Human Esophageal Carcinoma Cell Lines: Prostaglandin Production, Biological Properties, and Behavior in Nude Mice

Julia H. Botha,4,5 Kathy M. Robinson,6 Nirasha Ramchurren,4 Kogie Reddi,7 and Robert J. Norman7,8

ABSTRACT—Prostaglandin production by two continuous human esophageal carcinoma cell lines HCU 18 and HCU 39 derived from poorly and moderately differentiated source tumors, respectively, was investigated. Behavior of both lines in vitro and upon s.c. inoculation into athymic random bred BALB/c nude mice was also assessed. Approximately half the xenografts induced by HCU 18 cells were invasive, whereas those initiated by HCU 39 cells were all well encapsulated. Although metastases were not detected in mice given injections of HCU 39 cells, metastatic tumors developed in 2 mice inoculated with HCU 18 cells. In addition, HCU 18 cells produced significantly more prostaglandin E (PGE) and prostaglandin F (PGF) than HCU 39 cells. These findings suggest a relationship between PGE and PGF production by human esophageal carcinoma cells and their invasive and metastatic potential in athymic mice.—JNCI 1986; 76:1053-1056.

Although PGE, particularly those of the E-series, have been shown to be elevated in various human and experimental tumors, the exact biological significance of this observation remains obscure. Although there have been suggestions that increased PG synthesis represents part of a homeostatic response directed toward limitation of tumor growth, other workers propose that PG are involved in the initiation and enhancement of growth (1). Certainly, there is evidence that PG may play a role in invasion and metastasis of malignant cells: Huang has reported results of studies that suggest that PGE2 (possibly derived from tumor cells) may be a necessary intermediate step in stimulating tumor stroma to produce proteolytic enzymes important in the breakdown of host connective tissues, thus creating a route for tumor cell invasion (2). Furthermore, in one investigation of human breast cancers, findings indicated a relationship between low PG production by the tumor and “walling in” of tumor cells by dense connective tissue. In the same study high PG production was associated with the presence of neoplastic cells in tumor lymphatic and blood vessels and in axillary lymph nodes. In addition, greater amounts of PG were produced by node metastases than by primary tumor sites (3).

Although there is considerable further evidence (particularly in the case of breast cancer) of a link between PG synthesis and the formation of metastases (1, 4–6), not all findings support such a relationship: For example, Kibbey et al. found in a series of rat mammary carcinomas that the only metastatic tumor contained much less PGE2 and PG synthetase than 3 other tumors, none of which metastasized (7). Although experiments with B16 malignant melanoma cell lines have also indicated that the highly metastatic line B16F10 forms less PGD2 than the moderately metastatic parent line B16F1, this finding may be related to the fact that PGD2 inhibits platelet aggregation, which may be one of the factors involved in B16 metastasis (1). Different PG do appear to vary in their influence on metastasis; e.g., when B16 amelanotic melanoma cells were injected into a syngeneic host, while PGE2 and F2a were both ineffective in reducing metastasis, PGF2 and D2 (both inhibitors of platelet aggregation) were antimetastatic (8).

Since PG synthesis by esophageal carcinoma cells has not previously been reported, this study aimed: a) to measure PGE and PGF synthesis by two continuous human esophageal carcinoma cell lines. b) to compare PG production with 1) the biological properties of the lines and of the source tumors and 2) invasiveness and metastatic potential of the lines in athymic nude mice, the nude mouse human tumor xenograft system providing a useful model in which tumor growth and behavior can be studied (9).

MATERIALS AND METHODS

The continuous human esophageal carcinoma cell lines HCU 18 and HCU 39 were derived as described by Robinson et al. (10) from poorly and moderately differentiated invasive squamous carcinomas of the esophageal carcinoma cells and their invasive and metastatic potential in athymic mice.

ABBREVIATIONS USED: EMEM=Eagle’s minimum essential medium; PA=plasminogen activator; PG=prostaglandin(s); PGE=prostaglandin E; PGF=prostaglandin F; PGFM=13,14-dihydro-15-keto-PGF2; sEMEM=EMEM supplemented with 10% fetal calf serum and antibiotics.

1 Received July 29, 1985; revised December 18, 1985; accepted February 18, 1986.
2 Supported by grants from the National Cancer Association of South Africa.
3 Animals were maintained under the guidelines set forth by the South African Association of Laboratory Animal Science.
4 Department of Pharmacology, University of Natal, South Africa.
5 Address reprint requests to Dr. Botha, Department of Pharmacology, University of Natal, P. O. Box 17059, Congella 4013, South Africa.
6 Department of Physiology, University of Natal, South Africa.
7 Department of Chemical Pathology and Medical Research Council Preclinical Diagnostic Research Unit, University of Natal, South Africa.
8 Prostaglandins were provided by Dr. J. Pike, Upjohn, Kalamazoo, MI.
demonstrated a 10% cross-reactivity with PGF\textsubscript{1a} (13, 14). Accordingly, results are reported as PGE and PGF, and for the compounds measured (anti-PGE antisera: EI00%, described previously (13). The anti-PGE\textsubscript{2} antibody has a 20% cross-reactivity with PGE\textsubscript{1} at 50% inhibition of maximum binding. Similarly, the anti-PGF\textsubscript{2α} antibody demonstrated a 10% cross-reactivity with PGF\textsubscript{1a} (13, 14). Accordingly, results are reported as PGE and PGF, and no distinction between the 1- and 2-series is drawn. To calculate the amounts of PG released into sEMEM by cells, control results obtained for sEMEM not exposed to cells were subtracted from all values. Cells of each of the two lines were at similar densities at the time of assay. However, to prevent any possible influence of difference in cell size and number on the results, the protein content of the cells (after they had been removed from the flasks and lysed osmotically with 0.1% KCl) was determined by the method of Lowry et al. (15). The PGE and PGF released into the medium by cells were then expressed as nanograms per milligram protein per 24 hours. Ten and nine pairs of flasks were assessed for PGE and PGF, respectively.

To determine tumorigenicity in athymic nude mice (16), we removed healthy cells of each of the lines by trypsinization from the flasks in which they were growing. Approximately 2×10\textsuperscript{6} cells in 0.25 ml EMEM were inoculated sc into the suprascapular regions of 6-week-old nu/nu mice of both sexes (50 mice received HCU 18 cells and 22 mice, HCU 39). All mice were of BALB/c origin, back crossed once to Ha 1cr, and randomly bred. Mice were maintained in a specific-pathogen-free-4 environment and observed twice weekly for up to 9 months post inoculation. Any growth that appeared at the inoculation site and increased in size was designated a tumor. To identify any metastatic tumor depots, we performed full autopsies on all mice and further examined histopathologically any enlarged lymph nodes or other organs showing abnormalities. Confirmation of malignancy of both primary tumors and metastases was obtained by both light and electron microscopy following tumor removal.

The intracellular PA content of each of the lines as measured by the method of Halfpaje et al. (17) has been reported previously (18).

RESULTS

Mean values of PGE and PGF released into the medium by the two different esophageal cell lines are presented in table 1. Cells of the HCU 18 line produced more of both PG than did HCU 39 cells. Analysis of results by the Wilcoxon matched-pairs signed-ranks test showed the difference to be significant at a level of \(P<0.02\) and \(P<0.05\) for PGE and PGF, respectively, on a two-tailed test. A paired statistical analysis was performed to allow for possible slight day-to-day variations in the procedures, since it has been reported that the synthetic activity of a number of mammary tumor lines decreases with time in culture (6) and that PG production by macrophages is sensitive to the concentration of fetal calf serum in the culture medium (19). Properties of the two cell lines as well as details of behavior in the nude mouse can be seen in table 2. Although the number of mice that developed tumors is small due to the low take rates for both cell lines, differences were detected between the two lines with respect to invasiveness and metastatic ability. No primary tumors were observed in mice that developed metastases, and the secondary deposits were found in lymphatics, lymph nodes, liver, spleen, and kidney.
DISCUSSION

Some notable differences between the two esophageal cell lines were observed in terms of biological properties, behavior in the nude mouse, and ability to produce PGE and PGF.

A significantly greater amount of both PGE and PGF was produced by the line derived from the less differentiated tumor, which may suggest some relationship between degree of differentiation and PG production in carcinoma of the esophagus. Despite the difference in PG production by the two lines, they grew at similar rates in vitro. Although various workers have shown that addition of exogenous PG in experimental concentrations can influence growth of cells in culture (1), it is probable that the small differences observed in endogenous production between the lines in the present study were insufficient to affect in vitro growth rate.

Almost half the tumor xenografts induced in athymic mice by inoculation of cells of the HCU 18 line (which produced the larger amount of PG) were invasive in contrast to those initiated by HCU 39 cells, which were all well encapsulated. This finding supports the suggestion that the amount of PG produced may be related to the ability of a tumor to invade surrounding tissue (2, 3). Huang has proposed that such a role may involve stimulation of proteolytic enzyme production by tumor stromal connective tissue (2). Although no relationship was found in the present study between PG and PA production, PA measured was that released from tumor cells and not from connective tissue stroma.

The ability of the HCU 18 line to metastasize in nude mice was unexpected since xenografts, even those from metastatic tumors of origin, generally do not metastasize in this model (20). This is particularly so for esophageal carcinoma where no other incidence of metastasis in the nude mouse has been reported either in this laboratory in a study involving 256 mice (21) or at other institutions (22, 23). Metastatic invasion of kidney, liver, and spleen was also unusual in that the lungs, a site frequently involved in most other metastatic tumors in the nude mouse (24, 25), were unaffected. The fact that the HCU 18 line is derived from a poorly differentiated tumor may be related to its metastatic ability inasmuch as most tumors that have metastasized in nude mice have been derived from poorly differentiated tumors (25). Since not all such tumors metastasize (25), it is possible that some other factor such as production of PG may also be involved. Certainly, the metastatic line HCU 18 produced significantly more PGE and PGF than did HCU 39 cells. In a study of the relative PGE-synthesizing ability of a number of mammary tumor lines cultured in vitro, Fulton found metastatic tumor lines to produce more PGE than those that were nonmetastatic (6). There is, in addition, further evidence supporting an association between PGE and PGF production by the primary tumor and its metastatic ability, particularly in the case of skeletal metastases in breast cancer (1, 4, 5), although the mechanism by which PG from a primary tumor may influence metastasis remains obscure. Since almost 70% of an infusion of PGE is inactivated on a single passage through the lungs, it seems

TABLE 1.—PGE and PGF (ng) released into the surrounding medium by HCU 18 and HCU 39 cells (mg protein) in 24 hr comparing HCU 18 to HCU 39

<table>
<thead>
<tr>
<th>PG type</th>
<th>Mean difference within pairs (No. of pairs)</th>
<th>PG released by cells into the medium, ng/mg protein/24 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HCU 18&gt;HCU 39</td>
<td>HCU 39&gt;HCU 18</td>
</tr>
<tr>
<td>PGE&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.20 (9)</td>
<td>0.75 (1)</td>
</tr>
<tr>
<td>PGE&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.69 (6)</td>
<td>0.15 (3)</td>
</tr>
<tr>
<td>PGF&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.96 (1)</td>
<td>0.15 (3)</td>
</tr>
<tr>
<td>PGF&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.89 (3)</td>
<td>0.15 (3)</td>
</tr>
</tbody>
</table>

<sup>a</sup> P.<sub>.02</sub> (10 expts).
<sup>b</sup> P.<sub>.05</sub> (9 expts).

The ability of the HCU 18 line to metastasize in nude mice was unexpected since xenografts, even those from metastatic tumors of origin, generally do not metastasize in this model (20). This is particularly so for esophageal carcinoma where no other incidence of metastasis in the nude mouse has been reported either in this laboratory in a study involving 256 mice (21) or at other institutions (22, 23). Metastatic invasion of kidney, liver, and spleen was also unusual in that the lungs, a site frequently involved in most other metastatic tumors in the nude mouse (24, 25), were unaffected. The fact that the HCU 18 line is derived from a poorly differentiated tumor may be related to its metastatic ability inasmuch as most tumors that have metastasized in nude mice have been derived from poorly differentiated tumors (25). Since not all such tumors metastasize (25), it is possible that some other factor such as production of PG may also be involved. Certainly, the metastatic line HCU 18 produced significantly more PGE and PGF than did HCU 39 cells. In a study of the relative PGE-synthesizing ability of a number of mammary tumor lines cultured in vitro, Fulton found metastatic tumor lines to produce more PGE than those that were nonmetastatic (6). There is, in addition, further evidence supporting an association between PGE and PGF production by the primary tumor and its metastatic ability, particularly in the case of skeletal metastases in breast cancer (1, 4, 5), although the mechanism by which PG from a primary tumor may influence metastasis remains obscure. Since almost 70% of an infusion of PGE is inactivated on a single passage through the lungs, it seems

TABLE 2.—Characteristics of source tumor, in vitro characteristics, and behavior in nude mice of esophageal carcinoma cell lines

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>HCU 18</th>
<th>HCU 39</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor</td>
<td>Moderate</td>
<td></td>
</tr>
<tr>
<td>Celiac nodes, liver</td>
<td>Celiac nodes</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>Moderate</td>
<td></td>
</tr>
<tr>
<td>9.7</td>
<td>10.25</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>2.5-4</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Poor-well</td>
<td>Well</td>
<td></td>
</tr>
<tr>
<td>5/12</td>
<td>0/5</td>
<td></td>
</tr>
<tr>
<td>2/50</td>
<td>0/22</td>
<td></td>
</tr>
</tbody>
</table>
unlikely that PGE and PGF released from a primary tumor could affect the metastatic site directly (4). In addition, in the present study no primary tumor was detected in the mice in which metastases were observed. In the nude mouse model a well-developed primary tumor is not always essential for metastatic spread since increased incidence of metastasis has been reported following removal of primary tumors (26). An alternative possibility is that PG released from malignant cells present at the metastatic site may help to establish metastases. In a study involving an ascites hepatoma cell line in DONRYU rats, Nakazawa et al. found that cells metastasizing to the liver produced significantly more PGE than those that metastasized to the kidney, indicating that PGE-producing capacity of metastatic cells may be related not only to the mechanism but also to the site of metastasis (27). In the present study, the cell line that produced the larger amount of PGE and PGF (HCU 18) was that derived from a tumor that originated in a patient who exhibited liver metastases. In summary, the findings of this study indicate that the capacity of human esophageal carcinoma cells grown in vitro to produce PGE and PGF is correlated with their invasive and metastatic potential when inoculated in athymic nude mice. Such observations support the proposals of other authors that PG play a part in the invasion and metastasis of malignant cells.

REFERENCES

(9) ROBINSON KM, MAISTRY L. Tumorigenicity and other properties of cells from ten continuous human esophageal carcinoma cell lines in nude mice. JNCI 1983; 70:89-95.