Synergistic Anti-tumor Effects of PEGylated TRAIL and Docetaxel Combination Therapy

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ABSTRACT

SUMMARY

To develop an effective anti-tumor therapy with sustained biological activity, we researched the cytotoxic effect of PEGylated TRAIL in combination with docetaxel by MTT assay and tumor xenograft animal model. The combination of PEGylated TRAIL with docetaxel turns out to be a promising drawing to the colon cancer therapy.

INTRODUCTION

TNF-related apoptosis-inducing ligand (TRAIL), a type 2 trans-membrane protein, is considered a good candidate for cancer therapy. Because it induces cell death by apoptosis in a number of human cancer cells but not in normal cells. Among the TRAIL receptors discovered to death receptors 4 (DR4) and 5 (DR5) are known to be connected with the cytoplasmic death domain, and this binding is responsible for transducing the cell death signaling via caspase-dependent apoptotic pathway.

Despite its efficacy at killing tumor cells, TRAIL has a short biological half-life, low stability & solubility, rapid inactivation and drug resistance in cancer cells. These issues have been considered as crucial barriers for clinical application. To solve these problems, we developed a new version of modified TRAIL by site-specific N-terminal PEGylation. Especially, TRAIL strongly synergizes with conventional chemotherapeutic drugs to induce tumor cell death. Thus, a number of studies have been concentrated the anti-tumor therapy by TRAIL based combinations.

Therefore, our goal of this study was to maximize the therapeutic effects, docetaxel was combined to the PEG-TRAIL based colon cancer therapy. Then, in vitro and in vivo anti-tumor effects were researched.

EXPERIMENTAL METHODS

The trimeric TRAIL was expressed and isolated from human TRAIL plasmid transformed E. coli by Ni-affinity chromatography. Then, the trimeric TRAIL was modified by PEG (MW 5,000 Da) with site-specific N-terminal PEGylation.

The HCT-116 cells were pretreated with various concentrations of docetaxel for 6 hr followed by treatment with PEG-TRAIL for 12-18h. The cytotoxic effects were determined by MTT assay. The cells were cultured in DMEM with 10 % FBS and 1 % penicillin at 37 °C in a humidified 5 % CO2 atmosphere.

Activation of caspase in HCT-116 cells during sample drugs induced apoptosis were estimated by western blot analysis. Simply, cells were harvested and lysed with lysis buffer. Each equal amounts of protein were separated through SDS-PAGE and transferred to PVDF membranes. The membranes were incubated overnight at 4 °C caspase-8 and caspase-3 antibody (abcam, USA). The membranes were incubated for 1h with peroxidase conjugated goat polyclonal Rabbit IgG antibody and developed by chemiluminescence.

For in vivo study, HCT-116 colon cancer cells were inoculated (3 x 10⁶ cells/mouse, 50 μl injection) in athymic nude mice and tumor growth was measured with drug treatments (docetaxel, PEG-TRAIL and their combinations were injected 5 times 4 day intervals).

RESULTS AND DISCUSSION

The PEG-TRAIL combined with various dose of docetaxel treatment in HCT-116 cancer cells enhanced the induction of cytotoxicity and apoptosis, as shown in Figure 1. The sequential introductions of Docetaxel and following PEG-TRAIL treatments showed the most effective
cytotoxic effects on colon tumor HCT-116 cell line.

**Figure 1. Dose-dependent synergistic cytotoxic effect of the combinations of PEG-TRAIL & docetaxel.**

In addition, treatment of combinations of PEG-TRAIL and docetaxel induced synergistic effects of apoptosis mediators such as caspase-8 and caspase-3 in HCT-116 cells by western blot. These results suggest that synergistic cytotoxic effects of the combinations from increased powerful apoptosis role through that caspase activation.

![Western blot image](image1)

**Figure 2. Synergistic apoptosis effect of the combinations of PEGy-TRAIL & docetaxel.**

In our previous study, while drug administered 5 consecutive days, TRAIL based combination and PEG-TRAIL based combination treatment resulted similar efficiency. But in this study, while drug treated 5 times 4 day intervals, PEG-TRAIL based combination is more cytotoxic effects than TRAIL based combination treatment. It suggests that PEG-TRAIL is more stable and anti-tumor effect for a longer drug treated interval.

**CONCLUSION**

The advantages of PEG-TRAIL based combination therapy show the maximum anti-tumor effects that can be achieved by synergistic actions. It is more remarkable in 4 day interval than 1 day interval drug treatment. Thus, this PEG-TRAIL based combination therapy can be expected reducing numbers of injection and side effect. These issues contribute to increasing clinical efficacy. Therefore, the combination of PEG-TRAIL and docetaxel might be utilized a promising good applicant for colon cancer therapy.

**REFERENCES**