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THE ROLE OF PROLONGED MITOTIC CHECKPOINT ACTIVATION IN THE FORMATION AND TREATMENT OF CANCER

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SUMMARY

Mitotic abnormalities are a common feature of human cancer cells, and recent studies have provided evidence that such abnormalities may play a causative, rather than merely incidental role, in tumorigenesis. One such abnormality is prolonged activation of the mitotic checkpoint, which can be provoked by a number of the gene changes which drive tumor formation. At the same time, antimitotic chemotherapeutics exert their clinical efficacy through the large-scale induction of prolonged mitotic checkpoint activation, indicating that mitotic arrest is influential in both the formation and treatment of human cancer. However, how this influence occurs is not well-understood. In this perspective, we will discuss the current evidence in support of the potential mechanisms by which prolonged activation of the mitotic checkpoint affects both tumorigenesis and antimitotic chemotherapy.

Keywords

Aneuploidy; Antimitotic chemotherapy; Apoptosis; Cell cycle arrest; Centrosomes; Chromosomal instability; Checkpoint; DNA damage; Mitosis; Polypoidy; Tumorigenesis

Mitosis is the process whereby a eukaryotic cell divides to produce two identical copies of itself. Such division is vital to the development and homeostasis of multicellular organisms. However, the necessity of mitosis comes at a price, as errors in this process can lead to the generation of abnormal cells. In fact, such errors have long been associated with cancer, ever since the German pathologist David von Hansemann first described abnormal mitotic figures in human tumors more than a century ago [1]. Since that time, the list of mitotic abnormalities observed in cancer cells has expanded, and now includes defects in chromosome-spindle attachment, chromosome congression, spindle structure, mitotic checkpoint signaling, chromosome segregation, and cytokinesis [2–5]. Moreover, recent functional studies have shown that, rather than representing mere bystanders in the transformation process, many of these mitotic abnormalities likely promote tumorigenesis [3–5]. At the same time, a class of drugs used widely in the chemotherapeutic treatment of cancer—the antimitotics—exert their toxicity by directly disrupting mitosis [6,7]. Thus, disturbances of mitosis can play important roles in both the formation and treatment of cancer. However, the mechanisms underlying these roles are incompletely understood [4,6]. In this review, we will examine the importance of one

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type of mitotic abnormality in the biology of tumorigenesis and antimitotic chemotherapy: prolonged activation of the mitotic checkpoint.

Prolonged activation of the mitotic checkpoint in cancer cells

Mitosis is traditionally divided into five phases. In prophase, chromosomes condense and centrosomes move to opposite poles of the cell. In prometaphase, the nuclear envelope disassembles, and mitotic spindles capture and congress chromosomes to the equator of the cell. In metaphase, congression is completed, and chromosomes signal the cell that they are ready to divide. In anaphase, sister chromatids separate and egress to opposite poles of the cell. And in telophase, the nuclear membrane reassembles, chromosomes decondense, and cytokinesis produces two equivalent daughter cells.

A central regulator of this process is the mitotic checkpoint, a signaling mechanism which arrests the progression from metaphase to anaphase until all chromosomes have achieved proper attachment to mitotic spindles [3,6]. A diffusible “wait anaphase” signal is generated at the kinetochores of unattached chromosomes, and this signal is extinguished once all kinetochores properly attach to spindles [3,6]. Thus, sister chromatids are separated only once they are in a position to be distributed equally to daughter cells. Accordingly, the mitotic checkpoint serves to prevent chromosome missegregation.

In cancer cells, activation of this checkpoint is frequently prolonged [8,9]. The first evidence of this came from pathologists, who recognized that tumors not only possess higher mitotic indices than normal tissue, but they also exhibit a higher ratio of prometaphase/metaphase to prophase cells [8]. Because this ratio should reflect the relative durations of these phases of mitosis, this observation suggested that prometaphase/metaphase is prolonged in cancer cells [8]. Subsequently, timelapse videomicroscopy studies revealed that mitosis can indeed be lengthened up to 4-fold in transformed cells, as compared to normal cells [10,11]. More recently, Yang et al. have confirmed these initial findings and further demonstrated that the prolongation is dependent on the mitotic checkpoint, as impairment of mitotic checkpoint signaling reduced the length of mitosis in transformed cells [9]. Taken together, these studies demonstrate that cancer cells commonly possess defects which prolong mitosis by delaying satisfaction of the mitotic checkpoint.

Recently, several causes of prolonged activation of the mitotic checkpoint in human cancer cells have been described (Table 1). Inactivation of the Rb tumor suppressor has been shown to transcriptionally induce supernormal expression of the mitotic checkpoint protein Mad2, which in turn prolongs prometaphase by directly inhibiting the anaphase promoting complex [12,13]. Analogously, inactivation of the hCDC4 tumor suppressor decreases the degradation of cyclin E, whose abnormal accumulation lengthens mitosis through inhibition of the anaphase promoting complex [14,15]. Downregulation of other putative tumor suppressors, such as p120, B-Myb, CHC, and SBDS, have similar effects [16–19]. Likewise, activation of oncogenes, like c-Myc, B-Raf, Hec1, and E7, has also been reported capable of inducing mitotic arrest [20–23]. Finally, Yang et al. recently found that prolongation of the mitotic checkpoint can result from the simple presence of extra chromosomes and/or centrosomes, both of which are common features of cancer cells [9]. By extension, processes which create supernumerary chromosomes and/or centrosomes, such as polyploidization and centrosome overduplication, could be considered to indirectly increase the frequency of prolonged mitosis. Thus, multiple mechanisms can delay timely satisfaction of the mitotic checkpoint in cancer cells.

As is the case for other abnormalities observed in cancer cells, it is important to consider whether prolonged mitotic checkpoint activation is a causative factor in, rather than an incidental consequence of, tumorigenesis. Although events like inactivation of the Rb, oncogenic activation of c-Myc, and development of polyploidy and centrosome instability are

common events in human cancers [9,12,20], prolonged mitotic checkpoint activation is only one of many potentially oncogenic phenotypes created by these genetic and cellular changes. That said, Sotillo et al. have shown that isolated overexpression of Mad2, a protein whose principal function is to participate in the maintenance of the "wait anaphase" signal, induces not only prolonged activation of the mitotic checkpoint, but also dramatic incidences of spontaneous tumors in mice [13]. Similarly, overexpression of the kinetochore protein Hec1, which is observed in human tumors, induces both hyperactivation of the mitotic checkpoint and increased incidences of cancer in mice [23]. These studies thus provide evidence that prolonged activation of the mitotic checkpoint can directly promote tumorigenesis. The mechanisms by which this tumor promotion occurs, however, are poorly understood.

Prolonged activation of the mitotic checkpoint by antimitotic drugs

In addition to cellular and genetic defects which arise spontaneously to disrupt mitosis in cancer cells, antimitotic drugs can also cause prolonged activation of the mitotic checkpoint [6,7]. Agents like nocodazole, colchicine, and the vinca alkaloids induce mitotic arrest by destabilizing mitotic spindles [7]. Others, like taxanes and epothilones, cause arrest by pathologic stabilization of spindles [7]. Moreover, newer agents have been developed which provoke arrest through inhibition of mitotic motor proteins, such as the kinesins Eg5 and CENP-E [7]. In each case, these antimitotic agents not only induce prolonged activation of the mitotic checkpoint, but they also exhibit significant cytotoxicity. Indeed, many of these agents are used routinely in cancer chemotherapy, and others are in various stages of clinical trials [7]. Interestingly, induction of mitotic arrest appears necessary for the cytotoxicity of these agents, demonstrating that prolonged mitotic checkpoint activation is important in the treatment, as well as formation, of cancer [24]. However, like tumorigenesis, the mechanisms by which prolonged mitotic arrest provoke cytotoxicity are not well defined.

Cell fate following prolonged activation of the mitotic checkpoint

In order to understand how prolonged activation of the mitotic checkpoint might affect cancer formation and treatment, it is important to first consider the fate of cells following mitotic arrest. Recent studies with antimitotic agents have shown that several possible fates can befall cells that endure prolonged activation of the mitotic checkpoint [24–26]. First, cells may eventually complete cell division after a mitotic delay [26]. Such divisions are frequently accompanied by missegregation of chromosomes, thereby leading to aneuploidy [26,27]. Second, cells can undergo "mitotic slippage," a process whereby mitotic cells return to interphase without completing anaphase [6,26]. Because in this case cell division does not occur, the resulting interphase cells, which are often referred to as "postmitotic," will possess twice the normal number of chromosomes, making them tetraploid [6]. After either of these first two fates, cells will then realize one of three additional fates: continuation of the cell cycle, cell death, or cell cycle arrest [6,25,26]. Finally, a third fate following prolonged checkpoint activation is mitotic cell death, where cells die directly from the mitotic state [6,25,26].

What determines which of these fates a cell will meet? Gascoigne and Taylor have recently shown that the answer to this question is surprisingly complex, as both genetic and nongenetic factors create profound variation in the fates of cells following prolonged mitotic arrest [26]. Amid this complexity, however, these authors provide evidence that the first fate decision point —whether a cell will exit from, or die in, prolonged mitosis—is controlled by stochastic competition between progressive degradation of cyclin B1 and progressive activation of a caspase-dependent death pathway [26]. Cyclin B1 is required for maintenance of the mitotic state, and its degradation is controlled by the anaphase promoting complex. Thus, if cyclin B1 is degraded to a critical threshold level first, the mitotic cell will undergo slippage or, if possible, cell division; if activation of caspase-dependent cell death reaches a critical threshold first, the

cell will die in mitosis [26]. A plausible explanation for the progressive degradation of cyclin B1 during mitotic arrest is “leaky” activity of the anaphase promoting complex [28]. The mechanisms responsible for progressive activation of a mitotic cell death pathway are less understood, although progressive dephosphorylation of caspase 9 is an attractive possibility [26,29]. Additionally, Gascoigne and Taylor suggest that accumulation of DNA damage during mitotic arrest might be one cause of such a death signal [26], an idea which will be discussed in greater detail later.

For those cells which exit from, rather than die in, prolonged mitotic arrest, knowledge of the determinants of their subsequent fates is rather limited [25]. However, there is one node of regulation which has been characterized in several cell systems: control of the decision between postmitotic cell cycle arrest and continuation of the cell cycle by the tumor suppressor p53 [30]. Numerous studies have shown that in both human and mouse cells of various tissue origin, p53 induces cell cycle arrest in postmitotic cells made tetraploid through mitotic slippage following antimitotic-induced mitotic arrest [31–34]. This p53-dependent mechanism is known as the “postmitotic checkpoint,” and biochemical investigations have revealed that elements of the p53-dependent DNA damage checkpoint, such as transcriptional induction of p21, inhibition of cyclin E/cdk2, and hypophosphorylation of Rb, are also activated and/or required in the postmitotic checkpoint [32,35–38]. Thus, like its role in activating growth arrest following DNA damage, p53 inhibits the cell cycle of cells made tetraploid following prolonged mitotic arrest.

Of note, the role of p53 in the regulation of apoptosis following prolonged mitotic arrest is more complex. Although some studies have found that p53 promotes cell death following prolonged mitotic checkpoint activation [39–42], others have found that p53 is uninvolved in death signaling [35,43,44]. To complicate matters further, another study has found that p53 promotes cell survival, not death, following mitotic arrest [45]. The discrepancies between these studies may be attributable to cell-type differences in p53 activity, as tissue of origin has been shown to greatly influence the consequences of p53 inactivation [46]. Thus, it can be concluded that while the p53-dependent imposition of growth arrest following mitotic arrest appears to be a ubiquitous phenomenon, the role of p53 in postmitotic cell death appears to depend on the genetic background of the postmitotic cells.

Consequences of postmitotic cell fates

We have seen that a variety of cell fates can follow prolonged activation of the mitotic checkpoint, and that these fates are controlled by apoptotic and cell cycle arrest pathways. Mammalian cells therefore appear to have evolved mechanisms for suppressing or eliminating cells which have experienced prolonged activation of the mitotic checkpoint. As a consequence, these observations suggest that the cytotoxicity of antimitotic agents results, at least in part, from activation of these intrinsic checkpoint mechanisms. However, these data also raise a more fundamental question: what is it about prolonged activation of the mitotic checkpoint that necessitates the suppression or elimination of cells which have experienced it? By extension, why might cells that escape these controls be at greater risk of transformation? As we shall see, there are at least three, non-mutually exclusive answers to this question.

Aneuploidy and prolonged mitotic checkpoint activation

One consequence of prolonged mitotic checkpoint activation that may contribute to tumorigenesis is an increased frequency of chromosome missegregation, or aneuploidy. Indeed, many of the oncogenic changes which induce prolonged mitotic checkpoint activation also increase aneuploidy [13,14,23]. In some of these cases, such as overexpression of Mad2 or inactivation of hCDC4, the precise cause of increased chromosome missegregation is not understood [13,14]. In others, such as the presence of supernumerary centrosomes, the cause

appears to be an increased frequency of merotely [47]. Regardless of their source, such alterations in chromosome number could, in turn, produce cells with increased copy numbers of oncogenes and/or decreased copies of tumor suppressors [48,49]. Although shuffling chromosomes into the "right" oncogenic combination might be an infrequent event, even its rare occurrence might be sufficient to promote tumorigenesis over time [48,49]. In support of this concept, mouse studies have shown that aneuploidy can, in and of itself, facilitate tumor growth in vivo [48]. Thus, one way that prolonged mitotic checkpoint activation may promote cancer is by altering the copy numbers of genes which regulate tumorigenesis.

While aneuploidy might occasionally create such tumorigenic combinations of gene dosages, chromosome imbalances might be expected more frequently to result in neutral or even cytotoxic gene combinations [48,49]. Thus, in many cases, chromosome missegregations following prolonged mitotic checkpoint activation might be expected to impair, rather than promote, the growth of cancer cells. This may be of relevance to antimitotic therapy, as shorter-term and lower-dose treatments with antimitotic agents can induce not only apoptosis, but also aneuploidy through an increased frequency of merotely [26,27]. Indeed, Weaver et al. have demonstrated that aneuploidy can both promote and inhibit tumor growth, depending on the biological context [48], and Thompson et al. have shown that short-term antimitotic treatments can produce aneuploid cells that, while initially viable, show poor long-term proliferative capacity [27]. Thus, in addition to induction of mitotic cell death, introduction of aneuploidy following prolonged mitotic checkpoint activation may represent one of the cytotoxic mechanisms of antimitotic drugs.

Polyploidy and prolonged mitotic checkpoint activation

Analogous to aneuploidy, the induction of polyploidy may also represent a tumorigenic consequence of prolonged activation of the mitotic checkpoint. As we have seen, the formation of tetraploid cells through mitotic slippage is one outcome of mitotic arrest, and the long-term proliferation of these cells is facilitated by the inactivation of p53 [6,26]. Interestingly, several studies have provided evidence that, like aneuploidy, tetraploidy can itself directly promote tumorigenesis, particularly in the context of p53 mutation [50–52]. While the mechanism of this tumor promotion is unclear, it may result from an increased chance of oncogenic mutations due to extra copies of proto-oncogenes, the provision of a "genetic buffer" that allows better toleration of otherwise deleterious mutations, and/or the promotion of aneuploidy resulting from an increased number of centrosomes [5,47,53].

Also analogous to aneuploidy, polyploidy induced by mitotic arrest may be of relevance to antimitotic chemotherapy. Although it can, as discussed above, promote tumorigenesis in some contexts, polyploidy is a condition which, as a general rule, restricts the proliferative potential of cells, even many cancer cells [50,54]. Thus, polyploidization through mitotic slippage may represent another the mechanism by which antimitotics exert their cytotoxicity.

DNA damage and prolonged mitotic checkpoint activation

In addition to inducing changes in chromosome number, conditions which prolong activation of the mitotic checkpoint have also been associated with structural chromosome aberrations [13,14,20]. For example, mice overexpressing Mad2 exhibit not only aneuploidy, but also an increase in chromosome fragments and translocations [13]. Similarly, inactivation of the hCDC4 tumor suppressor produced not only prolonged mitosis and aneuploidy, but also an increase in micronuclei which contained acentric chromosome fragments [14]. Similar associations have been observed with activation of the c-Myc oncogene and overexpression of cyclin E [15,20,55]. These studies thus demonstrate that defects which elicit prolonged activation of the mitotic checkpoint also produce evidence of increased spontaneous DNA damage.

Are prolonged mitosis and DNA damage merely correlated, or are they causally related? Recently, several lines of evidence have supported the latter possibility. In their investigation of the mechanism of the p53-dependent postmitotic checkpoint, Wong and Stearns reported that human cells which had been presynchronized with double-thymidine and nocodazole later possessed foci of γ-H2AX, the phosphorylated form of H2AX that forms around sites of double-stranded DNA breaks [56]. Because nocodazole is a microtubule-destabilizing drug which prolongs activation of the mitotic checkpoint, this study prompted our laboratory to investigate whether prolonged activation of the mitotic checkpoint can directly provoke DNA damage [57]. We found evidence that, in multiple human cell types, DNA breaks were indeed elicited by mitotic arrest, whether induced by antimitotic drugs, knockdown of a mitotic kinesin protein, or spontaneously-arising defects of spindle pole number [57]. Moreover, we found that these breaks occurred independently of cell death, and could subsequently manifest as structural chromosome aberrations [57]. Similarly, other groups have reported that prolonged activation of the mitotic checkpoint is accompanied by evidence of DNA damage [24,58,59]. Taken together, these studies indicate that mitotic arrest, induced through multiple mechanisms, may provide a source of structural, as well as numerical, chromosome alterations. As it is well-established that structural DNA changes, ranging from single-base mutations to chromosome translocations, are primary drivers of tumorigenesis [53], these observations suggest that another way that prolonged activation of the mitotic checkpoint may contribute to tumorigenesis is by introduction of DNA damage.

Because infliction of DNA damage is also a well-known and clinically-exploited stimulus of cancer cell death, the provocation of DNA damage by mitotic arrest may represent an additional cytotoxic mechanism of antimitotic agents. This finding may have several important clinical implications. First, it is possible that the sensitivity of a tumor to antimitotic chemotherapy could be influenced by its propensity to acquire DNA damage during mitotic arrest, as well as its ability to repair such damage. Indeed, Swanton et al. have recently reported that the expression level of several genes involved in DNA repair may be determinants of tumor sensitivity to the antimitotic paclitaxel [60]. Second, strategies to maximize the impact of DNA damage during mitotic arrest may improve the efficacy of antimitotic therapy. Along these lines, combining antimitotics with specific inhibitors of DNA repair, such as the recently-developed PARP inhibitors, might be promising [61]. Finally, the ability of antimitotic agents to induce structural chromosomal instability suggests that a long-term consequence of these drugs may be the acceleration of further tumor evolution in those cells which survive treatment, as well as a carcinogenic risk to non-cancerous cells. Indeed, there is evidence that chemotherapeutic use of the antimitotic agent paclitaxel may increase the secondary risk of developing acute myelogenous leukemias which bear predictable chromosomal translocations [62–64].

CONCLUSION

Abnormalities of mitosis frequently occur in cancer cells, and there is now evidence that causally implicates these abnormalities in the process of tumorigenesis. One such abnormality is prolonged activation of the mitotic checkpoint, which is among the effects of several oncogenic changes important in the pathogenesis of human tumors. At the same time, antimitotic chemotherapeutics kill cancer cells by provoking the deleterious consequences of prolonged mitotic checkpoint activation. Precisely how mitotic arrest contributes to the formation and chemotherapeutic treatment of cancer is incompletely understood, but current evidence suggests that at least three mechanisms may be important: aneuploidy, polyploidy, and structural DNA damage. Further characterization of these phenomena should improve our understanding of tumorigenesis, and may suggest novel diagnostic and therapeutic approaches to human cancer.

FUTURE PERSPECTIVE

Although extensive research in recent years has provided great insight into how prolonged activation of the mitotic checkpoint affects the formation and treatment of cancer, much future work is needed to answer important mechanistic questions. We anticipate that progress will be made in identifying additional molecular mechanisms responsible for mitotic arrest in human cancer cells, as well as any clinically-exploitable phenotypic subtleties that may be unique to individual mechanisms. We also anticipate that greater insight will be attained into the biochemical pathways which determine cell fate decisions following prolonged mitotic checkpoint activation, the mechanisms which generate DNA damage during mitotic arrest, and the relative importance of aneuploidy, tetraploidy, and structural chromosome aberrations in tumorigenesis and antimitotic chemotherapy. If such progress can be made, we further anticipate that elucidation of these mechanisms will aid in the development of more precise diagnostic and prognostic approaches to—as well as more rational and individualized treatment of—human cancer.

EXECUTIVE SUMMARY

Prolonged activation of the mitotic checkpoint in cancer cells

- Mitotic abnormalities are a common feature of human cancer cells, and recent studies have provided evidence that such abnormalities may play a causative, rather than merely incidental role, in tumorigenesis.
- One such abnormality is prolonged activation of the mitotic checkpoint, which can be provoked by a number of the gene changes which drive tumor formation.

Prolonged activation of the mitotic checkpoint by antimitotic drugs

- At the same time, antimitotic chemotherapeutics exert their clinical efficacy through the large-scale induction of prolonged mitotic checkpoint activation.

Cell fate following prolonged activation of the mitotic checkpoint

- Several fates can befall cells which experience prolonged mitotic checkpoint activation, including cell death, cell cycle arrest, aneuploidization, and polyploidization.

Consequences of postmitotic cell fates

- Current evidence suggests that at least three consequences of prolonged mitotic checkpoint activation may contribute to tumorigenesis and antimitotic cytotoxicity: aneuploidy, polyploidy, and structural DNA damage.
- Further characterization of these phenomena should improve our understanding of tumorigenesis, and may suggest novel diagnostic and therapeutic approaches to human cancer.

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Table 1

Cancer-associated causes of prolonged activation of the mitotic checkpoint.

Tumor suppressors	**Mechanism**	**References**
Rb	Upregulation of Mad2	12,13
hCDC4	Impaired cyclin E degradation	14,15
SBDS	Destabilization of mitotic spindles	16
p120	Spindle disorganization?	17
B-Myb	Disruption of Myb-Clafi complex	18
CHC fusion proteins	Destabilization of mitotic spindles	19
Oncogenes		
c-Myc	Upregulation of Mad2 & BubR1	20
B-Raf	Stabilization of Mps1	21
E7	Dynein & NuMA delocalization	22
Hec1	Destabilization of chromosome-spindle interactions?	23
Other		
Extra chromosomes	Increased attachment time?	9
Extra centrosomes	Resolution of spindle intermediates?	9

? represents speculated mechanism.