Effect of Dietary Essential Fatty Acids on Pulmonary Metastasis of Ascites Tumor Cells in Rats

TAKAMITSU Hori,a ATSUKO MORIUCHI,a HARUMI OKUYAMA*,a TAKESHI SOBAJIMA,b KEIKO TAMII-KOIZUMI,b and KIYOHIDE KOJIMA

Department of Biological Chemistry, Faculty of Pharmaceutical Sciences, Nagoya City University,a Tanabedori, Mizuho-ku, Nagoya 467, Japan and Laboratory of Cancer Cell Biology, Nagoya University School of Medicine,b Tsurumai, Showa-ku, Nagoya 466, Japan

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Rats were fed a semi-purified diet supplemented either with a linoleic acid-rich oil (saflower oil) or with an α-linolenic acid-rich oil (perilla oil) from weanling to 7 weeks of age. Rats fed a conventional diet were also used as controls. Ascites tumor cells were injected intravenously into these rats and the numbers of metastatic foci on the pulmonary surface were determined macroscopically. The average number of metastatic foci was significantly less in the rats fed the perilla oil diet than in the rats fed the saflower oil diet or the conventional diet. Combined with previous results on the metastatic potentials of tumor cells modified with different fatty acids, these results indicate that the modification of host animal cells by dietary fatty acids also affects the metastatic potentials of the ascites tumor cells in rats, apparently independently of the tumor cell modifications caused by the supplemented fatty acids.

Keywords—metastasis; tumor; linoleate; α-linolenate; platelet; diet; rat; fatty acid composition

Epidemiologically, mammary tumorigenesis has been positively correlated with the amount of fat intake. Although polyunsaturated fatty acids were once supposed to lead to an increase in tumorigenesis through the action of lipid peroxide, it was later shown that the intake of higher amounts of saturated fats also increased the incidence of mammary tumor in animals. Polyunsaturated fatty acids in animal tissues are classified mainly into the n-6 series derived from linoleic acid (18:2 n-6) and then n-3 series derived from α-linolenic acid (18:3 n-3). The essentiality of linoleic acid has been established and its physiological functions appear to be mediated through the hormone-like eicosanoids derived from arachidonic acid (20:4 n-6). Some eicosanoids from arachidonate are inhibitory for tumor cell growth while others appear to be stimulatory, indicating the complicated involvement of arachidonate in the division, growth and differentiation of tumor cells. As to the metastasis of tumor cells, the administration of prostaglandin I2 (PGI2) or a drug inducing an increase in PGI2 inhibited the metastasis of tumor cells, leading to the hypothesis that platelet–tumor cell interaction is important in the metastatic phenomena.

In contrast to the n-6 series fatty acids, the essentiality of α-linolenic acid for higher animals had been questioned, until we recently showed that it is essential for maintaining proper learning ability in rats. Eicosanoids appear to be synthesized also from an n-3 fatty acid, eicosapentaenoic acid (20:5 n-3), but only to a limited extent. Their physiological activities are generally lower than those of the eicosanoids from n-6 fatty acids, and no specific functions have been ascribed to the eicosanoids from n-3 acids. However, n-3 polyunsaturated fatty acids in general can compete with n-6 fatty acids and regulate their metabolism in...
animal tissues. One of the consequences of increasing n-3 fatty acids in the diet is supposed to be a decrease in platelet aggregability and thrombotic tendency. The decrease in platelet aggregability may decrease the metastatic potentials of tumor cells through platelet–tumor cell interaction. To test this possibility, we examined the metastatic potentials of ascites sarcoma cells in rats fed either with a linoleate-rich diet or with an α-linolenate-rich diet.

Materials and Methods

Diets — The basic components of a semi-purified diet (Nippon Clea Corp., Tokyo) were 24.6% milk casein, 47.0% corn starch, 2.0% α-starch, 8.0% cellulose, 5.0% sucrose, 2.0% vitamin mixture, 6.0% minerals, 0.4% methionine, and 5.0% oil (safflower oil or perilla oil). A conventional diet (Nippon Clea, CE-2) was also used as a control. Fatty acid compositions were 10.6% saturated, 12.3% oleic, 12.8% linoleic and 64.0% α-linolenic acids in the perilla oil diet, 11.3% saturated, 10.4% oleic, 78.0% linoleic and 0.05% α-linolenic acids in the safflower oil diet, and 18.3% saturated, 22.2% oleic, 49.7% linoleic and 4.1% α-linolenic acids in the normal diet; the details are described elsewhere.

Animals — Donryu strain rats at 3 weeks of age were purchased and fed the test diets for 4 weeks prior to use in the experiment on metastasis.

Assay of Metastatic Potential — Yoshida sarcoma cells were maintained by intraperitoneal transplantation in inbred Donryu strain rats. A suspension of 10⁴ cells was injected into the iliac vein of a male Donryu rat at 5 weeks of age. After 2 weeks, the number of metastatic foci on the lung surface was determined macroscopically.

Results and Discussion

The number of pulmonary metastatic foci was significantly less in the perilla group than in the safflower group, and also significantly less than in the normal diet group. No statistically significant difference was observed between the safflower group and the normal diet group. The difference in metastatic foci between the perilla group and the safflower group was statistically significant (p < 0.05) (Table I). Two other experiments with rats fed the perilla oil diet and safflower oil diet through two generations showed similar results; the metastatic foci in the perilla group amounted to 60% of those in the safflower group (data not shown).

The difference in the fatty acid compositions of diets was reflected in the fatty acid compositions of plasma lipids and tissue lipids; these results have been reported in part elsewhere. Generally, the proportions of 20:5 n-3 and 22:6 n-3 were higher and those of 18:2 n-6 and 20:4 n-6 were lower in the perilla group as compared with the safflower group. In the platelet phospholipids, the 20:5 n-3/20:4 n-6 ratios were 0.826 and 0.002 in the perilla group and safflower group, respectively.

Previously, we showed that the tumor cells supplemented with 20:5 n-3 or 22:6 n-3 exhibited higher stickiness to a glass surface, lower ability to pass through capillary vessels and a larger number of metastatic foci as compared with controls. In contrast to those results, the metastatic potential of the tumor cells was less in the rats enriched with 20:5 n-3 (Table I). Thus, not only the modification of tumor cells but also the modification of host

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a) Values are means ± S.E. Numbers in parentheses indicate the numbers of rats used. b) Statistically significant differences between the safflower oil group and the perilla oil group (p < 0.05 in Student’s t-test).
animal cells with different fatty acids had a significant influence on the metastatic potential of the tumor cells. The enrichment of n-3 fatty acids in the host cells and tumor cells affected metastasis differently.

The mechanisms inducing such a difference in the metastatic potential of tumor cells can not be easily explained. Since the membrane lipid acyl chains of endothelial cells are known to be modified by dietary fatty acids, eicosanoids formed in these cells are probably different, and might have affected the metastatic potential of tumor cells. Possible differences in platelet aggregability, expected from the changes in platelet fatty acids, might also affect the metastasis.

The present results, however, may have an etiological and practical significance, since one of the major dietary changes in the past 30 years in Japan has been characterized as a decrease in the n-3/n-6 ratio of the dietary fats (data not shown). Although the animal model used in the present experiments may be only partially relevant to the actual metastasis of syngenic tumors, the supplementation of n-3 series fatty acids may have a preventive effect on metastasis of tumor cells in humans. In this context, it seems interesting to note that tumorigenesis and tumor growth are also inhibited by dietary n-3 fatty acids in animal models.

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References and Notes

1) Fatty acids are designated by carbon number: number of double bonds, and the position of the double bond (indicated as n-3 or n-6).