Research Article

Diosgenin decrease proliferation of human lung cancer cells by promoting cholesterol efflux via up-regulation of LXR-α and its target ABCA1 expression

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Abstract

Background: Several reports are available on the antitumor effects of diosgenin in various cancer cells. However, the effect of diosgenin on LXR-α and its target ABCA1 has not been studied in lung cancer cells. **Objective**: To inspect the effect of diosgenin reduce cell proliferation in lung cancer cell line by the induction of LXR-α and ABCA1 in lung cancer cell line. **Materials and Methods:** Cell viability was determined by MTT assay, Cells proliferation was determined by Wound healing/scratch assay and mRNA and proteins expression were examined by Semi-Quantitative RT- PCR. **Results:** Diosgenin inhibits A549 cell viability in a dose dependent manner and was proved by MTT assay. Diosgenin inhibits proliferation of A549 cell wound healing/scratch assay. Diosgenin (45 µM) induces mitochondrial mediated apoptosis by the activation of caspase-3, up-regulation of LXR-α, ABCA1, Bax, caspase-3and down-regulation of Bcl-2. **Conclusion:** Diosgenin reduce proliferation in A549 cells by promoting cholesterol efflux via up-reguatinof LXR-α and ABCA1 expression.

Keyword: ATP-binding cassette transporter A1, Diosgenin, Lung cancer, Liver x receptor-α

Introduction

Lung cancer is the most widespread type of cancer and is also one of the major causes of death in the world. The two extensive types of lung cancer are non-small-cell lung cancer (NSCLC) (85%) and small-cell lung cancer (SCLC) (15%). The 5-year continuity rate for lung cancer patients is alone 8–14% due to initial metastasis and the absence of effective remedial treatment for the metastasis (Lim et al., 2017; Liu et al., 2017) Lung cancer is a multi-step movement of acquire genetic and epigenetic change caused by perpetual presentation to cancer-causing agents. The apprehension of lung cancer and unique treatments form extreme advances in the past few decades, notably for NSCLC. Targeted therapies with exhibited advantage for NSCLC patients have been received in clinic application (Yuan et al., 2017).

Findings of some studies have that discovered many drugs of Traditional Chinese Medicine (TCM) are effective in the treatment of malignant tumors, also including HCC. Diosgenin, a steroidal saponin, which is mostly separated from root of wild yam (*Dioscorea villosa*) which is a customary medication though activities of anti-inflammatory, antitumor, anti-diabetes and anti-atherosclerosis (Nie et al., 2016; Gao et al., 2013). Diosgenin inhibits the proliferation of prostate cancer by the induction of apoptosis and autophagy, and mechanism behind this induction occurs via PI3K/Akt/mTOR signaling pathways (Rahmati et al., 2014; Jiang et al., 2016). Diosgenin has enacted ROS-subordinate autophagy and cytotoxicity via mTOR signaling pathway in chronic myeloid leukemia cells (Li et al., 2014). Autophagy plays a dual aspect in cancer and can act as a tumor silencer or tumor initiator. Dysfunction of autophagy is related with chromosome anxiety, DNA harm, cell death, cell proliferation, evaluation and innate immunity (Jiang et al., 2016; Jeyamohan et al., 2016). Diosgenin-induced autophagic cell death endure unclear and the definite mechanism behind its involvement with apoptosis and autophagy is not clearly prominent.

Diosgenin induces apoptosis in HCC cell by inducing caspase -3, -8 and -9 (Li et al., 2014). Liver X receptor (LXR) is a transcription factor markedly communicated the liver, adipocytes, macrophages and intestine. It reside to the nuclear receptor superfamily and forms heterodimers with

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retinoid X receptor (RXR) (Dai et al., 2016). LXR can also be activated by synthetic agonists, such as TO901317 in MCF-7 cell line (El Roz et al., 2012). And that LXR inhibited the proliferation, and induced the apoptosis of melanoma cells (Zhang et al., 2012). A study on diosgenin revealed that it increases ABCA1-dependent cholesterol efflux and inhibits aortic atherosclerosis evolution by suppressing macrophage miR-19b expression (Lv et al., 2015). LXRs induced through T0901317 prohibit the aggression and metastasis by repressing the NF-κB/MMP-9 signaling pathway in NSCLC (Liver X receptors agonist T0901317). Gefitinib resistance by inhibition of vimentin using LXR ligands, along with GW3965 in NSCLC (Hu et al., 2017). Enacted LXR/RXR heterodimers persuade genes implicated in the regulation of cholesterol metabolism, with the ABCA1 and ABCG1, which arouse cholesterol efflux starting cells to apo lipoprotein A-I (APOA-I) also high-density lipoprotein (HDL) separately. A new pharmacological approach has been developed by the use of LXR ligands, has led to retarding atherosclerosis and cardiovascular diseases (Lv et al., 2015). We introduce that the LXR deprives cancer cell membranes of lipids crucial for their growth, through provocative cholesterol efflux.

Hence, we investigated the inhibitory effect of diosgenin that diminished the proliferation of A549 cells in a dose-dependent manner and exerted an anti-proliferative effect in the A549 cells by apoptosis and autophagy. Moreover, Diosgenin upregulated LXR-α and ABCA1 expression which activates the Caspase and ATG5 that leads to apoptosis and autophagy through the suppression of PI3K/AKT signlling pathway in A549 cells. The results imply that diosgenin potentially exhibits chemopreventive effects on the death receptors of apoptotic and autophagy pathways.

Materials and methods

Chemicals and reagents

Diosgenin ($C_{27}H_{42}O_3$; MW 414.62 kDa), were purchased from Sigma–Aldrich Co. Diosgenin stock solution was prepared in ethanol. Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), MTT [3-(4, 5-dimethylthiazol- 2yl)-2, 5-diphenyltetrazolium bromide], were purchased from Invitrogen. Antibodies against LXR-α, ABCA1, Bcl2, Bax, Caspase and GAPDH were purchased from Sigma–Aldrich Co.

Cell culture

Human lung cancer cell line, A549, was purchased from the National Centre for Cell Science (NCCS), India. Cells were consequently grown in DMEM supplemented with 10 % (v/v) FBS and 1 % (w/v) penicillin/streptomycin at 37°C in a humidified atmosphere 95 % air and 5 % CO_2.

MTT Assay

Cell viability was fixed by the MTT assay. The cells were implanted in 96-well plates at a density of 5 × 10^3 cells/well and treated with Diosgenin at various concentrations (1– 45 μM) for 24h. After the liability period, media were evacuated. Following, the medium was changed and incubated with MTT (45µl) for 3 h. The possible cell number per dish is directly proportional to the synthesis of formazan, which was solubilized in isopropanol and deliberate spectrophotometrically at 570 nm (Aggarwal et al., 2006).

Wound healing/scratch Assay

A549 cells were seeded in 6 well plates, after reaching 80% of confluence, cells were treated with or without diosgenin. A wound was initiated by using micropipette p100 tip in the center area of confluent cells. Cell migration was appraised with time and images were taken using phase-contrast microscopy (Floid cell imaging station).

Quantitative real-time PCR

A549 cells were incubated with 45µM diosgenin for 24 hr behind incubation, total RNA was isolated with Trizol. Equal quantities of RNA (2µg) from each sample were used to synthesize cDNA with a cDNA synthesis kit. RT- PCR assay was carried out using PCR master mix in 5µl by the Step one plus RT-PCR (Applied Biosystem). Quantitative RT-PCR primers are listed in (Table 1). The GAPDH gene was used for RNA template normalization.

Statistical analysis

One-way ANOVA using GraphPad Prism software (version 6.0) was used for all the statistical analysis. A difference with $P<0.05$ is considered statistically significant.

Results

Cytotoxic effect of Diosgenin on A549 cells

MTT assay was performed to assess the cytotoxicity effect of diosgenin on A549 cells. A549 cells were treated with

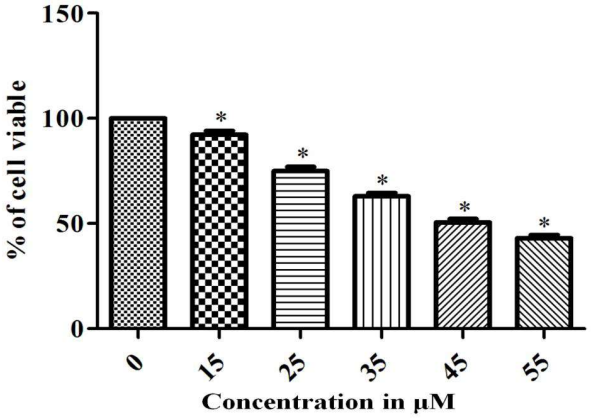

Figure 1. MTT assay: Cells were treated with diosgenin (0-100 µM) for 24 h to determine the cytotoxicity of diosgenin using MTT assay. Cell viability was significantly reduced at 45 µM diosgenin concentration compared with the control and treated cells (*P< 0.05).

diosgenin (0–45μM) and 45μM IC_{50} value was determined. Diosgenin obviously instigated cell death in A549 cells in a dose-dependent manner when compared to control (Figure 1).

Diosgenin inhibits proliferation of A549 cells

Wound healing/scratch assay was implemented with time and images were taken (Figure 2). In control cells, scratch was approximately closed at 24 h. However, in Diosgenin treated cells, the scratch did not closed at 24 h. These data proposed that diosgenin suppressed the proliferation of A549 cells.

Diosgenin upregulates the LXR-α and ABCA1 signaling pathway in A549 cell line

To investigate the aspect of LXRα and ABCA1 expression in A549 cell, we first studied the expressions of LXR and ABCA1 in lung cancer A549 cells using reverse transcription-PCR (Figure 3a, b). Both LXRα and ABCA1 were upregulated in A549 cells. Upon diosgenin treatment, expressions of LXR target genes, ABCA1, were increased in A549 indicating ABCA1 is the direct target of LXR-α in lung cancer.

Diosgenin reduce the cell prolifertion of A549 cell line via LXR-α mediated caspase pathway

The effect of diosgenin on the expression of antiapoptotic Bcl-2, pro-apoptotic Bax and caspase-3 were analyzed by reverse transcriptase PCR (Figure 4a-b). Antiapoptotic Bcl-2 mRNA expression was decreased and Bax and caspase-3 mRNA expressions were increased in A549 cells. Our results indicated that diosgenin triggers the intrinsic pathway-mediated apoptosis by decreased Bcl-2 expression and increased Bax and caspase-3 expressions in A549 cells.

Discussion

In the present study, we investigated the effect of diosgenin in lung cancer A549 cell line. The results demonstrated that diosgenin exerted a strong growth inhibitory activity against A549 human lung cancer cells. Diosgenin reduce the cell proliferation in A549. Further, our study showed that the up-regulation of LXR-α, ABCA1, Bax, down

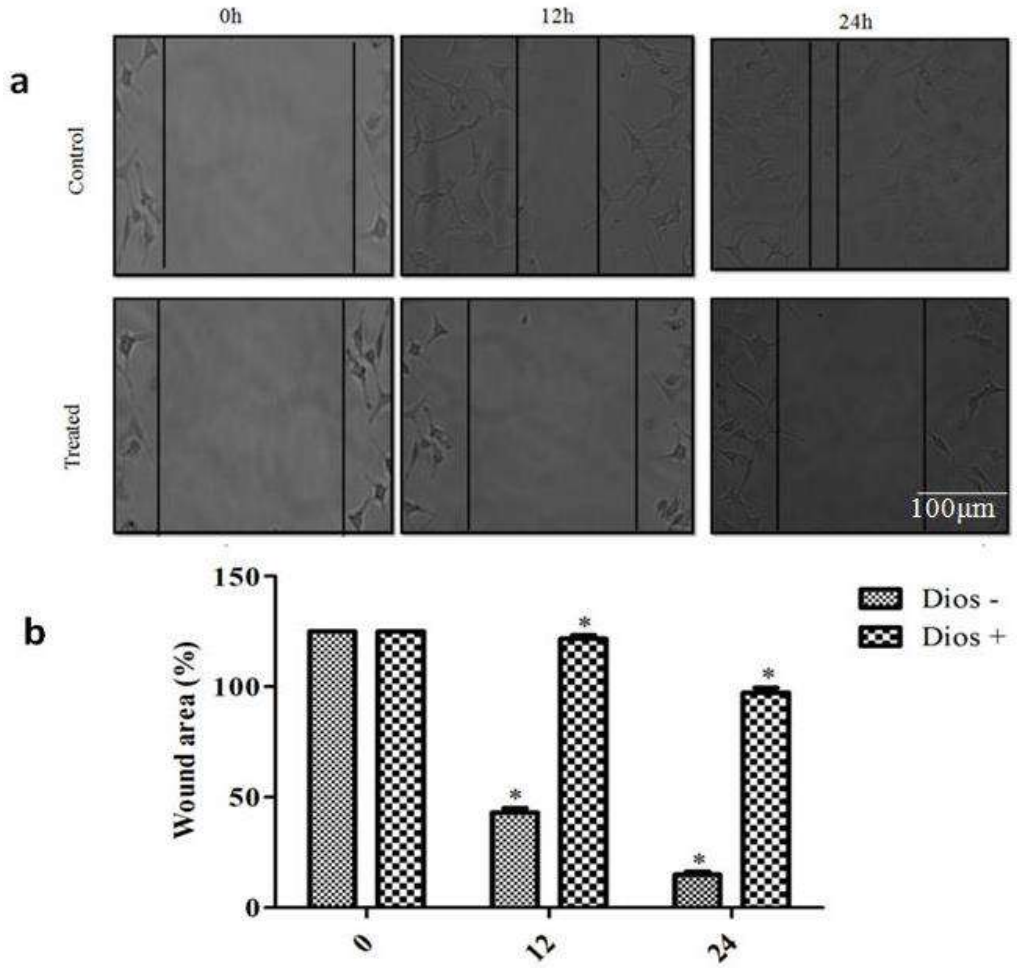

Figure 2. Wound healing/scratch assay. A549 cells were treated with or without 45µM Diosgenin. (a) Wounds were created in the cultured cells and pictures were taken using a cell imaging station (×20) after 0, 12 and 24 h of diosgenin treatment respectively. (b) Wound closure area quantification data (*P<0.05)

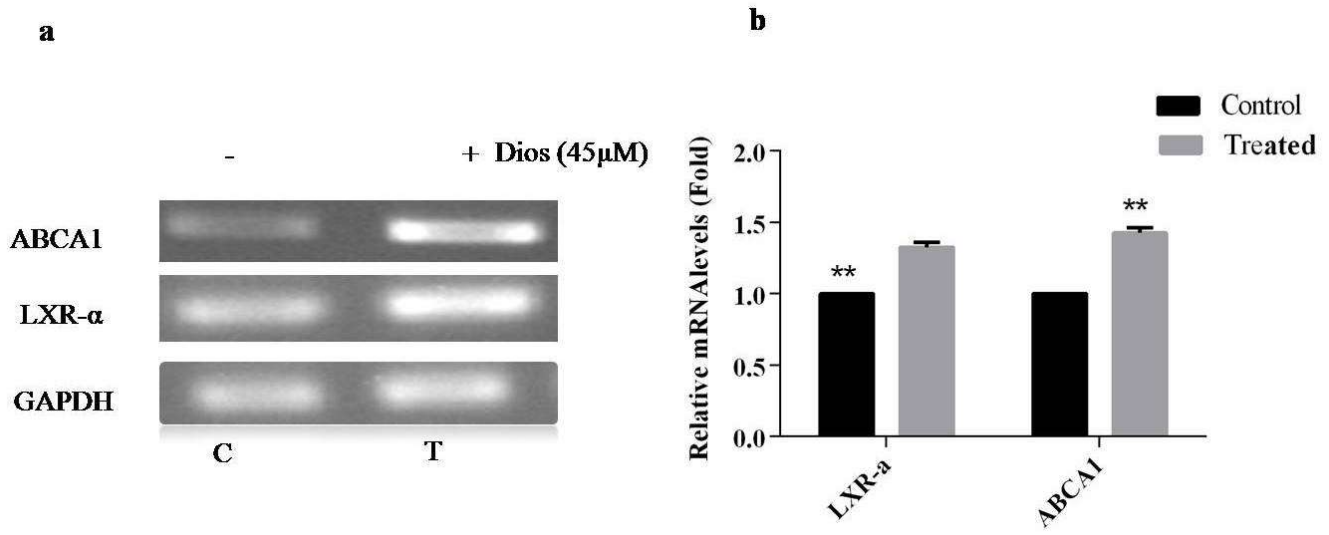

Figure 3. Diosgenin upregulates LXR-α, ABCA1, (**a**) mRNA. (**b**) mRNA quantification data (*P\0.05) in A549 cell. Cells were treated with 45µM diosgenin for 24 h. The expressions of LXR-α, ABCA1, mRNA were determined by reverse transcription-PCR. GAPDH were used to normalize the gene expressions

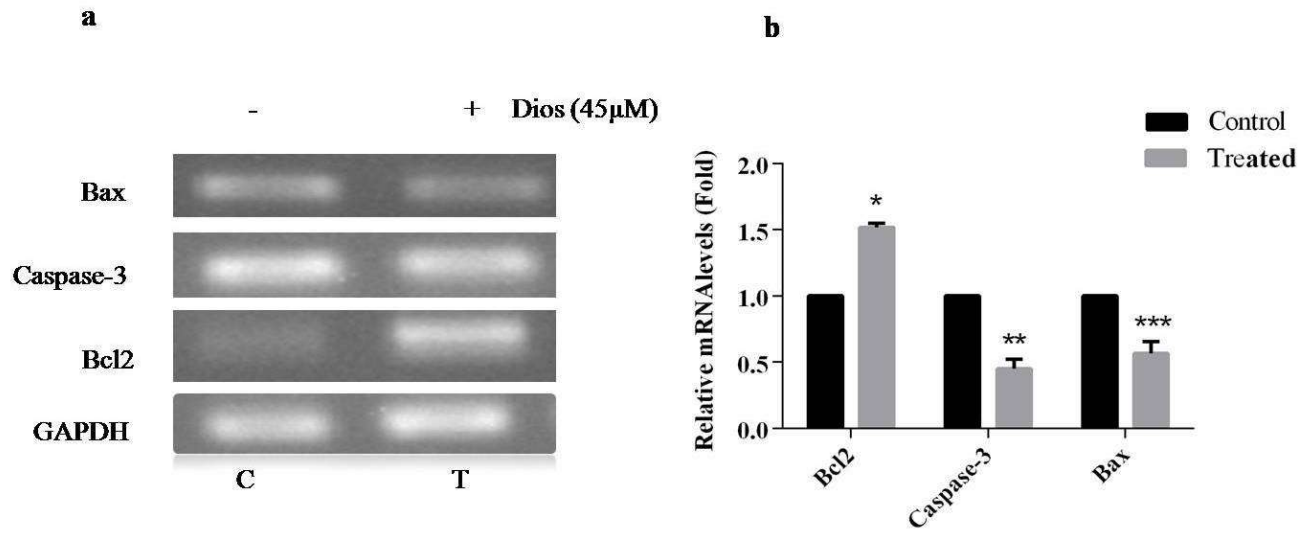

Figure 4. Diosgenin upregulates Bax and caspase-3 and down regulation of Bcl-2 (a) mRNA expression. (b) mRNA quantification data (*P\0.05) in A549 cell. Cells were treated with 45µM diosgenin for 24 h. The expressions of Bax, caspase-3 and Bcl-2, protein were determined by reverse transcription-PCR. GAPDH were used to normalize the gene expressions.

regulation of Bcl-2activation of caspase. Diosgenin reduces telomerase exploit by the down regulation of hTERT gene expression in A549 cancer line (Mohammad et al., 2013). Diosgenin overcomes the migration and invasion of PC-3 cells that restrain the MMPs action. It also prohibited ERK, JNK and PI3K/Akt signaling pathways as well as NF-kB activity (Chen et al., 2011). Diosgenin showed possible anti-proliferative effect in HCC cells by stimulating apoptosis and G2/M cell cycle arrest. Further, upregulation of p27 and p21 expression and activation the caspaseca Mohammad scade has been observed with diosgenin. Upregulation of p27 and p21 induced by diosgenin was independent of p53 (Jiang et al., 2016).Diosgenin induces apoptosis in IGF-1-stimulated primary human thyrocytes via caspase-dependent pathways and also prohibit the FLIP and activates caspase-8 through PI3K signals. Diosgenin generates ROS, regulates the balance between Bcl-2 and Bax and activates caspase-9. Diosgenin induces the autophagy through the mTOR signaling pathway. And also diosgenin strongly elevates ROS level in CML Cells (Rahmati et al., 2014). Diosgenin inhibits actin polymerization, Vav2 phosphorylation and reduces Cdc42 activation in human breast cancer (MDA-MB-231) cells (He et al., 2014). Diosgenin reduces the HGF-induced Mdm2 and vimentin by down-regulating phosphorylated

Akt and mTOR in prostate cancer (Chang et al. 2011). Effects of the two synthetic LXR ligands (T0901317 and GW3965) on the evolution of acquired refuse to EGFR TKI (Wu et al., 2015).

LXR agonist T0901317 and 22 (R) – hydroxycholesterol treatment suppresses the proliferation of progression stage of LNCaP human prostate cancer cell and other frequent human cancer cell line (Chuu et al., 20110). Diosgenin enhances ABCA1-dependent cholesterol efflux and suppresses aortic atherosclerosis progression by silencing macrophage miR-19b expression (Lv et al., 2015). LXR agonist T0901317 can reverse EGFRTKI EGFRTKI resistance of lung cancer cell lines A549 and H1650 (Haixia Cao). Gas 6 stimulated the activity of LXR and was enhanced through an interaction between LXR-α and STAT-1 on the DNA- promoter of ARG 2. Gas 6 suppresses lipopolysaccharide that stimulates nitrite production in a STAT-1 and LXR pathway dependent manner in bone marrow derived macrophages. And it also reduced the Mer- neutralizing antibody in LXR and Arg 2 expression in lung tissue and acute lung injury, the Gas 6- Mer- PI3k/AKT, STAT- 1, LXR, Arg 2 pathway plays an essential role (Kim et al., 2016). LXR agonist GW3965 affect, gefitinib defiance of HCC827/GR-8-2 cells (Hu et al., 2017). Accordingly, further analysis will be mandatory in the future to confirm the applicability of these findings in vivo.

Conclusion

Diosgenin inhibits the proliferation of human lung cancer cells (A549) in a dose dependent way. To the best of our insight, these are new discoveries we recommend that diosgenin reduce the cell proliferation by promoting cholesterol efflux via up-regulation of LXR-α and ABCA1 expression in A549 cells and accordingly may be used as a novel therapeutic agent for the treatment of lung cancer.

Conflict of interest

The authors have no conflict of interest to declare.

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