Note

Effects of Spinach Extract on the Differentiation of the Human Promyelocytic Cell Line, HL-60

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The effects of a non-dialyzable extract of spinach on the differentiation of HL-60 leukemia cell line were examined. The non-dialyzable extract of fresh and freeze-dried spinach induced the ability of nitroblue tetrazolium reduction and alpha-naphthyl butyrate esterase activity of HL-60 cells. These results suggest that non-dialyzable extract of spinach induces the differentiation of HL-60 cells into monocytes.

A human myeloid leukemia cell line, HL-60, 1) can be differentiated by a variety of agents into granulocytes or monocytes/macrophages, such as dimethylsulfoxide (DMSO), 1) 12-O-tetradecanoylphorbol-13-acetate, 2) and arginase. 3) Cytokines such as GM-CSF and interferon-γ, 4) and gangliosides 5) on the cell surface are known to induce differentiation of HL-60 cells and some other myeloid leukemia cells into granulocytes or monocyte/macrophages. A vitamin A derivative, all-trans retinoic acid, which induces HL-60 cells into granulocytes, has been used for clinical therapy of patients with acute promyelocytic leukemia. 6) 7) 8) Although many kinds of compounds that induce the differentiation of HL-60 cells have been found, no differentiation inducing compounds have been detected from edible plants. We have now found that a non-dialyzable extract of fresh and freeze-dried spinach induced the differentiation of HL-60 cells.

HL-60 cells provided from Japanese Cancer Research Resources Bank (JCRB0085) were usually maintained in RPMI1640 medium (Nissui Pharmaceutical Kogyo Co., Tokyo, Japan) with 10% fetal bovine serum (FBS; Irvine Scientific, U.S.A.) in a 5% CO₂ humid incubator at 37°C. Fresh Spinacia oleracea cv. Okame (500 g fresh weight) was homogenized in 1.5 liters of phosphate buffered saline (PBS, pH 7.4) with a Waring blender. After filtration through a cotton cloth, the homogenate was centrifuged at 10,000 × g for 30 min at 4°C. The supernatant was fractionated by 20-80% saturated ammonium sulfate and dialyzed against PBS with seamless cellulose tubing (Viskase Sales Corp., U.S.A.) with a molecular weight cut of 12,000–14,000, for 24 h at 4°C. Extract of freeze-dried spinach was prepared as follows. Freeze-dried spinach leaves (500 g dry matter) were homogenized in 5 liters of 40% ethanol with a Waring blender and kept at 4°C overnight. The homogenate was filtered through a cotton cloth and centrifuged at 10,000 × g for 30 min at 4°C. The supernatant was concentrated and dialyzed against running tap water with seamless cellulose tubing at room temperature overnight, and lyophilized. The extracts of freeze-dried spinach were dissolved in PBS.

Differentiation of HL-60 cells by the spinach extract was examined by elevation of the ability of nitroblue tetrazolium (NBT) reduction and alpha-naphthyl butyrate esterase activity of HL-60 cells. In the former, HL-60 cells (5 × 10⁶ cells/ml) were seeded in a 60 mm culture dish and cultured with PBS or non-dialyzable extract prepared from freeze-dried or fresh spinach for 2, 4, or 6 days, and NBT positive cells in the 200 HL-60 cells treated with spinach extract were counted under a microscope of 200 magnifications by the methods of Collins et al., 9) and Breitman et al. 10) Alpha-naphthyl butyrate esterase activity of HL-60 cells was stained with 2-methyl-4-[(2-methylphenylazo)benzenediazonium (fast garnet GBC) by the method of Li et al., 10) after the cells (5 × 10⁶ cells/ml) was cultured with PBS or spinach extract in RPMI1640 medium with 10% FBS for 5 days.

When the HL-60 cells (5 × 10⁶ cells/ml) were cultured with 0.1–0.5 mg protein/ml of fresh spinach extract or 0.25–1.0 mg dry wt/ml of freeze-dried extract for 6 days, these concentrations of spinach extract inhibited the proliferation of HL-60 cells. NBT positive cells also appeared in the HL-60 cells treated with 0.25–1.0 mg/ml of freeze-dried extract (Fig. 1). About 20–40% in the 200 HL-60 cells treated with 0.5 and 1.0 mg/ml of extract for 4 or 6 days were NBT-positive. Treatment of HL-60 cells with 0.1 or 0.5 mg protein/ml of fresh spinach extract also induced 10–50% NBT-positive cells. These results were reproducible. Furthermore, an increase in the level of alpha-naphthyl butyrate esterase activity, a key enzyme for monocyte/macrophage cells, was observed in the HL-60 cells treated with 0.5 mg/ml of freeze-dried spinach extract, but not in the HL-60 cells without treatment. HL-60 cells treated with extract were clearly stained with fast garnet GBC (Fig. 2). A similar result was obtained when the HL-60 cells were treated with fresh spinach extract. The activity of AS-d chloroacetate esterase in the HL-60 cells treated with freeze-dried spinach extract was almost the same as that of the untreated HL-60 cells. A Giemsa staining of HL-60 cells cultured for 5 days with the extract showed that morphology of nuclei of the cells was not significantly different from that of untreated HL-60 cells, although some of the cells reduced NBT dye and induced alpha-naphthyl

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Fig. 1. Effects of Freeze-dried Spinach Extract on NBT Reducing Ability of HL-60 Cells.

HL-60 cells (5 × 10⁶ cells/ml) were cultured with PBS (●), or 0.25 mg/ml (□), 0.5 mg/ml (▲), and 1.0 mg/ml (▼) of freeze-dried spinach extract. NBT reducing ability of the HL-60 cells was measured by the method of Collins et al. 9)
butyrate esterase activity of HL-60 cells without inducing a clear morphological change.

It was thus found that about 10–50% of total HL-60 cells, which had NBT reduction ability or alpha-naphthyl butyrate esterase activity, were induced by spinach extract. These findings indicate that the compounds which have the ability to induce the differentiation of HL-60 cells into monocyte cells exist in the spinach extract. This is the first observation of HL-60 differentiation into monocyte cells by high molecular weight compounds from a plant. Although the active compounds in the non-dialyzable extract of spinach are not clear, arginase is a possible candidate for the HL-60 differentiation inducing factor in the extract, because arginase from bovine serum is known to be an inducer of differentiation of HL-60 cells into macrophage cells and because it may exist in the non-dialyzable extract of spinach. However, the activity of arginase in the non-dialyzable extract of freeze-dried and fresh spinach was quite low. Accordingly, the active compounds in the extracts could be other than arginase. Isolation of the differentiation inducing factor(s) from the spinach extract and the more detailed actions on HL-60 cells are now in progress.

Fig. 2. Alpha-naphthyl Butyrate Esterase Activity Stain of HL-60 Cells Cultured with or without Spinach Extract. HL-60 cells (5 x 10^6 cells/ml) were cultured with fresh 0.5 mg/ml of spinach extract (A) or PBS (B) in RPMI1640 medium with 10% FCS for 5 days. Alpha-naphthyl butyrate esterase activity was stained with fast garnet GBC by the method of Li et al. and microscope photographs (x 500) were taken.

References