Procoagulant Activity of Sarcoma Sublines With Different Metastatic Potential

By M. Colucci, R. Giavazzi, G. Alessandri, N. Semeraro, A. Mantovani, and M. B. Donati

It has been suggested that cancer cell procoagulant activity influences metastasis formation by promoting fibrin deposition around tumors. We have investigated the procoagulant activity of various tumor cell sublines with different metastatic capacity derived from metastatic nodules of a murine fibrosarcoma. All the cells tested possessed a marked thromboplastin-like activity; they were, however, heterogeneous as regards the degree of procoagulant activity: the two cell lines with virtually no metastatic capacity showed 6-8 times higher procoagulant activity than the cells from the parent line; in contrast, the procoagulant activity of the two sublines with higher metastatic capacity did not differ significantly from that of the parent line. These findings support the hypothesis that fibrin is part of a defense reaction against cancer cell invasiveness.

It has been suggested that cancer cell procoagulant activity influences metastasis formation by promoting fibrin deposition around tumors. This is mainly derived from studies of "artificial" metastasis models where, upon i.v. injection of huge amounts of cancer cells, lung colonies rather than real metastases are formed. Virtually no information is available, in contrast, on the possible role of cancer cell procoagulant activities in "spontaneous" metastasis processes, which develop upon i.m. or s.c. implantation of the tumor. The latter process appears to mimic more closely the pathogenetic sequence of metastasis formation from solid primaries in patients.

Very recently, sublines were derived from individual lung metastases of a transplanted murine sarcoma, which are heterogeneous as regards several biologic properties, including the capacity for spontaneous dissemination.

We have investigated the procoagulant activity of cells from sarcoma sublines with different metastatic capacity derived from the same parent line. We report that these cells are heterogeneous also as regards their degree of procoagulant activity—the cells with the lowest metastatic capacity showing the highest procoagulant activity.

MATERIALS AND METHODS

Animals and Tumor

The benzo(a)pyrene-induced mFS6 sarcoma of C57BL/6 mice was obtained as previously described. Of the sublines derived from spontaneous lung nodules of this tumor, M1, M2, M3, and M4 were studied. These tumor sublines were chosen because they differed significantly from the primary tumor, M5 and M6, being more metastatic, and M7 and M8, being less metastatic.

No significant difference was observed among the various lines studied in the growth rate of primary i.m. growing neoplasms and of in vitro cultured cells.

Table 1 summarizes the in vivo characteristics of the tumor sublines: the number and weight of secondary lung deposits was measured as previously described. Cells stored in liquid nitrogen were first passaged once in vivo by s.c. implantations into C57BL/6 mice. After 20-25 days from implantation of a 40-mg tumor fragment s.c. with trocars or of 10^7 tumor cells i.m., tumors were disaggregated with trypsin, deprived of host macrophages (the major component of the lymphoreticular infiltrate of these neoplasms) by adherence on plastic, and placed in vitro as previously described. Tumor cells 3 x 10^6 in 6 ml RPMI 1640 medium with 20% fetal bovine serum (growth medium) were seeded in tissue culture flasks (3050 Costar, Cambridge, Mass.). In each experiment, sublines transplanted in the same way (i.m. or s.c.) were tested in parallel. In vitro cultures, maintained in growth medium, consisted of tumor cells, as assessed morphologically and by lack of contact inhibition. Sublines were tested within two in vitro passages.

In each experiment, tumor cells from in vitro growing sublines were harvested at the same time by brief exposure (1-5 min) to 0.1% trypsin-0.02% ethylene diamine tetraacetic acid (EDTA) in CA+-free phosphate-buffered saline (PBS). Cells were washed in 20 ml growth medium and then extensively with PBS. After resuspension in PBS, viability, assessed by Trypan blue exclusion, exceeded 90% and was similar for all cell lines.

Evaluation of Cell Procoagulant Activity

Tumor cell procoagulant activity was measured by a one-stage clotting time in plastic tubes at 37°C using the following test system: 0.1 ml cell suspension or PBS, 0.1 ml autologous normal plasma, 0.1 ml 0.025 M CaCl₂.

In some experiments, human plasma from normal subjects or patients with congenital deficiency of factor X, VIII, or VII was used as substrate (Dade Inc., Pharmaseal, Trieste, Italy).

RESULTS

In a first series of experiments we attempted to characterize the type of procoagulant activity of the fibrosarcoma lines, using a human plasma substrate. Table 2 shows that all cell sublines tested shortened the recalcification time of normal or factor-VIII-

From the Istituto di Ricerche Farmacologiche Mario Negri, Milan, Italy.

Supported in part by Grant ROI-CA-12764 from the National Cancer Institute (Bethesda, Md.), by Italian National Research Council (Contract 80.01621.96) and by the Italian Association for Cancer Research.

Submitted September 16, 1980; accepted December 5, 1980.

Address reprint requests to Maria Benedetta Donati, M.D., Ph. D., Istituto di Ricerche Farmacologiche Mario Negri, Via Eritrea, 62, 20157 Milan, Italy.

© 1981 by Grune & Stratton, Inc.

0006-4971/81/5704-0016$01.00/0

Blood, Vol. 57, No. 4 (April), 1981 733
Table 1. Metastasizing Capacity of the mFS6 Sarcoma and Cell Lines From Metastases

<table>
<thead>
<tr>
<th>Tumor Line</th>
<th>MST*</th>
<th>No./Total</th>
<th>Percent</th>
<th>Metastasis Number (± SE)†</th>
<th>Metastasis Weight (± SE)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>mFS6</td>
<td>33</td>
<td>17/32</td>
<td>53</td>
<td>3.3 ± 0.3</td>
<td>18.2 ± 5.4</td>
</tr>
<tr>
<td>(25-50)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M₄</td>
<td>36</td>
<td>13/14†</td>
<td>92</td>
<td>16.7 ± 3.6§</td>
<td>122.5 ± 38.5§</td>
</tr>
<tr>
<td>(30-49)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M₅</td>
<td>44</td>
<td>15/15†</td>
<td>100</td>
<td>13.8 ± 2.6§</td>
<td>170.2 ± 12.7§</td>
</tr>
<tr>
<td>(33-52)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M₆</td>
<td>35</td>
<td>1/16†</td>
<td>6</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>(26-47)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M₇</td>
<td>36</td>
<td>0/15†</td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>(25-52)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

10⁶ tumor cells were injected i.m. and spontaneous metastases were examined at death.4
*Median survival time with range.
†Number and weight of lung metastases/mouse.
‡p < 0.01 compared to primary mFS6 (Fisher exact test).
§p < 0.01 compared to mFS6 (Duncan’s new multiple range test).

Table 2. Effect of mFS6 Cells and Related Sublines on the Recalcification Time of Various Human Plasma Substrates

<table>
<thead>
<tr>
<th>Cells (10⁴/ml)</th>
<th>Normal</th>
<th>Factor-VIII-Deficient</th>
<th>Factor-VII-Deficient</th>
<th>Factor-X-Deficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>mFS6</td>
<td>105-138</td>
<td>107-141</td>
<td>220-241</td>
<td>-300</td>
</tr>
<tr>
<td>M₄</td>
<td>110-135</td>
<td>110-150</td>
<td>207-236</td>
<td>-300</td>
</tr>
<tr>
<td>M₅</td>
<td>99-146</td>
<td>102-144</td>
<td>210-250</td>
<td>-300</td>
</tr>
<tr>
<td>M₆</td>
<td>73-96</td>
<td>74-99</td>
<td>200-212</td>
<td>-300</td>
</tr>
<tr>
<td>M₉</td>
<td>68-91</td>
<td>71-96</td>
<td>197-220</td>
<td>-300</td>
</tr>
<tr>
<td>Buffer</td>
<td>-.300</td>
<td>-.300</td>
<td>220-266</td>
<td>-300</td>
</tr>
</tbody>
</table>

For each cell line the range of values obtained from duplicate experiments on 3 different cell preparations is reported.

deficient plasma to a similar extent but had no activity on factor-VII-deficient plasma. As cells have been reported to display greater procoagulant activity in autologous plasma, mouse platelet-poor plasma (PPP) was used to assess the potency of the procoagulant activity of our sublines. These results are shown in Fig. 1: mFS6, M₄, and M₅ did not differ significantly, whereas M₆ and M₉ had a significantly higher procoagulant activity (shorter clotting times) than each of the remaining lines (p < 0.01 by Duncan’s new multiple range test).

The coagulant activity of each subline was quantified in relation to that of a parent line sample prepared and tested simultaneously. Clotting times of different dilutions of each cell subline were plotted against the corresponding cell number on a double logarithmic scale. Since the straight lines obtained were parallel in each instance, it was possible to express the procoagulant activity as a percentage of the parent line. As shown in Fig. 2, M₄ and M₅ had a greater and M₆ and M₉ a much lower

DISCUSSION

This study shows that cells derived from nodules of a murine metastasizing tumor had qualitatively similar procoagulant activity, which was identified as tissue factor. They were, however, heterogeneous as regards the degree of such activity. M₄ and M₅ consistently showed significantly higher procoagulant activity then the other sublines and the parent line. It has been previously shown that of the different sublines, M₄ and M₅ had a greater and M₆ and M₉ a much lower

Fig. 1. Effect of mFS6 cells and related sublines on the recalcification time of autologous plasma. Cell count in the preparations tested: 5 x 10⁴/ml. Each point represents the mean of 3 replications of the clotting time (variability never exceeded 5%).
capacity for disseminating spontaneously to the lung as compared to the primary mFS6 tumor. It is not possible to establish a simple correlation between the procoagulant activity and the metastatic capacity of the cells studied. However, it was striking that the cell sublines virtually unable to give metastases were the ones with the highest procoagulant activity.

Clot-promoting substances produced by cancer cells are thought to represent the main cause of fibrin deposition around tumors, and fibrin, at least in some experimental tumors, might be part of a defence reaction against cell invasiveness; a fibrin coating would indeed impair detachment of cancer cells from the primary, which represents the first step for dissemination. Our observation that the two sarcoma sublines with no metastatic potential had the highest clot-promoting activity supports this concept. It is noteworthy in this respect that the different metastasizing capacity of the various lines could not be related to differences in their immunogenicity.

REFERENCES

Procoagulant activity of sarcoma sublines with different metastatic potential

M Colucci, R Giavazzi, G Alessandri, N Semeraro, A Mantovani and MB Donati