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ORIGINAL ARTICLE

Dammarenediol suppresses the growth, migration and invasion of human osteosarcoma cells by modulating PI3K/Akt and STAT-3 signaling pathways

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Summary

Purpose: *The present work was aimed at exploring the anticancer potential of dammarenediol against human osteosarcoma cells together with exploration of its mechanism of action.*

Methods: *The osteosarcoma cell line HOS was used in this study. The proliferation rates of cancer cells were determined through CCK-8 kit. The colony forming assay was carried out to analyze the viability of osteosarcoma cells. The migration and invasion of osteosarcoma cells was examined by the transwell chamber method. Western blotting was used to elucidate the protein expression levels.*

Results: *The osteosarcoma cell growth was inhibited by dammarenediol in a dose-dependent manner, while the normal bone cell line was not affected much. Dammarenediol further declined the viability of cancer cells along with restricting their metastatic potential. The anticancer effects of dammarenediol were attributed to the blockage of PI3K/Akt and STAT-3 signaling pathways.*

Conclusion: *Dammarenediol effectively restricted the growth and metastasis of osteosarcoma cells in vitro by inhibiting the PI3K/Akt and STAT-3 signaling pathways*

Key words: *osteosarcoma, dammarenediol, proliferation, migration, invasion, metastasis.*

Introduction

In the past few years, a number of studies have focused on the elucidation of anticancer properties of various chemical compounds [1]. These studies have not only enhanced our understanding towards the human cancer but also have led to identification of a series of chemical compounds with anticancer properties [2]. Also, the studies have highlighted in depth the crucial targets of these chemical compounds mediating their anticancer effects [3]. Terpenoid type of compounds are among the most prevalent natural compounds and a battery of terpenoid molecules were confirmed to possess significant anticancer properties against different types of human cancers [4,5]. Dammarenediol is a teracyclic terpenoid compound [6]. In the present study, we tried to infer the effects of dammarenediol against human osteosarcoma cells. Osteosarcomas is the most dominant type of primary malignancy of bone [7]. Osteosarcoma results from the primary bone forming cells [8]. This malignancy is comparatively more frequent in children and adolescents [9], while cases in adults are rare, but adults with Paget's bone disease have been found to be more susceptible to osteosarcoma [10]. The treatment methods used against osteosarcoma include chemotherapy, radiotherapy and surgery [11]. However, the currently applied treatment methods are unsatisfactory, creating a need for newer therapeutic approaches. This study was undertaken to investigate the anticancer effects of Dammarenediol against human osteosarcoma cells and attempted to explore the underlying mechanism.

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Methods

Cell lines and cell culture

The normal bone cells were obtained from the Second Xiangya Hospital (Changsha, China) and osteosarcoma cell line (HOS) was purchased from the American Type Collection Center (ATCC, USA). Both the normal bone and osteosarcoma cell line were maintained at 37°C in humidified incubator. For their culturing, the RPMI medium added with fetal bovine serum (FBS) (10%) were used.

Cell proliferation assay

The normal bone cells and HOS cells were cultured in 96-well plates for 24h. Afterwards, the dammarenediol was added at 0, 7.5, 15, 22.5, 50 or 100 μM concentrations and the cells were incubated for 24h at 37°C. After 24h, 15 μl CCK-8 reagent (Thermo Scientific, Waltham, Mass, USA) were added and the cells were again incubated for another 4h. A spectrophotometer was then used to determine the absorbance from the cell cultures at 570nm. The recorded values were used to infer the relative cell growth.

Cell viability assay

Colony formation assay was performed for detecting the viability of HOS cancer cells treated with 0, 7.5, 15 or 22.5 μM dammarenediol. Briefly, after being treated with different concentrations of dammarenediol for 24h in 6-well plates, the cell cultures were drained of the culture medium and then were fixed with ethanol. Staining with 0.1% crystal violet was performed and the relative colony number was determined using light microscope to assess the relative cell viability.

Migration and invasion assays

The migration of HOS cancer cells treated with 0, 7.5, 15 or 22.5 μM dammarenediol was analyzed by the transwell assay. Briefly, after trypsinization, about 10^5 cells from each concentration set up were added to the upper chamber of the transwell plate. The lower chamber was supplemented with 10% FBS only. After 24h incubation, the cells sticking to the upper surface of the transwell filter were swabbed away carefully and the cells migrated to the lower side of the filter were fixed with methanol, stained with 0.1% crystal violet and examined under 100x light microscope. The invasion of cells was determined in the same fashion except matrigel was added with the cell cultures. Almost 10 microscopic fields were randomly selected for cell counting to assess the percent relative number of migrated and invaded cells.

Western blotting

The HOS cancer cells treated with or without dammarenediol were centrifuged and cell pellets were lysed with the RIPA lysis buffer. The extracted proteins were quantified with the Bradford's method. Equal concentrations of extracted proteins were loaded on SDS-PAGE gels and separated proteins were transferred to nitrocellulose membranes which were then blocked using skimmed milk. The membranes were then incubated with primary antibodies designed against p-Akt, Akt, p-PI3K, PI3K, p-STAT3 and STAT3 proteins at 4°C overnight in the dark. Afterwards, the membranes were exposed to secondary antibodies conjugated with horseradish peroxidase. After being washed by PBS, the membranes were processed with ECL Plus Kit method for detecting the specific protein bands. Human β-actin was used as internal control.

Statistics

The values obtained were presented as mean± SD of 3 independent experiments. Student's t-test was performed for estimating the level of inter-value difference using SPSS 16.0 software. The difference between two values was considered significant where p values were lower than 0.05.

Results

Dammarenediol selectively inhibits the proliferation of osteosarcoma cells

The chemical structure of dammarenediol is presented in Figure 1. When the normal bone cells

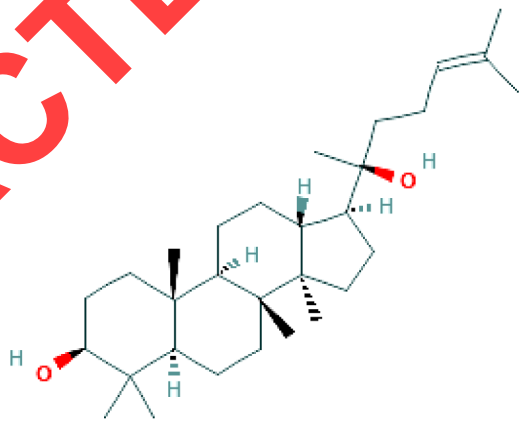

Figure 1. Chemical structure of dammarenediol.

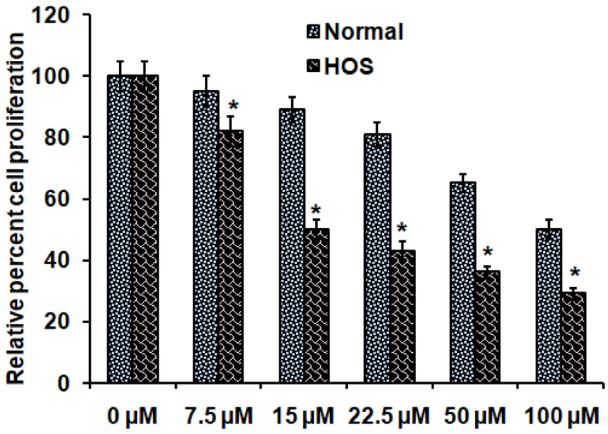

Figure 2. Dammarenediol inhibits selectively the osteosarcoma cell proliferation. Percent proliferation rates of normal bone cells and HOS osteosarcoma cells treated with 0, 7.5, 15, 22.5, 50 or 100 μM dammarenediol. The experiments were performed in triplicate and expressed as mean±SD (*p<0.05).

and HOS osteosarcoma cells were treated with this terpenoid compound, the proliferation was inhibited in a dose-dependent manner (Figure 2). However, dammarenediol inhibited the proliferation of HOS osteosarcoma cells to a greater extent than the normal bone cells with an IC_{50} value of 12.5 μM vs 100 μM for the normal bone cells, showing selective inhibition of osteosarcoma cells.

Dammarenediol reduced the viability of osteosarcoma cells

The anticancer effects of dammarenediol against the HOS osteosarcoma cells were analyzed by estimating the relative number of colonies formed by the HOS cells treated with or without dammarenediol. The results showed that HOS cancer cells formed significantly less colonies when treated with dammarenediol (Figure 3). The relative percentage of number of colonies formed by HOS decreased with the increase of dammarenediol dosage. The results thus infer that dammarenediol is effective in declining the viability of osteosarcoma cells *in vitro*.

Migration and invasion of osteosarcoma cells was restricted by dammarenediol

The migration and invasion of HOS osteosarcoma cells treated without or with different concentrations of the molecule were determined by transwell assay. The migration of HOS cells was seen to decrease dose-dependently under the dammarenediol treatment (Figure 4). Similar results were obtained for the invasion of HOS osteosar-

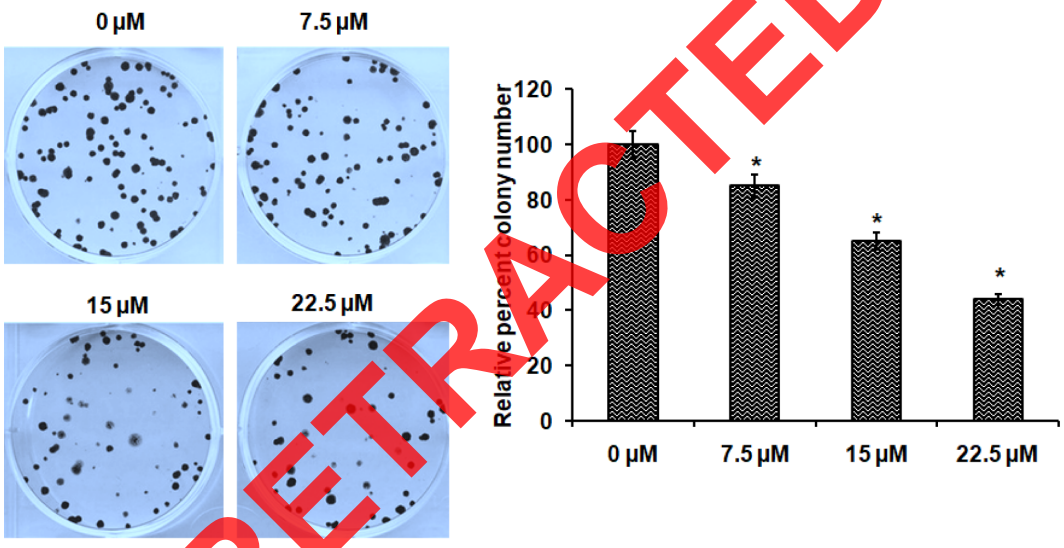

Figure 3. Dammarenediol inhibits the osteosarcoma cell viability. Colony forming assay and percent number of cell colonies formed by HOS carcinoma cells treated with 0, 7.5, 15 or 22.5 μM dammarenediol. The experiments were performed in triplicate and expressed as mean±SD (*p<0.05).

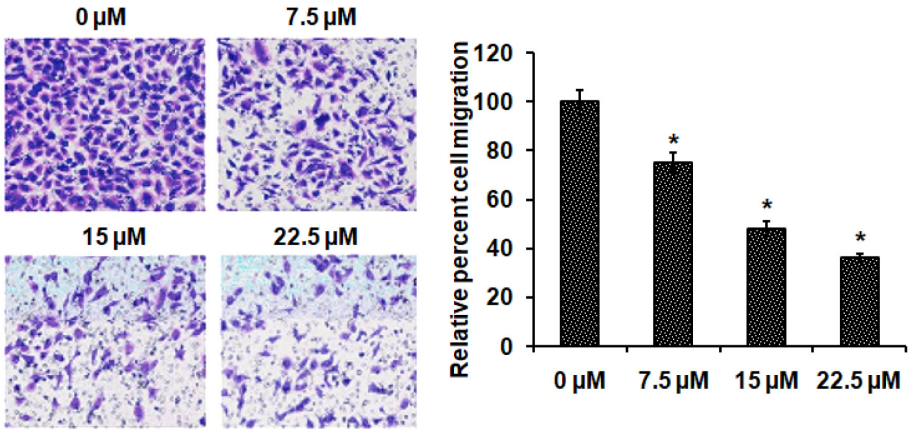

Figure 4. Dammarenediol reduces the migration of osteosarcoma cells. Transwell assay for assessment of migration of HOS carcinoma cells and percent cell migration of HOS cells treated with 0, 7.5, 15 or 22.5 μM dammarenediol. The experiments were performed in triplicate and expresses as mean±SD (*p<0.05).

coma cells (Figure 5). Thus, it can be stated that dammarenediol has a potential to restrict the osteosarcoma cell metastasis.

Dammarenediol inhibited PI3K/Akt and STAT-3 signaling pathways of osteosarcoma cells

To investigate the effect of dammarenediol on the PI3K/Akt and STAT-3 signaling pathways of HOS osteosarcoma cells, these cells were exposed to 0, 7.5, 15 or 22.5 μM dammarenediol. The results showed that the concentrations of phosphorylated proteins i.e., p-Akt, p-PI3K and p-STAT3 decreased under dammarenediol treatment. However, there was little or no effect on the concentrations of normal proteins, Akt, PI3K and STA-3 (Figure 6). Therefore, dammarenediol inhibits the PI3K/Akt and STAT-3 signaling pathways of osteosarcoma cells to exert its anticancer effects.

Discussion

Osteosarcoma is the primary malignancy of bone frequently affecting the children and adolescents [12]. Osteosarcoma accounts for about 20% of the cancer mortality among children [13]. Although the recent advancements in the treatment strategies and curative measures has improved the survival rate of osteosarcoma patients, still almost 35% of patients detected with this malignancy die within 5 years [13]. This calls for urgent exploration of more possible ways for treating osteosarcoma. In our study, we explored the effects of a tetracyclic terpenoid compound, dammarenediol, against human osteosarcoma cells. The results were conclusive that dammarenediol-treated osteosarcoma cells proliferate at much lower rates than the control cells. Interestingly, the dammarenediol-treated normal bone cells were not affected much and they proliferated at significantly higher rates than the cancer cells. Such selective targeting is also true for other chemical compounds as reported in a previous study [14]. Dammarenediol treatment in osteosarcoma cells led to decreased viability in dose-dependent manner. The loss of cancer cell viability by chemical compounds has also been found for many other chemical compounds [15]. The anticancer effects of dammarenediol on osteosarcoma cells were also seen in terms of decrease of their migration and invasion potentials. Other terpenoid compounds have been shown to exhibit similar effects against human cancer cells [16]. The PI3K/Akt signaling pathway is important for regulating the proliferation of cancer cells as it has been proposed by a research study [17]. This pathway has prominent role in regulating the growth of cancer cells. The PI3K/Akt pathway has been elucidated to be deregulated in human cancers which is responsible

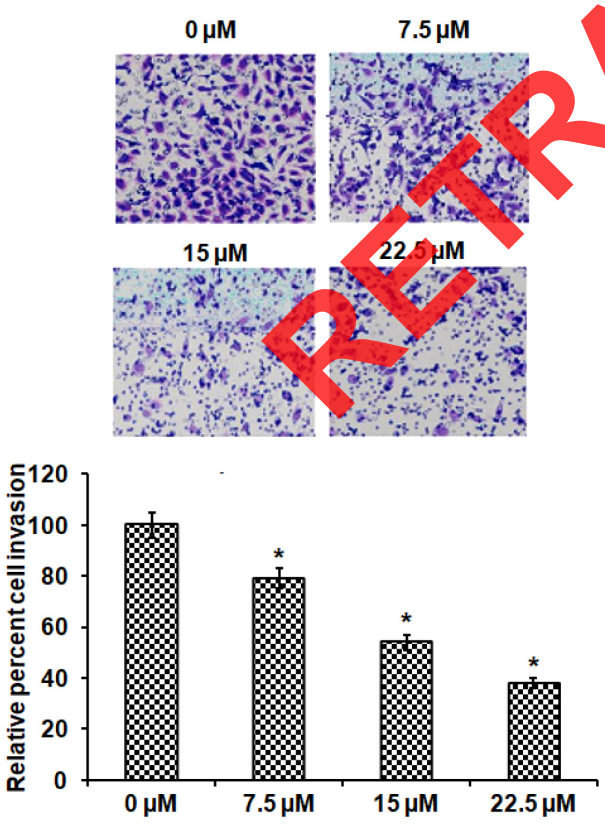

Figure 5. Dammarenediol (μM) restricts the invasion of osteosarcoma cells. Transwell assay for assessment of invasion of HOS carcinoma cells and percent cell invasion of HOS cells treated with 0, 7.5, 15 or 22.5 μM dammarenediol. The experiments were performed in triplicate and expressed as mean±SD (*p<0.05).

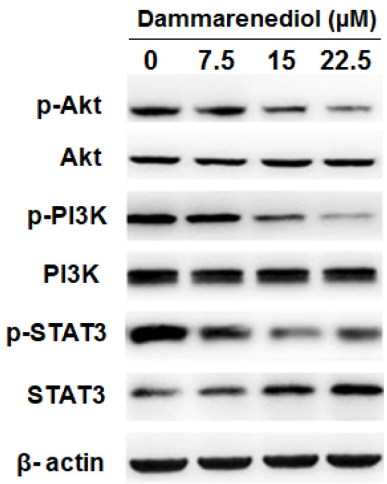

Figure 6. Dammarenediol inhibits the PI3K/Akt and STAT-3 signaling pathways in osteosarcoma cells. The western blot analysis depicts the expression levels of p-Akt, Akt, p-PI3K, PI3K, p-STAT3 and STAT3 proteins in HOS osteosarcoma cells treated with 0, 7.5, 15 or 22.5 μM dammarenediol.

for tumorigenesis [18]. The signal transducer and activator of transcription (STAT3) signaling pathway is one of the crucial pathways whose constitutive activation has been proven to be associated with induction of a number of human cancers [19]. The activation of this pathway is responsible for either inhibting the apoptosis of cancer cells or has role in cell proliferation and metastasis. In our research, we found that dammarenediol treatment of osteosarcoma cells led to inhibition of both PI3K/Akt and STAT-3 signaling pathways in cancer cells and thus reduced their proliferation and metastasis.

Conclusion

The results of the present study are indicative of anticancer effects of dammarenediol against the human osteosarcoma cells. The anticancer effects were evident as decrease of proliferation, loss of viability and decline of cell metastasis and the anticancer effects were exerted through inhibition of PI3K/Akt and STA-3 signaling pathways.

Conflict of interests

The authors declare no conflict of interests.

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