Differences in the Peripheries of Walker Cancer Cells Growing in Different Sites in the Rat

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ABSTRACT

Walker 256 cancer cells growing in the ascitic form and following direct injection in the livers and in s.c. sites in rats had significantly higher anodic electrophoretic mobilities than did cells derived from the same source but growing in kidneys and spleens. Following incubation with neuraminidase, the cancer cells from the kidneys and spleen lost significantly less net surface negativity than did cells growing in the other 3 sites. These kidney- and spleen-associated differences were not demonstrably due to preexisting, electrophoretic subpopulations of cancer cells within the original ascites tumor; they were maintained on organ-to-organ passage and were reversed on reconstitution of the tumors to ascitic form. The evidence favors cell-induced modulation to account for the differences between primary cancers and their metastases are conceivably due to site-induced modulation as distinct from preexisting metastatic subpopulations.

Evidence which will be discussed later suggests that the cancer cells in some primary tumors are different from those in their metastases in at least some organs. The nonexclusive possibilities arise of whether these organs were selectively seeded by preexisting subpopulations of cancer cells from within the primary tumor or whether the seeding was random. In either case, the cells in the metastases were different because they were located in specific metastatic sites.

INTRODUCTION

In this communication, we describe reversible changes in the net surface charge of Walker 256 cancer cells growing at different sites in rats. It will be shown that our observations support the concept of site-induced modulation of the surfaces of these cancer cells.

MATERIALS AND METHODS

Animal Inoculation. Ether-anesthetized rats (young, adult femaleSprague-Dawley, weighing 150 to 200 g) received 106 Walker 256 'carcinosarcoma.(W-256) cells by i.p. injection. Ascitic fluid was harvested after 7 days and washed once in calcium-magnesium-free Dulbecco's PBS3 (pH 7.2), and erythrocytes were largely removed by hypotonic lysis. In this procedure, 1 part of ascitic fluid was added to 3 parts of half-strength HBSS and centrifuged for 1 min at 100 x g. The washing was repeated a total of 4 times in a maximum of 10 min, and the remaining cells were washed and resuspended in isotonic HBSS. Compared with cells treated with isotonic HBSS alone, hypotonic lysis changes neither the viabilities (32) nor the electrophoretic mobilities of the W-256 cells.

Cells were suspended in HBSS at a concentration of 107 trypan blue-excluding cells/μl. By direct injections through 25-gauge needles, anesthetized animals received 106 dye-excluding cancer cells in a total volume of 0.1 ml. Each animal received an injection into 1 organ (or lobe of the liver) only, and its abdominal cavity was closed in 2 layers with nylon sutures.

Cell Preparation. After 7 days, the animals were exsanguinated by decapitation, and the appropriate organs were removed. The tumors were measured, trimmed free of necrotic regions, minced with scissors, and squeezed through 80 mesh stainless steel gauze. The resulting suspension was washed once, resuspended in HBSS, and then passed through 200 mesh gauze. The resulting single cell suspensions were used for injections into fresh animals or were incubated at 37° for 30 min in T-flasks, during which time most of the macrophages present adhered to the T-flask surface. The suspensions were divided into 2 x 2 ml volumes containing approximately 3 x 106 total cells/ml. To 1 tube was added 1 unit of neuraminidase (Grand Island Biological Co., Grand Island, N. Y.) per 106 cells; the other tube received an equal volume of Dulbecco's PBS. After 30 min incubation at 37°, the cells were washed 3 times and resuspended in Dulbecco's PBS to give final volumes of 10 ml for cell electrophoresis.

Cell Electrophoresis. Electrophoretic mobilities were measured with the cells suspended in HBSS in a cylindrical tube apparatus at 37°. A voltage was applied through gray sintered platinum electrodes and reversed after each cell transit. Human erythrocytes were used as standard particles (—1.38 μm·sec−1·V−1·cm) in each group of experiments.

RESULTS

Electrophoretic Mobilities of Cells from Different Sites. The results are summarized in Table 1. Compared (by means of Student's t test) with the original ascites form, cells obtained from all other 4 sites are different at the 1% (or less) level of significance. This reflects the large numbers of observations. However, if the more usual criteria are applied, i.e., that differences of 3% or less are not meaningful, then only the mobilities of the cancer cells obtained from the kidney and spleen are different from the parent ascites cells.

Effects of Neuraminidase on Electrophoretic Mobilities. The neuraminidase-induced changes in electrophoretic mobilities of the cells obtained from the 5 different sites and shown in Table 1, reveal comparatively minor reductions in those from peritoneal, s.c., and liver sites. The enzyme-induced reductions in cells from kidneys and spleens were substantially different from the parent ascites form, whereas those obtained from s.c.

1 This work was partially supported by Grant CA-17609-04 from The National Cancer Institute, NIH.
2 To whom requests for reprints should be addressed.
3 The abbreviations used are: Dulbecco's PBS, Dulbecco's phosphate-buffered saline; HBSS, Hanks' balanced salt solution.

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Table 1

Electrophoretic mobilities of cells from a common ascites source, growing in the different sites indicated

The reductions in mobilities brought about by incubation with neuraminidase are shown.

<table>
<thead>
<tr>
<th>Site</th>
<th>Controls (μm·sec⁻¹·V⁻¹·cm)</th>
<th>Neuraminidase-induced change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascites form (% ascites control)</td>
<td>-1.20 ± 0.01⁺ (868)</td>
<td>-0.87 ± 0.01 (840)</td>
</tr>
<tr>
<td>s.c. (% ascites control)</td>
<td>-1.16 ± 0.01 (300)</td>
<td>-0.90 ± 0.01 (300)</td>
</tr>
<tr>
<td>Liver (% ascites control)</td>
<td>-1.17 ± 0.01 (250)</td>
<td>-0.88 ± 0.01 (250)</td>
</tr>
<tr>
<td>Kidney (% ascites control)</td>
<td>-1.12 ± 0.01 (438)</td>
<td>-1.03 ± 0.01 (432)</td>
</tr>
<tr>
<td>Spleen (% ascites control)</td>
<td>-1.06 ± 0.01 (450)</td>
<td>-0.92 ± 0.01 (462)</td>
</tr>
</tbody>
</table>

⁺ Mean ± S. E.
⁻ Numbers in parentheses, number of observations.

Table 2

Mean diameters of W-256 tumors growing in the sites indicated 7 days after the injection of 10⁶ cancer cells

<table>
<thead>
<tr>
<th>Site</th>
<th>Mean tumor diameter (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>s.c.</td>
<td>1.14 ± 0.13⁺ (10)</td>
</tr>
<tr>
<td>Liver</td>
<td>0.78 ± 0.1 (20)</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.71 ± 0.07 (23)</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.97 ± 0.06 (22)</td>
</tr>
</tbody>
</table>

⁺ Mean ± S.E.
⁻ Numbers in parentheses, number of measurements.

Discussion

Metastasis involves interactions of cancer cells with their environment; the initial site for these interactions is the cell periphery. It is therefore reasonable to seek correlation between the physicochemical nature of the cancer cell periphery and metastasis.

The suggestion that the cancer cells in metastases are in some way different from those in the primary tumor generating them is based on 4 main types of experimental data. First, there is the general observation on animal tumors that large numbers of injected cancer cells give rise to relatively small

sites and livers were not. Enzyme treatment reduced the mobilities of the cells from the spleens, but not the kidneys, to a common level. Histograms are given in Chart 1 of the mobilities of the ascites tumor cells, with and without neuraminidase treatment. Analysis of these curves reveals that both fall into Pearson’s type IV (16) with unimodal distribution. The distributions are also characterized by kurtosis and skew.

Table 1 provides the means and standard errors of the mean for the s.c. tumors was not significantly different for that of the spleen (p = 0.2).

Stability of Site-associated Changes. The stability of site-associated changes in cancer cells maintained in the kidney and spleen was studied by organ-to-organ passage as shown in Table 3. The results show that compared with cells passaged in the ascites form, the cells passaged in the spleen and kidneys maintained a lower absolute net surface negativity and a reduced susceptibility to neuraminidase, as assessed by electrophoretic mobility measurements.

Reversibility of Site-associated Changes. The results of 2 separate experiments are summarized in Table 4. When W-256 cells growing in the spleen or kidney were transplanted into the peritoneal cavities of fresh rats after 1 passage, their electrophoretic mobilities, with or without neuraminidase treatment, were very similar to those of cells maintained continuously in the ascites form and measured during the same experiments. These similarities between cells of different immediate origin, growing in the ascites form, were maintained after 6 i.p. passages. At this time, the mobilities of spleen and kidney-derived cells were 97 and 98% of the continuous ascites values. Thus, site-associated reversibility occurred.

Discussion

Metastasis involves interactions of cancer cells with their environment; the initial site for these interactions is the cell periphery. It is therefore reasonable to seek correlation between the physicochemical nature of the cancer cell periphery and metastasis.

The suggestion that the cancer cells in metastases are in some way different from those in the primary tumor generating them is based on 4 main types of experimental data. First, there is the general observation on animal tumors that large numbers of injected cancer cells give rise to relatively small
numbers of metastases. Even in people having long histories of cancer, the numbers of metastases discovered at autopsy are quite small. Thus, metastasis appears to be associated with high growth rate may be associated with increased cell surface negativity described in statistical terms. Another mechanism to account for the differences is the hypothesis that metastases arise from the metastatic event and are site-induced modulations of the type discussed by Weiss (36) in connection with cellular differentiation in developmental systems. One example of such a site-induced change is provided by sarcoma 37 cells in mice, in which morphological changes between cells present as distinct from a selective process.

In the present work we have examined some physicochemical properties of the surfaces of W-256 cells taken from a common (ascitic) source and growing at different sites in rats, and we have tested the hypothesis that differences between primary cancers and their metastases are critically reviewed elsewhere (31).

Although the differences referred to above exist and are of potential therapeutic importance, the mechanisms underlying them need clarification (31). Thus, in the natural history of cancer, metastases may arise by means of a random survival of cells going through all the complex sequence of events in the metastatic cascade (29). This type of chance survival is common to many biological events and is perhaps best described in statistical terms. Another mechanism to account for the differences is the hypothesis that metastases arise from preexisting subpopulations within primary tumors as discussed by Greene (11), Leighton (14), and Zeidman (38), and as supported by the evidence from combined in vitro-in vivo experiments with one or 2 mouse tumors by Fidler (6), Fidler and Kripke (7), Nicolson et al. (15), and Tao and Burger (24).

Another hypothesis is that differences between primary cancers and their metastases after the metastatic event and are site-induced modulations of the type discussed by Weiss (36) in connection with cellular differentiation in developmental systems. One example of such a site-induced change is provided by sarcoma 37 cells in mice, in which morphological changes between the solid and ascitic forms, which are due to a change in cells present as distinct from a selective process (13), are demonstrably associated with reversible changes in cell electrophoretic mobility and neuraminidase sensitivity (2).

In the present work we have examined some physicochemical properties of the surfaces of W-256 cells taken from a common (ascitic) source and growing at different sites in rats, and we have tested the hypothesis that differences between them were due to site-induced modulation.

On the evidence of cellular electrophoretic mobilities, with and without incubation with neuraminidase (Table 1), the surfaces of the W-256 cells growing in kidneys and spleens were different from their "parent" ascites form, whereas those growing in livers and s.c. sites were not. A number of previous studies have shown that high growth rate may be associated with increased cell surface negativity.

### Table 3

**Mobilities of Wp256 cells, with and without neuraminidase treatment, following the indicated site-to-site passages**

<table>
<thead>
<tr>
<th>Origin</th>
<th>Site of growth</th>
<th>Control</th>
<th>Ascites control (%)</th>
<th>+ Neuraminidase</th>
<th>Neuraminidase-induced change</th>
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<tr>
<td>Ascites</td>
<td>Kidney (1)</td>
<td>-1.12 ± 0.01 b (438)</td>
<td>93</td>
<td>1.03 ± 0.01 (432)</td>
<td>-8</td>
</tr>
<tr>
<td>Ascites</td>
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<td>-1.06 ± 0.01 (450)</td>
<td>88</td>
<td>0.92 ± 0.01 (462)</td>
<td>-13</td>
</tr>
<tr>
<td>Ascites</td>
<td>Ascites control (1)</td>
<td>-1.20 ± 0.01 (868)</td>
<td>80</td>
<td>0.87 ± 0.01 (840)</td>
<td>-28</td>
</tr>
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### Table 4

**Mobilities of W-256 cells, with and without neuraminidase treatment, after return to the ascites form**

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<tr>
<th>Origin</th>
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<tr>
<td>Ascites</td>
<td>Ascites</td>
<td>-1.16 ± 0.01 a (402)</td>
<td>100</td>
<td>0.85 ± 0.01 (356)</td>
<td>-27</td>
</tr>
<tr>
<td>Kidney</td>
<td>Ascites</td>
<td>-1.15 ± 0.02 (204)</td>
<td>99</td>
<td>0.85 ± 0.02 (160)</td>
<td>-26</td>
</tr>
<tr>
<td>Spleen</td>
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<td>-1.14 ± 0.02 (202)</td>
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a Numbers in parentheses, number of sites.
b Mean ± S.E.
c Numbers in parentheses, number of observations.

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**Surface Modulation in Cancer Cells**

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**Table 3**

**Mobilities of Wp256 cells, with and without neuraminidase treatment, following the indicated site-to-site passages**

The differences in mobilities between the ascites cells and those growing in the other sites are maintained.

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**Table 4**

**Mobilities of W-256 cells, with and without neuraminidase treatment, after return to the ascites form**

The previous differences are lost.

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<tr>
<th>Origin</th>
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a Mean ± S.E.
b Numbers in parentheses, number of observations.
(5), which is partially due to increased expression of sialic acid moieties at the cellular electrophoretic surface (26, 34). It has also been observed that W-256 tumors at different sites produce different general effects on the host animal (12) and that the tumors themselves exhibit different rates of proliferation at different sites (1). The question has been raised of whether the differential response of metastases and primary cancers to chemotherapy is a reflection of differential growth rate (4). In the present experiments, on a size basis (Table 2), tumors in s.c. sites and the spleen grow faster than do those in the liver or kidney. However, the mean sizes in the s.c. sites and spleen were not significantly different, and those in the kidney and liver were also similar. Therefore, the differences in the electric properties of cancer cells growing in the spleen and kidney on the one hand and those growing s.c. and in the liver on the other cannot be directly ascribed to differences in growth rate in the different sites.

It is of interest (Table 1) that treatment with neuraminidase should have reduced the mobilities of the cancer cells from the spleen to a common level with those from the ascites, s.c., and hepatic forms. This suggests that one difference between the splenic and latter forms is a comparative lack of neuraminidase-sensitive, surface, anionic sites in W-256 cells growing in the spleen. In contrast, the difference in the electrokinetic surfaces of kidney-derived cells is not accountable in terms of neuraminidase-susceptible anionic sites because following enzyme treatment, the mobilities of these cells are not reduced to the common level of the cells growing in the other 4 sites. Although the technique of electrophoretic mobility permits measurements to be made on individual cancer cells within suspensions containing other cell types, it is not possible to make meaningful chemical analyses on such fresh, mixed populations containing macrophages, erythrocytes, etc., which can then be related exclusively to the cancer cells. The present studies, therefore, do not permit additional comment on the chemical nature of the cell surfaces.

If the observed site-associated, electrophoretic differences between the W-256 cells were due to preexisting subpopulations within the ascites form of the tumor, then these might well be revealed in histograms of the mobility measurements, with and/or without neuraminidase treatment. However, the histograms shown in Chart 1, based on the individual measurements on ascites cancer cells given in Table 1, demonstrate nonnormal distributions of mobilities with skewness and positive kurtosis. They are unimodal (Pearson type IV) and provide no direct evidence for an explanation of the site-associated differences in terms of preexisting subpopulations of cancer cells, since on the basis of similar tumor size in the different sites appreciable proportions of these subpopulations should have been present in the original ascites tumors.

An essential feature of a site-induced modulation of cancer cell properties is that they should be stable while the cells remain in the site inducing them and reversible when returned to their original environment. The electrokinetic data given in Table 3 show that, regardless of mechanism, the site-associated changes in W-256 cells growing in the kidney or spleen are maintained in organ-to-organ passage.

The data given in Table 4 show that when W-256 cells growing in either the kidney or the spleen are transferred back to the peritoneal cavity they assume the electrokinetic characteristics of the original ascites cells on first passage and maintain these properties on continuous peritoneal transfer. The experimental evidence therefore supports the view that cancer cells growing in certain organs are different from those growing in other organs because of site-induced changes in the original cancer cell population.

It must be emphasized that we claim only to have demonstrated reversible, site-induced differences in the peripheries of W-256 cancer cells growing in different organs of rats by measurements of their electrophoretic mobilities before and after neuraminidase treatment. We are not prepared to speculate on the functional significance of these changes in terms of metastasis-related cell interactions (27, 28), since even "simple" cell contact interactions require a knowledge of the spatial distribution of peripheral ionogenic sites (33, 35) which cannot be derived solely from electrokinetic data of the present type.

It is important to put the present results into perspective in relation to metastasis. The growth of cells in an organ following direct injection is not a model for the whole metastatic process. At most, these experiments are relevant to the growth of cancer cells after delivery to an organ; they give no information on the antecedent history of the cancer cell in naturally occurring metastasis (27). The sequence of events leading to "delivery" is demonstrably traumatic to cancer cells (21), and this probably contributes to the low overall efficiency of the metastatic process (30). It is conceivable that selection, either at random or by virtue of the metastatic subpopulation described by Fidler et al., operates at this level. However, on present evidence, differences at the cell periphery associated with survival and growth in different organs could be accounted for without involving the concept of subpopulations but rather in terms of environmental interactions. However, although the present work establishes the feasibility of site-induced, interactive changes in cancer cells growing in different sites, it neither adds to nor detracts from the feasibility of the role of subpopulations of cancer cells in the metastatic process.

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