# DOPAMINE ACTIVATES ACUTE MYELOID LEUKAEMIA NLRP3 INFLAMMASOME THROUGH THE DR5 RECEPTOR PATHWAY TO PROMOTE CANCER AND INDUCE CHEMOTHERAPY RESISTANCE

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***ABSTRACT***

***Objective:*** *To explore the role and mechanism of dopamine (DA)-activating acute myeloid leukaemia (AML) NOD-like receptor family pyrin domain (NLRP3) inflammasomes on tumour progression and chemotherapy resistance through the dopamine receptor 5 (DR5) pathway.*

***Methods:*** *AML cell line U937 was divided into a control group, DA group, SKF82958 group and chlorprothixene group. The control group was treated with 1μL/mL DMSO, the DA group was treated with 50μmol/L DA, the SKF82958 group was treated with DR1/5 agonist SKF82958 and the chlorprothixene group was treated with 50 μmol/L DA. Moreover, 30 minutes before DA treatment, 36 nmol/L of the DR1/5 antagonist chlorprothixene was used for intervention. Proliferation and cleaved caspase-1, IL-1β protein expression levels and IL-1β levels were compared at 24, 48 and 72 h in each group. Then, 30μg/L Adriamycin (ADR) was added to each group for 48 h, and the IC50 values of ADR were compared.*

***Results:*** *At 24, 48 and 72 h, the proliferation OD values of U937 cells in the DA group and SKF82958 group were significantly higher than those in the control group. The proliferation OD values of U937 cells in the chlorprothixene group were significantly lower than those in the DA group (P<0.05). Expression levels of NLRP3, cleaved caspase-1 and cleaved IL-1β protein in U937 cells in the DA and SKF82958 groups were significantly higher than those in the control group (P<0.05). The expression levels of NLRP3, cleaved caspase-1 and cleaved IL-1β in U937 cells in the chlorprothixene group were significantly lower than those in the DA group (P<0.05). The levels of IL-1β in U937 cells in the DA and SKF82958 groups were significantly higher than those in the control group (P<0.05). The IL-1β level of U937 cells in the chlorprothixene group was significantly lower than that in the DA group (P<0.05). The ADR values of U937 cells in the DA and SKF82958 groups were significantly higher than those in the control group (P<0.05). The ADR and Ara-C IC50 values of U937 cells in the chlorprothixene group were significantly lower than those in the DA group (P<0.05).*

***Conclusion:*** *DA can activate the AML NLRP3 inflammasome through the DR5 receptor pathway to promote cancer and induce chemotherapy resistance.*

***Keywords:*** *DA, DR5, AML, NLRP3.*

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## Introduction

Chemotherapy and haematopoietic stem cell transplantation are the primary means of clinical treatment for acute myeloid leukaemia (AML) and prolong the survival of patients to a certain extent. However, chemotherapy has high toxicity and numerous side effects, with especially poor tolerance among elderly patients; thus, its therapeutic effect is not ideal[1, 2]. Current studies on the pathogenesis of AML have mainly focused on gene mutations and related signalling pathways, while other reports have shown that inflammatory immune responses were involved in the pathogenesis of AML[3]. Under the stimulation of infections and tumours, the body's inflammatory response was defensive. However, a long-term chronic inflammatory state could induce tumour formation[4]. The mechanism of tumorigenesis induced by inflammatory response is extremely complex and involves gene instability

of inflammatory tissues[5]. The Nod-like receptor domain-containing protein (NLRP3) family pyrin domain is the most thoroughly studied inflammasome, whose overactivation could accelerate advanced tumour progression[6, 7]. Dopamine (DA), a type of catecholamine, serves an important role in the regulation of human immunity[8]. Clinical studies have confirmed that the immunomodulatory effect of DA mainly occurs through its receptors. Notably, dopamine receptor 5 (DR5) could increase the expression of cyclic adenosine monophosphate (cAMP) and activate protein kinase A (PKA)[9].

Moreover, DA can significantly block the growth and metastasis of many types of malignant tumours[10]. However, the roles and mechanisms of DA, its receptor DR5 and the NLRP3 inflammasome in AML have not been reported. Therefore, this study aimed to explore the role and mechanism of DA in activating the AML NLRP3 inflammasome through the DR5 receptor pathway in tumour progression and chemotherapy resistance.

## Methods

### *Experimental materials*

AML cell line U937 was purchased from Wuxi NewGain Biotechnology Co., LTD.

### *Main reagents and instruments*

*Reagents*

NLRP3 antibody was purchased from Wuhan Fine Biotech Co., Ltd.; Cleaved caspase-1 antibody was purchased from AmyJet Scientific Inc.; Cleaved IL-1β antibody was purchased from Shanghai Xinyu Biotech Co., LTD.; RPMI-1640 medium was purchased from Wuhan Churuike Pharmaceutical Technology Co., LTD.; SKF82958 was purchased from Beijing Mairuibo Biotechnology Co., LTD.; Chlorprothixene was purchased from TargetMol China.

*Instruments*

A $CO_2$ incubator was purchased from Shanghai Tusen Vision Technology Co., LTD.; A multifunctional microplate reader was purchased from Shenzhen Enke Biotechnology Co., LTD.; A chemiluminescence gel imager was purchased from Alite Life Science Co., LTD.; A low-temperature high-speed centrifuge was purchased from Shanghai Tusen Vision Technology Co., LTD.; A clean bench was purchased from Shanghai Fuze Trading Co., LTD.

### *Methodology*

• The U937 cell line was suspended for culture on RPMI-1640 complete medium (10% FBS+1 U/mL penicillin +1 μg/mL streptomycin) and incubated in a constant temperature cell culture box. After growing to the logarithmic growth stage, they were passed on and finally frozen in liquid nitrogen tanks.

• The U937 cell line was taken and its concentration was adjusted to 5×105/mL; 2 mL of cell suspension was then implanted into a six-well plate. The cells were divided into the control group, DA group, SKF82958 group and chlorprothixene group. DMSO was added to the control group and 50 μmol/L DA was applied to the DA group. The SKF82958 group was treated with the DR1/5 agonist SKF82958, while the chlorprothixene group was initially treated with 50 μmol/L DA and then treated with 36 nmol/L of the DR1/5 antagonist chlorprothixene 30 minutes before DA treatment.

• The proliferation capacity of each group was detected using the CCK-8 method at 24, 48 and 72 h after treatment. Then, 100 μL of cell suspension was implanted into 96-well plates. Overall, three multiple wells were set in each group and 10 μL of CCK-8 reagent was added. The OD value of each group was detected after 3 h.

• NLRP3, cleaved caspase-1 and interleukin-1β (IL-1β) protein expression levels were detected by western blot 48 h after treatment. U937 cells were collected for lysis and centrifugation, and the supernatant was taken. Protein concentration was detected by a BCA protein quantitative kit. After electrophoresis, the U937 cells were transferred to a PVDF membrane sealed with 5% skim milk powder and incubated with primary antibodies. They were then incubated with secondary antibodies for colour processing and observed under a microscope.

• IL-1β levels were detected by ELISA. The supernatant of each group and 200 μL of the standard with adjusted concentration were placed in each well and incubated naturally at room temperature for 2 hours. After the supernatant was discarded, 200 μL of IL-1β conjugate was added and incubated naturally at room temperature for 1 hour. Then, 400 μL of washing solution was added to each well. Pre-configured A+B substrate-binding solution was then added and incubated for 20 min at room temperature. Thereafter, 50 μL of stop solution was added for termination. Then, the OD value of each group was measured at 450 nm using a microplate reader.

• 30 μg/L Adriamycin (ADR) was added to each well in each group and incubated in a 24-well

plate for 48 h. The OD value was detected by CCK-8 and the IC50 value was also calculated.

***Statistical methods***

Measurement data with a normal distribution, such as the OD values of U937 cell proliferation in each group, were expressed by ($\bar{x}\pm s$). One-way ANOVA was used for comparison between multiple groups. The SNK-q test was used for pair comparison. Also, SPSS 23.0 was used for data analysis. P<0.05 was considered a significant difference.

## Results

***Comparison of the proliferation ability of U937 cells in each group***

At 24, 48 and 72 h, the OD values of U937 cells in the DA and SKF82958 groups were significantly higher than those in the control group. The OD value for U937 cell proliferation in the chlorprothixene group was significantly lower than that in the DA group (P<0.05) (see Table 1 and Figure 1).

| Group | 24 h | 48 h | 72 h |
|---|---|---|---|
| Control | 0.63±0.01 | 0.74±0.05 | 1.15±0.09 |
| DA | 1.29±0.03[a] | 1.25±0.09[a] | 1.43±0.04[a] |
| SKF82958 | 0.83±0.05[a] | 1.19±0.06[a] | 1.34±0.04[a] |
| Chlorprothixene | 0.73±0.02[b] | 0.85±0.05[b] | 1.09±0.01[b] |
| *F* | 1308.720 | 225.720 | 133.460 |
| *P* | <0.001 | <0.001 | <0.001 |

**Table 1:** Comparison of the OD values for U937 cell proliferation in each group ($\bar{x}\pm s$).
*Note: [a]compared with the control group at the same time, P<0.05; [b]compared with the DA group at the same time, P<0.05.*

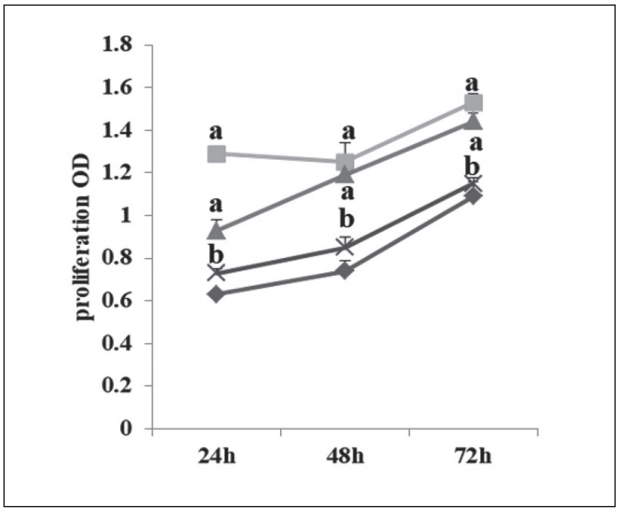


**Figure 1:** Comparison of the OD values of U937 cell proliferation in each group.
*Note: [a]compared with the control group at the same time, P<0.05; [b]compared with the DA group at the same time, P<0.05.*

***Comparison of NLRP3, cleaved caspase-1 and cleaved IL-1β protein expression levels in U937 cells in each group***

Expression levels of NLRP3, cleaved caspase-1, and cleaved IL-1β protein in U937 cells in the DA group and SKF82958 group were significantly higher than those in the control group (P<0.05). The expression levels of NLRP3, cleaved caspase-1, and cleaved IL-1β in U937 cells in the chlorprothixene group were significantly lower than those in the DA group (P<0.05) (see Figure 2).

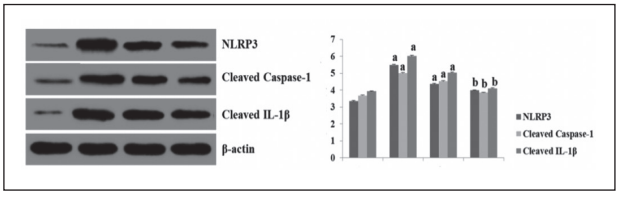


**Figure 2:** Comparison of NLRP3, cleaved caspase-1 and cleaved IL-1β protein expression levels in the U937 cells of each group.
*Note: [a]compared with the control group, P<0.05; [b]compared with the DA group, P<0.05.*

***Comparison of IL-1β levels in the U937 cells of each group***

The levels of IL-1β in U937 cells of the DA and SKF82958 groups were significantly higher than that in the control group (P<0.05). The IL-1β level of U937 cells in the chlorprothixene group was significantly lower than that in the DA group (P<0.05) (see Table 2).

| Group | IL-1β (pg/mL) |
|---|---|
| Control | 3.52±0.91 |
| DA | 10.26±0.69[a] |
| SKF82958 | 12.76±1.32[a] |
| Chlorprothixene | 1.23±0.68[b] |
| *F* | 508.380 |
| *P* | <0.001 |

**Table 2:** Comparison of the IL-1β levels of U937 cells in each group ($\bar{x}\pm s$).
*Note: [a]compared with the control group, P<0.05; [b]compared with the DA group, P<0.05.*

***Analysis of the chemotherapy resistance of U937 cells in each group***

The ADR IC50 values of U937 cells in the DA and SKF82958 groups were significantly higher than those in the control group (P<0.05). The ADR IC50 values of U937 cells in the chlorprothixene group were significantly lower than those in the DA group (P<0.05) (see Table 3).

| Group | ADR IC50 value (μg/L) |
|---|---|
| Control | 125.13±9.23 |
| DA | 154.17±15.62[a] |
| SKF82958 | 149.88±11.33[a] |
| Chlorprothixene | 132.05±4.43[b] |
| *F* | 569.53 |
| *P* | <0.001 |

**Table 3:** Comparison of the IC50 values of U937 cells in each group ($\bar{x}\pm s$).
*Note: [a]compared with the control group, P<0.05; [b]compared with the DA group, P<0.05.*

## Discussion

The survival of approximately 40% of non-elderly AML patients could be extended to 5 years after treatment, while the remaining elderly patients have a very poor clinical prognosis and lack an active and effective treatment. Therefore, targeted therapy has become the focus and hotspot in the treatment of AML patients.

Current reports on AML involved fusion genes, signalling pathways, the bone marrow microenvironment, etc.[11]. Hussain et al.[12] showed that infection and persistent inflammatory response can lead to the initiation of many tumours. In particular, the inflammatory response can play important roles in tumour vascular formation and invasion. NLRP3 gene polymorphism was widely found in healthy people, and its changes can lead to the abnormal activation of NLRP3, which results in increased IL-1β and IL-18 content[13]. Goldberg et al.[14] stimulated the activation of NLRP3 inflammasome in mesotheliocytes through asbestos and then induced inflammatory responses, which ultimately led to tumour initiation and progression. Additionally, Wang et al.[15] induced the activation of the NLRP3 inflammasome in lung adenocarcinoma cell line A549 and found that the expression levels of IL-1β and IL-18 significantly increased, which was conducive to enhancing the proliferation, migration and invasion ability of A549 cells. Furthermore, Kristin[16] found that both doxorubicin and erythromycin can promote the expression of IL-1β precursor protein and stimulate the release of mature IL-1β in macrophages treated with lipopolysaccharide. Moreover, IL-1β release was blocked when macrophages were deficient in apoptosis-related speck-like protein, caspase-1, NLRP3, etc., suggesting that activation of the NLRP3 inflammasome pathway is closely related to IL-1β release. Oral administration of DA receptor antagonists to psychiatric patients could significantly reduce the risk of colon cancer and prostate cancer, indicating that DA receptors and pathways may induce tumour cell proliferation[17]. Colombo et al.[18] found that in the peripheral blood mononuclear cells of healthy people, low DA concentrations can block the ability of cell apoptosis; conversely, this can enhance the ability of apoptosison. Simultaneously, the ER1 receptor antagonist SCH23390 can block DA inhibition. The expression of the DA receptor was not found in the normal cord blood, red blood cells and macrophages of AML patients.

Instead, it was found on the surface of monocytes, while the overexpression of DA receptors was closely related to the poor clinical prognosis of AML patients. Chlorpromazine and other DA receptor antagonists can significantly reduce the number of leukaemia cells in DA-positive AML patients. Moreover, it can induce the generation of protocell colonies and decrease the value of cytarabine EC50 in these patients. In this study, the proliferation ability of U937 AML cells was detected via the CCK-8 method, and it was found that both DA and the DR1/5 agonist SKF82958 could induce the proliferation of U937 cells, while adding DR1/5 antagonist before DA treatment significantly blocked the ability of DA to promote cell proliferation. These results indicate that DA can induce the proliferation of DR1/5 chlorprothixene antagonist U937 cells through the DR1/5 receptor pathway. We used western blot to detect the expression levels of inflammasome associated proteins in U937 cells and found that DA and SKF82958 significantly increased the expression levels of NLRP3, cleaved caspase-1 and cleaved IL-1β, while the level of IL-1β in supernatant also increased. Notably, chlorprothixene could promote the expression of these indicators. These results suggest that DA activates AML NLRP3 inflammasome through the DR5 receptor pathway. Additionally, we also found that both DA and SKF82958 could induce chemotherapeutic resistance in U937 cells, while chlorprothixene could block the generation of chemotherapeutic resistance in U937 cells. This suggests that DA could induce chemotherapeutic resistance in U937 cells through the DR1/5 receptor pathway.

In conclusion, DA can activate the AML NLRP3 inflammasome through the DR5 receptor pathway, thereby promoting cancer and inducing chemotherapy resistance.

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