# The association between expression of hypoxia inducible factor-1α and multi-drug resistance of acute myeloid leukemia

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**Background:** Acute myeloid leukemia (AML) is the most common acute leukemia in adults, and chemotherapy is the main treatment. However, patients with AML poorly respond to chemotherapy because of multi-drug resistance (MDR); MDR may mainly involve hypoxia.

**Methods:** To investigate the expression levels of hypoxia inducible factor-1α (HIF-1α), MDR1, multi-drug resistant associate protein (MRP), breast cancer resistance protein (BCRP), and lung resistance-associated protein (LRP) in MDR of AML, we detected the blood of 118 patients with AML and 20 healthy people through quantitative real-time polymerase chain reaction (PCR). Data were statistically analyzed in SPSS v12.0 (SPSS Inc., Chicago, IL, USA).

**Results:** In these hypoxia-induced genes, HIF-1α could be significantly correlated with (P<0.05) the expression of MDR1 ($r^2$=0.114) and MRP ($r^2$=0.086). In our study, the post-chemotherapy survival rate of patients with AML was significantly related to HIF-1α expression (P<0.05), and the disease free survival (DFS) of patients with low HIF-1α expression was longer than those with high HIF-1α expression.

**Conclusions:** This finding indicated that HIF-1α could be considered as a prognosis indicator of patients with AML.

**Keywords:** Hypoxia inducible factor-1α (HIF-1α); acute myeloid leukemia (AML); multi-drug resistance (MDR)

## Introduction

Acute myeloid leukemia (AML) is the most common form of acute leukemia affecting adults. Approximately 10,500 new cases are recorded each year in the United States, and this condition accounts for 1.2% of all cancer deaths in the United States (1). Although hematopoietic stem cell transplantation has been widely employed in clinical settings, chemotherapy remains the first line of treatment for AML. However, failure of chemotherapy and post-remission relapse of leukemia likely occur mainly because of multi-drug resistance (MDR). The number of remaining leukemic cells is related to recurrence (2). Cancer cells may exhibit MDR induced by a specific drug, but cancer cells may become resistant to several other anti-cancer drugs with different structures and mechanisms (3). Various hypotheses have been presented, but the mechanisms of MDR remain unclear (4).

Hypoxic tumor microenvironment can affect the susceptibility to chemotherapy drugs via the following

mechanisms (5). First, hypoxia can stimulate the aggressiveness of neoplasms and induce distant metastasis. Second, the cell cycle of tumors can be slowed down or even terminated by hypoxia. Hypoxia likely weakens the cytotoxicity of chemotherapeutic drugs because these drugs elicit strong cytotoxic effects on actively proliferating cells. Extracellular pH becomes lower than intracellular pH if hypoxia occurs; as such, a transmembrane pH gradient can be established. Under these circumstances, the aggregation of alkaline drugs, such as Adriamycin, in cells can be inhibited. As a result, the resistance of tumor cells to radiotherapy and chemotherapy increases and curative effects are impaired.

As a relevant transcription factor in hypoxia induction, hypoxia inducible factor-1α (HIF-1α) is involved in various hypoxia-induced target gene expression. HIF-1α is considered a new anti-neoplasm target overproduced in tumor tissues and participates in the oncogenesis, development, and progression of tumors. HIF-1α functions through several steps, including protein expression, dimerization, combination to target DNA sequences, and transcriptional activation. The whole process is regulated by many factors. For example, PI3K/Akt and MAPK signal transduction pathways are implicated in HIF-1α protein expression and HIF-1transcriptional activation, respectively (6). Numerous post-transcriptional modification enzymes, including deubiquitinase and phosphatase, stimulate protein expression and regulate HIF-1α activity. However, HIF-1α production can decrease when these signal pathways are inhibited. Various compounds, such as PD98059 signal pathway inhibitor, Wortmannin, Topotecan, and other molecular compounds, including 2ME2 and YC-1, can inhibit HIF-1α. With enhanced understanding of the biological mechanisms of cellular adaptation in hypoxic environments, novel strategies can be developed to treat neoplasms (7,8).

HIF-1α is related to the MDR of patients with different kinds of tumors. For instance, HIF-1α mediates the MDR of osteosarcoma by activating the P-glycoprotein (P-gp) expression and by regulating the expression of c-Myc and p21 (9). In the meantime, HIF-1α plays a major role in the MDR of laryngeal cancer cells with similar mechanisms (10). However, the role of HIF-1α in the MDR of malignant hematological tumors remains unclear. Few studies have been carried out to evaluate the correlation between HIF-1α and MDR of hematological malignancy. Therefore, this study aimed to investigate the correlation of HIF-1α and other hypoxia-induced gene expression with the MDR of

patients with AML and to discuss the potential underlying mechanisms, which could possibly provide additional information for AML treatment.

## Methods

### Patient characteristics

A total of 118 patients with AML (apart from M3) in accordance with French-American-Britain (FAB) criteria but without a history of other cancers or hematological malignances were enrolled in the study from January 2010 to November 2015. Donors signed the questionnaire and informed consent form in accordance with Chinese guidelines for blood donation. This study was also conducted in accordance with the declaration of Helsinki and was approved by the Ethics Committee of Drum Tower Hospital affiliated with Nanjing University.

Of the total number of the enrolled patients with AML, 10 had M0 (minimally differentiated AML), 17 had M1 (AML without maturation), 28 had M2 (AML with maturation), 18 had M4 (acute myelomonocytic), 26 had M5 (acute monocytic leukemia), 20 had M6 (erythroleukemia), and 1 had M7. The characteristics of the patients are shown in *Table 1*. Cytogenetic risk groups were defined as follows, by WHO criteria: unfavorable, –7/del(7q), –5/del(5q), abn3q, abn9q, abn11q, abn-17p; favorable, inv(16)/t(16;16)/del(16q), and t(8;21); and intermediate risk, all other karyotypic aberrations or a normal karyotype (11).

### Evaluation of chemotherapy regimens and therapeutic response

All of the patients with AML accepted cytosine arabinoside-based chemotherapy, which included Mitoxantrone + Ara-C(cytosine arabinoside) (MA), Daunorubicin (DNR) +Ara-C(cytosine arabinoside) (DA), and Idarubicin + Ara-C (IA) regimens (*Table 2*). Of the 118 patients, 78 were treated with DA induction chemotherapy, 24 were subjected to IA induction chemotherapy, and 16 patients were administered with MA induction chemotherapy regimen. Complete response (CR) was defined as the absolute values of granular leukocytes and platelets in peripheral blood higher than $1.5 \times 10^9$/L and $100 \times 10^9$/L, respectively; blast cell count in the bone marrow was less than 5% for at least 4 weeks. The symptoms and signs of leukemia disappeared.

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**Table 1** Clinical features of enrolled AML patients (118 cases)

| Variables | Names | Cases | Percentage (%) | Mean ± standard deviation |
|---|---|---|---|---|
| Gender | Male | 60 | 50.80 | – |
| | Female | 58 | 49.20 | – |
| FAB subtypes | M0 | 10 | 8.50 | – |
| | M1 | 15 | 12.80 | – |
| | M2 | 28 | 23.70 | – |
| | M4 | 18 | 15.30 | – |
| | M5 | 26 | 22.00 | – |
| | M6 | 20 | 16.90 | – |
| | M7 | 1 | 0.80 | |
| Age | – | 118 | – | 43.19±16.88 |
| WBC (×10⁹/L) | – | 118 | – | 2.52±0.92 (1.1–3.8) |
| Hb (g/dL) | – | 118 | – | 8.83±1.01 (7.1–11.9) |
| PLT (×10⁹/L) | – | 118 | – | 3.83±1.01 (2.4–6.9) |
| Cytogenetic risk | Favorable | 39 | 33.05 | – |
| | Intermediate | 42 | 35.59 | – |
| | Unfavorable | 37 | 31.36 | – |
| Cytogenetics | Normal | 66 | 55.93 | – |
| | Abnormal | 52 | 44.07 | – |
| Regimen | DA | 78 | 66.10 | – |
| | MA | 16 | 13.56 | – |
| | IA | 24 | 20.34 | – |

DA, Daunorubicin (DNR) + Ara-C(cytosine arabinoside); MA, Mitoxantrone + Ara-C(cytosine arabinoside); IA, Idarubicin + Ara-C(cytosine arabinoside); FAB, French-American-Britain; WBC, white blood cell; Hb, Hemoglobin; PLT, platelets; AML, acute myeloid leukemia.

Partial remission (PR) required similar criteria, with the exception of the presence of 6–20% marrow blasts. Non-remission (NR) was described on the basis of three clinical manifestations, including blood analysis, bone marrow biopsy inconsistent with the standard criteria for CR, and >20% promyelocytic cells in the bone marrow. Early death was noted if a patient died within 8 weeks from the start of the first induction of the therapeutic course. CR and PR were considered good responses, and NR and early death were recorded as poor response (12).

### RNA extraction and quantitative real-time polymerase chain reaction (PCR)

RNA was isolated from the blood samples of the participants and stored at –20 ℃ for later use. The RNA was then reverse-transcribed to obtain cDNA. PCR primers were designed by Primer Premier 6.0 (*Table 3*). The experiments were performed in triplicate and normalized to β-actin. PCR was performed under the following conditions: 40 cycles with a three-cycle loop in each cycle; initial temperature at 95 ℃ for 2 min; followed by 95 ℃ for 10 s, 60 ℃ for 3 s, and 70 ℃ for 45 s. The program lasted 1 min at 60 ℃.

### Statistics

Data were statistically analyzed in SPSS v12.0 (SPSS Inc., Chicago, IL, USA). The correlation of the HIF-1α expression with MDR1, multi-drug resistant associate protein (MRP), breast cancer resistance protein (BCRP), and lung resistance-associated protein (LRP) gene expression was analyzed through Pearson's $\chi^2$. Significant differences of the HIF-1a expression among various responders were calculated with least-significant difference analysis. Survival curves were plotted with Kaplan-Meier method. In all of the tests, P value of <0.05 was considered statistically significant.

**Table 2** Chemotherapy regimens

| Regimens | Contents |
|---|---|
| DA | Daunorubicin (DNR) 45 mg/m²/day d1–3 + Ara-C 100 mg/m²/day d1–7 |
| MA | Mitoxantrone 4 mg/m²/day d1–5 + Ara-C 100 mg/m²/day d1–7 |
| IA | Idarubicin 12 mg/m²/day d5–7 + Ara-C 100 mg/m²/day d1–7 |

DA, Daunorubicin (DNR) +Ara-C(cytosine arabinoside); MA: Mitoxantrone + Ara-C(cytosine arabinoside); IA, Idarubicin + Ara-C(cytosine arabinoside).

**Table 3** Primer sequence

| Genes | Primer Sequence (5'-3') | Band size (bp) |
|---|---|---|
| HIF-1α | F-ACCTATGACCTGCTTGGTGCTGAT | 196 |
| | R-TTGGCAAGCATCCTGTACTGTCCT | |
| MDR1 | F-TGGTTTGATGTGCACGATGTTGGG | 104 |
| | R-AGATCAGCAGGAAAGCAGCACCTA | |
| MRP | F-TGGGACTGGAATGTCACG | 118 |
| | R-AGGAATATGCCCCGACTTC | |
| LRP | F-GTCTTCGGGCCTGAGCTGGTGTCG | 106 |
| | R-CTTGGCCGTCTCTTGGGGGTCCTT | |
| BCRP | F-TTAGGATTGAAGCCAAAGG | 124 |
| | R-TAGGCAATTGTGAGGAAAATA | |
| β-actin | F-CGCCGCGCTCGTCGTCGACA | 120 |
| | R-GTCACGCACGATTTCCCGCT | |

HIF-1α, hypoxia inducible factor-1α; MRP, multi-drug resistant associate protein; BCRP, breast cancer resistance protein; LRP, lung resistance-associated protein; P-gp, P-glycoprotein; MDR, multi-drug resistance.

## Results

### Clinical features of the enrolled patients with AML

The clinical features of the enrolled patients are presented in *Table 1*. The patients' ages ranged from 15 to 81 years, and the median patient age was 43.19±16.88 (14–78) years. The Patients with AML included 60 males and 58 females, and the male-to-female ratio was close to 1:1 (50.80% and 49.20%). Most of the enrolled patients had M2 (23.70%), and a few of the patients had M0 (8.5%). The mean of white blood cell (WBC), Hb, and PLT counts were $2.52±0.92×10^9$/L, $8.83±1.01$ g/dL, and $3.83±1.01×10^9$/L, respectively. In terms of cytogenetic risk, 39 patients exhibited favorable cytogenetic risk, 42 patients manifested intermediate risk, and 37 patients showed unfavorable risk. The effective rates of the chemotherapeutic methods used in this study did not significantly vary (P>0.05). Hence, the efficacy of different chemotherapy regimens did not significantly differ.

### Analysis of the mRNA expression Of HIF-1a, MDR1, BCRP, MRP, and LRP in the patients with AML and the correlation between expression and chemotherapy

RT-qPCR revealed that the median mRNA expression
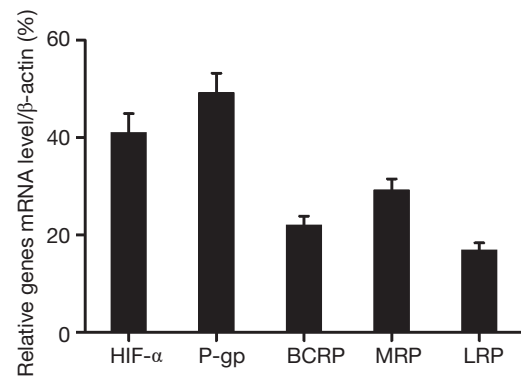


**Figure 1** HIF-1α, MDR1, BCRP, MRP and LRP mRNA expression in AML patients. HIF-1α, hypoxia inducible factor-1α; MRP, multi-drug resistant associate protein; BCRP, breast cancer resistance protein; LRP, lung resistance-associated protein; P-gp, P-glycoprotein; AML, acute myeloid leukemia; MDR, multi-drug resistance.

levels of HIF-1α, MDR1, BCRP, MRP, and LRP were 40.81%±21.60%, 48.74%±20.61%, 21.67%±15.76%, 30.31%±14.10%, and 17.99%±15.12%, respectively (*Figure 1*). The correlation coefficients of HIF1-α, MDR1, BCRP, MRP, and LRP were 0.354, 0.333, 0.451, and 0.182, respectively. These results indicated that HIF-1α could be significantly correlated with (*Table 4*; *Figure 2A,B*; P<0.05) the expression of MDR1 ($r^2$=0.114) and MRP ($r^2$=0.086). By contrast, HIF-1α was not significantly associated (*Table 5*; *Figure 2C,D*; P>0.05) with the expression of BCRP ($r^2$=0.059) and LRP ($r^2$=0.016). Therefore, HIF-1α was correlated with the mRNA expression of drug-resistant genes, namely, MDR1 and MRP, in patients with AML after chemotherapy.

### Effects of the mRNA expression of HIF-1a, MDR1, BCRP, MRP, and LRP on the survival rates of patients

The evaluation of the mRNA expression of HIF-1α in different chemotherapy regimens indicated that DA regimen (41.32±20.44) was more efficient than HA regimen (49.49±22.27) and MA regimen (25.31±20.32; *Table 5*; P<0.05). Kaplan-Meier test was performed to estimate the survival rate of the patients on the basis of HIF-1α expression. The patients with low HIF-1α expression showed a significantly higher survival rate than those with high HIF-1α expression (HR =4.63; *Figure 3A*; P<0.05). Nevertheless, the expression levels of MDR1 (HR =0.35),

**Table 4** Correlation analysis for HIF-1α and MDR1, BCRP, MRP, LRP

| Variables | Cases | Correlation coefficients | Standardized correlation coefficients | P value |
|---|---|---|---|---|
| HIF-1α-MDR1 | 118 | 0.354 | 0.338 | 0.009* |
| HIF-1α-BCRP | 118 | 0.333 | 0.243 | 0.063 |
| HIF-1α-MRP | 118 | 0.451 | 0.295 | 0.023* |
| HIF-1α-LRP | 118 | 0.182 | 0.127 | 0.336 |

HIF-1α, hypoxia inducible factor-1α; MDR, multi-drug resistance; MRP, multi-drug resistant associate protein; BCRP, breast cancer resistance protein; LRP, lung resistance-associated protein.
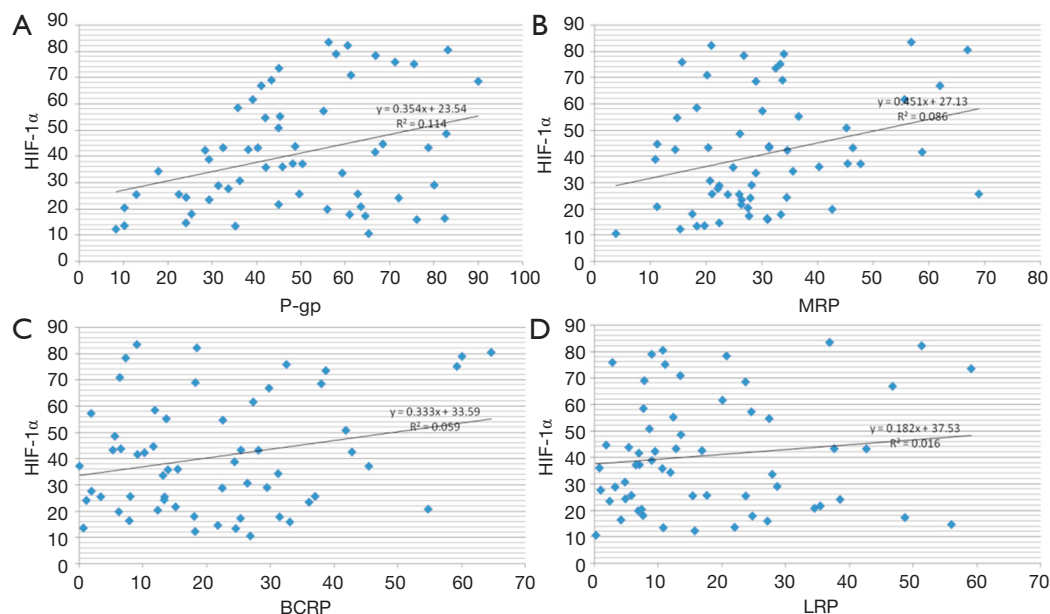


**Figure 2** Scatter diagram of correlation analysis for HIF-1α and MDR1, BCRP, MRP and LRP. HIF-1α, hypoxia inducible factor-1α; MRP, multi-drug resistant associate protein; BCRP, breast cancer resistance protein; LRP, lung resistance-associated protein; P-gp, P-glycoprotein; MDR, multi-drug resistance.

**Table 5** Evaluation of HIF-1α mRNA expression by different chemotherapy regimens

| Regimens | Cases | Mean ± standard deviation | Median | Minimum | Maximum | F | P value |
|---|---|---|---|---|---|---|---|
| DA | 78 | 41.32±20.44 | 35.96 | 13.56 | 83.41 | 3.278 | 0.045* |
| IA | 24 | 49.49±22.27 | 43.22 | 21.63 | 82.14 | – | – |
| MA | 16 | 25.31±20.32 | 17.08 | 10.53 | 68.94 | – | – |
| Total | 118 | 40.81±21.60 | 37.13 | 10.53 | 83.41 | – | – |

Comparison with HA and MA had statistical difference; DA, Daunorubicin (DNR) + Ara-C(cytosine arabinoside); MA, Mitoxantrone + Ara-C(cytosine arabinoside); IA, Idarubicin + Ara-C(cytosine arabinoside).
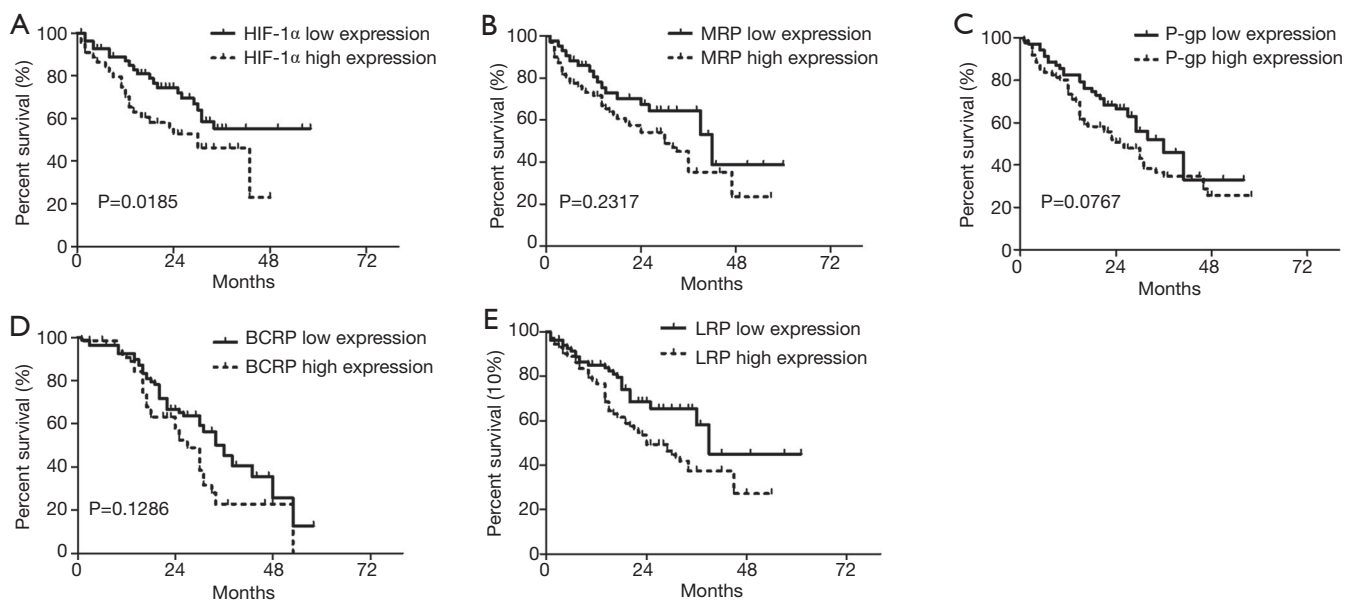
**Figure 3** Association between HIF-1α, MDR1, BCRP, MRP, LRP mRNA expression and survival rate of AML patients was analyzed by Kaplan-Meier curve analysis. P<0.05 were considered statistically significant. HIF-1α, hypoxia inducible factor-1α; MRP, multi-drug resistant associate protein; BCRP, breast cancer resistance protein; LRP, lung resistance-associated protein; P-gp, P-glycoprotein; AML, acute myeloid leukemia; MDR, multi-drug resistance.

BCRP (HR =0.406), MRP (HR =0.413), and LRP (HR =0.49) were not associated with the survival rate of patients with AML (*Figure 3B-E*; P>0.05). Therefore, high HIF-1α expression at a transcription level is related to the prognosis of patients with AML after chemotherapy.

## Discussion

Hypoxia is considered the main cause of MDR and chemotherapy failure; however, the underlying mechanisms are complicated. (I) The bone marrow of patients with AML is in a state of chronic physical hypoxia, recent study demonstrated sever hypoxia of 1% $O_2$ could induce decreased cellular proliferation which cause S phase significantly decreased, this mechanism is thought to be related with cytarabine resistant because the cytotoxic activity is linked to the proliferative activity and hence the numbers of cells in the S phase (13); (II) the structural and functional abnormality of vessels in solid tumors and tumor necrosis can induce insufficient blood flow, which prevents drugs to reach and kill tumor cells; (III) hypoxia can promote the proliferation of tumor cells through rapid cell division and thus reduce tumor chemo-sensitivity; (IV) the intracellular and extracellular distribution of anti-cancer

drugs can be affected by changes in pH of tumor cells, while pH of tissues under low oxygen conditions is lower than pH of normal tissue (14); (V) hypoxia increases the expression of MDR gene, P-gp, MRP, BCRP, and LRP (15). These mechanisms illustrate that hypoxia is associated with MDR of tumor cells.

HIF-1α is overexpressed in pancreatic cancer cells, especially under hypoxic conditions; the inhibition of HIF-1α can induce a more efficient response to chemotherapy; therefore, HIF-1α expression is related to the therapeutic effect of pancreatic cancer (16). For example, a recombinant lentivirus containing HIF-1α small interfering RNA (siRNA) can reverse the MDR of breast cancer xenograft models by downregulating the expression of HIF-1α; this finding suggested that HIF-1α also participates in MDR of breast cancer (17). It is also be found that patients with advanced colon carcinoma and with expressed HIF-1α and P-gp are more resistant to chemotherapy than those without HIF-1α and P-gp expressed in their system. The sensitivity of chemotherapy can be restored after the HIF-1α gene in colon cancer cells is silenced. The inhibition of HIF-1α can significantly decrease the mRNA or protein expression of MDR1/P-gp in colon cancer cells; therefore, MDR1/P-gp expression is associated with the expression of

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HIF-1α (18).

Although the correlation between HIF-1α and MDR in solid tumors has been comprehensively described, the correlation between these factors in hematological malignancy remains unclear. Nevertheless, our study demonstrated that the HIF-1α expression was correlated with MDR in AML. Therefore, HIF-1α may be implicated in MDR of hematological malignancy. However, further studies should be conducted to clarify the underlying mechanisms of this phenomenon.

Our results demonstrated that HIF-1 can be used as an independent indicator of the prognosis of tumor patients because this indicator can transmit cellular hypoxia signals and induce "hypoxic effect." As an important hypoxia-induced transcription factor, HIF-1 participates in angiogenesis, anaerobic metabolism, chemotherapy and radiotherapy resistance, and tumor cell invasion and metastasis (19). Increased HIF-1 expression can be considered as an indicator of poor prognosis of different solid tumors (20-22). Recent study also suggested that the HIF-1 expression is related to the poor survival of patients with AML (23). In our study, Kaplan-Meier curve analysis suggested that the post-chemotherapy survival rate of patients with AML was significantly related to HIF-1α expression (P<0.05), and the DSS of patients with low HIF-1α expression was longer than those with high HIF-1α expression. This finding indicated that HIF-1α could be considered as a prognosis indicator of patients with AML. Our study also indicated that the MDR-associated proteins, including MDR1, BCRP, MRP, and LRP, were not significantly correlated with the survival rate of patients with AML. This finding showed that MDR may not directly cause poor prognosis, and HIF-1α expression might be accounted for the poor prognosis of AML through other mechanisms. Recent researches demonstrated that high HIF-1α expression is associated with the extramedullary infiltration of AML, and this process can be considered as a possible mechanism (24). Nevertheless, the mechanisms of the relationship between HIF-1α expression and poor prognosis of AML should be further investigated.

## Acknowledgments

## Footnote

*Conflicts of Interest:* All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2017.01.10). The authors have no conflicts of interest to declare.

*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of Drum Tower Hospital affiliated with Nanjing University (No. 2010-11) and written informed consent was obtained from all patients.

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