Understanding the role of aneuploidy in tumorigenesis

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Abstract

The role of aneuploidy in tumorigenesis remains poorly understood, although the two have been known to be linked for more than 100 years. Recent studies indicate that aneuploidy can promote tumour cell growth and cell death and that the cellular outcome is dependent on the extent of aneuploidy induced. The mitotic checkpoint plays a pivotal role in the maintenance of genome stability and has been the focus of work investigating the distinct outcomes of aneuploidy. In the present article, we review the molecular mechanisms involved and discuss the potential of the mitotic checkpoint as a therapeutic target in cancer therapy.

Introduction

Disruption of mitosis often leads to defects in chromosome segregation and the production of genetically unstable aneuploid cells. Although a link between cancer and aneuploidy has been established for decades, much debate has surrounded the precise role of aneuploidy in cancer. In 1914, Theodor Boveri proposed that aneuploid cells produced from defective mitosis are tumour progenitor cells [1]; however, this original hypothesis remained untested for decades. In recent years, new evidence has emerged which supports the hypothesis that aneuploidy is critically important in cancer initiation. Further investigation into the link between mitosis and aneuploidy has revealed that, in addition to a role for aneuploidy in oncogenesis, it can also act as a tumour suppressor, and the outcome is dependent on the extent of mitotic checkpoint silencing and aneuploidy induced. Thus manipulation of the molecular processes that contribute to tumour initiation to induce tumour cell death represents a novel therapeutic approach in cancer therapy.

The mitotic checkpoint

The mitotic checkpoint, also known as the spindle checkpoint, is the major cell-cycle-control mechanism. During mitosis, DNA that is replicated during S-phase of the cell cycle undergoes segregation whereby one chromosome copy is delivered to two cells, ensuring the production of genetically identical daughter cells. This is achieved through a process whereby sister chromatids are organized on the mitotic bipolar spindle and are attached through their kinetochores. Stable bipolar attachment of kinetochores to microtubules leads to the dissociation of the kinetochore-associated protein E; MTA, microtubule-targeting agent.

Key words: aneuploidy, centrosome, genome stability, mitotic checkpoint.

Abbreviations used: APC, anaphase-promoting complex; Bim, Bcl-2-interacting mediator of cell death; BRCA1, breast cancer 1 early-onset; Cdc20, cell division cycle 20; CENP-E, centromere-associated protein E; MTA, microtubule-targeting agent.

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The mitotic checkpoint protects against chromosome missegregation by delaying sister chromatid separation until all kinetochores are bound to the spindle. The checkpoint is activated by sensing kinetochores that lack bound microtubules or by lack of tension across attached kinetochores. Components of the mitotic checkpoint, which include members of the Bub and Mad family, generate a ‘wait anaphase’ signal, which delays the irreversible process of sister chromatid separation [2,3]. Conserved components of the mitotic checkpoint include Bub1, BubR1, Bub3, Mad1 and Mad2 [4]. Recruitment of Mad and Bub proteins to the unattached kinetochores is essential to delay mitotic progression by inhibiting the activity of Cdc20 (cell division cycle 20) which is required for activation of the APC (anaphase-promoting complex) [5]. When all kinetochores are completely attached to the spindle microtubules, mitotic checkpoint signals are relaxed and Cdc20 is released from an inhibitory complex. Cdc20 subsequently activates the APC E3 ubiquitin ligase activity, leading to ubiquitination of securin and activation of its binding partner separase, which cleaves the cohesins that links sister chromatids, leading to sister chromatid separation and the onset of anaphase [6].

Mitotic checkpoint status influences cell fate

The mitotic checkpoint was discovered in 1991 following the depolymerization of the mitotic microtubules leading to kinetochore detachment and generation of a checkpoint signal [7]. Thus the ability to activate the mitotic checkpoint in response to mitotic spindle depolymerizing agents has remained a common test of checkpoint competence. In contrast with the long-held view that the mitotic checkpoint is either ‘on’ or ‘off’, recent investigations into the molecular links between mitosis and cancer have revealed that partial loss of the mitotic checkpoint results in low levels of aneuploidy and promotes tumour initiation and progression, whereas complete loss of the mitotic checkpoint induces high levels of aneuploidy and lethality [8]. Thus differential checkpoint...
silencing has a profound outcome on cell fate; however, the molecular mechanisms underlying this phenomenon are not fully understood. Examination of mitotic checkpoint responses in a wide variety of tumour cells indicated that two thirds have a reduced capacity to maintain mitotic arrest when challenged with spindle poisons [9]. However, surprisingly, genes encoding checkpoint components are infrequently mutated in cancer, and altered expression of mRNA does not correlate well with chromosomal instability [10], therefore it is likely that checkpoint activity is affected by other mechanisms that remain unclear. It has been found that cells in which the mitotic checkpoint is weakened cannot prevent defective segregation of a single or a few chromosomes during mitosis. Examples include cells which have reduced levels of the centromere-associated protein CENP-E [11], and checkpoint proteins Bub1 [12] and Mps1 [10]. Reduced levels of CENP-E, which stimulates the mitotic checkpoint through the activation of BubR1 [13,14], leads to missegregation of one or a few chromosomes and is accompanied by an increase in spontaneous tumour formation \textit{in vivo} [11]. Furthermore, mice with a weakened checkpoint due to heterozygous loss of \textit{Mad2} [15] or \textit{BubR1} [16] develop spontaneous tumours with enhanced frequency. Thus a weakened mitotic checkpoint contributes to tumorigenesis and possibly promotes resistance to chemotherapy by allowing genetically defective cells to escape mitotic arrest and repopulate the tissue [9]. In contrast with this, complete inhibition of the mitotic checkpoint following depletion of essential checkpoint proteins, BubR1 and Mad2, results in large-scale chromosome loss and death in a variety of human cancer cells [17]. In addition, heterozygous loss of \textit{Cenp-E} increased the incidence of lymphomas of the spleen and adenomas of the lung \textit{in vivo}, however, resulting in decreased incidence of spontaneous tumours formed by carcinogens and loss of the p19/ARF (alternative reading frame) tumour suppressor, confirming that aneuploidy can act either oncogenically or as a tumour suppressor depending on the cell type and on the presence of additional genetic damage [8]. Thus defining the fundamental mechanisms that regulate checkpoint activity and the extent of aneuploidy induced is of paramount importance in order to delineate precisely how aneuploidy can lead to tumour initiation and tumour suppression.

The role of centrosome proteins in the maintenance of genome stability

The centrosome is a structurally conserved and complex organelle that acts as the major microtubule nucleating centre in mammalian cells and comprises centrioles embedded in the pericentriolar matrix which contains the γ-tubulin ring complex [18]. The centrosome plays an essential role in cell division by nucleating mitotic spindle microtubules and by acting as a scaffold for many cell-cycle-regulatory proteins [19]. During S-phase of the cell cycle, centrosome duplication takes place, and the centrosomes establish the mitotic spindle poles required for cell division. In addition to a role in mitosis, centrosomes nucleate microtubules in interphase cells to maintain cell morphology and polarity. Centrosome abnormalities have been observed in a wide range of tumour cells and include an increase in number and volume, supernumerary centrioles, accumulation of excess pericentriolar material and aberrant phosphorylation of centrosomal proteins [20]. These changes have been observed in early stages of cancer development and are believed to create a phenotype that promotes neoplastic transformation and correlate with poor clinical outcome in some cases. Furthermore, several oncogenes and tumour suppressors affect centrosome function [19,20]. Hence, aberrant centrosome function has serious consequences for genetic integrity and cellular transformation.

The identification and functional characterization of additional centrosome components is important for delineating mechanisms of genome stability and understanding precisely how deregulated centrosome function may contribute to tumour initiation. The mammalian centrosome contains at least 150 proteins [21], including cell-cycle proteins and tumour suppressors [p53, Rb (retinoblastoma protein), LAPSER (named after the single-letter codes of the amino acids in which it is rich), katanin, BRCA1 (breast cancer 1 early-onset)] [22–25] that prevent proliferation of genetically defective cells, thus emphasizing its role as ‘guardian of the genome’. Centrosome defects that lead to genome instability emerge as a result of cytokinesis failure, mitotic slippage, cell fusion and DNA replication or repair defects [20,21,24]. For example, Aurora A and C are localized to the centrosome and are key regulators of cytokinesis [26,27]. The breast cancer tumour suppressor BRCA1 is localized to centrosomes, and BRCA1 inhibition leads to abnormal centrosome number and defective function [25]. Disruption of the death inducer-obliterator (\textit{Dido}) gene, which causes myelodysplastic syndrome, leads to centrosome amplification and mitotic checkpoint defects, leading to compromised chromosome stability [28]. Furthermore, the peptidylprolyl isomerase, pin1, is located at the centrosome in mammalian cells where it regulates centrosome duplication. Pin1 binds and regulates a number of cell-cycle regulators [29–31]. This discovery has raised renewed interest in the importance of prolyl \textit{cis-trans} isomerization as a regulator of cell proliferation and as a new target for therapeutic intervention [32,33].

Targeting mitosis in cancer therapy

During M-phase of the cell cycle, the replicated chromosomes are segregated on the mitotic spindle and the cell subsequently undergoes nuclear division (mitosis) and cytoplasmic division (cytokinesis), producing two identical daughter cells. The mitotic spindle is made up of microtubules and microtubule-associated proteins that play an essential role in spindle pole assembly and chromosome segregation. Microtubules are dynamic cylindrical structures that comprise polymers of α- and β-tubulin, bound in a head-to-tail manner by non-covalent interactions. Microtubules assemble and disassemble in a GTP-dependent process and transmit signals throughout the cell. The orientation of α- and β-subunits...
creates polar polymers with distinct plus and minus ends [34]. The minus end of the microtubules associate with the centrosome MTOC (microtubule-organizing centre), which forms the mitotic spindle poles, whereas the plus end of the microtubules are free in the cell cytoplasm where they play a role in the maintenance of cell polarity and structure and act as an important intracellular protein transport mechanism [34].

The essential role of microtubules in mitosis has made them an attractive target for cancer chemotherapy, and MTAs (microtubule-targeting agents) have been used in cancer treatment for decades and are effective against a broad range of tumours [35,36]. MTAs are divided into two main groups: the vinca alkaloids which inhibit microtubule polymerization and the taxanes which promote microtubule polymerization (including paclitaxel and docetaxel) [34]. The action of MTAs, through suppression or stabilization of microtubule polymerization, disrupt microtubule dynamics, and thereby microtubule function, and block cell-cycle progression from prometaphase/metaphase to anaphase, leading to cell-cycle arrest. Failure to proceed through the cell cycle leads to death of the cells, either through apoptosis induced following mitotic arrest or cell death can occur following aberrant mitotic exit, also called mitotic slippage, in a G1-like multinucleated state. Alternatively, defective cells that survive the G1 block can re-enter mitosis and die through mitotic catastrophe [34–37]. The precise mechanisms of cell death are being explored, but are thought to be dependent on the concentration of MTA, time of exposure and the cell type [34]. Elucidation of the precise signalling mechanisms that connect mitotic arrest to the proteolytic cell death machinery will unveil new sites of potential intervention. For example, microtubule disruption is thought to cause the release of important signalling molecules that are sequestered in the cell microtubule network such as survivin [34,38] and Bim (Bcl-2-interacting mediator of cell death) [34,39,40]. Bim is a pro-apoptotic BH3 (Bcl homology 3)-only member of the Bcl-2 family, which is associated with DLC (dynein light chain) and is sequestered to the microtubule-associated dynein motor complex [39]. The release of Bim from microtubules causes it to translocate to mitochondria where it promotes apoptotic cell death by inhibition of the anti-apoptotic Bcl-2 proteins [40].

Because microtubules are involved in a wide array of cellular functions, such as intracellular protein transport, membrane scaffolding and cell signalling, microtubule-interfering agents affect important processes during interphase as well as mitosis. The lack of selectivity towards tumour cells leads to toxic side effects and a low therapeutic index for many MTAs. Thus targeting cell components that only play a role in mitosis could display fewer side effects than observed with MTAs. The identification of additional mitotic targets that could be exploited in novel drug design programmes will provide new opportunities in cancer research. The Aurora kinase protein family represent a family of conserved serine/threonine kinases that play multiple roles in mitosis, and loss of activity affects a variety of mitotic events, including bipolar spindle formation, histone H3 phosphorylation, chromosome segregation and cytokinesis [41–44]. Cycling cells have been shown to rapidly lose viability in the presence of Aurora inhibitors whereas non-dividing cells remain viable, suggesting that Aurora kinase inhibitors may be selectively toxic to proliferating tumour cells [45]. In addition, small-molecule inhibitors of the mitotic spindle motor protein, kinesin-5 (KSP (kinesin spindle protein)), which is essential for centrosome separation and bipolar spindle formation, exhibit anti-tumour activity superior to paclitaxel in vivo and have entered clinical trials for the treatment of taxane-refractory cancer [46].

Targeting the mitotic checkpoint is a promising therapeutic strategy that can sensitize cancer cells to genotoxic stress due to a compromised checkpoint response. Synthetically lethal interactions between two genes lead to cell death when both are mutated; however, mutation in either alone is compatible with viability [47]. Many tumour cells harbour defects in their mitotic checkpoint machinery [9,10], therefore targeting another component of that pathway may induce tumour cell lethality while having little effect on normal cells. Thus the discovery of additional synthetic lethal interactions in the mitotic checkpoint machinery of a given tumour type represents an important development that will provide new therapeutic opportunities in cancer therapy.

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References
