ORIGINAL ARTICLE

Cytotoxicity A549 Cancer Cells Apoptotic

Effect of GW9508 Cytotoxicity on A549 Cancer Cells and Expression Bax Apoptotic Gene

SIMA OROUEI[1], MALIHEH ENTEZARI[1*], MEHRDAD HASHEMI[1], KIAVASH HUSHMANDI[2], ABOLFAZL MOVAFAGH[3*] .

[1]*Department of Genetic Faculty of Advanced Science and Technology, Islamic Azad University, Tehran Medical SciencesTehran, Iran.*

[2]*PhD student of epidemiology, Department of food hygiene, Faculty of Veterinary Medicine, Tehran University, Tehran, Iran.*

[3]*Department of Medical Genetics, cancer Research Center, School of Medicine, ShahidBeheshti University of Medical Sciences,*

Correspondence to Dr. Maliheh Entezari, Emai, Iran. mentezari@iautmu.ac.ir and Dr. AbolfazlMovafagh, E-mail: Movafagh_a@yahoo.com, Telephone: +98 912 130 7881,

ABSTRACT

Background: Small molecules such as GW9508 can easily cross the cell membrane and reach the target area because of their very low weight. Also, due to the high costs of treatment and cancer drugs, the need to replace pharmacologic factors and cost-effective anticancer drugs is felt.

Aim: To investigate the anticancer and cytotoxic effects of GW9508 on A549 lung cancer cells.

Methods: In this experiment, cell line (A549) was first cultured in DMEM medium containing 10% FBS and then treated with different concentrations of both compounds. MTT assay was performed on days 1, 3 and 5 to determine IC50 and to compare the viability of cells treated with different concentrations of GW9508. QRT-PCR assay was used to evaluate the effect of GW9508 with IC50 concentration on apoptosis induction and Bax gene expression.

Results: The results showed that GW9508 in a dose and time-dependent manner significantly reduced the viability and proliferation of A549 cells ($P \leq 0.001$). The control group showed a significant decrease compared to the first- and third-day samples ($P < 0.05$). Morphological changes such as decreased chromatin density and cell rounding were also observed in the cells. Also, molecular results showed that GW9508 was able to increase Bax gene expression.

Conclusion: GW9508 small molecule induces cell death in lung cancer cells by decreasing cell viability and increasing Bax gene expression. As a result, it has the potential to induce cell death in cancer cells and cancer treatment.

Keywords: GW9508, Lung Cancer, Apoptosis

INTRODUCTION

Lung cancer was initially prevalent in men, but nowadays, death from lung cancer in women has surpassed deaths from breast cancer[1]. In 2013, the United States estimated 229,000 cases of lung cancer and 16,000,000 deaths, accounting for about 27% of all deaths from all types of cancer[2]. While the relationship between smoking and lung cancer is well known, statistics show that lung cancer is a multifactorial process[3]. Various researchers have proven that more than twenty different genetic and epigenetic alterations must be assembled to cause lung cancer. Among the tumor-generating genes, oncogenes were the first genes to be identified. They need other factors such as mutations in other genes or environmental factors such as viral infection to cause cancer[4]. The tumor suppressor protein is one of the proteins that is involved in lung cancer, which inhibits growth by attachment to DNA. Mutations in this protein gene increase its expression, which leads to cell overgrowth, DNA damage, and cell cancer, with approximately 70% of lung cancer due to mutations in its expression[5]. In the apoptosis process, regulatory molecules include the anti-apoptotic Bcl2 and proapoptotic members (Bax and Bad genes), the Bcl-2 gene inhibiting apoptosis, whereas the Bad, Bax and P53 genes induce it.

Lung cancer was initially prevalent in men, but nowadays, death from lung cancer in women has surpassed deaths from breast cancer[1]. In 2013, the United States estimated 229,000 cases of lung cancer and 16,000,000 deaths, accounting for about 27% of all deaths from all types of cancer[2]. While the relationship between smoking and lung cancer is well known, statistics show that lung cancer is a multifactorial process[3]. Various researchers have proven that more than twenty different genetic and epigenetic alterations must be assembled to cause lung cancer. Among the tumor-generating genes, oncogenes were the first genes to be identified. They need other factors such as mutations in other genes or environmental factors such as viral infection to cause cancer[4]. The tumor suppressor group is one of the groups that is involved in the flow of the tumor, which grows by attachment to high DNA. Mutations in this protein gene increase its expression, which leads to indiscriminate cell growth, DNA damage and cell cancer, with approximately 70% of lung cancer due to mutations in its expression[5]. In the apoptosis process, regulatory molecules include the anti-apoptotic Bcl2 and proapoptotic members (Bax and Bad genes), the Bcl-2 gene inhibiting apoptosis, whereas the Bad, Bax and P53 genes inducing it.Depending on how the message of death is delivered to the cell, the pathway of activation of cell death is different. If the messages are internal, the first activated organ of the mitochondria will be transmitted to the caspase cascade if the message reaches the cell's surface receptors. The rate of death induced by the mitochondrial pathway is more severe, leading to proteolytic activity that causes and enhances the phenotype of cell death and subsequently is associated with DNA fragmentation, chromatin compaction, cell shrinkage, and plasma membrane[6]. In the mitochondrial

pathway or the intrinsic pathway of apoptosis, cytochrome c is released from the space between the two mitochondrial membranes into the cytoplasm. Cytochrome c interacts with apoptotic protease activating factor 1 and procaspase-9 to produce apoptosomes[7]. The apoptosome activates the caspase cascade and the apoptosis process occurs. The Bax protein leads to the release of cytochrome c, which in turn induces apoptosis[8]. Programmed cell death or apoptosis is the basis for new therapeutic targets that induce cancer cell death or their susceptibility to apoptosis. Cytotoxic agents increase the effectiveness of radiation therapy, so this process has been extensively studied by cancer researchers. Therefore, substances capable of inducing cell death and expression of pro-apoptotic genes may be used in the treatment of cancer[9]. Recent studies have shown that unsaturated fatty acids, such as linoleic acid, play an important role in the treatment of cancer. GPR40 is a receptor for long-chain unsaturated fatty acids (FFAR1), which is a type of G protein-coupled receptor. Generation and enhancement of oxidative stress of mitochondrial origin can lead to cell apoptosis and autophagy[10,11]. Much evidence suggests that FFAR4 free fatty acids FFAR1 and FFAR1 play an important role in metastasis and migration and tumor formation, and FFAR4 and FFAR1 appear to have opposite functions in cancer biology[12]. Lack of FFAR1 in pancreatic cancer stimulates cancer cell migration, whereas FFAR4 deficiency Inhibits metastasis so the GPR40 receptor inhibits locomotor activity, invasion, and tumor characteristics of pancreatic cancer cells[13], and a study has shown that activation of the GPR40 receptor by GW9508 enhances the activity of inflammatory cells such as neutrophils[14]. GW9508 as a GPR40 fatty acid receptor agonist specifically induces the death of bone marrow progenitor cells by mitochondrial oxidative stress[15]. Therefore, due to the anticancer properties and cell death induction of unsaturated fatty acids such as linoleic acid after binding to GPR40 receptors, the GW9508small molecule is a good option for investigating the anticancer effects, induction of cell death and its mechanism of action in cancer treatment. In the present study, we sought to investigate the anticancer effects and induction of apoptosis and autophagy death of this small molecule on lung cancer cell line or A549.

MATERIALS AND METHOD

GW9508 solution preparation: GW9508 was purchased as a powder from Sigma and dissolved in DMSO to give the original stock solution in sterile conditions. Then concentrations of 1, 10, 50 and 100 μM of GW9508 were prepared as doses used in cell culture and then filtered. The extracts were maintained by a 0.22 μm syringe filter (Bio Fact BSS20-PE2-Korea) at 4°C until use.

Cell culture and passage: A549 lung carcinoma cell line was purchased from Pasteur Institute of Tehran and cultured in DMEM medium (Gibco, USA) containing 10% FBS (Gibco, USA) and incubated (Sina Co., Iran). They were maintained with 5% CO_2, 95% humidity at 37°C. When the cell density in the flask reached 80%, cell passage was performed and cells were cultured with approximately 1×10^4 in a 96 well plate containing complete culture medium. After 24 h, cells were treated with different concentrations of small molecules and cell viability was evaluated at 1, 3 and 5 days.

Evaluation of cell viability by MTT assay: Cell viability and IC50 were determined by MTT (Dimethylthiazol-2-yl) - 2,5-Diphenyltertrazolium Bromide)[3-5]. Cell count was 1×10^4 cells/cm 2 on a plate. 96 wells were cultured in normal medium and incubated for 24 h, then cells were treated with 1, 10, 50 and 100 μM of GW9508 concentrations and cell viability was evaluated at 1, 3, and 5 days. Then a control group was considered untreated, then MTT assay in which the culture medium was withdrawn from the wells containing cell and at each time approximately 100 μl fresh medium containing 10 μM MTT solution (with 5 mg / m concentration 1 ml) was added, the cells were incubated for 3 h at 37°C, then the MTT solution was extracted, and 100 μM DMSO (Merck, USA, 100%) was added to each well and the optical absorption of the samples was measured at 570 nm using a microplate reader (Stat fax 2100, Florida, USA).

Morphological and staining studies of Giemsa: For morphological study of A549 cells from control groups treated with IC50 concentrations of the small molecule at days 1, 3 and 5 in 96 well plates were imaged using a digital camera connected to an inverted microscope with a lens of 20 and 40 microscopic images. Each cell is examined and compared in control and patient groups. Then, after the preparation of Giemsa 4% staining, cells were stained and the morphology of treated cells was compared with control cells.

RNA extraction and cDNA synthesis: RNA extraction was performed according to the kit protocol of CinnaGen. All steps were performed according to the protocol using kit solutions. Finally, a nano-drop spectrophotometer was used to measure RNA and measure the properties and size of RNAs at wavelengths 260/280 nm was used. Once the RNA was purified, cDNA was synthesized using the HyperScript kit (GeneAll - Portugal) and the steps were performed according to the instructions. And 0.5μm Forward Primer, 0.5μm Reverse Primer, 0.5μm SYBR Green, and 5μm distilled water using Power SYBER Green master mix (Qiagen, Japan) in a final volume of 10μm and by Real-Time PCR will be performed.

Real-Time PCR: To measure gene expression, the time-heat program is performed in 3 steps in the Thermal Cycler apparatus as follows. Stage one is denaturation reaction at 95°C for 30 seconds, followed by melting at 95°C for 1 second and finally the third application stage at 60°C for 33 seconds for 40 cycles. Data analysis (CT) of each sample is performed using StepOne software and normalization will be performed using the GAPDH gene (housekeeping control gene) in order to estimate the fold change rate of Bax, Bad, Bcl2, and p53 were compared to control (untreated) samples in comparison to the small molecule-treated sample in comparison to the GAPDH gene threshold cycle, and each experiment was repeated three times.

Design and synthesis of primers: The primers used in this study are based on the corresponding gene sequence obtained from the NCBI site and direct and reverse primers are designed using Gen runner and primer express software.

Table 1: Initiator primer sequences

Name	Primer Sequence(5'-3')	Tm9(C)
BAX(F)	GCTTGGACTTCCTC	58/5
BAX(R)	ACCACTGTGCCTGCTCCA	
GAPDH(F)	GCAAGAGCACAAGAGGAAGA	57
GAPDH(R)	ACTGTGAGGAGGGGAGATTC	

Statistical analysis: The Livak method is used to analyze the data obtained from this reaction. GraphPad Prism, One-way ANOVA and t-test software were also used for statistical analysis. P <0.05 was considered as a significant difference for samples.

RESULTS

Evaluation of Cell Viability in Lung Cells Treated with GW9508 Small Molecule: The figure below shows the viability rate of A549 cancer cells treated with different concentrations of GW9508 tested compared to the control sample. Using MTT assay after 24 h, the IC50 concentration of GW9508 small molecule was determined to be 25 μM for A549 cells (P <0.05). Figure 2 shows the lethal effects of this compound on the viability of A549 cells on day 1, day 3 and day 5. As shown in the figure, the lethal effect of this compound is dose-dependent and decreases with increasing concentration of cell viability. According to the results of this test, the lethal effect of GW9508 small molecule is dose and time-dependent, with its lethality increased on the third day compared to the first day and on the fifth day compared to days 3 and 1 (P <0.05).

Morphological changes of A549 cells treated with GW9508 small molecule: Morphological observations of A549 cancerous cells by invert microscopy showed that different concentrations of the GW9508 small molecule caused noticeable morphological changes in the cancerous cells. These changes included a significant decrease in cell size with their rounding and shrinkage, which was noticeable when compared to control samples, and the granulation of cells was consistent with other changes (Fig. 3-D). Morphological changes of A549 cells using Giemsa staining and fluorescence microscopy showed shrinkage and increase in vacuole size, decrease in the cytoplasm, nucleation pigmentation and chromatin fragmentation (Figure B-3) resulting from morphological changes. And as a result, there are signs of A549 apoptosis, which is quite evident.

Figure 1 - Effects of different concentrations of GW9508 small molecule on the viability of A549 cells by MTT assay and determination of 25μM concentration as the IC50 of A549 cells (different letters indicate significant differences between groups) (P <0.05). The arrow indicates the concentration of IC50).

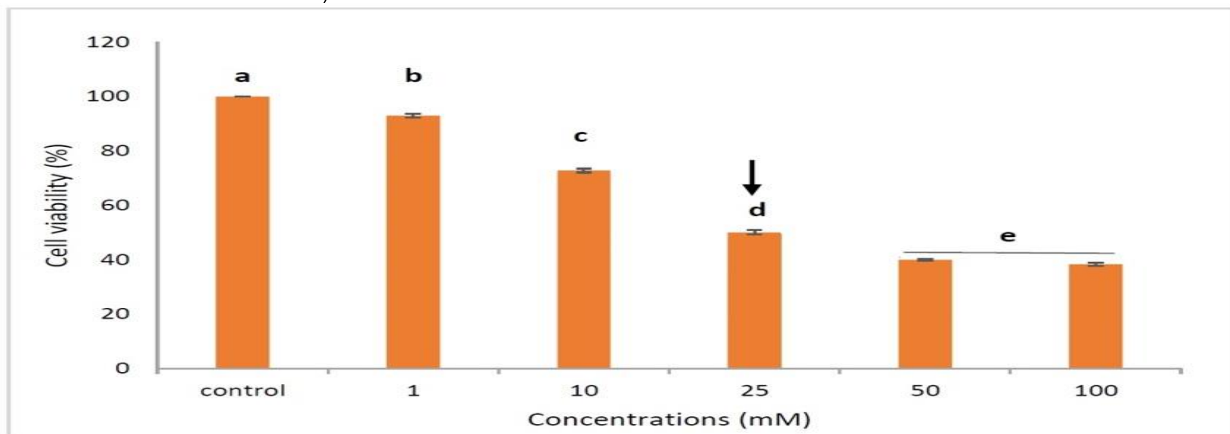


Figure 2:- Effects of IC50 concentration of GW9508 (G2) on A549 cell viability on days 1, 3 and 5 after treatment - (P <0.05).

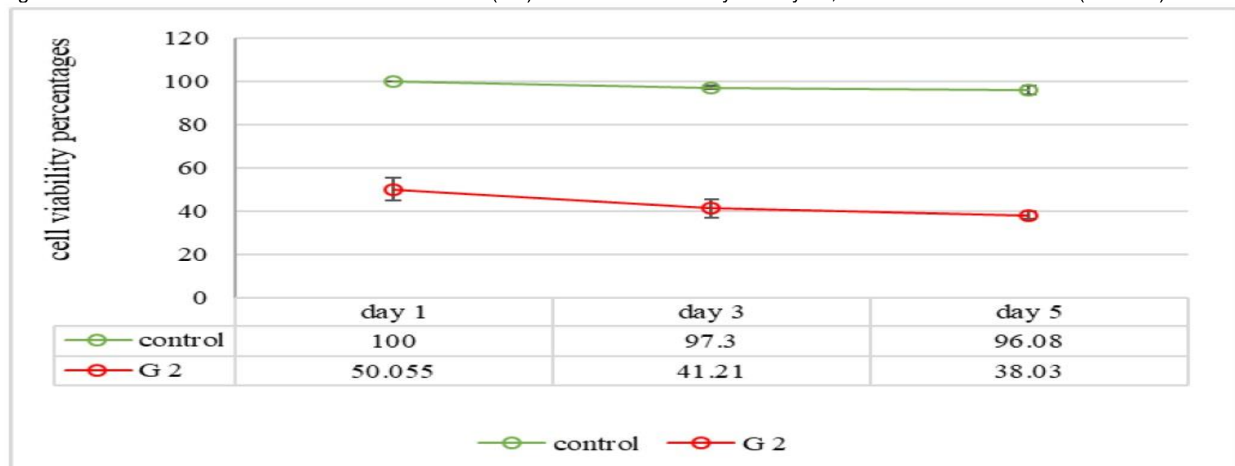
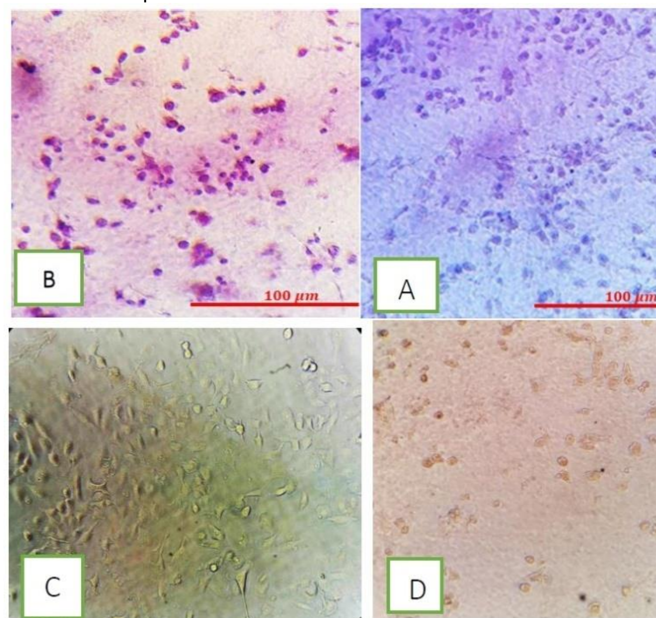
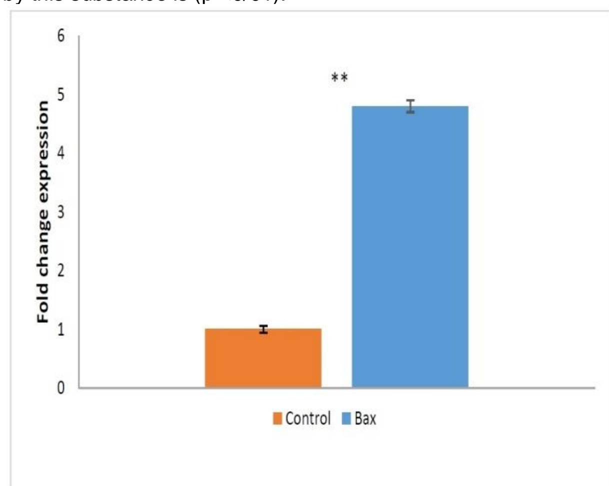


Figure 3 - Morphology of A549 cells treated with IC50 concentration of GW9508 small molecule -A- control group B- cells treated with Gw9508 stained with Giemsa C- Control group with conventional invert microscope -D- group treated with Gw9508 by invert microscope



Evaluation of Bax Gene Expression in A549 Cells Treated with IC50 GW9508 Small Molecule: Gene analysis results show that Bax apoptotic gene expression is increased in cells treated with GW9508 compared to the control group since increased Bax gene expression induces apoptosis, so GW9508 small molecule causing cell death in cancer cells.

Figure 4 - Changes in Bax gene expression in 24-hour A549 cells treated with low IC50 concentration of GW9508: Increased expression of Bax gene in A549 cells treated with IC50 concentration of small molecule in A549 cell line compared to control group indicating induction of apoptotic death in cancer cells by this substance is (p <0/01).



DISCUSSION

In this study, A549 lung cancer cells were treated with different concentrations of the GW9508small molecule. The results of this study showed that GW9508 can cause significant changes in the morphology of A549 cells. This combination significantly reduced the growth and proliferation of A549 cells compared to the control group.
The induction of cell death by GW9508 was directly related to its increase in concentration and duration of treatment. In addition, analysis of apoptosis and Bax gene analysis by PCR showed that the tested compound stimulated and increased Bax gene expression in A549 cells. Finally, the results showed that GW9508 enhances the lethal potency and induces apoptosis in cancer cells. This may be due to the GW9508 agonist being a long-chain free fatty acid receptor or GPR40 and induction of mitochondrial oxidative stress that causes cell death in the cells[15]. Since the apoptotic process has two internal pathways of mitochondrial origin and an outer pathway induced by death receptors, and the rate of death induced by surface receptors of death is more severe than the mitochondrial pathway[6], GW9508 stimulates this pathway by causing cell death and cell death. DNA fragmentation, chromatin aggregation, cell shrinkage. The permeability of the mitochondrial membrane to cytochrome c is determined by the relative ratio of proapoptotic and antiapoptotic mediators. The pro-apoptotic molecules Bax and Bak increase the permeability of the mitochondrial membrane and neutralize the coupling of pro-apoptotic molecules with antiapoptotic factors (Bcl2, Bcl-xl). Thus, the relative amount of proapoptotic mediators (Bax, Bad, Bak, and Bid) and antiapoptotic (Bcl2, Bcl-xl) determines the amount of cytochrome c available for apoptosome formation[16]. Extensive studies on the anticancer effects and induction of apoptosis by unsaturated fatty acids such as linoleic acid and omega-3s have been performed with activation of the GPR40 receptor in cancer cells, which is consistent with the findings of the present study, including Pierre (2013) studied the effects of free fatty acid trans-10, cis-12 CLA on the induction of cell death in human colon cancer and stated that it increases apoptosis in endoplasmic reticulum through increased ROS production (oxidative stress). Their studies showed that CLA-treated tumor cells suppressed growth by inducing apoptosis and inhibiting cell cycle and angiogenesis. Their studies also showed that the apoptosis studied is caspase-dependent, in fact by enhancing Bax gene expression and releasing cytochrome C from the mitochondrial interstitial space, it activates the caspase cascade and induces apoptosis[17]. Clinical studies have recently shown the potential link between long-chain unsaturated fatty acids and some cancers. They exert their anticancer and cytotoxic properties through different metabolic pathways[18,19]. Long and medium-chain free fatty acids, especially the relative acids between omega-3 and omega-6, are widely discussed in modern diets due to their antitumor properties. In malignant melanoma, omega-3s prevent tumor growth and prevent malignant melanoma invasion and metastasis by activating proapoptotic and blocking angiogenesis[20]. In 2002, Briscoe, T, and colleagues first identified free fatty acids such as omega-3 and omega-6 as the G protein-coupled receptor ligand, or GPR40[21]. Several clinical and pre-clinical agonists were also tested as GPR40 agonists, including TAK-875,

LY2922470 and SHR0534[22]. The role of the GPR40 receptor in several cancer cells has been investigated[23]. In breast cancer, the inhibitory and stimulatory effects of the tumor with different GPR40 receptor agonists indicate that the use of omega-3 fatty acids or synthetic GPR40 agonists such as GW9508 and TUG-891 inhibit tumor growth[24]. Researchers have also studied the effects of omega-3 fatty acid inhibitors on the proliferation of prostate cancer cells by the F-protein coupled receptor (FFA4) of the fatty acid receptor family (FFAR)[25]. A strong evidence of the relative effect of fatty acid inhibitors. Omega-3 is present in many human cancers[26], including breast cancer [27]. In a study of the effect of FFAR agonists including TUG-891 and GW9508 small molecule on proliferation MDA-MB-231 and MCF-7 breast cancer cells have been studied. The results of the study indicate that both of these compounds inhibit the proliferation of cancer cells. GW9508 as a specific agonist of the FFA1 receptor, its lethality (IC50 = 16nm) is much stronger than the combination of TUG-891 with IC50 = 403nm.

GW9508 also prevented the migration and metastasis of MCF-7 breast cancer cells. The PCR results of this study also indicate that FFAR free fatty acid receptor genes and proteins, including GPR40, are expressed in breast cancer. And activation of FFAR receptors by each of its agonists, including GW9508, inhibits proliferation and migration of MDA-MB-231 and MC7-7 cancer cells [28]. Other studies on this small molecule have shown that GW9508 induces apoptosis by binding to the GPR40 receptor as its unsaturated free fatty acids[12,13].

Small molecules, because of their very low weight, can easily cross the cell membrane and reach the target region. Also, because of the high costs of treatment and cancer drugs, the need to replace pharmacological factors and anti-cancer drugs that are cost-effective is felt, and the use of small molecules in cancer treatment imposes a lower cost on the patient. Thus, with these features, the GW9508 small molecule has the therapeutic potential to induce cell death in cancer cells and to treat cancer[29].

CONCLUSION

The study of the effects of GW9508 small molecule on apoptosis induction in lung cancer cells was investigated. Results from MTT assay, morphological and PCR changes confirmed that GW9508 small molecule induces apoptosis in cancer cells by decreasing cell viability and enhancing Bax gene expression.

REFERENCES

1. Von Dincklage JJ, Ball D, Silvestri GA. A review of clinical practice guideline for lung cancer. Journal of Thoracic Disease. 2013; 5(suppl 5): S607-S622
2. American Cancer Society. Cancer Facts & Figure 2013. Atlanta, GA: American Cancer Soc; 2013.
3. Zappa C, Mousa SA. Non-small cell lung cancer: current treatment and future advances. Translational lung cancer research. 2016; 5(3): 288-300
4. Cooper WA, Lam DC, O'toole SA, et al. Molecular biology of lung cancer. J Thorac Dis. 2013; 5 (suppl 5): S479- S490.
5. Baldi A, De Luca A, Esposito V et al. Tumor Suppressors and Cell-Cycle Proteins in Lung Cancer. Patholog Res Int; 2011. 605042.
6. Honardoost M, Soleimanjahi H, Rajaei F. Apoptosis: programmed cell death. J Qazvin Univ Med Sci 2013, 17(3): 48-57
7. Adrian C, Martin, SJ. The mitochondrial apoptosome: a killer unleashed by the cytochrome seas. Trends BiochemSci, 2001; 26 (6): 390-397.
8. Torki-Boldaji B L Azadi, Tavalaee M, Nasr- Esfahani MH. Human sperm cryopreservation update in treatment of infertility: a review study. J Cell and Tissue, 1995; 8: 332-351.
9. Fesik SW. Promoting apoptosis as a strategy for cancer drug discovery. Nature Reviews Cancer,2005; 5(11): 876.
10. Gao B, Huang Q, Jie Q. Dose-response estrogen promotes osteogenic differentiation via GPR40 (FFAR1) in murine BMMSCs. Biochimie, 2015;110:36-44.
11. Philippe C, Wauquier F, Léotoing L, et al. GW9508, a free fatty acid receptor agonist, specifically induces cell death in bone resorbing precursor cells through increased oxidative stress from mitochondrial origin. Experimental cell research, 2013;319(19): 3035-3041.
12. Movafagh A, Mirfakhraei R, Mousavi-Jarrahi A. Frequent incidence of double minute chromosomes in cancers, with special up-to-date reference to leukemia. Asian Pacific Journal of Cancer Prevention. 2011;12: 3453-3456.
13. Fukushima K, Takahashi K, Fukushima N, Honoki K,. Different effects of GPR120 and GPR40 on cellular functions stimulated by 12-Otetradecanoylphorbol-13-acetateinmelanomacells.Biochem Biophys ResCommun, 2016; 475:25–30.
14. Movafagh A, Maleki F, Fadaie S, AzarGashb E. Persistent unstable chromosomal aberrations in lymphocytes of radiotherapy workers after 1st mitotic division in Tehran, Iran. Pakistan Journal of Medical Sciences. 2007;23: 254-258.
15. Gao B, Huang Q, Jie Q, et al. Dose-response estrogen promotes osteogenic differentiation via GPR40 (FFAR1) in murine BMMSCs. Biochimie, 2015; 110: 36-44.
16. Ashkenazi A, Dixit VM. (1998). Death receptors: signaling and modulation. Science, 1998; 281 (5381): 1305-1308.
17. Pierre AS, Minville-Walz M, Fèvre C, et al. 2013. Trans-10, cis-12 conjugated linoleic acid induced cell death in human colon cancer cells through reactive oxygen species-mediated ER stress. Biochimicaet Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids, 2013; 1831(4): 759-768.
18. Doll R, Peto R. The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. JNCI: Journal of the National Cancer Institute. 1981; 66 (6): 1192–1308.
19. Milligan G, Shimpukade B, Ulven T, et al. Complex pharmacology of free fatty acid receptors. Chemical Reviews.2016; 117 (1): 67.
20. Serini S, Fasano E, Celleno L, et al. Potential of long-chain n-3 polyunsaturated fatty acids in melanoma prevention. Nutrition Review. 2014;72; 255–266.
21. Briscoe C, Tadayyon M, Andrews J, et al. Theorphan G protein-coupled receptor GPR40 is activated by medium and long chain fatty acids.Journal of Biological Chemistry. 2003;278; 11303.
22. Li Z, Qiu Q, Geng X, et al. Free fatty acid receptor agonists for the treatment of type 2 diabetes: drugs in preclinical to phase II clinical development. Expert Opinion on Investigational Drugs. 2016;25; 871–890.
23. Hardy S, St-Onge GG, Joly E, et al. Oleate promotes the proliferation of breast cancer cells via the G protein-coupled receptor GPR40. Journal of Biological Chemistry. 2005;280; 13285–13291.
24. Lin DC, Guo Q, Luo J, et al. Identification and pharmacological characterization of multiple allosteric binding sites on the free fatty acid receptor. Molecular Pharmacology. 2012;82: 843–859.
25. Liu Z, Hopkins MM, Zhang Z, et al. ω-3 Fatty acids and other FFA4 agonists inhibit growth factor signaling in human prostate cancer cells. J Pharm ExpTher, 2015; 352: 1–15.
26. Hardman WE. ω-3 Fatty acids to augment cancer therapy. J Nutr, 2002, 132, 3509S–3512S.
27. ZhengJS, Hu XJ, Zao YM,et al. Intake of fish and marine n-3 polyunsaturated fatty acids and risk of breast cancer: Meta-analysis of data from 21 Independent Prospective Cohort Studies. Br Med J, 2013; 346: 3706.
28. Hopkins M M, Zhang Z, Liu Z, et al. Eicosopentaneoic acid and other free fatty acid receptor agonists inhibit lysophosphatidic acid-and epidermal growth factor-induced proliferation of human breast cancer cells. Journal of clinical medicine,2016;*5*(2): 16.
29. Veber DF, Johnson SR, Cheng HY, et al. (2002) Molecular properties that influence the oral bioavailability of drug candidates. J Medicinal Chemistry, 2002; 45(12):2615–262