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学術論文

Changes in Serum and Liver Lipid Levels Observed in RCN-9-Implanted Rats: An Observation of the Difference in Implanted Cell Numbers

大腸癌細胞 RCN-9 移植ラットで観察された血清および肝臓脂質レベルの変化 —移植細胞数の違いの観察

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Changes in serum and liver lipid levels were investigated in transplantable rat colon adenocarcinoma RCN-9-implanted rats. The rats of tumor-bearing groups were implanted with 5×10^6 or 5×10^7 RCN-9 cells and maintained for 21 days. Total serum cholesterol concentration, and especially low-density lipoprotein cholesterol, free cholesterol and esterified cholesterol concentrations, were significantly increased following the implantation of 5×10^7 RCN-9 cells compared to the control group (no cells implanted). Serum triglyceride concentration was significantly decreased following the implantation of 5×10^7 RCN-9 cells compared to the control group. Serum and liver thiobarbituric acid-reactive substance (TBARS) values were significantly increased after the implantation of 5×10^7 RCN-9 cells compared to the control group. Solid tumors were first observed on days 2-3 after the implantation of 5×10^7 RCN-9 cells, and continued to grow with time. These results suggest that in RCN-9-implanted rats, serum cholesterol and triglyceride concentrations and serum and liver TBARS values undergo changes resulting in an abnormal lipid metabolism corresponding with increases in the number of tumor cells implanted.

Keywords: *Cancer, Serum and liver lipids, Tumor bearing*
癌，血清および肝臓脂質，担癌

INTRODUCTION

Tumor cells have a very distinctive metabolism. Several previous studies have examined the metabolic changes that take place in the host after tumor implantation. Metabolic abnormalities arising from malnutrition and malignancy are seen in animals and patients with cancer cachexia. Various cancers affect the lipid metabolism.[1-7] Rats subcutaneously implanted with the AH109A ascites hepatoma cell line show hyperlipidemia with a notable decrease in the high-density lipoprotein (HDL) fraction and an enormous increase in the very-low-density lipoprotein (VLDL) plus low-density lipoprotein (LDL) fractions[5-7] during the growth of solid tumor.[5] AH109A is a hepatoma that originates in the liver. Major lipid metabolism occurs in the liver; this includes cholesterol, fatty acid, and triglyceride biosyntheses as well as VLDL production. It is therefore believed that hepatoma cells may affect the lipid metabolism in the host body and that the state of the cancer affects the lipid metabolism.

RCN-9, a transplantable rat colon adenocarcinoma, was induced in the colon of a male Fisher F344 rat by the subcutaneous administration of 1,2-dimethylhydrazine. RCN-9 metastasizes spontaneously, and when RCN-9 cells were injected subcutaneously or into the cecal subserosa of syngeneic rats, carcinomas with progressive growth were obtained and lung and liver metastases developed.[8]

In the present study, to investigate the effects of RCN-9 implantation on the lipid metabolism, changes in lipid levels in the serum and liver were examined in RCN-9-implanted rats. RCN-9 is a colon cancer that originates from the colon; the effects of the implanted RCN-9 tumor cells on lipid metabolism may be small compared to those of a hepatoma,[5-7] which originates from the liver and is active in various types of lipid metabolism. A previous study reported that RCN-9-bearing rats implanted with 5×10^6 cells showed an increase in their serum and liver thiobarbituric acid-reactive substance (TBARS) values.[9] A solid tumor was observed in each rat within a short time after RCN-9 implantation, though the tumors stopped growing and their weight was less than 1% of the body weight of the host rat on the 21st day after RCN-9 implantation.[9] In the present study, rats were implanted with 5×10^6 or 5×10^7 RCN-9 cells to investigate the effects of the number of implanted tumor cells on serum and liver lipid levels.

MATERIALS AND METHODS

Animals and diets. This animal experiment was conducted with the approval of the Iwate Prefectural University Research Ethics Committee.

Male F344 rats (four weeks of age, Charles River Laboratories Japan, Inc., Kanagawa, Japan) were individually housed in stainless steel cages with wire bottoms in an air-conditioned room with a temperature of 22±2°C, a relative humidity of 55±5%, and an 8:00 a.m. to 8:00 p.m. light cycle and were fed a stock pellet diet (MF; Oriental Yeast Co., Ltd., Tokyo, Japan) followed by a basal diet containing 20% casein.[10] The composition of the basal diet was as described in a previous report.[9]

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Abbreviations: HDL, high-density lipoprotein; LCAT, lecithin-cholesterol acyl transferase; LDL, low-density lipoprotein; NEFA, nonesterified fatty acid; PBS(-), phosphate-buffered saline; TBARS, thiobarbituric acid-reactive substance; VLDL, very-low-density lipoprotein.

Table 1. Initial body weight, food intake, body weight gain, and weights of liver and epididymal adipose tissue in normal (Control) and tumor-bearing rats.

Measurement	Control	Tumor-bearing	
		5×10^6 cells	5×10^7 cells
Initial body weight (g)	79.0 ± 1.2	79.0 ± 1.4	79.0 ± 1.4
Food intake (g/21d)	285.1± 4.6	258.3 ± 5.1	252.8 ± 4.5
Body weight gain (g/21d)	97.4 ± 2.8	98.3 ± 2.1	96.1 ± 2.8
Liver weight (g/whole body)	6.95 ± 0.36	7.05 ± 0.22	5.75 ± 0.36 *
Epididymal adipose tissue weight (g/whole body)	2.83 ± 0.13	2.83 ± 0.06	2.40 ± 0.09 *

Values represent the means ± standard errors for seven rats. * Significantly different from the control group at $p < 0.05$ by one-way analysis of variance followed by Dunnett's pairwise multiple comparison t-test.

After preliminary feeding, the rats were divided into three groups (n=7, each group) of similar body weights. The RCN-9 cells were prepared by means of a cell culture system. The RCN-9 cells were cultured in RPMI-1640 medium supplemented with 10% fetal calf serum. Two of the groups received a subcutaneous implantation of 5×10^6 (T6 group) or 5×10^7 (T7 group) RCN-9 cells (provided by the RIKEN BRC through the National Bio-Resource Project of MEXT, Japan) suspended in phosphate-buffered saline (PBS(-)) (0.5 ml/rat) in the back to produce a solid tumor (tumor-bearing groups), and the last group received a sham injection of PBS(-) alone (0.5 ml/rat); the latter group was designated as normal rats (control group). All rats were then maintained for 21 days on the basal diet. The solid tumor diameter was measured every day to estimate the tumor volume. The tumor volume was calculated by the following formula:

Tumor volume (cm^3) = $4/3\times\pi\times r^3$ (r: radius of solid tumor (cm))

Water and diet were available at all times every day. The animals were deprived of their diet at 9:00 a.m. on the 21st day, but were allowed free access to water until they were sacrificed 4 hours later. Blood was collected and left to clot at room temperature to obtain serum. The liver, epididymal adipose tissue, and solid tumor were quickly removed, washed with cold 0.9% NaCl, blotted on filter paper, and weighed. The serum and liver were stored at -30°C until lipid concentration analyses were performed. Aliquots of the liver were also preserved in methanol and stored at 4°C until analyses of the lipid contents were performed.

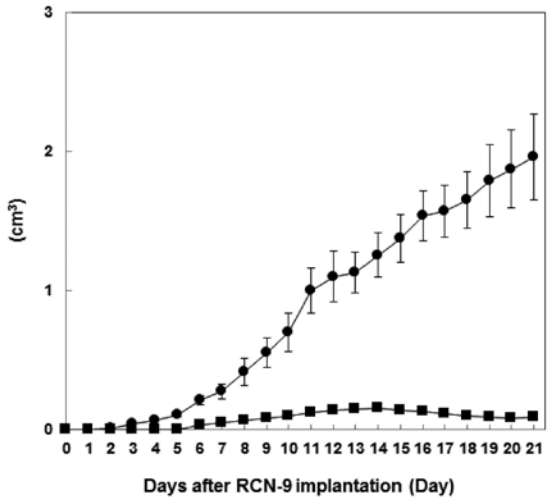

Fig. 1. Estimated solid tumor volume in tumor-bearing rats implanted with 5×10^6 (■) and 5×10^7 (●) RCN-9 cells. Each value represents the means for seven rats. Vertical bars indicate standard errors.

Lipid analyses. The lipoprotein separation of serum was as follows, HDL was separated from VLDL plus LDL by precipitation method using sodium phosphotungstic acid and MgCl,[11] and VLDL was separated from LDL plus HDL by ultracentrifugation.[12]

Serum total, HDL-, and (HDL+LDL)-cholesterol, free cholesterol, triglyceride, phospholipid, and nonesterified fatty acid (NEFA) concentrations were determined by an enzymatic method using a Cholesterol E-test, Free cholesterol E-Test, Triglyceride E-test, Phospholipid C-test, and NEFA C-test (Wako Pure Chemical Industries, Ltd.), respectively. The difference between the total cholesterol concentration and HDL-cholesterol concentration was regarded as the (VLDL+LDL)-cholesterol concentration. The difference between the (HDL+LDL)-cholesterol concentration and the HDL-cholesterol concentration was regarded as the LDL-cholesterol concentration. The difference between the (VLDL+LDL)-cholesterol concentration and the LDL-cholesterol concentration was regarded as the VLDL-cholesterol concentration. The ratio of the (VLDL+LDL)-cholesterol concentration to HDL-cholesterol concentration is designated as the atherogenic index. The difference between the total cholesterol concentration and free cholesterol concentration was regarded as the esterified cholesterol concentration, and the ratio of the esterified cholesterol concentration to total cholesterol concentration is designated as the cholesterol ratio.

The liver (0.5 g) was homogenized with total 50 volume of chloroform-methanol mixture (2:1, v/v) and total lipids were extracted according to the procedure of Folch *et al.*[13]. The contents of cholesterol,[14] triglyceride,[15] and phospholipid[16] were determined using 2ml, 1ml, or 0.1ml of chloroform-methanol extract, respectively.

The serum and liver TBARS values were measured according to the method described by Yagi[17] and Mihara *et al.*,[18] respectively.

Statistical analysis. Results were expressed as mean ± standard error. Statistical analysis was carried out by one-way analysis of variance followed by Dunnett's pairwise multiple comparison *t*-test using the SPSS Statistics, version 26 (IBM Japan, Ltd., Tokyo, Japan). A

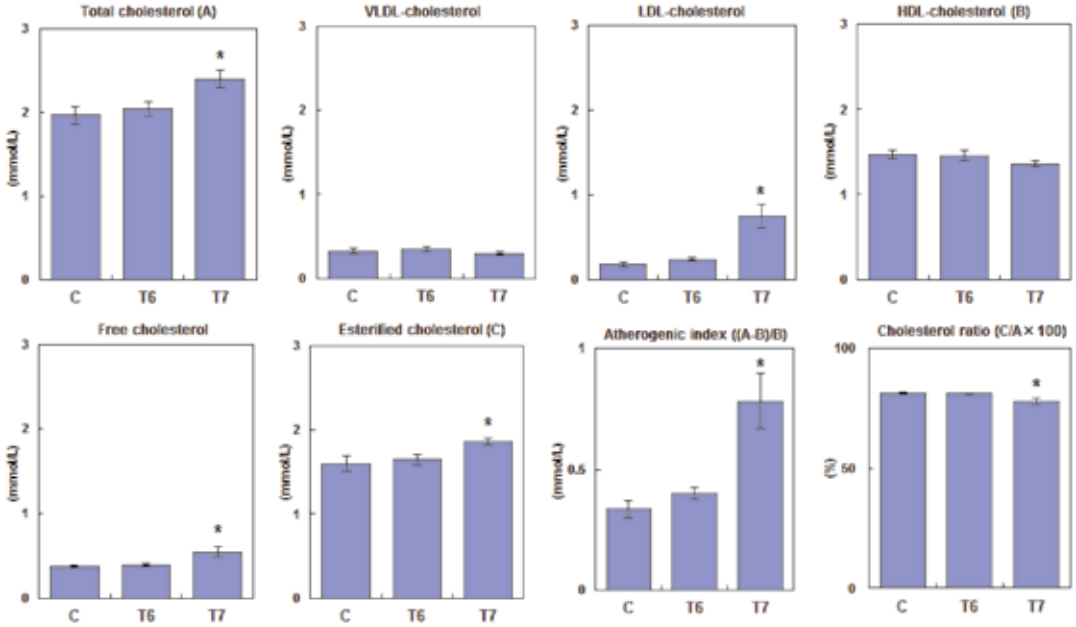

Fig. 2. Serum cholesterol concentration, atherogenic index, and cholesterol ratio in normal and tumor-bearing rats. Each value represents the means for seven rats. Vertical bars indicate standard errors. * Significantly different from the control group at $p < 0.05$ by one-way analysis of variance followed by Dunnett's pairwise multiple comparison *t*-test. C, normal rats group (Control); T6, tumor-bearing rats implanted with 5×10^6 RCN-9 cells group; T7, tumor-bearing rats implanted with 5×10^7 RCN-9 cells group.

significance level of $p < 0.05$ was used for all the comparisons.

RESULTS

Table 1 shows the initial body weight, food intake, and body weight gain for the 21-day duration of the experimental period, and the weights of the liver and epididymal adipose tissue at the end of the experimental period. There were no significant differences between the control group and each of the two tumor-bearing groups with regard to food intake and body weight gain, however, the liver and epididymal adipose tissue weights in the T7 group decreased significantly compared to the control group.

The absolute and relative weights of the solid tumors at the end of the experimental period were 0.10 ± 0.04 g and 0.06 ± 0.02 % of body weight, respectively, in the T6 group, and 2.56 ± 0.05 g and 1.48 ± 0.34 % of body weight, respectively, in the T7 group.

Figure 1 shows the estimated solid tumor volume in tumor-bearing

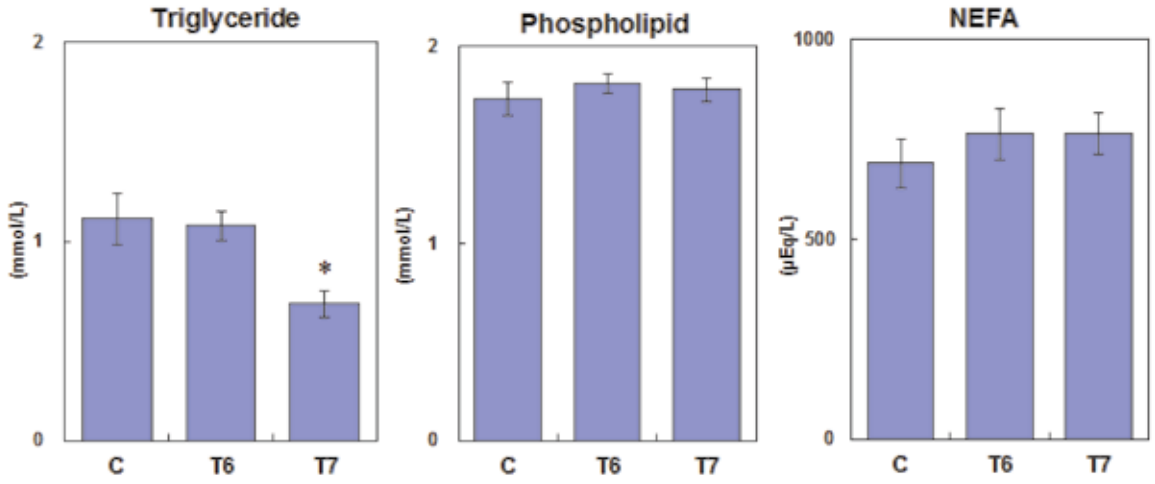

Fig. 3. Serum triglyceride, phospholipid, and nonesterified fatty acid (NEFA) concentrations in normal and tumor-bearing rats. Each value represents the means for seven rats. Vertical bars indicate standard errors. * Significantly different from the control group at $p < 0.05$ by one-way analysis of variance followed by Dunnett's pairwise multiple comparison *t*-test. C, normal rats group (Control); T6, tumor-bearing rats implanted with 5×10^6 RCN-9 cells group; T7, tumor-bearing rats implanted with 5×10^7 RCN-9 cells group.

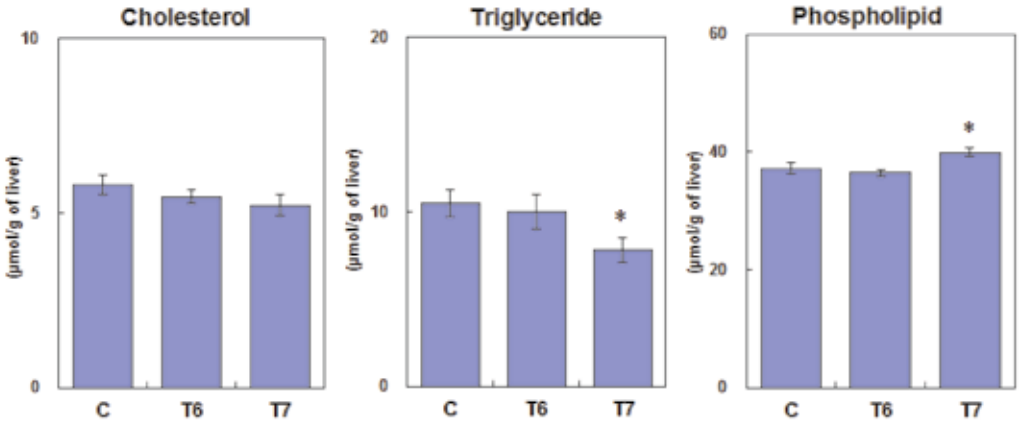

Fig. 4. Liver lipid contents in normal and tumor-bearing rats. Each value represents the means for seven rats. Vertical bars indicate standard errors. * Significantly different from the control group at $p < 0.05$ by one-way analysis of variance followed by Dunnett's pairwise multiple comparison *t*-test. C, normal rats group (Control); T6, tumor-bearing rats implanted with 5×10^6 RCN-9 cells group; T7, tumor-bearing rats implanted with 5×10^7 RCN-9 cells group.

rats after RCN-9 implantation. In the T7 group, the solid tumor was first observed on day 2-3 after RCN-9 implantation and continued to grow with time. In the T6 group, the solid tumor was first observed on day 5-6, and its growth was slow during the late period.

Serum cholesterol concentrations are shown in Fig. 2. The total cholesterol concentration in the T7 group increased significantly compared to the control group. In lipoprotein cholesterol, the LDL-cholesterol concentration in the T7 group was significantly higher than that in the control group, while the VLDL- and HDL-cholesterol concentrations did not change in response to tumor bearing. The atherogenic index in the T7 group was significantly higher than that in the control group. The free and esterified cholesterol concentrations in the T7 group were significantly higher than those in the control group. The cholesterol ratio in the T7 group was significantly lower than that in the control group.

Serum triglyceride, phospholipid, and NEFA concentrations are shown in Fig. 3. The serum triglyceride concentration in the T7 group decreased significantly compared to the control group. There were no significant differences between the control group and each of the two tumor-bearing groups with regard to serum phospholipid and NEFA concentrations.

Liver lipid contents are shown in Fig. 4. The liver cholesterol content was not significantly different between the control group and each of the two tumor-bearing groups, though in the T7 group, it tended to decrease compared to the control group. The liver triglyceride content in the T7 group decreased significantly compared to the control group. The liver phospholipid content in the T7 group increased significantly compared to the control group.

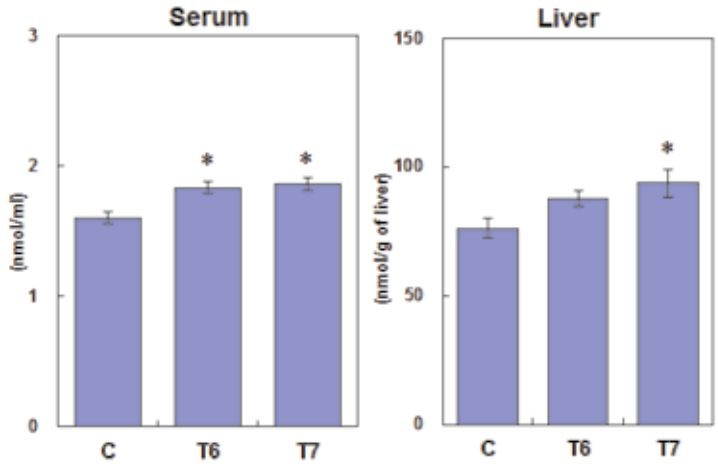

Fig. 5. Serum and liver thiobarbituric acid-reactive substance (TBARS) values in normal and tumor-bearing rats. Each value represents the means for seven rats. Vertical bars indicate standard errors. * Significantly different from the control group at $p < 0.05$ by one-way analysis of variance followed by Dunnett's pairwise multiple comparison *t*-test. C, normal rats group (Control); T6, tumor-bearing rats implanted with 5×10^6 RCN-9 cells group; T7, tumor-bearing rats implanted with 5×10^7 RCN-9 cells group.

Serum and liver TBARS values are shown in Fig. 5. Both TBARS values in the T7 group increased significantly compared to the control group. The serum TBARS values in the T6 group was also increased significantly compared to the control group.

DISCUSSION

A significant decrease in the cholesterol ratio (the ratio of the esterified cholesterol concentration to total cholesterol concentration) was seen in the RCN-9-bearing rats. Free cholesterol esterification is catalyzed by lecithin-cholesterol acyl transferase (LCAT), which catalyzes the transfer of a fatty acid residue from the 2nd position of lecithin to cholesterol to form esterified cholesterol and is considered to be responsible for much of the esterified cholesterol in plasma lipoproteins. It is likely that, in the rats that received the RCN-9 implantation, the ratio of the esterification of free cholesterol might be reduced by the suppression of LCAT activity; hence the significant decrease in the cholesterol ratio.

The serum and liver TBARS values in the 5×10^7 RCN-9 implantation group increased significantly compared to the control group. TBARS value is an index of lipid peroxidation in the blood or tissues. Lipids peroxidize at double bonds between carbon atoms of fatty acids. Unsaturated fatty acids have double bonds between carbon atoms, and are therefore easily oxidized. Srivastava *et al.* reported a significant increase in plasma TBARS values in patients with oral cancer compared to control subjects. In cancer patients, unsaturated fatty acids in the cell membranes of erythrocytes in blood are thought to be highly susceptible to oxidative attack. Thus, in plasma, the erythrocyte cell membrane becomes a major substrate for reactive oxygen species-mediated damage.[19] This mechanism might account for the enhanced serum and liver TBARS values in RCN-9-implanted rats compared to the control group in the present study.

The study by Srivastava *et al.* also reported that levels of superoxide dismutase, reduced glutathione, glutathione peroxidase, and catalase, which are antioxidant enzymes, were significantly decreased in oral cancer patients compared to healthy subjects, and that cancer patients also showed increased plasma TBARS values.[19] It is likely that, in the present study, the antioxidation of unsaturated fatty acids in the erythrocyte cell membranes and liver in RCN-9-implanted rats might have been reduced by the suppression of these antioxidant enzyme activities, and thus, the serum and liver TBARS values were enhanced. The suppression of these antioxidant enzyme activities might have been the cause of the significant increases seen in the serum and liver TBARS values of the RCN-9-implanted rats.

It is well known that some cancers affect the lipid metabolism. For example, one study found that the serum total cholesterol and (VLDL+LDL)-cholesterol concentrations were increased in ascites hepatoma cell line AH109A-bearing rats, whereas the serum HDL-cholesterol concentration decreased.[5-7] Another study showed that in Lewis lung carcinoma-implanted mice, serum (VLDL+LDL)-cholesterol concentrations were increased, whereas the serum HDL-cholesterol concentration decreased.[20] Additionally, a decrease in the serum total cholesterol concentration, and especially in HDL- and esterified cholesterol concentrations, were observed in Sato lung carcinoma-bearing rats.[21] However, another previous study found that 5×10^6 RCN-9 implantation did not change most of the parameters related to the lipid metabolism.[9] In the tumor-bearing state, disorders of the lipid metabolism occur during the growth of the solid tumor.[5] A previous study of tumor-bearing rats found an increase in the plasma triglyceride concentration together with the suppression of the activities of tissue lipoprotein lipase, the hydrolytic enzyme in serum triglyceride, with increased tumor burden, and tumor removal completely reversed these changes.[22] These findings provide evidence that the tumor-induced changes in lipid concentrations in blood are stimulated by the presence of the tumor. In the present study, solid tumors were observed within a short time, and the growth of the solid tumors after the 5×10^7 RCN-9 implantation was rapid compared to that after the 5×10^6 implantation. On the 21st day after RCN-9 implantation, solid tumor weight in the 5×10^7 RCN-9 implantation group was higher than that in the 5×10^6 RCN-9 implantation group. Therefore, some of the parameters related to the lipid metabolism examined in the present study might have undergone a greater change due to the implantation of 5×10^7 RCN-9 cells rather than 5×10^6 cells.

Serum triglyceride concentration decreased significantly in the T7 group compared to the control group. A previous study found an increase in serum triglyceride concentration in rats implanted with the AH109A ascites hepatoma cell line.[7] The same study also showed the suppression of hepatic triglyceride lipase activity and the elevation of hepatic fatty acid oxidation.[7] The reason for the significant decrease in the serum triglyceride concentration remains unclear, but RCN-9 is a colon cancer that originates in the colon; the effects of these implanted RCN-9 tumor cells on the lipid metabolism may be different from those of a hepatoma,[7] which originates in the liver and is active in various aspects of lipid metabolism.

In conclusion, RCN-9 implantation in rats reduced the cholesterol ratio and serum triglyceride concentration, and enhanced serum and liver TBARS values. The mechanisms responsible for the observed changes after RCN-9 implantation were not identified, and remain to be investigated in future studies.

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REFERENCES

1) Gallagher, E. J., Zelenko, Z., Neel, B. M., Antoniou, I. M., Rajan, L., Kase, N., and LeRoith, D., Elevated tumor LDLR expression

accelerates LDL cholesterol-mediated breast cancer growth in mouse models of hyperlipidemia, *Oncogene*, **36**, 6462-6471 (2017).
2) Huang, J., Li, L., Lian, J., Schauer, S., Vesely, P.W., Kratky, D., Hoefler, G., and Lehner, R., Tumor-induced hyperlipidemia contributes to tumor growth, *Cell Rep.*, **15**, 336-348 (2016).
3) Hirasawa, A., Makita, K., Akahane, T., Yokota, M., Yamagami, W., Banno, K., Susumu, N., Aoki, D., Hypertriglyceridemia is frequent in endometrial cancer survivors, *Jpn. J. Clin. Oncol.*, **43**, 1087-1092 (2013).
4) Inamdar, P., and Mehta, G., Correlation between obesity and high density lipoprotein cholesterol (HDL-C) in breast cancer patients of Southern Rajasthan, *Indian J. Surg. Oncol.*, **2**, 118-121 (2011).
5) Irikura, T., Takagi, K., Okada, K., and Yagasaki, K., Effect of KCD-232, a new hypolipidemic agent, on serum lipoprotein changes in hepatoma-bearing rats, *Lipids*, **20**, 420-424 (1985).
6) Kawasaki, M., Yagasaki, K., Miura, Y., and Funabiki, R., Responses of serum lipids and adipose tissue lipases to lipopolysaccharide administration in normal and hepatoma-bearing rats, *Biosci. Biotechnol. Biochem.*, **60**, 528-529 (1996).
7) Kawasaki, M., Yagasaki, K., Miura, Y., and Funabiki, R., Comparison of the changes in lipid metabolism between hepatoma-bearing and lipopolysaccharide-treated rats, *Biosci. Biotechnol. Biochem.*, **68**, 72-78 (2004).
8) Inoue, Y., Kashima, Y., Aizawa, K., Hatakeyama, K., A new rat colon cancer cell line metastasizes spontaneously: biologic characteristics and chemotherapeutic response, *Jpn. J. Cancer Res.*, **82**, 90-97 (1991).
9) Kawasaki, M., Effects of tumor bearing on serum and liver lipid levels in RCN-9-implanted rats, *Bulletin of Morioka Junior College, Iwate Prefectural University*, **16**, 1-6 (2014).
10) Reeves, P. G., Nielsen, F. H., and Fahey, G. C. Jr., AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet, *J. Nutr.*, **123**, 1939-1951 (1993).
11) Burstein, M., Scholnick, H. R., and Morfin, R., Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions, *J. Lipid Res.*, **11**, 583-595 (1970).
12) Wu, L. L., Warnick, G. R., Wu, J. T., Williams, R. R., and Lalouel, J. M., A rapid micro-scale procedure for determination of the total lipid profile, *Clin. Chem.*, **35**, 1486-1491 (1989).
13) Folch, J., Lees, M., and Sloane-Stanley, G. H., A simple method for the isolation and purification of total lipides from animal tissues, *J. Biol. Chem.*, **226**, 497-509 (1957).
14) Zak, B., Simple rapid microtechnic for serum total cholesterol, *Am. J. Clin. Path.*, **27**, 583-588 (1957).
15) van Handel, E., Suggested modifications of the micro determination of triglycerides, *Clin. Chem.*, **7**, 249-251 (1961).
16) Chen, P. S., Toribara, T. Y., and Warner, H., Microdetermination of phosphorus, *Anal. Chem.*, **28**, 1756-1758 (1956).
17) Yagi, K., A simple fluorometric assay for lipoperoxide in blood plasma, *Biochem. Med.*, **15**, 212-216 (1976).
18) Mihara, M., and Uchiyama, M., Determination of malonaldehyde precursor in tissues by thiobarbituric acid test, *Anal. Biochem.*, **86**, 271-278 (1978).
19) Srivastava, K. C., Austin, R. D., Shrivastava, D., Sethupathy, S., and Rajesh, S., A case control study to evaluate oxidative stress in plasma samples of oral malignancy, *Contemp. Clin. Dent.*, **3**, 271-276 (2012).
20) Kawasaki, M., Time course changes in serum and liver lipid levels and lipid-related parameters in Lewis lung carcinoma-implanted mice, *Bulletin of Morioka Junior College, Iwate Prefectural University*, **12**, 1-7 (2010).
21) Kawasaki, M., Effects of tumor bearing on serum and liver lipid levels in Sato lung carcinoma-implanted rats: Effect of a difference of implanted cell numbers, *Bulletin of Morioka Junior College, Iwate Prefectural University*, **17**, 1-6 (2015).
22) Noguchi, Y., Vydelingum, N. A., Younes, R. N., Fried, S. K., and Brennan, M. F., Tumor-induced alterations in tissue lipoprotein lipase activity and mRNA levels, *Cancer Res.*, **51**, 863–869 (1991).

和文要旨　　大腸癌細胞 RCN-9 移植ラットにおける血清および肝臓脂質レベルの変化を検討した。担癌群のラットには，RCN-9 細胞を 5×10^6 または 5×10^7 移植し、21 日間飼育した。血清総コレステロール濃度ならびにその中で低密度リポタンパク質コレステロール，遊離コレステロールおよびエステル結合型コレステロール濃度が，5×10^7 の RCN-9 細胞の移植後対照群と比較して有意に上昇した。血清トリグリセリド濃度が，5×10^7 の RCN-9 細胞の移植により対照群と比較して有意に低下した。血清および肝臓チオバルビツール酸反応物質（TBARS 値）が，5×10^7 の RCN-9 細胞の移植により対照群と比較して有意に上昇した。固形癌が，5×10^7 の RCN-9 細胞移植後 2 から 3 日後に観察されるようになり，時間の経過とともに増殖していった。RCN-9 細胞移植ラットにおいては，血清コレステロールおよびトリグリセリド濃度，血清および肝臓 TBARS 値が変化し，移植細胞数の増加により脂質代謝に異常が生じることが示唆された。