Journal of Current Biomedical Reports
Volume 4, Number 1, 2023
jcbior.com
eISSN: 2717-1906

Original research

The comparative assessment of single dose versus co-administration of salinomycin and MK-2206 on viability, apoptosis, and gene expression of human prostate cancer cells

Mohamadreza Savaee[1], Ali Bakhshi[1], Mehran Dehghanian[1], Ghafour Yarahmadi[2], Fatemeh Yaghoubi[1], Fatemeh Pourrajab[3,4,5,*]

[1]*Department of Clinical Biochemistry, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran*
[2]*Department of Medical Genetics, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran*
[3]*Reproductive Immunology Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran*
[4]*Nutrition and Food Security Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran*
[5]*Hematology and Oncology Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran*

Abstract

Globally, the most prevalent medical condition affecting males is identified as prostate cancer (PCa). The aim of the present study was the comparative assessment of single dose versus co-administration of salinomycin and MK-2206 on several laboratory indicators of PCa. The probable anticancer potential of salinomycin and MK-2206 was assessed in a panel of PCa cell line. Several experiments were conducted to determine the primary underlying biological processes, which includes apoptosis induction, cancer cell survival, and gene expression count data. As compared to the negative control group, all salinomycin, MK-2206, and salinomycin + MK-2206 treatment groups showed significantly lower rates of cell viability, and both Akt and Bcl-2 expressions [($P < 0.001$), ($P < 0.001$), and ($P < 0.001$), respectively]. In addition, treatments with salinomycin, MK-2206, and salinomycin + MK-2206 was accompanied by noticeably higher level of apoptosis in PCa cells in comparison with the negative control group. Specifically, the concomitant use of salinomycin + MK-2206 resulted in synergistic advantages in the study experiments. Given that timely treatment is widely believed to play an important role in management of the PCa, our data suggest that salinomycin and MK-2206 therapy may be considered as a suitable strategy for PCa improvements. Thus, further clinical trial studies are required in order to obtain stronger evidence regarding the effectiveness of salinomycin and MK-2206 treatments in PCa.

Keywords: Neoplasm, Cell survival, Salinomycin, Mk 2206, Gene

1. Introduction

One of the most prominent medical conditions affecting men worldwide is prostate cancer (PCa) [1]. In the last decade, well-conducted investigations in the field of PCa have expanded our knowledge regarding its genetic and environmental risk factors such as radiation and toxic chemicals [2]. The unconventional activity of phosphatidylinositol 3 kinase (PI3K) has been reported to play an important role in cancer initiation and advancement [3]. In this regard, a key serine-threonine kinase that functions downstream of PI3K and is linked to the survival of body cells is Akt, also recognized as protein kinase B (PKB) [4]. Accordingly, clinically proper inhibitors of Akt may exhibit a great ability in cancer treatment [5]. There are several remedies including chemotherapy,

[*]**Corresponding author:**
Fatemeh Pourrajab, Ph.D
Reproductive Immunology Research Center,
Shahid Sadoughi University of Medical Sciences, Yazd, Iran
Tel/Fax: +98 35 38203410
Email: fatemehpourrajab1400@gmail.com
http://orcid.org/0000-0002-1724-5392

Received: October, 21, 2022
Accepted: February, 26, 2023

radiotherapy, and androgen deprivation therapy which have been suggested in order to derive maximum benefit for the patients suffering from PCa [6-8]. However, the effectiveness of all current therapies is limited relating to serious side effects, which harden the appropriate drug selection for these individuals [9]. Thus, many researchers have been focused on alternative treatments with fewer adverse effects in patients with PCa as a global necessity [10].

MK-2206 is a selective Akt inhibitor that increases apoptosis rate while inhibiting cell-cycle progression [4]. Binding of MK-2206 to Akt causes a structural deformation of Akt, which results in diminishing its subsequent activation [11]. Furthermore, previous investigations have documented that MK2206 synergized with chemotherapy in various type of cancers, providing a suitable therapeutic strategy for these patients [12, 13].

On the other hand, salinomycin is a monocarboxylic polyether ionophore antibiotic with a molecular weight of 751Da which derived from *Streptomyces albus* [14]. Former studies have shown favorable effects of salinomycin including antibacterial, antifungal, antiparasitic, antiviral, and anti-inflammatory actions [15]. Also, several investigations have recently indicated that salinomycin may have an antitumor potential in different forms of cancer [14, 16]. Salinomycin may lead to a higher sensitivity of cells to chemotherapeutic drugs through inhibiting the P-glycoprotein transporter, which involved in multidrug resistant by facilitating movement of drug out of cells [17]. Moreover, it has been observed that using salinomycin on cancer cells can diminish the body's activation signaling of Akt enzyme [18]. Hence, the purpose of the current investigation was the comparative assessment of single dose versus co-administration of salinomycin and MK-2206 on viability, apoptosis, and gene expression of human PCa cells.

2. Materials and Methods

2.1 Ethical approval

The procedure of the present investigation was conducted in Yazd, Iran, from April to June 2022. The Research Ethics Committee of Shahid Sadoughi University of Medical Sciences has been accepted all processes of the current study (IR.SSU.REC.1400.245).

2.2 Cell culture

At first, Pastor Institute (Tehran, Iran) provided the human prostate cancer cell line (PC-3) for this study. Then, cells were cultured in Dulbecco's modified Eagle's medium (INOCLON, LOT number: C1419005, Iran) together with 10% fetal bovine serum (FBS), 2 mM of L-glutamine, 100 U/ml of penicillin and 100 µg/ml of streptomycin (WelGENE Inc., Daegu, Korea) and eventually stored in a humidified incubator at 37 °C with 5% CO_2.

During the study cell's growth period, the logarithmic phase was chosen for implementation of the experimental procedures, which is linked to the higher proliferation and total number of PCa cells.

2.3 Cell viability

Cells were seeded at a density of 2 to 3 × 10^3 per well in 96-well plates for 24 h. Afterward, the study drugs were added to the wells for 72 h as follows: (a) salinomycin, 1.13 µM (Sigma-Aldrich Chemical Co., Cat. No. S4526, Germany); (b) MK-2206, 11 µM (Cayman Chemical Company, Item No. 11593, USA); (c) compound C, 10 µM (used as the positive control in order to suppress the PI3K/Akt signaling; Sigma-Aldrich Chemical Co., Cat. No. 171260, Germany); and (d) salinomycin + MK-2206, 7.8 µM. MTT solution, 0.5 mg/ml (Sigma-Aldrich Chemical Co., LOT number: MKCL9866, Germany) was then applied to the cells, and finally the precipitates were treated with dimethyl sulfoxide (DMSO, used as the negative control; Lifebiolab, Cat. No. LB17067, Heidelberg, Germany). An ELIZA Microplate Reader (Lx800, Biotech, Germany) was used to perform colorimetric examination at 570 nm. At last, the vitality of PCa cells was determined by comparing optical densities of treated and untreated cells.

2.4 Measurement of apoptosis

Treating cells with the DAPI solution (4, 6-diamidino-2-phenylindole, Sigma-Aldrich Chemical Co., LOT number: 066M4053V, Germany) was the procedure of investigating the occurrence of apoptosis without staining the nucleus. For 24 hours, 6-well plates containing PCa cells were treated either with salinomycin (5 µM), MK-2206 (10 µM), compound C (12 µM), or salinomycin + MK-2206 (7 µM). After multiple rounds of PBS (PH= 7.4) washing, PC-3 cells were stained for five minutes with 0.5 µg/mL of DAPI solution. A fluorescence microscope was employed to observe all images (Carl Zeiss, Jena, Germany).

2.5 The isolation of total RNA

The RNX-Plus kit (SinaClon BioScience, Cat. No. EX6101, Iran) was used in order to extract total RNA in accordance with given guidelines of its company. Then, a nanodrop spectrophotometer was applied to evaluate the concentration and absorbance of the isolated RNA at 260/280 nm.

The highest quality of RNA extraction was confirmed by examination of the 28s, 18s, and 5s rRNA bands on an agarose gel. At last, the RNAs were maintained in a freezer at −80 ˚C.

2.6 The synthesis of cDNA

The Easy cDNA Synthesis Kit (Pars tous, Tehran, Iran) was utilized in order to synthesis the cDNA. Afterwards, the aliquots were normalized, and then kept frozen (−80 ˚C) until the real-time polymerase chain reaction (RT-PCR) technique.

2.7 Real Time PCR (RT-PCR)

Due to the high specificity of the assay, sensitivity of the detection, and wide linear dynamic range, the RT-PCR is regarded as the gold standard among the scientific biological experiments for evaluating gene expression [19]. A reference/housekeeping gene called β-Actin was used in the investigation of gene expression, which the expression rate of target genes such as Akt and the anti-apoptotic protein Bcl-2 (also known as B-cell lymphoma 2) were compared to it. It is important to note that all body cells express the housekeeping gene, and that environmental factors have no impact on the frequency of that expression [20].

The determination of non-primer binding to non-specific genomic loci or non-formation of homodimer and heterodimer was obtained from Primer Blast, Gene Runner, and UCSC gene websites. Additionally, the Tm of the primers was verified using the Tm calculate webpage. The ROYAN organization (Stem Cell Technology, Tehran, Iran) provided the primer sequences for the genes including Akt [21], Bcl-2 [22], and β −Actin [23] (Table 1).

The PCR method was carried out utilizing an Opticon thermal cycler (settings: 95 ˚C for 15 min, then 40 cycles of 95 ˚C for 15 seconds, 55 ˚C for 15 seconds, 72 ˚C for 30 seconds, and finally 72 ˚C for 5 minutes) and a SYBR Green qPCR Master Mix Kit (Yektatajhiz Co; Cat No. YT2551, Tehran, Iran). Eventually, the study samples were conducted separately on various days to determine the SYBR Green real-time PCR reproducibility.

2.8 Statistical analysis

Statistical analysis was done using the SPSS statistical software, version 22 (SPSS Inc., Chicago, IL, USA). The quantitative study variables were compared using one-way ANOVA and Tukey's multiple range post-hoc tests. All the differences were considered statistically significant at P-values<0.05.

3. Results

3.1 The PCa cells viability

In comparison with the DMSO treated cells, the total viability of the PC-3 cells was significantly reduced via treatment of compound C (P<0.001). Similarly, cells treated with salinomycin, MK-2206, and salinomycin + MK-2206 showed a considerable reduction in viability of PC-3 cells (all P <0.001). Additionally, compared to salinomycin and MK-2206 administered separately, salinomycin + MK-2206 demonstrated a further reduction in the viability of cancerous cells (P <0.05) (Figure 1).

3.2 The assessment of apoptosis

All salinomycin, MK-2206, and salinomycin + MK-2206 indicated apoptotic activity based on visual observations of cancer cells stained with the DAPI solution (Supplementary Figure 1).

3.3 Gene Expression

3.3.1 Akt expression

Findings of the treatment with compound C, salinomycin, MK-2206, and salinomycin + MK-2206 regarding the expression of Akt in the study cancer cells have been shown in (Figure 2). Comparatively to the DMSO group, the compound c group had reduced expression of Akt (P <0.001). Likewise, a substantial decrease in Akt expression was observed in all salinomycin, MK-2206, and salinomycin + MK-2206 treatment groups (P <0.001). Also, the concomitant administration of salinomycin and MK-2206 showed a noticeably greater decrease in Akt expression than separately treated cells with both compounds (P <0.05).

Table 1. AKT, Bcl-2, and β-Actin primer sequences

Genes	Forward primer (5′ → 3′)	Reverse primer (5′ → 3′)	Product size (bp)
AKT1	AGCGACGTGGCTATTGTGAAG	GCCATCATTCTTGAGGAGGAAGT	96
Bcl-2	TTGTGGCCTTCTTTGAGTTCGGTG	GGTGCCGGTTCAGGTACTCAGTCA	114
β-Actin	CGCGAGAAGATGACCCAGATC	GATAGCACAGCCTGGATAGCAAC	77

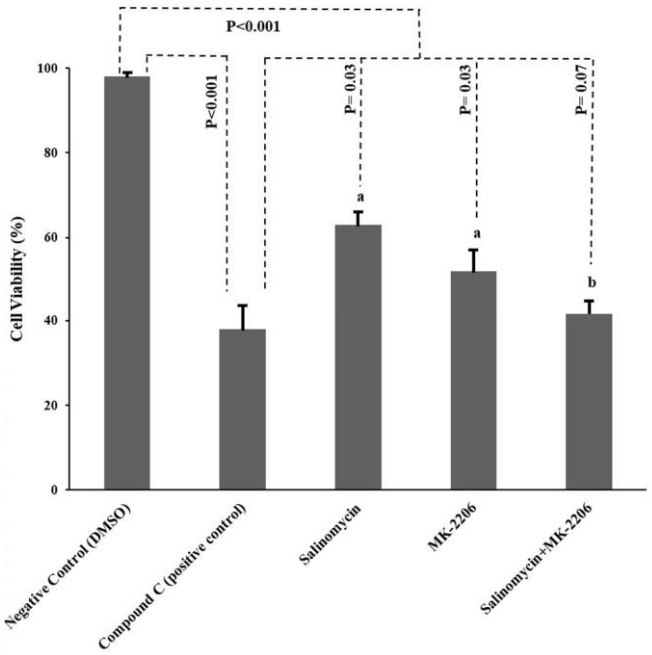

Figure 1. Prostate cancer cells' viability level.
All values expressed as Mean±SD. All P-values obtained from the One-way ANOVA, followed by Tukey's multiple range post-hoc tests. P-values <0.05 were regarded as statistically significant. P-value <0.05 indicates a significant difference between graph columns with various superscripts.

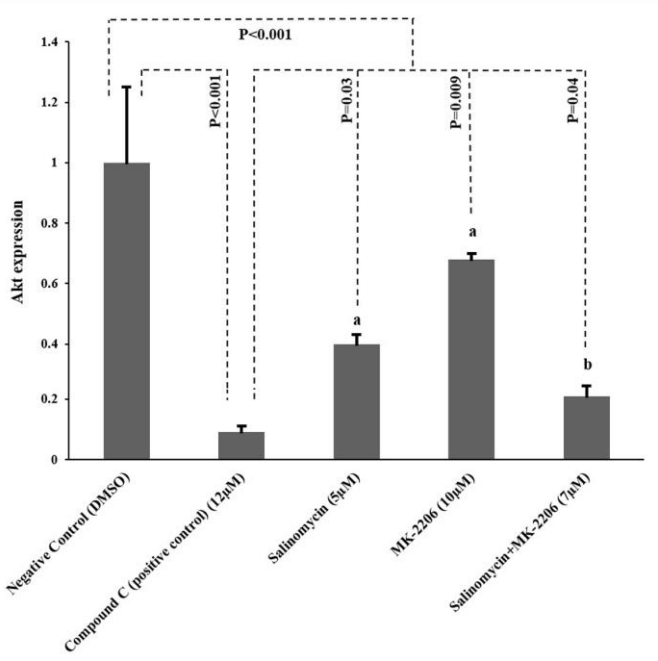

Figure 2. Akt expression levels in prostate cancerous cells. All values expressed as Mean±SD. All P-values obtained from the One-way ANOVA, followed by Tukey's multiple range post-hoc tests. P-values <0.05 were regarded as statistically significant. P-value <0.05 indicates a significant difference between graph columns with various superscripts

3.3.2 Bcl-2 expression

Bcl-2 expression levels in prostate cancerous cells have been presented in (Figure 3).

As the Figure 3 depicts, the greater expression of Bcl-2 was observed in the negative control group compared to the compound C treated cells (P <0.001). Additionally, as compared to the DMSO treatment, the level of Bcl-2 expression in the salinomycin, MK-2206, and salinomycin + MK-2206 therapies were notably lower (P = 0.01, P = 0.01, and P <0.001, respectively).

Furthermore, as compared to the negative control group, there was a substantial decrease in the rate of Bcl-2 expression in the salinomycin, MK-2206, and salinomycin + MK-2206 groups [(P = 0.01, P = 0.01, and P <0.001, respectively)].

Meanwhile, in comparison with the single dose therapy, the expression level of Bcl-2 was considerably down regulating in co-administration of salinomycin and MK-2206 (both P <0.05).

4. Discussion

According to the best of our knowledge, this is a novel experiment regarding the comparative effects of single and co-administration of salinomycin and MK-2206 on several laboratory factors of PCa cell line. According to the findings of the present investigation,

both single and combined treatment of salinomycin and MK-2206 triggered the reduction of PC-3 cells' survival, apoptosis enhancement, and decreased expression of both Akt and Bcl-2 genes.

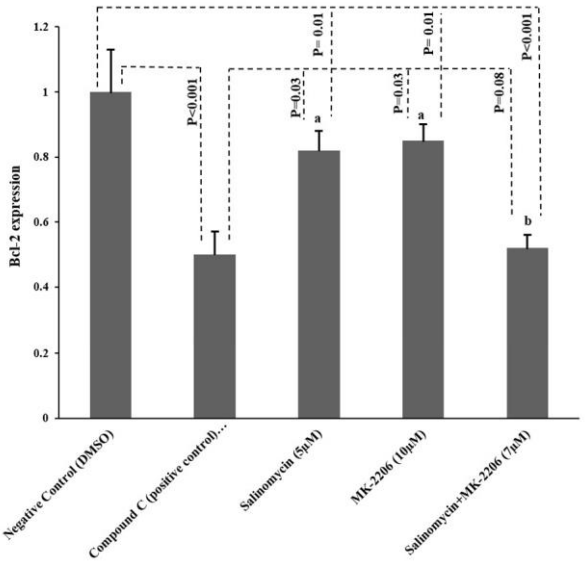

Figure 3. Bcl-2 expression levels in prostate cancerous cells. All values expressed as Mean±SD. All P-values obtained from the One-way ANOVA, followed by Tukey's multiple range post-hoc tests. P-values <0.05 were regarded as statistically significant. P-value <0.05 indicates a significant difference between graph columns with various superscripts.

There are convincing reports about the underlying mechanisms by which Akt may exert its antiapoptotic effects, which are based on the balance between pro- and anti-apoptotic proteins as follows: 1) down-regulation of Bad expression as a pro-apoptotic factor; 2) Development of cell survival due to up-regulation of Bcl-2 expression as an anti-apoptotic factor [24, 25].

In one study, Choi et al. [18] found reduced total Akt level in breast cancer cells treated with salinomycin and MK-2206, which is consistent with the results of the present investigation. Additionally, the researchers claimed that the simultaneous administration of salinomycin and MK-2206 enhanced cancer cell death through a decrease in overall Akt expression [26-28]. As well, it has been documented that MK-2206 may play a key role in reducing the Akt signaling in different types of human cancer [3]. Specifically, in 2012, the investigators of an experimental study observed that treatment with MK-2206 inhibited Akt activation and cell-cycle development in breast cancer cell lines in a dose-dependent manner [4].

In line with our results, in a study by Neri et al. [29] the researchers found an underlying molecular pathway between cell's caspase-dependent death and co-administration of MK-2206 with RAD001 (a mTOR enzyme inhibitor) in B-precursor acute lymphoblastic leukemia cells. Similarly, an investigation in 2013 revealed that salinomycin may lead to an accelerated cell death process when co-treatment with a PI3K inhibitors as compared to independent therapy [30]. Salinomycin has also been shown to lower oxidative stress and cell motility, while reducing MYC, AR, and ERG expression in PCa cells [31]. While the exact molecular mechanisms regarding the functions of salinomycin are yet unknown, a number of studies have proposed the inhibition of cancerous cell growth through autophagy enhancement and disrupting Wnt/β-catenin signaling pathway, which is necessary for the preservation of progenitor cells in a variety of cancer cells [32, 33].

It is important to interpret our findings by consideration of the major limitations. Due to a lack of financing, it was not possible to compare the effects of other relevant cytotoxic drugs such carboplatin, doxorubicin, and gemcitabine. Therefore, it is required to conduct further planned researches to assess additional particular compounds that could provide information on the critical underlying processes driving the actions of salinomycin and MK-2260 on PCa cells.

Given that timely treatment is widely believed to play an important role in management of the PCa, our data suggest that salinomycin and MK-2206 therapy may be considered as a suitable strategy for PCa improvements. Thus, further clinical trial studies are required in order to obtain stronger evidence regarding the effectiveness of salinomycin and MK-2206 treatments in PCa.

Authors' contributions

MS: Original draft, Data curation, Formal analysis, Investigation, Software, Visualization; AB: Original draft, Data curation, Formal analysis, Investigation, Software, Visualization; MD, GY, and FY: Original draft, Data curation, Formal analysis, Investigation, Software, Visualization; FP: Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Supervision,

Validation, Visualization, Original draft, Review & Editing. All authors read and approved the final manuscript.

Conflict of interests

None to declare.

Ethical declarations

The Research Ethics Committee of Shahid Sadoughi University of Medical Sciences has been accepted all processes of the current study (IR.SSU.REC.1400.245).

Financial support

Financial support for this research was provided by Shahid Sadoughi University of Medical Sciences, Yazd, Iran (Grant No. 12438).

Supplementary files

Supplementary file 1.

References

1. Swami U, McFarland T, Nussenzveig R, Agarwal N. Advanced Prostate Cancer: Treatment Advances and Future Directions. Trends Cancer. 2020; 6(8):702-15.
2. Li J, Xu C, Lee HJ, Ren S, Zi X, Zhang Z, et al. A genomic and epigenomic atlas of prostate cancer in Asian populations. Nature. 2020; 580(7801):93-9.
3. Ma Y, Sender S, Sekora A, Kong W, Bauer P, Ameziane N, et al. The inhibitory response to PI3K/AKT pathway inhibitors MK-2206 and Buparlisib is related to genetic differences in pancreatic ductal adenocarcinoma cell lines. Int J Mol Sci. 2022; 23(8):4295.
4. Sangai T, Akcakanat A, Chen H, Tarco E, Wu Y, Do K-A, et al. Biomarkers of Response to Akt Inhibitor MK-2206 in Breast CancerAntitumor Activity of MK-2206. Clin Cancer Res. 2012; 18(20):5816-28.
5. Iida M, Harari P, Wheeler D, Toulany M. Targeting AKT/PKB to improve treatment outcomes for solid tumors. Mutat Res. 2020; 819-820:111690.
6. Pérez-Gracia JL, Gúrpide A, de Fata Chillón F, Villacampa F. The role of chemotherapy in the treatment of hormone sensitive metastatic prostate cancer. Arch Esp Urol. 2018; 71(3):276-80.
7. Rizzo A, Mollica V, Cimadamore A, Santoni M, Scarpelli M, Giunchi F, et al. Is there a role for immunotherapy in prostate cancer? Cells. 2020; 9(9):2051.
8. Teo MY, Rathkopf DE, Kantoff P. Treatment of advanced prostate cancer. Annu Rev Med. 2019; 70:479-499.
9. Oun R, Moussa YE, Wheate NJ. The side effects of platinum-based chemotherapy drugs: a review for chemists. Dalton trans. 2018; 47(19):6645-6653.
10. Prakash O, Kumar A, Kumar P. Anticancer potential of plants and natural products. Am J Pharmacol Sci. 2013; 1(6):104-15.
11. Ma BB, Lui VW, Hui CW, Lau CP, Wong C-H, Hui EP, et al. Preclinical evaluation of the AKT inhibitor MK-2206 in nasopharyngeal carcinoma cell lines. Invest New Drugs. 2013; 31(3):567-75.
12. Meng J, Dai B, Fang B, Bekele BN, Bornmann WG, Sun D, et al. Combination treatment with MEK and AKT inhibitors is more effective than each drug alone in human non-small cell lung cancer in vitro and in vivo. PloS one. 2010; 5(11):e14124.
13. Ji S, Lin W, Wang L, Ni Z, Jin F, Zha X, et al. Combined targeting of mTOR and Akt using rapamycin and MK-2206 in the treatment of tuberous sclerosis complex. J Cancer. 2017; 8(4):555-562.
14. Dewangan J, Srivastava S, Rath SK. Salinomycin: A new paradigm in cancer therapy. Tumor Biol. 2017; 39(3):1010428317695035.
15. Huczyński A, Janczak J, Antoszczak M, Wietrzyk J, Maj E, Brzezinski B. Antiproliferative activity of salinomycin and its derivatives. Bioorg Med Chem Lett. 2012; 22(23):7146-50.
16. Kim K-Y, Yu S-N, Lee S-Y, Chun S-S, Choi Y-L, Park Y-M, et al. Salinomycin-induced apoptosis of human prostate cancer cells due to accumulated reactive oxygen species and mitochondrial membrane depolarization. Biochem Biophys Res Commun. 2011; 413(1):80-6.
17. Riccioni R, Dupuis ML, Bernabei M, Petrucci E, Pasquini L, Mariani G, et al. The cancer stem cell selective inhibitor salinomycin is a p-glycoprotein inhibitor. Blood Cells Mol Dis. 2010; 45(1):86-92.
18. Choi A-R, Kim J-H, Yoon S. Sensitization of cancer cells through reduction of total Akt and downregulation of salinomycin-induced pAkt, pGSk3β, pTSC2, and p4EBP1 by cotreatment with MK-2206. Biomed Res Int. 2014;2014:295760.
19. Arikawa E, Sun Y, Wang J, Zhou Q, Ning B, Dial SL, et al. Cross-platform comparison of SYBR® Green real-time PCR with TaqMan PCR, microarrays and other gene expression measurement technologies evaluated in the MicroArray Quality Control (MAQC) study. BMC Genomics. 2008; 9:328.
20. Nozari E, Moradi A, Samadi M. Effect of atorvastatin, curcumin, and quercetin on miR-21 and miR-122 and their correlation with TGFβ1 expression in experimental liver fibrosis. Life Sci. 2020; 259:118293.
21. Huang D, Wang F, Wu W, Lian C, Liu E. MicroRNA-429 inhibits cancer cell proliferation and migration by targeting the AKT1 in melanoma. Cancer Biomark. 2019; 26(1):63-8.
22. AlMalki FA, Hassan AM, Klaab ZM, Abdulla S, Pizzi A. Tannin Nanoparticles (NP99) Enhances the Anticancer Effect of Tamoxifen on ER [supercript]+[/supercript] Breast Cancer Cells. J Renew Mater. 2021; 9(12):2077-92.
23. Liu B, Sun X. miR-25 promotes invasion of human non-small cell lung cancer via CDH1. Bioengineered. 2019; 10(1):271-81.
24. Al-Bazz YO, Underwood JC, Brown BL, Dobson PR. Prognostic significance of Akt, phospho-Akt and BAD expression in primary breast cancer. Eur J Cancer. 2009; 45(4):694-704.
25. Zhou L, Zhao Y. B7-H3 induces ovarian cancer drugs resistance through an PI3K/AKT/BCL-2 signaling pathway. Cancer Manag Res. 2019; 11:10205-10214.
26. Balasis ME, Forinash KD, Chen YA, Fulp WJ, Coppola D, Hamilton AD, et al. Combination of farnesyltransferase and Akt inhibitors is synergistic in breast cancer cells and causes significant breast tumor regression in ErbB2 transgenic mice. Clin Cancer Res. 2011; 17(9):2852-62.

27. Cheng Y, Zhang Y, Zhang L, Ren X, Huber-Keener KJ, Liu X, et al. MK-2206, a Novel Allosteric Inhibitor of Akt, Synergizes with Gefitinib against Malignant Glioma via Modulating Both Autophagy and ApoptosisSynergistic Antitumor Effect of MK-2206 and Gefitinib. Mol Cancer Ther. 2012; 11(1):154-64.
28. Chen K-F, Chen H-L, Tai W-T, Feng W-C, Hsu C-H, Chen P-J, et al. Activation of phosphatidylinositol 3-kinase/Akt signaling pathway mediates acquired resistance to sorafenib in hepatocellular carcinoma cells. J Pharmacol Exp Ther. 2011; 337(1):155-61.
29. Neri L, Cani A, Martelli A, Simioni C, Junghanss C, Tabellini G, et al. Targeting the PI3K/Akt/mTOR signaling pathway in B-precursor acute lymphoblastic leukemia and its therapeutic potential. Leukemia. 2014; 28(4):739-48.
30. Kim J-H, Choi A-R, Kim YK, Kim HS, Yoon S. Low amount of salinomycin greatly increases Akt activation, but reduces activated p70S6K levels. Int J Mol Sci. 2013; 14(9):17304-18.
31. Ketola K, Hilvo M, Hyötyläinen T, Vuoristo A, Ruskeepää A-L, Orešič M, et al. Salinomycin inhibits prostate cancer growth and migration via induction of oxidative stress. Br J Cancer. 2012; 106(1):99-106.
32. Mao J, Fan S, Ma W, Fan P, Wang B, Zhang J, et al. Roles of Wnt/β-catenin signaling in the gastric cancer stem cells proliferation and salinomycin treatment. Cell Death Dis. 2014; 5(1):e1039.
33. Li T, Su L, Zhong N, Hao X, Zhong D, Singhal S, et al. Salinomycin induces cell death with autophagy through activation of endoplasmic reticulum stress in human cancer cells. Autophagy. 2013; 9(7):1057-68.