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Hericium erinaceus polysaccharide-protein HEG-5 inhibits SGC-7901 cell growth *via* cell cycle arrest and apoptosis

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ABSTRACT

HEG-5 is a novel polysaccharide-protein purified from the fermented mycelia of *Hericium erinaceus* CZ-2. The present study aims to investigate the effects of HEG-5 on proliferation, cell cycle and apoptosis of human gastric cancer cells SGC-7901. Here, we first uncover that HEG-5 significantly inhibited the proliferation and colony formation of SGC-7901 cells by promoting apoptosis and cell cycle arrest at S phase. RT-PCR and Western blot analysis suggested that HEG-5 could decrease the expressions of Bcl2, PI3K and AKT1, while increase the expressions of Caspase-8, Caspase-3, p53, CDK4, Bax and Bad. These findings indicated that the Caspase-8/-3-dependent, p53-dependent mitochondrial-mediated and PI3k/Akt signaling pathways involved in the molecular events of HEG-5 induced apoptosis and cell cycle arrest. Thus, our study provides in vitro evidence that HEG-5 may be taken as a potential candidate for treating gastric cancer.

1. Introduction

Mushrooms are ubiquitous in nature and have high nutritional/pharmaceutical attributes. Many species such as *Ganoderma lucidum* and *Hericium erinaceum* have long been used as traditional Chinese medicines or functional foods in China, Japan and other Asian countries for the treatment of gastroenteric disorder, lymphatic disease and various cancers [1]. In particular, low-molecular-weight (e.g. cerebrosides, isoflavones, catechols, amines, triacylglycerols, sesquiterpenes and steroids) and high-molecular-weight (e.g. polysaccharides, polysaccharide-proteins/peptides and proteins) compounds are considered as the major functional constituents responsible for mushrooms' biological activities [2–5].

Generally, polysaccharide–proteins/peptides complexes have different structures and bioactivities from those of polysaccharides in mushrooms. Polysaccharides possess the pure carbohydrate chains and are considered as biological response modifiers (BRMs) and used as the nonspecific immune-stimulator for cancer treatment. For example, the homogeneous *Grifola frondosa* polysaccharide GFPBW2 and *Phellinus igniarius* extracellular polysaccharides (EPS) showed their antitumor activities by activating the macrophages or balancing the numbers of White blood cells (WBC), Red blood cell (RBC), platelet (PLT) and Hemoglobin (HGB) in vitro and in vivo [6,7]. However, polysaccharide-proteins/peptides complexes contain the protein/polypeptide covalently and specifically bound with carbohydrates as the side-chains [8,9]. In most cases, polysaccharide–proteins/peptides inhibit the tumor cells growth *in vitro* by activating multiple signal pathways including cell cycle arrest, DNA damage, and alteration of death inhibitors/promoters expression [10]. Previous studies had revealed that *Ganoderma lucidum* glycopeptide GLPS-SF1, *Grifola frondosa* polysaccharide–peptide GFPS1b, *Pleurotus citrinpileatus* glycoprotein PCP-3A and pea glycoprotein could kill the tumor cells directly *via* inducing cell cycle arrest, up-regulating *Bax* gene expression and/or down-regulating *Bcl-2*/*Bcl-xl* genes expression [11–14].

The edible mushroom *Hericium erinaceus* belongs to the order *Aphyllophorales* and family *Hycnaecae* [15]. Some biological components such as polysaccharides, lectins, amycenone, and aromatic compounds have been obtained from *H. erinaceus* mycelia or fruiting body and showed the pharmacological properties [16–19]. In our previous study, a hemagglutinating/antitumor

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0141-8130/

Table 1
Sequence of primers used in quantitative RT-PCR.

Target gene	primer	Nucleotide sequence
p53	F	CACGTACTCTCCTCCCCTCAATA
	R	TCTTCCAGTGTGATGATGGTAAGG
CDK4	F	GAGGCGACTGGAGGCTTTT
	R	GGATGTGGCACAGACGTCC
Bcl 2	F	GGATTGTGGCCTTCTTTGAG
	R	CAGCCAGGAGAAATCAAACAG
Bax	F	TCCACCAAGAAGCTGAGCGA
	R	GTCCAGCCCATGATGGTTCT
Bad	F	CGAGTGAGCAGGAAGACTCC
	R	CTGTGCTGCCCAGAGGTT
Caspase 3	F	AACCTCAGGGAAACATTCAG
	R	GGCTCAGAAGCACACAAAC
Caspase 8	F	GGATGCCTTGATGTTATTCC
	R	AGTTCCCTTTCCATCTCCTC
AKT1	F	GGCGAGCTGTTCTTCCACCT
	R	ATTGTCCTCCAGCACCTCGG
PI3K	F	ATTCCCAGTCAGAGGCGCTAT
	R	GAACTTGTCTTCCCGTCGTGT
β-Actin	F	GTCCACCGCAAATGCTTCTA
	R	TGCTGTCACCTTCACCGTTC

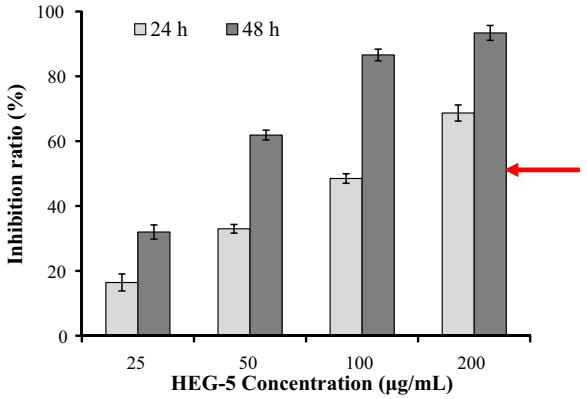

Fig. 1. Growth inhibitory effects of HEG-5 on SGC-7901 cells. SGC-7901 cells in their exponential growth phase were plated into a 96-well culture plate at the density of 5×10^4 cells per well and incubated for 24 h. Then, the cells were exposed to 25, 50, 100 and 200 μg/mL HEG-5 sample for 24 h and 48 h. The effects of HEG-5 on the growth of SGC-7901 cells were determined by MTT assay. Data represent the mean ±SD of three replicates.

polysaccharide-protein (glycoprotein) HEG-5 with the novel and thermo-unstable structure was purified from the *H. erinaceus* fermented mycelia [20–22]. To further elucidate the mechanism of HEG-5 on inhibiting tumor cells, the present study aims to evaluate the inhibitory effect of HEG-5 and its role on cell cycle arrest and apoptosis of SGC-7901 cells, and reveal a possible signal pathway for elucidating the HEG-5′ antitumor molecular mechanisms.

2. Materials and methods

2.1. Materials

All the cell culture reagents were purchased from Hyclone laboratories (South Logan, Utah, USA). Hoechst dye 33258, agrose, ethidium bromide (EtBr), 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethyl sulfoxide (DMSO),

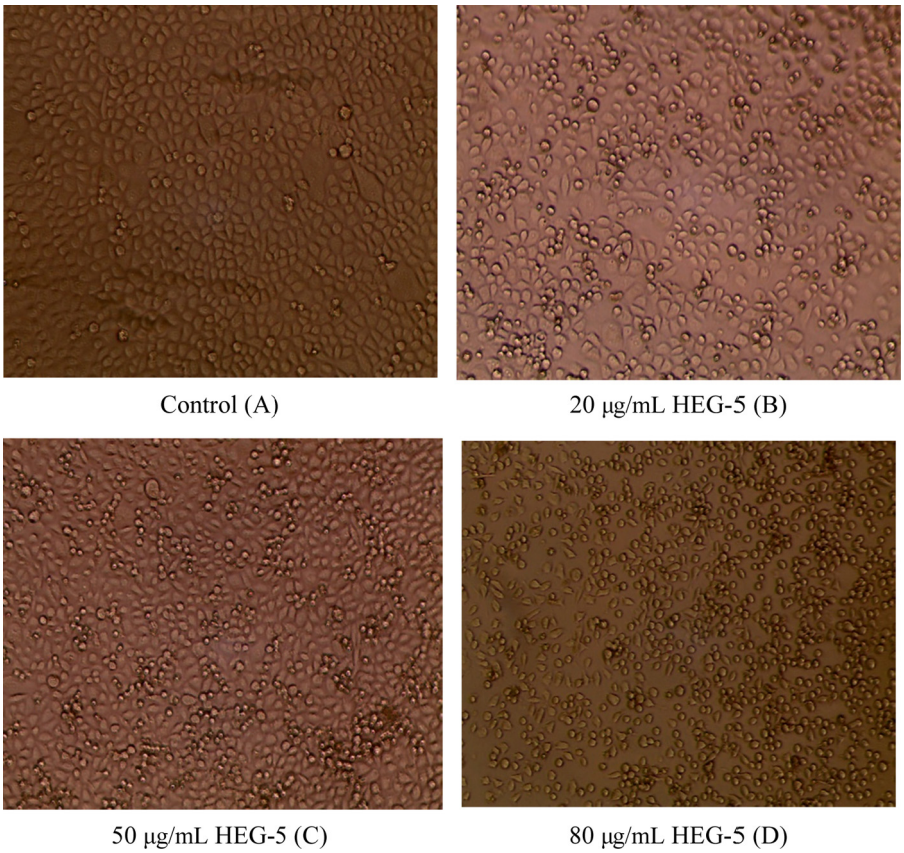

Fig. 2. Inverted light micrographs of SGC-7901 showing morphological changes of cells treated without (A) and with 20 μg/mL (B), 50 μg/mL (C), and 80 μg/mL (D) of HEG-5 for 48 h (×10). With increasing concentrations of HEG-5, the number of normal cells gradually decreased. Additionally, the cell morphology began to change as round-shaped cells shrinkage and detachment from culture plate.