EFFECTS OF GINSENOSIDES ON CULTURED HEPATOMA CELLS

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Recently, the chemical structure of ginseng saponin and its biological effects have been widely studied, and it has been revealed that ginseng saponin stimulates various metabolic reactions of normal tissues *in vivo*[1-3].

However, the elucidation of the mechanism responsible for the stimulation of various metabolic reactions by ginseng saponin has remained illusive. For study of cellular and molecular mechanism of the ginseng saponin's effects it seems desirable to use a culture cell system in which direct effects of ginseng saponin can be exactly observed.

On the basis of this consideration, systemic studies on effects of ginseng saponin to various cultured cells are undertaken in our laboratory. In this symposium, we will report the effects of ginseng saponin on cultured Morris hepatoma cells.

Morphological transformation of Morris hepatoma cells by ginseng saponin

The cultured Morris hepatoma cells (MH_1C_1) used in this experiment were derived from the transplantable rat Morris hepatoma tissue. They were established as a clonal strain by Richardson *et al.*[4]. The cells were grown in Leibovitz L-15 medium supplemented with 10% fetal calf serum. Cultured cells were incubated at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. The medium was refreshed two times a week, and the subculture was regularly performed at weekly intervals with 0.1% trypsin in saline phosphate buffer.

The effects of ginseng saponin on cultured Morris hepatoma cells were examined using a growth medium containing 20 μg/ml or 100 μg/ml of ginseng saponin under the same conditions as stated above.

After about 24 subcultures in growth medium with ginseng saponin, some morphologically transformed cells were found among the Morris hepatoma cells. These cells are distinguishable from Morris hepatoma cells forming as a compact monolayer by light microscopical observation since they are larger cells and have abundant cytoplasm containing numerous small particles and clear intercellular spaces. As shown in Figure 1, they form a typical epithelial pattern that resembles the appearance of normal hepatocyte. This process was named "reverse transformation," since it appeared to reverse many of the characteristics assumed by cells treated with malignant transforming agents[5]. Number of reversely transformed cells are increased gradually and after 30 subcultures, all cells were reversely transformed.

Colony formation of reversely transformed cells in soft agar suspension culture

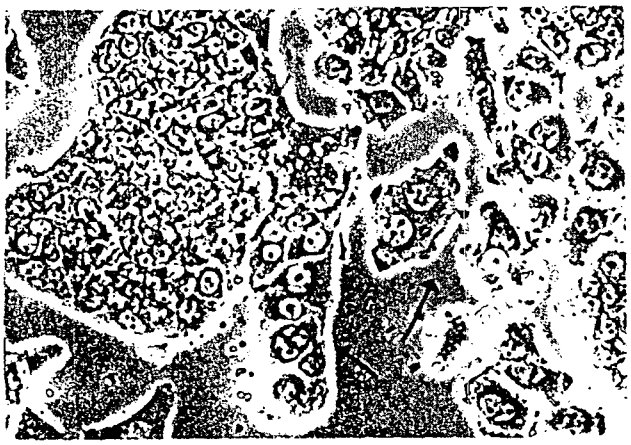

Fig. 1. Phase-contrast micrograph showing Morris hepatoma cells cultured in a medium with 20 μg/ml of ginsenosides.
Arrows indicate transformed hepatoma cells. These transformed cells are relatively large, form the typical epithelial pattern and the cytoplasm is granular. (× 400)

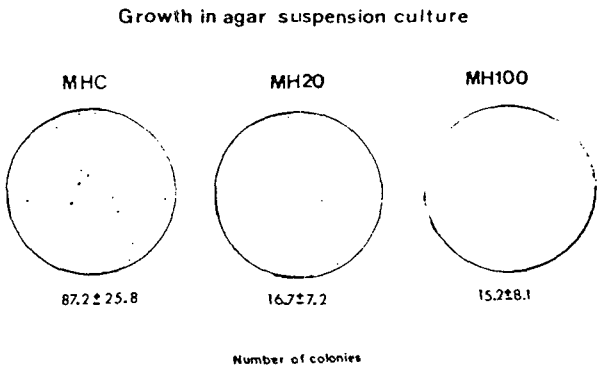

Fig. 2. Colony formation of hepatoma cells in soft agar suspension culture.
MHC: after 21 days of seeding of Morris hepatoma cells.
MH20: after 21 days of seeding of hepatoma cells transformed by presence of 20 μg/ml of ginsenosides in growth medium.
MH100: after 21 days of seeding of hepatoma cells transformed by presence of 100 μg/ml of ginsenosides in growth medium.

The ability of the reversely transformed cells to grow in 0.33 % soft agar suspension culture was examined according to the method of Macpherson and Montagnier[6].

When seeded at a density of 10^3 cells per 60mm dishes in 0.33 % soft agar made up with growth medium, macroscopic colonies appeared within 8 days. The colony formation rate of reversely transformed cells in soft agar suspension culture is less than one-fourth of the original Morris hepatoma cells, although the doubling time of these cells is 30 hours which is similar to original Morris hepatoma cells (Fig. 2).

Macpherson *et al.* reported that this method of soft agar suspension culture is one of the assay for neoplastic transformation and that the number of colonies is remarkably increased in malignant transformation of normal cells with polyoma virus. The decrease of colony number of cells reversely transformed by ginseng saponin in soft agar suspension culture is a very interesting phenomenon because this result suggests the possible normalization action of ginseng saponin in cultured Morris hepatoma cells.

Ornithine uptake of cells reversely transformed by ginseng saponin

The incorporation of L-^{3}H-ornithine in trichloroacetic acid (TCA) precipitable material from cultured cells was determined by the method of Odashima *et al.*[7].

The cultured cells were incubated for four hours in a medium containing 4×10^{-4} M L-^{3}H-ornithine (5 μCi/ml). After incubation, the cells were washed several times, suspended with 0.1 % trypsin in saline phosphate buffer solution, and then TCA was added. The radioactivities of TCA insoluble fraction were measured.

Urea cycle is a specific metabolic cycle in mammalian liver cells and Morris hepatoma cells used in this experiment maintain this metabolic cycle. As shown in Table 1, the uptake of L-^{3}H-ornithine of Morris hepatoma cells is significantly increased in an arginine deficient medium.

Particularly in the cells cultured in the medium with 100 μg/ml of ginseng saponin the uptake of ornithine was remarkably increased in arginine deficient medium.

From this result, it is clear that ginseng sapo-

nin stimulates the metabolism of urea cycle. Marker enzyme activities for endoplasmic reticulum, mitochondria and plasma membrane in cells was reversely transformed by ginseng saponin.

The enzyme activities of endoplasmic reticulum, mitochondria and plasma membranes were measured respectively on homogenates of both Morris hepatoma cells and cells reversely transformed by ginseng saponin. Glucose-6-phosphatase activity as a marker enzyme for endoplasmic reticulum was measured according to the method of Swanson[8], using glucose-6-phosphate disodium salt. Succinate-cytochrome C reductase activity as a marker enzyme for mitochondria was measured by the method of King[9] and 5'-nucleotidase activity was determined by the method of Wright[10].

Table 2 summarizes the results of cellular enzyme activities of these cells. The activities of succinate-cytochrome C reductase increased approximately 1.5 fold in cells reversely transformed by ginseng saponin and 5'-nucleotidase activity of the same cells decreased by approximately 50%. However, we did not find a change of glucose-6-phosphatase activity in cells reversely transformed by ginseng saponin. These results indicate that the metabolic activities of Morris hepatoma cells were changed with morphological transformation by ginseng saponin.

Serum protein synthesis of cell reversely transformed by ginseng saponin

Cells in confluent growth were washed three times with Hanks solution and reincubated in a medium without fetal calf serum for 24 hours. The medium was harvested, dialyzed extensively against glycine buffer and lyophilized.

The dry powder of medium was dissolved in distilled water and analyzed by immunoelectrophoresis using antiserum to normal rat whole serum.

The medium harvested from control Morris hepatoma cell cultures yields two precipitation lines in the β-region, but it does not form precipitation line in albumin fraction.

The medium harvested from reversely transformed cell cultures form several precipitation lines characteristic of albumin, α- and β-globulin regions (Figure 3). These results indicate that ginseng saponin stimulates protein synthesis, particularly production of albumin and globulin.

Table 1. Incorporation of L-^{3}H-ornithine into cultured cells in media with and without arginine

Medium	MHC*1	MH20*2	MH100*3
Complete medium	620.3 ± 156.6	1057.5 ± 232.5	1376.0 ± 346.2
Arginine deficient medium	2573.3 ± 405.5	5772.9 ± 1223.0	7258.2 ± 668.4

Radioactivity determined from TCA precipitable material from cell cultures. Each value represents mean dpm/10^5 cells ± standard error from 3 aliquots of cells.

*1 Control MH_1C_1 cells

*2 MH_1C_1 cells cultured in a medium with 20 μg/ml of ginsenosides

*3 MH_1C_1 cells cultured in a medium with 100 μg/ml of ginsenosides

Table 2. Effects of ginsenosides on succinate-cytochrome c reductase, 5'-nucleotidase and glucose-6-phosphatase activities

	MHC*1	MH20*2	MH100*3
Succinate-cyt. creductase (μmoles/mg protein/hour)	0.188 ± 0.035	0.284 ± 0.016	0.316 ± 0.022
5'-nucleotidase (μmoles/mg protein/hour)	0.540 ± 0.081	0.203 ± 0.046	0.211 ± 0.025
Glucose-6-phosphatase (μmoles/mg protein/hour)	0.619 ± 0.052	0.569 ± 0.051	0.572 ± 0.079

*1 Control MH_1C_1 cells

*2 MH_1C_1 cells cultured in a medium with 20 μg/ml of ginsenosides

*3 MH_1C_1 cells cultured in a medium with 100 μg/ml of ginsenosides

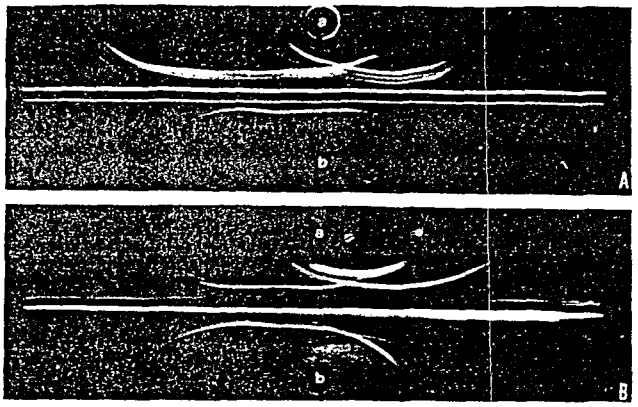

Fig. 3. Immunoelectrophoresis of Morris hepatoma cell culture fluid and transformed cell culture fluid. The electrophoresis was run for 2 h and the antiserum used was against rat whole serum protein.
A: wells a and b contained, respectively, rat whole serum and fluid obtained from Morris hepatoma cell culture.
B: wells a and b contained, respectively, culture fluid of cells transformed by presence of 20 μg/ml of ginsenosides and fluid of cells transformed by presence of 100 μg/ml of ginsenosides in growth medium. The trough between wells a and b contained antiserum to rat whole serum in both a and b.

Conclusion

After 24 subcultures with growth medium containing ginseng saponin, Morris hepatoma cells were morphologically transformed into relative larger cells with clear outline and with abundant granular cytoplasm, which resembled the appearance of hepatocyte of a normal liver. These morphological reverse transformations of Morris hepatoma cells are not accompanied by a change of proliferative ability, but the ability of these cells to grow in 0.33% soft agar suspension culture remarkably decreased. These effects of ginseng saponin on cultured Morris hepatoma cells were associated with a remarkable increase of uptake of ornithine in an arginine deficient medium, an increase of activity of succinate-cytochrome C reductase and a decrease of activity of 5'-nucleotidase. Moreover, these reversely transformed cells by ginseng saponin show a prominent increase of serum protein synthesis, particularly of the production of albumin and α-globulin.

From these results, it is clear that ginseng saponin induced a good differentiation of Morris hepatoma cells. But it is uncertain whether the reverse transformation of Morris hepatoma cells is a step toward normalization.

Many chemicals which induce neoplastic transformation have been extensively investigated, but there has not been found any chemical substance which induces a good differentiation of cancer cells. The study of the effects of ginseng saponin on cancer cells will prove valuable in understanding neoplastic transformation and in devising effective therapies for cancer.

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