A Difference in Prostaglandin-producing Ability between Cancer Cells Metastasized into Liver and Kidney

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NAKAZAWA, I., IWAIZUMI, M. and OHUCHI, K. A Difference in Prostaglandin-producing Ability between Cancer Cells Metastasized into Liver and Kidney. Tohoku J. Exp. Med., 1991, 165 (4), 299-304 — In order to study the mechanism of cancer metastasis AH100B cells, a rat hepatoma cell line, were injected into the left carotid artery of male Donryu rats to form metastatic lesions. Each metastatic nodule in the liver and kidney was collected and injected into the peritoneal cavity of normal rats. About 3 weeks later, intact metastatic cancer cells were collected from each ascites that was not bloody. After washing in Dulbecco's phosphate-buffered saline (PBS, Ca²⁺ and Mg²⁺-free, pH 7.2), 1 x 10⁶ cancer cells were incubated in the PBS containing [1-¹⁴C]-arachidonic acid (AA) at 24°C for 5 min. AA metabolites formed during the incubation period were extracted and subjected to thin layer chromatography, followed by autoradiography. Each radioactive spot was scraped off the plate and its radioactivity was measured. In the cancer cells which metastasized to the liver, the ability to produce prostaglandin (PG) E₂ was higher (p < 0.05) but those to produce PGF₂α and 6-keto-PGF₁α were lower (p < 0.01) than in the cancer cells which metastasized to the kidney. These results suggest that cancer cells metastasizing to the liver and the kidney are different from each other in the ability to produce PG. ——— cancer metastasis；PG production；liver；kidney

Fidler showed that only 1% of B16 melanoma cells injected intravenously had survived to form actual pulmonary metastasis (1973a), and succeeded in obtaining highly metastatic strains of B16 melanoma (1973b). Bosmann et al. (1973) clarified the biochemical features of the most highly metastatic cell line (F10). Nakazawa et al. (1978) studied chemical features of lipids of metastatic lesions in the human liver and noticed a difference in the phospholipid fatty acid

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Abbreviations: PG, prostaglandin; AA, arachidonic acid; TX, thromboxane; PBS, phosphate-buffered saline; EGF, epidermal growth factor.
*Address for reprints: Dr. Ichiro Nakazawa, The Third Department of Internal Medicine, Tohoku University School of Medicine, Seiryo-machi, Aobaku, Sendai 980, Japan.
Nakazawa and Goto (1978) recognized that the percentage of arachidonic acid in phospholipid fatty acid composition of the cancer cells metastasized to the liver was much higher than that of the original cancer cells (AH100B strain). Furthermore, the same authors (1981) recognized that the percentage of polyunsaturated fatty acids such as linoleic acid and arachidonic acid in phospholipids of the cancer cells metastasized to the liver was higher than that of the original cancer cells (AH66F strain, a rat hepatoma cell line). Then, Nakazawa et al. (1985) studied a role of arachidonic acid which was increased in the metastatic cancer cells and recognized that prostagrandin (PG) E$_2$, synthesis in AH100B cancer cells which metastasized to the liver was much more accelerated as compared with that of the cells which metastasized into the kidney, when incubated in Eagle's minimal essential medium with 10% calf serum at 37°C for 24 hr. Furthermore, Nakazawa et al. (1989) reported that the cancer cells (AH100B strain) metastasizing to the liver have a quite higher ability to metabolize [1-$^{14}$C]-arachidonic acid (AA) into PGF$_{2\alpha}$ than the control cancer cells. The present work was intended to clarify whether there is a difference in the ability to produce arachidonic metabolites between the cancer cells which metastasized to the liver and the kidney, and to gain further insight into the mechanism of cancer metastasis from the view point of AA metabolism.

In this paper, we will describe that the ability to produce PGs such as PGE$_2$, PGF$_{2\alpha}$, and 6-keto-PGF$_{1\alpha}$ of the cancer cells metastasizing to the liver is different from that of the cancer cells metastasizing to the kidney.

**Materials and Methods**

*Animals.* Male Donryu rats (Nihon Rat Co., Ltd., Urawa) weighing 100 to 120 g were used in this experiment. They were fed on a pellet diet NF (Oriental Yeast Co., Ltd., Tokyo).

*Tumor.* The AH100B rat hepatoma cell line was obtained from the Department of Oncology, the Research Institute for Tuberculosis and Cancer, Tohoku University. AH100B cells were transplanted to rats intraperitoneally. About 1 week later, ascites was collected from each rat and centrifuged at 1,600 x g for 5 min at 4°C under sterile conditions. Sedimented cancer cells were washed three times with a sterilized physiological saline. Then, 1 x 10$^7$ cancer cells were suspended in 1 ml of the saline solution and 0.1 ml of the cell suspension was injected into the left carotid artery of rats, which were sacrificed about 3 weeks later, after fasting for 16 hr. White small metastatic nodules, diameter of which was 1 mm or less, were recognized sporadically on the surface of liver and kidney of the rats. Then, ten metastatic nodules each were collected from the liver and kidney. Each specimen thus obtained was divided into two parts. One part was provided for patho-histological examination and the other half was injected into the peritoneal cavity of normal rats to make free cancer cells from each metastatic nodule. Further details for obtaining intact metastatic cancer cells have been described elsewhere (Nakazawa and Iwaizumi 1982, 1989; Nakazawa et al. 1989).

*Measurements of PG production.* Metastatic cancer cells were suspended in 500 µl of Dulbecco's phosphate-buffered saline (Ca$^{2+}$ and Mg$^{2+}$-free, pH 7.2, Gibco, Long Island, NY, USA) at 2 x 10$^7$ cancer cells/ml. Then, each cell suspension containing 1 x 10$^6$ cancer cells
was mixed with 50 μl of the PBS containing 0.1 μCi of [1-14C]-AA (New England Nuclear, Boston, MA, USA) and incubated at 24°C for 5 min. After the incubation, PGs formed were extracted and separated by means of thin layer chromatography (TLC) as described previously (Nakazawa et al. 1989). As authentic samples, AA (Merk, Germany), 6-keto-PGF1α, PGF2α, PGE2, PGD2 and thromboxane (TX) B2 (Ono Pharmaceutical Co. Ltd., Osaka), 5 μg each per plate, were used. TLC plates were subjected to autoradiography at 4°C for 2 weeks. Then, radioactive spots corresponding to authentic standards and also other radioactive parts were separately scraped off the plate and transferred into counting vials (Wheaton, NY, USA). After addition of 0.5 ml of ethyl acetate and 10 ml of Aquasol 2 (New England Nuclear, Boston, USA) to each vial, radioactivity was measured using a liquid scintillation counter (LSCT 1000, Aloca Co., Ltd., Tokyo).

RESULTS

In Table 1, radioactivities of 6-keto-PGF1α, PGF2α, TXB2, PGE2 and PGD2 synthesized by the cancer cells which metastasized to liver and kidney are shown.

| TABLE 1. Prostaglandin producing ability of metastatic cancer cells in AH100B (radioactivity, dpm) |
|--------------------------------------------------|--------------------------------------------------|
| Cancer cells metastasized to                     |                                                  |
| Liver (n = 3)a                                   | Kidney (n = 3)                                   |
| 6-keto-PGF1α                                     | 931 ± 66                                         |
| PGF2α                                            | 920 ± 142                                        |
| TXB2                                             | 2612 ± 297                                       |
| PGE2                                             | 1092 ± 103                                       |
| PGD2                                             | 1079 ± 290                                       |
| 2561 ± 452 dpmb                                  | 1826 ± 330                                       |
| 3216 ± 369                                       | 753 ± 76                                         |
| 996 ± 262                                        |                                                  |

*a n represents numbers of samples examined.

b Mean ± s.d.

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<th>TABLE 2. Pattern of prostaglandin producing ability of metastatic AH100B cancer cells (percentage)*</th>
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<tr>
<td>Cancer cells metastasized to</td>
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<tr>
<td>Liver (n = 3)b</td>
</tr>
<tr>
<td>6-keto-PGF1α</td>
</tr>
<tr>
<td>PGF2α</td>
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<tr>
<td>TXB2</td>
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<td>PGE2</td>
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<td>11.73 ± 2.07</td>
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<td>14.73 ± 1.69</td>
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*a Percentage was calculated as described in the text. The total radioactivity recovered from the TLC plate was not significantly different between in the cancer cells which metastasized to liver (21,800 ± 460 dpm) and to kidney (22,000 ± 720 dpm, mean ± s.d).

b n represents numbers of samples examined.

*Mean ± s.d.

c n.s., not significant (Student’s t-test).
Table 2 shows percentage of radioactivity of 6-keto-PGF\(_{1\alpha}\), PGF\(_{2\alpha}\), TXB\(_2\), PGE\(_2\) and PGD\(_2\) synthesized by the metastatic cancer cells mentioned above. 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 thus calculated represents the relative ability to synthesize respective PGs in the metastatic cancer cells. The percentage of PGE\(_2\) in the cancer cells which metastasized to the liver was higher than that of the cancer cells which metastasized to the kidney \((p < 0.05)\), whereas those of PGF\(_{2\alpha}\) and 6-keto-PGF\(_{1\alpha}\) in the cancer cells which metastasized to the kidney were remarkably higher than those of the cancer cells which metastasized to the liver \((p < 0.01)\). The control cancer cells produced only negligible amounts of PGs under the experimental conditions as stated before (Nakazawa et al. 1989).

**DISCUSSION**

Cancer metastasis is one of the characteristic behaviors of the cancer cells. It is thus important to study the in vivo characteristics of the metastatic cancer cells in order to clarify the mechanism of the cancer metastasis. From this standpoint, the characteristics that the tumor cells are easily changed to an ascitic form would be an advantage for the study of the solid tumors. Thus, AH100B ascitic cancer cell line was used as the experimental model in this study. Since the cell surface of cancer cells has an important role in metastasis, the metastatic cancer cells were isolated in the abdominal cavity of rats where AH100B cells had been maintained by means of repeated transplantation; this procedure allows to obtain intact metastatic cancer cells.

As reported previously (Nakazawa et al. 1989), the synthesis of PGs in the cancer cells metastasized to the liver was markedly accelerated as compared with that of the control. Furthermore, in this study, the difference in prostaglandin synthesis was observed between the cancer cells which metastasized to the liver and kidney. That is, PGE\(_2\) production was more accelerated in the cancer cells which metastasized to the liver than those to the kidney, while production of PGF\(_{2\alpha}\) and 6-keto-PGF\(_{1\alpha}\) in the cancer cells which metastasized to the liver was lower as compared with that of those to the kidney. PGE\(_2\) has many kinds of biological activities. Above all, the immunosuppressive action is important for cancer metastasis. In tumor bearers, the immunosuppression frequently occurs and the macrophages act to suppress T-lymphocytes and NK activities (Jessup et al. 1985; Young et al. 1986). An elevated secretion of PGE\(_2\) is associated with this macrophage-mediated suppression (Glaser 1980; Young et al. 1986). In addition, NK cells are important in limiting tumor metastatic formation (Wiltrout et al. 1985). On the other hand, PGF\(_{2\alpha}\) is one of competence factors in Swiss 3T3 cells (Macphee et al. 1984; Macara 1985) and cooperates with a promoter effect of
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12-O-tetradecanoylphorbol 13-acetate in the carcinogenesis of mouse skin (Fischer et al. 1980). Handler et al. (1990) reported that epidermal growth factor (EGF)-stimulated expression of c-myc in BALB/c 3T3 fibroblasts was enhanced by PGF2α in a concentration dependent manner up to 600% of the levels observed in the presence of EGF alone. Furthermore, several investigators demonstrated the antimetastatic effects of PGI2 (Honn et al. 1981; Honn 1983). As shown in Tables 1 and 2, different abilities to synthesize PGs were recognized between the cancer cells which metastasized into liver and kidney. These results suggest that the metastatic cancer cells are heterogenous in prostaglandin production. In addition, it would be suggested that the metastatic cancer cells are heterogenous in a metastatic ability.

Further study is necessary to clarify the role of prostaglandins produced by the metastatic cancer cells in the cancer metastatic formation.

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References


