

## Growth-inhibitory Effects of the Ketone Body, Monoacetoacetin, on Human Gastric Cancer Cells with Succinyl-CoA: 3-Oxoacid CoA-Transferase (SCOT) Deficiency

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**Abstract.** *Background:* Monoacetoacetin (MAA) has been used experimentally as a physiological energy source in parenteral nutrition. Succinyl-CoA: 3-oxoacid CoA transferase (SCOT) is a key enzyme in the metabolism of MAA. In this study, the effect of MAA on the growth of human gastric cancer cells was examined in relation to SCOT expression. *Materials and Methods:* Four gastric cancer cell lines, OCUM-2M, MKN-28, MKN-45 and MKN-74, and two fibroblast cell lines were used in this study. The proliferation of gastric cancer cells was determined by MTT assay, by calculating the number of cancer cells, and by [<sup>3</sup>H]-thymidine uptake. Cells were cultured in DMEM containing 10% FBS with glucose (4.5 g/L) as the control, or with MAA (4.5 g/L). SCOT mRNA expression was examined by RT-PCR. *Results:* The growth of OCUM-2M and MKN-28 cells was significantly suppressed in MAA medium compared with glucose medium. In contrast the growth of MKN-74, MKN-45 and normal fibroblasts was not suppressed in MAA medium. SCOT mRNA was expressed in MKN-45, MKN-74 and normal fibroblasts, but not in MKN-28 or OCUM-2M. *Conclusion:* Parenteral nutrition with MAA may provide preferential energy for patients with some types of gastric cancer with SCOT deficiency.

Total parenteral nutrition (TPN) produces nutritionally satisfactory results for patients with advanced cancer (1). The supply of nutrition for patients with carcinoma has been achieved mainly with glucose in clinical practice (2-4).

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Although TPN using glucose maintains the nutritional condition of patients with carcinoma, the growth of cancer cells is promoted by glucose (3-5). Therefore, instead of glucose, the development of nutrition which has growth-inhibitory effects for tumor cells, but not for normal cells, is needed.

Ketone bodies constitute nutritional material (6). TPN with ketone bodies has advantages over glucose (7,8). Ketone bodies are maintained more easily than glucose (7). Many kinds of tissues use ketone bodies as a preferred energy source, as well as glucose (8). Monoacetoacetin (MAA) is a ketone body composed of glycerol and acetoacetate. This substance is a transparent liquid with a molecular weight of 176, highly soluble in water and with an energy value 4.4 Kcal/g (5). MAA has been used experimentally as a physiological energy source in parenteral nutrition, but it is not metabolized in tumor cells (9-11). The growth of xenografted sarcoma cells was significantly reduced by parenteral nutrition with MAA as compared to glucose (5), while body weight was not different. However, the mechanism of tumor growth inhibition by MAA is still unclear. The utilization of ketone bodies is mediated by two enzymes, succinyl-CoA: 3-oxoacid CoA transferase (SCOT) which catalyzes CoA transfer from succinyl-CoA to acetoacetate, and mitochondrial acetoacetyl-CoA thiolase (12). SCOT is a key mitochondrial enzyme in the metabolism of ketone bodies in various organs (13,14). Deficiency of SCOT activity inhibits peripheral ketone body utilization. In this study, the effect of MAA on the growth of human gastric cancer cells was examined in relation to SCOT expression.

### Materials and Methods

*Cell lines and cell culture.* Four gastric cancer cell lines and two fibroblast cell lines were used in this study. The human gastric cancer cell lines, OCUM-2M (15), MKN-28 (16), MKN-45 (16) and MKN-74 (16) were placed in culture medium in 100-mm culture dishes (Falcon, Lincoln Park, NJ, USA) and incubated at 37°C in a

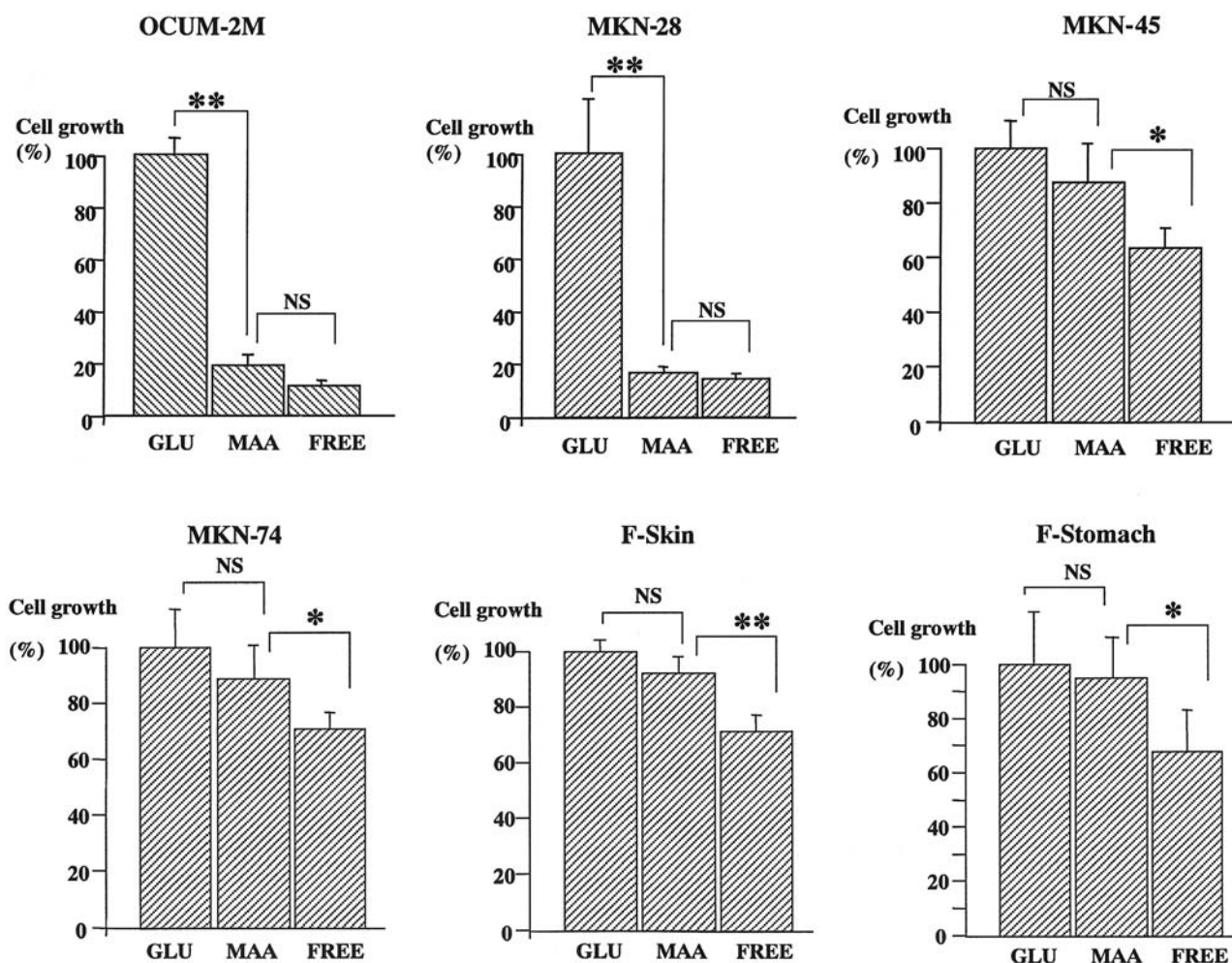


Figure 1A.

humidified atmosphere of 5% CO<sub>2</sub> in air. Human fibroblast cell lines were obtained from the stomach (F-Stomach) and skin (F-Skin). Human fibroblasts were used as normal cells. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Bioproducts, Walkersville, MD, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA), 100 IU/ml penicillin (ICN Biomedicals, Costa Mesa, CA, USA), 100 µg/ml streptomycin (ICN Biomedicals) and 0.5 mM sodium pyruvate (Bioproducts) at 37°C for 5 days until they became semi-confluent on the culture dish.

*Effect of ketone body on the growth of cancer cells or normal cells.* The proliferation of gastric cancer cells was determined by MTT assay, by calculating the number of cancer cells and by [<sup>3</sup>H]-thymidine uptake. Each of the three culture media used was prepared in DMEM as follows: GLU: 10% FBS with glucose (4.5 g/L) as the control; MAA: 10% FBS with MAA (4.5 g/L); FREE: 10% FBS. Growth activity was studied by culturing each cell line using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, as described previously (17). Briefly, cells in 150 µl of cell

suspension (5.0x10<sup>4</sup> cells/ml) with 10% FBS were incubated for 24 h. After centrifugation, the supernatant was aspirated. The cells were suspended in 100 µl culture medium and inoculated into a 96-well plate (Falcon). After 3 days, 20 µl of MTT (5 mg/ml in PBS; Wako Pure Chemical Industries, LTD., Osaka, Japan) were added to each well and the plates were incubated at 37°C for 3 h. Dimethyl sulfoxide (DMSO) (200 µl) was added. The plates were then read on the microplate reader (EAR-400; SLT Labinstrument, Grodig, Austria) using a test wavelength of 550 nm and a reference wavelength of 630 nm. The direct proportionality between dye reduction and cell number extended over a wide range, from 10<sup>3</sup> to 10<sup>5</sup> cells/well.

The number of cancer cells was calculated following the addition of culture medium using a Coulter counter (INDUSTRIAL D; Coulter Electronics, Luton, UK). Each cell line (10<sup>4</sup> each) was seeded into 24-well microplates (Falcon) with 2 ml of DMEM containing 10% FBS and incubated for 24 h. After centrifugation, the supernatant was aspirated. The cells were treated with 2 ml of various media, and incubated for 24, 48, 72, 96 and 120 h. The density of cells was determined using a Coulter counter.

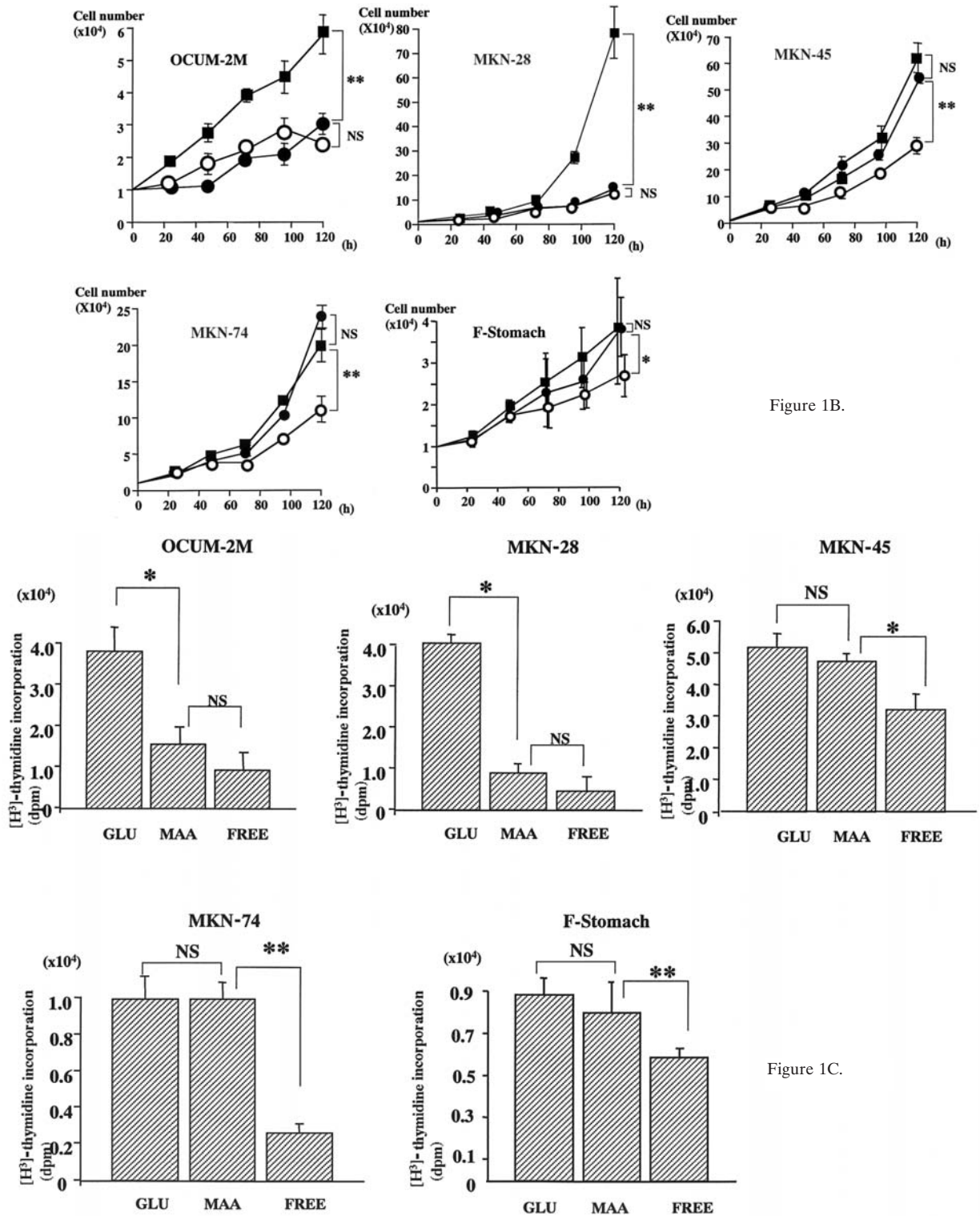


Figure 1B.

Figure 1C.

Figure 1. Effect of monoacetoacetin on the growth of cancer cells and normal cells. (A), The growth of OCUM-2M and MKN-28 cells was significantly suppressed by MAA medium, but MKN-74, MKN-45, F-Stomach and F-Skin were not compared to GLU medium. (B), The numbers of OCUM-2M and MKN-28 cells were significantly suppressed in MAA medium, while MKN-74 and MKN-45 were not. GLU, (■); MAA, (●); FREE, (○). (C), MAA significantly suppressed DNA synthesis of OCUM-2M and MKN-28 cells in MAA medium, but not that of MKN-74, MKN-45 cell and F-Stomach. The growth of MKN-28 and OCUM-2M was not significantly different between MAA medium and FREE medium. Results are presented as the mean of three independent experiments and the bars indicate the SD. \*,  $p < 0.05$ ; \*\*,  $p < 0.01$  versus control.

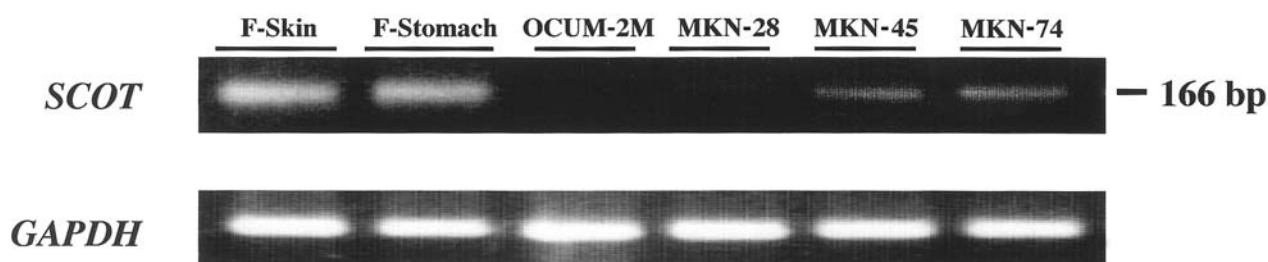


Figure 2. *SCOT* expression of each cell line. The PCR product of *SCOT* was 166 bp in length. *SCOT* was detected in MKN-45 cells, MKN-74 cells and fibroblasts, whereas MKN-28 cells and OCUM-2M cells did not express *SCOT*.

[<sup>3</sup>H]-thymidine uptake was determined as follows. Cells ( $10^4$  each) were seeded into 24-well microplates (Falcon) with 2 ml of DMEM containing 10% FBS and incubated at 37°C in a 5% CO<sub>2</sub> atmosphere for 24 h. After centrifugation, the supernatant was aspirated. Then, the cells were treated with 2 ml of various media with a pulse of 1  $\mu$ Ci per well [<sup>3</sup>H]-thymidine (28 Ci/mmol; Amersham, Tokyo, Japan) for 24 h at 37°C. Then the cells were washed three times with PBS, detached by trypsinization and collected by centrifugation. Radioactivity incorporation into DNA was measured with a liquid scintillation counter (Aloka, Tokyo, Japan).

**Reverse-transcription polymerase chain reaction (RT-PCR).** Total cellular RNA was extracted from gastric cancer cells with Trizol (Gibco) according to the manufacturer's protocol, and cDNA was synthesized by M-MLV-RT (reverse transcriptase of mRNA; Gibco) using oligo dt primer. The cDNA was amplified by PCR for 25 cycles with Taq DNA polymerase (Nippon Gene, Tokyo, Japan) on a thermal cycler. The following primers were used: *SCOT*, forward primer: 5'-GTTCAATGGCAGCACTTTA-3' and reverse primer, 5'-CTTCAACCTCTACCTACTGT-3'. To ensure that the RNA was of sufficient purity for RT-PCR, a PCR assay with primers specific for a housekeeping gene, *glyceraldehyde-3-phosphate dehydrogenase (GAPDH)* cDNA, was carried out in each sample, using 35 cycle conditions: GAPDH, forward primer: 5'-ACCTGACCTGCCGTCTAGAA-3', reverse primer: 5'-TCCACCACCCTGTTGCTGTA-3'. The conditions were as follows; pre-denaturation: 1 min at 94°C; 1 min at 55°C; and 1 min at 72°C, for 35 cycles. The PCR product of *SCOT* was 166 bp in length. The amplified products were analyzed by 2% agarose gel electrophoresis. The gel was stained with ethidium bromide (Bio-Rad Laboratories, Hercules, CA, USA) and illuminated using ultraviolet (UV) light.

**Statistical analysis.** Data are expressed as the mean  $\pm$  standard deviation (SD). Significant differences were analyzed using the unpaired Student's test. Values of *p* less than 0.05 indicated statistical significance.

## Results

**Inhibitory effect of monoacetoacetin on the growth of each cell line.** The growth of OCUM-2M and MKN-28 cells was significantly suppressed in MAA medium by 79% and 82%

compared with GLU medium, respectively. Growth of MKN-74 and MKN-45 was not significantly suppressed. The growth of normal cells, F-Stomach and F-Skin, was not suppressed in MAA medium (Figure 1A). The numbers of OCUM-2M and MKN-28 cells were significantly reduced in MAA medium by 51% and 79%, respectively, compared to GLU medium (Figure 1B). MAA significantly suppressed DNA synthesis of OCUM-2M and MKN-28 cells by 67% and 73%, respectively, but not MKN-74, MKN-45 cell and F-Stomach (Figure 1C). The growth of MKN-28 and OCUM-2M was not significantly different between MAA medium and FREE medium.

***SCOT* mRNA expression.** *SCOT* mRNA was expressed in MKN-45 and MKN-74, but not in MKN-28 or OCUM-2M. Normal cells, F-Stomach and F-Skin expressed *SCOT* (Figure 2). The 166 bp product was confirmed to be *SCOT* by direct sequence (data not shown).

## Discussion

In this study, the growth of two gastric cancer cell lines, MKN-28 and OCUM-2M, was significantly suppressed in MAA medium compared to GLU medium, while that of normal cells, F-Stomach and F-Skin was not. The growth of MKN-28 and OCUM-2M in MAA medium was not significantly different compared to FREE medium. These results suggest that MAA was not metabolized in MKN-28 or OCUM-2M cells. In contrast, the growth of MKN-45, MKN-74 and fibroblasts were not different between MAA medium and GLU medium. MAA has growth-inhibitory effects for MKN-28 and OCUM-2M cells, but not for normal fibroblasts. These results suggest that parenteral nutrition with MAA might provide preferential energy for patients with some types of gastric cancer. *SCOT* has been reported to be deficient in tumor cells, including hepatoma and neuroblastoma (18,19). MKN-28 and OCUM-2M cells did not express *SCOT* mRNA, whereas MKN-45 cells,

MKN-74 cells and fibroblasts expressed *SCOT*. Utilization of MAA in gastric cancer cells might be mediated by *SCOT*. These findings suggest that the deficiency of *SCOT* in tumor cells was closely associated with the growth-inhibitory effects of MAA for cancer cells. This is the first report that growth of tumor cells is suppressed by MAA medium in gastric carcinomas with *SCOT* deficiency. OCUM-2M and MKN-45 were derived from a poorly-differentiated adenocarcinoma, while MKN-28 and MKN-74 were derived from a well-differentiated adenocarcinoma (15,16). Histological types of gastric cancer cells may not correlate with *SCOT* deficiency. No report of TPN which provide energy for host cells but not for tumor cells was found. The genetic evaluation of *SCOT* mRNA expression in biopsy specimen might be useful to determine whether TPN using MAA is beneficial for patients with gastric cancer or not. TPN using MAA in accordance with *SCOT* activity could be a novel therapy for patients with gastric cancer.

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