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Tangeretin, a citrus pentamethoxyflavone, antagonizes ABCB1-mediated multidrug resistance by inhibiting its transport function

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Daunorubicin (PubChem CID: 30323)
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ABSTRACT

Multidrug resistance (MDR) and tumor metastasis are the main causes of chemotherapeutic treatment failure and mortality in cancer patients. In this study, at achievable nontoxic plasma concentrations, citrus flavonoid tangeretin has been shown to reverse ABCB1-mediated cancer resistance to a variety of chemotherapeutic agents effectively. Co-treatment of cells with tangeretin and paclitaxel activated apoptosis as well as arrested cell cycle at G2/M-phase. Tangeretin profoundly inhibited the ABCB1 transporter activity since it significantly increased the intracellular accumulation of doxorubicin, and flutax-2 in A2780/T cells and decreased the efflux of ABCB1 substrates in Caco2 cells without altering the expression of ABCB1. Moreover, it stimulated the ATPase activity and inhibited verapamil-stimulated ATPase activity in a concentration-dependent manner, indicating a direct interaction with the transporter. The molecular docking results indicated a favorable binding of tangeretin with the transmemberane region site 1 of homology modeled ABCB1 transporter. The overall results demonstrated that tangeretin could sensitize ABCB1-overexpressing cancer cells to chemotherapeutical agents by directly inhibiting ABCB1 transporter function, which encouraged further animal and clinical studies in the treatment of resistant cancers.

1. Introduction

Multidrug resistance (MDR) is the major factor leading to the failure of cancer chemotherapy, and reversing MDR has been an important goal for clinical and investigational oncologists [1,2]. The most established mechanism for MDR is the overexpression of ATP-binding cassette (ABC) family membrane transporters. Up to now, ABC transporters have 49 members [3], among which ABCB1, ABCG2 and ABCCs are known as the most important members that result in MDR in cancer cells [4,5].

ABCB1, also known as glycoprotein P (P-gp) encoded by *MDR1* gene, was the first cloned human ABC transporter that can transport a large number of compounds including most chemotherapeutic drugs such as taxanes (e.g. paclitaxel (PTX), docetaxel) and anthracyclines (e.g. doxorubicin (DOX) and mitoxantrone) [2,6]. In cancerous tissue, the highest expression of ABCB1 was conventionally found in tumors that are derived from tissues that normally express ABCB1, such as epithelial cells of the colon, kidney, adrenal, pancreas, and liver, resulting in chemotherapeutic drug resistance [7,8]. Developing inhibitors that either down-regulate the expression of ABC proteins or inhibit the efflux function of ABC transporters would have potential clinical benefit. However, the first, second and third generation of ABC modulators such as quinine, verapamil, cyclosporine-A, tariquitor, PSC 833, LY335979, and GF120918 required high doses to reverse MDR and were associated with adverse effects [8]. These limitations have spurred efforts to search for new, multi-target compounds from natural products with higher efficacy and lower toxicity [9,10].

Flavonoids are a large group of poly-phenolic antioxidants found in fruits and vegetables. Many evidence indicated that flavonoids interact with ABC transporters and modulate MDR in tumors

Abbreviations: MDR, Multidrug resistance; P-gp, glycoprotein P; ABC, ATP-binding cassette; PTX, paclitaxel; DOX, doxorubicin; NSCLC, non-small cell lung cancer; CI, combination index; Rho 123, rhodamin123.

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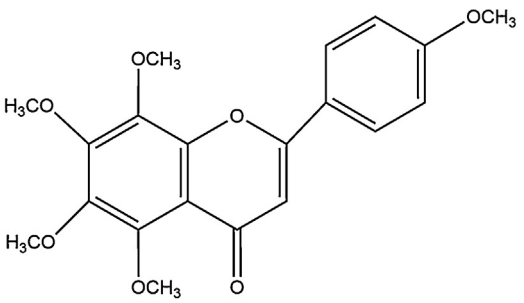
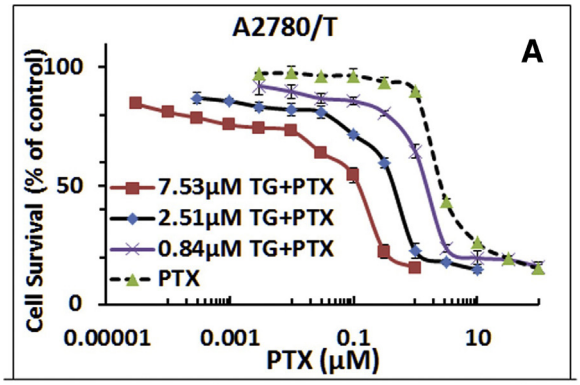
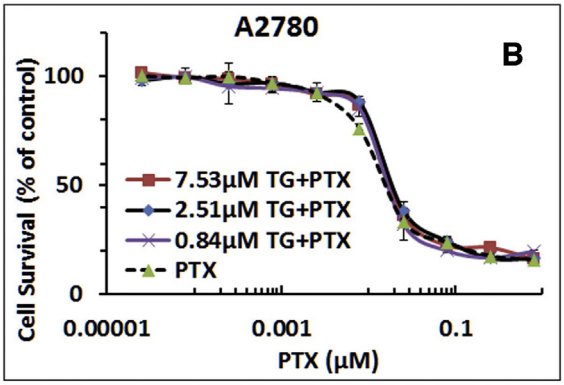


Fig. 1. Chemical structure of tangeretin.

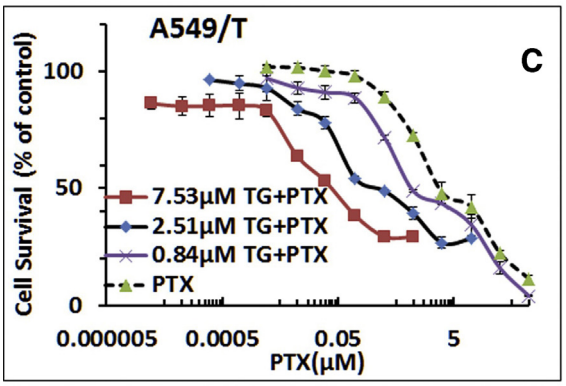
[9,11–13]. In previous studies, we screened a self-built library comprised of flavonoids from natural products against human ovarian paclitaxel resistance cancer cell A2780/T to identify the most suitable candidates. Tangeretin (5,6,7,8,4′-pentamethoxyflavone; Fig. 1) was identified as one of the most effective MDR reversing agents.

Tangeretin is a non-toxic dietary bioflavonoid found in citrus peel (*Citrus sinensis*) as well as orange juice [14,15]. It has been reported to exhibit biological effects *via* its anti-inflammatory, anti-tumor, cholesterol lowering, and neuroprotective activities [16–18]. As a potent chemopreventive agent, tangeretin inhibited the growth of several prostate cancer cell lines with IC_{50} values around 50 μM through induction of G_0/G_1 phase cell-cycle arrest [19,20]. Moreover, in combination, tangeretin synergisti-





	IC50 of PTX(μM)	
Drug	A2780/T	Fold reversal
PTX	2.51±0.08	1.00
0.84μM TG+PTX	1.58±0.04	1.59
2.51μM TG+PTX	0.45±0.07*	5.66
7.53μM TG+PTX	0.14±0.02**	18.88



	IC_{50} of PTX (μM)	
Drug	A549/T	Fold reversal
PTX	2.53±0.41	1.00
0.84μM TG+PTX	1.03±0.33*	2.67
2.51μM TG+PTX	0.27±0.02**	9.52
7.53μM TG+PTX	0.03±0.01***	80.05

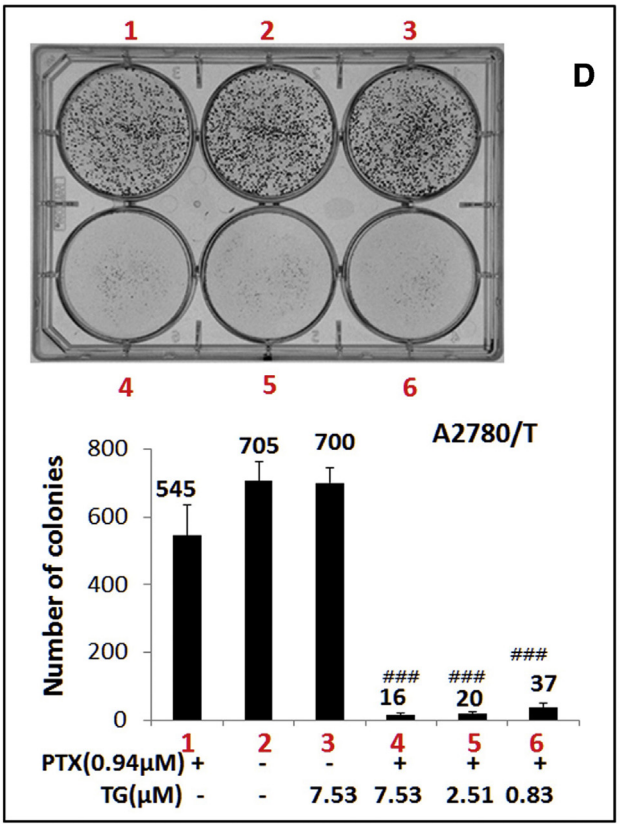


Fig. 2. The effect of tangeretin (TG) on the sensitivity of resistant cells to paclitaxel (PTX) (A) Tangeretin reduces the IC_{50} of paclitaxel in resistant cancer cells (A2780/T) but not in drug sensitive (A2780) (B). (C) Tangeretin reduces the IC_{50} of paclitaxel in resistant cancer cells (A549/T). Cells were treated with the indicated drugs for 48 h and subjected to SRB assay. (D) Colony formation assay of PTX in the presence or absence of tangeretin. Colony numbers were counted after Giemsa staining using the software of Quantity one-Colony counting. IC_{50} values are represented as means ±SD of three independent experiments performed in triplicate. ## or **, $P < 0.01$., ### or ***, $P < 0.001$, significantly different from those obtained in the absence of tangeretin.