Aurora A inhibition limits centrosome clustering and promotes mitotic catastrophe in cells with supernumerary centrosomes

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Running Title: AurA dependent spindle pole clustering

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Abstract

The presence of supernumerary centrosomes is prevalent in cancer, where they promote the formation of transient multipolar mitotic spindles. Active clustering of supernumerary centrosomes enables the formation of a functional bipolar spindle that is competent to complete a bipolar division. Disruption of spindle pole clustering in cancer cells promotes multipolar division and generation of non-proliferative daughter cells with compromised viability. Hence molecular pathways required for spindle pole clustering in cells with supernumerary centrosomes, but dispensable in normal cells, are promising therapeutic targets. Here we demonstrate that Aurora A kinase activity is essential for spindle pole clustering in cells with extra centrosomes. While cells with two centrosomes are ultimately able to build a bipolar spindle and proceed through a normal cell division in the presence of Aurora A inhibition, cells with supernumerary centrosomes form multipolar and disorganized spindles that are not competent for chromosome segregation. Instead, following a prolonged mitosis, these cells experience catastrophic divisions that result in grossly aneuploid, and non-proliferative daughter cells. Aurora A inhibition in a panel of Acute Myeloid Leukemia cancer cells has a similarly disparate impact on cells with supernumerary centrosomes, suggesting that centrosome number and spindle polarity may serve as predictive biomarkers for response to therapeutic approaches that target Aurora A kinase function.

Keywords
Aurora A
Centriole
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Centrosome

Introduction

The formation of a mitotic spindle with two spindle poles is integral to proper chromosome segregation. During mitosis a pair of centrosomes, the microtubule nucleating and organizing centers of the cell, serve as focal points at which molecular motors and crosslinkers cluster microtubules (reviewed in (1) and (2)). In cancer, centrosome amplification is common, where it contributes to aneuploidy, and is correlated with high tumor grade and poor patient prognosis (3-7). A direct consequence of having more than two centrosomes is the capacity to form a mitotic spindle with more than two spindle poles(8). Multipolar divisions that can result from such spindle geometry, lead to daughter cells with dramatic changes in chromosome number, and decreased viability (5, 9). To limit multipolar divisions, cells with excess centrosomes actively cluster excess centrosomes into functional bipolar spindles (7, 10-13). Cells with clustered centrosomes complete bipolar anaphases with only moderate chromosome segregation errors, and remain viable (8, 14). In this way, the molecular players involved in centrosome clustering have an important role in determining the fate of cancer cells with supernumerary centrosomes and are therefore attractive candidates for therapeutic approaches (11, 15).

Aurora A (AurA) is a serine/threonine kinase that is important for proper bipolar spindle formation. Localized to the centrosome during interphase and to spindle poles during mitosis, AurA phosphorylates protein targets to regulate centrosome maturation and spindle assembly such that loss of AurA kinase–dependent phosphorylation of key mitotic targets disrupts mitotic spindle formation (16, 17). Some targets of AurA kinase, including Eg5, contribute to spindle formation by promoting anti-poleward forces that push

centrosomes apart (18, 19). Other targets of AurA kinase, including TACC, have roles in stabilizing microtubule dynamics and promoting spindle pole organization (20-22). Interestingly, inhibition of AurA kinase activity has been shown to both impair separation of spindle poles to result in spindle pole collapse and formation of a single monopole (23-25), and alternatively to promote spindle disorganization (26-28). Nevertheless, the underlying cause of this disparate response to AurA inhibition remains unclear.

Here we show that the response to AurA kinase inhibition is influenced by centrosome number and the capacity to form multipolar spindles during mitosis. Inhibition of AurA kinase activity in cells with normal centrosome content induces a transient mitotic spindle pole collapse that ultimately resolves to enable a bipolar division and sustained proliferation. In contrast, AurA kinase activity is essential in cells with supernumerary centrosomes such that AurA inhibition here compromises centrosome clustering, promotes abortive cell division, and results in multinucleated, highly aneuploidy daughter cells with reduced proliferative capacity. Together, these findings suggest that therapeutic approaches that exploit AurA inhibition will be most advantageous in cancers with supernumerary centrosomes.

Results

AurA promotes clustering of supernumerary centrosomes

To explore the relationship between spindle polarity and response to AurA inhibition we utilized two experimental systems to manipulate centrosome number. First, we induced supernumerary centrosomes using a previously characterized human epithelial cell line

carrying a doxycycline-regulated PLK4 expression construct (indPLK4) (8). PLK4 is a regulator of centrosome biogenesis that when overexpressed leads to a cell-cycle dependent over-duplication of centrosomes (Supplemental Figure 1A & B). In the absence of PLK4 induction, the hTERT-immortalized human retinal pigment epithelial (RPE) cell line is diploid, contains a single pair of centrioles (one centrosome) during interphase that is duplicated in preparation for mitosis, and forms a bipolar mitotic spindle. Following induction of PLK4 overexpression for 36h, the majority of RPE cells have 8+ centrioles, as indicated by centrin-2 staining, and exhibit an increased frequency of multipolar spindles (Figure 1A and 1B). In a second, complementary approach, the near-diploid HCT116 colon cancer cell line was treated with Cytochalasin B (CytoB) to induce cytokinesis failure. The resulting cells have double the DNA content and double the centrosome number, and an increased incidence of multipolar mitotic spindles, independent of PLK4 expression level (Figure 1C and 1D).

Alisertib (MLN8237) is an orally bioavailable inhibitor of AurA kinase that is ~200 fold more selective for AurA than the closely related Aurora B (29). Pharmacological inhibition of AurA kinase activity can be monitored through loss of AurA auto-phosphorylation of threonine residue 288 in its activation loop (30). We show that 100nM alisertib is sufficient to inhibit AurA kinase activity and prevent threonine 288 phosphorylation (p-AurA) similarly in both control cells, and those with centrosome amplification (Figure 1E). Furthermore, both immunofluorescence and FACs analysis indicate that AurA inhibition with alisertib for 16 hours results in a similar mitotic enrichment in populations of cells with and without supernumerary centrosomes (Figure 1F, Supplemental Figure 1D).

However, while over a third of mitotic cells with two centrosomes exhibit spindle pole collapse following alisertib dependent inhibition of AurA kinase activity, cells with supernumerary centrosomes predominantly form multipolar or disorganized spindles (Figure 1B & D). This disparate response of cells with supernumerary centrosomes is not restricted to inhibition of AurA with alisertib, as similar results were seen in response to a panel of AurA inhibitors (Supplemental Figure 1C, D & 1E).

Cell fate in the presence of AurA inhibition is influenced by centrosome number

Regardless of centrosome number, following extended AurA inhibition, mitotic enrichment does not surpass ~10% of the cell population (Figure 1F), suggesting that mitotic defects imposed by AurA inhibition are either transient, or lethal. Therefore, to determine the consequences of collapsed and unclustered spindle poles in cells with two, or supernumerary centrosomes, respectively, we performed live cell imaging immediately after addition of alisertib to assess mitotic progression following AurA inhibition. Images of RPE cells expressing an RFP-tagged histone (RFP-H2B) to enable monitoring of chromosome movement and cell cycle progression were captured every 5 minutes for 36 hours. Untreated RPE cells progressed from nuclear envelope breakdown (NEB) to anaphase onset in ~20 minutes. Although cells with extra centrosomes (indPLK4) took on average nearly twice as long (Figure 2A & 2B; ctrl: 19.3 +/- 3.2 minutes vs indPLK4: 37.7 +/- 7.2 minutes; p=0.016), clustering of supernumerary centrosomes ultimately enabled over 80% of these cells to exit mitosis with a bipolar division (Figure 2C). The minority (~15%) of cells that were unable to cluster spindle poles spent more time in mitosis before ultimately proceeding to a multipolar division (Figure 2: indPLK4).

Consistent with a higher incidence of collapsed spindle poles (Figure 1B), following AurA inhibition, control cells exhibit a prolonged mitotic delay (Figure 2A & 2B) before eventually achieving metaphase alignment and completing a bipolar division (Figure 2C). In contrast, only half of cells with supernumerary centrosomes are able to complete a bipolar division in the presence of AurA inhibition. However, cells that are able to cluster centrosomes to achieve a bipolar division are just as likely as their counterparts with normal centrosome content to proceed through a subsequent cell cycle and enter into a second mitosis (Figure 2D & 2E: ~90% enter a second mitosis, with an average cell cycle ~19 hours).

For the >40% of cells with supernumerary centrosomes that are unable to complete a bipolar division in the presence of AurA inhibition, mitotic exit is catastrophic. In agreement with the high incidence of multipolar and disorganized spindles seen in fixed cell analysis (Figure 1), many cells with supernumerary centrosomes exit mitosis via a multipolar or abortive division. Consistent with previous reports (8, 14), we find the progeny of multipolar divisions exhibit decreased proliferative capacity and decreased viability. Cells that instead undergo an abortive mitosis attempt cytokinesis in the absence of metaphase alignment and anaphase segregation (Figure 2A, B & C). Consistent with cytokinesis failure, following a single cell cycle in the presence of AurA inhibition, immunofluorescence analysis demonstrates that nearly 25% of these cells are multinucleated (Figure 3 A & B). Fluorescence *in situ* hybridization (FISH)- based approaches using centromere-targeted enumeration probes for chromosomes 6 and 8

similarly demonstrate that cells with supernumerary centrosomes exhibit a >6-fold increase in tetraploid cells following AurA inhibition (Figure 3C & D). Importantly, none of the cells that experienced multipolar or abortive mitotic divisions were competent to re-enter the cell cycle within the duration of our movies, contributing to a 40% decrease in proliferation of cells with supernumerary centrosomes following AurA inhibition (Figure 2D).

Centrosome number corresponds with response to Aurora A inhibition

To determine if centrosome number similarly corresponds with response to AurA inhibition in a cancer cell context we utilized a panel of Acute Myeloid Leukemia (AML) cells. AML is a cancer context where AurA kinase is often amplified, and where its inhibition is actively being exploited in preclinical and clinical approaches (31-33). Western blot and qPCR analyses demonstrate that AurA expression in these cell lines, independent of cell cycle distribution, range from levels comparable to that of the non-transformed RPE cell line (ie HL60), to levels over 8-fold higher that that seen in RPE-1 cells (ie K562) (Figure 4A & 4B, Supplemental Figure 2A). Importantly, irrespective of AurA protein level, exposure to 100nM alisertib was sufficient to completely inhibit AurA kinase activity, as judged by AurA auto-phosphorylation (Figure 4B), and similarly resulted in a dose-dependent enrichment of mitotic cells in all AML cell lines (Figure 4E and Supplemental Figure 2B).

Immunofluorescence analysis of centrin-2 indicated that over 70% of interphase U937 and K562 cells contain more than one centrosome (2 centrioles) (Figure 4C & D). Nevertheless,

mitotic spindles in each cell line are predominantly bipolar, indicating that these cells can efficiently cluster supernumerary centrosomes (data not shown). As seen with the RPE and HCT116 cells, inhibition of AurA promotes spindle pole collapse in AML cells with two centrosomes (HL60, THP1, KG1a), and a multipolar or disorganized spindle phenotype in AML cells with supernumerary centrosomes (K562) (Figure 4F). Consistent with the abortive mitoses seen following experimental induction of supernumerary centrosomes in RPE cells, FACXs analysis indicate that AML cells with supernumerary centrosomes and disorganized spindles also exhibit an increased DNA content following AurA inhibition (Figure 4G).

Discussion

Our data support a model whereby the presence of extra centrosomes, acquired either through over-duplication or previous failed cytokinesis, can alter the response of cells to inhibitors of mitotic spindle assembly and subsequently impact the efficacy of such drugs. We find that following AurA inhibition, cells with supernumerary centrosomes exhibit multipolar and disorganized spindles that cannot be resolved prior to mitotic exit. Instead, such cells are subject to abortive mitoses where cytokinesis is attempted in the absence of metaphase alignment, and anaphase chromosome segregation is lacking. Daughter cells that result from such catastrophic mitoses are multinucleated and exhibit dramatic changes in chromosome content. Importantly, such progeny are not competent for continued proliferation. In contrast, following AurA inhibition, cells with normal centrosome number ultimately overcome a transient spindle pole collapse to form bipolar spindles and complete near-normal cell division. Progeny from these divisions remain proliferative and

proceed through the cell cycle to enter a second mitosis with near wild type frequency and timing. In this way, differential response to AurA inhibition in cancer cells stems from an essential and cancer-specific dependence on AurA-driven spindle-pole clustering activity in cells with supernumerary centrosomes.

Proteins that are required for centrosome clustering are often overexpressed in human cancers with extra centrosomes (reviewed in: (34)). Indeed, supernumerary centrosomes, and the need to cluster them to achieve proper cell division, has been proposed to be a driving force in the selective overexpression of proteins with pole-clustering functions. Consistent with this model, we find that AurA expression scales with centrosome number in our panel of AML cell lines (Figure 4A & E). Furthermore, AurA has been reported to be amplified or overexpressed in a number of cancer contexts, including colon, breast, and hematological cancers (35-39), where its expression corresponds with centrosome amplification, genetic instability, and aneuploidy (40-43).

Together with cell biological evidence that inhibition of AurA compromises mitotic progression, an increased Aurora A expression profile in cancer supports that AurA is a clinically relevant drug target (44). Pharmaceutical companies have begun to exploit this possibility, bringing numerous targeted inhibitors to the market as anti-cancer drugs, and spurring clinical trials (33, 45-51). However, while the efficacy and safety of inhibiting AurA have been supported by preclinical and early stage clinical trials, response to AurA inhibition has been limited (33, 51-53) and it remains unclear which patients may best benefit from Aurora A inhibitor treatment either alone, or in combination with standard

chemotherapeutic approaches. We now show that cells with extra centrosomes are susceptible to catastrophic cell divisions when AurA activity is inhibited. Furthermore, consistent with the documented tolerance of patients to AurA inhibitors in the clinic (33, 52, 54), our data demonstrate that normal cells with two centrosomes are not susceptible to the mitotic catastrophe and abortive divisions that are induced in cancer cells with supernumerary centrosomes. Together, these findings suggest that AurA inhibition can selectively corrupt proliferation of cancer cells with extra centrosomes, and that clinical assessment of mitotic structures may be useful in identifying patients most likely to benefit from their use.

Materials and Methods

Cell culture, induction, and inhibition:

Cells were grown in DMEM/F12 medium (RPE-1) or RPMI 1640 medium (HL60, K562, KG1α, THP1, and U937) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin and maintained at 37C with 5% CO2. For experiments where PLK4 expression was induced, media was supplemented with 2 μg/mL Doxycycline for 36 hours. Alternatively, cells were treated with 30 μM Cytochalasin B for 16h to induce cytokinesis failure and generate tetraploid cells with double the normal centrosome number (8). Drug treatments to subsequently inhibit AurA kinase (alisertib; MLN8054, Aurora A inhibitor 1, MK-5108 (VX-689): Selleckchem) were performed at the indicated concentrations for 16-18 hours for immunofluorescence and FACs analysis, or as otherwise indicated. Unless otherwise noted, all experiments to inhibit Aurora A kinase were performed with the highly specific Aurora A kinase inhibitor alsiertib at a concentration of 100nM.

FACS and expression analysis

To assess cell cycle differences and response to mitotic inhibitors, cells were treated with 100 ng/mL Nocodazole (to induce mitotic arrest) and/or 100 nM alisertib for 16-18 hours, fixed and stained with propidium iodide, and processed for FACs analysis as previously described (55). To assess AurA expression levels, RNA extraction was performed with Qiagen's RNeasy kit, and qRT PCR with Applied Biosystem's SYBR green kit according to the respective manufacturers' guidelines. Primers used for amplification of GAPDH and AurA were 5'-ccctctggtggtggcccctt-3' & 5'-ggcgcccagacacccaatcc -3' and 5'-ttttgtaggtctcttggtatgtg-3' & 5'-gctggagagcttaaaattgcag-3' respectively. Inhibition of AurA kinase activity was confirmed by western blot analysis of total AurA (Cell Signaling) and phosphorylated AurA levels (Cell Signaling), using tubulin as a loading control (dm1α, Sigma).

FISH, Immunofluorescence and live cell imaging

Cells were prepared and fixed as described previously (55) and α-satellite probes for chromosomes 6 and 8 hybridized to chromatin. Numerical Heterogeneity (NH) was determined by scoring a minimum of 500 cells per population for copy number of each chromosome probe, for each of three independent experiments. Percent variation from the modal copy number was calculated and compared across conditions.

For immunofluorescence imaging, cells were cultured on glass coverslips (RPE), or spun onto coverslips by centrifugation at 1000 RPM for 3 minutes (AML cell lines) prior to

fixation for 10 min in ice-cold methanol, blocked in TBS-BSA (10mM Tris at pH 7.5, 150mM NaCl, 1% bovine serum albumin), and stained for α-tubulin (dm1α, Sigma), and/or centrin-2 (Santa Cruz) as indicated. DNA was detected with 0.2μg/mL DAPI (Sigma), and coverslips mounted with Prolong Antifade Gold mounting medium (Molecular Probes). Cells were plated in 12 well imaging bottom tissue culture dishes for live cell imaging. Images of fixed and live cells were captured with a Zyla sCMOS camera mounted on a Nikon Ti-E microscope with a 60x Plan Apo oil immersion objective, or a 20x CFI Plan Fluor objective. A minimum of 50 mitotic cells or 1000 interphase cells were analyzed per condition for each of 3 biological replicates. Live cell images were captured at 10 coordinates per condition at 5 min intervals for 36 hours, for each of 3 biological replicates. 100 individual cells were tracked through mitosis and analyzed for each condition and replicate. Error bars throughout represent standard deviation unless otherwise indicated. Pairwise analyses were performed using a one-way ANOVA to determine statistical significance.

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Conflict of Interest

The authors declare that they have no competing financial interests in relation to the work described herein.

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Figure Legends

Figure1. Aurora A kinase promotes clustering of supernumerary centrosomes. A & B) RPE cells induced to overexpress PLK4 (indPLK4) to generate supernumerary centrosomes and C & D) HCT116 p53-/- cells treated with cytochalasin B (CytoB) to induce cytokinesis failure and generate cells with extra centrosomes were compared to their respective untreated controls. In both cell lines, induction of supernumerary centrosomes promotes transient multipolar spindle structures that are efficiently clustered into bipolar spindles (see also supplemental figure 1). Centrin-2, a marker of centrioles is shown in green, tubulin in red, and chromatin in blue. The scale bar is 10 μM. Treatment with 100nM of the Aurora A inhibitor alisertib compromises spindle pole clustering in cells with supernumerary centrosomes. E & F) Treatment with 100nM alisertb similarly inhibits Aurora A kinase auto-phosphorylation in cells with two, or supernumerary centrosomes and promotes enrichment of mitotic cells.

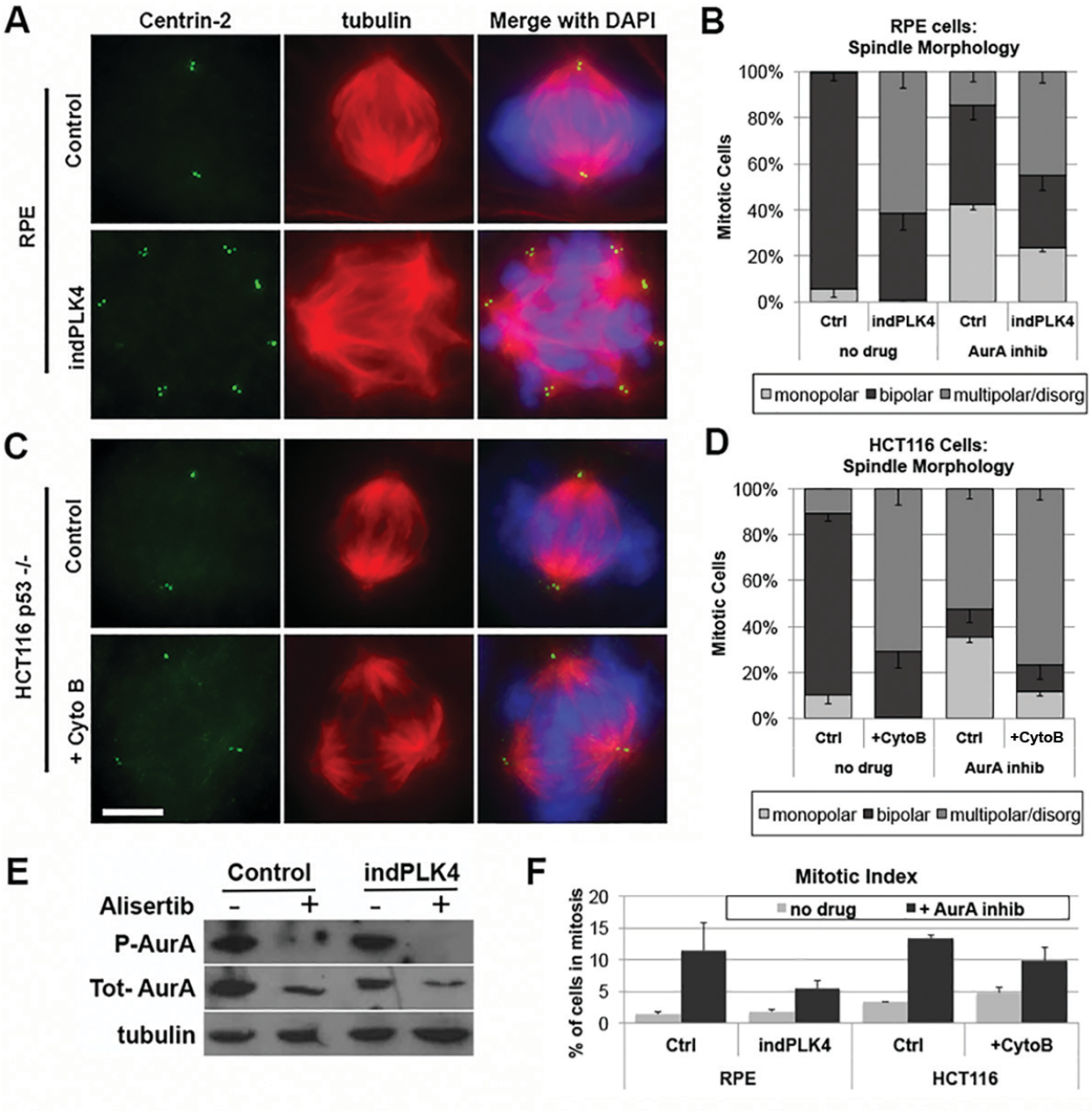
Figure 2: Centrosome number influences mitotic outcome following Aurora A kinase inhibition. A) Representative still frames from live cell phase contrast and fluorescence imaging of RFP-labeled Histone 2B (in red)-expressing cells. Nuclear envelope breakdown (NEB) to anaphase onset and cytokinesis are indicated. Note that 10min increments are represented for cells treated with Aurora A inhibition, 5 min increments for those not treated. Scale bars represent 10μM. B) The presence of supernumerary centrosomes promotes an increase in the time from NEB to anaphase onset following Aurora A kinase inhibition. C) Both control cells and those with PLK4 induction alone divide to form two

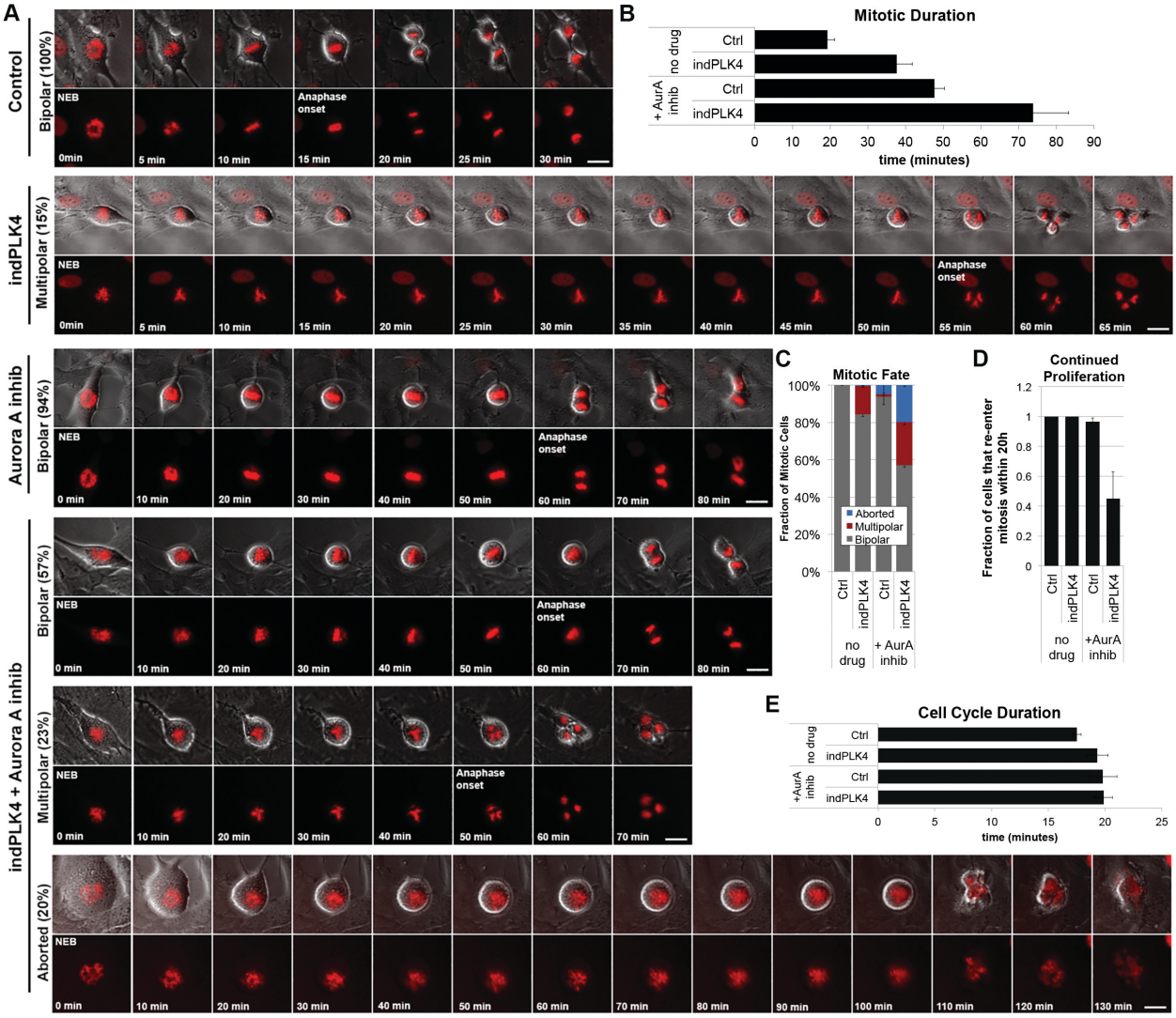
daughter cells following metaphase alignment of chromosomes. In the presence of Aurora A kinase inhibition, while the vast majority of control cells also achieve metaphase alignment and divide into two daughter cells, cells with PLK4 induction often fail cytokinesis and exit mitosis as a single cell. D) In the presence of Aurora A inhibition, cells with PLK4 induction are less likely than cells without Aurora A inhibition and/or without PLK4 induction to proceed to a second mitotic division within 20 hours of a first division. E) Cells in all conditions that do complete a bipolar division continue to a second division with comparable cell cycle timing.

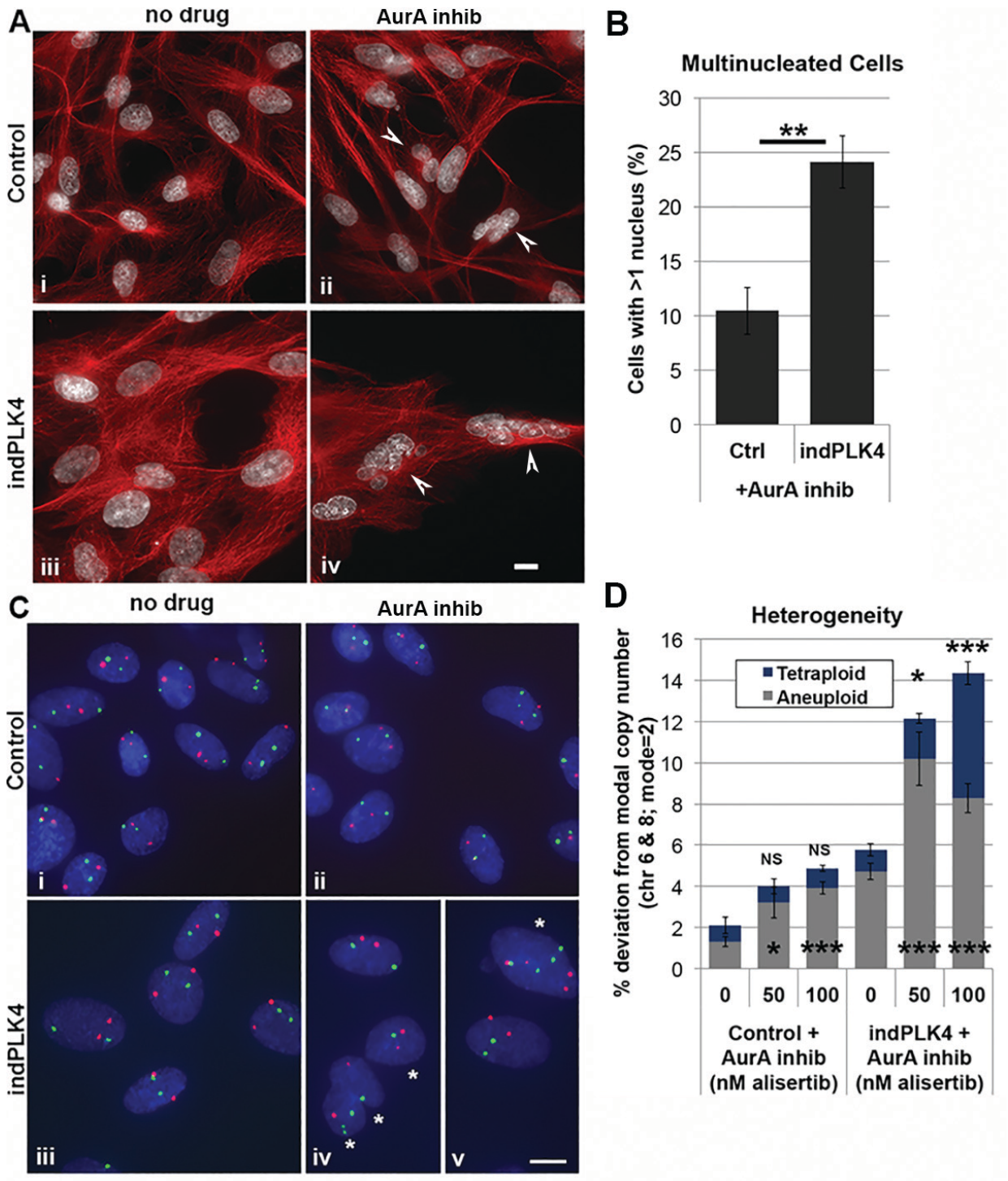
Figure 3: Aurora A inhibition compromises genome stability in cells with supernumerary centrosomes. A & B) Cells with supernumerary centrosomes exhibit high levels of multinucleated and C & D) tetraploid cells following inhibition of Aurora A kinase activity. A) white arrow heads indicate multinucleated cells. Tubulin shown in red, chromatin in white. C) Stars in iv and v indicate a multinucleated and mononucleated tetraploid cell, respectively. Probe for chromosome 6 shown in red and chromosome 8 shown in green. Scale bars are 10μM.

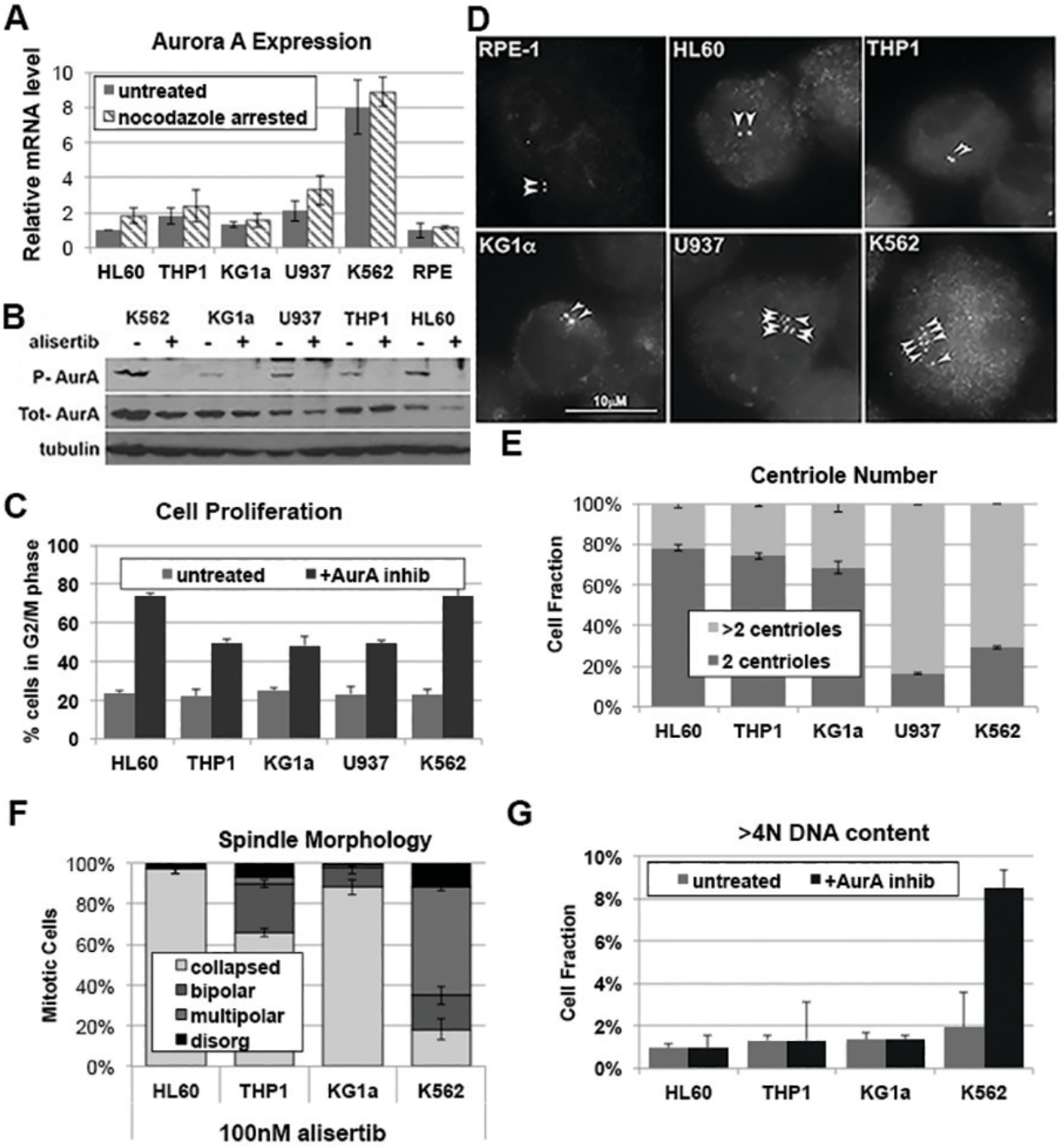
Figure 4. Response to Aurora A inhibition corresponds with centrosome number in Acute Myeloid Leukemia cells. A) A panel of five AML cell lines exhibit a range of Aurora A expression levels, irrespective of differences in cell cycle distribution or proliferation between cell lines. B & C) Treatment with 100nM alisertib in all five cell lines is similarly sufficient to suppress auto-phosphorylation of Aurora A and promote enrichment of cell in G2/M. D & E) Immunofluorescence analysis of centrin-2 staining indicates that U937 and

K562 cells have supernumerary centrosomes (2 centrioles /centrosome). F) Although AML cells are typically non-adherent, four of the five cell lines were sufficiently adherent to enable immunofluorescence-based assessment of mitotic spindle structure. Following Aurora A inhibition, spindle pole clustering is compromised in K562 cells, which have supernumerary centrosomes, but not in cell lines with two centrosomes. G) FACs analysis of DNA content indicates that cells with supernumerary centrosomes also exhibit an increase in DNA content following Aurora A inhibition.


A
Centrin-2
tubulin
Merge with DAPI
RPE
Control
indPLK4
B
RPE cells:
Spindle Morphology
Mitotic Cells
100%
80%
60%
40%
20%
0%
Ctrl
indPLK4
Ctrl
indPLK4
no drug
AurA inhib
monopolar
bipolar
multipolar/disorg
C
HCT116 p53 -/-
Control
+ Cyto B
D
HCT116 Cells:
Spindle Morphology
Mitotic Cells
Ctrl
+CytoB
Ctrl
+CytoB
no drug
AurA inhib
monopolar
bipolar
multipolar/disorg
E
Control
indPLK4
Alisertib
-
+
-
+
P-AurA
Tot- AurA
tubulin
F
Mitotic Index
no drug
+ AurA inhib
% of cells in mitosis
20
15
10
5
0
Ctrl
indPLK4
Ctrl
+CytoB
RPE
HCT116


A
Control
Bipolar (100%)
NEB
Anaphase onset
0min
5 min
10 min
15 min
20 min
25 min
30 min
B
Mitotic Duration
no drug
Ctrl
indPLK4
+ AurA inhib
Ctrl
indPLK4
0
10
20
30
40
50
60
70
80
90
time (minutes)
indPLK4
Multipolar (15%)
35 min
40 min
45 min
50 min
55 min
60 min
65 min
Aurora A inhib
Bipolar (94%)
0 min
70 min
80 min
C
Mitotic Fate
100%
80%
60%
40%
20%
0%
Fraction of Mitotic Cells
Aborted
Multipolar
Bipolar
Ctrl
indPLK4
Ctrl
indPLK4
no drug
+ AurA inhib
D
Continued Proliferation
1.2
1
0.8
0.6
0.4
0.2
0
Fraction of cells that re-enter mitosis within 20h
Ctrl
indPLK4
Ctrl
indPLK4
no drug
+AurA inhib
indPLK4 + Aurora A inhib
Bipolar (57%)
Multipolar (23%)
E
Cell Cycle Duration
no drug
Ctrl
indPLK4
+AurA inhib
Ctrl
indPLK4
0
5
10
15
20
25
time (minutes)
Aborted (20%)
90 min
100 min
110 min
120 min
130 min


A
no drug
AurA inhib
Control
indPLK4
i
ii
iii
iv
B
Multinucleated Cells
Cells with >1 nucleus (%)
30
25
20
15
10
5
0
**
Ctrl
indPLK4
+AurA inhib
C
no drug
AurA inhib
Control
indPLK4
i
ii
iii
iv
v
D
Heterogeneity
% deviation from modal copy number (chr 6 & 8; mode=2)
16
14
12
10
8
6
4
2
0
Tetraploid
Aneuploid
NS
NS
*
***
*
***
***
0
50
100
0
50
100
Control + AurA inhib (nM alisertib)
indPLK4 + AurA inhib (nM alisertib)


A
Aurora A Expression
Relative mRNA level
untreated
nocodazole arrested
HL60 THP1 KG1a U937 K562 RPE
B
K562 KG1a U937 THP1 HL60
alisertib
P- AurA
Tot- AurA
tubulin
C
Cell Proliferation
% cells in G2/M phase
untreated
+AurA inhib
HL60 THP1 KG1a U937 K562
D
RPE-1
HL60
THP1
KG1α
U937
K562
10μM
E
Centriole Number
Cell Fraction
>2 centrioles
2 centrioles
HL60 THP1 KG1a U937 K562
F
Spindle Morphology
Mitotic Cells
collapsed
bipolar
multipolar
disorg
HL60 THP1 KG1a K562
100nM alisertib
G
>4N DNA content
Cell Fraction
untreated
+AurA inhib
HL60 THP1 KG1a K562