Toxicology Letters 220 (2013) 219–228

Contents lists available at SciVerse ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

Naringin inhibits growth potential of human triple-negative breast cancer cells by targeting β-catenin signaling pathway

Hongzhong Li[a], Bing Yang[a], Jing Huang[a], Tingxiu Xiang[a], Xuedong Yin[a], Jingyuan Wan[b], Fuling Luo[b], Li Zhang[c], Hongyuan Li[a], Guosheng Ren[a,*]

[a] Molecular Oncology and Epigenetics Laboratory, The First Affiliated Hospital of Chongqing Medical University, Chongqing, PR China
[b] Department of Pharmacology, Chongqing Medical University, Chongqing, PR China
[c] Department of Pathophysiology, Chongqing Medical University, Chongqing, PR China

HIGHLIGHTS

- Naringin inhibits cell growth in triple-negative breast cancer cells.
- Naringin regulates p21 and survivin in triple-negative breast cancer cells.
- Naringin inhibits the activation of β-catenin pathway.
- Inhibiting β-catenin pathway is responsible for the antitumor activity of naringin.

ARTICLE INFO

Article history:
Received 22 March 2013
Received in revised form 10 May 2013
Accepted 12 May 2013
Available online 18 May 2013

Keywords:
Naringin
Flavonoid
Breast cancer
Apoptosis
β-Catenin

ABSTRACT

Triple-negative (ER-/PR-/HER2-) breast cancer (TNBC) is a severe clinical problem because of its relatively poorer prognosis, aggressive behavior and lack of targeted therapies. Naringin, a major flavonoid extracted from citrus fruits, has been reported to exert promising anticancer activities. However, the detailed antitumor mechanism of naringin still remains enigmatic. In this study, TNBC cell lines-based *in vitro* and *in vivo* models were used to explore the anticancer effect and mechanism of naringin. Our data demonstrated that naringin inhibited cell proliferation, and promoted cell apoptosis and G1 cycle arrest, accompanied by increased p21 and decreased survivin. Meanwhile, β-catenin signaling pathway was found to be suppressed by naringin. In contrast, over-expressing β-catenin by adenoviral vector system in TNBC cells reversed the antitumor activity of naringin, and regulated p21 and survivin. Correspondingly, the antitumor potential of naringin was also observed in naringin-treated MDA-MB-231 xenograft mice, while immunohistochemical analysis of tumors from naringin-treated mice showed higher expression of p21 and lower expression of survivin and active β-catenin. Taken together, these results indicate that naringin could inhibit growth potential of TNBC cells by modulating β-catenin pathway, which suggests naringin might be used as a potential supplement for the prevention and treatment of breast cancer.

1. Introduction

Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death in females worldwide (DeSantis et al., 2011). Triple-negative breast cancer (TNBC), characterized by a lack of expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor (HER)-2, accounts for 15% of all types of breast cancer. Epidemiologic studies demonstrate that TNBC, showing a tendency toward early metastasis and poor prognosis, represents a significant clinical challenge (Irvin and Carey, 2008). Since TNBC does not respond to endocrine therapy or other available targeted agents, drug treatment options are limited to traditional chemotherapy which is frequently trapped in the drug resistance (DeSantis et al., 2011; Reddy, 2011). Therefore, to explore new drugs or treatments against TNBC has been very imperative and attracted extensive attention.

Furthermore, as it is widely accepted that carcinogenesis is a complex and multi-stage process, cancer prevention by the use

Abbreviations: TNBC, triple-negative breast cancer; ER, estrogen receptor; PR, progesterone receptor; HER, human epidermal growth factor receptor; DMSO, dimethyl sulfoxide; PI, propidium iodide; IHC, immunohistochemistry; SCID, severe combined immunodeficiency; CDK, cyclin-dependent kinase; PCNA, proliferating cell nuclear antigen; IAP, inhibitors of apoptosis.

* Corresponding author at: Molecular Oncology and Epigenetics Laboratory, The First Affiliated Hospital of Chongqing Medical University, No. 1 Youyi Road, Yuzhong District, Chongqing 400016, PR China. Tel.: +86 2389012305; fax: +86 2389012305.
E-mail address: rgs726@163.com (G. Ren).

0378-4274/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.toxlet.2013.05.006

of pharmacological agents, especially naturally occurring dietary substances has been considered as a practical approach to reduce the incidence of breast cancer. Numbers of natural substances in human diet have been studied to evaluate the antitumor activities. It has been revealed that many dietary materials such as curcumin, diallyl disulfide, and extracts from bitter melon or blueberry exert inhibitory effects on the growth and/or metastasis of breast cancer cells, showing a good application prospect in cancer prevention and treatment (Adams et al., 2010; Nagaraj et al., 2010; Ray et al., 2010; Sun et al., 2012).

Naringin is a major flavonoid extracted from grapefruit and other citrus fruits. Its structure contains the aglycone moiety named naringenin, which is linked to a dioside, the neohesperidoside. In the human body, naringin is mainly metabolized to naringenin and its conjugates, which are eliminated by renal excretion (Fuhr and Kummert, 1995). Studies have shown that naringin exerts a variety of pharmacological effects such as antioxidant, cholesterol-lowering, antiatherogenic, anti-inflammatory and antiviral activities (Kumar et al., 2010; Nie et al., 2012; Xulu and Oroma Owira, 2012; Zandi et al., 2011). Recently, several

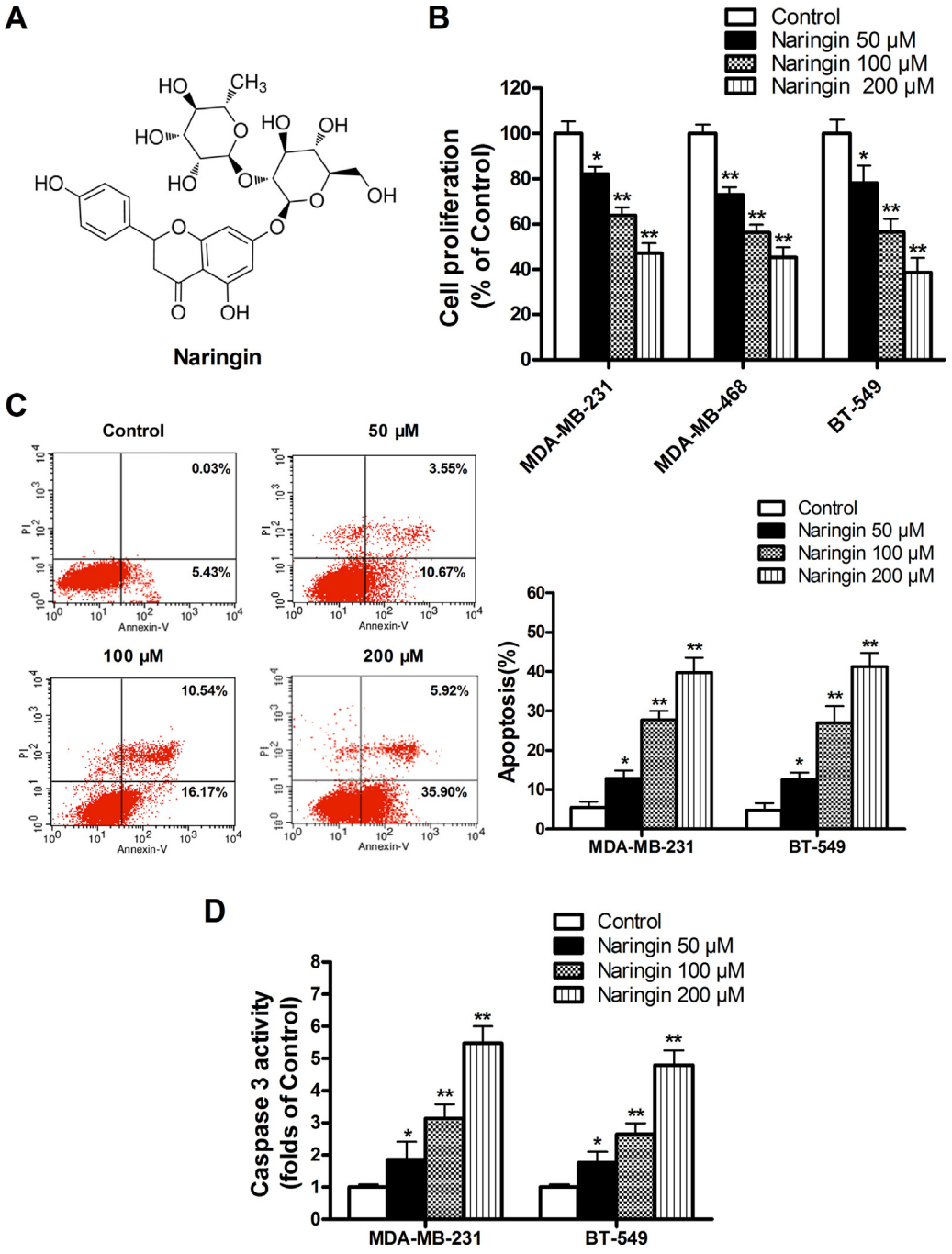

Fig. 1. Naringin inhibits cell proliferation and induces cell apoptosis in TNBC cells. (A) Chemical structure of naringin. (B) Breast cancer cell lines were treated with various concentrations of naringin for 48 h, cell viability was measured by a CCK-8 kit. Data were expressed as ratios (treated *vs.* control). (C) The cell apoptosis of MDA-MB-231 cells and BT-549 cells induced by naringin was examined by flow cytometry analysis of annexin V-FITC/PI staining. Numbers inside dot plots indicate the percentages of apoptotic MDA-MB-231 cells. (D) Caspase 3 activity was measured using a colorimetric assay kit. Data were expressed as ratios (treated *vs.* control). All the data were presented as the mean ± SD, $n \geq 9$, * $p < 0.05$ and ** $p < 0.01$, compared with control group.