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ORIGINAL ARTICLE

Antitumor effects of Farnesol in optic nerve sheath meningioma cell line and its effects on cell cycle progression, autophagy, cell migration and invasion

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Summary

Purpose: *Farnesol has been shown to exhibit important anticancer potential. However, its antiproliferative effects have not been examined against the optic nerve sheath meningioma cells. In this study the potential of Farnesol in the treatment of optic nerve meningioma was evaluated by examining its antiproliferative effects against the HBL-52 cells.*

Methods: *The MTT assay was used to determine cell viability of HBL-52 cells. Autophagy was detected by transfection assay. The cell migration and invasion of HBL-52 cells was determined by transwell assay. Protein expression was checked by western blot assay.*

Results: *The results showed that Farnesol decreased significantly the viability of HBL-52 cells and showed an IC_{50} of 25 μM. The antiproliferative effects were due to the activation of the autophagy in the HBL-52 cells. The autophagy was also accompanied by upsurge of LC3 II and Beclin 1 expression. Farnesol also triggered the cell cycle arrest of the HBL-52 at the G2/M phase of the cell cycle which was accompanied by suppression of cyclin B1. The cell migration and invasion of the HBL-52 cells was also suppressed by Farnesol via inhibition of MMP-2 and 9 expressions.*

Conclusions: *To sum up, Farnesol may prove beneficial in the treatment of optic nerve sheath meningioma as it has shown significant antiproliferative effects against this rare form of tumor.*

Key words: *Farnesol, meningioma, autophagy, cell cycle arrest, invasion*

Introduction

Nature is an amazing repository of chemical entities with exceptional properties to alleviate human diseases [1]. A wide array of drugs has been isolated from natural sources for the treatment of diseases such as diabetes, malaria and cancer to name a few [2]. Out of all natural sources, plants have developed enormous potential to synthesize diverse chemical scaffolds with potent bioactivities [3]. Farnesol is prevalently found in plants and has also been reported from several fungal species [4]. Chemically, Farnesol is a hydrophobic acyclic sesquiterpene containing 15 carbon atoms and has a remarkable potential to halt the growth of cancer cells [5]. Farnesol has been shown to suppress the proliferation of multiple myeloma and has been shown to augment the anticancer effects of bortezomib [6]. This molecule has also been reported to trigger cell cycle arrest of pancreatic adenocarcinoma cells [7]. In pancreatic cancer, Farnesol has also been shown to modulate the expression of the biomarker proteins of apoptosis [8]. This compound has been shown to cause suppression of cervical cancer by induction of apoptosis and loss of mitochondrial membrane potential (MMT) [9] and also to block the PI3K/AKT signalling cascade in cervical cancer [9]. In yet another study, Farnesol de-

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creased the survival of the pancreatic cancer cells [10]. It also prompted apoptosis is accompanied to endoplasmic reticulum stress in lung cancer [11]. Although the anticancer effects of Farnesol are well explored, there is no report on the its antiproliferative effects against the optic nerve sheath meningioma. Optic nerve sheath meningioma is one of the lethal types of meningioma and is often associated with loss of vision and even death [12]. This study was therefore undertaken to examine the antiproliferative effects of Farnesol against the optic nerve sheath meningioma cell lines along with examining its effects on cell cycle phase distribution, autophagy induction, cellular migration and invasion.

Methods

Cell viability assay

The viability of the meningioma cells was measured by MTT assay. In brief, as the confluence of the HBL-52 cells reached 70% they were seeded in 96-well plates and treated with 0-200 µM of Farnesol. After an incubation of 24 h, the cells were incubated with MTT for another 4 h. After this, Dulbecco's Modified Eagle Medium (DMEM) was removed and the colored formazan product was solubilized by 200 µl of dimethyl sulfoxide. The viability of the HBL-52 cells was then determined by taking absorbance at 570 nm.

GFP-LC3 transfection for the detection of autophagy

For detection of the autophagy, the HBL-52 cells were grown to 70% confluence and transfected with GFP-LC3 plasmids using Lipofectamine 2000 (Invitrogen, Waltham, Massachusetts, USA) as per the manufacturer's instructions. The transfected cells were then treated with varied concentrations of Farnesol (0, 12.5, 25 and 50 µM) for 24 h and subsequently monitored by fluorescent microscopy.

Cell cycle, migration and invasion analysis

Following incubation of the HBL-52 meningioma cells with varied concentrations of Farnesol (0, 5, 10 and 20 µM) for 24 h, the cells were washed with phosphate buffered saline (PBS). Afterwards, the HBL-52 cells were stained with propidium iodide (PI) and the distribution of the cells in cell cycle phases was assessed by FACS flow cytometer. The cell migration and invasion assays were performed as described previously [14].

Western blotting

The HBL-52 cells were harvested and washed with ice-cold PBS. The pellet was then suspended in a lysis buffer at 4°C and then shifted to 95°C. Thereafter, the protein content of each cell extract was checked by Bradford assay. About 40 µg of protein was loaded from each sample and separated by SDS-PAGE before being shifted to polyvinylidene fluoride membrane. The membranes were then subjected to treatment with Tris-buffered saline (TBS) and exposed to primary antibodies at 4°C. Thereafter, the cells were treated with appropriate secondary antibodies and the proteins of interest were visualised by enhanced chemiluminescence reagent.

Statistics

Data is shown as mean ± SD. Assessment of differences was performed with one way ANOVA, after Bonferroni and Dunnet *post hoc* tests were used for multiple comparisons. In comparison to control, $p<0.05$ was considered statistically significant.

Results

Farnesol inhibits the growth of HBL-52 cells

The effects of Farnesol (Figure 1A) were examined on the HBL-52 cell viability at concentrations ranging from 0-200 µM. It was found that Farnesol caused significant decline in the viability of HBL-52 cells (Figure 1B). The IC_{50} of Farnesol against the HBL-52 meningioma cells was found to be 25 µM. These growth inhibitory effects on the HBL-52 cells were found to be dose-dependent.

Farnesol induces autophagy in HBL-52 cells

The HBL-52 cells were transfected with GFP-LC3 vectors and treated with different concentrations of Farnesol. The results revealed that Farnesol treatment caused increase in the expression of the LC3 expression as indicated by fluorescence

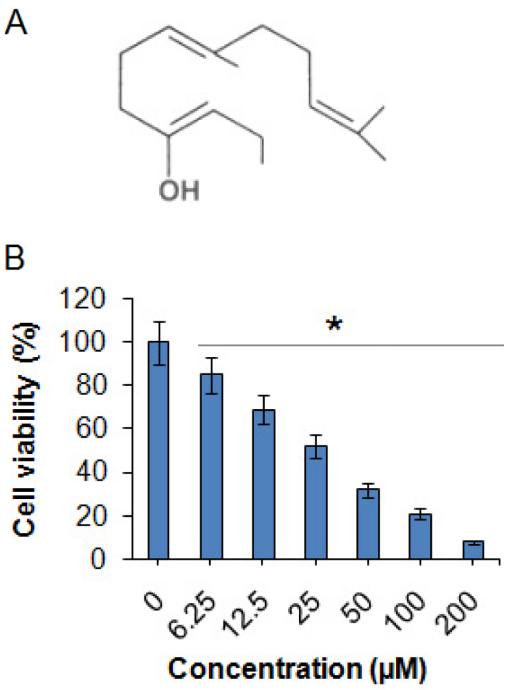

Figure 1. A: Chemical structure of Farnesol. **B:** MTT assay showing the effects of Farnesol on the viability of the HBL-52 cells. The cell viability decreased significantly as the Farnesol dose increased. The experiments were carried in triplicate (*p<0.05).

microscopy suggesting that Farnesol induced autophagy in the HBL-52 cells (Figure 2). To confirm the induction of autophagy in the HBL-52 cells, the proteins of the Farnesol-treated HBL-52 cells were extracted and subjected to western blot analysis. The results showed this molecule caused enhancement of the expression of LC3 II and Beclin-1 confirming the Farnesol-induced autophagy (Figure 3).

Farnesol triggers G2/M phase arrest of HBL-52 cells

The effects of Farnesol were also examined on the cell cycle distribution of HBL-52 cells at 0, 12.5, 25 and 50 μM concentrations by flow cytometry. The results showed that Farnesol produced G2/M arrest of the cell cycle. The percentage of the G2/M phase cells increased from 16% in control to around 40% at 20 μM (Figure 4). These effects of Farnesol were concentration-dependent. Moreover, this compound also caused upregulation of p27 while the expression of cyclin B1 was significantly decreased (Figure 5).

Farnesol inhibits cell migration of cancer cells

Next, the effect of Farnesol on the migration of the HBL-52 cancer cells was investigated by transwell assay. The results showed that at IC_{50}, Farnesol could inhibit the migration of the HBL-52 cancer cells (Figure 6).

Farnesol inhibits the invasion of HBL-52 cells

The inhibitory effects of Farnesol were also examined on the HBL-52 cells by transwell assay at 0, 12.5, 25 and 50 μM concentrations. The results showed that Farnesol suppressed the invasion of

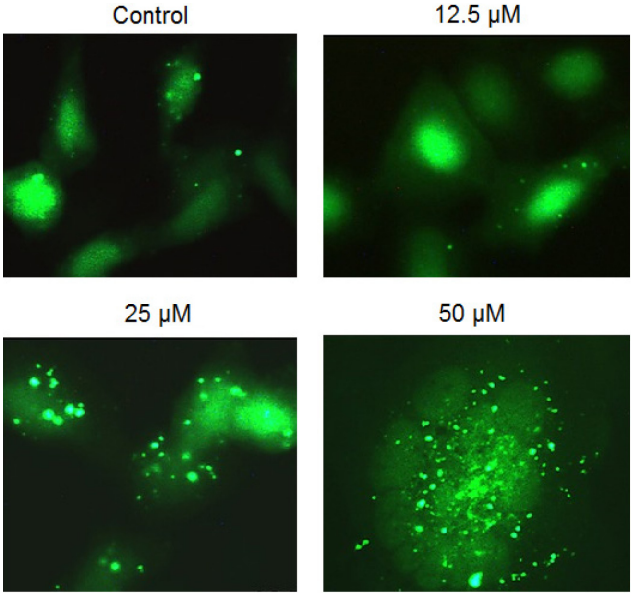

Figure 2. Effect of Farnesol on the LC3 expression as depicted by fluorescence microscopy of the Farnesol-treated HBL-52 cells. Farnesol treatment caused increase of the LC3 expression as indicated by fluorescence microscopy. The experiments were performed in triplicate.

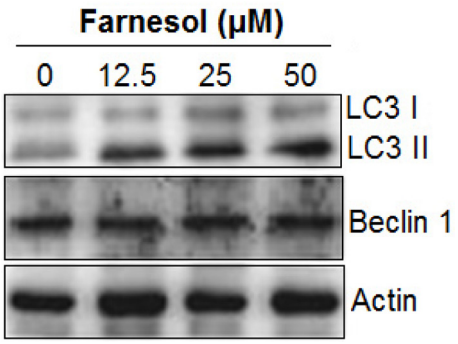

Figure 3. Effect of Farnesol on the expression of autophagy proteins in HBL-52 cells as depicted by western blot analysis. The results showed this molecule caused enhancement of the expression of LC3 II and Beclin-1. The experiments were performed in triplicate.

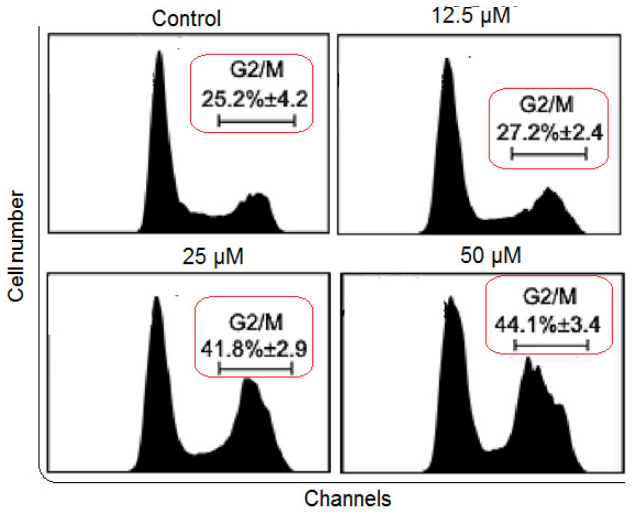

Figure 4. Cell cycle phase distribution of HBL-52 cells upon treatment with varied concentrations of Farnesol as depicted by flow cytometry. The results indicate an increase in G2/M phase cells with increasing Farnesol dose. The experiments were performed in triplicate.

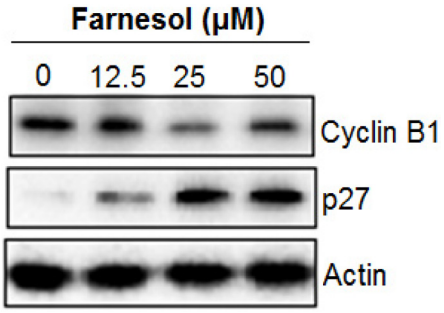

Figure 5. Effect of Farnesol on the expression of cell cycle proteins in HBL-52 cells as depicted by western blot analysis. The expression of cyclin B1 decreased while that of p27 increased. The experiments were performed in triplicate.

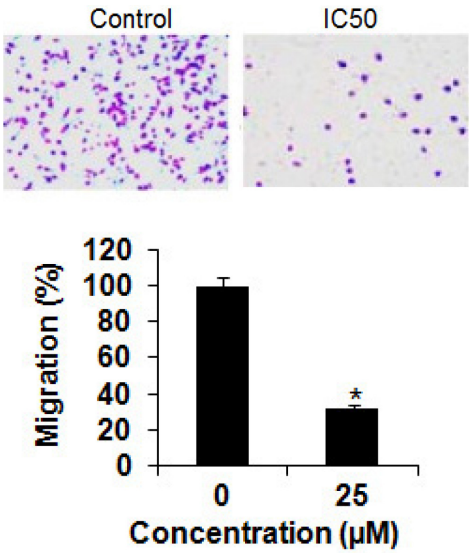

Figure 6. Inhibition of migration of the HBL-52 cells by Farnesol at indicated concentrations as depicted by transwell assay. The values represent the mean of three experiments ± SD (*p<0.05).

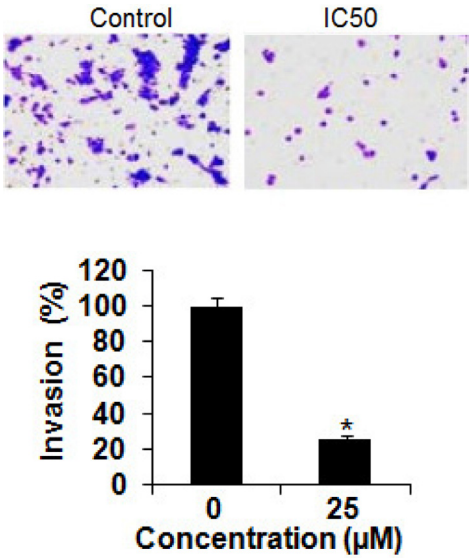

Figure 7. Inhibition of invasion of HBL-52 cells by Farnesol at indicated concentrations as depicted by transwell assay. The values represent the mean of three experiments ± SD (*p<0.05).

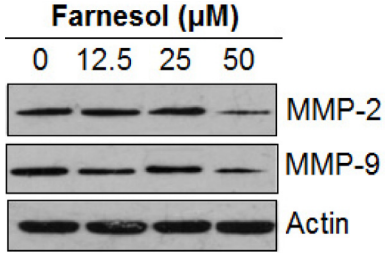

Figure 8. Effect of Farnesol on the expression of MMP-2 and 9 proteins in HBL-52 cells as depicted by western blot analysis. The expressions of both MMP-2 and MMP-9 decreased with increasing dose of Farnesol. The experiments were performed in triplicate.

HBL-52 cells at IC_{50} (Figure 7). Moreover the inhibition of the HBL-52 cell invasion was also associated with the inhibition of MMP-2 and MMP-9 (Figure 8).

Discussion

Optic nerve sheath meningioma accounts for 1-2% of all meningiomas [15]. Herein, the antiproliferative effects of the naturally occurring Farnesol were evaluated on the HBL-52 optic nerve bundle sheath cells. Plants have provided a number of pharmaceutical agents for the alleviation of human diseases [3]. Drugs such as etoposide, vincristine and others which are used for the treatment of cancer are of plant origin [16]. These metabolites have also been screened by researchers to develop drugs for the treatment of diseases [2]. In this study Farnesol was evaluated against the HBL-52 meningioma cells. The results showed this molecule could inhibit the growth of the HBL-52 cells concentration-dependently. The anticancer effects of Farnesol were mainly due the induction of autophagy. This was associated with upregulation of autophagy-related LC3 II and Beclin expression. Autophagy helps in the elimination of the diseased and harmful cells from the body of an organism [17]. Previous studies have also shown that Farnesol induces cell death in prostate cancer cells via modulation of PI3K/AKT and MAPK pathway [18]. Besides autophagy, cell cycle arrest is another mechanism by which anticancer agents exert their anticancer effects [19] and herein we observed that Farnesol triggered the arrest of HBL-52 cells at the G2/M phase of the cell cycle. Previous studies have also shown that Farnesol triggers cell cycle arrest of pancreatic adenocarcinoma cells [7]. Cell migration and subsequent invasion to distant parts of the body is the initial requirement for metastasis of cancers [13] and herein we observed that Farnesol caused suppression of cell migration and invasion of the HBL-52 cells, suggestive of its antimetastatic potential.

Conclusion

The findings of this study show that Farnesol suppresses the growth of the HBL-52 meningioma cells via induction of autophagy and cell cycle arrest. Moreover, Farnesol could also suppress the migration and invasion of the HBL-52 cells, indicative of its potential in the treatment of optic nerve sheath meningioma.

Conflict of interests

The authors declare no conflict of interests.

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