Short Report

Some Features in Prostaglandin Synthesis of the Cancer Cells Which Metastasized into Liver from Intestinal Cancer Lesions

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NAKAZAWA, I., IWAIZUMI, M. and OHUCHI, K. Some Features in Prostaglandin Synthesis of the Cancer Cells Which Metastasized into Liver from Intestinal Cancer Lesion. Tohoku J. Exp. Med., 1993, 170 (2), 131-133 — In order to study the mechanism of cancer metastasis, AH100B cells, an ascitic hepatoma cell line, were transplanted into the small intestine of male Donryu rats. Each metastatic nodule in the liver was collected with the respective intestinal lesion. Each sample thus obtained was injected into the peritoneal cavity of male Donryu rats to make free cancer cells. Then, the cancer cells, having an intact cell surface, of the metastatic and primary intestinal lesion were collected respectively. After washing in Dolbecco's PBS (Ca²⁺ and Mg²⁺-free, pH 7.2), the definite numbers of cancer cells of the metastatic and primary intestinal lesion were incubated in the PBS containing [1-14C]-AA at 25°C for 30 min, respectively. AA metabolites formed during the incubation period were extracted and subjected to TLC, followed by autoradiography. Each radioactive part was scraped off the plate and measured for its radioactivity. The pattern of the ability to synthesize PGs was different between the cancer cells which metastasized to the liver and those of the primary lesion, that is, percentage values of PGE₂ and PGF₂α were higher (p < 0.01) in the cancer cells which metastasized to liver as compared with those of the primary intestinal lesion. These results suggest that PGs produced by hepatic metastatic cancer cells might play an important role in cancer metastasis.

cancer metastasis; prostaglandin; liver; intestinal cancer

Authors (Nakazawa et al. 1985, 1989, 1991) have reported features of PG synthesis of the metastatic cancer cells, using an animal model in which AH100B (a rat hepatoma cell line) cells were injected into the carotid artery of male Donryu rats.

In this article, we will deal with some features of PGs synthesized by cancer cells which metastasized into liver from the small intestinal cancer lesion.

Materials and methods. As experimental animals, male Donryu rats (Nihon Rat Co. Ltd., Urawa) weighing 100 to 120 g were used and fed with a pellet diet, NF (Oriental Yeast Co. Ltd., Tokyo).

Tumor. AH100B (ascitic hepatoma cell line) cells were obtained from the Department of Oncology, the Research Institute for Tuberculosis and Cancer, Tohoku University. After

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they were treated as described before (Nakazawa et al. 1989), 10^6 cancer cells suspended in 0.05 ml of sterilized physiological saline were transplanted into muscles of the small intestine of rats, respectively. About one month later, they were sacrificed and metastatic nodules sized 1 mm or less in a diameter were collected from the liver with the intestinal tumor tissues. Each sample was pathohistologically examined and determined the ability to synthesize PGs according to the procedure described previously (Nakazawa et al. 1991). Each tissue of the metastatic and primary lesion which was used for studying PG synthesis was injected into the peritoneal cavity of the rats to make free intact cancer cells respectively. The nature of cell surface would be closely related with cancer metastasis. Further details for obtaining intact free cancer cells were described elsewhere (Nakazawa and Iwaizumi 1982).

**Experimental procedure.** Cancer cells which metastasized into the liver and of the intestinal lesion, obtained by the treatment mentioned above, were suspended in Dulbecco’s PBS (Ca^{2+} and Mg^{2+}-free, pH 7.2, Gibco, Long Island, NY, USA) at 4 x 10^6 cancer cells per 1 ml. Then, each cell suspension containing 0.2 μCi of [1-14C]-AA (New England Nuclear, Boston, MA, USA) and incubated at 25°C for 30 min. Then, PGs formed during the incubation were extracted and separated by means of TLC as described before (Nakazawa et al. 1989). As authentic standard samples, AA (Merck, Germany), PGF_{2α}, 6-keto-PGF_{1α}, PGE_{2}, PGD_{2}, and TXB_{2} (Ono Pharmaceutical Co. Ltd., Osaka), 5 μg each per plate, were used. TLC plates were subjected to autoradiography at 4°C for 2 weeks. After radioactive parts, including those corresponding to authentic standards, were scraped off the plate separately, each radioactivity was measured by means of a liquid scintillation counter (LSC 1000, Aloca Co. Ltd., Tokyo) as described previously (Nakazawa et al. 1991).

**Results and discussion.** Conversion of [1-14C]-AA into each PG was examined by incubating the cells at 25°C for 15, 30 and 60 min. Since the conversion rate showed a plateau at 30 min, the following experiments were performed by incubating the cells for 30 min at 25°C. In the incubation for 30 min, more than 50% of [1-14C]-AA was metabolized. Table 1 shows percentages of radioactivity of 6-keto-PGF_{1α}, PGF_{2α}, TXB_{2}, PGE_{2} and PGD_{2} synthesized by the cancer cells which metastasized into liver and the cancer cells of the intestinal tumor. The percentage was calculated as follows:

\[
\text{Percentage} = \frac{\text{radioactivity of each spot recovered from the TLC plate}}{\text{total radioactivity recovered from the TLC plate}}
\]

The percentage of PGF_{2α} or PGE_{2} in the cancer cells which metastasized into the liver was significantly higher than the corresponding percentage of the cancer cells of the intestinal

| Table 1. Pattern of prostaglandin synthesis of the cancer cells in the hepatic metastatic and primary intestinal lesions (AH100B, Percentage\(^a\)) |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Cancer cells metastasized to liver (n = 5)\(^b\) | Cancer cells transplanted to intestine (n = 5) | \(p\)\(^c\) |
| 6-keto-PGF\(_{1α}\) | 2.54 ± 0.12% d | 2.59 ± 0.21 | n.s. |
| PGF\(_{2α}\) | 3.67 ± 0.55 | 0.77 ± 0.13 | 0.01 |
| TXB\(_2\) | 16.34 ± 2.96 | 13.59 ± 0.69 | n.s. |
| PGE\(_2\) | 2.61 ± 0.56 | 0.59 ± 0.11 | 0.01 |
| PGD\(_2\) | 1.44 ± 0.42 | 1.73 ± 0.61 | n.s. |

\(^a\)Percentage was calculated as described in the text. The total radioactivity recovered from the TLC plate was not significantly different between the cancer cells metastasized to liver (38,700 ± 3,600 dpm) and the primary lesion (32,500 ± 3,900 dpm, mean ± s.d.).

\(^b\)n represents numbers of samples assayed.

\(^c\)p values were not significant.

\(^d\)mean ± s.d.
tumor. PGE\(_2\) has broad kinds of biological activities. Above all, the immunosuppressive action would be the most important for cancer metastatic formation. The immunosuppressive state frequently observed in tumor bearers was reported to be due to PGE\(_2\) secreted by tumor cells and the host macrophages acting to suppress T-lymphocytes and NK activities (Jessup et al. 1985; Young et al. 1986). In addition, NK cells are important for limiting cancer metastatic formation (Wiltrout et al. 1985). PGF\(_2\alpha\) also has many kinds of biological activities. That is, PGF\(_2\alpha\) is one of competence factors in Swiss 3T3 cells (Macphee et al. 1984; Macara 1985) and enhances epidermal growth factor (EGF)-stimulated expression of c-myc in Balb/c 3T3 fibroblasts (Handler et al. 1990). The results obtained here shows that cancer cells which metastasized into liver from intestinal cancer lesions can produce PGs in a way different from those of intestinal primary lesions, and suggest that PGs produced by the cancer cells which metastasized might play some important roles in cancer metastatic formation. A further study tackling the problem of PGs receptors, however, is necessary to clarify the role of PGs produced by the metastatic cancer cells in cancer metastatic formation.

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References


