Inhibition of mTOR Pathway Sensitizes Acute Myeloid Leukemia Cells to Aurora Inhibitors by Suppression of Glycolytic Metabolism

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Abstract

Aurora kinases are overexpressed in large numbers of tumors and considered as potential therapeutic targets. In this study, we found that the Aurora kinases inhibitors MK-0457 (MK) and ZM447439 (ZM) induced polyploidization in acute myeloid leukemia (AML) cell lines. The level of glycolytic metabolism was significantly increased in the polyploid cells, which were sensitive to glycolysis inhibitor 2-deoxy-D-glucose (2DG), suggesting that polyploidy cells might be eliminated by metabolism deprivation. Indeed, inhibition of mTOR pathway by mTOR inhibitors (rapamycin and PP242) or 2DG promoted not only apoptosis but also autophagy in the polyploid cells induced by Aurora inhibitors. Mechanically, PP242 or 2DG decreased the level of glucose uptake and lactate production in polyploid cells as well as the expression of p62/SQSTM1. Moreover, knockdown of p62/SQSTM1 sensitized cells to the Aurora inhibitor whereas overexpression of p62/SQSTM1 reduced drug efficacy. Thus, our results revealed that inhibition of mTOR pathway decreased the glycolytic metabolism of the polyploid cells, and increased the efficacy of Aurora kinases inhibitors, providing a novel approach of combination treatment in AML.

Introduction

Anticancer drug that targets cell-cycle regulators has been widely applied in the treatment of the hematologic malignancies (1–3). Aurora kinases A–C, which play important roles in the process of mitosis, have been implicated in the tumorigenesis (1–3). Aurora A and B proteins were overexpressed in a number of tumors, including leukemia cells, which were correlated with aneuploidy in tumor tissues and poor prognosis (4, 5). Previous report showed that Aurora kinases inhibitors could suppress the growth of leukemia cells (1). ZM447439 (ZM), the dual Aurora A and B inhibitor, inhibited the cell proliferation, induced polyploidization, and promoted apoptosis of acute myeloid leukemia (AML) cells (6). Our previous study showed that VX-680 (MK-0457), a relatively specific Aurora A inhibitor, induced apoptotic cell death in AML cells, especially in the primary leukemic blasts with high Aurora A expression (4, 7). Although Aurora kinases inhibitors have significant therapeutic potential in AML, single-agent activity seems to be uniformly modest and the efficacy needs improving (6). Recently, we also showed that Aurora A inhibition triggered drug resistance could be reversed by autophagy inhibitors in breast cancer cells (8). In addition, combination of Aurora kinases inhibitors with vincristine or dasatinib presented synergistic effect to inhibit cell viability in human pre-B acute lymphoblastic leukemia (9). Thus, new strategy for drug combination is urgently required to overcome drug resistance induced by Aurora A inhibition.

Polyploidy is a prominent characteristic of human cancers and proposed as a driver of tumorigenesis (10). Study showed that somatic polyploidy was associated with readjustment of main metabolic pathways. For example, mitochondrial process was depressed, and carbohydrate degradation and lipid biosynthesis were increased in the polyploid cells (11). Warburg reported that tumors preferred aerobic glycolysis to mitochondrial oxidative phosphorylation (12). Based on the reports, suppression of the aerobic glycolytic metabolism might help for restraining the proliferation of polyploidy cancer cells. Aurora kinases are crucial for cytokinesis in a number of reports. Aurora kinases deletion induced mitosis arrest and promoted polyploidy formation (13). Therefore, readjustment of the metabolic...
status in polyploid cells induced by Aurora kinases inhibition could also be a promise approach to cancer therapy. The mTOR, a well-conserved serine/threonine kinase with 2 complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2), is essential for cell growth, protein translation, autophagy, and metabolism (14). To adapt the condition of hypermetabolic demand and thrive rapidly, oncogenes in growth and nutrient-sensing signaling pathways were activated (15). In numerous cancer types, mTOR signaling pathway was activated by deregulation of its upstream factors, such as Akt activation or deficiency of tuberous sclerosis complex 2 (TSC2; refs. 16 and 17). Activation of mTORC1, a metabolic hallmark of cancer, increased glycolysis, and lipid biosynthesis (15). Although mTORC2 was not thoroughly studied in the metabolism of tumor, RNAi against one of mTORC2 components also impaired the glucose and lipid metabolism in muscle and fat cells (18, 19). Furthermore, mTOR was associated with the development of polyploidization. Ma and colleagues showed that S6K1, a downstream of mTOR, was involved in polyploidization through its phosphorylation at Thr421/Ser424 (20). In addition, activation of mTOR pathway was required for TSC2 (ref. 17). In polyploidization through its phosphorylation at Thr421/Ser424 (20). In addition, activation of mTOR pathway was required for TSC2 (ref. 17).

Peripheral blood mononuclear cells (PBMC) were enriched by Ficoll–Hypaque density gradient centrifugation.

Cell-proliferation assay

Cell viability was assessed by Cell counting kit-8 (CCK8; Dojindo Biotechnology) according to the manufacturer’s instructions. The optical density at 450 nm was measured using a multiwell plate reader (Micro-plate Reader; Bio-Rad).

Cell-cycle analysis

Cell cycle was analyzed by flow cytometry. Cells were treated with drugs for 24 hours. Then, cells were harvested and stained with mixture of 50 μg/mL propidium iodide (PI), 0.2% Triton X-100, and 100 μg/mL RNAase inhibitor. Cell-cycle analysis was conducted using a FACS flow cytometer equipped with Modfit LT for Mac V2.0 software (BD Biosciences).

Microscopic analysis of cells and nuclear morphology

Cells were treated with Aurora kinases inhibitors for 24 hours, followed by PP242 or 2DG for another 24 hours. After 48 hours, the cells were photographed under an inverted microscopy (Olympus). Nuclear fragmentation was stained by Hoechst 33342 (Sigma) with 10 μg/mL for 15 minutes at 37°C. Slides were viewed using a fluorescence microscope.

Monodansylcadaverine staining

Cells were incubated with the monodansylcadaverine (MDC; Sigma) with a final concentration of 0.05 mmol/L for 10 minutes at 37°C. Then, cells were viewed on slides under a laser scanning confocal microscope (ZEISS, Germany), and tested by flow cytometry. MDC dye was applied to stain the late autophagic vacuoles.

Western blotting analysis

Total cellular proteins were isolated with RIPA buffer. lysates were subjected to SDS-PAGE gel electrophoresis, and were transferred to nitrocellulose membranes. The membranes were blocked with 5% bovine serum albumin and then incubated overnight with LC3 (Novus), p62 (Santa Cruz), Aurora A (Upstate), p-mTOR (Ser2448, Ser2481), mTOR, p-Aurora A (Thr288), cleaved caspase 3 (Asp175), cleaved PARP (Asp214), and β-actin antibodies (Cell Signaling Technology). p-4EBP1/2/3 (Thr45) and 4EBP1/2/3 antibodies were purchased from Epitomics, Inc. Subsequently, the membranes were incubated with an HRP-conjugated secondary antibody (Protein Tech Group) at room temperature for 1 hour. The protein bands were detected with an enhanced chemiluminescence reagent (Sigma), according to the manufacturer’s instructions.

Measurement of glucose uptake and lactate production of cells in the media

Cells were counted using trypan blue staining before measurement of the glucose uptake and lactate production. The media were collected, and the glucose and lactate levels were measured by a cellulose filter (Macherey-Nagel).
were immediately measured using Automatic Biochemical Analyzer (Olympus AU5400). The glucose consumption and lactate production were normalized to the number of cells and results were presented as pmol/cell (22).

**Plasmid construction and retrovirus packaging**

The coding sequence of p62/SQSTM1 was amplified from human leukemia cells. The primers were as followed: 5′ Bgl II: TTAGATCTATGGCGTCGCTACCGTGAGG; 3′ Sal I: GTGTCGACTGGGCAAAAGTGGTCAACG. The cDNA was cloned into MSCV-IRES-GFP plasmid. 293FT cells were transfected with packaging vectors and MSCV-p62-IRES-GFP or MSCV-IRES-GFP using Lipofectamine 2000 (Invitrogen). Media containing retrovirus were collected 48 hours after transfection for U937 cells infection. The short hairpin RNA (shRNA) targeting p62 (NM_003900), Aurora A (NM_003600), and the nontarget shRNA (SHC002) were obtained from Sigma. 293FT cells were used for packaging lentivirus. After infection with the lentivirus, infected U937 cells were selected with puromycin.

**Statistics**

Data were expressed as mean ± SD of 3 determinations with GraphPad Prism software. Statistical analysis was analyzed using the Statistical Package for Social Sciences (SPSS) software (version 16.0). *P*-value < 0.05 was considered statistically significant.

**Results**

Aurora kinases inhibitors induce polyploidization of AML cell lines

To study the effect of Aurora kinases inhibitors on AML cell lines, the proliferation of cells was tested. U937, NB4, HL-60, and KG1a cell lines treated with Aurora kinases inhibitors (MK or ZM) for 24 and 48 hours were subjected to CCK8 assay (Fig. 1A and Supplementary S1A). The 2 drugs had slight effects on leukemia cell lines viability. For example, MK 1 μmol/L suppressed the proliferation of U937 cells by 17.3% ± 5.4% and 22% ± 6.3% at 24 and 48 hours respectively, and inhibited the proliferation of NB4 cells by 18.7% ± 1.8% and 20.4% ± 1.8% at 24 and 48 hours, respectively (Fig. 1A). As Aurora kinases were critical for the regulation of mitosis
process, the cell-cycle distribution of these cell lines was analyzed. Flow cytometry analysis showed that treatment of MK 1 µmol/L or ZM 2 µmol/L could induce polyploidization of both U937 and NB4 cells, as well as HL-60 and KG1a cells (Fig. 1B and Supplementary Fig. S1B). Western blotting confirmed the effect of Aurora kinases inhibition by testing the level of T288 phosphorylated Aurora A protein, and the results showed that the phosphorylated Aurora A decreased in a dose-dependent manner (Fig. 1C). Moreover, knockdown of Aurora A in U937 cells reduced the cell viability (Supplementary Fig. S2), indicating that the antiproliferation effect of MK and ZM was largely due to Aurora A inhibition.

Polyploidy cells induced by Aurora inhibition have high level of glycolytic metabolism

After 24 hours incubating with MK or ZM, U937 and NB4 cells converted to polyploidy cells. Study showed that metabolism was altered in polyploidy cells (23). So next, glucose uptake and lactate production in culture media were measured to evaluate the level of glycolytic metabolism. Compared with that of control cells, the level of glucose uptake and lactate production of U937 polyploidy cells was increased [Fig. 2A(a, b); glucose uptake, 8.3 ± 0.8 pmol/cell for control vs. 14.0 ± 0.4 pmol/cell for MK, 13.3 ± 2.8 pmol/cell for ZM and lactate production, 15.1 ± 2.2 pmol/cell for control vs. 25.4 pmol/cell for MK].

![Figure 2. Polyploidy cells induced by Aurora inhibition have higher metabolism level than control cells. A, U937 and NB4 cells were treated with MK 1 µmol/L or ZM 2 µmol/L for 24 hours, and the level of glucose uptake (a, c) and lactate production (b, d) in the culture media was examined. The results were normalized to the cells number, and conducted as pmol/cell. B, U937 and NB4 cells were treated with MK 1 µmol/L or ZM 2 µmol/L for 24 hours, followed by 2DG for another 24 hours, and CCK8 assay was conducted. Data were mean ± SD (n = 3). *P < 0.05, **P < 0.01.](image-url)
± 0.1 pmol/cell for MK, 24.4 ± 1.7 pmol/cell for ZM]. Similar results were observed in NB4 cells [Fig. 2A(c, d)]. 2DG, a glucose analog, was used to confirm whether the polyploidy cells were sensitive to energy deprivation. 2DG transiently inhibited glycolysis, which was considered as the mainly energy source of tumor cells (24). Figure 2B(left) showed that 2DG inhibited the proliferation of U937 polyploidy cells by ~30% at 2 mmol/L and ~40% at 5 mmol/L. In Fig. 2B(right), the inhibition rate of NB4 polyploidy cells by 2DG was ~30% at 2 mmol/L and ~50% at 5 mmol/L. Two drugs combination test was also conducted in normal PBMCs and we found minimal cell death in PBMCs with either single drug or 2 drugs combination treatment, indicating the observed phenotypes were tumor specific (Supplementary Fig. S3). Our data suggested that the polyploidy cells induced by Aurora kinases inhibitors required higher level of glycolytic metabolism than control cells.

**Inhibition of mTOR pathway decreases the level of glycolytic metabolism in AML cell lines**

Rapamycin is the specific inhibitor of mTORC1, and PP242 inhibits mTORC1 and mTORC2. U937 and NB4 cells were treated with different concentrations of rapamycin or PP242 for 24 hours, respectively. The activity of mTOR and its substrate 4EBP1 was detected by Western blotting. As shown in Fig. 3A, rapamycin inhibited the mTOR activation, as well as phosphorylated 4EBP1. The same results were also detected in PP242 treated cells. Rapamycin 0.05 μmol/L or PP242 1 μmol/L could inhibit mTOR activation, and the 2 concentrations were chosen for further...
In Fig. 3B, rapamycin decreased the level of glucose uptake, whereas PP242 significantly downregulated the glucose uptake and lactate production in U937 and NB4 cells. In addition, we also tested the inhibition of mTOR pathway in U937 and NB4 cells with increasing dose of Aurora kinases inhibitors. However, there was no change for the expression of phosphorylated mTOR and 4EBP1, suggesting that the mTOR pathway could not be suppressed by Aurora inhibitors (Fig. 3C).

**Polyploidy cells induced by Aurora inhibition are sensitive to the mTOR inhibitors**

Then we examined the effect of mTOR inhibitor on polyploidy cells. After exposed to MK 1 μmol/L or ZM 2 μmol/L for 24 hours, U937 and NB4 cells were treated with PP242 for another 24 hours. As shown in Fig. 4A, PP242 significantly suppressed the viability of polyploidy cells. Under the microscopy, the polyploidy cells exposed to PP242 broke into pieces [Fig. 4B(a)]. These morphological changes were confirmed by flow cytometry. For U937 cells treated with PP242, the sub-G1 rates of polyploidy cells induced by MK or ZM were 33.5 ± 3.0% or 43.4 ± 2.5% versus 2.15 ± 0.55% of control cells. For NB4 cells treated with PP242, the sub-G1 rates of polyploidy cells induced by MK or ZM were 24.3 ± 1.1% or 22.5 ± 1.6% versus 2.0 ± 0.3% of control cells [Fig. 4B(b)]. As mentioned in Fig. 3B, inhibition of mTOR decreased the metabolism level of the control cells. Moreover, the metabolism level significantly declined after the polyploidy cells were treated with PP242 (Fig. 4C). In U937 cells, the glucose uptake decreased from 23.1 ± 2.5 to 14.7 ± 1.1 pmol/cell, and lactate production decreased from 33.9 ± 0.8 to 18.6 ± 2.8 pmol/cell. In NB4 cells, the glucose uptake decreased...
from $14.8 \pm 2.0$ to $8.3 \pm 1.1$ pmol/cell, and lactate production decreased from $31.2 \pm 5$ to $21.8 \pm 4.4$ pmol/cell. To further investigate the combinational effect, U937 and NB4 cells were treated with Aurora kinases inhibitors (MK or ZM) and mTOR inhibitors (rapamycin or PP242) simultaneously for 48 hours. The metabolism examination also showed the same outcomes (Supplementary Fig. S4). Of note, as shown in Supplementary Figs. S5 and S6, the combination had synergistic effect on the proliferation inhibition of AML cells, but showed little effect on the normal PBMCs. These results indicated that, compared with control cells, the polyploidy cells had higher metabolism level and were more sensitive to mTOR inhibitors.

mTOR inhibitors or the glycolysis inhibitor induce not only apoptosis but also autophagy in polyploidy cells

Next, control cells and polyploidy U937 cells induced by Aurora inhibitors were treated with PP242 or 2DG, respectively. After 24 hours, the cells were stained with Hoechst 33342 dye and observed under fluorescence microscopy. Figure 5A showed that much more apoptotic bodies in polyploidy cells than that in control cells, and the percentage of apoptotic bodies was presented (MK, $14.0\% \pm 5.1\%$ for control, $56.3\% \pm 5.7\%$ for PP242, $47.3\% \pm 11.0\%$ for 2DG; ZM, $15.7\% \pm 5.5\%$ for control, $56.0\% \pm 15.6\%$ for PP242, $46.7\% \pm 10.7\%$ for 2DG). MDC dye was applied to stain the late autophagic vacuoles.

**Figure 5.** PP242 or 2DG induces not only apoptosis but also autophagy in polyploidy cells. U937 cells were treated with MK $1 \mu$mol/L or ZM $2 \mu$mol/L for 24 hours, followed by PP242 $1 \mu$mol/L or 2DG $5 \text{mmol/L}$ for another 24 hours. The apoptotic bodies in the cells were stained by Hoechst 33342 dye and the percentage of apoptosis cells was tested by flow cytometry (A). Autophagic vacuoles in the cells were detected by MDC dye and the percentage of MDC staining in cells was quantified by flow cytometry (B). The apoptosis and autophagy-related proteins in U937 cells were determined by Western blotting (C). $^* P < 0.05$; $^{**} P < 0.01$; $^{***} P < 0.001$. 

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**Supplementary Figures**

S4: [Supplementary Fig. S4](#).

S5: [Supplementary Fig. S5](#).

S6: [Supplementary Fig. S6](#).
As shown in Fig. 5B, inhibition of mTOR or the glycolysis in polyploidy cells increased the appearance of autophagic vacuoles, which seemed as distinct dot-like structures distributed within the cytoplasm or localized in the perinuclear regions in cells. Moreover, the percentage of autophagic vacuoles in cells tested by flow cytometry also showed the same results (MK, 36.5% vs. 17.3% for control, 79.2% vs. 10.0% for PP242, 77.7% vs. 18.7% for 2DG; ZM, 64.8% vs. 6.0% for control, 80.4% vs. 9.6% for PP242, 85.3% vs. 6.9% for 2DG; Fig. 5B).

The expression of apoptosis-associated proteins and autophagy markers was further determined. As shown in Fig. 5C, PP242 or 2DG inhibited mTOR activation at serine 2448 and 2481 phosphorylated sites in polyploidy cells. Either PP242 or 2DG increased the expression of cleaved caspase 3 and PARP (poly ADP-ribose polymerase) in polyploidy cells. P62/SQSTM1 was downregulated and LC3II were upregulated in polyploidy cells with PP242 or 2DG treatment, indicating autophagy occurred (Fig. 5C). Moreover, in Supplementary Fig. S7, the expression of p62 decreased and cleaved caspase 3 expression increased in cells with drug combination simultaneous for 48 hours.

**P62/SQSTM1 acts as a regulator in the metabolism of polyploidy cells induced by Aurora kinase inhibitors**

P62 is a key regulator of nutrient sensing in the mTOR pathway (25). The glucose deprivation was applied to further study the function of p62 in polyploidy cells. We found that the expression of p62 in polyploidy cells was significantly decreased when cultured in glucose-free media, suggesting that p62 was correlated with the metabolism of polyploidy cells (Fig. 6A). Expression of cleaved caspase 3 and PARP was significantly increased when compared with cells cultured with glucose-containing media (Fig. 6A). Furthermore, we chose one plasmid which showed great efficiency to knockdown p62 expression for virus packaging and U937 infection (Supplementary Fig. S8). P62 knockdown-U937 cells were treated with MK and subjected to Western blotting analysis with indicated antibodies.

Discussion

In this study, we found that Aurora kinases inhibitors could induce polyploidization in various AML cell lines. The induced polyploidy cells had higher level of glycolytic metabolism than control cells. Importantly, mTOR inhibitors could target p62 to promote apoptotic and autophagic death in Aurora kinases inhibitors induced polyploidy cells. Our data suggested that suppression the metabolism of polyploidy cells by mTOR inhibition was a novel approach to improve the therapeutic effect of Aurora kinases inhibitors in AML.

Aurora kinases were participated in a wide range of cell-cycle events and overexpressed in a numerous of tumors. Aurora kinases inhibitors have been considered as the attractive anticancer regents (4, 6, 26). In our study, MK and ZM inhibited Aurora A phosphorylation in a dose-
indicating that the polyploidy cells with high metabolism and result in apoptotic and autophagic death (Fig. 5), polyploidy cells (Fig. 2B), suppress the mTOR pathway, our study, 2DG could inhibit the proliferation of the decrease intracellular ATP, and induce autophagy (36). In glucose analog, had been reported to inhibit hexokinase, glycolytic metabolism than control cells (Fig. 2A). 2DG, inhibitors might display elevated glycolysis. Consistent with (35). Therefore, polyploidy induced by Aurora kinases inhibitors had been showed polyploidy might be independent of p53 pathways (data not show). The study of ZM on leukemia cells also indicated that polyploidy induced by Aurora kinases inhibitors did not inevitably result in apoptosis (29). Thus, polyploidy induced by Aurora kinases inhibitors seemed to be the cause of modest effect on proliferation. Eliminating polyploidy cells might enhance the effect of Aurora kinase inhibitors in AML treatment.

Metabolic pathways are activated in tumor cells, as well as polyploid tumor cells (30). In the study of comparison in the whole genomic transcripts of polyploidy and nonpolyploidy decidual cells in mouse, genes involved in metabolic process were upregulated (23). In addition, immortalized nontumorigenic human prostate and mammary epithelial cells which overexpressed pim-1, could gradually convert to polyploidy cells, with increase of reactive oxygen species (ROS) level (31). In aneuploid cells, the metabolic properties were also altered with increased glucose and glutamine consumption (32). Anatskaia and colleagues reported that the rearrangement of the mainly metabolic pathways such as depression of mitochondrial processes and increase of carbohydrate degradation and lipid biosynthesis in polyploidy cells, triggered pentose–phosphate pathway and depressed oxidative stress (33). Furthermore, these metabolic changes could increase metabolic plasticity and protect replicating DNA from oxidative damage (33), suggesting that polyploidy cells might be sensitive to metabolic inhibitors. Polyploidy induced by Aurora kinases inhibitors had been showed with more than 4N chromosomes (Fig. 1B and Supplementary Fig. S1B). The most obvious phenomenon of polyploidy-related changes was the decrease of nuclear surface-to-volume ratio, which modified chromatin architecture and exerted spectacular effects on genes activity (33, 34). A great of ATPase and energy were required for stemming from the metabolic shift from tricarboxylic acid cycle to glycolysis (35). Therefore, polyploidy induced by Aurora kinases inhibitors might display elevated glycolysis. Consistent with the previous reports, we showed that polyploidy cells induced by Aurora kinases inhibitors had higher level of glycolytic metabolism than control cells (Fig. 2A). 2DG, glucose analog, had been reported to inhibit hexokinase, decrease intracellular ATP, and induce autophagy (36). In our study, 2DG could inhibit the proliferation of the polyploidy cells (Fig. 2B), suppress the mTOR pathway, and result in apoptotic and autophagic death (Fig. 5), indicating that the polyploidy cells with high metabolism were susceptible to 2DG.

The mTOR signaling pathway integrates various metabolic pathways in cells. mTORC1 positively regulates the genes encompassed glycolysis, pentose phosphate pathway, lipid/sterol biosynthesis, and mitochondrial function (37, 38). For glycolysis, mTORC1 signaling stimulates glucose uptake, promotes glycolysis flux, and increases lactate secretion (37, 38). mTORC2 modulates cells metabolism through phosphorylating Akt/PKB and activating the downstream such as FoxO3a (19, 39). Furthermore, knockout of rictor (one of the mTORC2 components) in mice liver (40), adipose (19), or muscle (20) cells impaired glycolysis and lipogenesis. These studies suggested that mTOR pathway played an important role in activating cellular bio-energetic processes contributed to cancer. In this study, mTOR inhibitors decreased the glucose uptake and lactate production of AML cell lines (Fig. 3B). Numerous studies reported that rapamycin activated Akt signaling pathway via a negative feedback loop by inhibiting mTORC1 activation (41, 42). Low concentrations of rapamycin increased Akt and Erk phosphorylation through an mTORC1-dependent mechanism, but high doses of rapamycin inhibited Akt and Erk phosphorylation mainly via mTORC2 signaling pathway (43). Compared with mTORC1 inhibitor rapamycin, the dual mTORC1/2 inhibitor PP242 showed more effective to reduce cellular metabolic level in cancer cells (Fig. 3B). Furthermore, PP242 significantly decreased the proliferation of polyploidy cells, as well as the glucose uptake and lactate production, compared with that of control cells (Fig. 4), providing evidence that mTOR pathway regulated the metabolism of polyploidy cells. Thus, mTORC1/2 inhibition might be a potential approach to eliminate the polyploidy cells. Indeed, the improved anticancer effect of mTOR inhibitors and Aurora kinases inhibitors combination was reported in HCT116 cells recently (44). In our study, we elucidated a novel mechanism of the synergistic combination by suppression of cell metabolism.

Previous study reported T-cell lymphomas that overexpressed Myc were sensitive to VX-680 (MK), and the synthetic lethal interaction was executed by sequential apoptosis and autophagy. Exposed to VX-680 (MK) for 3 days, ~30% of the cells were in apoptosis, and the survived cells entered a new killing phase induced by autophagy after 3 days (45). In our study, apoptosis slightly increased and autophagy elevated in AML cells incubating with Aurora kinases inhibitors for 2 days. Significantly, PP242 and 2DG increased the apoptosis and autophagy of the polyploidy cells (Fig. 5). The results implied that inhibition the metabolism of polyploidy cells provoked not only apoptotic cell death, but also significant autophagy.

Autophagy, an evolutionary highly conserved process, is involved in degrading proteins and organelles in response to cellular stress (46). However, the function of autophagy in tumorigenesis is complex and paradoxical. On the one hand, autophagy protects tumor cells from stress including nutrient starvation, radiotherapy, and certain cytotoxic drugs (47). On the other hand, autophagy plays a vital role in suppressing tumorigenesis. Overexpression Beclin-1 gene exhibited increased susceptibility to cancer (48). Prolonged stress and...
excessive cellular damage might lead to cell death by over-stimulating autophagy (49). In this study, AML cell lines were shown increased autophagy level with polyploidization after treating with Aurora kinases inhibitors (Fig. S5B). Furthermore, either mTOR inhibitor PP242 or glycolysis inhibitor 2DG promoted the polyoidy cells death by significantly inducing apoptosis and autophagy (Fig. 5). We previously showed that Aurora inhibition induced autophagy and triggered drug resistance, which could be reversed by autophagy inhibitors (8). These paradoxical data suggested that cell death or survival promoted by autophagy depended on the physiologic state of the cell (47). In our present system, mTOR inhibition might overstimulate autophagy in polyoidy cells and eventually induce the polyoidy cells death.

The adaptor p62 is initially identified as a partner of the atypical PKC (protein kinase C), interacting with various signaling proteins to regulate multiple cellular functions, including apoptosis and autophagy (50–52). UBA and LIR domains of p62 confer the protein as a function as an adaptor between autophagy and ubiquitinated proteins (53). As an autophagy substrate, p62 is recruited to the autophagosomal membrane dependent or independent of LC3 for degrada-

tion (54, 55). Upregulation of p62 by defective autophagy was a key factor in tumorigenesis, and elimination of p62 might benefit to cancer therapy (56, 57). Deficiency of p62 increased ROS levels, enhanced cell death, and reduced tumorigenicity of Ras (58). mTORC1 activation inhibited autophagy by abolishing phosphorylated ATG13 protein binding to ATG1, promoting the accumulation of p62 protein via reducing its degradation (59). Indeed, decrease the p62 expression by mTOR inhibitor was showed in some reports (8, 60). Similarly, we found that expression of p62 decreased in mTOR inhibitors treated polyoidy cells (Fig. 5C), implying that p62 might be a target for oncotherapy. In addition, our data showed that p62 knockdown U937 cells treated with MK were observed with more apoptosis, whereas p62 overexpressed U937 cells reversed MK-induced apoptosis, indicating that p62 might act as a potential target for cancer treatment (Fig. 6B).

In summary, we found that polypoidy cells induced by Aurora inhibitors required more energy than that of the control cells. Suppression of metabolism by mTOR pathway inhibition increased the apoptosis and autophagy of the polyoidy cells. Moreover, p62 expression was decreased by mTOR inhibitor or glucose deprivation in polyoidy cells and might act as an attractive target. Thus, inhibition of mTOR pathway could increase the potential efficiency of Aurora kinases inhibitors in AML treatment.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors' Contributions
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): L.-L. Liu, Z.-J. Long, L.-X. Wang, Q. Liu
Writing, review, and/or revision of the manuscript: L.-L. Liu, Z.-J. Long, F.-M. Zheng, D.-J. Liu, Q. Liu
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