REVIEW

In Vitro-Screening for Cancer-Suppressive Effect of Food Components

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Abstract

Some diets have been shown to be correlated with the development and progression of cancer. In contrast, many kinds of foods are expected to prevent the development and progression of cancer. To identify such cancer-preventive food components and elucidate their cancer-preventive functions, we investigated important anti-cancer effects, that is, growth-inhibitory and apoptosis-inducing effects of some food and food components on cancer cells. Among the vegetables studied, bitter gourd was the most effective in inhibiting the growth of HL60 human leukemia cells. Bitter gourd extract induced apoptosis in the cells. The bitter gourd extract also inhibited growth and induced apoptosis in B16 mouse melanoma 4A5 cells, whereas it inhibited the growth of BALBc/3T3 mouse fibroblast cells only to a small extent. Berries are rich sources of the naturally occurring phenolic pigments anthocyanins, which show a potent antioxidant activity. Bilberry ethanol extract strongly inhibited the growth of HL60 and HCT116 human colon carcinoma cells, and also induced apoptosis in HL60 cells but not in HCT116 cells. Of the berry extracts tested, that from bilberry contained the largest amount of phenolic compounds, including anthocyanins, and exhibited the highest DPPH radical-scavenging activity. The anthocyanidins, delphinidin and malvidin, inhibited cell growth and induced apoptosis in HL60 cells. Delphinidin, but not malvidin inhibited the HCT116 cell growth. These effects of food components probably contribute to cancer prevention.

Discipline: Food

Additional key words: apoptosis, growth-inhibition, cancer cells, bitter gourd, berries

Introduction

Food and nutrient intake has been examined in relation to many types of cancer. It is generally recognized that some diets are correlated with the development and progression of cancer. In contrast, vegetables and fruits have been reported to reduce the risk of cancer in epidemiological studies. Some food factors, such as vitamins and polyphenols, are listed as possible cancer suppressors. They display antioxidant activities, which prevent the formation of highly reactive lipid peroxidation products and reduce the deleterious effects of reactive oxygen species, in addition to anti-mutagenicity, anti-tumor activity or other anti-cancer effects.

Tumors proliferate beyond the constraints limiting growth in normal tissues. Therefore, the inhibition of cell growth and the induction of apoptotic cancer cell death are important mechanisms of cancer prevention. Apoptosis is one of the major mechanisms of cancer suppression. The apoptotic cells are fragmented and removed by phagocytosis. To elucidate the cancer preventive effects of food components, we investigated the growth-inhibiting and apoptosis-inducing effects of some food and food components in cultured cancer cells.

Materials and methods

1. Materials

Carrot (Daucus carota L.), Welsh onion (Allium t Fistulosum L.), Chinese radish (Raphanus sativus L.), bitter gourd (Momordica charantia L.), cabbage (Brassica oleracea L.) and sweet potato (Ipomoea batatas Poir.) were cultivated in Kagoshima prefecture. Lowbush blueberry
Cells were cultured at 37°C in 5% CO₂ in air in a medium supplemented with 10% heat-inactivated fetal calf serum (FCS; ICN Biomedicals, USA). Viable cells were counted with a hemocytometer by trypan blue exclusion. Among the extracts, the bitter gourd extract was the most effective inhibitor of HL60 cell growth (Fig. 1). The bitter gourd extract decreased the viable cell number by 60% at a concentration of 200 µg/mL (Fig. 1). The extract induced apoptotic cell bodies and oligonucleosomal DNA fragmentation typical of apoptosis in HL60 cells (data not shown, Fig. 2a). The results indicated that the bitter gourd extract induced apoptosis in HL60 cells. The bitter gourd extract inhibited the growth of B16 melanoma cells at a concentration of 200–400 µg/mL after 24 h incubation. The extract induced nuclear and DNA fragmentations typical of apoptosis in the B16 cells (Fig. 2b). Thus the bitter gourd extract inhibited cancer cell growth and induced apoptosis in cancer cells, whereas it hardly inhibited the growth of BALBc/3T3 mouse fibroblast cells. The extract decreased the viable cell number only by 20% at a concentration of 400 µg/mL after 24 h incubation (Fig. 3).

Berries are known to contain the phenolic pigments, anthocyanins, which display an antioxidant activity. We also screened the growth inhibitory effects of berry ethanol extracts in human cancer cells⁶. Berry extracts were added to the culture medium of HL60 and HCT116 human carcinoma cells and incubated for 24 and 48 h, respectively. The bilberry extract markedly inhibited the growth of HL60 and HCT116 cells. The bilberry extract decreased the number of viable HL60 cells by 84 and 88% at concentrations of 4 and 6 mg/mL, respectively.

Results and discussion

First, we examined the effects of ethanol extracts of vegetables, which were grown in Kagoshima prefecture in the Kyushu area, on cancer cell growth³. We prepared extracts from 5 vegetables (carrot, Welsh onion (2 varieties), Chinese radish, bitter gourd and cabbage) and 4 varieties of sweet potato, and investigated the inhibitory effects of the vegetable extracts on the growth of HL60 human leukemia cells. The HL60 cells were seeded at a concentration of 2 × 10⁴ cells/mL on 24 well plates. Each extract was added to the medium of HL60 cells, incubated for 24 h and then the viable cell number was counted. Among the extracts, the bitter gourd extract was the most effective inhibitor of HL60 cell growth (Fig. 1). The bitter gourd extract decreased the viable cell number by 60% at a concentration of 200 µg/mL (Fig. 1). The extract induced apoptotic cell bodies and oligonucleosomal DNA fragmentation typical of apoptosis in HL60 cells (data not shown, Fig. 2a). The results indicated that the bitter gourd extract induced apoptosis in HL60 cells. The bitter gourd extract inhibited the growth of B16 melanoma cells at a concentration of 200–400 µg/mL after 24 h incubation. The extract induced nuclear and DNA fragmentations typical of apoptosis in the B16 cells (Fig. 2b). Thus the bitter gourd extract inhibited cancer cell growth and induced apoptosis in cancer cells, whereas it hardly inhibited the growth of BALBc/3T3 mouse fibroblast cells. The extract decreased the viable cell number only by 20% at a concentration of 400 µg/mL after 24 h incubation (Fig. 3).

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Fig. 1. Effect of vegetable extracts on the growth of HL60 human leukemia cells

HL60 cells were incubated with the vegetable extracts (■, 50 µg/mL; □, 100 µg/mL; △, 200 µg/mL) for 24 h. Viable cells were counted with a hemocytometer by trypan blue exclusion (n = 3). Vertical bars indicate SD.

Fig. 2. Analysis of DNA fragmentation patterns by agarose gel electrophoresis

a : DNA was extracted from the HL60 cells treated with the bitter gourd extract (A, none; B, 50 µg/mL; C, 100 µg/mL; D, 200 µg/mL) for 24 h.

b : DNA was extracted from the B16 cells treated with the bitter gourd extract (A, none; B, 100 µg/mL; C, 200 µg/mL; D, 300 µg/mL; E, 400 µg/mL) for 24 h.

Fig. 3. Effect of the bitter gourd extract on the growth of BALB/c3T3 mouse fibroblast cells

BALB/c3T3 cells were incubated with the extract of bitter gourd for 24 h. Viable cells were counted with a hemocytometer by trypan blue exclusion (n = 3). Vertical bars indicate SD.
and the number of viable HCT116 cells by 66 and 97% at 2 and 4 mg/mL (Fig. 4). The bilberry extract induced apoptotic cell bodies and nuclear fragmentation typical of apoptosis in HL60 cells at the concentration of 4 mg/mL after 6 h incubation (Figs. 5a & 5c). Apoptotic cells with fragmented nuclei were also observed in HCT116 cells treated with the 4 mg/mL bilberry extract, but the proportion of apoptotic cells was much lower than in HL60 cells (Figs. 5b & 5d).

Among the 10 berry extracts, the bilberry extract showed the highest DPPH radical-scavenging activity and the highest total phenolic and anthocyanin contents (Figs. 6a & 6b). We next fractionated the bilberry extract by Oasis HLB column chromatography. Our results suggested that the anthocyanins contained in the bilberry extract inhibited the growth of HL60 and HCT116 cancer cells and induced apoptosis in HL60 cells (data not shown). Therefore, we investigated the growth-inhibitory and apoptosis-inducing effects of pure anthocyanidins on cancer cells. Anthocyanidins are aglycons of anthocyanins. Fig. 7 shows the structure of the anthocyanidins contained in the berries. Delphinidin markedly inhibited the growth of HL60 and HCT116 cells over a concentration range of 50–200 µM. The number of viable HL60 and HCT116 cells was reduced by 100 µM del-
Fig. 6. DPPH radical-scavenging activity and total phenolic and anthocyanin contents in berry extracts

a: DPPH radical-scavenging activity of berry extracts. Each value is the mean ± SD of triplicate experiments.
b: Total phenolic and anthocyanin contents in berry extracts. Total phenolic compounds ( ) in the berry ethanol extract were determined by the Folin-Ciocalteu’s method⁹ and the contents were expressed as milligram of D-catechins equivalents per g of extract. Anthocyanins ( ) in the berry extract were analyzed by the HPLC method of Ando et al.¹. Total anthocyanin content was expressed as milligram of cyanidin equivalents per 1 g of extract. Each value is the mean ± SD of triplicate experiments.

Fig. 7. Structure of anthocyanidins contained in berries
Fig. 8. Effects of anthocyanidins on the growth of HL60 and HCT116 cells
Anthocyanidins were incubated with HL60 cells for 24 h or with HCT116 cells for 48 h. Each value is the mean ± SD of triplicate cultures.

Fig. 9. Analysis of DNA fragmentation patterns by agarose gel electrophoresis
DNA was extracted from HL60 cells treated with 200 µM anthocyanidins for 24 h.

Fig. 10. Effects of flavonols on growth of HL60 and HCT116 cells
The flavonoids were incubated with HL60 cells for 24 h or HCT116 cells for 48 h. Each value is the mean ± SD of triplicate cultures.

Induction of apoptotic cell bodies and nuclear fragmentation was observed after 6 h of treatment with 200 µM malvidin, delphinidin or cyanidin, but not with peonidin or pelargonidin (data not shown). Malvidin, delphinidin and cyanidin, but not peonidin and pelargonidin induced the DNA fragmentation typical of apoptosis in HL60 cells (Fig. 9). Induction of apoptosis in HCT116 cells was minimal following treatment with 200 µM pure anthocyanidins.

The flavonols, myricetin, quercetin and kaempferol, were reported to be constituents of edible berries and to induce apoptosis in cancer cells. The growth inhibitory effects of malvidin and delphinidin on cancer cells were compared with those of flavonols. Myricetin, quercetin and kaempferol inhibited the growth of HL60 and HCT116 cells in the concentration range of 50–200 µM (Fig. 10). The inhibitory effects of malvidin and del-
phinidin were more pronounced than those of the flavonols in HL60 cells (Fig. 10). In contrast, the latter were more effective than the anthocyanidins in inhibiting HCT116 cells (Fig. 10). Bilberry has been reported to contain flavonols but only at about 1% of the anthocyanin content. The growth inhibitory and apoptosis-inducing effects of bilberry on cancer cells are, therefore, likely to be attributable to the anthocyanins.

We then isolated all the anthocyanins contained in the bilberry extract and investigated the growth-inhibitory and apoptosis-inducing effects on HL60 and HCT116 cells. The anthocyanins, with delphinidin or malvidin as the aglycon, inhibit the growth of HL60 cells through the induction of apoptosis (data not shown). On the other hand, only delphinidin-glycosides isolated from the bilberry extract, but not malvidin-glycosides inhibited the growth of HCT116 cells (data not shown). Thus, berries such as bilberry that contain a large amount of anthocyanins are likely to suppress cancer through the induction of apoptosis and/or inhibition of cancer cell growth.


References