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Parthenolide Induces Apoptosis and Cell Cycle Arrest by the Suppression of miR-375 Through Nucleolin in Prostate Cancer

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: To investigate the effect of parthenolide on nucleolin in controlling the expression of miR-375 that induces apoptosis and cell cycle arrest in prostate cancer.
Study Design: This study is an experimental study.
Methodology: The cytotoxicity effect of parthenolide was tested by MTT assay for 48 h. Microscopic techniques were used to identify the morphological changes of the cell line. The expression of apoptotic and cell cycle regulatory genes was analyzed by the Real-Time PCR. The phase of cell cycle arrest was identified by Flow cytometry.
Results: The obtained results indicated that parthenolide induced cytotoxicity and suppressed the proliferation by reducing the growth of LNCaP cells in 48 h. The microscopic analysis showed the alteration of cell morphology and increase of cytoplasmic reactive oxygen species. Parthenolide promotes apoptosis by the downregulation of nucleolin, Bcl-2, and up-regulation of Bax gene. Moreover, the flow cytometry assay showed the G1/G0 phase of cell cycle arrest.
Conclusion: Parthenolide induces apoptosis and cell cycle arrest through nucleolin by the suppression of miR-375 in prostate cancer cells.

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Keywords: Parthenolide; nucleolin; miR-375; apoptosis; cell cycle arrest; prostate cancer.

1. INTRODUCTION

Cancer is the second most cause of death and prostate cancer (PCa) is the major contributor to worldwide cancer morbidity and mortality and is the sixth most commonly diagnosed malignancy in men [1]. Nucleolin (NCL) is one of the phosphoprotein and expression of NCL on the cell surface is increased in numerous tumour cells and endothelial cells during angiogenesis. NCL interacts with several factors that favouring cell proliferation and inhibiting apoptosis [2]. Aberrant expression of NCL is correlated with different cancer types. Evidence has been found that there is a deregulated accumulation of NCL significantly observed in various cancer cells. It has also been found that NCL is highly expressed in colorectal cancer [3], gastric cancer [4], breast cancer [5], and leukemia [6]; circulating tumour cells displayed unique NCL expression in PCa [7]. As a result, nucleolin is thought to enhance mechanisms that alter cancer cell destiny and represents a novel and promising target for anticancer treatment.

MicroRNAs are small non-coding RNAs known to regulate gene expression by binding to 3'UTR of mRNA. MiR-375 is enriched in PCa and enhanced cellular proliferation, migration, invasion and reduced survival *in vivo* [8]. The previous studies report that NCL is involved in the biogenesis of miR-15a and 16 [5] and also regulates miR-21, miR-221, miR-222, and miR-103 [9]. Moreover, the miRNA expression signatures of prostate cancers have revealed that miR-375 is significantly overexpressed [10]. Although miR-375 has been implicated in PCa, its precise biological role and molecular mechanism of function are not yet fully understood.

Parthenolide (PTL) is a sesquiterpene lactone derivative isolated from feverfew (*Tanacetum parthenium*), exerts antioxidant, neuroprotective, anti-inflammatory, and anti-cancer properties [11,12]. We previously reported that parthenolide induces apoptosis and autophagy through activating PI3K/Akt pathway in cervical cancer [13]. It also activates B-Raf/MAPK/Erk pathway to inhibit migration and cell proliferation arrest in human non-small cell lung cancer cells [14]. Parthenolide induces apoptotic cell death via the formation of ROS, activation of caspase-3, and modulation of Bcl-2 family proteins [15]; increases reactive oxygen species (ROS) and mitochondrial Ca^{2+} to induce caspase-independent cell death [16]. Moreover, parthenolide activates p38 mitogen-activated protein kinase pathways and extracellular signal-regulated kinase and induces growth inhibition to promote apoptosis and cell cycle arrest in breast cancer [17]. In addition, the previous study shows that parthenolide binds with NCL and regulates miRNA biogenesis [5,9].

As parthenolide has a potent anticancer effect against various cancer cell lines, it is currently being subjected to clinical studies. In our earlier work, we found that parthenolide has an IC_{50} value of 6μM against cervical cancer cell line. [13]. Even parthenolide induces apoptosis in cancer cells by various mechanisms but how parthenolide induces apoptosis through NCL in prostate cancer cells remains unclear. Therefore, we aimed to investigate the role of parthenolide on NCL in controlling the expression of miR-375 to promote apoptosis and cell cycle arrest in prostate cancer.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Parthenolide was purchased from Sigma-Aldrich (St. Louis, MO, USA). Roswell Park Memorial Institute (RPMI) medium, fetal bovine serum (FBS), penicillin-streptomycin, trypsin-EDTA were obtained from Gibco-BRL (Invitrogen, Grand Island, NY, USA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma-Aldrich), 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolcarbocyanine iodide (JC-1) kit was purchased from Beyotime Biotechnology (Haimen, China).

2.2 Cell Culture

LNCaP cell line was purchased from National Center for Cell Science (NCCS), Pune, India. Cell line was immediately sub-cultured using RPMI medium with the supplementation of 10% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin antibiotics. Cells were incubated at 37°C under 5% CO_2.

2.3 MTT Assay for Cell Viability

Prostate cancer cells were seeded at 1×10^4 cells/well in 96 well plates. After overnight, cells were incubated with different concentrations of

parthenolide (0-10μM) for 48 h. DMSO (0.01%) (v/v) was used as control. After 48 h, the medium was removed and 200μl of fresh medium and 20μl freshly prepared MTT (5 mg/ml PBS) was added to each well and the plate was incubated in a dark at 37°C for 4 h. The culture medium was removed and 200μl of DMSO was added to each well to dissolve the crystal formation and the absorbance was read at 595 nm using a microplate reader (Bio-Rad).

2.4 Morphological Changes using Phase-Contrast Inverted Microscope

Morphological changes of apoptotic cells were observed according to the protocol with slight modifications. Briefly, 5×10^5 cells were incubated for 48 h with or without parthenolide in a T25 tissue culture flask. The medium was discarded and cells were washed once with PBS. The morphological changes of the apoptotic cells were observed using phase-contrast inverted microscope at 20x magnifications.

2.5 Ethidium Bromide / Acridine Orange (EtBr / AO) dual staining

Cells were grown in 6 well plates and incubated with parthenolide (IC_{50}-8μM) for 48 h. The medium was removed and cells were washed with PBS and stained with 10μl of EtBr/AO (1 mg/ml) for 5 min. Morphological changes were visualized under fluorescence microscope (Floid Cell Imaging Station).

2.6 DAPI Staining

Nuclear fragmentation and chromatin condensation were analyzed using DAPI staining. Cells were cultured in 6 well plates and incubated with 8μM concentration of parthenolide for 48 h. The medium was removed and cells were washed with PBS and stained with 10μl of DAPI (100 μg/ml) and incubated for 30 min at 37°C. The stained cells were visualized under fluorescence microscope.

2.7 Estimation of Intracellular Reactive Oxygen Species (ROS) generation

LNCaP cells (8×10^3/well) were incubated with parthenolide alone at various times. After 48 h of the treatment, the medium was removed, 100 μl of 5μM H2-DCFDA were added and incubation was protracted for 30 min at 37°C. Then, the medium was replaced with PBS and after fluorescence intensity was directly observed under fluorescence microscope (excitation wavelength of 485 nm and emission wavelength of 530 nm).

2.8 qRT-PCR

Prostate cancer cells were incubated with parthenolide (IC_{50}-8μM) for 48 h. After incubation, total RNA was isolated using Trizol reagent. Equal quantities of RNA (2μg) from each sample were used to synthesize cDNA with a cDNA synthesis kit (Takara Bio companies). Real-time PCR was carried out using SYBER FAST qPCR master mix kit (Kappa Biosystems) in 20μl by Step one plus RT-PCR (Applied Biosystem): 40 cycles at 95°C for 15 s, 60°C for 45 s and 72°C for 15 s. The β-actin gene was used for RNA template normalization.

2.9 Cell Cycle Analysis

Cell cycle analysis was performed to determine parthenolide-induced cell cycle arrest in prostate cancer cell line. Briefly, Prostate cancer cells (1×10^6 cells per well) were treated with or without parthenolide for 48 h. Cells were then collected and centrifuged at 2000 rpm for 5 min, washed in PBS, and fixed with 75% EtOH. PI solution (10 μg/ml) was added at room temperature prior to cell cycle distribution analysis, which was conducted using a FACS Scan flow cytometer (BD Biosciences, Heidelberg, Germany).

2.10 Statistical Analysis

Statistical significance was analyzed for three independent experiments (n = 3) by One-way ANOVA using GraphPad Prism software version 6.0. Statistical significance was given at a level of $^*P<0.05$.

3. RESULTS

3.1 PTL Decreases Cancer Cell Viability in Prostate Cancer Cells

To investigate the potential anticancer effect of PTL on cancer cell growth or proliferation, LNCaP cancer cell lines were tested by MTT. LNCaP cells were treated with different concentrations (0-10 μM/ml) of PTL for 48 h. The IC_{50} value was determined based on cell viability rates. As shown in Fig 1, the survival rate of LNCaP cells markedly decreased with incremental doses of PTL up to 10 μM. (Fig. 1a).

These assay results showed a dose-dependent decrease in viability of LNCaP cells at 48 h time points. The IC_{50} values of parthenolide against LNCaP cells were 8µM respectively ($p \leq 0.05$). These results indicate that PTL can significantly decrease cell viability or proliferation within a suitable concentration range in a dose dependent manner.

3.1.1 PTL induced significant morphological changes in human prostate cancer cells (Morphological assay)

In order to determine the role of apoptosis in cell growth inhibition by PTL, morphological changes in LNCaP cells were examined by phase-contrast microscopy (Fig. 1b). The inhibitory effect of the PTL was assessed by observing morphological changes in LNCaP cells, using phase-contrast microscopy. The recognizable morphological variations were observed in cancerous cells that undergoing cell shrinkage and membrane blebbing in prostate cancer cell lines with PTL while control cells showed no morphological changes.

3.1.2 Parthenolide promotes apoptosis in prostate cancer cells

In order to prove whether PTL induced cell death mediated through apoptosis, cells were treated with parthenolide for 48 h and stained with EtBr/ AO and featured morphological changes were observed under fluorescence microscope. The bright green emission indicates the intact nuclei of viable cells and dead cells emits bright orange. No morphological changes were seen in control cells which appeared uniformly green in colour (Fig. 1c).

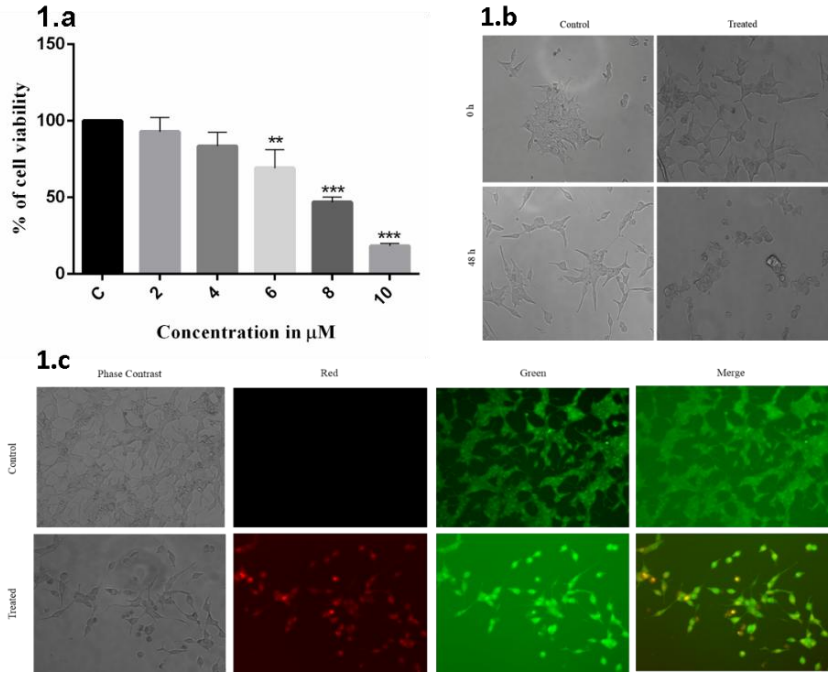


Fig. 1. a) Parthenolide (PTL) decreases the viability of prostate cancer cells and promotes apoptotic cell death. LNCaP cells were treated with PTL at concentrations of 0-10 µM. After 48 h of incubation, the MTT assay was performed to measure viability. Statistical analysis is done by ANOVA one-way test. Data represent the mean±SD of three independent experiments. *p < 0.05, **p < 0.01 compared with control; b) Morphological changes of LNCaP cells after PTL treatment. The cells were treated with PTL for 48 h, and then cellular morphological changes were studied in light microscopy (magnification 20x). After 48 h, cell aggregation, cell shrinkage, and apoptotic bodies were observed in PTL treated LNCaP cells; c) Morphological changes during PTL induced apoptosis in LNCaP cells. After treatment with PTL, LNCaP cells were stained with EtBr/AO (100 µg/µM) and observed under fluorescence microscopy (20×). Control cells appeared green in colour and parthenolide treated cells showed apoptotic bodies in orange colour

3.1.3 Detection of apoptotic cells by DAPI staining

DAPI was used to observe the chromatin condensation in the nucleus of apoptotic cells. Apoptotic cells showed smaller and brighter nuclei than normal cells. Parthenolide induced cell death by increasing the percentage of apoptotic cells compared to control (Fig. 2a).

3.1.4 PTL promotes apoptosis by triggering loss of mitochondrial membrane potential (ΛΨm) in LNCaP cells

Mitochondrial membrane potential loss is a hallmark for apoptosis. JC-1 (5,5″,6,6″-tetrachloro-1,1″,3,3″-tetraethylbenzimidazolylcarbocyanineiodide) is a specific Δψm-dependent fluorescent stain which is mostly used for detecting mitochondrial membrane depolarization changes in the early stages of apoptosis in both live and dead cells. It accumulates in mitochondria in a potential-dependent manner, as evidenced by a shift in fluorescence emission from green to red. In non-apoptotic cells, JC-1 exists as a dimer and accumulates as aggregates in the mitochondria which stains red. JC-1 is found in monomeric form in the cytosol of apoptotic and necrotic cells, staining the cytoplasm green. So, Changes in the mitochondrial membrane potential (ΔΨm) were analyzed using JC-1 dye. Our results revealed that the PTL induced a potent and dose-dependent loss of mitochondrial membrane potential by decreasing the number of cells with intact membrane potential and increasing the number of cells with low potential after 48 h exposure of PTL. As shown in Fig. 2b, as compared to control cells, PTL treatment led to a substantial loss of membrane potential in PTL treated LNCaP cells.

3.1.5 Parthenolide induces apoptosis of prostate cancer cells by triggering intracellular ROS levels

Oxidative stress was evaluated by the measurement of intracellular levels of ROS by measuring the DCF (2′, 7′- dichlorofluorescein) fluorescence intensity. This is the most common method used in ROS investigations and is based on the application of H2DCFDA (acetylated form of DCF), which is consecutively deacetylated inside the cells by intracellular esterases. Further H2DCFDA is oxidized by intracellular ROS to generate a fluorescent product, DCF. As increased production of ROS was found to induce cell death, we examined whether PTL affected ROS levels in LNCaP cancer cells **(**Fig. 2c). It was found that PTL significantly induced ROS generation (bright green) in a dose-dependent manner. These results indicate that PTL induced apoptosis by generating ROS in LNCaP cells.

3.1.6 Parthenolide regulates mRNA expression in LNCaP cells

The effect of parthenolide on apoptosis, angiogenesis, cell cycle, and metastasis regulatory mRNA expressions were analyzed by Real-Time PCR. Bcl-2, an anti-apoptotic gene expression was decreased and pro-apoptotic protein, Bax was increased. Besides, angiogenic promoting components such as VEGF, HIF-1α were downregulated. Moreover, MMP-9 is a highly elevated metastasis promoter, was decreased. Cell cycle regulatory factors were analyzed. CDK-4 and Cyclin D1 expression was suppressed in parthenolide-treated LNCaP cells. In addition, nucleolin and miR-375 expression were also decreased (Fig. 3a).

3.1.7 PTL induces cell cycle accumulation in G1 Phase

We examined the effect of PTL on cell cycle distribution because the induction of apoptosis could be mediated through cell cycle arrest. PTL treatment resulted in a significant increase in cell population in the G1 phase of the cell cycle in LNCaP cells, as shown in Fig. 3b. The increase in cell population in the G1 phase was observed to be accompanied by a decrease in cell population in the S phase and the population of cells in the G2/M phase also decreased but not substantially compared with control.

4. DISCUSSION

Prostate cancer remains a severe concern, owing to a lack of understanding of the elements that influence its development and progression [17]. Finding compounds to improve the therapeutic efficacy of existing medications is a viable treatment strategy because of its high proliferative and invasive characteristics. Natural products have long been used in cancer research, with several plant extracts and pure substances demonstrating excellent potency in cancer treatment [18]. As a result, parthenolide causes apoptosis in cancer cells although the mechanism by which it induces cell death in prostate cancer cells is still unknown. So the

effect of parthenolide via modulation of nucleolin and miR-375 axis in prostate cancer has been demonstrated in this study.

Apoptosis is an active fundamental modality of natural cell death in the maintenance of tissue homeostasis and embryonic development in all living organisms. Cell rapture, Cell swelling, cell shrinkage, plasma membrane blebbing, chromosomal DNA fragmentation, nuclear condensation, and the production of apoptotic bodies are some of its morphological hallmarks [19].

In our study, we found that cell shrinkage, cell damage, and chromosomal DNA fragmentation in parthenolide-treated LNCaP cells, which are morphological hallmarks of apoptosis (Fig 1, 2, 3). The result showed that parthenolide suppressed cancer cell survival rate by significantly inducing cell death, which denotes that the LNCaP cells were more sensitive to parthenolide treatment.

Furthermore, increased intracellular ROS generation plays a vital role in the early stages of cell death; generating oxidative stress and ultimately causes cell death including apoptosis [20]. During the stimulation of apoptosis by anticancer medicines, ROS production is critical. Increased ROS production can result in the loss of ΛΨm, which leads to apoptosis. In cervical cancer cells, parthenolide causes apoptosis via reactive oxygen species (ROS) production [13]. In our study, parthenolide increased the ROS level in treated cells compared to control in agreement with previous studies. These findings primarily revealed that parthenolide potentially suppressed LNCaP prostate cancer cell line through ROS production.

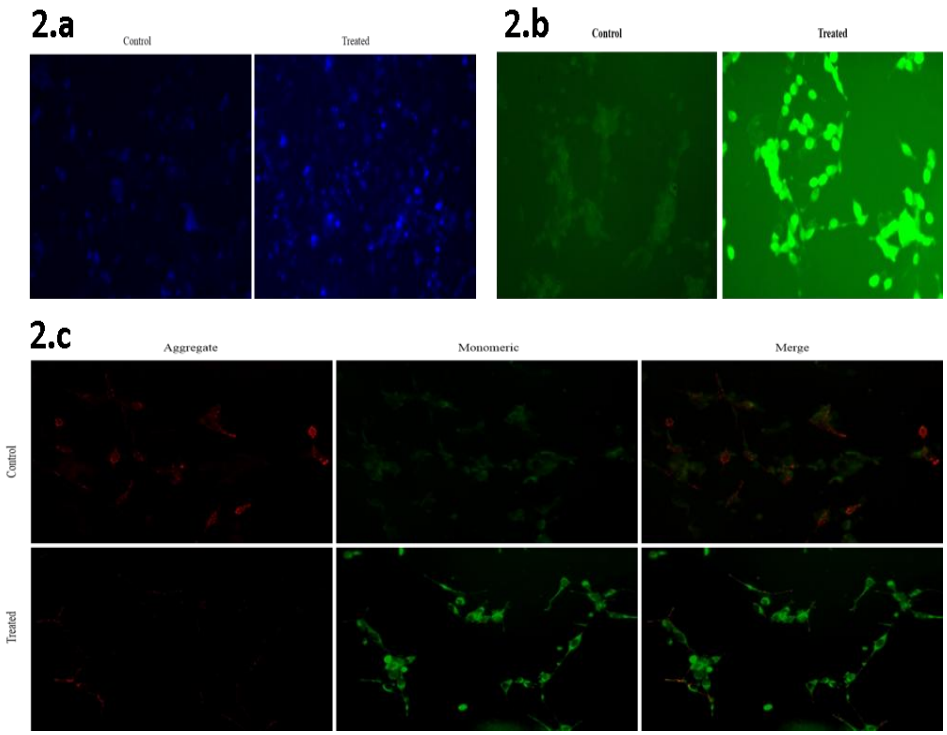
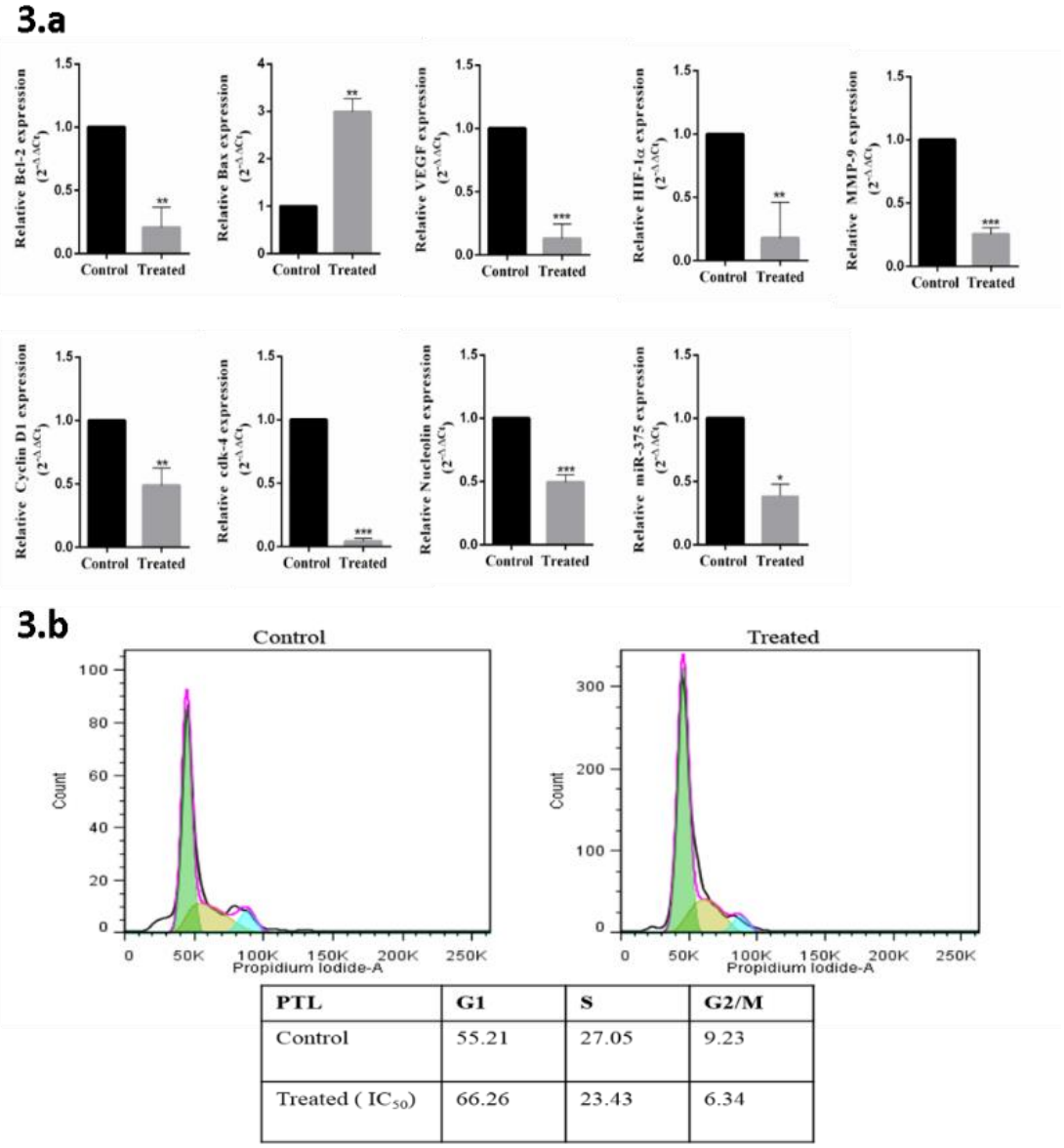


Fig. 2. a) PTL treatment causes DNA condensation and fragmentation in LNCaP cells. DNA condensation was analyzed through apoptotic cell death using DAPI stain (1µg/ml). Apoptotic nuclei stained with DAPI showed intense blue colour fluorescence corresponding to chromatin condensation and fragmentation; b) PTL triggers ROS production in LNCaP cells. After treatment with PTL, LNCaP cells were stained with DCFH-DA (100 µg/µM) and observed under fluorescence microscopy (20×). Control cells appeared low green and parthenolide treated cells showed high emission of green colour which indicates the ROS generation. c) Determination of mitochondrial membrane potential (Δψm). LNCaP cells were treated with parthenolide for 48 h. Cells were labelled with JC-1 and visualized with a cell imaging station (20×). The change from red to green indicates mitochondrial membrane depolarization



PTL	G1	S	G2/M
Control	55.21	27.05	9.23
Treated (IC_{50})	66.26	23.43	6.34

Fig. 3. a) Quantitative Real-time PCR analysis of mRNA levels in LNCaP cell line. Expression of Bcl-2, Bax, VEGF, HIF-1α, Cyclin D1, Cdk-4, Nucleolin and miR-375 genes in parthenolide-treated LNCaP cells. Cells were incubated for 48 h with PTL at IC_{50} concentrations and the expression was evaluated in qRT-PCR. Data represent mean ± SD of three independent experiments. *p < 0.05, **p < 0.05 compared with control;. Statistical significance was assessed using one-way ANOVA; b) Effect of PTL on cell cycle changes in LNCaP cells. After treatment with PTL for 48 h, LNCaP cells were harvested and the percentage of cells at each stage of the cell cycle was analyzed by flow cytometry, following DNA staining with propidium iodide and compared with control cells. The sub-G1 population percentage is reported in each histogram and the total number of events analyzed for each condition was 10,000

Generally, apoptosis (programmed cell death) is a complicated process that alleviates many oncogenes and stimulates many tumour suppressor genes. Apoptosis can be achieved by both intrinsic (mitochondrial-dependent) and extrinsic (death receptor-dependent) pathway activation. Bcl-2, an anti-apoptotic gene, and Bax, a pro-apoptotic gene, have been studied in a variety of malignancies. They play a vital role in the progression of cancer. The activation of Bax or the inhibition of Bcl-2 promotes mitochondrial malfunction and apoptosis [21,22]. Bcl-2 is a key

regulator of apoptosis mediated by mitochondria. So, we investigated whether PTL may trigger apoptosis in LNCaP prostate cancer cells. In our study, Bcl2, Bax mRNA expression levels dramatically changed after PTL treatment in LNCaP cells. So this finding demonstrated that PTL triggered apoptosis in LNCaP prostate cancer cell line.

Inhibition of new blood vessel formation (angiogenesis), which is necessary for tumour growth, invasion, and metastasis, is one of the most important targets in cancer treatment [23]. Angiogenesis is implied to vascular endothelial cells and angiogenesis-inducing factors in cancer [24]. Vascular endothelial growth factor (VEGF) and hypoxia-inducible factor-1a (HIF-1α) are the key regulators in angiogenesis and consider as a potential target for anti-angiogenic therapy [25]. Hypoxia-inducible factors stimulate the expression of angiogenic growth factors like VEGF, which causes neovascularization in tissues under physiologic and pathologic conditions [26]. So, we evaluated whether PTL deregulates angiogenic factors in LNCaP prostate cancer cells. In our study, PTL treatment inhibited the expression of both VEGF and HIF-1α expression in LNCaP cells. These observations indicated that PTL significantly could inhibit angiogenesis in LNCaP through VEGF and HIF-1α [27]. Metastasis and invasion are the leading cause of death in cancer patients. The early stage of the metastatic process is characterized by tumour cell migration and invasion from surrounding tissue to the circulation, a process known as EMT [28]. MMPs are a group of zinc-containing endopeptidases that are calcium-dependent [29] in which MMP-9 is a member of the MMP family that contributes to cancer progression by destroying the extracellular matrix, allowing cancer cells to move out of the primary tumour and form metastasis. MMP-9 may also degrade type IV collagen, the most prevalent component of the basement membrane, which is an important stage in most malignancies' metastatic growth [30]. Therefore, a compound that efficiently inhibits cancer cell migration, and invasion could be created as a new medicine to prevent or treat progressive and metastatic tumours. Hence, in this study, we focused on MMP-9 that is involved in metastasis processes. In consistent with the previous report [31], PTL significantly reduced the function of migration and invasion associated protein matrix metalloproteinase-9 (MMP-9) in LNCaP cells. These findings suggest that PTL could be a promising antagonist for prostate cancer patients with metastases.

The cell cycle and apoptosis are inextricably associated and they both have a role in cancer cell's sensitivity to chemotherapy [32]. In addition, cell cycle arrest also plays an important role in the upstream factors that induce apoptosis. Li *et al.,* have revealed that PTL can cause apoptosis by inducing cell cycle arrest [33]. We further examined the expression of cell cycle-associated protein at the mRNA level. When the chromosomal DNA of the cell is damaged, P53 binds to the corresponding part of the DNA and acts as a special transcription factor [23], which activates p21 transcription [34] and multiple Cyclin-CDK complexes bind to P21 and inhibit the corresponding protein kinase activity [35]. In the present study, we found that the mRNA levels in LNCaP cell cycle inhibitory regulatory proteins CDK-4 and cyclin D1 are decreased after PTL treatment, We further evaluated the effect of PTL on cell cycle-relevant proteins in the G1 phase. The major regulators of the G1–S phase transition, cyclin D1, and CDK4 were studied. We found that PTL treatment resulted in a significant reduction of cyclin D1, and CDK-4 expression. This could be explained by the possibility that PTL alters the cell cycle in a cell type-specific manner. We further studied the cell cycle distribution following PTL treatment and discovered that PTL caused cell cycle arrest in the G1 phase. Previous research has shown that PTL causes cancer cells to accumulate in the S or G2/M phases [36,37]. Moreover, the FACS experiment showed that the proportion of G1 phase of LNCaP cells in the PTL treatment group was significantly increased, and the proportion of the S phase was significantly decreased. This indicates that PTL can block the cell cycle of LNCaP in the G1 phase.

Nucleolin is primarily a nucleolus-localized protein; nevertheless, nucleolin is detected mostly in the cytoplasm, and overexpression of nucleolin promotes leads tumour progression in a variety of malignancies including breast cancer [38,39]. Evidence suggests it may possibly play a function in miRNA production as an auxiliary protein. Parthenolide, a natural product small molecule that impacts many signal transduction pathways, causes a substantial increase in cytoplasmic nucleolin levels in MOLM-13 acute myelogenous leukemia cells [5]. Also, the all-*trans*-retinoic acid treatment decreased cytoplasmic nucleolin level thereby enhanced the miR-15a and miR-16 expression in acute

promyelocytic leukemia cell lines and patients [40,41]. Furthermore, few studies have examined whether nucleolin stabilizes the mRNA of Bcl-2, inhibiting apoptosis [42]. Our data revealed that parthenolide reduced nucleolin expression levels in LNCaP cells. MiR-375 has been implicated in carcinogenesis in a number of cancers and it has both oncogenic and tumour suppressor functions depending on the cellular context. Many diseases, including prostate cancer, have been linked to dysregulated miRNA signatures. miRNAs have been widely employed as a new biomarker to predict prognosis and therapy effectiveness. miRNAs have been found to act as tumour suppressors and over-expressed in a variety of human cancers, contributing to tumour development. Also, MiR-375 has been reported to function as an oncogenic and tumour suppressor miRNA in various cancers. Although most studies are considered miR-375 as a tumour suppressor in gastric and cervical cancers [43,44]; few studies have been implicated miR-375 upregulation in prostate cancer [45]. Elevation of miR-375 regulates cell proliferation and mesenchymal epithelium transition (MET) in prostate cancer [46]. Interestingly, MiR-375 develops docetaxel resistance in mCRPC patient tissues and leads to poor cell death [47]. These findings give light to the prospect of using miRNA as a gene therapy target. Previous reports revealed that anticancer agents alter the miRNA expression in cancer cell lines [48,49] and the role of PTL on miR-375 in prostate cancer has not been yet reported.

So, our study mainly focuses on whether nucleolin and miR-375 reduction could suppress LNCaP cell line growth in response to parthenolide treatment. In the present study, we found that parthenolide significantly decreased nucleolin and miR-375 expression in the prostate cancer cell line. However, the interaction between nucleolin and miR-375 to cause apoptosis is unknown. As a result, more research is needed to fully understand this occurrence. Furthermore, the implication among nucleolin and miR-375 requires further investigations and these are brand new findings to the best of our knowledge.

5. CONCLUSION

This current study was conducted to investigate the effect of parthenolide on nucleolin in controlling the expression of miRNA-375 in prostate cancer. Finally, parthenolide significantly decreased nucleolin expression thereby down-regulated miR-375 level. Hence, our findings explored that parthenolide triggered apoptosis and cell cycle arrest by modulating the expression of nucleolin and miR-375 in prostate cancer cells.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by the personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Jain S, Saxena S, Kumar A. Epidemiology of prostate cancer in India. Meta gene. 2014;2:596–605.
DOI:https://pubmed.ncbi.nlm.nih.gov/25606442.
2. Berger CM, Gaume X, Bouvet P. The roles of nucleolin subcellular localization in cancer. Biochimie. 2015;113:78–85.
DOI:http://dx.doi.org/10.1016/j.biochi.2015.03.023.
3. Hammoudi A, Song F, Reed KR, Jenkins RE, Meniel VS, Watson AJM, et al. Proteomic profiling of a mouse model of acute intestinal Apc deletion leads to identification of potential novel biomarkers of human colorectal cancer (CRC). Biochem Biophys Res Commun. 2013; 440(3):364–70.
DOI:http://dx.doi.org/10.1016/j.bbrc.2013.08.076.

4. Qiu W, Zhou F, Zhang Q, Sun X, Shi X, Liang Y, et al. Overexpression of nucleolin and different expression sites both related to the prognosis of gastric cancer. APMIS. 2013;121(10):919–25.
DOI: http://dx.doi.org/10.1111/apm.12131.
5. Pickering BF, Yu D, Van Dyke MW. Nucleolin protein interacts with microprocessor complex to affect biogenesis of microRNAs 15a and 16. J Biol Chem. 2011/11/02. 2011;286 (51):44095–103.
DOI:https://pubmed.ncbi.nlm.nih.gov/22049078.
6. Shen N, Yan F, Pang J, Wu L-C, Al-Kali A, Litzow MR, et al. A nucleolin-DNMT1 regulatory axis in acute myeloid leukemogenesis. Oncotarget. 2014;5(14): 5494–509.
DOI:https://pubmed.ncbi.nlm.nih.gov/25015109.
7. Chalfin HJ, Verdone JE, van der Toom EE, Glavaris S, Gorin MA, Pienta KJ. Nucleolin Staining May Aid in the Identification of Circulating Prostate Cancer Cells. Clin Genitourin Cancer. 2017; 15(3):e477–81.
DOI:http://dx.doi.org/10.1016/j.clgc.2016.12.004.
8. Pickl JMA, Tichy D, Kuryshev VY, Tolstov Y, Falkenstein M, Schüler J, et al. Ago-RIP-Seq identifies Polycomb repressive complex I member CBX7 as a major target of miR-375 in prostate cancer progression. Oncotarget. 2016;7(37):59589–603.
DOI:https://pubmed.ncbi.nlm.nih.gov/27449098.
9. Palmieri D, De Luca L, Consiglio J, Rocci A, Talabere T, Piovan C, et al. Abstract 1122: In vivo NCL-targeting affects breast cancer aggressiveness through miRNA regulation. . Molecular and Cellular Biology. American Association for Cancer Research; 2013.
DOI: http://dx.doi.org/10.1158/1538-7445.am2013-1122.
10. Costa-Pinheiro P, Ramalho-Carvalho J, Vieira FQ, Torres-Ferreira J, Oliveira J, Gonçalves CS, et al. MicroRNA-375 plays a dual role in prostate carcinogenesis. Clin Epigenetics. 2015; 7(1):42.
DOI:https://pubmed.ncbi.nlm.nih.gov/25977730.
11. Mathema VB, Koh Y-S, Thakuri BC, Sillanpää M. Parthenolide, a Sesquiterpene Lactone, Expresses Multiple Anti-cancer and Anti-inflammatory Activities. Inflammation. 2011;35(2):560–5.
DOI: http://dx.doi.org/10.1007/s10753-011-9346-0.
12. Orofino Kreuger MR, Grootjans S, Biavatti MW, Vandenabeele P, D'Herde K. Sesquiterpene lactones as drugs with multiple targets in cancer treatment. Anticancer Drugs. 2012;23(9):883–96.
DOI:http://dx.doi.org/10.1097/cad.0b013e328356cad9.
13. Jeyamohan S, Moorthy RK, Kannan MK, Arockiam AJV. Parthenolide induces apoptosis and autophagy through the suppression of PI3K/Akt signaling pathway in cervical cancer. Biotechnol Lett. 2016;38(8):1251–60.
DOI: http://dx.doi.org/10.1007/s10529-016-2102-7.
14. Lin M, Bi H, Yan Y, Huang W, Zhang G, Zhang G, et al. Parthenolide suppresses non-small cell lung cancer GLC-82 cells growth via B-Raf/MAPK/Erk pathway. Oncotarget. 2017; 8(14):23436–47.
DOI:https://pubmed.ncbi.nlm.nih.gov/28423582.
15. Kim IH, Kim SW, Kim SH, Lee SO, Lee ST, Kim DG, et al. Parthenolide-induced apoptosis of hepatic stellate cells and anti-fibrotic effects in an in vivo rat model. Exp Mol Med. 2012;44(7):448–56.
DOI:https://pubmed.ncbi.nlm.nih.gov/22581380.
16. D'Anneo A, Carlisi D, Lauricella M, Emanuele S, Di Fiore R, Vento R, et al. Parthenolide induces caspase-independent and AIF-mediated cell death in human osteosarcoma and melanoma cells. J Cell Physiol. 2013;228(5): 952–67.
DOI: http://dx.doi.org/10.1002/jcp.24131.
17. Shen MM, Abate-Shen C. Molecular genetics of prostate cancer: new prospects for old challenges. Genes Dev. 2010;24(18):1967–2000.
DOI:https://pubmed.ncbi.nlm.nih.gov/20844012.
18. Talib WH. Consumption of garlic and lemon aqueous extracts combination reduces tumour burden by angiogenesis inhibition, apoptosis induction, and immune system modulation. Nutrition. 2017;43–44:89–97.
DOI:http://dx.doi.org/10.1016/j.nut.2017.06.015.
19. Bertheloot D, Latz E, Franklin BS. Necroptosis, pyroptosis and apoptosis: an intricate game of cell death. Cell Mol

Immunol. 2021/03/30. 2021;18(5):1106–21.
DOI:https://pubmed.ncbi.nlm.nih.gov/33785842.

20. Gao H-E, Sun Y, Ding Y-H, Long J, Liu X-L, Yang M, et al. Antineoplastic effects of CPPTL via the ROS/JNK pathway in acute myeloid leukemia. Oncotarget. 2017;8(24):38990–9000.
DOI:https://pubmed.ncbi.nlm.nih.gov/28473664.

21. Dong M, Zhou J-P, Zhang H, Guo K-J, Tian Y-L, Dong Y-T. Clinicopathological significance of Bcl-2 and Bax protein expression in human pancreatic cancer. World J Gastroenterol. 2005; 11(18):2744–7.
DOI:https://pubmed.ncbi.nlm.nih.gov/15884114.

22. Wang W, Adachi M, Zhang R, Zhou J, Zhu D. A Novel Combination Therapy with Arsenic Trioxide and Parthenolide against Pancreatic Cancer Cells. Pancreas. 2009;38(4):e114–23.
DOI:http://dx.doi.org/10.1097/mpa.0b013e3181a0b6f2.

23. Talib WH, Al Kury LT. Parthenolide inhibits tumour-promoting effects of nicotine in lung cancer by inducing P53 - dependent apoptosis and inhibiting VEGF expression. Biomed Pharmacother. 2018;107:1488–95.
DOI:http://dx.doi.org/10.1016/j.biopha.2018.08.139.

24. Musumeci G, Loreto C, Giunta S, Rapisarda V, Szychlinska MA, Imbesi R, et al. Angiogenesis correlates with macrophage and mast cell infiltration in lung tissue of animals exposed to fluoro-edenite fibers. Exp Cell Res. 2016; 346(1):91–8.
DOI:http://dx.doi.org/10.1016/j.yexcr.2016.06.017.

25. Byrne AM, Bouchier-Hayes DJ, Harmey JH. Angiogenic and cell survival functions of vascular endothelial growth factor (VEGF). J Cell Mol Med. 2005;9(4):777–94.
DOI:https://pubmed.ncbi.nlm.nih.gov/16364190.

26. Tang N, Wang L, Esko J, Giordano FJ, Huang Y, Gerber H-P, et al. Loss of HIF-1α in endothelial cells disrupts a hypoxia-driven VEGF autocrine loop necessary for tumourigenesis. Cancer Cell. 2004;6(5):485–95.
DOI:http://dx.doi.org/10.1016/j.ccr.2004.09.026.

27. KIM S-L, LEE S-T, TRANG KTHITHU, KIM SHUN, KIM INHEE, LEE SOK, et al. Parthenolide exerts inhibitory effects on angiogenesis through the downregulation of VEGF/VEGFRs in colorectal cancer. Int J Mol Med. 2014;33(5):1261–7.
DOI:http://dx.doi.org/10.3892/ijmm.2014.1669

28. Wu J-S, Jiang J, Chen B-J, Wang K, Tang Y-L, Liang X-H. Plasticity of cancer cell invasion: Patterns and mechanisms. Transl Oncol. 2020/10/17. 2021;14(1):100899.
DOI:https://pubmed.ncbi.nlm.nih.gov/33080522

29. Verma RP, Hansch C. Matrix metalloproteinases (MMPs): Chemical–biological functions and (Q)SARs. Bioorg Med Chem. 2007;15(6):2223–68.
DOI:http://dx.doi.org/10.1016/j.bmc.2007.01.011

30. Mook ORF, Frederiks WM, Van Noorden CJF. The role of gelatinases in colorectal cancer progression and metastasis. Biochim Biophys Acta - Rev Cancer. 2004;1705(2):69–89.
DOI:http://dx.doi.org/10.1016/j.bbcan.2004.09.006

31. Liu YC, Kim SL, Park YR, Lee S-T, Kim SW. Parthenolide promotes apoptotic cell death and inhibits the migration and invasion of SW620 cells. Intest Res. 2017/04/27. 2017;15(2):174–81.
DOI:https://pubmed.ncbi.nlm.nih.gov/28522946.

32. Evan GI, Vousden KH. Proliferation, cell cycle and apoptosis in cancer. Nature. 2001; 411(6835):342–8. Doi: http://dx.doi.org/10.1038/35077213.

33. Li X, Yang H, Ke J, Liu B, Lv X, Li X, et al. Smad4 re-expression increases the sensitivity to parthenolide in colorectal cancer. Oncol Rep. 2017;38(4):2317–24.
DOI:http://dx.doi.org/10.3892/or.2017.5929

34. Li C, Ge Q, Liu J, Zhang Q, Wang C, Cui K, et al. Effects of miR-1236-3p and miR-370-5p on activation of p21 in various tumours and its inhibition on the growth of lung cancer cells. Tumour Biol. 2017;39(6):101042831771082.

DOI:http://dx.doi.org/10.1177/1010428317710824.

35. Liu B, Zhou Z, Zhou W, Liu J, Zhang Q, Xia J, et al. Resveratrol inhibits proliferation in human colorectal carcinoma cells by inducing G1/S‐phase cell cycle arrest and apoptosis through caspase/cyclin‐CDK pathways. Mol Med Rep. 2014/07/21. 2014;10(4):1697–702.
DOI:https://pubmed.ncbi.nlm.nih.gov/25050564

36. Shanmugam R, Kusumanchi P, Appaiah H, Cheng L, Crooks P, Neelakantan S, et al. A water soluble parthenolide analog suppresses in vivo tumour growth of two tobacco-associated cancers, lung and bladder cancer, by targeting NF-κB and generating reactive oxygen species. Int J cancer. 2011 May 15; 128(10):2481–94.
DOI:https://pubmed.ncbi.nlm.nih.gov/20669221

37. Cheng G, Xie L. Parthenolide induces apoptosis and cell cycle arrest of human 5637 bladder cancer cells in vitro. Molecules. 2011; 16(8):6758–68.
DOI:https://pubmed.ncbi.nlm.nih.gov/21829151

38. Joo EJ, Yang H, Park Y, Park NY, Toida T, Linhardt RJ, et al. Induction of nucleolin translocation by acharan sulfate in A549 human lung adenocarcinoma. J Cell Biochem. 2010; 110(5):1272–8.
DOI: http://dx.doi.org/10.1002/jcb.22643.

39. Mourmouras V, Cevenini G, Cosci E, Epistolato MC, Biagioli M, Barbagli L, et al. Nucleolin protein expression in cutaneous melanocytic lesions. J Cutan Pathol. 2009; 36(6):637–46.
DOI: http://dx.doi.org/10.1111/j.1600-0560.2008.01126.x.

40. Otake Y, Sengupta TK, Bandyopadhyay S, Spicer EK, Fernandes DJ. Retinoid-Induced Apoptosis in HL-60 Cells Is Associated with Nucleolin Down-Regulation and Destabilization of Bcl-2 mRNA. Mol Pharmacol. 2004; 67(1):319–26.
DOI:http://dx.doi.org/10.1124/mol.104.006080.

41. Garzon R, Pichiorri F, Palumbo T, Visentini M, Aqeilan R, Cimmino A, et al. MicroRNA gene expression during retinoic acid-induced differentiation of human acute promyelocytic leukemia. Oncogene. 2007;26(28):4148–57.
DOI:http://dx.doi.org/10.1038/sj.onc.1210186.

42. Otake Y, Soundararajan S, Sengupta TK, Kio EA, Smith JC, Pineda-Roman M, et al. Overexpression of nucleolin in chronic lymphocytic leukemia cells induces stabilization of bcl2 mRNA. Blood. 2007 Apr 1; 109(7):3069–75.
DOI:https://pubmed.ncbi.nlm.nih.gov/17179226.

43. Tsukamoto Y, Nakada C, Noguchi T, Tanigawa M, Nguyen LT, Uchida T, et al. MicroRNA-375 Is Downregulated in Gastric Carcinomas and Regulates Cell Survival by Targeting PDK1 and 14-3-3ζ. Cancer Res. 2010; 70(6):2339–49.
DOI: http://dx.doi.org/10.1158/0008-5472.can-09-2777.

44. Jayamohan S, Kannan M, Moorthy RK, Rajasekaran N, Jung HS, Shin YK, et al. Dysregulation of miR-375/AEG-1 Axis by Human Papillomavirus 16/18-E6/E7 Promotes Cellular Proliferation, Migration, and Invasion in Cervical Cancer. Front Oncol. 2019 Sep 9; 9:847.
DOI:https://pubmed.ncbi.nlm.nih.gov/31552174.

45. Huang X, Yuan T, Liang M, Du M, Xia S, Dittmar R, et al. Exosomal miR-1290 and miR-375 as prognostic markers in castration-resistant prostate cancer. Eur Urol. 2014/08/14. 2015; 67(1):33–41.
DOI:https://pubmed.ncbi.nlm.nih.gov/25129854.

46. Rajendiran S, Maji S, Haddad A, Lotan Y, Nandy RR, Vishwanatha JK, et al. MicroRNA-940 as a Potential Serum Biomarker for Prostate Cancer. Front Oncol. 2021;11:628094.
DOI:https://pubmed.ncbi.nlm.nih.gov/33816263.

47. Wang Y, Lieberman R, Pan J, Zhang Q, Du M, Zhang P, et al. miR-375 induces docetaxel resistance in prostate cancer by targeting SEC23A and YAP1. Mol Cancer. 2016;15(1):70.
DOI:https://pubmed.ncbi.nlm.nih.gov/27832783.

48. Li Y, Sun W, Han N, Zou Y, Yin D. Curcumin inhibits proliferation, migration, invasion and promotes apoptosis of retinoblastoma cell lines through modulation of miR-99a and JAK/STAT

pathway. BMC Cancer. 2018;18(1): 1230.
DOI:https://pubmed.ncbi.nlm.nih.gov/30526546.

49. Wang K, Tan S-L, Lu Q, Xu R, Cao J, Wu S-Q, et al. Curcumin Suppresses microRNA-7641-Mediated Regulation of p16 Expression in Bladder Cancer. Am J Chin Med. 2018;46(06):1357–68.
DOI:http://dx.doi.org/10.1142/s0192415x18500714.

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