against their overexpression would lead to suppression of replication stress that arise from causes such as DNA damage and oncogene activation. These two proteins play principal role in monitoring the DNA replication and the genome integrity. For example, the phosphatase Cdc25A is a key Chk1 downstream target that plays a key role in regulating both the entry and the progression of S phase. Activation of Chk1 leads to the proteasome-dependent degradation of Cdc25A followed by S phase progression inhibition. Chk1 polypeptide contains a number of critical regulatory mechanisms mediating its own phosphorylation, checkpoint activation and cell viability.
Chk1 is composed of a highly conserved kinase domain at the amino (N)-terminal half and a regulatory region at the carboxyl (C)-terminal half. The C-terminus contains a Ser/Gln (SQ) motif and two highly conserved motifs. Checkpoint activation requires phosphorylation of Chk1 at both Ser-317 and Ser-345 residues, with Ser-345 phosphorylation being the final determinant of checkpoint activation.[70] In fact, Chk1 activation requires its protein conformation change. Under normal growth conditions, Chk1 adopts a "closed" conformation through an intra-molecular interaction between the N-terminus and the C-terminus, leading to suppress the kinase activity of Chk1 and to stabilize the protein. In other words, the N-terminal kinase domain suppresses phosphorylation of residues located at the C-terminus of Chk1. Upon DNA damage, Chk1 undergoes ATR-dependent phosphorylation on chromatin, which disrupts the intra-molecular interaction, resulting in an "open" conformation of Chk1 followed by checkpoint activation.[70] Worthy to mention that ataxia-telangiectasia-mutated (ATM)-dependent activation of ATR only occurs in the presence of DNA damage. In addition, the two highly conserved motifs on the C-terminus contributes to high-level phosphorylation of Chk1 in response to DNA damage, which might be through providing a proper conformation for maximal Ser-345 phosphorylation by ATR.[70]

To design a therapeutic agent capable of inhibiting the activity of Chk1, such a compound must have a conformational control to provide appropriate spatial constraints on the C-terminal kinase of Chk1, or by transiently masking the kinase domain in order to suppress the phosphorylation of C-terminal kinase of Chk1 by ATR, which may prevent accidental activation of checkpoints by either the exposure of the catalytic domain or the phosphorylation of the SQ sites of Chk1. Such a proper conformation may give a compound unusual therapeutic properties, and may serve as a stimulus for the regulation of checkpoint protein kinases by enhancing and adjusting its function.

Another approach is the constraint of the cyclin N-terminal helix. Cyclin-dependent kinases (CDKs) are the major regulators of mitosis and their hyperactivation influences several components of mitotic chromosomes, leading to centrosome abnormalities and genomic instability. Cell cycle progression is regulated by the activity of cyclin-dependent kinases (CDKs) bound to an activating cyclin subunit, in which the cyclin subunit plays a key role in substrate recognition by the cyclin-CDK complex. The interaction between the cyclin N-terminal CBF (cyclin box fold) and the PSTAIRE motif in the N-terminal lobe of the CDK leads to the formation of a tight complex. The cyclin has a conserved N-terminal helix in its N-terminal domain (cyclin A and cyclin B) (Figure 11). The cyclin N-terminal helix contributes to the binding site of substrates differently *via* a conformational change in the complex, allowing it to recruit a specific substrate into the catalytic cleft. In other words, the substrate specificity depends upon binding specificity of the cyclin-CDK complex, which is due to the cyclin N-terminal helix.[71] Thus, if a bulky small molecule is introduced to shield the cyclin N-terminal helix from the recognition of a substrate, *e.g.* ATP, the hyperactivation of cyclin-dependent kinases would be reduced

and the signal activities of components of mitotic chromosomes would be controlled. The centrosome is a multifunctional organelle, and its aberration is associated with many diseases. Therefore, therapeutic interventions require a synergistic agent with combined activities to impose a wide range of spectrum to regulate the centrosome-associated proteins.

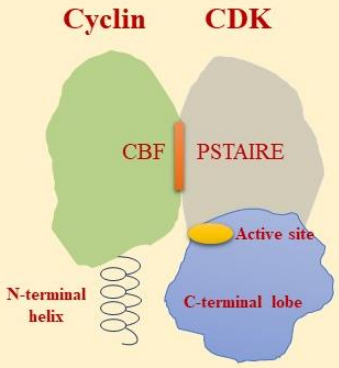
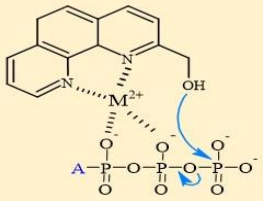


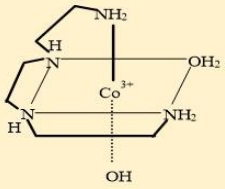
Figure 11. Schematic illustration of the location of the cyclin N-terminal helix in the cyclin-CDK complex.[71]

10.2. Proposals for therapeutic intervention

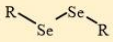
Among the several approaches targeting centrosome-associated proteins is the inhibition of Plk4. The Plk4 overexpression ultimately results in genomic instability and tumorigenesis. Most of the centrosome-associated proteins are serine/threonine protein kinases, which their activation occurs *via* the phosphorylation on serine and threonine residues. To affect their overexpression, there is a need for a compound capable of decreasing their levels by blocking the active site of kinase residues, or stabilizing the fold's conformation of the protein by removing a specific group or atom, which ultimately leads to centrosome inactivation.
Previously, I have reported conceptual approaches to influence the overproduction of TGF-β.[72] Among these approaches is the coordination of metal cations with the active sites of the kinases or the ligands bound to them which may lead to disruption of the local hydrogen bonding, the hydrophobic interactions and the conformational dynamics of the region. These cations, such as the redox responsiveness diselenide- or monoselenide containing hyperbranched polymers; the complex ion catalyst *cis* β-hydroxoaquatriethylene-tetramine cobalt(III), $[Co(trienH_2O)OH]^{2+}$, and 2-hydroxymethyl phenanthroline-M^{2+}, may play an active regulatory role, serving as physiological activators or inhibitors (Figure 12).[72]



2-hydroxymethyl phenanthroline-M^{2+}
$M^{2+} = Zn^{2+}$, A = adenosine



$[Co(trienH_2O)OH]^{2+}$

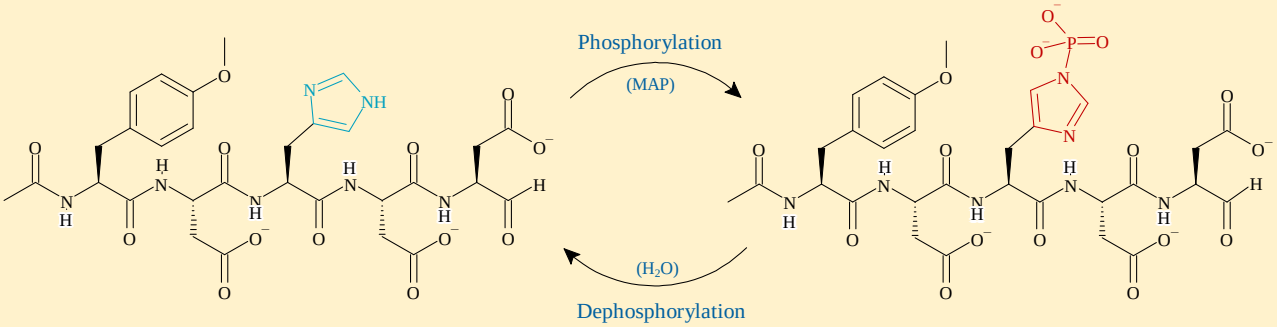


diselenide containing hyperbranched polymers

Figure 12. The coordination of metal cations with the active sites of the kinases or the ligands bound to them.[72]

10.2.1. L-2-thiohistidine

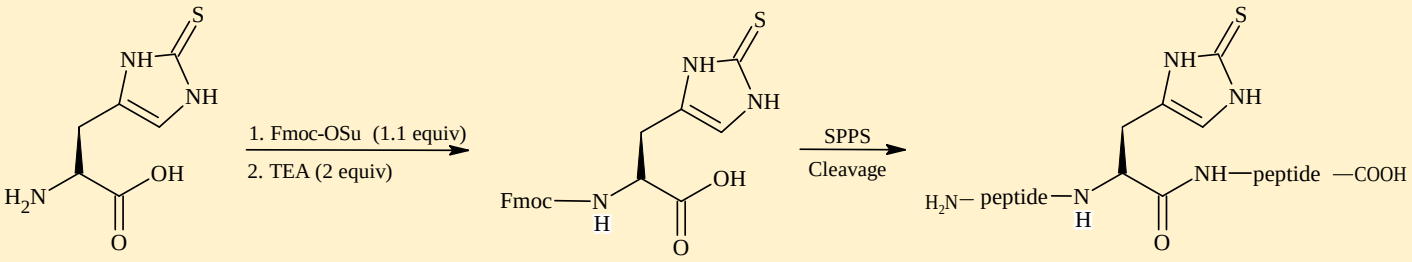
Here, I propose a new strategy to address the overexpression of PLK4 and other key kinases controlling centrosome cell-cycle fidelity. It is speculated that creating an environment that implies dynamic activation of the regulation of these processes, may lead to motivating these kinases to adjust their activates by transiently consuming their phosphorylation. That is, a competitive compound has the capability to be phosphorylated and hydrolyzes with a tunable half-life. Studies have shown that histidine is used as a phosphate shuttle in the active site of kinases and phosphatases (Scheme 1).[73,74] Boekhoven *et al.* have demonstrated that a phosphorylation molecule can be consumed by transiently phosphorylating histidine and histidine-containing peptides, in which the phosphorylated species are labile and deactivate through hydrolysis.[73] Furthermore, they revealed that the resulting phosphorylated products can interact with other amino acids (*e.g.* poly-arginine) to form liquid-liquid droplets, which these droplets dissolve upon dephosphorylation.[73]



Scheme 1. The dynamic phosphorylation of histidine-containing peptides. MAP : monoamidophosphate.

To achieve this hypothesized scenario, it is envisaged that 2-thiohistidine could be able to impose a dynamic environment of activation leading ultimately to motivate and adjust the centrosome cell-cycle proteins

activation. The 2-thiohistidine is featured with its unique antioxidant, radical scavenging and metal chelation properties.[75] Furthermore, 2-thiohistidine has the functional groups that allow coupling to peptides, paving the way for targeting to specific tissues and organelles (Scheme 2).[75] The 2-thiohistidine could also be incorporated into multifunctional hybrid nanoparticles. It can be readily synthesized from histidine (His) and cysteine (Cys) *via* a labile His-bromolactone intermediate on a gram scale.[75]



Scheme 2. Inserating 2-thiohistidine into peptides. Fmoc-OSu **:** Fmoc *N*-hydroxysuccinimide ester; SPPS : solid-phase peptide synthesis.

10.2.2. Integrin-blocking aptamers

The structural integrity of centrosomes is stabilized by integrin or the focal adhesion kinase (FAK) activity, which attachment of integrin to the extracellular matrix ligands is a key regulatory stimulus for FAK, leading to its tyrosine phosphorylation and enzyme activation. However, overexpression of such activation leads to destabilization of the structural integrity of centrosome, which is frequently caused detachment of pericentriolar materials from centrioles. Thus, reducing the activity of integrin would lead to limited FAK signaling, resulting in maintenance of cellular phenotypes. As a therapeutic option, selection of integrin-blocking aptamers may result in FAK-inhibiting functions. To protect such proposed aptamers from nuclease degradation and to improve their stability *in vivo*, it is proposed to modify them with a hydrophilic poly(ethylene glycol) (PEG) block or by conjugated polymers (*e.g.* PEG-PUSe-PEG) (Figure 13).[72]

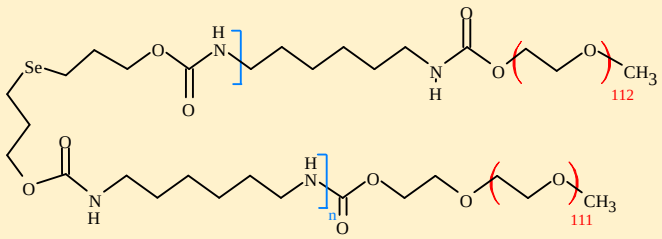


Figure 13. The structure of monoselenide-containing polymer PEG-PUSe-PEG, which is an amphiphilic triblock copolymer with hydrophobic monoselenide polyurethane blocks and hydrophilic poly(ethylene glycol) (PEG) blocks.[72]

10.2.3. Multifunctional hybrid nanoparticles

Multifunctional hybrid nanocomposites containing gold and carbon nanotube (AuNPs-CNT) provide two functional surfaces available for multipurpose use with different molecules without interferences between them due the presence of different discrete domains, allowing to construct multifaceted therapeutic agent. Therefore, it is proposed that employing such nanocomposites decorated with small molecules and aptamers may induce a collective and intrinsic therapeutic outcome. Furthermore, these nanocomposites may integrate the advantages of individual components to improve aptamers delivery potency. For instance, pH-responsive polymers, such as poly (β-amino ester), can be incorporated into hybrid nanoparticles to facilitate endosomal escape of aptamers. The performance of the formed nanocomposites depends on the morphology features, like size and shape, of the nanoparticles.

The synthesis of gold nanoparticles decorated multiwalled carbon nanotubes (MWCNTs) is readily achieved by a simple, cost-effective and greener method using cysteaminium chloride *via* the formation of a Zwitterionic reaction between the negative MWCNTs-$COOH^-$ with the positive, primary amino groups of cysteamine ^+H_3N-CH_2-CH_2-SH, followed by the decoration of AuNPs on the MWCNTs surface (Figure 14).[76] Such ionic features may allow electrostatic interactions between MWCNTs and biological molecules.

10.2.4. Amphiphilic bacterial cellulose nanocrystals (BCNCs)

The bacterial cellulose nanofibers are optimal carriers for metal nanoparticles, which can be considered as an effective strategy to expand the applications of carbon materials. They are produced by environmentally friendly biotechnological fermentation techniques, which are characterized by 10-50 nm in diameter and 100-1000 nm in length, and closely resembling the morphology of carbon nanotubes.[77]

BCNCs are characterized by their high hydrophilicity, good colloidal properties, non-toxic, biocompatibility and biodegradability. Given these attractive inherent properties, they are available for biomedical applications, especially the produced rod-like BCNCs, which can be generated from the bacterial cellulose (BC) and modified to alter the features of nanocrystals, depending on the desired field.[78] For example, a study demonstrated the potential of cationic-modified BCNCs produced by acid hydrolysis as nucleic acid delivery systems, which were completely complexed with siRNA, exhibiting their potential as vehicles in nucleic acid delivery.[79]

In fact, the properties of BC can be enhanced *via* their surface functionalization. Increasing the hydrophobic and electrostatic interactions with cells can be, for example, modified chemically by grafting methyl terminated octadecyltrichlorosilane (OTS) or amine terminated 3-aminopropyltriethoxy-silane (APTES).[78] The poor solubility of BC due to its inter- and intra-hydrogen bonds present in its molecules can also be enhanced by chemical modification *via* an acid treatment.[80] Furthermore, forming nanocrystalline ZnO particles without destroying the 3D structure of the BC were prepared *via* ZnO particle-incorporated BC sheet by ultrasonic-assisted *in situ* synthesis, which exhibited excellent antibacterial activity against both Gram-positive and Gram-negative bacteria.[81]

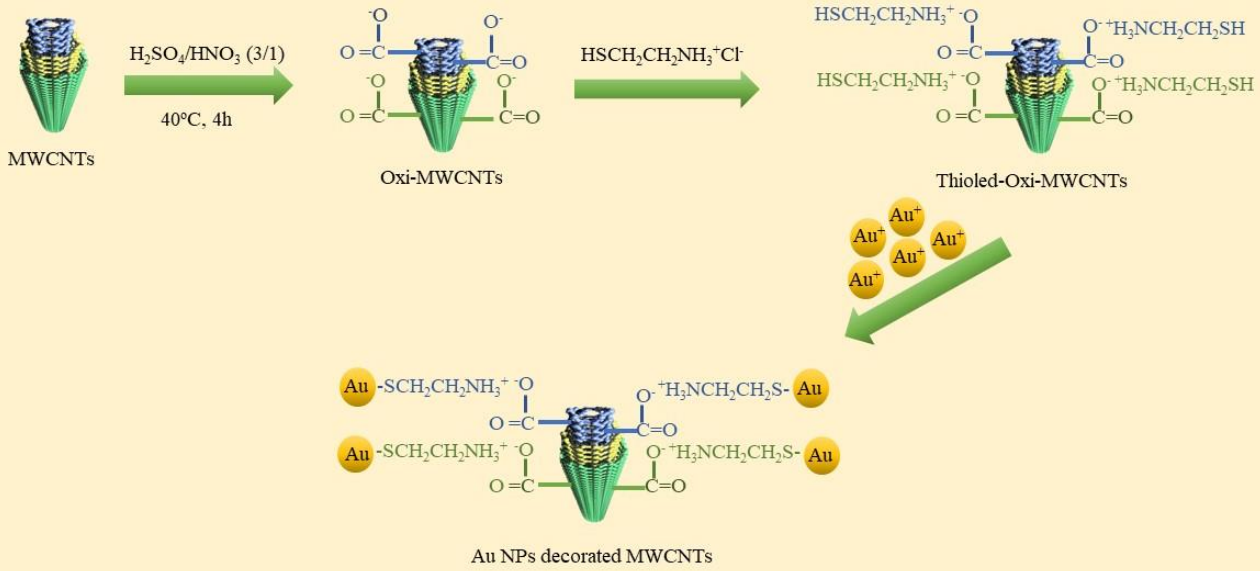
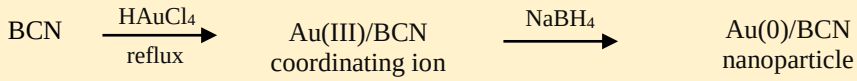


Figure 14. Schematic illustration for the synthesis of Au-MWCNTs.

The construction of highly dispersed gold nanoparticles loaded on BCNCs can be prepared through one-step *in situ* reduction, and monitored by changing the color of the chloroauric acid (HAuCl4) and BCN mixed solution from light yellow to brown and finally to light purple as a reducing agent ($NaBH_4$) was added dropwise (Scheme 3).[82] The strong interaction between the gold nanoparticles with a size of about 2-5 nm on the surface of BCNCs with a diameter of 30-50 nm were uniformly dispersed and did not alter the crystallography of the materials (Figure 15).



Scheme 3. Synthesis of Au/BCN nanoparticles.

It is hypothesized that the BCNCs can inhibit the phosphorylation of kinases by trapping ATP *via* interaction with the hydrogen bonds present in their molecules, and, hence, leading to limited the kinase's activations. In addition, they can disturb the conformation of the kinases in different pathways.

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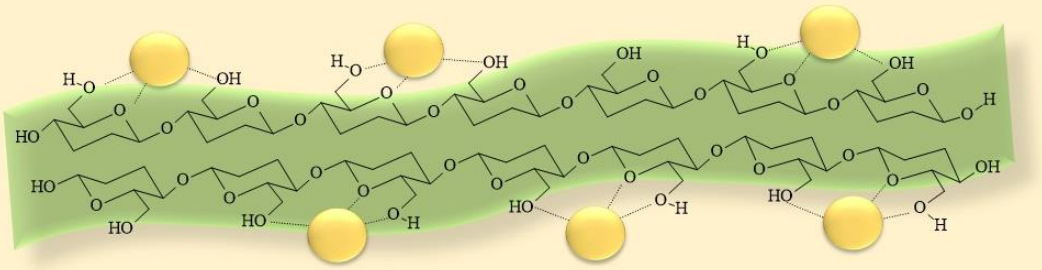

Figure 15. Schematic illustration of Au/BCNCs.

The solubility of Au-MWCNTs and Au/BCNCs can be improved by using a quaternary ammonium compound *via* two routes. The addition of the 3-sulfanylpropyltrimethoxysilane, ligand **1,** to these nanocomposites in propanol, in which the thiol would bind to the gold particle surface and the trimethylsilyl group would be available for further functionalization. Subsequently, polycondensation in the presence of relatively small amount of 3-aminopropyl-dimethylethoxysilane ligand **2** and ligand **3** functionalized with quaternary ammonium group in a ratio of 1:1 would yield multi-functionalized composites (Figure 16 A).[72] The amino group of ligand **2** would be available for coupling with biological molecules, while quaternary ammonium groups of ligand **3** would provide a positive charge to the particles over the entire pH range and give rise to the desired water solubility of the particles during the coupling with aptamers.[72]

To fabricate dual responsive selenide bridged mesoporous silica NPs (MSNs), the diselenide-bond-containing organosilica moieties, compound 4, can be incorporated into the silica network (Figure 16 B). The proposed small compounds, 2-thiohistidine, *cis*-β-hydroxoaquatriethylenetetramine cobalt(III), and 2-hydroxmethyl phenanthroline-Zn^{2+} can be encapsulated into the internal pores of MSNs through electrostatic interactions. Upon exposure to oxidative or reductive conditions, the MSN frameworks are degraded owing to the cleavage of diselenide bonds, and the complex compound can consequently be released.[72]

10.3. The expected outcomes of the hypothetical scenarios

The following aspects are anticipated from the proposed therapeutic interventions: 1) the 2-thiohistidine could inhibit the overexpression of Chk1 and stimulate the regulation and adjustment of its function by inducing a dynamic environment of activation, promoting a conformational control. They could also impose spatial constraints on its C-terminal. Furthermore, 2-thiohistidine could coordinate with the conserved sites of Chk1, shielding its phosphorylation by ATR; 2) the incorporation of 2-thiohistidine to Au-MWCNTs nanotubes or Au/BCNCs could affect Chk1 by transiently masking the kinase and suppressing the phosphorylation of its C-terminal by ATR; 3) the divalent metal cations could also coordinate with the active sites of Plk4 and other kinases that are involved in cell cycle regulation or the ligands bound to them, leading to disruption of the local hydrogen bonding, the hydrophobic interactions and the conformational dynamics of the region blocking their binding with cyclin, which ultimately resulting in centrosome inactivation; 4) the divalent metal cations have the susceptibility to coordinate with ATP in the presence of functionalized molecules, inhibiting its function

towards the kinases; 5) maintaining the structural integrity of centrosome can be sustained by controlling the focal adhesion kinase activity which is integrin-dependent. Thus, selecting an integrin-blocking aptamers could reduce the high levels of focal adhesion kinase activity. Aptamers have the capability to integrin-ligand binding. They are eligible to recognize the active conformational epitops, as well as an active or diseased-associated protein conformational state. So, they could affect the conformational environment of integrin. These proposed therapeutic agents could influence and inhibit the activity of major player proteins that controlling centrosome cell-cycle fidelity.

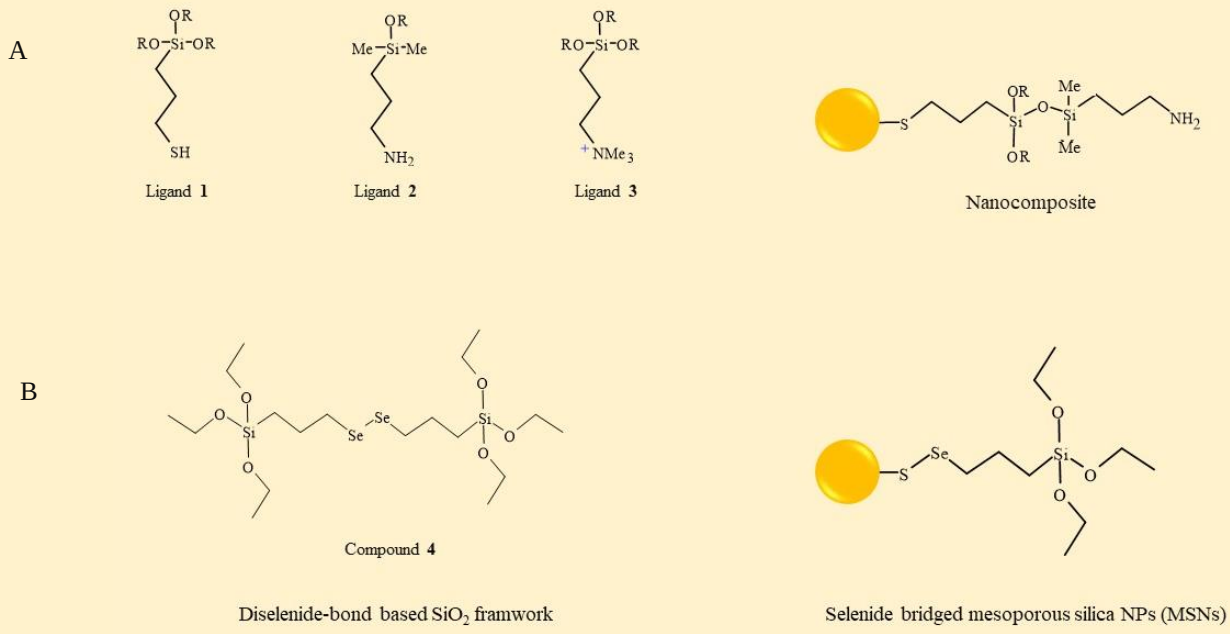

Figure 16. Schematic composition of the ligand shell.

11. Conclusions

The centrosome is a complex organelle, that acts as a signaling platform, mediating cell cycle transitions. Furthermore, several centrosomal interactions are involved in a variety of biological functions, including metabolism, protein synthesis, autophagy and inflammations.[83] Centrosome dysfunctions have been implicated in a wide range of diseases, including cancer. Centrosome defects are ubiquitous in cancer and are associated with dynamic CIN due to chromosome missegregation during mitosis. The combination of structural and numerical chromosome abnormalities triggered by centrosome dysfunction, ultimately leads to gene reshuffling and reprograming of the cancer genome. Centrosome dysfunction is linked to aneuploidy and CIN. Increasing

evidence reveals that centrosomes play a key role in the regulation of cell senescence, an irreversible type of growth arrest that takes place when cells suffer extensive intrinsic and/or extrinsic damage.[9]
In the course of this study, important topics are briefly discussed, emphasizing the point that Plk4 is the master regulation of centriole number, as any aberration of Plk4 activity leads to a variation of centriole numbers, and hence to chromosomal mis-segregation and cytokinesis failure. The key role of checkpoint proteins ATR/Chk1 and ATM/Chk2 pathways in DNA-damage-activated response is highlighted. The study also focuses on how the deficiency of p53 leads to unrestricted cell cycle and replication of damaged DNA. The cyclin-Cdks and integrin-dependent regulation of the cell cycle, among other topics are also discussed. In this study, I reasonably propose some inhibitors scenarios, including targeting checkpoint protein Chk1, Plk4 and other kinases that participated in cell cycle regulation, as well as targeting integrin. I hope the proposed nanocomposites would help in the design of potent and selective inhibitors.

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