Effect of Angelica keiskei Koidz Chalcone on PCNA and BCL-2 Protein Expression of Mice Hepatocarcinoma Cells

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ABSTRACT Objective: To investigate the effect of angelica keiskei koidz chalcone on the expression of PCNA and BCL-2 in mice hepatocarcinoma cells. Methods: Fifty mice were inoculated with hepatocarcinoma H22 cells and divided into five groups, with 10 mice per group. High, medium and low chalcone groups were given 40, 20, 5mg/kg/d of chalcone by mouth, respectively. The tumor control group was given saline by mouth and the cylophosphamide group was given 20mg/kg cyclophosphamide by intraperitoneal injection every other day. Ten days later all mice were sacrificed. The proliferation activity of hepatocarcinoma cells was determined by methy tetrazolium (MTT) assay, and the levels of the proliferating cell nuclear antigen (PCNA) and BCL-2 protein expression were detected by immunohisto chemistry method. Results: The cell proliferation activity of high dose chalcone group and tumor control group were (0.716± 0.018) and (1.135± 0.032). The difference was significant (P<0.05). The expression of PCNA and BCL-2 protein in high dose chalcone group were 28.33% and 16.77% respectively, while which of the tumor control group were 72.77% and 65.17%. The differences between two groups were significant (P<0.05). Conclusion: Chalcone can reduce the expression of PCNA and BCL-2, and inhibit the proliferative activity of mice hepatocarcinoma cells.

Key words: Chalcone; Hepatocarcinoma cell; Proliferation; PCNA; BCL-2

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Introduction

Angelica keiskei Koidzumi, hachijo ashitaba, is a species of umbelliferae, biennial or perennation, herbaceous plants and native to Izu islands in Japan, which is named as longevity grass because of its abundant vitamins, mineral substances and trace elements as well as its effects of enforcing the immune function, improving sleeping and deferring senility [1]. Chalcone, the active component of Angelica Keiskei Koidzumi, is characteristics as anti-tumor, oxidation resistance, removing oxygen free radicals, anti-gastric ulcer, bacteriostasis, inhibiting phosphodiesterase anti-human immunodeficiency virus (HIV), and anti-inflammatory according to overseas literatures[2-4], among which study on anti-tumor was reported singularly at home. This study was to detect PCNA and BCL-2 and observed negative effect of chalcone on proliferation of hepatocarcinoma cells with intragastric lavage to H22 liver cancer mice.

1 Materials and Methods

1.1 Materials

1.1.1 Animal Kunming species mice (SPF grade), 6-8 weeks old and 18-20g weight, were provided by lab center of Shandong Lu kang medicines co., ltd. H22 liver cancer mice were provided by Shandong experimental animal center.

1.1.2 Experimental sample Chalcone was made by our lab and its purity was more than 90% detected with ultraviolet spectrophotometry. Standard chalcone, purity>99%, was bought from Sigma.

1.1.3 Regents and Instruments Methyl thiazolyl tetrazolium (MTT), RPMI-1640 culture medium, fetal bovine serum (FBS) from GiBco, PCNA mono-antibody, BCL-2 poly-antibody and SABC immunohistochemistry kit from Wuhan Boster bio-engineering co., Ltd, Rosys Anthos 2010 auto enzyme-labeled instrument from Swiss, and Olympus XDS-1B inverted microscope and camera from Olympu

1.2 Methods

1.2.1 Inoculation of cancer cell Collect aseptically ascites of mice when H22 hepatocarcinoma cells were inoculated to them 6 days later; prepare cell suspension with normal saline; adjust cell concentration to 1×10^7 / ml; and inject to the subjects subcutaneously by right upper axillary fossa.

1.2.2 Animal groups and treatments The mice inoculated with H22 hepatocarcinoma cells were randomly divided into five groups (10mice/group). High, medium and low chalcone groups were given 40, 20, 5mg/(kg.bw) of chalcone by intragastric lavage once a day, respectively. The tumor control group was given equivalent normal saline with the same method, continuously for 10 days. The cylophosphamide control group was given 20mg/kg cyclophosphamide by intraperitoneal injection, once every other day. All the mice were sacrificed with dislocation of cervical vertebra the second day after the final administration. Hepatoma tissue was prepared aseptically for use later.

1.3 Detection

1.3.1 Proliferation of hepatocarcinoma cells detected by MTT assay The fresh tumor tissue was prepared as cell suspen-

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sion by cutting into pieces, digestion, filter and centrifugation，adjusted to 1 × 10^6 / ml in RPMI-1640 culture medium with 10% FBS, which was cultured with 96-well plate (0.1ml/well) in 5% CO_2 incubator at 37℃ for 16h. Proliferation was analyzed with MTT (detecting absorbency, A). Inhibitory rate of mice hepatocarcinoma cells in groups was calculated with the formula: inhibitory rate (%) = (tumor control group chalcone group)/ tumor control group× 100%

1.3.2 Expression of PCNA The expression of PCNA of hepatocarcinoma cells was detected by SABCimmunohistochemistry kit: embedment, section, deparaffinization, hydration, deactivation of enzyme with 3%H_2O_2, microwave method, incubation with the first antibody (1:50) to PCNA at 4℃ overnight, incubation with the second antibody for 30min, development with DAB, dehydration, and mounting. Expression of PCNA was observed in random fields under high power lens (400). 1000 hepatocarcinoma cells were studied and positive cells were recorded. PCNA proliferation index (%) =cells with PCNA+/total counting 100%

1.3.3 Expression of BCL-2 Be analogous with 1.3.2.

1.4 Statistics

Measurement variables were analyzed with one-factor analysis of variance and q test by using SPSS 17.0 software,; Nominal variables were done with chi-square test.

2 Results

2.1 Proliferation activity of hepatocarcinoma cells

Proliferation activity of hepatocarcinoma cells was 0.716 ± 0.018 in high chalcone group while which was 0.834 ± 0.027 in medium chalcone group, respectively. Both of them were significantly less than those in tumor control group ($p<0.05$) (Table 1).

Table 1 Proliferation activity of hepatocarcinoma cells in proups

Groups	A	Inhibitory rate(%)
Tumor control	1.135± 0.032	---
High chalcone	0.716 ± 0.018[a]	36.92
Medium chalcone	0.834 ± 0.027[a]	26.52
Low chalcone	1.106 ± 0.038[b]	2.56
Cylophosphamide control	0.079 ± 0.056[a]	40.18

Notes: a $P<0.05$ with tumor control; b $P<0.05$ with high chalcone group

2.2 Expression of PCNA

PCNA expressed endonuclearly and presented brownish yellow granula. This study found numerous cells with PCNA+ in tumor control group, which were hyperchromatic, brownish yellow or tan, and overexpressed (Fig.1). Expression of PCNA in high chalcone group, however, was lower than those in tumor control group (Fig.2). The rate of expression of PCNA was 72.77% in tumor control group and 28.33% in high chalcone group, which was significant ($P<0.05$) (Table 2).

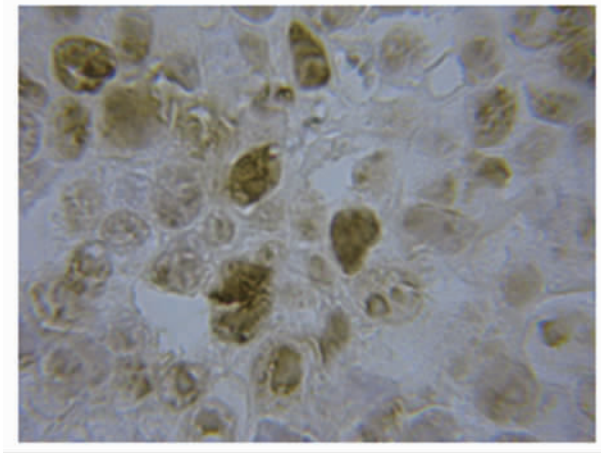

Fig.1 Over expression of PCNA in tumor control group (DAB, × 400)

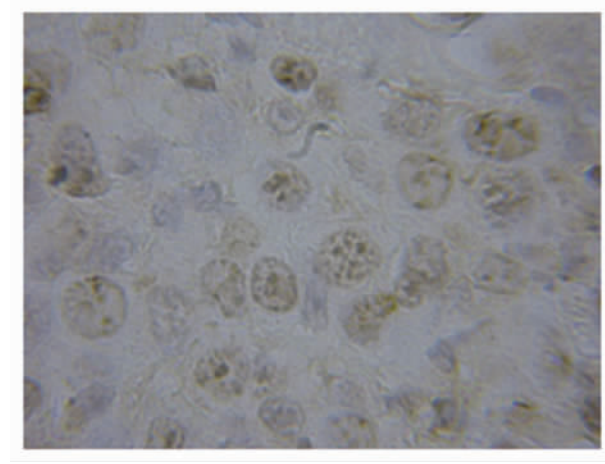

Fig.2 Low expression of PCNA in high chalcone group (DAB, × 400)

2.3 Expression of BCL-2

BCL-2 expressed in cytoplasm and presented brownish yellow granula under optical microscope. There were numerous cells with PCNA+ in tumor control group, which were hyperchromatic and over-expressed (Fig.3). PCNA in high chalcone group had low expression and presented light yellow yet (Fig.4). The rate of expression of PCNA was 65.17% and 16.17%, respectively, and the difference was significant ($P<0.05$) (Table 2).

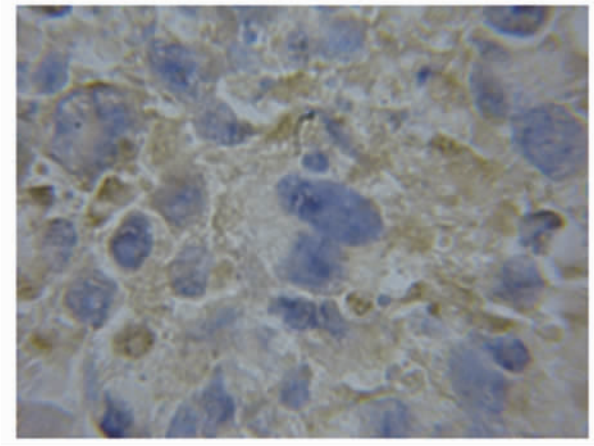

Fig.3 Over expression of BCL-2 in tumor control group (DAB, × 400)

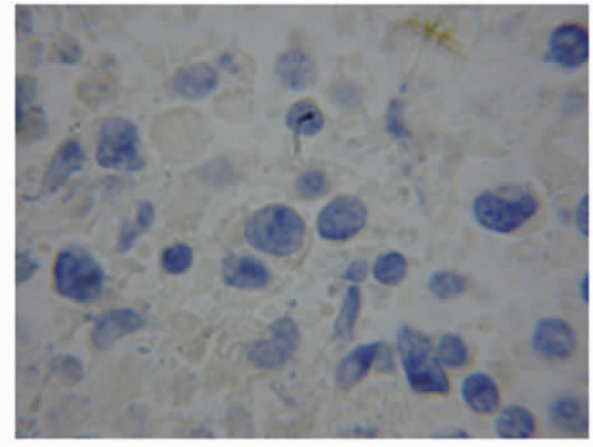

Fig.4 Low expression of BCL-2 in high chalcone group (DAB, × 400)

Table 2 Expression of PCNA & BCL-2 in groups

		PCNA		BCL-2	
Groups	Number	Positive cells	Positive rate (%)	Positive cells	Positive rate (%)
Tumor control	5000	3638	72.77	3258	65.17
High chalcone	5000	1417	28.33a	839	16.17a
Medium chalcone	5000	2168	43.35b	1867	37.32b
Low chalcone	5000	3610	72.04b	3124	62.55b
Cylophosphamide control	5000	1305	26.11a	788	15.77a
Tumor control	5000	3638	72.77	3258	65.17
High chalcone	5000	1417	28.33a	839	16.17a
Medium chalcone	5000	2168	43.35b	1867	37.32b
Low chalcone	5000	3610	72.04b	3124	62.55b
Cylophosphamide control	5000	1305	26.11a	788	15.77a

Notes: a P<0.05 with tumor control; b P<0.05 with high chalcone group

3 Discussion

Chalcone, 1, 3-Diphenyl-2-propennone, is ubiquitous in the nature and is the active component of many kinds of medical plants, such as hachijo ashitaba, safflower, liquorice, etc [5]. Recently, scientists have done research generally and deeply into its bioactivities: anti-tumor, bacteriostasis and anti-inflammatory. It was found that chalcone may inhibit proliferation of breast cancer cells by means of inducing apoptosis and blocking cell cycle progression [6]. Chalcone from hops had antiproliferative activity to colon cancer and ovarian cancer as well as inhibited growth of tumor cell lines[7].

Proliferative activity of cells is an important index reflecting their physical function and ability to proliferate. The injured cells display decreasing proliferative activity firstly. MTT assay is the routine method to detect proliferative activity of cells at home and aboard at the present [8, 9]. MTT is reduced to blue formazam by succinic dehydrogenase of chondrosome, which is associated closely with cell metabolism. There is attenuated reaction or no reaction if cells are injured or dead. In this study, proliferative activity of cells of high chalcone group lower than those of tumor control, medium and low chalcone groups, which demonstrated that chalcone can inhibit proliferative activity of H22 hepatocarcinoma cells. This study had the same results with those reported in literatures-chalcone could inhibit proliferative activity of tumor cells and induce apoptosis [10].

Proliferating cell nuclear antigen (PCNA) is the accessory protein of DNA polymerase, whose synthesis and expression has closely relationship with proliferation of cells. PCNA is over-expressed and its proliferative index rose when proliferative activity of cells increased and vise versa, which is known for reflecting proliferative activity of cell [11-12]. In this study, the expression rate of PCNA in high and medium chalcone group was significantly lower than that in tumor control, and the expression rate of PCNA in high chalcone group wase lower than that in medium group, which demonstrated that chalcone could inhibit proliferative activity of tumor cells.

BCL-2 family has extensive control on apoptosis, among which BCL-2 is the most important anti-apoptosis gene [13]. BCL-2 is low when the cell is injured, apoptosis and undergo antiprolifer-

ative activity [14-16]; However, BCL-2 over-expressed in most of tumor cells. In this study, the expression of BCL-2 of tumor control group was the highest in sorts of groups[17-20], which was agreement with anti-tumor activity of chalcone.

Associated with this study, it can be thought chalcone was ability to inhibit proliferative activity of H22 hepatocarcinoma cell, decrease expressions of PCNA and BCL-2 and inhibit the growth of mice Hepatocarcinoma Cells

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明日叶查尔酮对小鼠肝癌细胞 PCNA 和 BCL-2 蛋白表达影响

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摘要 目的 :研究明日叶 (Angelica keiskei Koidz) 查尔酮对小鼠肝癌细胞 PCNA 和 BCL-2 蛋白表达的影响。**方法 :**将 50 只皮下接种肝癌 H22 细胞株的小鼠随机分为 5 组,每组 10 只。高、中、低剂量组分别每日经口灌胃给予 40、20、5mg/kg 的查尔酮,肿瘤对照组给予等量生理盐水,连续 10d,环磷酰胺组隔天腹腔注射环磷酰胺 20mg/kg。取肝癌组织用四甲基偶氮噻唑蓝 (MTT) 法测各组小鼠肝癌细胞增殖活性,免疫组化法检测各组肝癌细胞增殖细胞核抗原 (PCNA) 和凋亡相关蛋白 BCL-2 表达水平。**结果 :**高剂量查尔酮组和肿瘤对照组的肝癌细胞增殖活性分别为(0.716± 0.018)和 (1.135± 0.032),差别有显著性 (P<0.05) 。高剂量组 PCNA 和 BCL-2 蛋白表达率分别为 28.33%和 16.77%,肿瘤对照组分别为 72.77%和 65.17%,差异均有显著性 (P<0.05) 。**结论 :**查尔酮可降低小鼠肝癌细胞 PCNA 和 BCL-2 表达水平,对肝癌细胞增殖有一定抑制作用。

关键词 :查尔酮 ;肝癌细胞 ;增殖活性 ;PCNA ;BCL-2

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