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Engaging Anaphase Catastrophe Mechanisms to Eradicate Aneuploid Cancers

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Abstract

Cancer cells often have supernumerary centrosomes that promote genomic instability, a pathognomonic feature of cancer. During mitosis, cancer cells with supernumerary centrosomes undergo bipolar cell division by clustering centrosomes into two poles. When supernumerary centrosome clustering is antagonized, cancer cells are forced to undergo multipolar division leading to death of daughter cells. This proapoptotic pathway, called anaphase catastrophe, preferentially eliminates aneuploid cancer cells and malignant tumors in engineered mouse models. Anaphase catastrophe occurs through the loss or inhibition of the centrosomal protein CP110, a direct cyclin dependent kinase 1 (CDK1) and CDK2 target. Intriguingly, CP110 is repressed by the *KRAS* oncoprotein. This sensitizes *KRAS*-driven lung cancers (an unmet medical need) to respond to CDK2 inhibitors. Anaphase catastrophe-inducing agents like CDK1 and CDK2 antagonists are lethal to cancer cells with supernumerary centrosomes, but can relatively spare normal cells with two centrosomes. This mechanism is proposed to provide a therapeutic window in the cancer clinic following treatment with a CDK1 or CDK2 inhibitor. Taken together, anaphase catastrophe is a clinically-tractable mechanism that promotes death of neoplastic tumors with aneuploidy, a hallmark of cancer.

Keywords

anaphase catastrophe; aneuploidy; CDK1; CDK2; *KRAS* oncoprotein; and antineoplastics

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Disclosure of conflict of interest

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Introduction

For duplicated chromosomes to segregate faithfully into two daughter cells during cell division, proper bipolar spindle formation is required (1,2). There must be precisely two centrosomes that serve as spindle poles during cell mitosis (1,2). While centrosome numbers are tightly controlled in normal cells, aberrant centrosome numbers are detected as frequent features of both solid and hematological cancers (3–7). This is associated with genomic instability (8), a hallmark of cancer (9,10). Excessive centrosomes in cancer cells lead to multipolar spindle assembly, causing asymmetric chromosome segregation and aneuploidy in daughter cells after multipolar cell division (4,11–13). This contributes to tumor initiation or evolution (14–19) by increasing the proliferative advantage of some cellular populations through the loss of a chromosome domain that contains tumor suppressor genes or by gain of a region containing oncogenes (8,20–23). The appearance of supernumerary centrosomes is associated with tumor progression and an unfavorable clinical outcome (7,11). This links centrosome alterations to cancer progression (24).

There is also another important aspect of the biology of supernumerary centrosomes. Daughter cancer cells with excessive aneuploidy after multipolar cell division of parental cells with supernumerary centrosomes can compromise their survival (4,25–27). Cancer cells circumvent these detrimental effects through several mechanisms. One involves clustering of supernumerary centrosomes into two spindle poles during mitosis so that they preserve bipolar spindle assembly and engage bipolar mitosis (25,28–31). Several other pathways and regulators are involved in this process including motor proteins, centrosomal proteins, kinetochore proteins, spindle assembly checkpoint proteins, microtubule associated proteins, and components of the actin cytoskeleton (25,29,32,33).

Herein, we describe a distinct type of mitotic catastrophe called anaphase catastrophe. This is conferred by inhibition of centrosome clustering in cells with supernumerary centrosomes. It causes death of daughter cells after forcing them to undergo multipolar cell division (34,35). As supernumerary centrosomes are not typically found in normal diploid cells with some exceptions like polyploid hepatocytes (36,37), targeting centrosome clustering would theoretically affect only chromosomally-unstable cancer cells while sparing normal cells from exhibiting anaphase catastrophe (25,33–35,38,39). Given this, inducing anaphase catastrophe is an attractive strategy to explore for cancer therapy (35,40–42). Interestingly, several agents that antagonize cyclin-dependent kinase 1 (CDK1) or CDK2 activities were reported to cause anaphase catastrophe in cancer cells (34,43,44).

In this review, the molecular mechanisms that are the basis for activating anaphase catastrophe following CDK2 or CDK1 antagonism are presented. Anaphase catastrophe confers apoptotic death of cancer cells while relatively sparing normal cells, as will be discussed. Intriguingly, this mechanism is preferentially engaged in *KRAS* mutant oncoprotein-expressing lung cancer (34,35,44). The mechanistic basis for this association will be discussed. Furthermore, the translational research implications of this finding will be highlighted.

Anaphase catastrophe

CDK1 or CDK2 inhibition induces anaphase catastrophe

The association between anaphase catastrophe and CDK2 inhibition was first uncovered after treating lung cancers with seliciclib (CYC202, Cyclacel), an orally bioavailable and fully reversible inhibitor of CDK2 activity with less evident effects against CDK5, CDK7, and CDK9 activity (IC₅₀ for CDK2: 100 nM, CDK5: 160 nM, CDK7: 540nM, and CDK9: 950 nM) (34,35,44–47). When aneuploid lung cancer cells were exposed to seliciclib, irreversible anti-proliferative effects were unexpectedly observed (34). In the pursuit of a mechanism responsible for these surprising irreversible actions, seliciclib was found to promote multipolar spindle formation during cell mitosis (34). This process does not disrupt the temporal sequence of cell mitosis, but forces cells to undergo multipolar cell division leading to apoptosis of daughter cells (34,35,48), as shown in Figure 1A. Since apoptosis was triggered by this aberrant mitosis after anaphase, this proapoptotic death program was called anaphase catastrophe (34,35). Live-cell imaging and cytochrome C staining of the progeny of seliciclib-treated cells revealed that affected cells with multipolar anaphases underwent apoptosis (43,48). This indicated that anaphase catastrophe caused apoptosis after engaging cell division (48).

The major molecular pharmacologic target of seliciclib is CDK2 activity (45–47). Given this, it was hypothesized that CDK2 inhibition was responsible for conferring anaphase catastrophe. To study the direct and specific effects of CDK2 inhibition, CDK2 was genetically-targeted for repression by transfection of siRNAs into lung cancer cells. Anaphase catastrophe was observed by this knockdown of CDK2 and this finding independently confirmed the pharmacologic effects of CDK2 antagonists (34,44). In addition to seliciclib, anaphase catastrophe engagement was observed using other selective CDK2 or pan-CDK inhibitors, that include dinaciclib (SCH727965, Merck) (IC₅₀ for CDK1: 3 nM, CDK2: 1 nM, CDK5: 1 nM, and CDK9: 4 nM) (43,49), CCT68127 (Cyclacel) (IC₅₀ for CDK2 and 30 nM, CDK9: 110 nM) (44) and CYC065 (PubChem ID: 24983461, Cyclacel) (IC₅₀ for CDK2: 5 nM and CDK9: 26 nM) (44). Notably, anaphase catastrophe induced after CDK2 inhibitor treatment was observed not only in lung cancer cells, but also in preliminary evidence from other aneuploid cancer cells (unpublished observations from Ethan Dmitrovsky laboratory). This indicates that anaphase catastrophe is a common pathway engaged in chromosomally-unstable cancer cells.

Because specific inhibitors can affect multiple CDKs in addition to CDK2, the potential contribution of other CDKs in mediating anaphase catastrophe were investigated directly by downregulating CDK1, CDK5, and CDK9 individually using independent siRNAs. Knockdown of CDK1 was shown to augment anaphase catastrophe as did CDK2 repression (43).

Anaphase catastrophe occurs by inhibiting centrosome clustering

Since multipolar spindle formation is linked to the presence of supernumerary centrosomes, spindle formation and centrosome number were each analyzed by respective α -tubulin and γ -tubulin staining of cancer cells following treatment with vehicle or with specific CDK2

inhibitors (50). Using these methods, it was found that anaphase catastrophe occurs via inhibition of supernumerary centrosomes clustering after treatment with CDK2 inhibitors, as shown in Figure 1B.

The examined lung cancer cells after the treatments were divided into three groups based on centrosome and spindle numbers at anaphase, as depicted in Figure 1C. The first includes bipolar normal cells with two centrosomes and spindles (N cells). The second describes cells with supernumerary centrosomes clustered into two poles with bipolar spindles formation (C cells). In this type of cell, chromosomes are segregated equally, despite an aberrant number of centrosomes. The third represents cells with supernumerary centrosomes and multipolar spindle formation without centrosome clustering (M cells). This type of cell undergoes multipolar cell division and anaphase catastrophe is subsequently conferred.

It was uncovered that the total population of cells with supernumerary centrosomes (the sum of the number of C and M cells) was unaffected by treatment with a selective CDK2 inhibitor, as indicated in pie charts in Figure 1C. This indicated that a CDK2 inhibitor does not cause *de novo* supernumerary centrosomes formation. However, the population of C cells, in which supernumerary centrosomes are clustered into two poles, was reduced and that of M cells, which have multipolar spindles without centrosome clustering, was correspondingly increased after the CDK2 inhibitor treatment, as depicted in the pie charts displayed in Figure 1C. This revealed that a CDK2 inhibitor inhibits clustering of supernumerary centrosomes. Thus, CDK2 inhibitors cause anaphase catastrophe by opposing the clustering of pre-existing supernumerary centrosomes and not by causing *de novo* supernumerary centrosomes to form. Therefore, anaphase catastrophe following CDK2 antagonism is detected preferentially in cells having supernumerary centrosomes while relatively sparing normal cells with two centrosomes. Consistent with this view, it was experimentally shown that anaphase catastrophe was not substantially observed after treatment of immortalized pulmonary epithelial cells that are not chromosomally unstable as are lung cancer cells (34,44). Normal cells like hematopoietic and gastric cells are affected by conventional chemotherapy because of their proliferative properties. Yet, anaphase catastrophe engagement is expected to spare even these proliferating normal cells. This is because anaphase catastrophe is conferred by the presence of aneuploidy rather than the rate of proliferation of a cancer cell (44).

Inhibition of CP110 centrosome protein mediates anaphase catastrophe

To elucidate the mechanisms underlying induced anaphase catastrophe after CDK2 antagonism, proteins reported as substrates of CDK2 phosphorylation were screened to uncover candidate mediators of anaphase catastrophe conferred by CDK2 inhibitor treatment (48). Amongst these proteins, the centrosomal protein CP110 was functionally highlighted (48). Indeed, CP110 knockdown can trigger multipolar anaphases in examined cancer cells (48).

CP110 is a direct phosphorylation target of cyclin E/CDK2, cyclin A/CDK2, and cyclin B/CDK1 (51), as summarized in Figure 2A. CP110 is not known to have enzymatic activity. However, it interacts with distinct protein complexes and regulates microtubule growth as well as centriole length (52–57). It plays pivotal roles in centrosome duplication (51),

maturation (54), separation (51), and cytokinesis (58,59). CP110 levels as well as its localization to the centrosome are each strictly controlled and depend on the phase of the cell cycle (51). During the G1/S cell cycle phase, CP110 is prominently expressed coincident with initiation of centrosome duplication, peaking during the S cell cycle phase; it is involved in centrosome duplication and maturation (51,54). CP110 protein expression diminishes substantially during the G2/M and G0/G1 cell cycle phases. During M phase, CP110 regulates centrosome separation and cytokinesis (58,59). CP110 knockdown or the mutation of its CDK2 phosphorylation sites each results in the unscheduled separation of centrosomes (51). From these findings, it is proposed that CP110 positively regulates centrosome duplication and inhibits premature centrosome separation. CP110 is also known to suppress primary cilia formation in non-cycling cells and cells in the G0 cell cycle phase (59–61).

CP110 is involved in centrosome function, especially centrosome separation, and it is a CDK1 and CDK2 phosphorylation target (51). Given this, CP110 was hypothesized to control supernumerary centrosome clustering and to trigger anaphase catastrophe after CDK2 antagonism (48). Engineered knockdown of CP110 conferred anaphase catastrophe and augmented this response after treatment with the CDK2 inhibitor, seliciclib (48). As expected, gain of CP110 expression antagonized this inhibition of centrosome clustering as well as anaphase catastrophe induction caused by either genetic or pharmacological antagonism of CDK2 (48). These findings were confirmed by use of different individual or combined CDK1 or CDK2 pharmacologic inhibitors including seliciclib (48), dinaciclib (43), CCT68127 (44), and CYC065 (44). Also, expression of a mutant CP110 species with all potential CDK2 phosphorylation sites replaced by alanine residues did not antagonize consequences of CDK2 inhibition (48). Based on these findings, it was hypothesized (48) that CDK2 antagonism disrupts effective clustering of supernumerary centrosomes via inhibition of CP110 phosphorylation, causing anaphase catastrophe.

There are ten potential CDK2 phosphorylation sites present in CP110. The role of each of these potential phosphorylation sites in CDK2 inhibitor-mediated anaphase catastrophe was interrogated by transversing each or all of these sites with alanine residues within CP110 expression vector constructs and independently by individually restoring these mutated sites to their respective wild-type sequences containing serine or threonine residues (50). Based on these experiments, serine 170 and threonine 194 residues were found as the sites responsible for causing anaphase catastrophe after CDK2 inhibition (50), as summarized in Figure 2B. It was also confirmed that these two sites were involved in the inhibition of centrosome clustering after CDK2 antagonism (50). CP110 serine 170 and threonine 194 residues are located between the CP110 coiled-coil and destruction box (D-box) domains (51) as shown in Figure 2B. These sites are likely important for interactions between CP110 and other centrosomal proteins that control centrosome clustering.

CP110 expression and the *KRAS* oncoprotein

CP110 protein levels are tightly controlled during the cell cycle to prevent errors in centrosome duplication, separation, or cytokinesis (51). CP110 expression is reduced at the early G1 cell cycle phase and is increased during the G1/S transition (51). These levels begin

to decline at the G2 cell cycle phase and diminish after cell mitosis (51). Ubiquitination mechanisms are engaged in regulating this CP110 protein expression (62,63). During the G2 phase of the cell cycle, CP110 associates with the F-box protein cyclin F and is ubiquitinated via the SCF (Skp1-Cul1-F-box protein)^{cyclin F} ubiquitin ligase complex and it is then degraded (62,63), as shown in Figure 2C. The siRNA-mediated depletion of cyclin F in the G2 cell cycle phase causes centrosome and mitotic abnormalities that are reversed by co-silencing CP110 (62). This indicates that SCF^{cyclin F}-mediated degradation of CP110 is necessary for proper mitosis and genomic integrity (62). Besides cyclin F, Neuralized homologue 4 (Neurl4), a member of Neuralized family, and EDD-DYRK2-DDB1^{VprBP}, an E3 ligase, can also regulate CP110 expression, respectively through ubiquitination (64–66).

Deubiquitination is critical for regulating CP110 protein expression (63). The ubiquitin-specific protease 33 (USP33), a deubiquitinating enzyme, deubiquitinates CP110 in a cell-cycle-dependent manner, thereby counteracting SCF^{cyclin F}-mediated ubiquitination (67). Ablation of USP33 destabilizes CP110 protein and by this inhibits centrosome amplification (67). The mitotic defects caused by siRNA-mediated depletion of SCF^{cyclin F} are reduced by co-depletion of USP33 (67). Pertinent to the molecular pharmacologic role of CP110, USP33 knockdown enhanced anaphase catastrophe caused by CDK2 inhibition (50).

Intriguingly, in *KRAS* mutant versus wild-type expressing lung cancer cells, the basal protein expression of cyclin F was detected as markedly elevated (50). Cyclin F is a key negative regulator of CP110 protein expression (62). Gain of expression of the *KRAS* oncoprotein led to reduced expression of CP110 protein (50), as summarized in Figure 2D. The direct effect of the *KRAS* oncoprotein on cyclin F and CP110 proteins was experimentally shown by loss of *KRAS* expression by use of siRNAs (50). When *KRAS* was repressed in lung cancer cells, cyclin F expression decreased and CP110 expression subsequently increased (50). This indicated that the *KRAS* oncoprotein downregulated CP110 protein by upregulating cyclin F expression (50). The precise mechanism through which the *KRAS* oncoprotein upregulates cyclin F expression is not yet elucidated, but cyclin F expression is affected by DNA damage and repair pathways (68,69). This links cyclin F expression to the *KRAS* oncoprotein that can confer intrinsic genotoxic stress to cancer cells (70).

CP110 immunohistochemical analysis revealed that both engineered murine lung cancer models and human lung cancer cases that expressed the *KRAS* oncoprotein substantially lowered CP110 expression as compared with *KRAS* wild-type expressing lung cancers (48). *KRAS* mutations are linked to centrosome amplification and chromosomal instability (71–73). These associations provide a mechanistic basis for the observed link between destabilization of CP110 protein and *KRAS* oncoprotein expression in lung cancer and potentially other cancer cell contexts (44).

Translational perspectives

Mutant *KRAS* species regulate expression of CP110 protein. In turn, CP110 is a mediator of anaphase catastrophe caused by CDK2 inhibition. Given this, the effect of *KRAS* mutations on activities of CDK2 inhibitors in cancers was analyzed. Notably, lung cancer cells with *KRAS* mutations were particularly sensitive to CDK2 inhibition (34,43,44). High

throughput pharmacogenomic analyses revealed that *KRAS* mutations expressed in lung cancer cells led to a statistically significantly more sensitive response to seliciclib (34) and CCT68127 (44) than *KRAS* wild-type expressing lung cancer cells. Since CP110 expression is reduced in *KRAS* mutant lung cancer cells (48), CDK2 inhibitors are proposed to inhibit CP110 activity more readily in these cancer cells. This is proposed to confer an efficient induction of anaphase catastrophe, as shown in Figure 3.

This hypothesis has translational research implications because the *KRAS* oncogene is often activated via mutation within diverse cancers. Likewise, the *KRAS* oncoprotein has been a challenging pharmacologic target; it is also associated with a poor clinical prognosis (74–77). This makes lung cancers driven by the *KRAS* oncoprotein an unmet medical need for innovative therapy (76,78). *KRAS* mutations are present in nearly 30% of lung cancers and are especially common in smokers, who develop pulmonary adenocarcinomas (74). These alterations frequently confer resistance to chemotherapeutic agents that are otherwise effective against *KRAS* wild-type cancers (75,79,80). Despite the considerable efforts already invested in targeting *KRAS* mutant cancers, this has not yet translated into clinical advances.

New insights are needed to address this refractory cancer problem (81). In this regard, the observed preclinical response of *KRAS* mutant lung cancers to CDK2 inhibitors has the prospect of producing substantial clinical benefits (34,35,44). Thus, clinical trials using either CDK1 or CDK2 inhibitors especially for combating *KRAS* mutant lung cancer cases are warranted. Since *KRAS* mutations are detected at a high frequency in other clinically-challenging cancers like pancreatic and colon cancers (82), the pharmacologic engagement of anaphase catastrophe following CDK1 or CDK2 inhibition could provide a new way to target broadly *KRAS* mutant-driven cancers. Added support for this view comes from *in vivo* evidence that found CDK2 inhibition substantially repressed growth of syngeneic murine lung cancers whether or not these models expressed wild-type or mutant *KRAS* species (34,35,44). Normal bipolar cells are less sensitive to the consequence of anaphase catastrophe than are aneuploid cells. Thus, anaphase catastrophe induction after treatment with a CDK1 or CDK2 inhibitor has a clinically-relevant therapeutic window.

Conclusions

This review summarized molecular insights and clinical implications of a previously unrecognized proapoptotic death program known as anaphase catastrophe. It preferentially eradicates aneuploid cancer cells with supernumerary centrosomes while relatively sparing bipolar cells. Specific CDK antagonists inhibit supernumerary centrosome clustering and trigger anaphase catastrophe via reduced phosphorylation of the centrosomal protein CP110, a direct CDK1 and CDK2 target (48). Notably, CP110 protein is regulated by the *KRAS* oncoprotein (50). Given this, induced anaphase catastrophe following treatment with CDK2 (or CDK1) inhibitors has therapeutic implications for otherwise therapeutically refractory cancers that harbor *KRAS* mutations, such as lung cancers. These antineoplastic agents engage a previously unrecognized mechanism that is worthy of study in the laboratory and in cancer patients.

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Figure 1. Summary of anaphase catastrophe

(A) The flow of mitosis with three spindle poles is shown to represent multipolar mitosis with normal bipolar mitosis for comparison. Although the temporal sequence of mitosis is maintained, chromosomes segregate inappropriately in multipolar cell mitosis, leading to apoptotic death of daughter cells. (B) CDK1 or CDK2 inhibition each can antagonize clustering of supernumerary centrosomes into two poles. This forces cells to undergo multipolar cell division. This leads to apoptotic death of daughter cells, known as anaphase catastrophe. The arrow size displays the relative extent of the indicated engaged pathway. The red rectangle indicates CDK1 or CDK2 inhibition blocks centrosome clustering. (C)

There are three types of indicated cancer cells identified after treatment with CDK2 inhibitors: normal cells with two centrosomes and spindles (N cells), cells with clustered supernumerary centrosomes with bipolar spindles (C cells), and cells with supernumerary centrosomes and multipolar spindles (M cells). In the left panel, representative immunofluorescence images of these different cell types and the respective schematic diagrams are displayed. Staining with DAPI (blue), α -tubulin (red), and γ -tubulin (green) are each shown. In the right panel, the pie charts indicate representative cell population changes for N cells, C cells, and M cells after treatment with a CDK2 inhibitor. Although cell populations of these cell types can differ between examined cancer cells, C cells are typically decreased and M cells are increased while the total cell population of cells with supernumerary centrosomes (C + M cells) is often not appreciably changed after treatment with a CDK2 inhibitor, as shown in these pie charts.

Figure 2. CP110 is a mediator of anaphase catastrophe

(A) CP110 is phosphorylated by CDK1 or CDK2. (B) The ten potential CDK2 phosphorylation sites within the CP110 amino acid sequence are displayed. Critical phosphorylation sites engaged in triggering anaphase catastrophe after CDK2 antagonism include residues serine 170 and threonine 194. These are highlighted with rectangles. (C) CP110 protein expression is regulated through proteasomal degradation after ubiquitination via the SCF^{cyclin F} ubiquitin ligase complex. (D) In *KRAS* mutant oncoprotein-expressing lung cancer cells, high levels of cyclin F protein, the F-box protein of SCF^{cyclin F}, promote ubiquitination and thereby proteasomal degradation of CP110 protein. This leads to reduced levels of CP110 protein as compared to *KRAS* wild-type expressing cancer cells. The font size is meant to display the relative contribution of the highlighted species. The arrow size displays the relative extent of the indicated engaged pathways.

Figure 3. Anaphase catastrophe induction in *KRAS* oncoprotein-driven cancer cells

In *KRAS* mutant oncoprotein-expressing lung cancer cells, basal expression levels of CP110 protein are reduced. As a result, anaphase catastrophe is readily conferred after inhibiting CP110 activity by use of a CDK2 inhibitor. This increases sensitivity of *KRAS* mutant lung cancer cells to treatment with a CDK1 or CDK2 inhibitor. The font size is meant to convey the relative contribution of the highlighted species. The arrow size displays the relative extent of the indicated engaged pathway.