JQ1 Inhibits Proliferation and Induces Apoptosis of Leukemia Cells Through BCL-2 Regulated Pathway

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Abstract

Objective To explore the mechanism of JQ1 on leukemia cells.

Methods This study takes two myeloid leukemia cell lines as a research model. Cells treated with high concentration of JQ1 were collected for quantitative real-time PCR, immunoblot and flow cytometry to verify the effects of JQ1 on myeloid leukemia tumor cells. Combined with mRNA sequencing of cell lines to identify the differences in mRNA expression of different cell lines.

Results Two cell lines changed cell morphology under JQ1 treatment. The cell membrane appeared in varying degrees of wrinkled internal subsidence. K562 cell lines can maintain stable proliferation after being induced by a specific concentration of JQ1. However, JQ1 cannot induce the death of the K562 cells. Although the MYC and BCL2 gene expression decreased, JQ1 did not affect the c-Myc targeted genes to affect the cell cycle, nor did it trigger the BCL2-mediated apoptosis pathway. On the contrary, after JQ1 induced the MV-4-11 cells, the MYC-mediated cell cycle significantly slowed down and arrested at the G0/G1 phase. The death of MV-4-11 tumor cells through the apoptosis pathway regulated by BCL-2 family.

Conclusion JQ1 has different pharmacological effects on two myeloid leukemia cell lines. For MV-4-11, JQ1 mainly inhibited cell cycle by regulating MYC pathway and induced BCL-2-mediated apoptosis to kill myeloid leukemia tumor cells and thus perform anti-tumor effects. K-562 cells showed drug resistance to JQ1 which confirmed that the K-562 cell line has a feedback mechanism that prevents JQ1-induced apoptosis.

Introduction

Acute myeloid leukemia is the most common acute leukemia in adults, and its morbidity increases with age[1]. The occurrence of myeloid leukemia is due to the accumulation of undifferentiated and poorly differentiated myeloid cells, which derived from the mutation of genes related to hematopoietic cell proliferation and differentiation and the translocation of large chromosomal segments[2,3]. Despite advances in prognostic risk stratification and supportive treatment that have improved current therapies, overall long-term survival remains poor. Although several targeted drugs have been developed according to specific target sites, including FLT3 tyrosine kinase inhibitors[4,5], IDH1 and IDH2 inhibitor[2,1]. However, it is difficult to deal with the molecular diversity of myeloid leukemia merely with the help of some single targeted drugs to achieve real individualized treatment. In particular, the majority of diagnosed elderly patients have a larger possibility of having adversative cytogenetic characteristics[6,7]. Therefore, finding a new therapeutic approach for myeloid leukemia is essential.

Bromodomain proteins are of substantial biological interest, as components of transcription factor complexes and determinants of epigenetic memory. Sharing a characteristic molecular structure containing a different C-terminal recruitment domain and two N-terminal bromodomains, the family of BET (bromodomain and extra-terminal) proteins (BRD4, BRD2, BRD3, and BRDT) exhibits high levels of

sequence conservation. The function of BRD4 proteins is tightly bound to the acetylated lysine on histone proteins, considered as chromatin 'reader' proteins to regulate the expression of genes [8,9]. BET proteins recruit co-regulatory complexes such as chromatin-modifying enzymes, the chromatin mediator elements, and chromatin remodeling factors, to regulate the levels of gene transcription with the help of extra-terminal (ET) protein-interacting domain in the C-terminus [10,11,9]. JQ1, the selective inhibitor of BET, is highly effective against different subtypes of myeloid leukemia[12]. Interrupting the binding of the bromodomains of BRD4 to acetylated lysine, treatment with JQ1 displaces the BET proteins and elongation factors as well as the associated transcript initiation from the enhancers and promoters of several oncogenes[9].

Apoptosis refers to the spontaneous and orderly death of cells controlled by genes in order to maintain the stability of the internal environment[13]. It can orderly and effectively remove damaged cells caused by DNA damage, external stimulus, or remove redundant cells in the development process to maintain the stable and healthy development of the body as well as remove potentially harmful cells to prevent tumor growth. The mechanism of apoptosis is involved and participates in many signal transduction pathways[14]. Caspase-mediated exogenous or endogenous pathways can trigger apoptosis. These pathways converge to activate the apoptosis of caspases in the effector, leading to morphological and biochemical cell changes and apoptosis characteristics. In general, the balance between pro-apoptotic and anti-apoptotic protein regulators is the crucial point to determine whether apoptosis occurs[15-17]. In this study, we have utilized two kinds of myeloid leukemia cell lines to investigate the mechanism underlying the anti-tumor activity of JQ1. We observed the effects of JQ1 on the biological phenotype of myeloid leukemia cells and illuminated the fundamental molecular mechanism of JQ1 induced apoptosis.

Materials And Methods

Reagents

(S)-JQ1 (active enantiomer, hereafter referred to as JQ1) obtained from Sigma Aldrich (St. Louis, MO). Fetal bovine serum obtained from AusGeneX, USA. IMDM medium, penicillin/streptomycin mixture, dimethyl sulfoxide (DMSO), FITC-AnnexinV apoptosis assay kit, FITC-PI kit, Tris-Base, NaCl, glycine, Tween-20 and ammonium persulfate obtained from Sigma. Trizol total RNA extraction reagent, PrimeScriptTM RT reagent Kit with gDNA Eraser kit SYBR Premix ExTaq (TliRNaseH Plus) reagent was obtained from Takara Bio. PVDF membrane, SDS-PAGE gel kit, chemical coloring solution Western ECL Substrate, protein loading buffer obtained from BIO-RAD. Pre-stained protein Marker obtained from Thermo Scientific. BCA protein content determination kit, RIPA tissue/cell lysate, protease inhibitor Cocktail Set I obtained from Millipore. BSA obtained from Genevieve. All compounds were prepared as ten mM stocks in 100% DMSO and frozen at -20°C to -80°C in 10 µL aliquots to allow for single-use, thus avoiding multiple freeze-thaw cycles that could result in compound decomposition and loss of activity. BCL-2, C-MYC, β-actin, CASSASE-3 antibody, murine secondary antibody, and rabbit secondary antibody obtained from Sigma. P53 antibody obtained from Millipore.

Cell Culture

The myeloid leukemia cell lines MV4-11 and K-562 were obtained from Procell Life Science & Technology (Co. Ltd) on June 2018. All experiments with cell lines were performed within six months after thawing or obtaining from Procell Life Science & Technology. MV-4-11 and K-562 cells were cultured in 10% fetal bovine serum, 1% penicillin/streptomycin, and IMDM medium. All cells were cultured in a 5% CO₂ humidified incubator at 37°C.

Apoptosis analyses

Untreated and drug-treated cells were washed with PBS and centrifuged to remove supernatant, which was repeated three times. Then, cells were resuspended in annexin V binding buffer and incubated with annexin V-FITC and Propidium iodide for 15 minutes before flow cytometry analysis.

Cell-cycle analyses

Untreated and drug-treated cells were washed with PBS and centrifuged to remove supernatant, which was repeated three times. Then, cells were resuspended in 250ul PBS, then added 750ul Absolute ethanol slowly as a fixative solution. The mixture was shaken for 30 minutes at 4 ° C and then put it at -20 ° C for 1 hour. The cells were incubated with Propidium iodide and RNaseA for 15 minutes before flow cytometry analysis.

RNA isolation and quantitative chain reaction

Following the designated treatments with JQ1 or DMSO, total RNA was isolated from cultured cells with a PrimeScriptTMRT reagent Kit with gDNA Eraser kit SYBR Premix Ex Taq (TliRNaseH Plus) reagent and reverse transcribed. Quantitative real-time PCR analysis for the expression of c-MYC, BCL-2, HEXIM1, and p21 was performed on cDNA using SYBR GREEN. Relative mRNA expression was normalized to the expression of GAPDH.

Cell lysis, protein quantitation, and immunoblot analyses

Untreated or drug-treated cells were centrifuged, and the cell pellets were lysed, and the protein quantitation and immunoblot analyses were performed. Immunoblot analyses were performed at least twice. The expression levels of β -Actin served as the loading control. Representative immunoblots were subjected to densitometry analysis. Densitometry was performed using ImageQuant 5.2 (GE Healthcare, Piscataway, NJ).

Statistical Analysis

Significant differences between values obtained in a population of myeloid leukemia cells treated with different experimental conditions were determined using a two-tailed, paired t-test or one way ANOVA analysis within an analysis package of Microsoft Excel 2010 software or using GraphPad Prism (GraphPad Software, Inc., CA). P values of less than 0.05 were assigned significance.

Following the designated treatments with JQ1 or DMSO, total RNA was isolated from cultured cells and subjected to pretreatment and quality inspection, followed by double-end (PE) sequencing in illumine NovaSeq 6000 sequencer to analyze differential expression between untreated and drug-treated groups.

Results

1.JQ1 changed the viability of myeloid leukemia cell lines

Our study found that JQ1 has different changes in morphology and physiological functions of different myeloid leukemia cell lines (Fig. 1). Myeloid leukemia cells K-562 and MV-4-11 were treated with different concentrations of JQ1 (experimental group) and DMSO (control group) for ten days, respectively. The concentrations of JQ1 were 10nM, 100nM and 500nM. In K-562 cells, after JQ1 treatment, the cell proliferation ability slightly decreased. Although the number of dead cells did not change too much, the cell morphology changed significantly, which showed that there were differences in cell size, even some cells were several times larger than normal cells, and some cells had vacuoles in cells, and these effects were more obvious with the increase of JQ1 drug concentration. Mv-4-11 showed that the number of cells decreased significantly, the overall state of cells was poor, and the number of dead cells increased significantly, which may induce apoptosis or necrosis. These effects were obvious with the increase of JQ1 concentration.

2.JQ1 arrested cell cycle of MV-4-11 cell lines at G0/G1 phase

To further determine whether JQ1 attenuated cell viability by affecting the cell cycle distribution or not. Flow cytometry was performed to analyze the cell cycle distribution. In MV-4-11 cells, compared with the DMSO control group and blank control group, the percentage of cells in the G1/G0 phase increased while their S phase and G2 phase decreased after JQ1 treatment(Fig. 2a).These effects also proved by the researches of Jennifer A. Mertz[18]. However, the treatment of JQ1 cannot arrest the cell cycle of K562 cell line at any phase (Fig. 2b). To test the possibility that whether BET inhibitor can explicitly retract cell cycle transcription, we utilized global transcriptional profiling and unbiased gene set enrichment analysis (GSEA)[19] to deeply analysis the result of RNA-seq. We evaluated a canonical transcriptional signature of cell cycle gene sets obtained from Molecular Signatures Database (MSigDB)[20,21] and found the signature strongly correlated with downregulated of expression by JQ1 (Fig. 2c and 2e). Next, we have performed quantitative real-time PCR analysis to validate the hypothesis that JQ1 reduces the proliferation of MV-4-11 by arresting cell cycle at the G0/G1 phase. Cyclin D1 is an essential regulator of the G1–S transition in response to growth factor stimulation in cells[22], CDK4 and CDK6 are also critical factors for G1-S transition.[23] We found that the relative mRNA expression of CDK6 and CYCLIN D1 dramatically decreased with the treatment of JQ1 (Fig. 2d and 2f). Besides, BRD4 is also an essential mediator of transcriptional elongation, interacting with pTEFb (positive transcription elongation factor b) [24]. CDK9(cyclin-dependent kinase-9), a core component of pTEFb, can be recruited to mitotic chromosomes contributing to increased expression of growth-promoting genes, its relative mRNA

expression also decreased. The above results suggest that JQ1 may block cell proliferation from G1/G0 into the S phase; these findings are complementary with the research of Lee, D. H. and Wang, T.[25,26]. However, K-562 cells have little change in the cell cycle, which can be considered that JQ1 does not limit the viability of K-562 cells by affecting cell growth ability.

3.The effects of JQ1 on MYC of myeloid leukemia

c-Myc is a master regulatory factor of cell proliferation[27]. In cancer, pathologic activation of c-Myc plays an essential role in disease pathogenesis by the coordinated upregulation of a transcriptional program influencing cell division, metabolic adaptation, and survival[27,28]. Therefore, we next investigated whether JQ1 has different effects on MV-4-11 and K562, respectively, which resulting in different changes in cell cycle and morphology. After a series of different time treatment of both MV-4-11 and K562 cell lines with 500nM JQ1, we performed qPCR to detect the relative mRNA levels and found that JQ1 induced and sustained decreases in MYC transcriptional levels (Fig. 3a). This was accompanied by a loss of detectable c-Myc protein in whole-cell lysates at both 48 and 72hours after treatment (Fig. 3e). We evaluated two canonical transcriptional signatures of MYC pathway's gene sets obtained from Molecular Signatures Database (MSigDB) and found two signatures strongly correlated with the downregulated expression by JQ1 (Fig. 3d). To define whether the changes of MYC transcription level with JQ1 treatment have further influence c-Myc target genes, we utilized the data from RNA-seq and selected 32 known c-Myc target genes. After 72 hours of treatment of MV-4-11 and K562 with DMSO and 500nM JQ1, collecting total RNA and performing pre-processing and quality inspection, followed by two-ended (PE) sequencing in illumine NovaSeq 6000 sequencer. We found that the MYC gene expression of MV-4-11 with the treatment of 500nM JQ1 decreased significantly as well as the c-Myc target genes also had the same significant downward trend (Fig. 3b). This effect of JQ1 is similar to the research of Ott, C. J. and Delmore, J. E. [29,30]. However, although the K562 cell line under JQ1 treatment had decreased expression of MYC gene, c-Myc target Genes did not show a significant downward trend while the expression of c-Myc target genes was similar to the DMSO control group (Fig. 3c). Therefore, the above results indicate that MV-4-11 may affect the cell cycle by reducing the expression of MYC gene, thereby affecting cell proliferation. However, JQ1 did not significantly affect the expression of c-Myc downstream genes of K562 cells though the expression of MYC had reduced. There may exist compensation pathways for the expression of MYC gene in K562 cells to escape the effects of JQ1, which verified that JQ1 did not significantly change cellular proliferation and cell cycle of K562 cell line.

4.Effects of JQ1 are associated with Bcl2 family

After 72 hours of treatment of MV-4-11 and K562 with DMSO and 500nM JQ1, collecting total RNA and performing pre-processing and quality inspection, followed by two-ended (PE) sequencing in illumine NovaSeq 6000 sequencer. We analyzed the RNA-seq data and did KEGG analysis of the genes that differential significantly, and got top30 pathways, including apoptosis pathway (Fig. 4a). Besides, we selected 80 cancer-related genes and tested their relative mRNA expression. Excellent concordance was observed between JQ1 treatment group and the control group. There existed the difference in expression

of the BCL-2 gene after JQ1 and DMSO treatment, respectively. Because BCL-2 gene is the core regulatory gene of apoptosis, which explaining that the effect of JQ1 on MV-4-11 is related to apoptosis, and the results are consistent with KEGG's analysis (Fig. 4b). However, the analysis of the cancer-related genes of K562 found no significant difference in the expression of bcl2 in the JQ1 treatment group and the control group (Fig. 4c).

In general, the balance between pro-apoptotic and anti-apoptotic protein regulators is the crucial point to determine whether apoptosis occurs[1]. Therefore, we selected the core genes associated with anti-apoptosis and pro-apoptosis[31,32]. In the apoptosis-related genes of the MV-4-11 cell line, all anti-apoptosis genes were down-expressed after JQ1 treatment, including BCL2, BCL2A1, BCL2L1, BCL2L2, except for the MCL1 gene. While most of the significant pro-apoptosis related gene expressions are uplifted, including APAF1, BAK, BCL2L11, BCL2L12, BCL2L13, BMF, PMAIP1, therefore, the effect of JQ1 on MV-4-11 cell line is most likely achieved by overexpression of pro-apoptotic proteins and downregulation of anti-apoptotic proteins to promote apoptotic proteins (Fig. 5a). After K562 cell line treated by JQ1, the expression of other anti-apoptosis genes increased, although the expression of the core gene BCL2 decreased. Furthermore, the trend of pro-apoptosis gene changes is difficult to determine whether to promote or prevent apoptosis (Fig. 5b). To prove that the effect of JQ1 is related to apoptosis, we used qPCR to detect the mRNA expression of the BCL2 gene after JQ1 treatment at 24h, 48h, 72h, 96h and found that the expression of BCL2 gene in both cell lines significantly decreased (Fig. 5c). Besides, we used immune blot to detect caspase-3, BCL2, P53 protein expression of MV-4-11 and K562 after JQ1 treatment for 24 hours, 48 hours, found that the MV-4-11 cell line after JQ1 treatment. The expression of the executive protein caspase-3, which must be performed during the apoptosis process, increased significantly. However, the amount of BCL-2 protein increased slightly (Fig. 5d), suggesting that there may be a possible way for cells to compensate for the reduction of bcl2 protein reduction caused by apoptosis. K562 is entirely different. The cell line failed to detect the rise of the caspase-3 protein, while BCL2 protein expression also increased to resist apoptosis (Fig 5e).

5.Effects of JQ1 on apoptotic program

To further determine whether the effects of JQ1 on MV-4-11 cell line and K562 cell line are associated with apoptosis. MV-4-11 and K562 cells were exposed to a specific concentration of JQ1(500uM) or DMSO for 48h. DMSO-treated and JQ1-treated cells were collected and incubated with Annexin V-FITC and PI staining, then the apoptosis changes were detected by flow cytometry. It can be seen that there are no significant changes in K-562 cells (Fig. 5g). However, the JQ1 treatment group of MV-4-11 is significantly different from the DMSO control group, showing an increase in apoptosis rate. Besides (Fig. 5f), fetal bovine serum deprivation medium will increase the inactivation of cells in the control group. Therefore, this may be considered as the reason for PI-positive Annexin V positive in the control group.

We next evaluated a canonical transcriptional signature of the apoptosis pathway's gene sets obtained from Molecular Signatures Database (MSigDB). For MV-4-11 cell line, Gene set defined by the apoptotic related pathway is significantly enriched in JQ1-upregulated genes. However, JQ1 treatment did not exert

significant changes for K562 cell lines (Fig. 6a). To further compare the differences between the performance of the two cell lines after JQ1 treatment, we further analyzed core genes obtained by GSEA analysis, which made a significant contribution to gene sets enrichment score. It was found that after JQ1 was treated in the MV-4-11 cell line, CFLAR (CASP8 and FADD like apoptosis regulator)[33], TNF (tumor necrosis factor)[34], CASP3, CASP6, CASP9 is upregulated significantly (Fig. 6b). It is proved that the death of MV-4-11 cells was subjected to the apoptosis pathway under the role of JQ1.

On the contrary, after JQ1 operated the K562 cell line, there was no change in the core genes in the apoptosis pathway (Fig. 6c). Besides, through the heatmap and scatter plots of differentiation genes of K562 cell line between JQ1-treatment and DMSO-control group, there is still a considerable amount of changes in K562 cell line after JQ1 treatment (Fig. 6d and 6e). The above results indicate that the effects of JQ1 on K562 cell line maybe not through the apoptosis pathway. This hypothesis is complementary with researches of Ott, C. J., and Zuber, J.[35,29].

Discussion

To determine the death pathway and drug resistance mechanism of myeloid leukemia cells in response to JQ1, we selected the myeloid leukemia cell lines K-562, MV-4-11, as research models. K-562 was a human erythroleukemia line. K-562 blasts are multipotential, malignant hematopoietic cells that spontaneously differentiate into recognizable progenitors of the erythrocytic and monocytic series. In this research, although K-562 cells weaken the capability of growth and proliferation after JQ1 treated, there is no apparent the fact that JQ1 can induce the apoptosis of K-562 cells. With further verification, we found that, in K-562 cells, treatment with JQ1 reduces MYC, CYCLIND1, CDK6 relative mRNA levels. However, although the K562 cell line under JQ1 treatment had decreased expression of MYC gene, c-Myc target Genes did not show a significant downward trend.

What is more, with the results of apoptosis and immunoblot assays, we demonstrate that K-562 cells exhibit the resistance of JQ1 while it performed significantly decreased in BCL-2 mRNA level. K-562 cells induced by JQ1 show stress in the early stage that exists a tendency to apoptosis, but then it shows drug resistance to JQ1, which shows that cell death may be arrested while cell proliferation slow down. On conclusion, we believe that the K-562 cell line has a feedback mechanism that prevents JQ1-induced death outcomes.

MV-4-11 cells were established by Rovera and associates from the blast cells of a 10-year-old male with biphenotypic B-myelomonocytic leukemia. In the present study, MV-4-11 has a very typical cell death after JQ1 treatment, which is characterized by a sharp change in cell morphology and physiological function. Further experiments have confirmed that MV-4-11 is extremely sensitive to JQ1; it may promote the death of MV-4-11 tumor cells through the apoptosis pathway regulated by BCL-2 family.

Based on the above results, we believe that the BCL-2 family protein is a target for JQ1 to regulate apoptosis of myeloid tumor cells and thereby kill blood tumors. Although BCL-2 protein does not affect cell proliferation, its overexpression can block cell apoptosis. When BCL-2 protein is down-regulated by

upstream genes or other cytokines, it loses its inhibition of BAX/BAK, which triggers mitochondrial outer membrane permeabilization (MOMP), releasing cytochrome C (CytC) into the cytoplasm, thereby activating the Caspase protein family and causing apoptosis.

JQ1 is a BRD4 protein inhibitor, and BRD4 protein is a chromatin "reader" that has the function of opening chromosomes, thereby regulating gene expression. In this study, the JQ1-sensitive cell line promoted programmed cell death by regulating the BCL-2 protein family. For JQ1 resistant cell lines, JQ1 may not continue to suppress BCL-2 expression through compensation or negative feedback mechanism. After BCL-2 is up-regulated, apoptosis is inhibited. However, whether the JQ1-sensitive cell line inhibits the slackening of chromosome structure by inhibiting BRD4, thereby reducing the expression of the target gene or alter the ability of the BCL-2 gene transcription factor to bind to its promoter, thereby rendering BCL-2 expression inactive? This will be the problem we will continue to explore, based on which we can further study the production of molecular resistance of myeloid leukemia cells to JQ1 and its molecular mechanism.

Tumor patients have not only extensive inter-tumor heterogeneity but also intra-tumor heterogeneity in each individual's tumor tissue. The formation and development of tumors derived from a large number of genetic variants, each of which often has hundreds of genetic variations. Therefore, even with the same drug, the response mechanisms in different types of tumor cells are different. myeloid leukemia is a disease caused by suspended tumor cells in the circulatory system. It is not possible to choose to remove a tumor mass or to kill tumor cells with a high dose and targeted radiation as well as stable tumor patients. Even with large doses of anti-tumor drugs, it can cause significant damage to the patient. Our studies have confirmed that how myeloid leukemia cell lines die in vitro under JQ1 treatment is mainly apoptosis, not other forms of death such as necrosis and autophagy. Besides, this study also demonstrated that the same drug showed different biological effects when treating cells of different genetic backgrounds of the same disease type. That is, two cell lines derived from different myeloid leukemia patients have different genes expression and phenotypes under JQ1 induction. Therefore, we believe that whether clinically effective tumor treatment can be achieved, depends on whether individualized treatment plans can be developed according to specific patients just as the current development of pharmacogenomics, the genetic SNPs of patients are used to guide the types of clinical drugs and concentrations of drugs to achieve the purpose of optimizing the medication regimen.

In conclusion, the results of this study will provide a more supportive theoretical basis for the prognosis assessment of JQ1-based treatment regimens for myeloid leukemia suspension cells, and predict the possible individual resistance, which will help the treatment plan to achieve the effective clinical treatment. How to establish accurate detection of JQ1 detection indicators for blood tumor patients at the genomic level will be the core issue for us to continue this study in-depth, and also an essential step from basic research to clinical detection applications.

Declarations

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Conflicts of interest/Competing interests

The authors report no conflicts of interest.

Availability of data and material

Not applicable

Code availability

Not applicable

Authors' contributions

ZYF and HWY conducted most of the experiments, analyzed the results, and wrote most of the paper. LL and LXH conducted sample collection and statistics. ZYF was responsive to the clinic consultant. XY conceived the idea for the project and wrote the paper with HWY.

Ethics approval

Ethical approval was obtained from the ethical committees of The Third Affiliated Hospital of Guangzhou Medical University, Guangdong, People's Republic of China. All methods in this study were performed in accordance with the relevant regulations.

Consent to participate

Consent to participate were obtained from all authors.

Consent for publication

Consent for publication were obtained from all authors.

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Figures

Figure 1

The cytotoxic effects of JQ1 on myeloid leukemia cell lines in vitro. Two types of human myeloid leukemia cells MV-4-11 and K562 were exposed to various concentrations of JQ1(10-500nmol/L) for 10 days. JQ1 inhibited the viability of MV-4-11 cell line in a dose dependent manner in vitro. Scale bars, 50um

Figure 2

JQ1 arrested cell cycle of myeloid leukemia cell lines at G0/G1 phase. Two types of human myeloid leukemia cells MV-4-11 and K562 were exposed to a specific concentration of JQ1(500nmol/L) or DMSO for 24 hours. (a-b) JQ1 treated cells were analyzed by flow cytometric, which show significant accumulation of cells at G0/G1 phase on MV-4-11 cells. Corresponding histograms were shown on the

right. (c and e) Gene set enrichment analysis of cell cycle signatures. (d and f) Quantitative PCR of CDK6, CDK9, CYCLIND1 transcript levels in K562 and MV-4-11 cells treated with 500nM JQ1.

Figure 3

The effects of JQ1 on MYC of myeloid leukemia. (a) Quantitative PCR of MYC transcript levels in K562 and MV-4-11 cells treated with 500nM JQ1 at indicated time points. (b-c) Heatmaps of MYC and c-Myc target genes expression in MV-4-11 and K562 cells treated with 500nM JQ1 for 72 hours. HK indicates

housekeeping controls. (d) Gene set enrichment analysis of two typical MYC signatures. (f) Immunoblotting for c-Myc in whole cell lysates of cells treated with 500nM JQ1.

Figure 4

Effects of JQ1 are associated with apoptosis pathway. (a) Obtained data from RNA-seq in MV-4-11 and K562 cells treated with 500nM JQ1, then performed KEGG analysis of the genes that differential

significantly and got top30 pathways. (b) Heatmap of cancer-related genes expressed in myeloid leukemia cells, treated with 500nM JQ1 for 72 hours.

Figure 5

(a-b) Heatmap of the core genes associated with anti- and pro-apoptosis in MV-4-11 and K562 cells treated with 500nM JQ1 for 72 hours. (c) Quantitative PCR of BCL-2 transcript levels in K562 and MV-4-11 cells treated with 500nM JQ1 at indicated time points. (d-e) Immunoblotting for caspase-3, bcl-2, P53

in whole cell lysates of cells treated with 500nM JQ1. (f-g) Apoptotic tests were analyzed by flow cytometric, which show significant accumulation of cells at PI (+) AnnexinV (+) in MV-4-11 cells while K562 cells have no significant changes. Corresponding histograms were shown on the right.

Figure 6

K562 resistant to JQ1 (a) Gene set enrichment analysis of apoptosis signatures of MV-4-11 and K562 cells. (b-c) Heatmap of core genes of apoptosis obtained from GSEA analysis, which made a major

contribution to gene sets enrichment score. (d-e) heatmap and scatter plots of differentional genes of K562 cell line between JQ1-treatment and DMSO-control group