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# Gambogic acid sensitizes breast cancer cells to TRAIL-induced apoptosis by promoting the crosstalk of extrinsic and intrinsic apoptotic signalings

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ABSTRACT

Due to the ability of tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) to induce cancer cell apoptosis selectively, TRAIL has attracted significant interest in the treatment of cancer. However, although TRAIL triggers apoptosis in a broad range of cancer cells, most primary cancers are often intrinsically TRAIL-resistant, or can acquire resistance after TRAIL treatment, evocating new strategies to overcome TRAIL resistance. Gambogic acid (GA), an active constituent of Garcinia Hanburyi (Teng Huang in Chinese), has been applied for thousands of years for medicinal uses, however, the potential effect of GA in combating cancer resistance remains poorly investigated. In this study, we found that GA could increase the sensitivity of breast cancer cells to TRAIL and enhance TRAIL-induced apoptosis. GA cooperated with TRAIL to decrease the levels of anti-apoptotic proteins and activate Bid (BH3 interacting-domain death agonist) to promote the crosstalk of extrinsic and intrinsic apoptotic signaling, rather than increasing the expression of TRAIL receptors DR4 and DR5. These findings may open a new window in the treatment of breast cancer using TRAIL in combination with GA.

## 1. Introduction

Cell apoptosis is mainly initiated by the intrinsic pathway, also called the mitochondrial pathway, and the extrinsic pathway. The intrinsic apoptosis pathway is triggered in response to cell damage, as in DNA damage and oxidative stress, which results in permeabilization of mitochondrial membrane and release of proteins from the interspace of mitochondrial membranes (Green and Kroemer, 2004). The extrinsic apoptosis pathway is activated through the binding of death ligands to death receptors (DRs), thereby transmitting death signals from the outside to inside of the cell (Wajant et al., 2013). Tumor necrosis factor (TNF), a death ligand, was first discovered in 1975 (Carswell et al., 1975). However, TNF also promotes inflammatory responses and causes many clinical problems (Aggarwal et al., 2012). In 1995, a new member of the TNF superfamily, designated as TNF-related apoptosis-inducing ligand (TRAIL) or Apo2L, was identified based on the homologic sequence to TNF and CD95L (Wiley et al., 1995). Unlike TNF, TRAIL induces apoptosis in various human cancer cells but does not kill normal cells. TRAIL mainly interacts with two distinct receptors, namely TRAILR1 (also known as DR4) and TRAILR2 (also known as DR5), and recruits Fas-associated death domain protein (FADD) and procaspase-8/10 to form the death-inducing signaling complex (DISC) (Kischkel et al., 2000, 2001). The formation of DISC activates caspase 8, and the cleaved caspase 8 directly induces cell apoptosis via activation of the downstream substrates of the apoptotic pathway. Besides, TRAIL could induce necroptosis, while necroptosis is a caspase-independent cell death program which depends on the formation of receptor-interacting protein kinase 1 (RIP1)-RIP3 complex (Jouan-Lanhouet et al., 2012).

Data from clinical trials of recombinant human TRAIL showed well tolerability in patients, but low therapeutic outcomes (Farooqi et al., 2016). While TRAIL triggers apoptosis in many cancer cell lines, most primary cancers are resistant to TRAIL monotherapy (Lemke et al., 2014). The molecules involved in TRAIL-mediated apoptosis, such as cellular FADD-like IL-1b-converting enzyme (FLICE)-inhibitory protein (c-FLIP), the Bcl-2 family proteins, IAPs, MAPK and transcription factor

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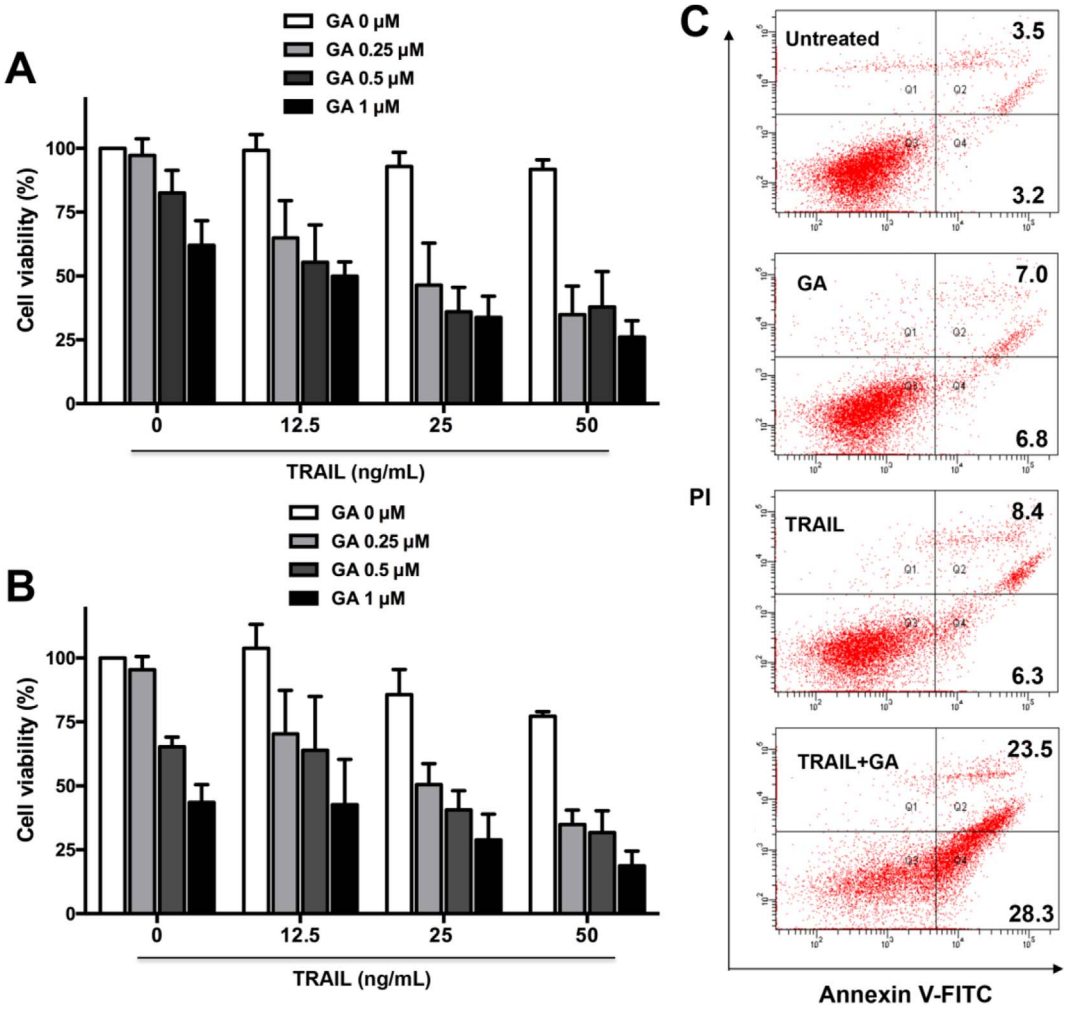


**Fig. 1. GA increases sensitivity of breast cancer cells to TRAIL.** MCF-7 (A) and MDA-MB-231 (B) cells were treated with indicated concentrations of TRAIL, GA, or their combinations for 24 h and cell viability was determined by MTT assay. (C) MCF-7 cells were treated with TRAIL (25 ng/mL), GA (0.25 μM) and their combination for 24 h and cell apoptosis was analyzed by flow cytometry. All values represent mean ± SEM, n = 3.

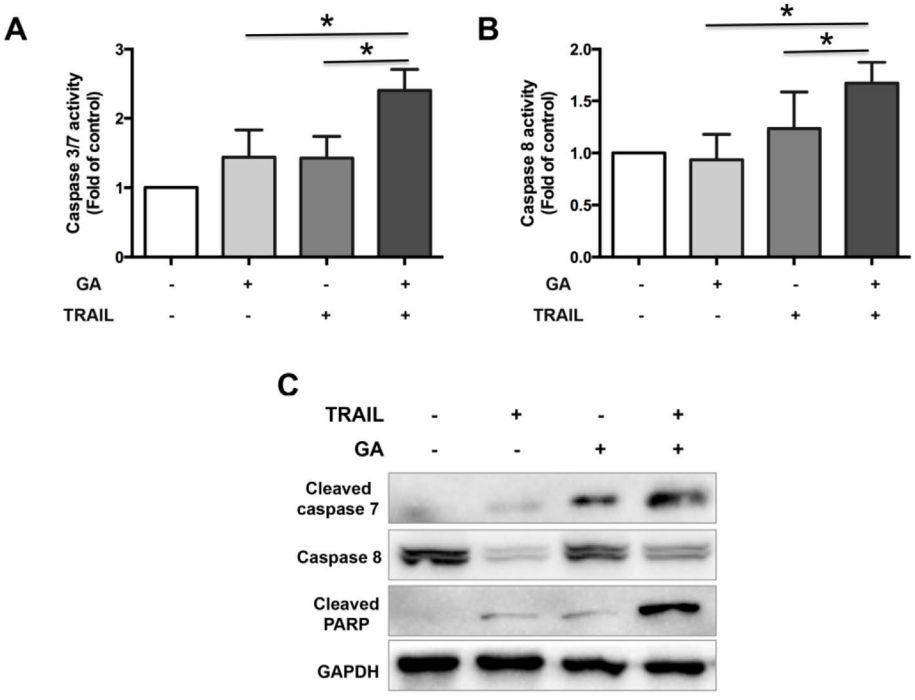


**Fig. 2. GA enhances TRAIL-induced apoptosis in breast cancer cells.** MCF-7 cells were treated with TRAIL (25 ng/mL), GA (0.25 μM) and their combination for 24 h. Caspase 3/7 (A) and caspase 8 (B) activities of MCF-7 cells were evaluated using Caspase-Glo assay kits. (C) Western blotting of lysates from MCF-7 cells that had been treated as described above using the indicated antibodies. All values represent mean ± SEM, n = 3. *$P < 0.05$.