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Combined use of low-molecular weight fucoidan extract and an anti-cancer drug enhances their anti-tumor effects

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Introduction

Fucoidan is a uniquely-structured sulfated polysaccharide found in the cell walls of several types of brown seaweed. Recently, fucoidan has attracted a lot of clinical attention due to its strong anti-tumor potential, which has been intensively investigated. Fucoidan suppresses the growth of tumor cells *in vivo* and activates the immune system against tumors [1-6]. Sulfation of fucoidan enhanced its antitumor activity [2]. Koyanagi *et al*. reported that fucoidan inhibited tube formation following migration of human umbilical vein endothelial cells [7]. Ye *et al*. reported that enzyme-digested fucoidan extract inhibited invasion and angiogenesis of tumor cells [8]. Zhang *et al*. reported that enzyme-digested fucoidan induced apoptosis in MCF-7 Cells via a JNK activation and mitochondria-mediated pathways [9]. In this study, we examined that combined use of low molecular weight fucoidan extract and sword bean extract enhanced their anti-tumor effects.

Methods

Anti-proliferative Assay

Cells were inoculated at a final concentration of 1×10^5 cells/mL in culture medium in a 96-well microplate. After 24 h pre-incubation, the cells were exposed to various concentrations of LMF and sword bean extract for 48 h. After cultivation, the cell number in each well was measured using WST-1 assay.

Analysis of Cell Cycle and Apoptosis

Cell cycle was determined by PI staining and flow cytometry as described [10]. Briefly, HT1080 cells (2×10^5 cells/mL) were incubated in culture medium containing LMF. After one gentle rinse by PBS, the cells were collected by trypsinization and centrifugation at 200 ×*g* for 5 min. Then the cell pellets were gently resuspended in 150 µL of PBS, and added 350 µL of cold ethanol for 30 min at 4°C. Fixed cells were collected by centrifugation at 200 ×*g* for 5 min, and were resuspended in 500 µL of PBS. The cell pellets were incubated in 500 µL of PI/RNase A/PBS staining solution (10 µg/mL PI and 10 µg/mL RNase A in PBS) at 25°C for 20 min in a dark place. Stained cells were analyzed by a flow cytometer (excitation at 488 nm; emission at 610 nm). Ten thousand cells were evaluated for each sample.

References

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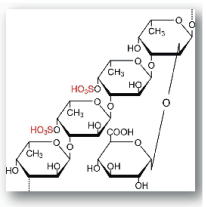
Fucoidan is a uniquely-structured sulfated fucose-rich polysaccharide derived from brown algae (such as kombu, wakame and mozuku).

Structure



Structure of fucoidan derived from mozuku (*Cladosiphon* sp.)
Berteau &Mulloy, *Glycobiology* **13**, 29R-40R, 2003.

Anti-tumor potential

Activation of the immune system against tumors
Suppression of the angiogenesis abilities of tumor cells
Suppression of the growth of tumor cells *in vivo*

→ ***Induction of apoptosis***

About Low molecular weight fucoidan extract (LMF)

Preparation

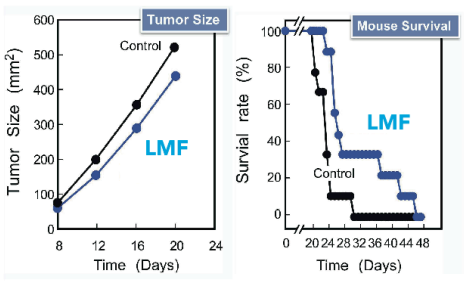
Mozuku
Cladosiphon novae-caledoniae Kylin, (originated in the Kingdom of Tonga)
↓ Extraction by organic acid/Desalting
↓ High molecular weight fucoidan (M.W.: about 800 kDa)
↓ *Glycosidase digestion*
↓ **Low molecular weight fucoidan extract (LMF)**

Components

Fucose	**62.1%**
Xylose	10.5%
Mannose	6.0%
Glucose	3.5%
Galactose	3.3%
Uronic acid	14.6%
Sulfate group	14.5%

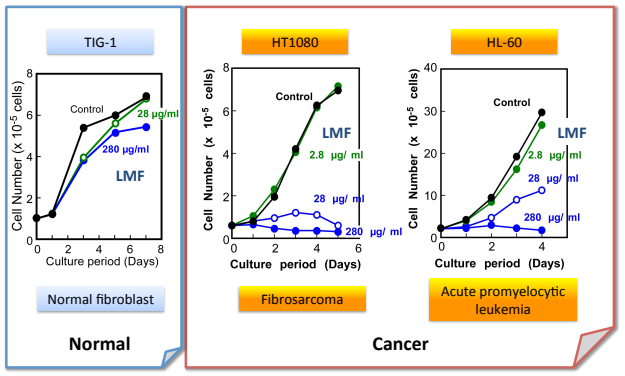
Enzyme-digested fucoidan extract includes fucoxanthine, amino acids, minerals etc.

LMF suppressed mouse colon cancer cells *in vivo*



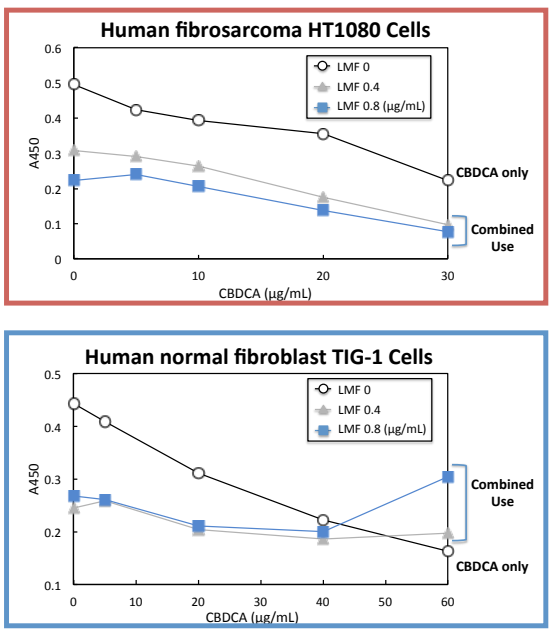
Balb/c mice (n = 9), Transplantation of Colon-26 cells (Day 0), LMF (oral administration, 700 µg/day for 2 weeks)

LMF suppressed growth of human cancer cells



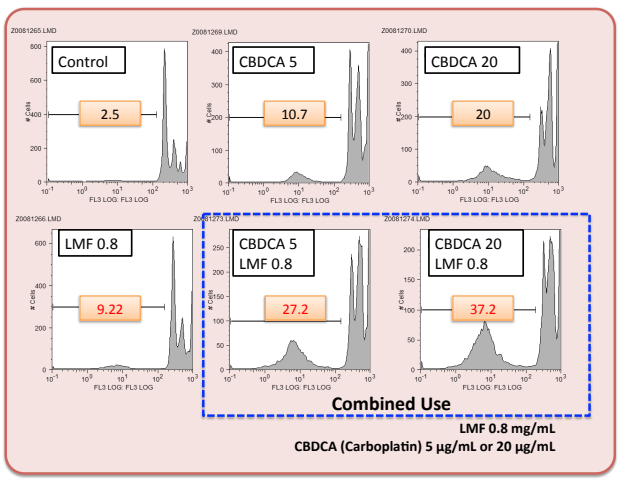
Results

Anti-proliferative activity of combined treatment of LMF and CBDCA

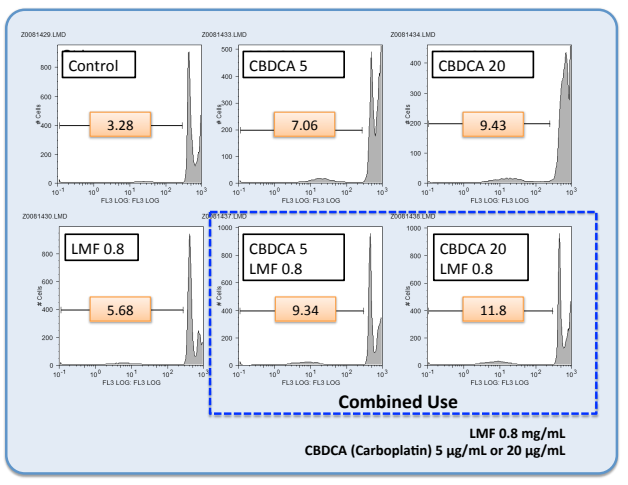


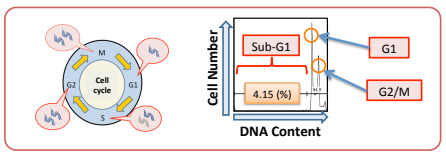
Cell cycle analysis of combined treatment of LMF and CBDCA

Human fibrosarcoma HT1080 Cells



Human normal fibroblast TIG-1 Cells





Conclusion

The combined use of LMF and carboplatin (CBDCA) caused decrease of cell viability of human fibrosarcoma HT1080 cells compared with a single use.

The Sub-G1 analysis revealed that dead cells of HT1080 were increased by the combined use of LMF and CBDCA.

Single use of CBDCA exhibited the cytotoxicity into human normal fibroblast TIG-1 cells. However, the LMF treatment protected TIG-1 cells from damages of CBDCA in the combined use.

These suggested that LMF had not only the enhancing effect for cancer cell death by CBDCA but also protecting effect for normal cells from damages of CBDCA.

COI Disclosure Information

Sanetaka Shirahata

I have the following financial relationships to disclose.

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