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Ginsenoside exhibits concentration-dependent dual effects on HepG2 cell proliferation via regulation of c-Myc and HNF-4α

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ABSTRACT

Although ginsenoside can generally promote cell proliferation, it is reported to have anti-proliferative effects in hepatocellular carcinoma (HCC). Whether ginsenoside has concentration-dependent effects on HCC cell proliferation have not been clarified. Transcription factors c-Myc and hepatocyte nuclear factor (HNF)-4α are the most important opposite controllers of HCC cell proliferation. Whether and how ginsenoside regulates c-Myc and HNF-4α as well as their recruitment of the co-activator p300 to exhibit its effects on HCC cell proliferation are pending. In this study, it was found that low concentration ginsenoside promoted HepG2 cell proliferation while high concentration ginsenoside exhibited anti-proliferation effect. For low concentration ginsenoside treatment, c-Myc was up-regulated and the binding of p300 to c-Myc was promoted with obvious co-localization to activate HepG2 cell proliferation. However, for high concentration ginsenoside treatment, besides c-Myc, HNF-4α was also up-regulated might to exhibit an alternative effect. Furthermore, in contrast to the weakened binding and co-localization of c-Myc and p300, the binding of p300 to HNF-4α was enhanced with distinct co-localization to inhibit HepG2 cell proliferation for high concentration ginsenoside treatment. The results manifested that ginsenoside with low and high concentrations may differentially regulate c-Myc and HNF-4α as well as their recruitments of p300, to exhibit concentration-dependent dual effects on HepG2 cell proliferation.

1. Introduction

Hepatocellular carcinoma (HCC) is a major liver malignancy and searching for specific therapy still remains a struggle in medical research (Han 2012; Yong et al., 2013; Yu et al., 2013; Singh et al., 2014; Sudan and Rupasinghe 2014).

Ginseng, the root of *Panax ginseng* C.A. Meyer (Araliaceae), is one of the most famous Chinese herbs for thousands of years and its main active components are ginsenoside (Bae et al., 2014; Wang et al., 2016). Besides cardiovascular beneficial effects, ginsenoside is generally believed to promote cell proliferation (Karmazyn et al., 2011; Park et al., 2015). However, ginsenoside is reported to have anti-proliferative effects on cancer cells, including HCC cells (Lee et al., 2013; Oh et al., 2015; Park et al., 2016). Whether ginsenoside has concentration-dependent effects on HepG2 cell proliferation and the underlying mechanisms have not been clarified.

In HCC, transcription factor c-Myc is a master controller of cell proliferation, which can bind the E-box to activate expression of genes, which are involved in cell proliferation (Dang 2012; Pannem et al., 2014). In contrary, hepatocyte nuclear factor (HNF)-4α is well known as an opposite controller of hepatocyte proliferation (Li et al., 2015). Over-expression of HNF-4α could inhibit proliferation and induce differentiation of hepatoma cells. The p300 family is the most important histone acetyltransferase, participating in the regulation of gene transcription. Both c-Myc and HNF-4α need recruit the co-activator p300 to ensure transactivation (Vervoorts et al., 2003; Roth et al., 2004). Whether ginsenoside regulates c-Myc and HNF-4α, as well as their binding to p300 to exhibit its effects on HCC cell proliferation is unclear.

In this study, HepG2 cells were treated with a series of concentrations of ginsenoside and their proliferation was evaluated to explore the concentration-dependent effects of ginsenoside on HepG2 cell proliferation. Then the mRNA expressions and protein contents of c-Myc and HNF-4α, the bindings of p300 to c-Myc or HNF-4α and their co-

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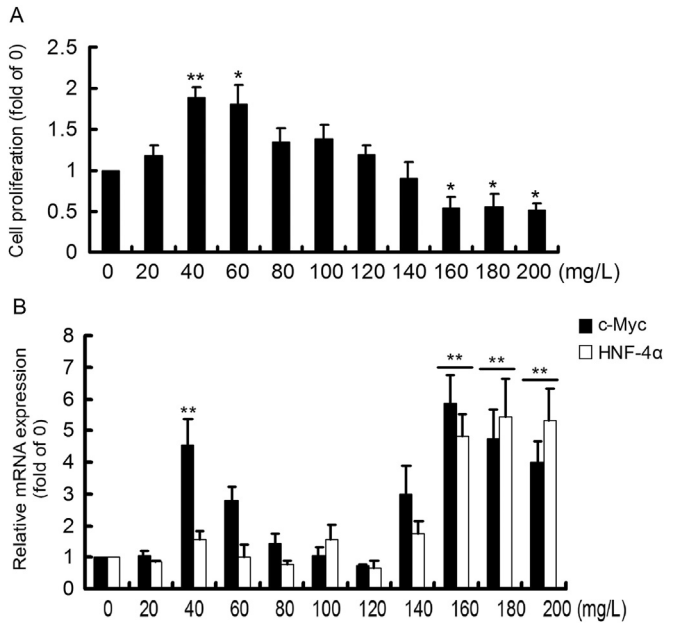

Fig. 1. HepG2 cell proliferation, c-Myc and HNF-4α expressions after ginsenoside treatments. A. HepG2 cell proliferation was evaluated by MTT. B. Expression of c-Myc and HNF-4α in treated HepG2 cells. * $P < 0.05$ or ** $P < 0.01$, vs 0 group. The experiments were repeated three times.

localizations were measured to investigate the underlying mechanism.

2. Materials and methods

2.1. Cells and treatment

HepG2 cells (human hepatoma cell line) were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China), and cultured in Dulbecco modified Eagle medium supplemented with 10% (v/v) fetal bovine serum (Gibco, Waltham, USA) with a temperature of 37 °C, 5% CO_2 concentration and 95% relative humidity. Ginsenoside was provided by Yiling Medical Research Institution of Hebei (purity 99%, No.20070203) with the composition of 50.96% Rb1, 6.81% Rg1, 20.8% Re, 5.23% Rd, 8.97% Rc. As no other drugs can mimic the unique effects of ginsenoside, positive control was not designed in this study, and instead, a series of concentrations of ginsenoside was adopted. After HepG2 cells were seeded in 6-well plate (1×10^5) and treated with 0, 20, 40, 60, 80, 100, 120, 140, 160, 180, 200 mg/L ginsenoside for 48 h, the cells were photographed, and mRNA expressions of c-Myc and HNF-4α as well as the metabolic enzyme Cyp3a4 were detected. And the proliferation of HepG2 cells (5×10^3) in 96-well plate was evaluated by MTT. Then HepG2 cells were divided into control, low concentration ginsenoside (40 mg/L) and high concentration ginsenoside (160 mg/L) groups, based on the distinct effects of ginsenoside on cell proliferation and gene expressions. After HepG2 cells were seeded in 6-well plate (1×10^5) and treated for 0, 24 h, 48 h and 72 h, changes of c-Myc and HNF-4α expressions were ascertained. After HepG2 cells were seeded in flasks (1×10^6) and treated for 48 h, protein contents of c-Myc and HNF-4α, and bindings of p300 to c-Myc or HNF-4α were measured. After HepG2 cells on cover slips in 6-well plate (5×10^4) were treated for 48 h, the co-localization of p300 with c-Myc or HNF-4α was evaluated.

2.2. Quantitative real-time reverse transcription-PCR

Total RNA of treated HepG2 cells was isolated using Trizol reagent (Takara, Dalian, China) and reverse transcribed into cDNA using RevertAid First Strand cDNA synthesis Kit (Fermentas, Shanghai, China), followed by real-time PCR amplification using specific primers. c-Myc, sense: 5' CGGGGCTTTATCTAACTCGC 3' and anti-sense: 5' GCTATGGGCAAAGTTTCGTG 3', with product 224 bp; HNF-4α, sense: 5' GATGACAATGAGTATGCCTACCT 3' and anti-sense: 5' GTCGTTGATGTAGTCCTCCAA 3', with product 132 bp; Cyp3a4, sense: 5' ACGGGACTATTTCCACCACC 3' and anti-sense: 5' TGTGCAGGAAAGCATCTGATA 3', with product 234 bp; β-actin, sense: 5' CTCCATCCTGGCCTCGCTGT 3' and anti-sense: 5' GCTGTCACCTTCACCGTTCC 3', with product 268 bp. The conditions were 95 °C 10 min and 40 cycles of 95 °C 10 s, 60 or 65 °C 20 s and 72 °C 20 s. And the relative gene expressions were calculated using the delta-delta-CT method.

2.3. Western blotting

Protein contents of c-Myc and HNF-4α in HepG2 cells were detected by Western blotting, with the protocol previously described (Li et al., 2013). The anti-c-Myc (Santa Cruz, Texas, USA), anti-HNF-4α (Santa Cruz, Texas, USA) and anti-actin antibodies (Santa Cruz, Texas, USA) were used.

2.4. Co-immunoprecipitation

The bindings of p300 to c-Myc or HNF-4α were analyzed by co-immunoprecipitation (Co-IP) assay. After cells were sonicated and protein content was determined, Co-IP assay was performed as described (Li et al., 2013). At first, an aliquot of the sample was used to detect total p300, c-Myc or HNF-4α (their respective Input) by Western blotting. When anti-p300 (Millipore, Darmstadt, Germany) antibody was used for IP, the co-immunoprecipitated c-Myc and HNF-4α were detected by Western blotting. When anti-c-Myc (Santa Cruz, Texas, USA) or anti-HNF-4α (Santa Cruz, Texas, USA) antibody was

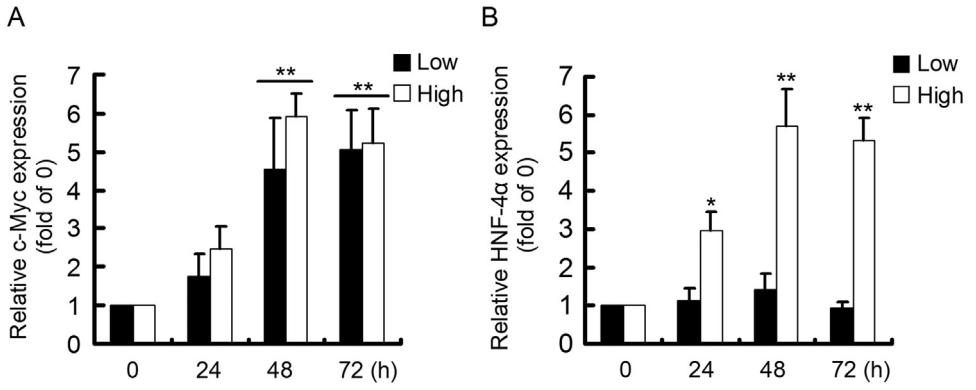

Fig. 2. The expressions of c-Myc and HNF-4α for ginsenoside treatments. The expressions of c-Myc (A) and HNF-4α (B) in treated HepG2 cells for different time. * $P < 0.05$ or ** $P < 0.01$, vs 0 group. The experiments were repeated three times. Low, low concentration ginsenoside; High, high concentration ginsenoside.