Exp Oncol 2020
42, 3, 188–191

Experimental ONCOLOGY

OSTEOPONTIN siRNA DOES NOT CONFER RESISTANCE TO TOXIC EFFECTS OF PARTHENOLIDE IN JURKAT CELLS

S. Mehri[1], S. Mohammadi[2], M. Nikbakht[2], M. Sahmani[1], M. Zahedpanah[1,]*
[1]Qazvin University of Medical Sciences, Qazvin, 34197-59811, Iran
[2]Hematology, Oncology and Stem Cell Transplantation Research Center, Tehran University of Medical Sciences, Tehran 14155-6559, Iran

***Background*: Osteopontin (OPN) plays a critical role in cell proliferation and drug resistance in cancer treatment and hematological malignancies. In T cell acute lymphoblastic leukemia, most initial therapies can induce remission while some patients then relapse and do not respond well to chemotherapy. The sesquiterpene lactone parthenolide (PTL) can induce apoptosis in a variety of cancer cell lines via inhibition of pro-inflammatory transcription factor nuclear factor kappa B and has anti-tumor activity in acute lymphoblastic leukemia treatment. *Aim*: To study the role of OPN in conferring *in vitro* resistance to PTL in Jurkat cells. *Methods:* Jurkat cells were cultured with 8–20 μm PTL for 48 h. Transfection with OPN siRNA was provided. Apoptosis assays were performed with Annexin V-Alexa Fluor-488/PI. Quantitative real-time polymerase chain reaction was used to measure *OPN* gene expression using the $2\text{-}2^{-\Delta\Delta Ct}$ method. *Results:* PTL has cytotoxic and apoptotic effect on Jurkat cells with IC_{50} values of 16.1 μm, and growth inhibition effect of PTL does not differ significantly in combination with OPN-siRNA. *OPN* gene expression is not affected by PTL. *Conclusions:* Parthenolide induces apoptosis in Jurkat cells, but inhibition of osteopontin gene expression with siRNA does not reduce apoptotic effect of parthenolide.**

***Key Words*: Jurkat cells, osteopontin, parthenolide.**

DOI: 10.32471/exp-oncology.2312-8852.vol-42-no-3.15180

Osteopontin (OPN), also known as secreted phosphoprotein 1 is a 34kD multidomain secreted glycoprotein that functions as a soluble cytokine and an adhesive component of the extracellular matrix [1, 2]. It is a member of small integrin-binding ligand N-linked glycoproteins (siblings), a family of five integrin binding glycophosphoproteins [3]. OPN interacts mainly with various α_v (particularly $\alpha_v\beta_1$, $\alpha_v\beta_3$, $\alpha_v\beta_5$) integrins and with the CD44 splice variants. Due to these properties, OPN induces the activation of signal transduction pathways leading to cell proliferation, adhesion, invasion and migration. Hence, its role in cancer is important, as it is involved in angiogenesis, and metastasis [4–7]. OPN, also called early T cell activation gene 1 (*Eta-1*), is expressed after activation of T cells, macrophages and in multiple tissue types. OPN is cleaved by some proteases such as thrombin, cleaved product participating in various cellular functions. Jurkat cells originating from T cell acute lymphoblastic leukemia (T-ALL) have enhanced cell adhesion to thrombin-cleaved OPN compared with full-length OPN [8, 9].

Given that the human *OPN* gene is encoded by a single-copy gene on chromosome 4q21-q25 and has an alternative translation start site, there are two major variants of OPN, intracellular and extracellular [1]. It is also subjected to alternative splicing as well as post-translational modifications such as phosphorylation, glycosylation and proteolytic cleavage.

Submitted: September 30, 2019.

*Correspondence: E-mail: zhdpnh@yahoo.com

Abbreviation used: ALL – acute lymphocytic leukemia; DMSO – dimethyl sulfoxide; OPN – osteopontin; PI – propidium iodide; PCR – polymerase chain reaction; PTL – parthenolide; siRNA – short interfering RNA; T-ALL – T-cell acute lymphoblastic leukemia.

Parthenolide (PTL)-containing herbs are highly bioactive and have been traditionally used for treatment of inflammations. The anti-inflammatory activities of these herbs are partially due to their anti-NF-kB effects [10]. Currently PTL is used extensively in cancer treatment [11–14]. It can induce oxidative-stress-mediated apoptosis in T cells at high doses (up to 10 μm) [15].

T-ALL represents approximately 12% to 15% of all newly diagnosed acute lymphocytic leukemia (ALL) cases in pediatric patients and about 25% of adult ALL. This aggressive malignancy is associated with a significant risk of disease relapse and does not respond well to chemotherapy [16–18].

Herein, we attempted to study the role of OPN in conferring *in vitro* resistance to PTL in Jurkat cells and to examine the effect of knockdown of *OPN* gene expression via OPN-siRNA on PTL-treated Jurkat cell survival.

MATERIALS AND METHODS

Reagents. Parthenolide was purchased from Sigma-Aldrich (USA) and dissolved in dimethyl sulfoxide (DMSO) as a 50 mM stock solution, stored at –20 °C, and diluted in DMSO before use. The Annexin V-FITC/PI kit was purchased from BD Biosciences (USA). Tripure Isolation Reagent was purchased from Roche Applied Science (Germany). The cDNA synthesis kit and SYBR® Premix Ex Taq™ were purchased from Takara Biotechnology Co. (Otsu, Japan).

Cell culture. The human leukemic Jurkat cell line was obtained from the Iranian Biological Resource Center. RPMI 1640 supplemented with 10% fetal bovine serum medium (Invitrogen, USA) was used for culturing. The medium was supplemented with 2 mM L-glutamine, 100 units/ml penicillin, and 100 μg/

ml streptomycin. Cells were incubated at 37 °C in a humidified atmosphere containing 5% CO_2.

MTT assay. Cells were cultured in triplicate at 5 · 10^3/100 µl in 96-well culture plates (SPL Life sciences, Korea) with 8, 10, 12, 15, or 20 µm PTL for 48 h. Following the culture, the cells were incubated for 4 h with 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT, 5 mg dissolved in 1 ml of PBS, Sigma, USA). The plates were centrifuged (10 min at 350 g), and the purple formazan crystals of metabolized yellow tetrazolium salt by viable cells were dissolved in DMSO. The absorbance was quantified at 570 nm using the ELISA plate reader (Microplate Reader; Bio-Rad, USA). The results were expressed as a percentage of viability, with 100% representing control cells treated with 0.1% DMSO alone.

Annexin V/Propidium iodide (PI) assay. Cells were stained with Annexin V-Alexa Fluor-488/PI according to the manufacturer's instructions and examined by flow cytometry (Partec, Germany). Discrimination of cells was performed as apoptosis (Annexin V^+/PI^- [early apoptosis] and Annexin V^+/PI^+ [late apoptosis]).

Quantitative real-time polymerase chain reaction (qRT-PCR). Total RNA of the PTL-treated and untreated cells was extracted with Tripure Isolation Reagent according to the manufacturer's instructions, quantified on a NanoDrop ND-1000 (NanoDrop Technologies, USA), and stored at −80 °C. The cDNA synthesis kit was used to synthesize cDNA). A light cycler instrument (Roche Diagnostics, Germany) and SYBR Premix Ex Taq were used for quantitative real-time RT-PCR analysis. A final volume of 20 µl containing 2 µl of a two-fold diluted cDNA, 1 µl of 10 pmol primers (0.5 µl each forward and reverse primers), 10 µl of SYBER, and 7 µl of distilled water were used. Data were normalized to HPRT expression in each sample. Relative gene expression data were analyzed using the $2^{-\Delta\Delta Ct}$ method.

Short interfering RNA (siRNA) transfection. The siRNA against OPN was applied to cell lines using lipofectamin 2000 reagent (LF2000; 10 µg/ml; Invitrogen, USA) according to the manufacturer's instruction. Cells were lysed 24 h post-transfection and quantitative real-time PCR was performed using following sequences of OPN siRNA primers: 5′-ggaauauuacugugggaaadtdt-3′ (sense) and 5′-uuucccacaguaauauuccdtdt-3′ (antisense). The highest transfection efficiency was obtained in the experiments within 24 h post-transfection at a final concentration of 30 pmol/ml. This optimal condition was used for subsequent experiments tested with siRNA.

Statistical analysis Using IBM SPSS Statistics 19 software, the groups of data was presented as means ± SDs and compared by one-way analysis variance (ANOVA) or *t*-test.

RESULTS

Jurkat cells viability. Various concentrations of PTL were used to determine its toxic potency in Jurkat cells. The IC_{50} values at 48 h were estimated to be 16.1 µM (Fig. 1). 15 µM concentration was chosen for other assays.

OPN-siRNA does not influence significantly growth inhibition and apoptosis-inducing effects of PTL in Jurkat cells. The viability of OPN siRNA-treated cells with or without treatment with PTL was measured using MTT assay (48 h). OPN siRNA (30 pmol/ml) did not decrease cell viability much more than control. In addition, subsequent PTL treatment decreased the cell viability up to 51% that is without any difference in comparison of PTL alone (Fig. 2).

Apoptosis assay was performed as an additional testing to contribute in evaluating the OPN function in PTL-induced death. Annexin-V/PI staining indicated that PTL induced apoptosis. The average percentage of apoptosis was 70% within 48 h (Fig. 3). OPN siRNA transfection and subsequent treatment with PTL did not increase the apoptotic cells compared to the PTL alone in Jurkat cells (see Fig. 3).

OPN gene expression is not affected by PTL in Jurkat cells. The expression of *OPN* was evaluated by qRT-PCR. As illustrated in Fig. 4, *OPN* mRNA expression in cells treated with PTL does not differ significantly from that in PTL-treated cells transfected with *OPN* siRNA. Therefore, it appears that OPN could not

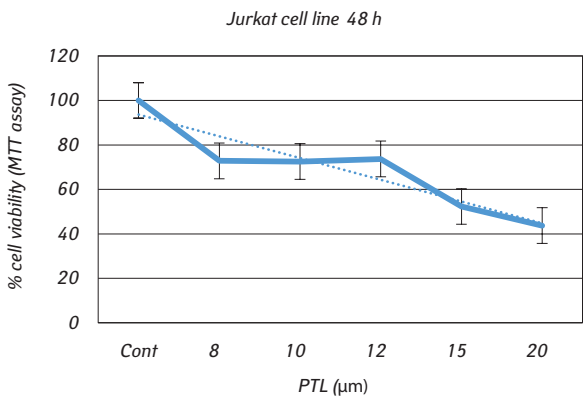

Fig. 1. Dose-response curves of Jurkat cell viability upon treatment with different PTL concentrations (MTT assay/48 h)

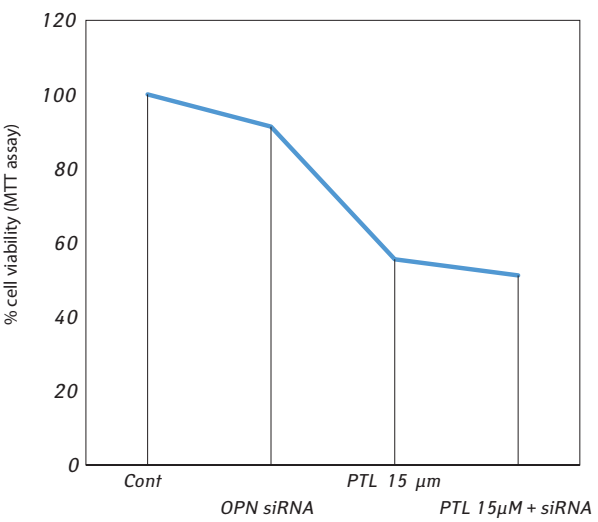

Fig. 2. OPN siRNA (30 pmol/ml) does not show significant decrease in cell viability of PTL (15 µM) treated Jurkat cells

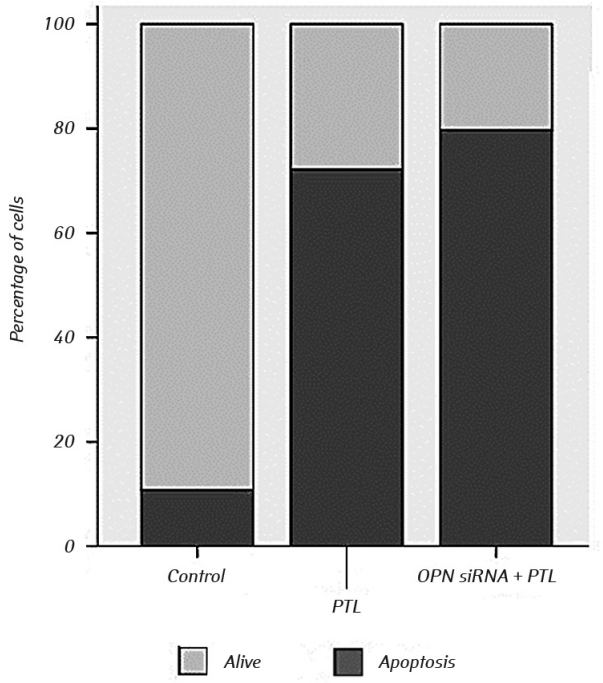

Fig. 3. Annexin-V/PI staining of PTL treated cells and cells incubated with OPN specific siRNA. The graphs represent three independent experiments

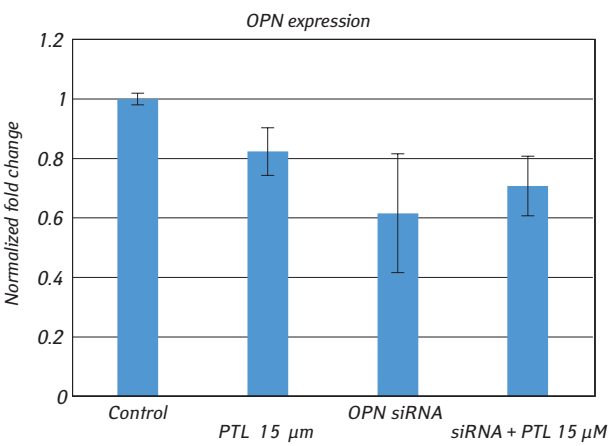

Fig. 4. *OPN* mRNA expression in cells treated with PTL and transfected with *OPN* siRNA. Three independent experiments were performed (mean ± SD)

be crucial for the survival of Jurkat cells in the setting of PTL treatment.

DISCUSSION

Regarding the role of OPN in chemotherapy resistance and leukemia treatment, our previous study demonstrated that PTL induces apoptosis in AML cell line via a reduction in *OPN* gene expression [19]. In another report, we suggested that silencing of *OPN* gene using siRNA significantly decreased $CD34^+/CD38^-$ human leukemia cells [20].

The results of this study showed that the transfection of Jurkat cells with OPN siRNA does not affect the viability of cells upon PTL treatment. It should be pointed out that OPN has three isoforms based on mRNA splice variants, including OPN-a, full-length transcribed mRNA containing all seven exons, OPN-b, with mRNA lacking exon 5, and OPN-c with mRNA lacking exon 4 [20]. Although in our own work, OPN siRNA degrades all three isoforms of the gene, this does not contribute to PTL toxicity for Jurkat cells [20].

While little evidence is available on the genetic basis of resistance of leukemic cells to chemotherapy, multidrug resistance genes and genes involving in cell-cycle progression and apoptosis, *OPN* is one of the candidate genes that has been recently investigated as a target in gene therapy projects and its spliced variants possibly have their unique place as cancer markers [6]. Following the related studies on OPN isoforms, it seems that two main isoforms including OPN-b and OPN-c have an important role in angiogenesis and chemoresistance [21]. Weber *et al.* [11] demonstrated that in T cells only OPN-a is expressed. Besides, in a study of OPN isoforms of macrophage, it was found that OPN isoforms increase macrophage survival and decrease macrophage apoptosis [2].

Previous research reported that drug resistance is an intrinsic feature of acute myeloid leukemia cells reflected in the gene expression pattern, which makes the resistance to chemotherapy predictable prior to the treatment [22]. The development of targeted therapies, including monoclonal antibodies and gene therapy and siRNA has shown great potential, particularly in the field of cancer treatment [17, 23].

On the other hand, Huang *et al.* [23] demonstrated that inhibition of *BCL11B* (B cell chronic lymphocytic leukemia (lymphoma11b) gene by siRNA selectively inhibited proliferation and effectively induced apoptosis in T-ALL cells. They revealed that the expression of *OPN* gene was significantly down-regulated by *BCL11B* gene silencing. Supplementary *OPN* gene silencing significantly increased mitochondrial cytochrome *c* release. Thus, the *OPN* gene may play a consistent role in anti-apoptotic effects by the *BCL11B* gene [23].

To sum up, if only OPN-a isoform would have existed in Jurkat cells [11], it would have been logical to hypothesize that the OPN-survival phenomenon might play a role in T-ALL cells similar to that of macrophages [2]. However, the role of this gene in T cell malignancies is unclear due to the low expression of *OPN* gene in Jurkat cells [23, 24]. It seems reasonable to hypothesize that *OPN* is not an appropriate gene for targeting in Jurkat cells. Further investigation on the role of OPN in malignant T cells is recommended.

REFERENCES

1. **Mazzali M, Kipari T, Ophascharoensuk V, *et al*.** Osteopontin — a molecule for all seasons. QJM 2002; **95**: 3–13.

2. **Lee GS, Salazar HF, Joseph G, *et al*.** Osteopontin isoforms differentially promote arteriogenesis in response to ischemia via macrophage accumulation and survival. Lab Invest 2019; **99**: 331–45.

3. **Boyerinas B, Zafrir M, Yesilkanal AE, *et al*.** Adhesion to osteopontin in the bone marrow niche regulates lymphoblastic leukemia cell dormancy. Blood 2013; **121**: 4821–31.

4. **Shevde LA, Samant RS**. Role of osteopontin in the pathophysiology of cancer. Matrix Biol 2014; **37**: 131–41.

5. **Nilsson SK, Johnston HM, Whitty GA, *et al*.** Osteopontin, a key component of the hematopoietic stem cell niche and regulator of primitive hematopoietic progenitor cells. Blood 2005; **106**: 1232–9.

6. **Mandarapu R, Prakhya BM**. Exposure to cypermethrin and mancozeb alters the expression profile of THBS1, SPP1, FEZ1 and GPNMB in human peripheral blood mononuclear cells. J Immunotoxicol 2016; **13**: 463–73.

7. **El-Tanani MK, Campbell FC, Kurisetty V, *et al*.** The regulation and role of osteopontin in malignant transformation and cancer. Cytokine Growth Factor Rev 2006; **17**: 463–74.

8. **Sharif SA, Du X, Myles T, *et al*.** Thrombin-activatable carboxypeptidase B cleavage of osteopontin regulates neutrophil survival and synoviocyte binding in rheumatoid arthritis. Arthritis Rheum 2009; **60**: 2902–12.

9. **Myles T, Nishimura T, Yun TH, *et al*.** Thrombin activatable fibrinolysis inhibitor, a potential regulator of vascular inflammation. J Biol Chem 2003; **278**: 51059–67.

10. **Steele A, Jones D, Ganeshaguru K, *et al*.** The sesquiterpene lactone parthenolide induces selective apoptosis of B-chronic lymphocytic leukemia cells in vitro. Leukemia 2006; **20**: 1073–9.

11. **Weber G**. Osteopontin-based cancer therapies. Patent USA 2004; US20060252684A1. Retrieved from: https://patents.google.com/patent/US20060252684

12. **Li-Weber M, Giaisi M, Treiber MK**, ***et al*.** The anti-inflammatory sesquiterpene lactone parthenolide suppresses IL-4 gene expression in peripheral blood T cells. Eur J Immunol 2002; **32**: 3587–97.

13. **Nakshatri H, Rice SE, Bhat-Nakshatri P**. Antitumor agent parthenolide reverses resistance of breast cancer cells to tumor necrosis factor-related apoptosis-inducing ligand through sustained activation of c-Jun N-terminal kinase. Oncogene 2004; **23**: 7330–44.

14. **Anderson KN, Bejcek BE**. Parthenolide induces apoptosis in glioblastomas without affecting NF-κB. J Pharmacol Sci 2008; **106**: 318–20.

15. **Li-Weber M, Giaisi M, Baumann S, *et al*.** The anti-inflammatory sesquiterpene lactone parthenolide suppresses CD95-mediated activation-induced-cell-death in T-cells. Cell Death Differ 2002; **9**: 1256–65.

16. **Li-Weber M, Palfi K, Giaisi M, *et al*.** Dual role of the anti-inflammatory sesquiterpene lactone: regulation of life and death by parthenolide. Cell Death Differ 2005; **12**: 408–9.

17. **Pozarowski P, Halicka DH, Darzynkiewicz Z**. Cell cycle effects and caspase-dependent and independent death of HL-60 and Jurkat cells treated with inhibitor of NF-6B parthenolide. Cell Cycle 2003; **2**: 374–80.

18. **Cario G, Stanulla M, Fine BM, *et al*.** Distinct gene expression profiles determine molecular treatment response in childhood acute lymphoblastic leukemia. Blood 2005; **105**: 821–6.

19. **Zahedpanah M, Shaiegan M, Ghaffari SH, *et al*.** Parthenolide induces apoptosis in committed progenitor AML cell line U937 via reduction in osteopontin. Rep Biochem Mol Biol 2016; **4**: 82–8.

20. **Mohammadi S, Zahedpanah M, Ghaffari SH, *et al*.** Osteopontin plays a unique role in resistance of CD34+/CD123+ human leukemia cell lines KG1a to parthenolide. Life Sci 2017; **189**: 89–95.

21. **Mirzaei A, Mohammadi S, Ghaffari SH**, ***et al*.** Osteopontin b and c splice isoforms in leukemias and solid tumors: angiogenesis alongside chemoresistance. Asian Pac J Cancer Prev 2018; **19**: 615–23.

22. **Panah MZ, Nikbakht M, Sajjadi SM**, ***et al*.** Anti-apoptotic effects of osteopontin via the up-regulation of AKT/mTOR/β-catenin loop in acute myeloid leukemia cells. Int J Hematol Oncol Stem Cell Res 2017; **11**: 148–57.

23. **Huang X, Chen S, Shen Q, *et al*.** Analysis of the expression pattern of the *BCL11B* gene and its relatives in patients with T-cell acute lymphoblastic leukemia. J Hematol Oncol 2010; **3**: 44.

24. **Holleman A, Cheok MH, den Boer ML**, ***et al*.** Gene-expression patterns in drug-resistant acute lymphoblastic leukemia cells and response to treatment. N Engl J Med 2004; **351**: 533–42.