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Nobiletin (NOB) suppresses autophagic degradation via over-expressing AKT pathway and enhances apoptosis in multidrug-resistant SKOV3/TAX ovarian cancer cells

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ABSTRACT

Chemotherapy could be used as an effective therapeutic treatment for ovarian cancer and subsequent peritoneal metastasis. However, the occurrence of drug resistance reduced the treatment effect originated from cancer chemotherapy. Accumulating evidences indicated the significant role of autophagy in tumor cell resistance to chemotherapy. Thus, inhibition of autophagy using natural compounds could be a promising candidate to overcome multidrug resistance in human ovarian cancer cells. Nobiletin (NOB), a polymethoxyflavonoid found in citrus fruits such as Citrus depressa and Citrus reticulate, exhibits a number of bioactivities. In the present study, NOB selectively suppressed the growth and proliferation of human SKOV3/TAX cells, inducing G0/G1 phase arrest and reducing G2/M phase, along with the increase of p53 and p21. In addition, NOB induced significant apoptosis in SKOV3/TAX cells through the intrinsic apoptosis pathway, as evidenced by the up-regulation of cleaved Caspase-9/-3 and PARP. Further, NOB impaired the autophagic degradation in SKOV3/TAX cells, resulting in autophagic flux inhibition. Moreover, the impaired autophagic flux enhanced NOB-induced apoptosis in SKOV3/TAX cells. Importantly, AKT signaling was activated by NOB, which was involved in autophagic degradation and apoptotic cell death. In conclusion, the findings here supplied the illustration that NOB could overcome multidrug resistance in human ovarian cancer cells through AKT-regulated suppression of autophagic degradation.

1. Introduction

Ovarian cancer is the leading cause of death among women with gynecological malignancies [1]. Acquired resistance to chemotherapy is a major limitation for the treatment of ovarian cancer [2]. Paclitaxel is recommended as a first-line chemotherapeutic agent against ovarian cancer, but drug resistance becomes a major limitation of its success clinically [3]. It is an important issue in ovarian cancer therapy to search an effective clinical treatment to improve curative effect of ovarian cancer patients [4,5]. A numbers of compounds derived from natural resources have provided novel leading compounds for drug development, and use as pharmacological tools [6]. Nobiletin (5,6,7,8,30,40-hexamethoxyflavone, NOB, Fig. 1A), a polymethoxyflavonoid, was found in citrus fruits such as Citrus depressa and Citrus reticulate, performs a series of bioactivities [7]. As previously reported, NOB could suppress inflammation-related tumorigenesis aberrant cell proliferation and colon carcinogenesis [8]. In addition, it has ability to suppress angiogenesis in human umbilical vein endothelial cells in vitro [9]. Of note, NOB is a decreases metastasis of human fibrosarcoma cells and gastric cancers [10,11]. Therefore, we supposed that NOB may display an effective anti-tumor role in suppressing human ovarian cancer, and is essential for multidrug-resistant ovarian cancer cells.

Increasing evidences have suggested that promotion of autophagy could result in tumor cell resistance to chemotherapy in a variety of tumors, including breast cancer, liver cancer, as well as leukemia [12]. Recently, researches have indicated that suppression of autophagy using specific inhibitors or genetic silence, could re-sensitize the drug-resistant cancer cells, and promote the efficiency of various chemotherapeutic agents [13]. Further, autophagic flux, also known as integrated autophagy, is a dynamic process, including cargo collection, autophagosome formation, the autophagosome-lysosome fusion, as well as the cargo degradation in the end [14]. Inhibition of autophagic flux

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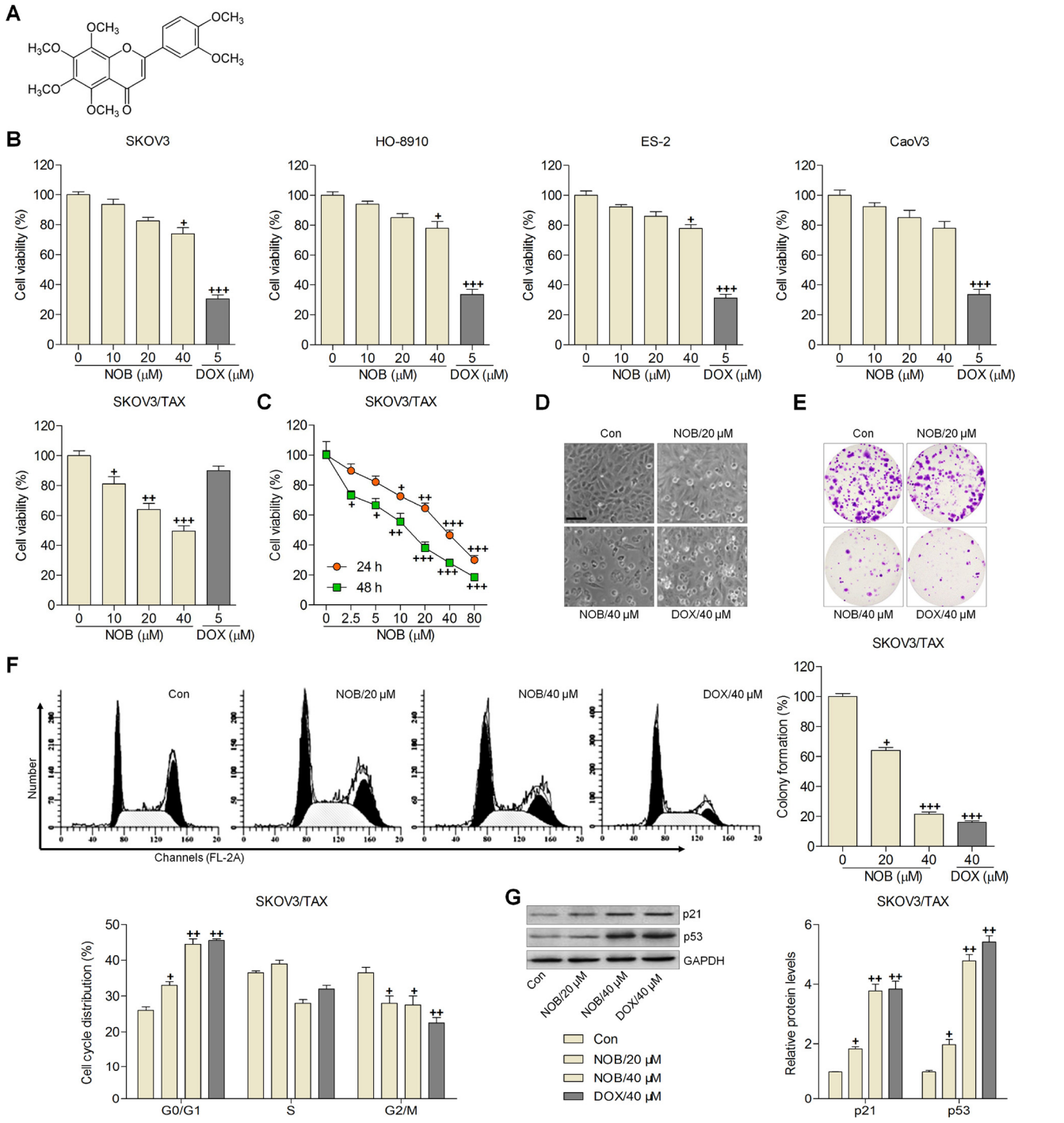

Fig. 1. Nobiletin selectively suppresses the growth and proliferation of drug-resistant ovarian cancer SKOV3/TAX cells. (A) The chemical structure of nobiletin (NOB). (B) Ovarian cancer cell lines, including SKOV3, HO-8910, ES-2 and CaoV3, as well as drug-resistant ovarian cancer cell line of SKOV3/TAX, were subjected to 0, 10, 20, and 40 μM NOB, or 5 μM DOX for 24 h, and then the cell viability was measured using MTT analysis. (C) SKOV3/TAX cells were cultured with the indicated doses of NOB for 24 or 48 h, followed by MTT analysis to calculate the cell viability. SKOV3/TAX cells were exposed to NOB or DOX at the indicated concentrations for 24 h. (D) The morphology of cells were observed using a light microscope. (E) Colony formation of cells was determined. (F) Cell cycle distribution was determined using flow cytometry analysis. (G) Western blot analysis of p21 and p53. Data are represented as mean ± SEM (n = 8/group). $^{+}P < 0.05$, $^{++}P < 0.01$ and $^{+++}P < 0.001$ versus the Con group.

by antitumor agents showed suppressive role in the growth of cancer cells [15]. Therefore, following the mentioned above, autophagy inhibition by natural agents might be the potential treatment against human ovarian cancer cells with drug resistance.

AKT is often activated in human cancer, and usually induces cancer cell proliferation and reduces apoptosis, which in turn facilitate the tumorigenesis [16]. It is also known that reactivation of AKT signaling confers resistance to chemotherapy using doxorubicin and etoposide [17]. AKT is well known to negatively modulate autophagosome formation through activating mTOR [18]. Moreover, the regulation of AKT activity has been implicated in drug resistance in several cancer cell lines [19,20]. Based on these, it is becoming clear that selective modulation of AKT signaling could improve the anti-cancer efficacy of chemotherapeutic agents.