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The synergistic killing of AML cells co-cultured with HS-5 bone marrow stromal cells by As_2O_3 and the PI3K/Akt signaling pathway inhibitor LY294002

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We aimed to investigate whether a combination of resistance to arsenic trioxide (As_2O_3) and the phosphoinositide 3-kinase (PI3K)/Akt signaling pathway inhibitor LY294002 can inhibit the proliferation of AML cells in the bone marrow microenvironment. Three AML cell lines were grown with HS-5 human bone marrow stromal cells in adherent co-cultures. The inhibitory effects of As_2O_3 alone or in combination with LY294002 on the proliferation of these co-cultured AML cells were observed. The PI3K/Akt signaling pathway was detected by Western Blot in co-cultured AML cells cultured alone or treated with As_2O_3 alone or in combination with LY294002. Our results demonstrate that AML cells adhered to stroma exhibited significantly reduced sensitivity to As_2O_3. The resistance can be partially abolished by inhibiting the PI3K/Akt pathway. The administration of As_2O_3 in combination with a PI3K/Akt signaling pathway inhibitor may be expected to become a new approach to the treatment of AML.

1. Introduction

Arsenic trioxide (As_2O_3) is the main component of arsenical traditional Chinese medicines; during the past three decades, this medicine has been widely used for the treatment of hematologic malignancies, particularly acute promyelocytic leukemia (Soignet et al. 2001; Carmosino et al. 2004; Leoni et al. 2002). Guo et al. (2010), who co-cultured cells from the K562 chronic myeloid leukemia (CML) cell line with cells from the HS-5 human bone marrow stromal cell line to simulate the growth and maintenance of CML cells in the bone marrow microenvironment, found that As_2O_3 produced the same rates of proliferation inhibition in K562 cells cultured alone and K562 cells co-cultured with HS-5 cells. This result suggested that As_2O_3 may inhibit the proliferation of leukemia cells supported by the bone marrow microenvironment. However, in an earlier study we demonstrated that the As_2O_3 sensitivity of cells from the NB4 acute promyelocytic leukemia (APL) cell line was significantly reduced by co-culturing with HS-5 bone marrow stromal cells (Chen et al. 2014). No prior reports have addressed whether this stromal cell-induced resistance to As_2O_3 exists in other acute myeloid leukemia (AML) cell lines or which mechanisms are involved in this resistance.

The phosphoinositide 3-kinase (PI3K)/Akt signaling pathway is an important intracellular signaling pathway. In cells, this pathway plays vital roles in inhibiting apoptosis and promoting proliferation. The abnormal activation of this pathway can be detected in many hematologic malignant cells (Osaki et al. 2004; Xu et al. 2003; Kubota et al. 2004). In addition, research has confirmed that the activation of this pathway is involved in the processes associated with As_2O_3 resistance in the HL60 and U937 AML cell lines (Chen et al. 2006). Previous studies have reported that treatment with a combination of the PI3K/Akt signaling pathway-specific inhibitor LY294002 and As_2O_3 may enhance the inhibitory effects of As_2O_3 on the proliferation of primary APL cells (West et al. 2004), HL60AR cells (HL60 cells with persistent activation of the PI3K/Akt signaling pathway), U937 cells, K562 cells (Tabellini et al. 2005), and cells from T-cell lymphoblastic leukemia (Ramos et al. 2005). To date, no reports have addressed whether a combination of LY294002 and As_2O_3 can synergistically inhibit cells from AML cell lines that have been co-cultured with HS-5 cells. Thus, in this study, cells from the NB4, HL-60, and U937 AML cell lines were co-cultured with stromal cells, and the cytotoxic effects of a combination of As_2O_3 and LY294002 on these co-cultured AML cells were investigated.

2. Investigation and results

2.1. For AML cells, adhesion inhibited proliferation and increased the proportion of cells in the G0/G1 growth phases

After adherent co-culture with HS-5 cells for 24 h, AML cells adherent to stromal cells were collected and sorted. BrdU assays revealed that after co-culture, the proliferation of NB4, HL-60, and U937 cells was inhibited. Specifically, as shown in Fig. 1A, the proliferative capacities of suspension cultures of NB4, HL-60, and U937 cells co-cultured with HS-5 cells were 73.23 ± 4.14 % ($P < 0.05$), 78.02 ± 3.72 % ($P = 0.05$), and 74.81 ± 5.15 % ($P < 0.05$), respectively, of the proliferative capacities of the corresponding monocultured cells. The

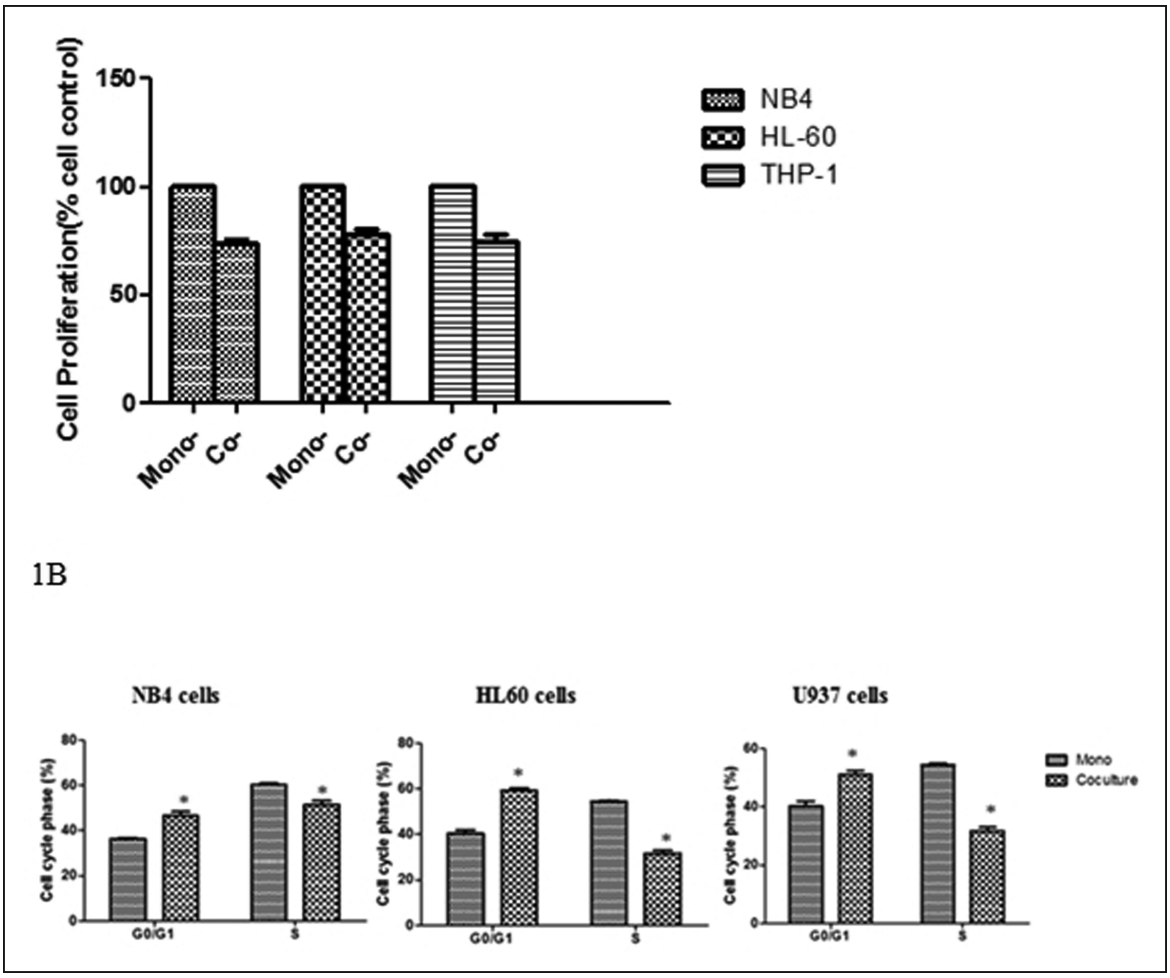

Fig. 1: Proliferation inhibition and cell cycle arrest in AML cells co-cultured with HS-5 bone marrow stromal cells. Suspension cultures of AML cells grown alone or in adherent co-culture with HS-5 cells for 24 h were assessed. A: BrdU assays were used to detect cell proliferation. Inhibition rates were defined as follows: inhibition rate (%) = (the OD value for the monocultured suspension culture group–the OD value for the co-cultured group)/the OD value for the monocultured suspension culture group × 100 %. The experiment was repeated three times, and the results were expressed as means ± standard deviation (X ± SD). B: Flow cytometry was used to detect cell cycle distributions. These experiments were repeated three times, and the results were expressed as X ± SD. *: $P < 0.05$.

proportion of cells in the G0/G1 growth phases was elevated for co-cultured AML cells relative to monocultured AML cells. Specifically, the proportions for monocultured cells *vs*. co-cultured cells were 35.55 ± 1.25 % *vs*. 46.5 ± 3.18 % for NB4 cells, 34.76 ± 3.38 % *vs*. 47.2 ± 2.1 % for HL-60 cells, and 40.3 ± 2.7 % *vs*. 51.1 ± 2.35 % for U937 cells (Fig. 1B).

2.2. Adhesion caused AML cells to become resistant to As_2O_3, whereas LY294002 and As_2O_3 may synergistically kill AML cells co-cultured with HS-5 cells

The proliferation-inhibiting effects of As_2O_3 were significantly reduced in AML cells co-cultured with HS-5 cells (Fig. 2A). In particular, the inhibition rates for co-cultured *vs*. monocultured cells were 15.86 ± 3.01 % *vs*. 62.92 ± 2.74 % for NB4 cells, 14.96 ± 1.40 % *vs*. 48.54 ± 4.54 %, for HL-60 cells, and 19.19 ± 2.39 % *vs*. 52.53 ± 2.99 % for U937 cells, with $P < 0.01$ for each group. Moreover, the induction of apoptosis by As_2O_3 was significantly decreased in co-cultured AML cells relative to monocultured AML cells (Fig. 2B). Specifically, the apoptosis rates for co-cultured *vs*. monocultured cells were 13.6 ± 0.85 % *vs*. 23.4 ± 2.3 % for NB4 cells, 9.83 ± 1.99 % *vs*. 17.97 ± 1.58 % for HL-60 cells, and 7.27 ± 1.77 % *vs*. 17.97 ± 2.05 % for U937 cells, with $P < 0.05$ for each group. The Q values for the cell proliferation inhibition rate of a combination of LY294002 and As_2O_3 were > 1.15 for all three examined AML cell lines, indicating that these two drugs synergistically inhibited the proliferation of AML cells; in addition, apoptosis rates were significantly elevated among cells treated with both drugs.

2.3. LY294002-induced inhibition of the PI3K/Akt signaling pathway in suspension cultures of monocultured AML cells

When suspension cultures of monocultured AML cells were treated with 10 μM of LY294002 for 2 h, the protein expression levels of p-Akt^{Ser473}, p-Akt^{Thr308}, and p-PDK1 were significantly reduced. Therefore, in subsequent analyses of the combined effects of LY294002 and As_2O_3, 2 h of LY294002 treatment was followed by treatment with As_2O_3. As the LY294002 treatment duration extended from 2 to 12 h, the down-regulation of p-Akt^{Ser473}, p-Akt^{Thr308}, and p-PDK1 protein expression and the upregulation of p-PTEN protein expression became increasingly apparent (Fig. 3).

2.4. Adhesion can activate the PI3K/Akt signaling pathway in AML cells, and LY294002 in combination with As_2O_3 can significantly inhibit this signaling pathway

After adherent co-culture with HS-5 cells for 24 h, the three examined AML cell lines exhibited up-regulated expression of the signaling molecules p-Akt^{Ser473}, p-Akt^{Thr308}, and p-PDK1 in

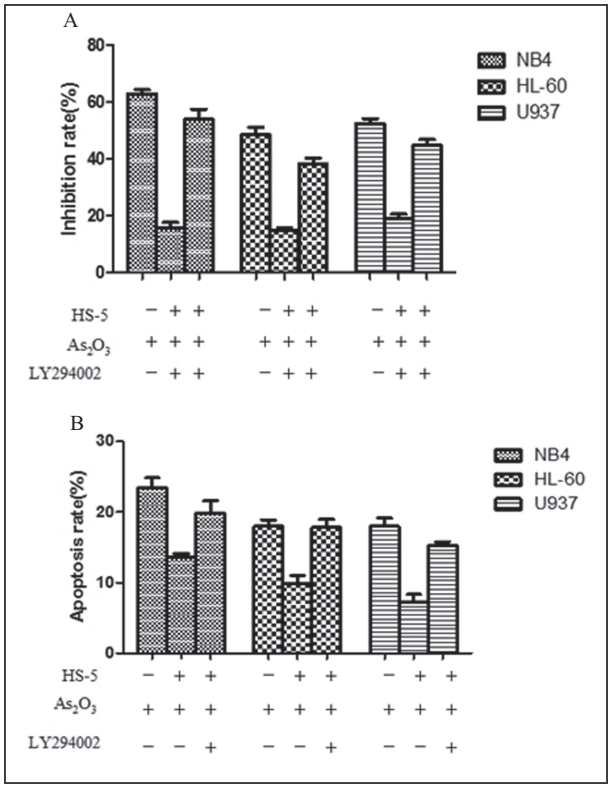

Fig. 2: A/2B: LY294002 and As2O3 can synergistically kill co-cultured AML cells. Suspension cultures of 1 × 105 NB4 cells/ml, 3 × 105 HL60 cells/ml, or 2 × 105 U937 cells/ml from monocultured AML cells or AML cells grown in adherent co-culture with HS-5 cells for 24 h were assessed. Treatment groups included the As2O3 treatment group (1 μM As2O3 for NB4 cells; 2 μM As2O3 for HL60 cells; and 4 μM As2O3 for U937 cells) and the LY + As2O3 treatment group (4 h of LY294002 treatment combined with incubation with As2O3 for 72 h). A: The detection of cell proliferation using a BrdU assay. B: Cell cycle analysis by flow cytometry.

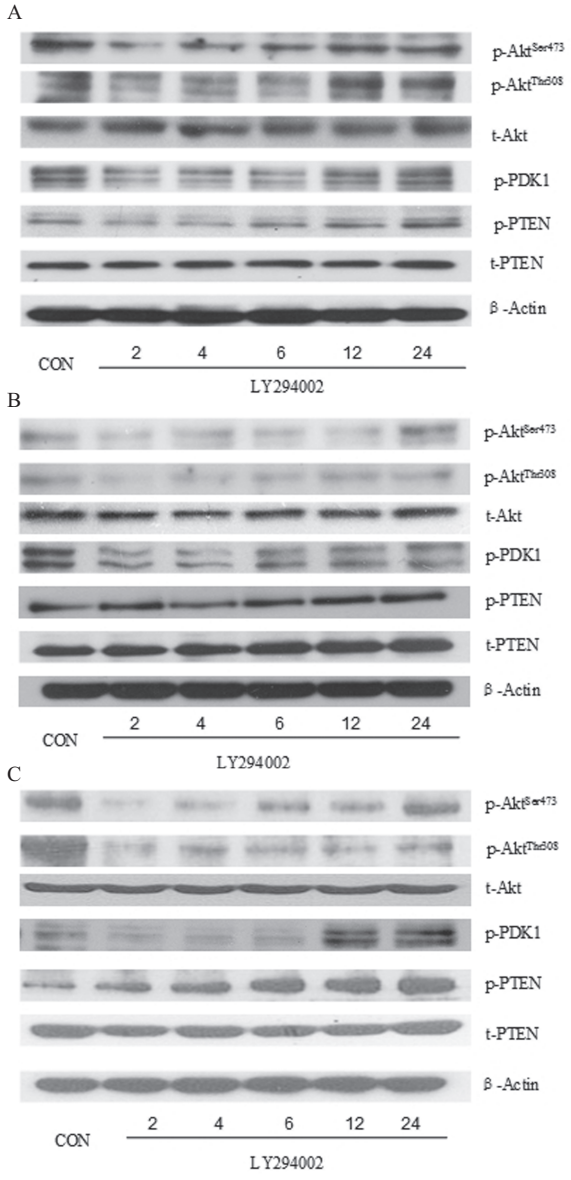

Fig. 3: A-C: Changes in important molecules in the intracellular PI3K/Akt signaling pathway of AML cells at different times after LY294002 treatment. After separate suspension cultures of 1 × 10^5 NB4 cells/ml (A), 3 × 10^5 HL-60 cells/ml (B), and 2 × 10^5 U937 cells/ml (C) were treated with LY294002 (10 μM of LY294002 for NB4 and HL60 cells; 20 μM of LY294002 for U937 cells) for 2-24 h, western blotting was used to detect the protein levels of various important molecules. The quantity of protein loaded into each well was 40 μg; CON indicates the results from a sample that was not treated with LY294002.

the PI3K/Akt pathway but down-regulated protein expression of p-PTEN. These findings indicated that adherent co-culture with HS-5 cells can activate the PI3K/Akt signaling pathway in AML cells; after LY294002 treatment, the activation of this signaling pathway was inhibited in co-cultured AML cells. In addition, the PI3K/Akt signaling pathway in AML cells was further suppressed by the synergistic effects of LY294002 and As_2O_3 (Fig. 4).

3. Discussion

The relapse of AML is a major problem that negatively impacts long-term disease-free survival among AML patients (Huntly et al. 2005; Chan et al. 2008; Taussig et al. 2010). Recent studies have suggested that the root cause of relapse is the presence of residual leukemia cells with multidrug resistance in the bone marrow microenvironment (Macanas-Pirard et al. 2012; Katsumi et al. 2011; Krause et al. 2013; Becker et al. 2009). The bone marrow microenvironment, which consists of stromal cells, adhesion molecules, and cytokines, may provide substantial signaling support for normal hematopoiesis (Nemeth et al. 2009, 2010; Goldman et al. 2009). However, it has also been demonstrated that this microenvironment provides substantial signaling support for the growth and maintenance of leukemia cells; these effects decrease the sensitivity of AML cells to conventional chemotherapy drugs, allowing these cells to escape the cytotoxic effects of these drugs and eventually cause relapse of AML (Zeng et al. 2009; Parmar et al. 2011; Konopleva et al. 2002). Therefore, the question of how to improve the sensitivity of AML cells to chemotherapy in this microenvironment and reverse the multi-drug resistance of these cells has become a frequently addressed topic in research examining AML treatment. In this study, AML cells were grown by adherent co-culture with cells from the HS-5 line of bone marrow stromal cells. The study results demonstrated that after co-culture, AML cells inhibited proliferation and exhibited arrest in the G0/G1 phases. Moreover, the co-cultured AML cells were resistant to As_2O_3, and the proliferation-inhibiting and apoptosis-inducing effects of As_2O_3 were weakened. Subsequently, the intracellular mechanisms underlying the As_2O_3 resistance of AML cells after co-culture were explored.

In vivo, the PI3K/Akt signaling pathway is an important pathway involved in cell proliferation, differentiation, and apoptosis. A major mechanism by which intracellular signal transmission occurs in this pathway is PI3K activation, which produces the second messenger phosphatidylinositol (3,4,5)-trisphosphate

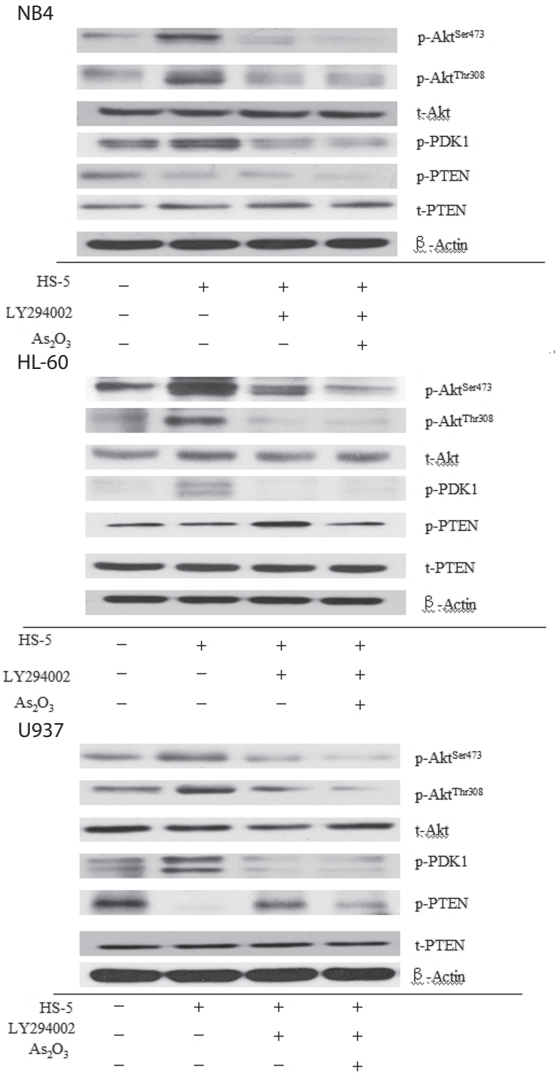

Fig. 4: Changes in important molecules in the intracellular PI3K/Akt signaling pathway of AML cells after different treatments. The following treatment groups were established for each type of AML cell: a separate suspension culture group; a group in which AML cells were subjected to adherent co-culture with HS-5 cells; a group in which AML cells were co-cultured with HS-5 cells followed by 12 h of LY294002 treatment (with 10 μM of LY294002 for NB4 and HL-60 cells and 20 μM of LY294002 for U937 cells); and a group in which AML cells were co-cultured with HS-5 cells and then treated for 2 h with LY294002 and for 12 h with a combination of LY294002 and As2O3 (with 1 μM, 2 μM, and 4 μM of As2O3 used to treat NB4 cells, HL-60 cells, and U937 cells, respectively). Cell densities were 1×10^5/ml for NB4 cells, 3×10^5/ml for HL-60 cells, and 2×10^5/ml for U937 cells. The quantity of protein loaded into each well was 40 μg, and western blotting was used to detect the.

(PIP3) at the plasma membrane. PIP3 then combines with the intracellular signaling proteins Akt and 3-phosphoinositide-dependent kinase-1 (PDK1), leading to the phosphorylation and activation of threonine-308 (Thr308) and serine-473 (Ser473) of Akt, which cause the activation or inhibition of numerous downstream target molecules. The abnormal activation of Akt, particularly if this activation is sustained, can cause various proteins, such as Bad and caspase-9, to be phosphorylated and consequently to loose activity; these effects block apoptosis *via* the mitochondrial pathway and promote the continued survival and proliferation of malignant clones (Kubota et al. 2004). The activation of the PI3K/Akt signaling pathway may be involved in the resistance of leukemia cells to many chemotherapeutic drugs (Martelli et al. 2006; Bortul et al. 2005; Longo et al. 2008; Tazzari et al. 2007), including As_2O_3 (Tabellini et al. 2005). Administering LY294002 or wortmannin, which inhibits this pathway, in combination with As_2O_3 can enhance the proliferation-inhibiting effects of As_2O_3 on suspension cultures of monocultured leukemia cells (West et al. 2002; Tabellini et al. 2005; Ramos et al. 2005). However, no studies have addressed whether a combination of As_2O_3 and a PI3K/Akt pathway inhibitor can synergistically kill AML cells grown by adherent co-culture with stromal cells. The results of this study indicated that adherent growth on the surface of HS-5 cells can activate the PI3K/Akt signaling pathway in AML cells. LY294002, which specifically inhibits the PI3K/Akt signaling pathway, may compete with ATP for binding sites on PI3K, thereby suppressing AKT activation and blocking signal transduction. This study's results indicated that 2 h of LY294002 treatment could significantly inhibit the PI3K/Akt signaling pathway in AML cells; thus, for experiments investigating the combined effects of LY294002 and As_2O_3, cells were treated with LY294002 for 4 h before As_2O_3 was added. Our results provide the first report indicating that the combined effects of LY294002 and As_2O_3 can synergistically inhibit AML cells co-cultured with HS-5 cells. Moreover, the extent to which the PI3K/Akt signaling pathway was inhibited in AML cells was significantly stronger among cells treated with both LY294002 and As_2O_3 than among cells treated with LY294002 alone. Notably, in this study, we attempted to increase the dose of LY294002 enough that the cytotoxic effects of the LY294002/As_2O_3 combination on co-cultured AML cells achieved the cytotoxic effects of As_2O_3 alone on suspension cultures of monocultured AML cells; however, the results of these experiments demonstrated that regardless of the LY294002 dose, the cytotoxic effects of the two-drug combination on co-cultured AML cells never achieved the cytotoxic effects of As_2O_3 alone on suspension cultures of monocultured AML cells. From another perspective, these results illustrated the multifaceted nature of signaling support for leukemia cells in a hematopoietic microenvironment; in particular, the blocking of a single pathway cannot completely remove residual leukemia cells in the bone marrow microenvironment, and therefore, the administration of a combination of pathway blockers and drugs may provide advantages relative to the administration of a single pathway blocker.

An extremely interesting finding from this study is that although AML cells co-cultured with bone marrow stromal cells exhibited arrest in the G0/G1 phases, many important proliferation signal transduction pathways are abnormally activated in these cells. These abnormally activated pathways include the Notch signaling pathway, as described in a prior report by our research group (Huang et al. 2012), and the PI3K/Akt signaling pathway, as indicated in this study. This phenomenon suggested that the arrest of co-cultured AML cells in the G0/G1 phases may be an effective measure by which bone marrow stromal cells protect AML cells. Specifically, this arrest causes reduced DNA synthesis in AML cells, inducing a status similar to a "dormant" state in which cellular energy consumption is decreased. In addition, important proliferative signal transduction pathways are activated in co-cultured AML cells, and the expression of adhesion molecules is up-regulated (Chen et al. 2013; Tabe et al. 2007; Funayama et al. 2010). Therefore, the effects produced by stromal cells may strengthen the proliferation and invasion capacities of AML cells that eventually leave the bone marrow microenvironment.

4. Experimental

4.1. Cell culture

NB4 cells were kindly provided by the Shanghai Institute of Hematology. U937 and HL-60 cells were purchased from Cell Bank of Chinese Academic of Science (Shanghai, China). HS-5 cells that did not express CD45

(cluster of differentiation 45), as demonstrated by flow cytometry, were purchased from the Biomedicine and Health of the Chinese Academy of Sciences (Guangzhou, China). The following cell culture conditions were utilized: cells were cultured in a humidified incubator at 37 °C and 5 % CO_2 in Roswell Park Memorial Institute (RPMI) 1640 medium (Gibco BRL, U.S.A) containing 10 % fetal bovine serum (FBS; Gibco BRL, U.S.A) and conventional doses of penicillin and streptomycin (Sigma,U.S.A). Cells in the logarithmic growth phase with cell viability > 95 % (as determined by trypan blue staining) were used for the experiments in this study.

4.2. Establishment of the co-culture system and magnetically activated cell sorting

HS-5 cells at a density of 5×10^4 cells/well were seeded into 12-well plates and cultured overnight to allow the cells to become adherent. Cells from an AML cell line (NB4, U937, or HL-60 cells) were seeded into these plates at a 1:1 ratio between AML cells and HS-5 cells, and the two types of cells were co-cultured for 72 h. Cells from each plate were separately collected and used to prepare single-cell suspensions with a nylon mesh (400 microns, BD). CD45 magnetic beads (Miltenyi, Germany) were utilized to sort AML cells in the cell suspensions; flow cytometry-based identification revealed that this process produced sorting purities > 95 %.

4.3. Reagents

As_2O_3 (Harbin Yida Pharmaceutical, Heilongjiang, China) was diluted with sterile phosphate-buffered saline (PBS) to a concentration of 8 mM and stored at − 80 °C. The PI3K/Akt signaling pathway inhibitor LY294002 was purchased from CST (Cell Signaling Tech, U.S.A), dissolved in dimethyl sulfoxide (DMSO) (Sigma, U.S.A) to a concentration of 10 mM, stored at − 20 °C, and diluted in RPMI 1640 medium to a working concentration immediately prior to use.

4.4. Cell cycle analyses

Approximately 1×10^5 AML cells were collected. These cells were then washed 2 times with pre-cooled PBS; they were centrifuged, and the supernatant was discarded after each wash. Subsequently, 0.5 ml of PBS was added, and cells were fixed overnight in 2 ml of 75 % ethanol. The collected cells were then washed with PBS and centrifuged at $1000 \times g$ for 5 min. The cells were resuspended in 0.5 ml of PBS, and propidium iodide (PI) and RNase were added to a final concentration of 50 μg/ml. The cells were incubated at 37 °C for 30 min, and modfit software was used with a Coulter Epics XL flow cytometer to analyze the cell cycle distributions in each group of cells.

4.5. Cell proliferation tests

AML cells were seeded into 96-well plates at a density of 5×10^3 cells/well, and cell proliferation assays using 5-bromo-2'-deoxyuridine (BrdU) (Roche, Shanghai) were performed in accordance with the manufacturer's instructions. A total of 3 parallel wells were established for each group. Inhibition rates were calculated using the following equation: inhibition rate (%) = (the optical density (OD) value of the untreated group–the OD value of the experimental group)/the OD value of the untreated group × 100 %. The experiment was repeated three times, and the experimental results were expressed as means ± standard deviation (X ± SD). When the two drugs LY294002 and As_2O_3 were used in combination, the Kim formula was used to calculate Q values to assess the combined effects of the drugs. Specifically, the equation used for these calculations was $Q = E(ab)/(Ea + Eb - Ea \times Eb)$, where Ea and Eb represent the inhibition rate of each drug when administered alone, and E(ab) is the inhibition rate when the two drugs are used in combination. $Q > 1.15$ indicates synergistic effects, $0.85 \leq Q \leq 1.15$ indicates additive effects, and $Q < 0.85$ indicates antagonistic effects.

4.6. Apoptosis detection

Apoptosis was detected by flow cytometry with Annexin V-fluorescein isothiocyanate (FITC)/PI double staining. Apoptosis assays were conducted in accordance with the instructions provided with the apoptosis detection kit (Roche, Shanghai), and detection was performed using a flow cytometer (BD, U.S.A).

4.7. Western blot analyses

A total of 2×10^6 AML cells were collected, and protein was extracted from these cells using specific steps described in previously published literature. Monoclonal anti-β-actin was obtained from Santa Cruz. Anti-Akt, anti-p-Akt^{Ser473}, anti-p-Akt^{Thr308}, anti-p-PDK1 (pyruvate dehydrogenase kinase, isozyme 1), anti-p-PTEN (phosphatase and tensin homolog), anti-PTEN monoclonal antibody, horseradish peroxidase-labeled anti-mouse secondary antibody, and anti-rabbit secondary antibody were purchased from CST.

4.8. Statistical analysis

The experimental data were expressed as $\bar{x} \pm s$. The SPSS 17.0 statistical software was used to perform univariate analyses of variance and significance tests. Differences with $P < 0.05$ were considered to be statistically significant.

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Conflicts of interest: None declared.

References

Becker PS, Kopecky KJ, Wilks AN, Chien S, Harlan JM, Willman CL, Petersdorf SH, Stirewalt DL, Papayannopoulou T, Appelbaum FR (2009) Very late antigen-4 function of myeloblasts correlates with improved overall survival for patients with acute myeloid leukemia. Blood 113: 866–874.

Bortul R, Tazzari PL, Billi AM, Tabellini G, Mantovani I, Cappellini A, Grafone T, Martinelli G, Conte R, Martelli AM (2005) Deguelin, A PI3K/AKT inhibitor, enhances chemosensitivity of leukaemia cells with an active PI3K/AKT pathway. Br J Haematol 129: 677–686.

Carmosino I, Latagliata R, Avvisati G, Breccia M, Finolezzi E, Lo Coco F, Petti MC (2004) Arsenic trioxide in the treatment of advanced acute promyelocytic leukemia. Haematologica 89: 615–617.

Chan WI, Huntly BJ (2008) Leukemia stem cells in acute myeloid leukemia. Semin Oncol 35: 326–335.

Chen D, Chan R, Waxman S, Jing Y (2006) Buthionine sulfoximine enhancement of arsenic trioxide-induced apoptosis in leukemia and lymphoma cells is mediated via activation of c-Jun NH2-terminal kinase and up-regulation of death receptors. Cancer Res 66: 11416–11423.

Chen F, Zhou K, Zhang L, Ma F, Chen D, Cui J, Feng X, Yang S, Chi Y, Han Z, Xue F, Rong L, Ge M, Wan L, Xu S, Du W, Lu S, Ren H, Han Z (2013) Mesenchymal stem cells induce granulocytic differentiation of acute promyelocytic leukemic cells via IL-6 and MEK/ERK pathways. Stem Cells Dev 22: 1955–1967.

Chen Ping, Wu Juan Y, Huang Hui F, Chen Yuan Z (2014) The effect to IL-3Rα, downstream PI3K/Akt signaling of all-trans retinoic acid and arsenic trioxide in NB4 cells. Pharmazie 69: 297–300.

Funayama K, Shimane M, Nomura H, Asano S (2010) An evidence for adhesion-mediated acquisition of acute myeloid leukemic stem cell-like immaturities. Biochem Biophys Res Commun 392: 271–276.

Goldman DC, Bailey AS, Pfaffle DL, Al Masri A, Christian JL, Fleming WH (2009) BMP4 regulates the hematopoietic stem cell niche. Blood 114: 4393–4401.

Guo-Bao W, Xiao-Qin C, Qi-Rong G, Jie L, Gui-Nan L, Yue L (2010) Arsenic trioxide overcomes cell adhesion-mediated drug resistance through down-regulating the expression of beta (1)-integrin in K562 chronic myelogenous leukemia cell line. Leuk Lymphoma 51: 1090–1097.

Huang HF, Lu R, Chen P, Lin ZX, Wu Y, Chen YZ (2012) Direct contact with bone stromal HS-5 cells reduces the sensitivity of HEL to drugs. Zhongguo Shi Yan Xue Ye Xue Za Zhi 20: 818–822.

Huntly BJ, Gilliland DG (2005) Leukemia stem cells and the evolution of cancer-stem-cell research. Nat Rev Cancer 5: 311–321.

Katsumi A, Kiyoi H, Abe A, Tanizaki R, Iwasaki T, Kobayashi M, Matsushita T, Kaibuchi K, Senga T, Kojima T, Kohno T, Hamaguchi M, Naoe T (2011) FLT3/□ITD regulates leukaemia cell adhesion through α4β1 integrin and Pyk2 signalling. Eur J Haematol 86: 191–198.

Konopleva M, Konoplev S, Hu W, Zaritskey AY, Afanasiev BV, Andreeff M (2002) Stromal cells prevent apoptosis of AML cells by up-regulation of anti-apoptotic proteins. Leukemia 16: 1713–1724.

Krause DS, Fulzele K, Catic A, Sun CC, Dombkowski D, Hurley MP, Lezeau S, Attar E, Wu JY, Lin HY, Divieti-Pajevic P, Hasserjian RP, Schipani E, Van Etten RA, Scadden DT (2013) Differential regulation of myeloid leukemias by the bone marrow microenvironment. Nat Med 19: 1513–1517.

Kubota Y, Ohnishi H, Kitanaka A, Ishida T, Tanaka T (2004) Constitutive activation of PI3K is involved in the spontaneous proliferation of primary acute myeloid leukemia cells:direct evidence of PI3K activation. Leukemia 18: 1438–1440.

Leoni F, Gianfaldoni G, Annunziata M, Fanci R, Ciolli S, Nozzoli C, Ferrara F (2002) Arsenic trioxide therapy for relapsed acute promyelocytic leukemia: a bridge to transplantation. Haematologica 87: 485–489.

Li H, Koh KR, Toramoto T, Nakamae H, Yamane T, Ohta K, Hino M (2010) Cytosine- arabinoside stimulates the capability of human marrow stromal cells to support normal hematopoiesis. Osaka City Med J 56: 11–20.

Li Y, Chen S, Yuan J, Yang Y, Li J, Ma J, Wu X, Freund M, Pollok K, Hanenberg H, Goebel WS, Yang FC (2009) Mesenchymal stem/progenitor cells promote the reconstitution of exogenous hematopoietic stem cells in Fancg-/- mice *in vivo*. Blood 113: 2342–2351.

Longo PG, Laurenti L, Gobessi S, Sica S, Leone G, Efremov DG (2008) The Akt/Mcl-1 pathway plays a prominent role in mediating antiapoptotic signals downstream of the B-cell receptor in chronic lymphocytic leukemia B cells. Blood 111: 846–855.

Macanas-Pirard P1, Leisewitz A, Broekhuizen R, Cautivo K, Barriga FM, Leisewitz F, Gidi V, Riquelme E, Montecinos VP, Swett P, Besa P, Ramirez P, Ocqueteau M, Kalergis AM, Holt M, Rettig M, DiPersio JF, Nervi B (2012) Bone marrow stromal cells modulate mouse ENT1 activity and protect leukemia cells from cytarabine induced apoptosis. PLoS One 7: e37203.

Martelli AM, Nyåkern M, Tabellini G, Bortul R, Tazzari PL, Evangelisti C, Cocco L (2006) Phosphoinositide 3-kinase/Akt signaling pathway and its therapeutical implications for human acute myeloid leukemia. Leukemia 20: 911–928.

Nemeth MJ, Mak KK, Yang Y, Bodine DM (2009) beta-Catenin expression in the bone marrow microenvironment is required for long-term maintenance of primitive hematopoietic cells. Stem Cells 27: 1109–1119.

Osaki M, Oshimura M. Ito H (2004) PI3K-Akt pathway: its functions and alterations in human cancer. Apoptosis 9: 667–676.

Parmar A, Marz S, Rushton S, Holzwarth C, Lind K, Kayser S, Döhner K, Peschel C, Oostendorp RA, Götze KS (2011) Stromal niche cells protect early leukemic FLT3-ITD + progenitor cells against first-generation FLT3 tyrosine kinase inhibitors.Cancer Res 71: 4696–4706.

Ramos AM, Fernández C, Amrán D, Sancho P, de Blas E, Aller P (2005) Pharmacologic inhibitors of PI3K/Akt potentiate the apoptotic action of the antileukemic drug arsenic trioxide via glutathione depletion and increased peroxide accumulation in myeloid leukemia cells. Blood 105: 4013–4020.

Soignet SL, Frankel SR, Douer D, Tallman MS, Kantarjian H, Calleja E, Stone RM, Kalaycio M, Scheinberg DA, Steinherz P, Sievers EL, Coutré S, Dahlberg S, Ellison R, Warrell RP Jr. (2001) United States multicenter study of arsenic trioxide in relapsed acute promyelocytic leukemia. J Clin Oncol 19: 3852–3860.

Tabe Y, Jin L, Tsutsumi-Ishii Y, Xu Y, McQueen T, Priebe W, Mills GB, Ohsaka A, Nagaoka I, Andreeff M, Konopleva M (2007) Activation of integrin-linked kinase is a critical prosurvival pathway induced in leukemic cells by bone marrow-derived stromal cells. Cancer Res 67: 684–694.

Tabellini G, Cappellini A, Tazzari PL, Falà F, Billi AM, Manzoli L, Cocco L, Martelli AM (2005) Phosphoinositide 3-kinase/Akt involvement in arsenic trioxide resistance of human leukemia cells. J Cell Physiol 202: 623–634.

Taussig DC, Vargaftig J, Miraki-Moud F, Griessinger E, Sharrock K, Luke T, Lillington D, Oakervee H, Cavenagh J, Agrawal SG, Lister TA, Gribben JG, Bonnet D (2010) Leukemia-initiating cells from some acute myeloid leukemia patients with mutated nucleophosmin reside in the CD34(-) fraction. Blood 115: 1976–1984.

Tazzari PL, Cappellini A, Ricci F, Evangelisti C, Papa V, Grafone T, Martinelli G, Conte R, Cocco L, McCubrey JA, Martelli AM (2007) Multidrug resistance-associated protein 1 expression is under the control of the phosphoinositide 3 kinase/Akt signal transduction network in human acute myelogenous leukemia blasts. Leukemia 21: 427–438.

West KA, Castillo SS, Dennis PA (2002) Activation of the PI3K/Akt pathway and chemotherapeutic resistance. Drug Resist Updates 5: 234–248.

Xu Q, Simpson SE, Scialla TJ, Bagg A, Carroll M (2003) Survival of acute myeloid leukemia cells requires PI3 kinase activation. Blood 102: 972–980.

Zeng Z, Shi YX, Samudio IJ, Wang RY, Ling X, Frolova O, Levis M, Rubin JB, Negrin RR, Estey EH, Konoplev S, Andreeff M, Konopleva M (2009) Targeting the leukemia microenvironment by CXCR4 inhibition overcomes resistance to kinase inhibitors and chemotherapy in AML. Blood 113: 6215–6224.