Some Features of the Metastatic Cancer Cells in Prostaglandin Production

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NAKAZAWA, I., IWAIZUMI, M. and OHUCHI, K. *Some Features of the Metastatic Cancer Cells in Prostaglandin Production.* Tohoku J. Exp. Med., 1989, **159** (1), 75-78 — In order to establish metastatic lesions, 2.5×10^6 AH100B cells were injected into the left carotid artery of male Donryu rats. Each metastatic nodule in the liver or kidney, 1 mm or less in diameter, thus obtained was then injected into the peritoneal cavity in which these metastatic cells come to free. About 3 weeks later, each ascites was collected from the rats, while not bloody. Then, cancer cells obtained from each ascites were suspended in Dulbecco's PBS without Ca^{2+} and Mg^{2+} (pH 7.2) after washing. Then, 10^6 metastasized or control cancer cells were incubated in 0.1 ml of PBS mentioned above together with $0.1 \mu\text{Ci}$ of ($1\text{-}^{14}\text{C}$)-AA at 24°C for 3 min, respectively. After the extraction procedure, AA metabolites formed were separated by means of TLC, and each TLC plate was subjected to autoradiography. In the metastasized cells, PG production ability was generally accelerated and especially in that of $\text{PGF}_{2\alpha}$ as compared with that of the control. ——— cancer metastasis; PG production; $\text{PGF}_{2\alpha}$; growth factor; promotor

Metastasis to other organs is one of characteristics of cancer which makes it difficult to treat cancer patients. Therefore, it is desirable to clarify the mechanism of cancer metastasis in order to cure patients. Fidler (1973a) reported that only 1% of B16 melanoma cells injected intravenously had survived to form actual pulmonary metastasis. Fidler (1973b) succeeded also in obtaining the highly metastatic strains of B16 melanoma by means of intravenous injection and tissue culture of the metastatic lesions. Bosmann et al. (1973) recognized the biochemical features of the most highly metastatic strain (F10), that is, the change in the electrophoretic mobility, surface glycoprotein, proteases and increasing degradative enzyme as compared with those of low metastatic B16 melanoma

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Abbreviations: PG, prostaglandin; AA, arachidonic acid; TX, thromboxane; TLC, thin-layer chromatography; PBS, phosphate buffered saline.

strain (F1). Miller et al. (1985) suggested that metastasis of human colonic cancer would be owing to the specific tumor cell phenotypes. Nakazawa et al. (1978) studied lipid-chemical features of metastatic lesions in the liver as compared with that of primary lesions.

In the present work, we deal with some features of the metastatic cancer cells in prostaglandin production.

MATERIALS AND METHODS

Animals. Donryu male rats (Nihon Rat Co., Ltd., Urawa) weighing 100 to 120 g were used. They were fed with a pellet diet, NF (Oriental Yeast Co., Ltd., Tokyo), and tap water freely.

Tumor. AH100B cell kept at the Department of Oncology, the Research Institute for Tuberculosis and Cancer, Tohoku University, was transplanted into the peritoneal cavity of rats. Ascites was collected about 7 days later, while not bloody, and centrifuged at 4°C for 5 min ($700\times g$). Each sediment was then washed three times with 0.9% NaCl solution. Then, 2.5×10^7 cancer cells were suspended in 1 ml of 0.9% NaCl solution and each 0.1 ml of the cell suspension was injected into the left carotid artery of rats retrogressively. The cell suspension was also transplanted intraperitoneally to rats as the control. The rats used for cancer metastatic formation were killed 3 weeks later, after fasting for 16 hr. Metastatic nodules, 1 mm or less, were collected from liver and kidney respectively. Each specimen thus obtained was divided into two parts. One part was provided for pathohistological examination and another was injected with a small amount of 0.9% NaCl solution into the peritoneal cavity of normal rats to make free cancer cells from each nodule, respectively. About 3 weeks later, each ascites was collected from each rat, while not bloody, and centrifuged at 4°C for 5 min ($700\times g$). Each sediment was washed three times with Dulbecco's PBS without Ca^{2+} and Mg^{2+} (pH 7.2, Gibco, Long Island, NY, USA). Further details for obtaining intact metastatic cancer cells have been described elsewhere (Nakazawa and Iwaizumi 1982; 1989).

Experimental procedure. Metastatic cancer cells thus obtained and the control were suspended in Dulbecco's PBS mentioned above at the concentration of 2×10^7 cells per 1 ml. Then, 50 μl of each cancer cell suspension (10^6 cancer cells) was transferred into a glass screw vial (4 ml) with silicon packing cap, and 50 μl of the PBS containing 0.1 μCi of ($1\text{-}^{14}\text{C}$)-AA (New England Nuclear, Boston, MA, USA) was added. After the mixture was incubated at 24°C for 3 min, 0.3 ml of a solution consisting of ethyl acetate, methanol and 1 M solution of citric acid (30 : 4 : 1, v/v) was added to each vial to stop the reaction. Afterwards, 0.5 g of anhydrous Na_2SO_4 was added to each vial and mixed violently using a mixer (Model TM-105, Thermonics, Tokyo), and 50 μl of the supernatant was applied to Silicagel F₂₅₄ glass plate (20 $\times$ 20 cm, Art. 11798, Merck, Darmstadt, FRG). As authentic samples, AA (Merck), 6-keto-PGF_{1 α} , PGF_{2 α} , PGE₂, PGD₂ and TXB₂ (Ono Pharmaceutical Co. Ltd., Osaka) were used. The plate was developed with a supernatant of a mixed solution of ethyl acetate, isooctane, acetic acid and distilled water (90 : 50 : 20 : 100, v/v) at room temperature in a height of 15 cm. After drying up in the atmosphere, the plate was developed again in the same solvent system, in order to separate TXB₂ and PGE₂. Then the plate was exposed to iodine vapor, and positions for authentic standards were marked, respectively. After that, each plate thus obtained was subjected to an autoradiography at 4°C for 2 weeks and each film was developed.

RESULTS AND DISCUSSION

As shown in Fig. 1, a separation of PGE₂ from TXB₂ on the TLC plate was

Fig. 1. Separation between authentic samples used in this study was well. Samp, sample applied to TLC; 1, 6-keto-PGF_{1 α} ; 2, PGF_{2 α} ; 3, TXB₂; 4, PGE₂; 5, PGD₂.

Fig. 2. Autoradiograms obtained from the hepatic metastatic and control cancer cells are presented. Abbreviation: Those of 1 to 5 are same with Fig. 1. 6, an unidentified cyclooxygenase product; 7, AA (substrate); 8, solvent front.

relatively good, as well as that of other authentic samples used in this study. In the autoradiograms, as shown in Fig. 2, PG production ability of metastatic cancer cells was generally accelerated as compared with that of the control cells originally injected into left carotid artery of the rat. That is, PG production ability of the control was almost negligible except for 6-keto-PGF_{1 α} and TXB₂. Above all, PGF_{2 α} production ability of metastatic cancer cells was quite remarkably accelerated as compared with that of the control. PGF_{2 α} is one of the cell growth factors in Swiss 3T3 cells (Macphee et al. 1984), and enhances TPA promotion of skin tumors (Fischer et al. 1980). Thus, it seems that an accelerated PGF_{2 α} production is closely related with cancer metastatic formation. Further study to clarify the causes of the accelerated PGF_{2 α} production in the metastatic cancer cells would be helpful for elucidating the mechanism of cancer metastasis.

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