Journal of Hainan Medical University

http://www.apjtm.net/JHMU/index.htm

Regulatory effect of parthenolide on proliferation and cell cycle of leukemia stem cell as well as study of its possible molecular mechanism

ZHANG Zhi−fu; TANG Li−bin; LIU Qian−ping; SONG Pei−yan; NING Yu

The Sixth Department of Internal Medicine, People's Hospital of Huadu District, Guzngzhou City, 510800, China

ARTICLE INFO

Article history:
Received
Received in revised form
Accepted
Available online

Keywords:
Leukemia stem cell
Parthenolide
Proliferation
Cell cycle
Signal pathway

ABSTRACT

Objective: To study the regulatory effect of parthenolide on proliferation and cell cycle of leukemia stem cell and its possible molecular mechanism. **Methods:** Leukemia stem cells were cultured and processed with different concentrations of parthenolide. Then cell viability, cell cycle and mRNA contents of related genes were detected. **Results:** (1) Cell viability and cycle: Parthenolide could decrease MTT values of leukemia stem cells in a dose dependent manner; 20μmol/L parthenolide could increase G2/M phase ratio and decrease G0/G1 phase and S phase ratios; (2) Proliferation and cell cycle regulating genes: Parthenolide could decrease mRNA contents of c-myc, bcl-2, cyclinA1 and cyclinE in a dose dependent manner; (3) Signal pathway: Parthenolide could decrease mRNA contents of SDF-1 and CXCR4 in a dose dependent manner. **Conclusion:** Parthenolide can inhibit leukemia stem cell proliferation and make cell cycle stagnated in G2 phase; possible molecular mechanism is inhibiting c-myc, bcl-2, cyclinA1 and cyclinE expressions and SDF-1-CXCR4 signal pathway.o

1. Introduction

Leukemia is a common disease of Hematology. It is a malignantly amplified monoclonal and heterogeneous disorder of hematopoietic cells at some stage during the course of normal development. Regular chemotherapy is mainly adopted for clinical treatment. However, chemotherapeutic drugs can only temporarily ease patients' condition. The ultimate outcome of the disease is still drug resistance and relapse. The effect is not ideal. Studies in recent years have shown that leukemic stem cell (LSC) is the most essential factor that causes the occurrence, development, drug resistance and relapse of leukemia. An ideal method for the treatment of leukemia should aim to eliminate LSC[1]. Parthenolide (PTL) has anti-tumor effect. Its killing effect on cancer cells have been confirmed in many malignant tumors. In the following research, the regulatory effect of parthenolide on proliferation and cell cycle of leukemia stem cell and its possible molecular mechanism were analyzed.

*Corresponding author: ZHANG Zhi-fu
Tel: 13760805284 Email: 3234658410@qq.com
Address: The Sixth Department of Internal Medicine, People's Hospital of Huadu District, Guangzhou City, No.48 Xinhua Road, Huadu District, Guangzhou City, 510800
Hospital: People's Hospital of Huadu District, Guangzhou City
Department: The Sixth Department of Internal Medicine

1. Materials and methods

1.1 Materials

Cell culturing materials were provided by Nest Company; cell culturing reagents were provided by Sigma Company; MTT detection reagents were provided by Promega Company; mRNA detection reagents were provided by Takara Company.

1.2 Methods

1.2.1 Cell culturing and processing methods

Peripheral blood of leukemia patients was drawn. Leukemia stem cells were sorted, regularly cultured and divided into control group and PTL group. Control group was processed with medium that didn't contain serum or drugs; PTL group was processed with 5μg/L, 10μg/L, 15μg/L and 20μg/L parthenolide respectively.

1.2.2 Cell viability and cycle detecting methods

After drug processing for 24h, MTT detection reagents were directly added in cell pores. After incubating for 3-4h, absorbance

at 490nm was read and used as cell viability; after processing was complete, cells were trypsin-digested, centrifuged, re-suspended with PBS that contained serum, and then fixed with 70% ethanol for 12h; then cells were washed with PBS twice and propidium iodide stained. FCM was used to detect percentages of cells in different cycles.

1.2.3 Gene mRNA content detecting methods

Cells were collected and Trizol lysate was added; mRNA was extracted from RT to cDNA; fluorescence quantitative PCR was carried out to amplify c-myc, bcl-2, cyclinA1, cyclinE, SDF-1 and CXCR4 respectively and mRNA contents were calculated.

1.2.4 Statistical methods

Detected data was input by SPSS18.0 software, multiple group comparison for variance analysis and pair wise comparison for LSD. Differences were considered to be significant at a level of P< 0.05.

2. Results

2.1 Cell viability and cycle

Different concentrations of PTL were used to process leukemia stem cells. After 24h, cell viability and cycle were detected. Variance analysis of detected results of cell viability and cycle showed that: (1) PTL could decrease MTT values of leukemia stem cells in a dose dependent manner; (2) 20μmol/L PTL could increase G2/M phase ratio and decrease G0/G1 phase and S phase ratios of leukemia stem cells.

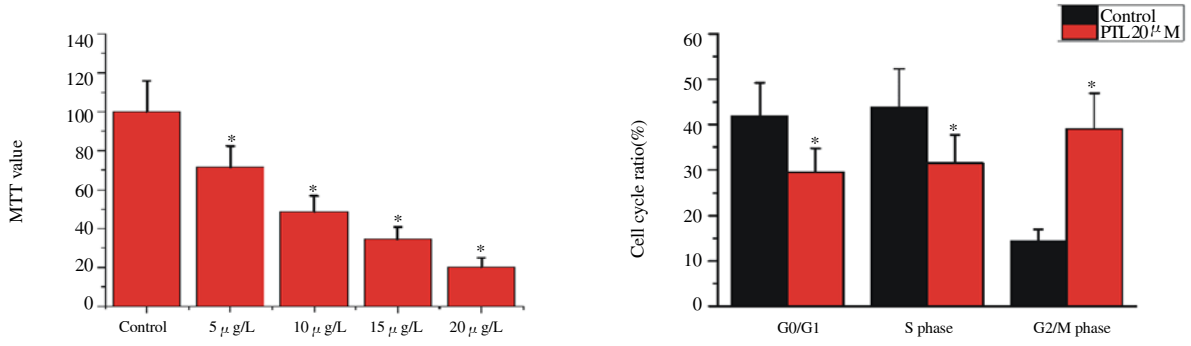
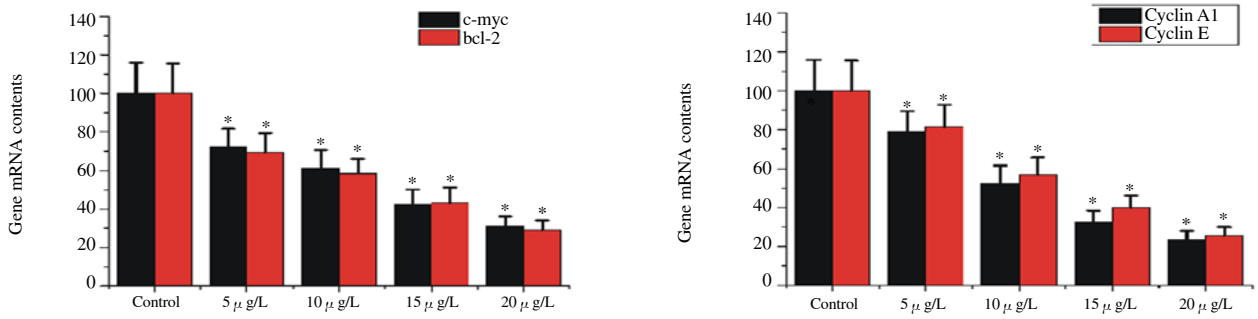


Figure 1. Effect of PTL processing on cell viability and cycle. (Left Fig.): PTL could decrease MTT values in a dose dependent manner; (Right Fig.): 20μmol/L PTL could increase G2/M phase ratio and decrease G0/G1 phase and S phase ratios. *: compared with control group, there were differences, $P<0.05$.

2.2 Proliferation and cell cycle regulating genes

Proliferation regulating genes c-myc and bcl-2 participate in cell viability regulation and cell cycle regulating genes cyclinA1 and cyclinE participate in cell cycle regulation. After processing leukemia stem cells with different concentrations of PTL, fluorescence quantitative PCR was used to detect mRNA contents of proliferation regulating genes and cell cycle regulating genes. Variance analysis of cell viability and cycle showed that PTL could decrease mRNA contents of c-myc, bcl-2, cyclinA1 and cyclinE in a dose dependent manner. Differences had statistical significance ($P<0.05$).



Figrue 2. Effect of PTL processing on mRNA contents of proliferation regulating genes and cell cycle regulating genes. (Left Fig.): PTL could decrease mRNA contents of c-myc and bcl-2 in a dose dependent manner; (Right Fig.): PTL could decrease mRNA contents of cyclinA1 and cyclinE in a dose dependent manner. *: compared with control group, there were differences, $P<0.05$.

2.3 SDF-1-CXCR4 signal pathway

SDF-1-CXCR4 pathway is an important factor that regulates the microenvironment of hematopoietic stem cells. After processing leukemia stem cells with different concentrations of PTL, fluorescence quantitative PCR was used to detect mRNA contents of SDF-1 and CXCR4. Variance analysis of cell viability and cycle showed that PTL could decrease mRNA contents of SDF-1 and CXCR4 in a dose dependent manner. Differences had statistical significance ($P<0.05$).

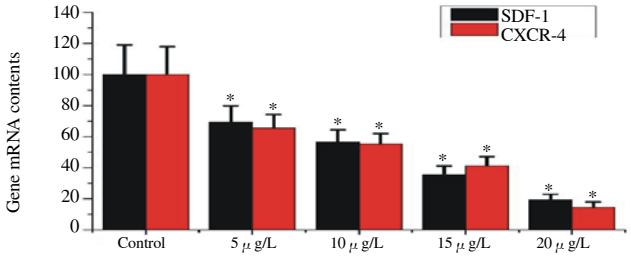


Figure 3. Regulatory effect of PTL processing on SDF-1-CXCR4 pathway. PTL could decrease mRNA contents of SDF-1 and CXCR4 in a dose dependent manner. *: compared with control group, there were differences, $P<0.05$.

3. Discussions

Leukemia is the most common malignant tumor of the blood system. Its relapse and drug resistance have been treatment challenges that trouble clinicians. Studies of domestic and foreign scholars about leukemia in recent years have shown that existence of leukemia stem cells (LSCs) is an important factor that causes the occurrence and development of leukemia and it is also the main cause of drug resistance and relapse of leukemia[2]. LSCs have many biological characteristics that are similar to those of normal hematopoietic stem cells, including self renewal, multi-directional differentiation, and infinite multiplication. LSCs that are in quiescent phase (G0 phase) can express ATP related transporters and have stronger tolerance to apoptotic stimuli. These biological characteristics enable LSCs with strong survival ability that can withstand intense chemotherapeutic drugs and continue to survive, thus becoming the root cause of drug resistance and relapse of leukemia. Therefore, LSC has to be cleared before it is possible to achieve the goal of curing leukemia [3]. Parthenolide (PTL) is the main active ingredient in medicine wild chamomile. It is a type of sesquiterpene lactones. Recent studies have shown that PTL has strong and broad anti-tumor activity. It can induce apoptosis of colon cancer, liver cancer, leukemia and other malignant tumors and increase sensitivity of cancer cells to other anti-cancer drugs[4-5]. Studies in recent years have shown that PTL has strong apoptosis-inducing effect on acute myeloid leukemic cells and in particular, can selectively kill leukemia stem/progenitor cells and have less impact on normal hematopoietic stem/ progenitor cells[6].

In the research, leukemia stem cells were isolated cultured and processed with PTL. Then cell proliferation condition was detected. MTT can be combined with live cells and form purple crystal while it does not react with apoptotic cells. The staining feature of MTT can accurately reflect cell viability; FCM can mark the cell surface markers to accurately reflect cell cycle. Analysis results of the research showed that PTL processing could decrease MTT values in a dose dependent manner and 20μm PTL could increase G2/M phase ratio and decrease G0/G1 phase and S phase cell ratios, which indicated that PTL could reduce leukemia stem cell viability and make cell cycle stagnated in G2 phase. Cell viability and cell cycle are regulated by proliferation regulating genes c-myc and bcl-2 as well as cell cycle regulating genes cyclinA1 and cyclinE[7]. Cyclin-dependent kinase (CDK) is the central link in the regulation of cell cycle. CyclinA1 regulates S phase through forming complex with CKD1; cyclinE regulates G1 phase through forming complex with CKD2[8]. C-myc is the crossroad of multiple signal pathways and it is also a key factor that regulates properties of stem cells[9-10]. Bcl-2 is a type of anti-apoptosis proteins of mitochondrial pathway and can play the anti-apoptosis role through inhibiting cyto C and Smac activation[11]. The research analyzed mRNA contents of related genes and found out that PTL could decrease mRNA contents of c-myc, bcl-2, cyclinA1 and cyclinE in a dose dependent manner, which indicated that PTL could inhibit expressions of proliferation regulating genes and cell cycle regulating genes.

Studies in recent years have found out that microenvironment of hematopoietic stem cells is the necessary factor that maintains cell self-renewal, multi-directional differentiation, and infinite multiplication. Foreign scholars use the word “niche” in ecology to represent microenvironment[12]. Osteoblast niche and tube sinus niche compose of the microenvironment of hematopoietic stem cells together. In the process of leukemia, microenvironment of leukemia cells changes. Hematopoietic stem cells are in a sick hematopoietic microenvironment. Leukemia stem cells can interact with bone marrow stromal cells and maintain the cells in physical barrier of tumor microenvironment, thus possessing the capability of apoptotic factor tolerance[13]. Studies have shown that SDF-1 and its receptor CXCR4 are important pathways in regulating hematopoietic microenvironment. SDF-1 mediated CXCR4 signal pathway has important regulatory effect on homing and migration of leukemia stem cells[14]. Stromal cell derived factor 1 (SDF-1) is a type of cytokines with chemotaxis function and it is an important component of bone marrow hematopoietic microenvironment; chemokine receptor of its ligand. CXCR4 is a type of G protein-coupled receptors, mainly expressed on hematopoietic stem cell surface[15]. Signal pathway formed by SDF-1-CXCR4 has the effect of promoting blood tumor cell migration and invasion. The research analyzed mRNA contents of related molecules in SDF-1-CXCR4 signal pathway and found out that parthenolide could

decrease mRNA contents of SDF-1 and CXCR4, which indicated that parthenolide could inhibit SDF-1-CXCR4 signal pathway.

In conclusion, parthenolide can inhibit leukemia stem cell proliferation and make cell cycle stagnated in G2 phase; possible molecular mechanism is inhibiting c-myc, bcl-2, cyclinA1 and cyclinE expressions and SDF-1-CXCR4 signal pathway.

References

[1] Zhou J, Chng WJ. Identification and targeting leukemia stem cells: The path to the cure for acute myeloid leukemia. *World J Stem Cells* 2014; **6**(4): 473-484.

[2] Zhao K, Yin LL, Zhao DM, Pan B, Chen W, Cao J, et al. IL1RAP as a surface marker for leukemia stem cells is related to clinical phase of chronic myeloidleukemia patients. *Int J Clin Exp Med* 2014; **7**(12): 4787-4798.

[3] Pollyea DA, Gutman JA, Gore L, Smith CA, Jordan CT. Targeting acute myeloid leukemia stem cells: a review and principles for the development of clinical trials. *Haematologica* 2014; **99**(8): 1277-1284.

[4] Liu ZP, Li YY, Gao B, Li J, Gao JP, Li P. Experimental study of parthenolide inducing autophagic death of hepatocellular carcinoma SMMC 7721 cells. *J Sichuan Univ* (Med Sci Edi) 2014; **45**(5): 587-590.

[5] Kim SL, Liu YC, Park YR, Seo SY, Kim SH, Kim IH, et al. Parthenolide enhances sensitivity of colorectal cancer cells to TRAIL by inducing death receptor 5 and promotes TRAIL-induced apoptosis. *Int J Oncol* 2015; **46**(3): 1121-1130.

[6] Liao K, Xia B, Zhuang QY, Hou MJ, Zhang YJ, Luo B, et al. Parthenolide inhibits cancer stem-like side population of nasopharyngeal carcinoma cells via suppression of the NF- κ B/COX-2 pathway. *Theranostics* 2015; **5**(3): 302-321.

[7] Liao Y, Ling J, Zhang G, Liu F, Tao S, Han Z, et al. Cordycepin induces cell cycle arrest and apoptosis by inducing DNA damage and up-regulation of p53 in Leukemia cells. *Cell Cycle* 2015; **14**(5): 761-771.

[8] Liang CW, Gong WX, Qu YL, Huang CZ, Chen H. The expression of cell cycle regulatory factors in acute leukemia and their clinical significance. *Chongqing Med* 2011; **40**(18): 1775-1778.

[9] Li L, Osdal T, Ho Y, Chun S, McDonald T, Agarwal P, et al. SIRT1 activation by a c-MYC oncogenic network promotes the maintenance and drug resistance of human FLT3-ITD acute Myeloid Leukemia stem cells. *Cell Stem Cell* 2014; **15**(4): 431-446.

[10]Pan XN, Fang ZG, Long ZJ, Chen JJ, Liu LL, Fan RF, et al. Effects of up-regulation of c-myc expression on U937 cell line. *Chin J Pathophysiol* 2013; **29**(11): 1984-1989.

[11]Ni J, Xie X, Xie J, Hu XY, Huang ZQ, Xia RX. Role of BCL-2, Caspase-3 and NF-κB in astragaloside inducing apoptosis of human NB4 cells [J]. *J Exp Hematol* 2014; **22**(3): 703-706.

[12]Chomel JC, Aggoune D, Sorel N, Turhan AG. Chronic myeloid leukemia stem cells: cross-talk with the niche. *Med Sci* (Paris) 2014; **30**(4): 452-461.

[13]Rovida E, Peppicelli S, Bono S, Bianchini F, Tusa I, Cheloni G, et al. The metabolically-modulated stem cell niche: a dynamic scenario regulating cancer cell phenotype and resistance to therapy. *Cell Cycle* 2014; **13**(20): 3169-3175.

[14]Ge J, Hu Y, Gui Y, Hou R, Yang M, Zeng Q, et al. Chemotherapy-induced alteration of SDF-1/CXCR4 expression in bone marrow-derived mesenchymal stem cells from adolescents and young adults with acute lymphoblastic leukemia. *J Pediatr Hematol Oncol* 2014; **36**(8): 617-623.

[15]Lucansky V, Krmencikova-Fliegl M, Stanek L, Vonka V. Administration of a plasmid that expresses SDF-1 affects the oncogenic potential of mouse bcr-abl-transformed cells. *Mol Med Rep* 2014; **10**(4): 2116-2122.