doi.org/10.36721/PJPS.2024.37.5.REG.1019-1025.1

The synergistic effect of miR-203 and cytarabine on the inhibition of cell proliferation and induction of apoptosis in chronic myelogenous leukemia cells

Shanshan Qi[1#], Jianghua Huang[2#] and Run Long[3*]

[1]Department of Oncology, Shijiazhuang Traditional Chinese Medicine Hospital, ShiJiaZhuang, HeBei, China
[2]Department of Pathology, Liuzhou Worker's Hospital, Liuzhou, Guangxi Zhuang Autonomous Region, China
[3]Digestive Endoscopy Department, Hebei Province Hospital of Traditional Chinese Medicine,
Hebei Key Laboratory of Turbidity Toxin Syndrome, ShiJiaZhuang, HeBei, China

Abstract: Cytarabine (Ara-C) is a commonly used chemotherapeutic drug for the treatment of leukemia, known for its significant tolerability. The down regulation of miR-203 in leukemia cells suggests its potential involvement in the pathogenesis of leukemia. In this study, we investigated the effects and possible mechanisms of miR-203 and Ara-C on proliferation and apoptosis of human leukemia K562 cells which were cultured with Ara-C and/or with transfection of miR-203 expression vectors. Our results showed that the combination of Ara-C and miR-203 synergistically inhibited the proliferation of K562 cells and the sensitivity of leukemia cells to Ara-C was increased by 2.5-fold with trasfection of miR-203. The proportion of apoptotic cells in the Ara-C and miR-203 combination group was higher than Ara-C or control plasmid group. Caspase-3 and caspase-9 activities were increased in Ara-C and miR-203 combination group. miR-203 down regulated the protein level of Bcr/abl in K562 cells compared with plasmid control. In conclusion, Ara-C in combination with miR-203 has a synergistic effect of proliferation inhibition and apoptosis induction in chronic myelogenous leukemia K562 cells, which may be associated with miR-203 down regulating Bcr/abl, thereby inhibiting cell proliferation and promoting cell apoptosis.

Keywords: Apoptosis, Bcr/abl, cytarabine, leukemia, miR-203.

Submitted on 17-08-2023 – Revised on 06-8-2024 – Accepted on 18-9-2024

INTRODUCTION

Chronic myelogenous leukemia (CML) involves malignant proliferation of bone marrow hematopoietic stem cells, mainly affecting the myeloid series. CML is characterized by a significant increase in peripheral blood granulocytes with maturation disorders, leading to symptoms such as infection, fever, bone pain, splenomegaly, anemia, and bleeding due to transformation (Cortes *et al*., 2021; Narlı Ozdemir *et al*., 2023), which poses threat to health and sometimes resulting in death. The most important diagnostic criterion for CML is the characteristic Philadelphia chromosome (Ph chromosome) (Soverini *et al*., 2019; Breccia *et al*., 2021; Cortes *et al*., 2021). Ph chromosome or Bcr/Abl gene positivity is often seen in bone marrow aspirates (Hidalgo-López *et al*., 2018). The appearance of the Philadelphia chromosome is a shorter chromosome 22 (Asnafi *et al*., 2019; Soverini *et al*., 2019). Simultaneously, the ABL gene fuses with the BCR gene, forming the Bcr/Abl fusion gene, which subsequently expresses the Bcr/Abl fusion protein (Younes *et al*., 2023). This abnormally expressed protein possesses aberrant tyrosine kinase activity, which can disrupt the proliferation (Bavaro *et al*., 2019; Liu *et al*., 2020; Wang *et al*., 2021), differentiation (Wang *et al*., 2019; Pinto *et al*., 2021) and apoptosis (Scherr *et al*., 2019; Wang *et al*., 2019; Zhang *et al*., 2022) of hematopoietic progenitor cells and is due to the development of CML. Thus, inhibiting the Bcr/Abl gene is of significant importance in halting the progression of leukemia.

MicroRNAs (miRNAs) can play an anti-leukemia role by regulating the expression of the Bcr/Abl fusion gene (Choi *et al*., 2016; Litwińska and Machaliński, 2017). Furthermore, miR-203 is found to be deleted or down-regulated in leukemia cells and patients (Fan *et al*., 2018; Zheng *et al*., 2019), indicating its potential involvement in leukemia development. Cytarabine (Ara-C) is a commonly used chemotherapy drug for treating leukemia, however, patients can develop tolerance to it (Shabashvili *et al*., 2022; Ling *et al*., 2023), necessitating its combination with other drugs. Clinical trials have shown that low-dose Ara-C combined with interferon therapy achieves complete hematologic and cytogenetic responses in CML patients (Hochhaus *et al*., 2017; Kumar *et al*., 2023). Low-dose Ara-C combined with imatinib treatment leads to molecular responses in CML patients (Gómez-Almaguer *et al*., 2016). Studies have revealed that the up regulation of Bcr/Abl gene expression in imatinib-resistant leukemia cells may be related to cellular resistance (Lu *et al*., 2021; Tadesse *et al*., 2021). Additionally, studies have indicated that silencing miR-203 can cause upregulation of Bcr/Abl gene expression (Bueno *et al*., 2008). Taking all of the above research into

[#]*These authors contributed equally to this work*
**Corresponding authors:* e-mails: longrunlr@163.com

consideration, it suggests that miR-203 may be related to leukemia cell resistance. To investigate whether targeted modulation of miR-203 can enhance the efficacy of Ara-C in treating leukemia, this study will transfect the eukaryotic expression vector of miR-203 into CML cell line K562 and evaluate the combined effect of both miR-203 and Ara-C, as well as explore their possible mechanisms of action. This study will shield light on the probability of using miRNA to avoid the tolerance of traditional chemotherapy drug for treating leukemia.

MATERIALS AND METHODS

Materials

The miR-203 eukaryotic expression vector ($_P$miR-203) was purchased from Gene Pharma Co., Ltd. Lipofectamine TM 2000 transfection reagent was obtained from Invitrogen (USA). MTT was purchased from Sigma Aldrich (USA). Annexin V/PI Kit was purchased from Kaiji Biotechnology Development Co., Ltd., Nanjing, China. Caspase-3 and Caspase-9 Spectrophotometric Assay Kits was purchased from Kaiji Biotechnology Development Co., Ltd., Nanjing, China. BCA Kit was purchased from BIOSS Biotechnology Co., Ltd., Beijing, China. Mouse anti-β-actin antibody was purchased from BOSTER Biological Technology Co., Ltd., Wuhan, China, mouse anti-Bcr/abl antibody was purchased from Abcam, USA. ECL Kit was purchased from Beyotime Biotechnology Co., Ltd., Shanghai, China.

Cell culture

The K562 cell line of chronic myeloid leukemia was from the Institute of Biological Sciences, Chinese Academy of Sciences (Shanghai). K562 cells were cultured in DMEM/F12 medium containing fetal bovine serum (10%) in a humidified incubator at 37°C with 5% CO_2 and subcultured every 3 days. Experiments were conducted once the cells reached exponential growth phase.

Methods

MTT assay for cell viability analysis

The experiment comprised of blank, Ara-C, plasmid control, $_P$miR-203, Ara-C combined plasmid control and Ara-C combined $_P$miR-203 group. K562 cells were seeded in 96-well plates with 6 replicates per group. The transfection of $_P$miR-203 and control plasmid was performed according to the protocol of Lipofectamine™ 2000 and final concentration of $_P$miR-203 or control vector transfected in each group was 0.05mg/L. Ara-C concentrations used for cell treatment were 0.8, 1.2, 1.6, 2.0 and 2.4μM. Cell proliferation was determined using the MTT method after 48 hours. The proliferation inhibition rate was calculated as: Proliferation inhibition (%) = $[A_{\text{control group}} - A_{\text{experimental group}}] / A_{\text{control group}} \times 100$ %. The drug sensitization fold was calculated as IC_{50} of single-drug group / IC_{50} of combination therapy group. Effect of combination therapy was quantized using the Q-value method as reported (Zhang *et al.*, 2018), where $Q = (E_{a+b}) / (E_a + E_b - E_a \times E_b)$. In the formula, E_a represents inhibition rate of Ara-C alone, E_b represents inhibition rate of $_P$miR-203 alone, E_{a+b} represents inhibition rate of Ara-C and $_P$miR-203. The Q value smaller than 0.85 represents an antagonistic effect, Q value between 0.85 and 1.15 represents an additive effect and Q value larger than 0.85 represents a synergistic effect.

Detection of the apoptosis of cells by flow cytometry

The experimental groups were divided and transfection methods are as mentioned above. Cells were seeded in the 24 well plates, with 3 replicates per group. The final concentration of plasmid transfection for both the $_P$miR-203 and empty vector groups was 0.05mg/L. Ara-C concentration was 1.6μM. After 48 hours, cells were collected by centrifugation and cell treatment and addition of assay reagents were carried out according to the protocol of the Annexin V/PI Kit for apoptosis detection. Flow cytometry was used for analysis of apoptosis.

Caspase-3 and Caspase-9 activity assay

The experimental groups, cell seeding and transfection procedures were the same as described above. After 48 hours, cells were collected by centrifugation, and cell treatment and addition of assay reagents were carried out following the instructions of Caspase-3 and Caspase-9 Spectrophotometric Assay Kits. Absorbance was measured using an ELISA reader.

Western blotting

The experiment consisted of a blank control group, an empty vector control group and a $_P$miR-203 group. The transfection method and cell seeding were the same as described above. After 48 hours, cells were collected by centrifugation and protein extraction was performed. Protein quantification was done using the BCA Kit, then separated by discontinuous polyacrylamide gel electrophoresis at 80V, followed by switching to 120V after entering the separating gel, for 40 minutes. Semi-dry transfer was employed to transfer proteins from the gel to a PVDF membrane at 15V for 18 minutes. The membrane was washed with TBST, then blocked with 5% bovine serum albumin and washed with TBST. The membrane was incubated with the primary antibody (mouse anti-β-actin antibody and mouse anti-Bcr/abl antibody) and secondary antibody in turn. The ECL Kit was used for signal detection.

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS 25.0. Data are presented as mean ± standard deviation. One-way ANOVA was used to analyze the differences between groups. $P<0.05$ was considered statistically significant.

RESULTS

Inhibition of K562 cells by treatment with Ara-C in combination with $_P$miR-203

The proliferation the chronic myelogenous leukemia

K562 cell line was investigated by MTT cell viability assay. Compared with control, the use of 0.8, 1.2, 1.6, 2.0, and 2.4μM Ara-C or $_P$miR-203 (0.05mg/L) alone inhibited proliferation of cells. The inhibition rates of cell proliferation in the Ara-C combined with $_P$miR-203 groups were significantly increased compared to the Ara-C combined with empty vector control group, with statistically significant differences (n=6, P<0.01) (fig. 1A). The IC_{50} for Ara-C alone in inhibiting K562 cell proliferation was 2.0μM, while the IC_{50} for the combination of Ara-C and $_P$miR-203 in inhibiting cell proliferation was 0.8μM. The drug sensitization fold was 2.5 and the combination of Ara-C and $_P$miR-203 displayed a synergistic effect (Q≥1.15) (fig. 1B).

Effect of the combination of Ara-C with $_P$miR-203 on apoptosis of K562 cells

Ara-C increased the apoptotic rate of cells compared with control, (P<0.01). Furthermore, transfection of $_P$miR-203 in K562 cells for 48 hours resulted in a significant increase in apoptosis rate compared with vector control (P <0.01). Notably, the combination of Ara-C and $_P$miR-203 demonstrated a significantly higher apoptotic rate of 32.01±2.95% compared to the combination of Ara-C and empty vector control group (13.32±3.24%) (P<0.01) (fig. 2).

Increase of Caspase-3 and Caspase-9 activity by Ara-C in combination with $_P$miR-203

Compared to the control group (0.11±0.01and 0.11±0.01), the activity values of Caspase-3 (0.43±0.06) and Caspase-9 (0.48±0.05) in the Ara-C group were increased, respectively (n=4, P<0.01). After transfection of $_P$miR-203 for 48 hours, the activity of Caspase-3 and -9 were increased compared to the empty vector control group, with values of 0.48±0.04 and 0.54±0.03 (n=4, P<0.01). In the Ara-C combined with $_P$miR-203 group, the activity of Caspase-3 and -9 were higher compared to the Ara-C combined with empty vector control group, with values of 0.88±0.07 and 0.94±0.04 (n=4, P<0.01) as shown in fig. 3.

Down-regulation of Bcr/abl protein by $_P$miR-203 in K562 cells

The protein level of Bcr/abl protein was not altered by transfection of the empty vector in K562 cells. Compared with control, the protein level of Bcr/abl in the empty vector control group and the $_P$miR-203 group were 0.98± 0.10 and 0.59±0.12, respectively. The level of Bcr/abl protein of cells trasfected with $_P$miR-203 was decreased compared to cells trasfected with plasmid vector (P<0.05) (fig. 4).

DISCUSSION

miRNAs play important roles in tumors, with approximately 50% of miRNA genes located in fragile sites within the tumor-related genome region, where these sites frequently undergo rearrangement in tumors (Menon *et al*., 2022). Some miRNAs are significantly down regulated or not expressed in tumor cells, and they inhibit the tumors by modulating the expression of cancer genes and other genes related to cell proliferation and apoptosis (Adlakha and Saini, 2016; Alizadeh *et al*., 2019; Darvish *et al*., 2023), often acting as tumor suppressor genes. miR-203 is one such miRNA that is absent or down regulated in leukemia cells (Fan *et al*., 2018; Zheng *et al*., 2019). When miRNAs are down regulated or lacking in tumors, researchers can simulate the function of endogenous miRNAs by over expressing them in cells based on the structural characteristics of endogenous miRNAs, which potentially exerting anti-tumor effects.

Studies have found that miRNAs can enhance the sensitivity of tumor cells to anti-cancer drugs by regulating the oncogenes (Sun *et al*., 2017), tumor suppressor genes (Gurbuz and Ozpolat, 2019), and proliferation and apoptosis-related genes (Adlakha and Saini, 2016; Alizadeh *et al*., 2019; Darvish *et al*., 2023). The combination of miRNA and conventional chemotherapy drugs used in tumor treatment can have a more positive anti-tumor effect (Zhang *et al*., 2020; Wu *et al*., 2022). In present study, miR-203 over-expression was achieved by transfecting a eukaryotic expression vector carrying miR-203 into K562 cells, simulating the function of endogenous miR-203. The combined use of conventional chemotherapy drug Ara-C and miR-203 significantly inhibited the proliferation of K562 cells, showing a synergistic anti-leukemia effect. The sensitivity to Ara-C was increased 2.5 folds compared to single-use of Ara-C in K562 cells, indicating that targeted regulation of miR-203 expression in combination with Ara-C may have a more effective anti-leukemia effect.

Caspase, a family of cysteine proteases, triggers a cascade reaction that is the central process of cell apoptosis. There are mainly two activation pathways, including the mitochondria-dependent pathway and the death receptor-mediated pathway (Van Opdenbosch and Lamkanfi, 2019). Caspase-9, located upstream of the caspase cascade reaction, is the initiator of apoptosis (Unnisa *et al*., 2023), while Caspase-3, located downstream, is the effector of apoptosis and can be activated by the upstream initiator (Asadi *et al*., 2022). The mitochondria-dependent pathway is the main pathway of programmed cell death in mammalian cells. Caspase-9 is located at the top of the caspase cascade reaction in the mitochondria-dependent pathway, forming an apoptosome with cytochrome C and Apaf-1, which undergoes self-cleavage and further activates members such as Caspase-3 with the participation of dATP, leading to apoptosis (Asadi *et al*., 2022; Unnisa *et al*., 2023). Caspase-3 is considered a key protease in the process of mammalian cell apoptosis. Once Caspase-3 is activated, it triggers downstream cascade reactions, making apoptosis inevitable (Asadi *et al*., 2022).

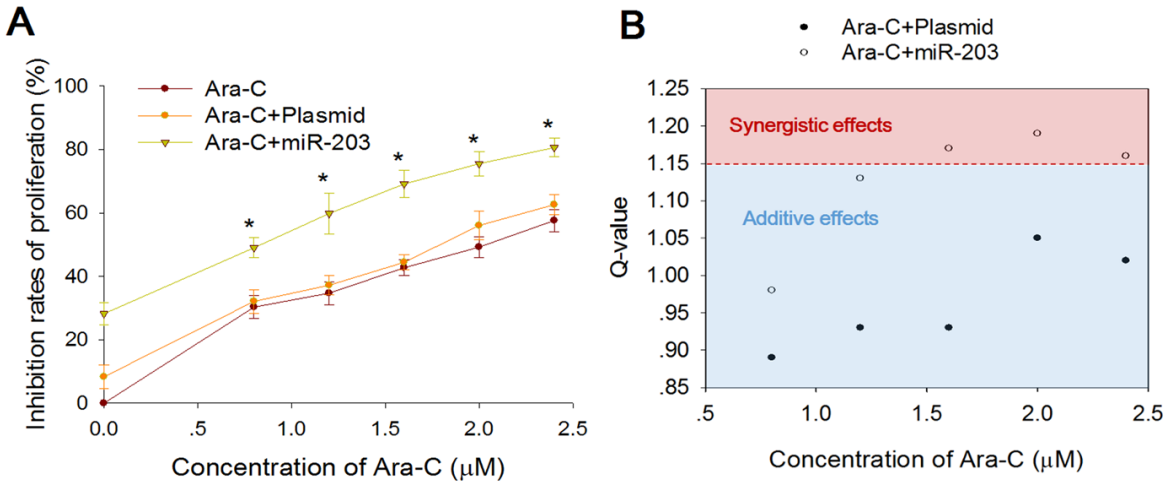

Fig. 1: Synergistic inhibition of proliferation of K562 cells by Ara-C and miR-203. (A) Inhibition of K562 cell proliferation. (B) Synergistic inhibition effects. $^{*}P<0.01$ compared with Ara-C group.

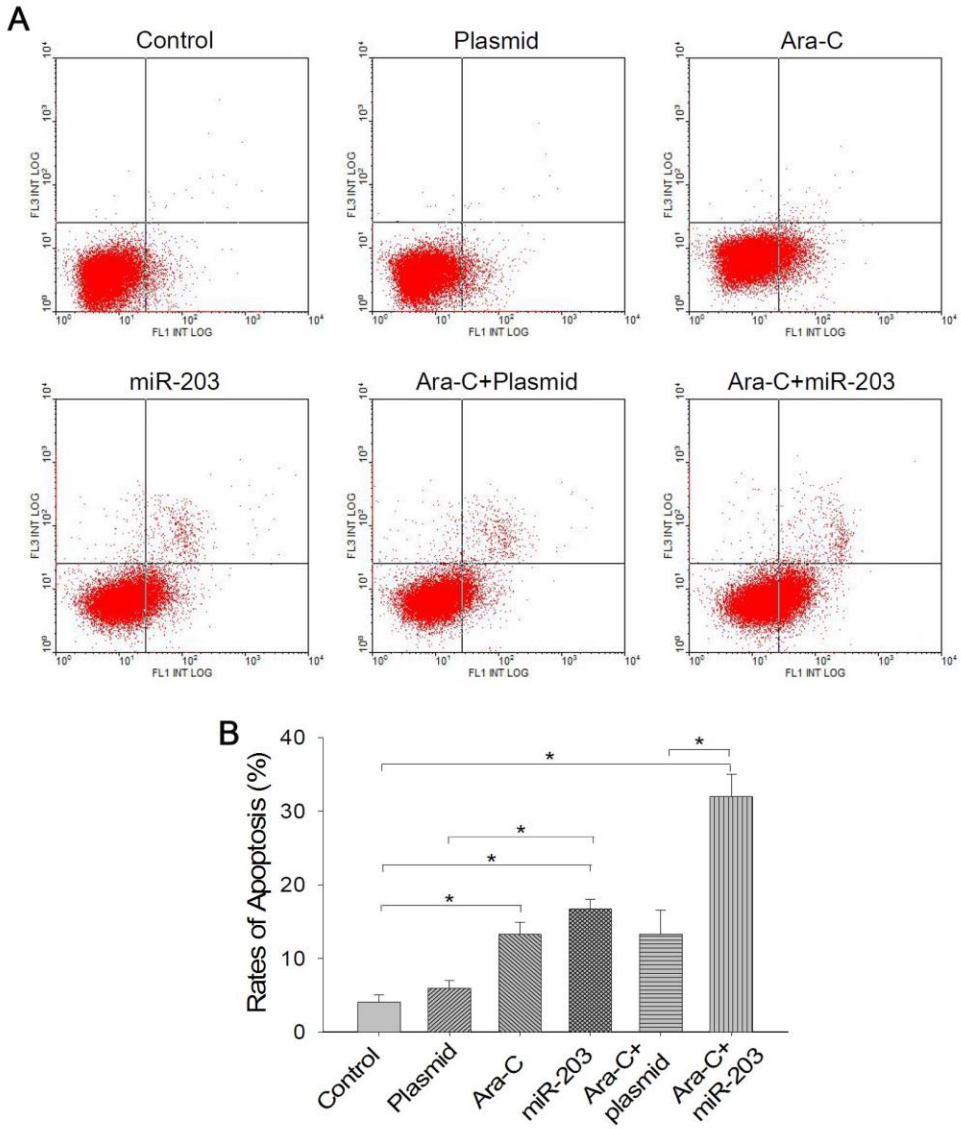

Fig. 2: Increased rates of apoptosis induced by Ara-C in combination with miR-203 in K562 cells. (A) Induction of apoptosis detected by flow cytometry. (B) Rates of apoptosis. $^{*}P<0.05$.

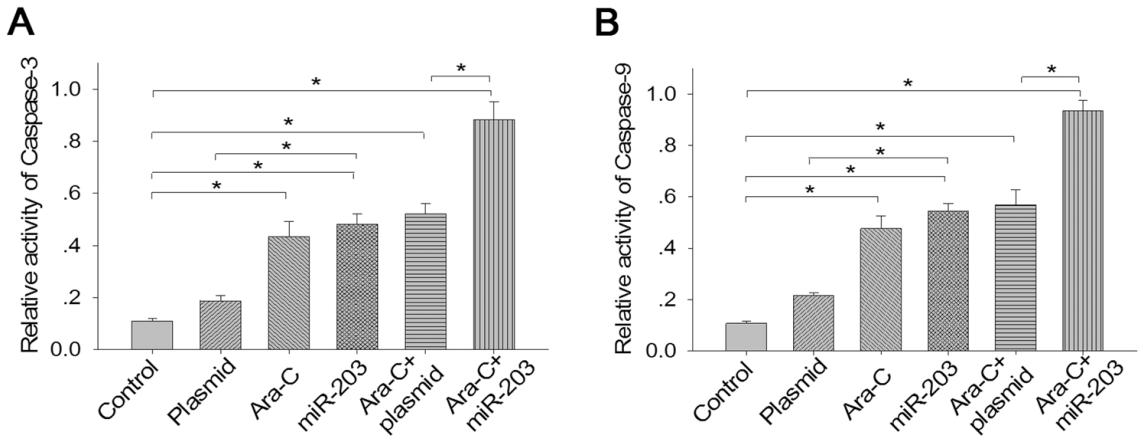

Fig. 3: Increase of Caspase activity by Ara-C in combination with miR-203. (A) Increased activity of Caspase-3. (B) Increased activity of Caspase-9. $^{*}P<0.01$.

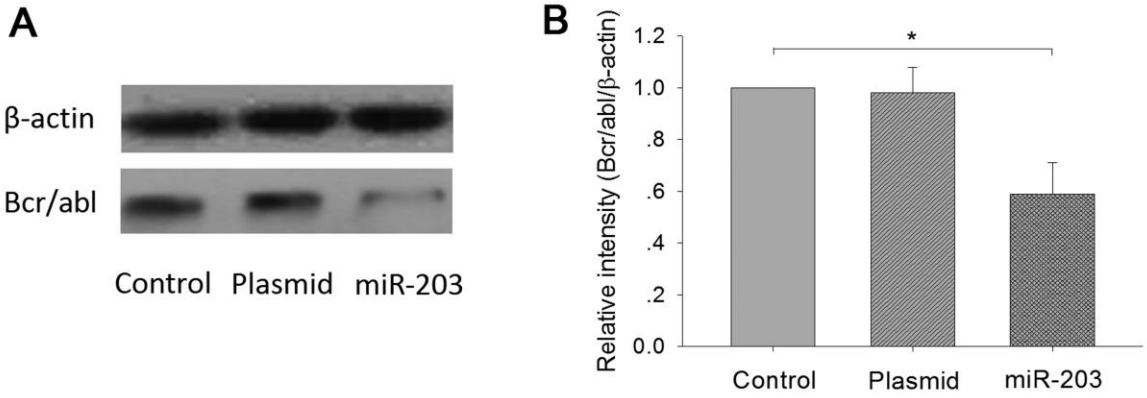

Fig. 4: Inhibitions of Bcr/abl protein expression by miR-203. (A) Typical Western blotting. (B) Relative intensity. $^{*}P<0.05$ compared with control.

Therefore, it is very important in cell apoptosis. In present study, the combined application of Ara-C and miR-203 increased apoptosis significantly, and the activity of Caspases was also significantly enhanced, suggested that up-regulation of miR-203 can promote Ara-C-induced apoptosis in K562 cells and the cascade reaction activated by Caspase-9 and Caspase-3 is involved in cell apoptosis, indicating that the mitochondria-dependent pathway is important in the process.

The main pathogenic mechanism of CML is the formation of the fusion gene Bcr/abl, which encodes the P210 protein (Asnafi *et al*., 2019; Liu *et al*., 2020). The abnormal elevation of tyrosine kinase activity caused by this fusion gene activates several signaling pathways, leading to uncontrolled proliferation and inhibition of apoptosis, thus contributing to the development of CML (Scherr *et al*., 2019; Wang *et al*., 2019; Zhang *et al*., 2022). In present study, we proved the downstream gene regulated by miR-203 and found that up-regulation of miR-203 down-regulated the protein level of the Bcr/abl in leukemia cells, suggesting a potential correlation between Bcr/abl down regulation and the synergistic effect of miR-203 combined with Ara-C in treating leukemia K562 cells.

Clinical research has shown that low-dose Ara-C combined with imatinib can achieve molecular responses in CML patients. However, studies have also indicated that when used in combination, imatinib inhibits the transport of Ara-C into cells, suggesting that further improvement is needed in the therapeutic effects of both drugs. Similar to imatinib, miR-203 exerts its inhibitory effect on tyrosine kinase activity by regulating the expression of the Bcr/abl fusion gene. Consequently, combining miR-203 with Ara-C may avoid the potential interactions between drug transport, thereby demonstrating a synergistic anti-leukemia effect and holding significant importance for the study of novel and effective anti- leukemia drugs.

CONCLUSION

This study demonstrates that the integration of targeted miRNA technology with conventional chemotherapy drugs can enhance the sensitivity of leukemia cells to chemotherapy, thus exerting a synergistic effect against leukemia cells. It provides new experimental evidence for the combined application of gene therapy and conventional chemotherapy in the treatment of leukemia.

ACKNOWLEDGMENTS

This study was supported by Hebei Health and Family Planning Commission Scientific Research Project (20240283), Shijiazhuang Science and Technology Research Program Project (221460333), Guangxi Zhuang Autonomous Region Health and Family Planning Commission Self-financed Scientific Research Project (Z20200928).

REFERENCES

Adlakha YK and Saini N (2016). MicroRNA: a connecting road between apoptosis and cholesterol metabolism. *Tumour. Biol.*, **37**(7): 8529-8554.

Alizadeh M, Safarzadeh A, Beyranvand F, Ahmadpour F, Hajiasgharzadeh K, Baghbanzadeh A and Baradaran B (2019). The potential role of miR-29 in health and cancer diagnosis, prognosis, and therapy. *J. Cell. Physiol.*, **234**(11): 19280-19297.

Asadi M, Taghizadeh S, Kaviani E, Vakili O, Taheri-Anganeh M, Tahamtan M and Savardashtaki A (2022). Caspase-3: Structure, function and biotechnological aspects. *Biotechnol. Appl. Biochem.*, **69**(4): 1633-1645.

Asnafi AA, Deris Zayeri Z, Shahrabi S, Zibara K and Vosughi T (2019). Chronic myeloid leukemia with complex karyotypes: Prognosis and therapeutic approaches. *J. Cell. Physiol.*, **234**(5): 5798-5806.

Bavaro L, Martelli M, Cavo M and Soverini S (2019). Mechanisms of disease progression and resistance to tyrosine kinase inhibitor therapy in chronic myeloid leukemia: An update. *Int. J. Mol. Sci.*, **20**(24) :6141.

Breccia M, Efficace F, Scalzulli E, Ciotti G, Maestrini G, Colafigli G and Martelli M (2021). Measuring prognosis in chronic myeloid leukemia: What's new. *Expert. Rev. Hematol.*, **14**(6): 577-585.

Bueno MJ, Pérez de Castro I, Gómez de Cedrón M, Santos J, Calin GA, Cigudosa JC, Croce CM, Fernández-Piqueras J and Malumbres M (2008). Genetic and epigenetic silencing of microRNA-203 enhances ABL1 and BCR-ABL1 oncogene expression. *Cancer Cell.*, **13**(6): 496-506.

Choi J, Kim YK, Park K, Nah J, Yoon SS, Kim DW, Kim VN and Seong RH (2016). MicroRNA-139-5p regulates proliferation of hematopoietic progenitors and is repressed during BCR-ABL-mediated leukemogenesis. *Blood.*, **128**(17): 2117-2129.

Cortes J, Pavlovsky C and Saußele S (2021). Chronic myeloid leukaemia. *Lancet.*, **398**(10314): 1914-1926.

Darvish L, Bahreyni Toossi MT, Azimian H, Shakeri M, Dolat E, Ahmadizad Firouzjaei A, Rezaie S, Amraee A and Aghaee-Bakhtiari SH (2023). The role of microRNA-induced apoptosis in diverse radioresistant cancers. *Cell. Signal.*, **104**: 110580.

Fan H, Li Y, Liu C, Liu Y, Bai J and Li W (2018). Circular RNA-100290 promotes cell proliferation and inhibits apoptosis in acute myeloid leukemia cells via sponging miR-203. *Biochem. Biophys. Res. Commun.*, **507**(1-4): 178-184.

Gurbuz N and Ozpolat B (2019). MicroRNA-based targeted therapeutics in pancreatic cancer. *Anticancer. Res.*, **39**(2): 529-532.

Gómez-Almaguer D, Cantú-Rodríguez OG, Gutiérrez-Aguirre CH and Ruiz-Argüelles GJ (2016). The treatment of CML at an environment with limited resources. *Hematology*, **21**(10): 576-582.

Hidalgo-López JE, Kanagal-Shamanna R, Quesada AE, Gong Z, Wang W, Hu S, Medeiros LJ, Bassett RL Jr, d'Orcy E, Yin CC, Cortes J, Jabbour EJ, Kantarjian HM and Bueso-Ramos CE (2018). Bone marrow core biopsy in 508 consecutive patients with chronic myeloid leukemia: Assessment of potential value. *Cancer*, **124**(19): 3849-3855.

Hochhaus A, Larson RA, Guilhot F, Radich JP, Branford S, Hughes TP, Baccarani M, Deininger MW, Cervantes F, Fujihara S, Ortmann CE, Menssen HD, Kantarjian H, O'Brien SG and Druker BJ (2017). Long-term outcomes of imatinib treatment for chronic myeloid leukemia. *N. Engl. J. Med.*, **376**(10): 917-927.

Kumar V, Jyotirmayee and Verma M (2023). Developing therapeutic approaches for chronic myeloid leukemia: a review. *Mol. Cell. Biochem.*, **478**(5): 1013-1029.

Ling VY, Straube J, Godfrey W, Haldar R, Janardhanan Y, Cooper L, Bruedigam C, Cooper E, Tavakoli Shirazi P, Jacquelin S, Tey SK, Baell J, Huang F, Jin J, Zhao Y, Bullinger L, Bywater MJ and Lane SW (2023). Targeting cell cycle and apoptosis to overcome chemotherapy resistance in acute myeloid leukemia. *Leukemia.*, **37**(1): 143-153.

Litwińska Z and Machaliński B (2017). miRNAs in chronic myeloid leukemia: Small molecules, essential function. *Leuk. Lymphoma.*, **58**(6): 1297-1305.

Liu Y, Shao Z, Liao Y, Xia X, Huang C, He J, Hu T, Yu C, Jiang L, Liu J and Huang H (2020). Targeting SKP2/Bcr-Abl pathway with Diosmetin suppresses chronic myeloid leukemia proliferation. *Eur. J. Pharmacol.*, **883**: 173366.

Lu T, Cao J, Zou F, Li X, Wang A, Wang W, Liang H, Liu Q, Hu C, Chen C, Hu Z, Wang W, Li L, Ge J, Shen Y, Ren T, Liu J, Xia R and Liu Q (2021). Discovery of a highly potent kinase inhibitor capable of overcoming multiple imatinib-resistant ABL mutants for chronic myeloid leukemia (CML). *Eur. J. Pharmacol.*, **897**: 173944.

Menon A, Abd-Aziz N, Khalid K, Poh CL and Naidu R (2022). miRNA: A promising therapeutic target in cancer. *Int. J. Mol. Sci.*, **23**(19):11502.

Narlı Özdemir Z, Kılıçaslan NA, Yılmaz M and Eşkazan AE (2023). Guidelines for the treatment of chronic myeloid leukemia from the NCCN and ELN: differences and similarities. *Int. J. Hematol.*, **117**(1): 3-15.

Pinto CA, DE Sousa Portilho AJ, Barbosa MC, DE Moraes MEA, DE Lemos JAR, Burbano RMR and

Moreira-Nunes CA (2021). Combined therapy of ATRA and imatinib mesylate decreases BCR-ABL and ABCB1/MDR1 expression through cellular differentiation in a chronic myeloid leukemia model. *In. Vivo.*, **35**(5): 2661-2667.

Scherr M, Kirchhoff H, Battmer K, Wohlan K, Lee CW, Ricke-Hoch M, Erschow S, Law E;Kloos A, Heuser M, Ganser A, Hilfiker-Kleiner D, Heidenreich O and Eder M (2019). Optimized induction of mitochondrial apoptosis for chemotherapy-free treatment of BCR-ABL+acute lymphoblastic leukemia. *Leukemia.*, **33**(6): 1313-1323.

Shabashvili DE, Feng Y, Kaur P, Venugopal K and Guryanova OA (2022). Combination strategies to promote sensitivity to cytarabine-induced replication stress in acute myeloid leukemia with and without DNMT3A mutations. *Exp. Hematol.*, **110**: 20-27.

Soverini S, Bassan R and Lion T (2019). Treatment and monitoring of Philadelphia chromosome-positive leukemia patients: Recent advances and remaining challenges. *J. Hematol. Oncol.*, **12**(1): 39.

Sun S, Wang Y, Zhou R, Deng Z, Han Y, Han X, Tao W, Yang Z, Shi C, Hong D, Li J, Shi D and Zhang Z (2017). Targeting and regulating of an oncogene via nanovector delivery of micro RNA using patient-derived Xenografts. *Theranostics.*, **7**(3): 677-693.

Tadesse F, Asres G, Abubeker A, Gebremedhin A and Radich J (2021). Spectrum of BCR-ABL mutations and treatment outcomes in ethiopian imatinib-resistant patients with chronic myeloid leukemia. *JCO. Glob. Oncol.*, **7**: 1187-1193.

Unnisa A, Greig NH and Kamal MA (2023). Inhibition of caspase 3 and caspase 9 mediated apoptosis: A multimodal therapeutic target in traumatic brain injury. *Curr. Neuropharmacol.*, **21**(4): 1001-1012.

Van Opdenbosch N and Lamkanfi M (2019). Caspases in cell death, inflammation, and disease. *Immunity.*, **50**(6): 1352-1364.

Wang F, Wang J, Chong Z and Mu Y (2019). A modified DAW-22 compound F-B1 inhibits Bcr/Abl and induces apoptosis in chronic myelogenous leukemia cells. *Anticancer. Drugs*, **30**(2): 159-166.

Wang J, Ma HL, Liu WR, Peng Y, Zhou JK and Yang JL (2021). CircBA1 derived from BCR-ABL fusion gene inhibits cell proliferation in chronic myeloid leukemia. *Cancer. Commun. (Lond).*, **41**(1): 79-82.

Wang T, Wen T, Li H, Han B, Hao S, Wang C, Ma Q, Meng J, Liu J and Xu H (2019). Arsenic sulfide nanoformulation induces erythroid differentiation in chronic myeloid leukemia cells through degradation of BCR-ABL. *Int. J. Nanomedicine*, **14**: 5581-5594.

Wu J, Zhang Y, Li X, Ren J, Chen L, Chen J and Cao Y (2022). Exosomes from bone marrow mesenchymal stem cells decrease chemosensitivity of acute myeloid leukemia cells via delivering miR-10a. Biochem. *Biophys. Res. Commun.*, **622**: 149-156.

Younes S, Ismail MA, Al-Jurf R, Ziyada A, Nasrallah GK, Abdulrouf PV, Nagy M, Zayed H, Farrell T, Sorio C, Morsi H, Qoronfleh MW and Al-Dewik NI (2023). Management of chronic myeloid leukaemia: Current treatment options, challenges, and future strategies. *Hematology*, **28**(1): 2196866.

Zhang B, Sun YF, Zhang XM, Jiang N and Chen Q (2020). TUG1 weakens the sensitivity of acute myeloid leukemia cells to cytarabine by regulating miR-655-3p/CCND1 axis. *Eur. Rev. Med. Pharmacol. Sci.*, **24**(9): 4940-4953.

Zhang H, Liu K, Xue Z, Yin H, Dong H, Jin W, Shi X, Wang H and Wang H (2018). High-voltage pulsed electric field plus photodynamic therapy kills breast cancer cells by triggering apoptosis. *Am. J. Transl. Res.*, **10**(2): 334-351.

Zhang H, Song T, Wang Z, Laura Bonnette U, Guo Y, Wang H, Gao Q and Zhang Z (2022). Bcr-Abl drives the formation of Hsp70/Bim PPI to stabilize oncogenic clients and prevent cells from undergoing apoptosis. *Biochem. Pharmacol.*, **198**: 114964.

Zheng Z, Rong G, Li G, Ren F and Ma Y (2019). Diagnostic and prognostic significance of serum miR-203 in patients with acute myeloid leukemia. *Int. J. Clin. Exp. Pathol.*, **12**(5): 1548-1556.