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ASTRAGALOSIDE III FROM *ASTRAGALUS MEMBRANACEUS* ANTAGONIZES BREAST CANCER GROWTH

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Abstract

Background: Astragaloside III has been used to treat different cancers; however their effect on breast cancer remains unknown.
Materials and methods: The present study examined the effects of Astragaloside III from *Astragalus membranaceus* on breast cancer cell lines *in vitro* as well as xenograft *in vivo*.
Results: The results showed that the Astragaloside III could effectively reduce cancer cell survival *in vitro* and inhibit the tumor growth *in vivo.* The potential mechanism is the induction of cell apoptosis signaling pathways.
Conclusion: We believe that Astragaloside III provides a new therapeutic tool to treat breast cancer.

Keywords: breast cancer; Astragaloside III; apoptosis; treatment;

Introduction

Breast cancer is one malignant cancer with serious results in female population (Cooney et al., 2013;Gelao et al., 2013;Ligibel and Strickler, 2013). There were more than 400,000 deaths caused by breast cancer per year in past few years (Despas et al., 2013;Khan et al., 2013;Makarem et al., 2013). The early diagnosis still represents the most powerful for necessary treatment at the early stage; while late stage prognosis is much worse (Corben, 2013;Gelao et al., 2013;Ligibel and Strickler, 2013;Ma et al., 2013). The current efforts in treating breast cancer target on multiple signaling pathways in tumor formation, growth and metastasis. Among these aspects, enhancing the cell apoptosis remains to be an important therapeutic target (Chen et al., 2013;O'Toole et al., 2013;Zhang et al., 2013).

Astragaloside III is extracted from *Astragalus membranaceus*, which has been adopted in combined treatment to multiple cancers (Astragaloside I & II has been studied in other reports and will not be detailed here). For instance, different extracts such as the total saponins or polysaccharides has been used to treat liver cancer, colon cancer, lung cancer, and gastric cancer (Liu et al., 2001;Auyeung et al., 2009;Su et al., 2009;Auyeung et al., 2012;Law et al., 2012;Li et al., 2012). In present study, we examined the potential effects of Astragaloside III on breast cancer cell lines in vitro as well as xenograft in vivo. We found that Astragaloside III could effectively antagonize the breast cancer cell growth by inducing cell apoptosis. We believe that this will provide a novel therapeutic tool in treating breast cancer.

Materials and methods

Drugs

Astragaloside III from *Astragalus membranaceus* was extracted by Lishengdu Med (Guangzhou, China). The purity was 99.99%.

Cell culture

A MCF-7 cell was from Gongji Biotech (Shanghai, China) and cultured in DMEM with 100 mg/ml penicillin, and 10% fetal bovine serum (FBS) at 37°C with 95%.

MTT assay

In order to examine the cell viability, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazo-lium bromide (MTT) assay was used. 6000 cells were seeded into 96-well plates for 24/48 hours in presence of 10, 50, and 100 ng/ml Astragaloside III, respectively.

After the incubation, 150 µl 5 mg/ml MTT solution was added for 2 hours. Finally the supernatants were removed and 150 µl DMSO was added into each well for absorbance reading at 570/630 nm with the plate reader (Bio Rad, Shanghai). The assay was repeated for 6 times.

TUNEL staining

In order to measure the cell apoptosis, the TUNEL kit (Roche, US) was adopted for staining and cell counting. The detailed procedures were performed as in the brochure of the kit. After the labeling with fluorescence, the cells were counted under 40 X microscope for positive cells (10 sites from each experiment, repeated 3 times).

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Animal study

The animal study has been approved by animal research ethic committee in The XIANGYA Hospital of Central south University, and all procedures have followed the guideline to animal research in XIANGYA Hospital of Central south University. A total of 24 nude mice were obtained from Animal center in XIANGYA Hospital of Central south University. They were assigned randomly into Saline, 10mg/kg or 20 mg/kg groups (8 animals in each group).

For tumor graft formation, the estrogen-independent breast cancer cell line MDA-MB-231 (Gongji Biotech, Shanghai, China) was employed as described before. 2×10^7 MDA-MB-231 cells separated with 170 µL DMEM medium were inoculated subcutaneously into the right flanks of nude mice (20-30 g, 2 months old).
For drug treatment, the Astragaloside III was given at a dose of 10 mg/kg or 20 mg/kg i.p. every 2 days. The insulin injection kit was used to prevent the harm caused by normal syringe needles. The experimental procedure is determined by preliminary experiments.

The tumor size was measured every 5 days (5, 10, 15, 20, 25, 30) by volume = 0.5 * length * width * width. At day 30 the mice were sacrificed and the tumor was harvested for histological analyses and the TUNEL staining.

Statistics

The data were represented by mean ± SD and analyzed SPSS 11.0 software (Chicago, US). The t test and ANOVA were used to compare differences between groups. P<0.05 was considered as statistically significant.

Results

Astragaloside III decreased cancer cell viability

We found that Astragaloside III treatment significantly decreased the cell viability. After 24 or 48 hours, the viable cells significantly decreased. In addition, this seems to be in a dose-dependent manner (Table 1).

Table 1: Astragaloside III decreased cancer cell viability

	24 hours				48 hours			
	Control	Astragaloside III			Control	ASTRAGALOSIDE III		
		10 ng/ml	50 ng/ml	100 ng/ml		10 ng/ml	50 ng/ml	100 ng/ml
Viable cells (%)	99.6±0.3%	57.0±7.4 %*	42.1±6.9 %**	27.7±5.3 %**	99.4±0.07 %	48.2±7.3 %*	36.6±3.9 %**	17.8±6.2%**

*suggests for P<0.05, ** suggests for P<0.01 in compared to control group.

Astragaloside III increased cancer cell apoptosis *in vitro*

Given the fact that ASTRAGALOSIDE III treatment decreased the cell viability, we wonder if the treatment leads to increased cell apoptosis. With TUNEL staining, in control group the apoptotic cells were 0.9±0.1%; while in 10, 50, 100 ng/ml ASTRAGALOSIDE III groups, the rates were 24.9±2.9% (P<0.01), 32.8±4.2% (P<0.01) and 44.9±7.7% (P<0.01) at 24 hours' time point.

ASTRAGALOSIDE III decrease the tumor xenograft growth by inducing apoptosis

We further examined if the drug treatment could be effective in antagonizing the tumor growth *in vivo*. We found that indeed the ASTRAGALOSIDE III treatment shifted the tumor growth curve by decreasing the xenograft size (Figure 1).

In addition, the decreased tumor growth is well correlated to the cell apoptosis at the end of the experiment. With TUNEL staining, we found that the ASTRAGALOSIDE III treatment at both 10 mg/kg and 20 mg/kg can increase the tumor cell apoptosis (Table 2).

Table 2: ASTRAGALOSIDE III induced the cell apoptosis inside the tumor (n=10 each)

	Saline control	ASTRAGALOSIDE III 10 mg/kg	ASTRAGALOSIDE III 20 mg/kg
Apoptotic cells (%)	2.4±0.5%	28.6±6.7%**	39.2±10.2%**

** suggests for P<0.01 in compared to control group.

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Discussion

Different extracts from *Astragalus membranaceus* such as the total saponins or polysaccharides has been used to treat liver cancer, colon cancer, lung cancer, and gastric cancer (Liu et al., 2001;Auyeung et al., 2009;Su et al., 2009;Auyeung et al., 2012;Law et al., 2012;Li et al., 2012). The Astragaloside has also been described; for instance, the total Astragaloside can induce the leukemia cell apoptosis (Hu et al., 2011). The Astragaloside II can reduce the drug-resistance in liver cancer lines (Huang et al., 2012), potentially through the modulation of C-jun pathway. However, the Astragaloside IV was found to increase MMP expression in lung cancer cells (Su et al., 2009), which might increase the metastasis ability of cancer cells. In present study we employed the Astragaloside III, another principle component isolated from the total Astragaloside from *Astragalus membranaceus*, and we found positive effect of this drug on breast cancer treatment: increase cell apoptosis after drug treatment.(Hu et al., 2011)

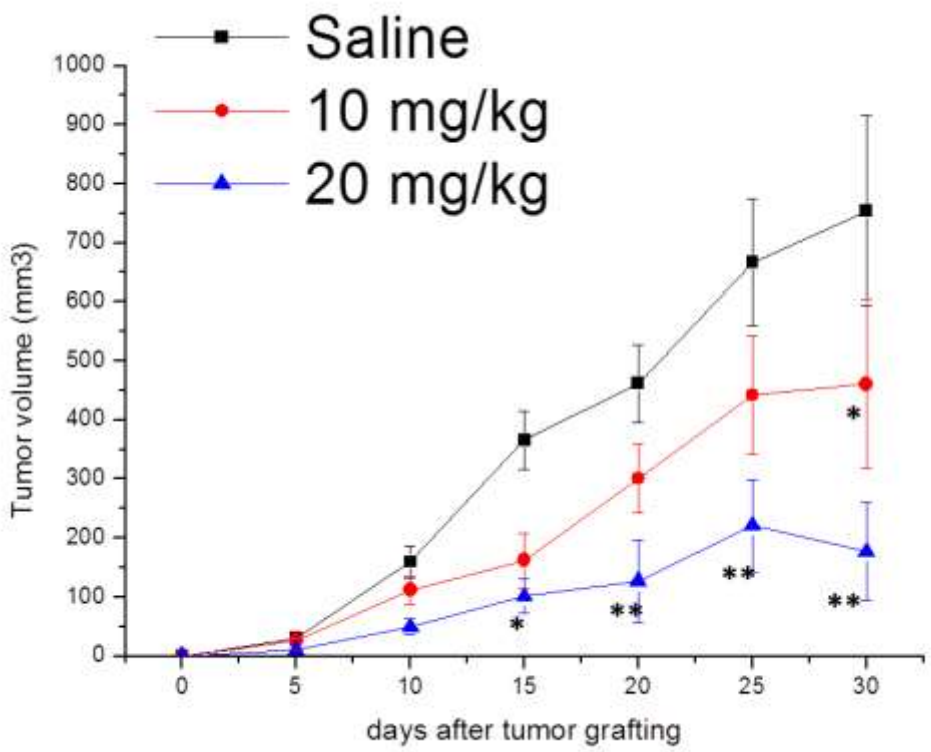

Figure 1 :ASTRAGALOSIDE III slowed down the xenograft growth

*suggests for P<0.05 and ** for P<0.01 in compared to the control group.

The mechanism underlying the drug effect has not been investigated. For the Astragaloside II, it was mediated by Akt - NF-kappaB signaling pathway (Hu et al., 2011). It is highly possible that Astragaloside III affects the same signaling pathway due to the similar molecular structure. This is yet to be examined in our future studies.

In addition, given the fact that Astragaloside II can reduce the tumor growth rate *in vivo*, it is conceivable to believe that the drug has high therapeutic potential. However as suggested above, the Astragaloside IV might promote cancer cell metastasis to certain extent (Su et al., 2009); this worth further investigations for breast cancer cell line. Last but not least, the combined effects of different Astragalosides should be investigated.

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Conflicts of interest: None declared

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