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Anticancer effects of ginsenoside Rg1, cinnamic acid, and tanshinone IIA in osteosarcoma MG-63 cells: Nuclear matrix downregulation and cytoplasmic trafficking of nucleophosmin

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Abstract

Ginsenoside Rg1, cinnamic acid, and tanshinone IIA are effective anticancer and antioxidant constituents of traditional Chinese herbal medicines of Ginseng (*Panax ginseng*), Xuanshen (*Radix scrophulariae*), and Danshen (*Salvia mitiorrhiza*), respectively. There was insufficient study on molecular mechanisms of anticancer effects of those constituents and their targets were unknown. We chose nucleophosmin as a candidate molecular target because it is frequently mutated and upregulated in various cancer cells. Nucleophosmin is a major nucleolus phosphoprotein that involves in rRNA synthesis, maintaining genomic stability, and normal cell division and its haploinsufficiency makes cell more susceptible to oncogenic assault. Ginsenoside Rg1, cinnamic acid, and tanshinone IIA treatment of osteosarcoma MG-63 cells decreased nucleophosmin expression in nuclear matrix and induced nucleophosmin translocation from nucleolus to nucleoplasm and cytoplasm, a process of dedifferentiating transformed cells. Using immunogold electro-microscopy, we found at the first time that nucleophosmin was localized on nuclear matrix intermediate filaments that had undergone restorational changes after the treatments. Nucleophosmin also functions as a molecular chaperone that might interact with multiple oncogenes and tumor suppressor genes. We found that oncogenes c-myc, c-fos and tumor suppressor genes, P53, Rb were regulated by ginsenoside Rg1, cinnamic acid, and tanshinone IIA as well. In present study, we identified nucleophosmin as a molecular target of the effective anticancer constituents of t Ginseng, Xuanseng, and Danseng that down-regulated nucleophosmin in nuclear matrix, changed its trafficking from nucleolus to cytoplasm, and regulated several oncogenes and tumor suppressor genes. Therefore, we postulate that Ginsenoside Rg1, cinnamic acid, and tanshinone IIA could serve as protective agents in cancer prevention and treatment.

Keywords: Ginsenoside; Nucleophosmin; Human osteosarcoma; Nuclear matrix; Induced differentiation

1. Introduction

Ginseng (*Panax ginseng*), Xuanshen (*Radix scrophulariae*), and Danshen (*Salvia mitiorrhiza*) literately mean in Chinese "essences of the earth in the form of a man, a saint, and a cardinal", respectively. They were highly

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treasured in Chinese traditional medicines for maintaining youth, promoting longevity, and balancing whole body ying and yang to prevent diseases. Ginsenoside Rg1, cinnamic acid, and tanshinone IIA (RCT) are effective constituents of Ginseng, Xuanshen, and Danshen, respectively. Previous studies showed that RCT prevent tumor growth through reversal of gene expression of transformed cells by antioxidant, anti-inflammatory and apoptotic mechanisms (Helms, 2004; Yun, 2001). However, RCT treatment of cancer is controversial because their adverse interactions with other anticancer drugs (Sparreboom, Cox, Acharya, & Figg, 2004). The molecular mechanisms of RCT anticancer effects were not well understood. We previously found that osteosarcoma MG-63 cells could be induced to differentiation by RCT. In this study, we used osteosarcoma MG-63 cells as a model to investigated RCT effect on a major nucleolus phosphoprotein, nucleophosmin (NPM), whose trafficking from nucleolus to nucleoplasm and cytoplasm was used to test effectiveness of anticancer drugs (Chan, Qi, Amley, & Koller, 1996; Yung, 2007; Yung, Yang, & Bor, 1991).

Nucleophosmin plays multiple roles in tumorogenesis by functions in rRNA processing (Savkur & Olson, 1998), histone chaperone (Okuwaki, Matsumoto, Tsujimoto, & Nagata, 2001; Szebeni & Olson, 1999), centrosome duplication (Okuda et al., 2000), negative regulation of the ARF-P53 tumor suppressor pathway (Colombo, Marine, Danovi, Falini, & Pelicci, 2002; Colombo et al., 2005; Li, Zhang, Sejas, & Pang, 2005). NPM mutations were frequently observed in acute myologenous leukemia with normal karyotype (AML-NK) and NPM cytoplasmic location in leukemic blasts forming a prognostically favorable subgroups to induction of chemotherapy (Falini et al., 2005; Falini, Nicoletti, Martelli, & Mecucci, 2007). Chromosomal translocations at NPM gene locus were found in anaplastic lymphoma with t(2;5)(p23;q35) (Morris et al., 1994), acute promyelocytic leukemia t(5;17)(q35;q12) (Redner, 2002), and acute myeloid leukemia t(3;5)(q25;q35) (Raimondi et al., 1989). NPM homozygous knockout mice were embryonic lethal and heterozygous knockout mice were NPM gene haploinsufficient with increased susceptibility to oncogenic transformation (Grisendi et al., 2005). NPM is tightly bond to nuclear matrix in association with induction of proliferation in lymphocytes (Feuerstein & Mond, 1987; Feuerstein, Spiegel, & Mond, 1988) and localized in nuclear matrix intermediate filament system in human hepatocarcinoma cells (Tang et al., 2007). In this study, we further explored differential expression and intermediate filament localization of nucleophosmin in the nuclear matrix during the differentiation of MG-63 cell induced by RCT.

2. Materials and methods

2.1. Cell culture and treatment

The osteosarcoma MG-63 cells, provided by China Center for Type Culture Collection (CCTCC), were maintained in RPMI-1640 medium supplemented with 15% heat-inactivated fetal calf serum, 100 U mL^{-1} penicillin, 100 $\mu g\,mL^{-1}$ streptomycin and 50 $\mu g\,mL^{-1}$ kanamycin at 37 °C, 5% CO_2 in air atmosphere. After being seeded for 24 h, MG-63 cells were treated with culture medium containing the combination of 33 $\mu g\,mL^{-1}$ ginsenoside Rg1, 0.3 $\mu g\,mL^{-1}$ cinnamic acid and 0.3 $\mu g\,mL^{-1}$ tanshinone IIA (shortened form RCT) for 7 days to induce differentiation. Meanwhile, MG-63 cells were cultured in RPMI-1640 medium as the control group. The components of RCT are the standards bought from National Institute of the Control for the Pharmaceutical and Biological Products (NCPBP). The concentration of RCT was decided by previous work which evaluated the effects of terminal differentiation on MG-63 cells treated with the components and the combination of RCT.

2.2. Extraction of nuclear matrix proteins (NMPs)

For 2-DE analysis, the extraction method used was modified from Fey and Nickerson et al. (Gao, Han, Jiao, & Zhai, 1994; Michishita et al., 2002; Nickerson, Krockmalnic, Wan, & Penman, 1997). Harvested MG-63 cells were firstly washed with ice-cold PBS twice and then extracted by cytoskeleton (CSK) buffer (100 mM KCl, 3 mM MgCl, 5 mM EGTA, 10 mM PIPES, pH 6.8, 300 mM sucrose, 0.5% Triton X-100, 2 mM PMSF) for 10 min at 0 °C. After being centrifuged at 600 × *g* for 5 min, the pellets were washed with ice-cold PBS to remove soluble cytoplasmic proteins. They were then re-centrifuged and suspended within digestion buffer (same as CSK buffer except with 50 mM NaCl instead of KCl) containing 400 mg mL^{-1} DNase I for 30 min at room temperature. Cold ammonium sulfate was added to a final concentration of 0.25 M to terminate the enzyme digestion. After centrifugation at 1000 × *g* for 10 min, the pellets were washed with CSK buffer and then dissovolved in lysis buffer (7 M urea, 2 M thiourea, 4% CHAPS, 1.5% Triton X-100, 1% Pharmalyte (pH 3–10, Amersham Biosciences), 65 mM DTT, 40 mM Tris, 5 mg/mL aprotinin, 1 mg/mL leupeptin, 1 mg/mL pepstatin, and 2 mM PMSF, 5 mM EDTA). The sam-