


ORIGINAL ARTICLE

Establishment and molecular characterization of decitabine-resistant K562 cells

Xiang-Mei Wen^{1,2}  | Ting-Juan Zhang^{2,3} | Ji-Chun Ma^{1,3} | Jing-Dong Zhou^{2,3} | Zi-Jun Xu^{1,3} | Xiao-Wen Zhu^{2,3} | Qian Yuan^{1,3} | Run-Bi Ji^{1,3} | Qin Chen^{1,3} | Zhao-Qun Deng^{1,3} | Jiang Lin^{1,3} | Jun Qian^{2,3}

¹Laboratory Center, Affiliated People's Hospital of Jiangsu University, Zhenjiang, Jiangsu, People's Republic of China

²Department of Hematology, Affiliated People's Hospital of Jiangsu University, Zhenjiang, Jiangsu, People's Republic of China

³The Key Lab of Precision Diagnosis and Treatment in Hematologic Malignancies of Zhenjiang City, Zhenjiang, Jiangsu, P.R. China

Correspondence

Jiang Lin, Laboratory Center, Affiliated People's Hospital of Jiangsu University, Zhenjiang, People's Republic of China.
Email: 2651329493@qq.com
and

Jun Qian, Department of Hematology, Affiliated People's Hospital of Jiangsu University, Zhenjiang, People's Republic of China.
Email: qianjun0007@hotmail.com

Funding information

Jiangsu University Medical Clinical Science and Technology Development Fund, Grant/Award Number: JLY20160011; China Postdoctoral Science Foundation funded project, Grant/Award Number: 2016M601748; 333 Project of Jiangsu Province, Grant/Award Number: BRA2016131; Social Development Foundation of Kun Shan, Grant/Award Number: KS1624; Zhenjiang Clinical Research Center of Hematology, Grant/Award Number: SS2018009; National Natural Science Foundation of China, Grant/Award Number: 81270630; Youth Medical Talents Project of "Ke Jiao Qiang Wei" project of Jiangsu province, Grant/Award Number: QNRC2016450 and QNRC2016449; Six talent peaks project in Jiangsu Province, Grant/Award Number: 2015-WSN-115; Social Development Foundation of Zhenjiang, Grant/Award Number: SH2016045, SH2016046 and SH2017040; Medical Innovation Team of Jiangsu Province, Grant/Award Number:

Abstract

The clinical activity of decitabine (5-aza-2-deoxycytidine, DAC), a hypomethylating agent, has been demonstrated in acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) patients. However, secondary resistance to this agent often occurs during treatment and leads to treatment failure. It is important to clarify the mechanisms underlying the resistance for improving the efficacy. In this study, by gradually increasing concentration after a continuous induction of DAC, we established the DAC-resistant K562 cell line (K562/DAC) from its parental cell line K562. The proliferation and survival rate of K562/DAC was significantly increased, whereas the apoptosis rate was remarkably decreased than that of K562 after DAC treatment. In K562/DAC, a total of 108 genes were upregulated and 118 genes were downregulated by RNA-Seq. In addition, we also observed aberrant expression of *DDX43/H19/miR-186* axis (increased *DDX43/H19* and decreased *miR-186*) in K562/DAC cells. Ectopic expression of *DDX43* in parental K562 cells rendered cells resistant to the DAC. Taken together, we successfully established DAC-resistant K562 cell line which can serve as a good model for investigating DAC resistance mechanisms, and *DDX43/H19/miR-186* may be involved in DAC resistance in K562.

KEYWORDS

DDX43, decitabine, *H19*, K562, resistance

Xiang-mei Wen and Ting-juan Zhang contributed equally to this work.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2019 The Authors. Journal of Cellular and Molecular Medicine published by John Wiley & Sons Ltd and Foundation for Cellular and Molecular Medicine.

CXTDB2017002; Postgraduate Research & Practice Innovation Program of Jiangsu Province, Grant/Award Number: KYCX17_1821, KYCX1, 8, _ and 2281

1 | INTRODUCTION

DNA methylation is a major contributor to epigenetics involved in carcinogenesis especially in leukemogenesis. The balance is needed to be precisely maintained between DNA hypermethylation and hypomethylation, and dysregulation of the balance may give rise to human diseases.¹ Abnormal DNA methylation changes, associated with DNA methyltransferases (DNMTs), are frequently observed in leukemia and supposedly contribute to disease occurrence and progression.^{2,3} Therapy targeting DNA methylation modifiers has been regarded as a success in the treatment of hematopoietic malignancies.^{4,5} Gene silencing caused by DNA hypermethylation can be reversed pharmacologically by prototypical DNMT inhibitors decitabine (5-aza-2-deoxycytidine, DAC) and 5-azacytidine (AZA), which have been recommended as one of the primary treatments for older acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) patients.⁶⁻⁸

The DAC is transported into the cell and then phosphorylated by deoxycytidine kinase (DCK) to the active metabolite 5-aza-dCTP, which incorporates into DNA during DNA replication to form a covalent complex with DNMTs, thereby inhibiting their activities followed by a reduction of DNA methylation, and consequently inducing anti-leukemia effects.⁹ However, increasing clinical studies have found that resistance to such drug can develop during treatment and lead to treatment failure. Drug resistance was the major clinical obstacle to successful treatment of leukemia patients compared to patients with relatively sensitive cells. The clinical outcome of patients after failure with hypomethylating therapy was poor.^{10,11} Insufficient incorporation into DNA was suggested to explain in vitro DAC resistance.¹² It was reported that *DNMT3b* was upregulated in hypomethylating agent-resistance cell lines.¹³ Also, high cytidine deaminase (CDA)/DCK ratio could be a mechanism of primary resistance to DAC in some patients.¹⁴

Nevertheless, the detailed mechanisms leading to DAC resistance still remains obscure. In this study, we induced K562 cell line for long periods of time using DAC to obtain the DAC-resistant K562 cell line and investigated the potential mechanisms of DAC resistance.

2 | MATERIALS AND METHODS

2.1 | DAC-resistant cell selection and cell culture

DAC-resistant K562 cell line (K562/DAC) was established from its parental K562 cell line. The parental K562 cells were exposed continuously to gradually increasing concentrations of DAC. Original inducing DAC concentration was 2.5 $\mu\text{mol/L}$ and then increased exponentially in each step till 320 $\mu\text{mol/L}$. The cells acquired resistance to DAC by a series of stepwise selections at last. Selected cells were cultured in DAC-free medium prior to the experiment for at

least 2 weeks. K562 and K562/DAC cells were incubated in Iscove's Modified Dulbecco's Medium (Wisent, Canada) containing 10% fetal bovine serum (FBS; ExCell Bio, Shanghai, China) and antibiotics at 37°C in a humidified, 5% CO₂ atmosphere.

2.2 | Morphology and measurement of drug sensitivity

An inverted light microscope (Nikon) and Wright-Giemsa's compound stain were used to observe K562 and K562/DAC cells during the exponential phase. The nuclear to cytoplasm ratio of the cells was measured, which was the ratio of the diameter of the nucleus to the thickness of the cytoplasm on both sides.

K562 and K562/DAC cells were collected and placed in 6-well plates at a density of $1 \times 10^5/\text{mL}$ with 2 mL medium. Fresh medium containing DAC at final concentration ranging from 0 to 2 $\mu\text{mol/L}$ was added immediately, then fresh DAC was supplemented every 24 hours. After 96 hours, the surviving cells were calculated by trypan blue exclusion. The concentration of DAC required for 50% growth inhibition was scored as half maximal (50%) inhibitory concentration (IC50) value. The degree of resistance was evaluated by IC50 value. Each experiment was repeated three times. IC50 value of DAC was analyzed by the method of probit analysis in SPSS21.0 (SPSS Inc, USA).

2.3 | Cell survival and proliferation assays

Cell viability of the K562 and K562/DAC cells were assessed. Briefly, cells were seeded in 6-well plates at a density of 1×10^5 cells/well with growth medium containing 0% FBS (cell survival assay) or 10% FBS (cell proliferation assay). DAC was added with the final concentration of 1 $\mu\text{mol/L}$ for 96 hours. The results were presented from three independent experiments.

2.4 | Cell apoptosis

To study cell apoptosis, cells were treated in 25 cm² tissue culture flasks without FBS. Then cell apoptosis was evaluated with Annexin-V-FITC and propidium iodide (PI) double staining using an Annexin V apoptosis detection Kit (556547, Annexin V-FITC Apoptosis Detection Kit I; BD, San Jose, CA, USA) according to the manufacturer's instructions, followed by flow cytometry analysis.

2.5 | RNA-Seq analysis

Total RNA was extracted from the cell samples by Trizol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. RNA was subjected to RNA-Seq analysis by Beijing BerryGenomics Institute, China.

2.6 | RQ-PCR

cDNA was reverse transcribed from the RNA. Real-time quantitative PCR (RQ-PCR) was conducted to evaluate the mRNA and miRNA expression levels in the DAC resistant cells as previously described using the primer sets (Table S1).¹⁵⁻¹⁹

2.7 | DNA isolation, chemical modification, RQ-MSP and BSP

Genomic DNA isolation, chemical modification, real-time quantitative methylation-specific PCR (RQ-MSP) and bisulfite sequencing PCR (BSP) were performed as our previous study.^{15,18}

2.8 | DDX43 stabled transfected K562 cell line

A lenti-virus vector containing *DDX43* cDNA sequence was used to generate stable *DDX43*-expressing K562 cell line. Then *DDX43* mRNA and protein were detected by real-time quantitative PCR and western blot, respectively.²⁰

2.9 | Statistical analysis

All experiments were performed in triplicate ($n \geq 3$) and the data were presented as mean \pm SD. The Student's *t* test for independent samples was applied to define differences in the experiments. The differences of results were determined statistically significant if *P* was less than 0.05.

3 | RESULTS

3.1 | Establishment of DAC-resistant cell line

Morphology differences between K562 and K562/DAC cells were surveyed using an inverted light microscope and Wright-Giemsa's compound staining, and the results were shown in Figure 1A. K562 cells were homogeneous, yet K562/DAC cells were more irregular with little atypia under the light microscope. Wright-Giemsa's compound staining showed that K562/DAC nucleus was more concentrated and the ratio of nucleus to cytoplasm became smaller compared with K562.

The IC50 value for DAC was 0.26 ± 0.02 $\mu\text{mol/L}$ in K562 and 3.16 ± 0.02 $\mu\text{mol/L}$ in K562/DAC (12-fold increase compared to the parental cell line) ($P < 0.05$) (Figure 1B).

To further explore biological property of K562/DAC cells, we found that after treatment with DAC, K562/DAC cells had significantly higher proliferation and survival rates and lower apoptosis rate as compared to K562 cells (Figure 1C-E). Meanwhile, the ratio of G0/G1 phase in K562/DAC increased (Figure 1F).

3.2 | Gene expression alterations identified in K562/DAC cells

To recognize genes associated with DAC resistance, the candidate genes differentially expressed in K562 and K562/DAC cells were

identified. RNA-Seq analysis was used to screen the candidates (Figure 2A, Figure S1). Then RQ-PCR was performed to validate the up-regulated oncogene in K562/DAC cells. Four up-regulated oncogenes in K562/DAC cells were validated by RQ-PCR. The levels of *H19*, *ID1*, *ID3* and *ITGA2* expressions dramatically increased in K562/DAC cells (Figure 2B). We also performed gene ontology (GO) enrichment analysis to classify differential genes into the categories of cellular component, molecular function and biological process, including extracellular space, protein binding and system development (Figure 2C). To gain deeper understanding the roles of these differentially expressed genes in K562/DAC, we further carried out KEGG pathway enrichment analysis. It was found that these genes were mostly enriched in hematopoietic cell lineage, NF-kappa B signaling pathway and many other pathways (Figure 2D).

3.3 | The role of DDX43/H19/miR-186 in DAC resistance

Our previous study had reported that overexpression of *DDX43* in K562 cell line upregulated *H19* through demethylation.²⁰ Also, *miR-186* was found to target *DDX43*, and *miR-186* was downregulated in *DDX43*-transfected cells.²⁰ Here, the density of *H19* and *DDX43* methylation was greatly decreased in K562/DAC cells (Figure 3A and B).

To further confirm the role of *DDX43* on the sensitivity of K562 cells to DAC, we performed RQ-PCR to detect the expression of *DDX43* and *miR-186*. *DDX43* expression level was increased and inversely correlated with *miR-186* level in K562/DAC cell line (Figure 3C). The IC50 value for DAC was calculated both in K562 transfected with *DDX43* (K562-*DDX43*) and the control (K562-NC). The results showed that upregulation of *DDX43* enhanced DAC resistance of K562 cells compared with K562-NC (IC50: 0.024 $\mu\text{mol/L}$ vs 0.161 $\mu\text{mol/L}$; Figure 3D, $P < 0.01$). Transfection with *DDX43* could reduce sensitivity to DAC (Figure 3E).

4 | DISCUSSION

The clinical outcome of patients after treatment failure with the DNA methylation inhibitors is poor in the clinics.^{11,21} Therefore, it is important to illuminate the resistance mechanism and to overcome this problem. Drug-resistant cell line models provide us with valuable in-vitro tools in clarifying the mechanisms underlying clinical anticancer drug resistance. Cellular or molecular alterations can be detected between a drug-resistant cell line and its drug-sensitive counterpart. Furthermore, cell line models with acquired resistance play an additional and important role in discovering the action mechanism of new, developmental anticancer agents.²² Until now, it was reported that DAC-resistant cells derived from HL-60 and MOLM-13 cells were investigated.^{12,13} Herein, we developed a DAC-resistant cell line by continuous exposure of K562 cell line to graded concentrations of the DAC. We also elucidated the phenotypic and molecular biology properties of our DAC resistance

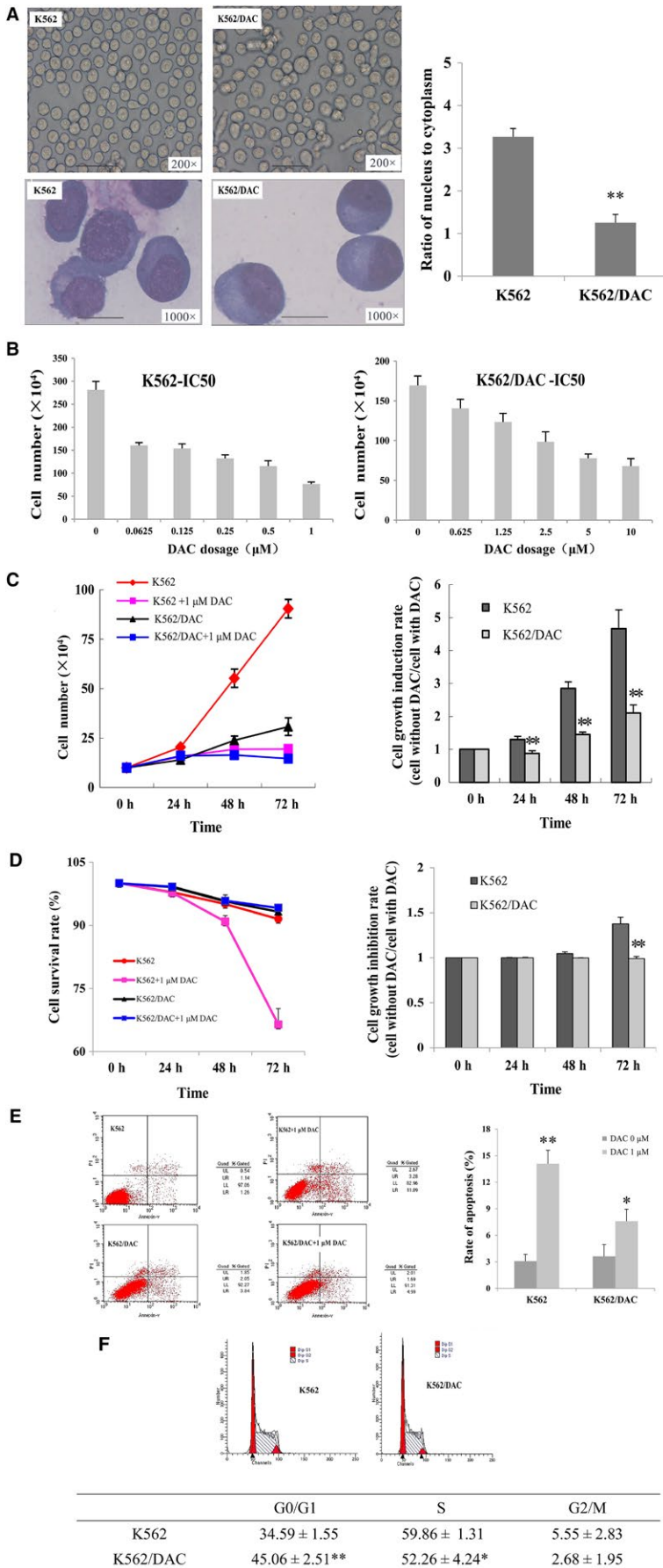


FIGURE 1 Establishment of DAC-resistant K562 cell line. A, Morphological observation of K562 and K562/DAC cells (×200 magnification, bar = 50 μm; and ×1000 magnification, bar = 10 μm). K562/DAC cells had little cytologic atypia with smaller ratio of nucleus to cytoplasm. B, The concentrations of DAC required for 50% growth inhibition were scored as IC50 values. The IC50 values of K562 cell line and K562/DAC cell line to DAC were 0.26 ± 0.02 μmol/L and 3.16 ± 0.02 μmol/L, respectively. C, The proliferation of cells was analysed by cell counting with trypan blue dyeing in study group (with 1 μmol/L DAC) and control group (without DAC), then results were compared. D, Cells were maintained in serum-free conditions. Surviving cells were harvested and counted for statistical analysis. E, Flow cytometry was performed after Annexin V-FITC/PI staining. Results showed the percentage of apoptotic cells. F, Cell-cycle distribution was measured by flow cytometry using PI, and the ratio of G0/G1 phase increased in K562/DAC cells. *P < 0.05, **P < 0.01 compared with control. Error bars indicate SD (n = 3)

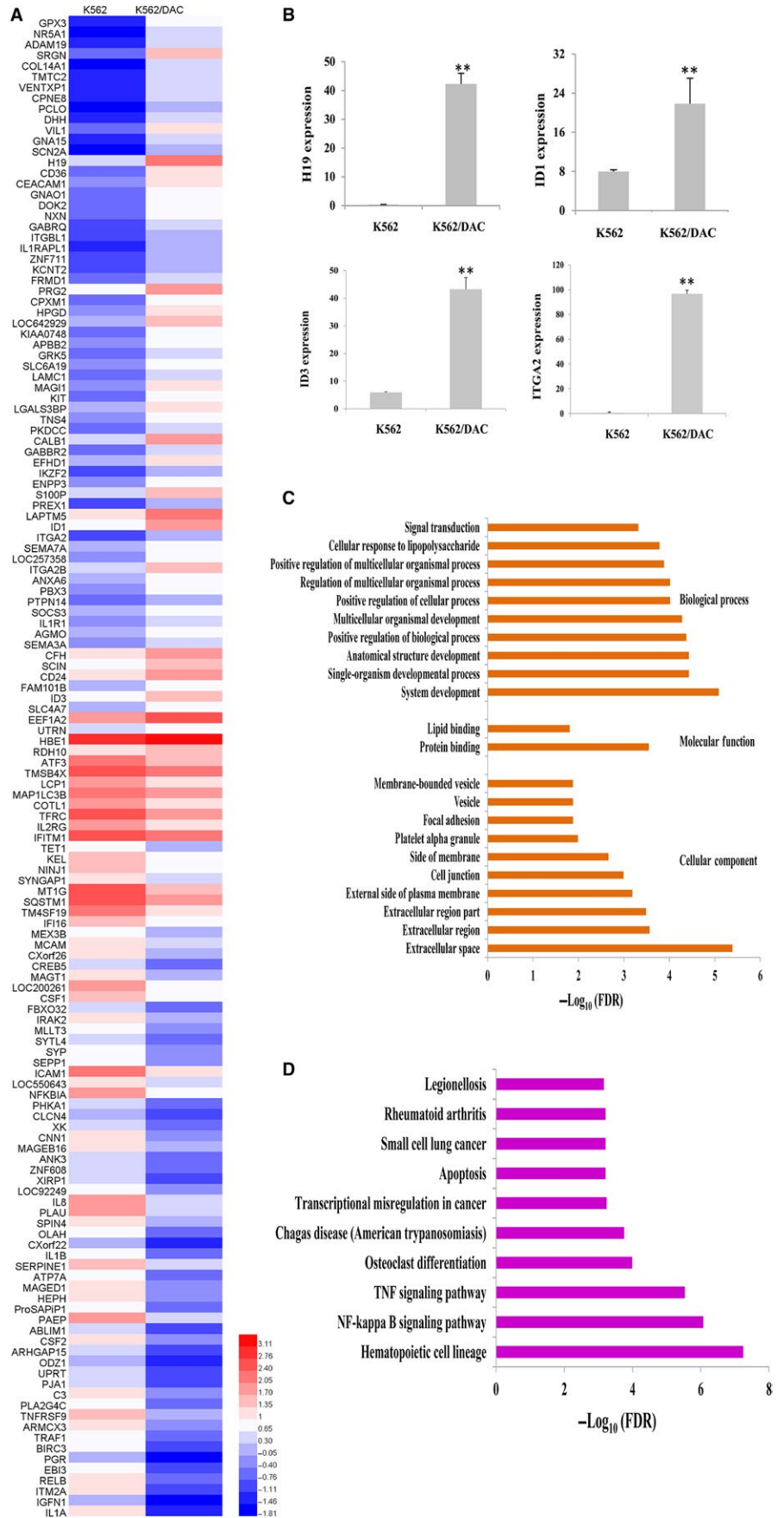


FIGURE 2 Analysis of the differentially expressed genes in K562/DAC cells. A, List of the top 140 differentially expressed mRNAs in K562/DAC cells compared to K562 cells. The color in each small boxes represents the expression level of the genes. Left lower panel: log values of reads per kilobase million in K562 and K562/DAC cells. B, Oncogene *H19*, *ID1*, *ID3* and *ITGA2* expression levels were confirmed with RQ-PCR. Expression of *H19*, *ID1*, *ID3* and *ITGA2* were increased in the K562/DAC cells. $**P < 0.01$, compared with K562 cells. Error bars indicate SD (n = 3). C, GO enrichment analysis of differential genes. The genes were clustered according to the biological process, molecular function and cellular component. FDR: false discovery rates, false discovery rates <0.05. D, KEGG analysis of the top 10 significantly altered pathways in DAC-resistant cells. FDR: false discovery rates, false discovery rates <0.05. The horizontal axis, $-\log_{10}(\text{FDR})$, denotes the significance of specific pathways in K562/DAC cells compared to K562 cells. GO, gene ontology; KEGG, Kyoto Encyclopaedia of Genes and Genomes

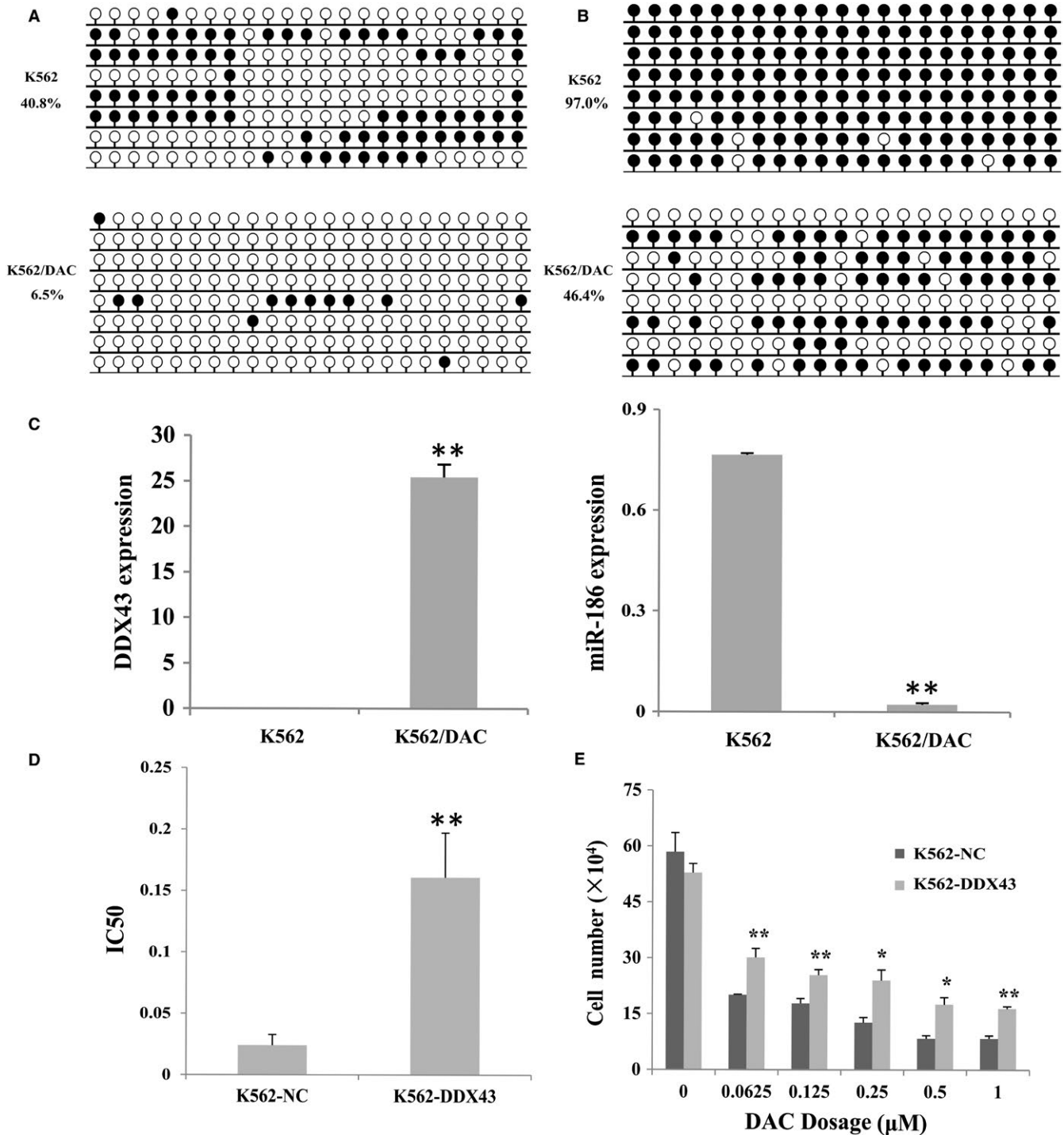


FIGURE 3 The role of DDX43/H19/miR-186 in DAC resistance. A, H19 methylation level detected by bisulfite sequencing in K562 and K562/DAC cells, respectively. B, Promoter methylation density of DDX43 in K562 and K562/DAC cells. White cycle: unmethylated CpG dinucleotide; black cycle: methylated CpG dinucleotide. C, DDX43 expression was up-regulated and inversely correlated with miR-186 level in K562/DAC cell line. D, DAC resistance of K562 cells transfected with DDX43 (K562-DDX43) and its control (K562-NC) were tested. IC50 value increased in K562-DDX43. E, The proliferation of K562-DDX43 was higher than that of control. * $P < 0.05$, ** $P < 0.01$, compared with control. Error bars indicate SD ($n = 3$)

model. The IC50 value for DAC in K562/DAC cells was higher than that of K562 cells. Also, K562/DAC cells showed stronger tolerance after treatment with DAC. Establishment of DAC-resistant cell line is not easy since half-life time was 21 hours for DAC at physiologic media.²³

In the study, we detected differentially expressed gene profiles to analyze whether a gene or signal pathway was involved using RNA-Seq analysis. Our data presented distinct gene expression between parental K562 and DAC-resistant cell line. The expression levels of oncogenes *H19*, *ID1*, *ID3* and *ITGA2* were upregulated in K562/

DAC cells. The DNA methylation inhibitors have no specificity to particular regions due to its extensive demethylation.²⁴ DNA methylation inhibitors not only up-regulate tumor suppressor genes, but also activate some oncogenes, accordingly counteracting the anti-tumor effect of tumor suppressor gene expression, and thus causing resistance in the tumor cells.² The expression of some cancer-related genes might be affected in the DAC-resistant cell line. Some cancer-related genes might play parts in the resistance by means of gene expression regulation. Meanwhile, GO and KEGG analysis illustrated that many fundamental genes and pathways were involved.

Enlighten by *DDX43/H19/miR-186* axis facilitating tumorigenesis and CML progression, we then detected the expression level of *DDX43* and *miR-186* in K562/DAC cells. Upregulated *DDX43* and downregulated *miR-186* were identified in K562/DAC cells. Additionally, ectopic expression of *DDX43* in parental K562 cells induced cells resistant to DAC *DDX43*, initially found as a cancer/testis antigen, which is overexpressed in many solid tumors but absent in normal tissues except testis.²⁵ *DDX43* was substantiated to stimulate oncogenic pathways responsible for cell proliferation.²⁶ *DDX43* provided critical support to the progression of CML by enhancing cell survival and colony formation, and inhibiting cell apoptosis *in vitro* and *in vivo*.²⁰ Moreover, DAC treatment in AML cell lines derepressed cancer/testis antigens localized on the X-chromosome readily and transiently, which implied long-term use of demethylated drugs may lead to genomic instability.²⁷ Besides, *DDX43* could possibly serve as a new potential therapeutic target for recurrent colorectal cancer patients with chemoresistance.²⁸ In selumetinib-resistant uveal melanoma cell lines, *DDX43* was obviously overexpressed and mediated the induction of RAS protein expression and activity.²⁹ In addition, *DDX43* inhibited IFN-induced PML expression by promoting the suppressor of cytokine signaling 1 protein expression that inactivated the Janus kinase–signal transducers and activators of transcription signaling, consequently causing resistance of ABCB5 β malignant melanoma-initiating cells (ABCB5 β MMICs) to IFN α .³⁰ *H19* is a long chain non-coding RNA with length of 2.3 kb. Several studies have reported that overexpression of *H19* was correlated with drug resistance in many tumors such as lung adenocarcinoma, ovarian cancer, human glioma, liver cancer.^{31–34} Our previous study revealed that *H19* expression level, associated with its promoter methylation status, was significantly upregulated in CML patients involving in disease progression.³⁵ Also, *H19* was identified to be upregulated by *DDX43* through demethylation related to CML progression.²⁰ Among AML, *H19* overexpression correlated with poor chemotherapy response and shorter overall survival.³⁶ Taken together, we deduced that *H19* may play a role in drug resistance during leukemogenesis. Thus, we detected the expression and methylation level of *H19* and *DDX43* in K562/DAC cells, and showed positive results. However, the relevance of methylation-associated *DDX43/H19* with DAC resistance need to be further explored. The axis of *DDX43/H19/miR-186* may be an attractive candidate for overcoming drug resistance in leukemia therapy.

In conclusion, a good *in vitro* model was successfully established, which can be used for elucidating the molecular mechanisms related to DAC resistance, and *DDX43/H19/miR-186* axis may be associated with DAC resistance.

ACKNOWLEDGEMENTS

This study was supported by National Natural Science foundation of China (81270630), Medical Innovation Team of Jiangsu Province (CXTDB2017002), 333 Project of Jiangsu Province (BRA2016131), Six talent peaks project in Jiangsu Province (2015-WSN-115), Zhenjiang Clinical Research Center of Hematology (SS2018009), Postgraduate Research & Practice Innovation Program of Jiangsu Province (KYCX17_1821, KYCX18_2281), China Postdoctoral Science Foundation funded project (2016M601748), Youth Medical Talents Project of “Ke Jiao Qiang Wei” project of Jiangsu province (QNRC2016450, QNRC2016449), Social Development Foundation of Zhenjiang (SH2016045, SH2016046, SH2017040), Jiangsu University Medical Clinical Science and Technology Development Fund (JLY20160011), Social Development Foundation of Kun Shan (KS1624), and Key Medical Talent Program of Zhenjiang City.

CONFLICTS OF INTEREST

The authors stated that there are no conflicts of interest regarding the publication of this article. Research support played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

ORCID

Xiang-Mei Wen  <https://orcid.org/0000-0003-0413-7912>

REFERENCES

1. Ehrlich M. DNA methylation in cancer: too much, but also too little. *Oncogene*. 2002;21:5400–5413.
2. Esteller M. Epigenetics in cancer. *N Engl J Med*. 2008;358:1148–1159.
3. Sharma S, Kelly TK, Jones PA. Epigenetics in cancer. *Carcinogenesis*. 2010;31:27–36.
4. Taby R, Issa JP. Cancer epigenetics. *CA Cancer J Clin*. 2010;60:376–392.
5. Dombret H, Seymour JF, Butrym A, et al. International phase 3 study of azacitidine vs conventional care regimens in older patients with newly diagnosed AML with >30% blasts. *Blood*. 2015;126:291–299.
6. Estey EH. Epigenetics in clinical practice: the examples of azacitidine and decitabine in myelodysplasia and acute myeloid leukemia. *Leukemia*. 2013;27:1803–1812.
7. Garcia-Manero G. Myelodysplastic syndromes: 2015 Update on diagnosis, risk-stratification and management. *Am J Hematol*. 2015;90:831–841.
8. Man CH, Fung TK, Wan H, et al. Suppression of SOX7 by DNA methylation and its tumor suppressor function in acute myeloid leukemia. *Blood*. 2015;125:3928–3936.
9. Hackanson B, Daskalakis M. Decitabine. *Recent Results Cancer Res*. 2014;201:269–297.

10. Steinhart L, Belz K, Fulda S. Smac mimetic and demethylating agents synergistically trigger cell death in acute myeloid leukemia cells and overcome apoptosis resistance by inducing necroptosis. *Cell Death Dis.* 2013;4:e802.
11. Lee JH, Choi Y, Kim SD, et al. Clinical outcome after failure of hypomethylating therapy for myelodysplastic syndrome. *Eur J Haematol.* 2015;94:546-553.
12. Qin T, Jelinek J, Si J, et al. Mechanisms of resistance to 5-aza-2-deoxycytidine in human cancer cell lines. *Blood.* 2009;113:659-667.
13. Hur EH, Jung SH, Goo BK, et al. Establishment and characterization of hypomethylating agent-resistant cell lines, MOLM/AZA-1 and MOLM/DEC-5. *Oncotarget.* 2017;8:11748-11762.
14. Qin T, Castoro R, El Ahdab S, et al. Mechanisms of resistance to decitabine in the myelodysplastic syndrome. *PLoS ONE.* 2011;6:e23372.
15. Zhang TJ, Zhou JD, Zhang W, et al. H19 overexpression promotes leukemogenesis and predicts unfavorable prognosis in acute myeloid leukemia. *Clin Epigenetics.* 2018;10:47.
16. Zhou JD, Yang L, Zhu XW, et al. Clinical significance of up-regulated ID1 expression in Chinese de novo acute myeloid leukemia. *Int J Clin Exp Pathol.* 2015;8:5336-5344.
17. Zhou JD, Ma JC, Zhang TJ, et al. High bone marrow ID2 expression predicts poor chemotherapy response and prognosis in acute myeloid leukemia. *Oncotarget.* 2017;8:91979-91989.
18. Chen Q, Lin J, Yao DM, et al. Aberrant hypomethylation of DDX43 promoter in myelodysplastic syndrome. *Br J Haematol.* 2012;158:293-296.
19. Zhang TJ, Wang YX, Yang DQ, et al. Down-regulation of miR-186 correlates with poor survival in de novo acute myeloid leukemia. *Clin Lab.* 2016;62:113-120.
20. Lin J, Ma JC, Yang J, et al. Arresting of miR-186 and releasing of H19 by DDX43 facilitate tumorigenesis and CML progression. *Oncogene.* 2018;37:2432-2443.
21. Zeidan AM, Kharfan-Dabaja MA, Komrokji RS. Beyond hypomethylating agents failure in patients with myelodysplastic syndromes. *Curr Opin Hematol.* 2014;21:123-130.
22. Coley HM. Development of drug-resistant models. *Methods Mol Med.* 2004;88:267-273.
23. Stresemann C, Lyko F. Modes of action of the DNA methyltransferase inhibitors azacytidine and decitabine. *Int J Cancer.* 2008;123:8-13.
24. Kelly TK, De Carvalho DD, Jones PA. Epigenetic modifications as therapeutic targets. *Nat Biotechnol.* 2010;28:1069-1078.
25. Martelange V, De Smet C, De Plaen E, et al. Identification on a human sarcoma of two new genes with tumor-specific expression. *Cancer Res.* 2000;60:3848-3855.
26. Van Tongelen A, Lorient A, De Smet C. Oncogenic roles of DNA hypomethylation through the activation of cancer-germline genes. *Cancer Lett.* 2017;396:130-137.
27. Almstedt M, Blagitko-Dorfs N, Duque-Afonso J, et al. The DNA demethylating agent 5-aza-2-deoxycytidine induces expression of NY-ESO-1 and other cancer/testis antigens in myeloid leukemia cells. *Leuk Res.* 2010;34:899-905.
28. Baharudin R, Ab Mutalib NS, Othman SN, et al. Identification of predictive DNA methylation biomarkers for chemotherapy response in colorectal cancer. *Front Pharmacol.* 2017;8:47.
29. Ambrosini G, Khanin R, Carvajal RD, Schwartz GK. Overexpression of DDX43 mediates MEK inhibitor resistance through RAS upregulation in uveal melanoma cells. *Mol Cancer Ther.* 2014;13:2073-2080.
30. Mathieu MG, Miles AK, Ahmad M, et al. The helicase HAGE prevents interferon-alpha-induced PML expression in ABCB5+ malignant melanoma-initiating cells by promoting the expression of SOCS1. *Cell Death Dis.* 2014;5:e1061.
31. Wang Q, Cheng NN, Li XF, et al. Correlation of long non-coding RNA H19 expression with cisplatin-resistance and clinical outcome in lung adenocarcinoma. *Oncotarget.* 2017;8:2558-2567.
32. Zheng ZG, Xu H, Suo SS, et al. The essential role of H19 contributing to cisplatin resistance by regulating glutathione metabolism in high-grade serous ovarian cancer. *Sci Rep.* 2016;6:26093.
33. Jiang PF, Wang P, Sun XL, et al. Knockdown of long noncoding RNA H19 sensitizes human glioma cells to temozolomide therapy. *Oncotargets and Therapy.* 2016;9:3501-3509.
34. Tsang WP, Kwok TT. Riboregulator H19 induction of MDR1-associated drug resistance in human hepatocellular carcinoma cells. *Oncogene.* 2007;26:4877-4881.
35. Zhou JD, Lin J, Zhang TJ, et al. Hypomethylation-mediated H19 overexpression increases the risk of disease evolution through the association with BCR-ABL transcript in chronic myeloid leukemia. *J Cell Physiol.* 2018;233:2444-2450.
36. Zhang TJ, Zhou JD, Zhang W, et al. H19 overexpression promotes leukemogenesis and predicts unfavorable prognosis in acute myeloid leukemia. *Clinical Epigenetics.* 2018;10:47.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Wen X-M, Zhang T-J, Ma J-C, et al. Establishment and molecular characterization of decitabine-resistant K562 cells. *J Cell Mol Med.* 2019;23:3317-3324. <https://doi.org/10.1111/jcmm.14221>