Isozyme Pattern in Serially Xenotransplanted Childhood Tumors

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ABSTRACT

Growth rate, histological course, and polymorphic enzyme pattern (glucose 6-phosphate dehydrogenase, glucose phosphate isomerase, and phosphofructokinase) were studied in eight childhood tumors xenotransplanted serially to nude mice. The growth rate of these tumors (three nephroblastomas, one hypercalcemic renal tumor, three rhabdomyosarcomas, and one malignant histiocytosis) appeared stable for any one particular tumor line. The time interval between two grafts varied from 1 to 3 weeks to 1 to 2 months in correlation with the clinical course of each malignant process. Histological changes were mostly in relation with a progressive dedifferentiation of the grafts. Immunoneutralization of glucose-6-phosphate dehydrogenase and glucose phosphate isomerase made possible the quantification of the stroma reaction in the grafts. A series of ten passages showed the amount of stroma to be constant for a given tumor type but variable from one tumor type to another, except for the malignant histiocytosis which showed an increase in stroma constituent after the sixth passage. One nephroblastoma tumor line showed, during the third passage, a sudden acceleration in the growth rate and complete transformation of the histological line showed, during the third passage, a sudden acceleration in the growth rate and complete transformation of the histological and isozymic patterns, which were interpreted as being the result of a murine lymphoma. The fibroblastic form of phosphofructokinase increased in every tumor line, whatever the tumor type. This change may be linked to a progressive dedifferentiation during the passage.

INTRODUCTION

Since the first successful heterotransplantation of a human cancer to the nude mouse (19), most types of cancers, have been xenografted, although childhood cancers to a lesser extent (8). Biological activity between initial and subsequent successive passages has been monitored regularly by morphological chromosomal and biochemical markers, but quantitative evaluation of these criteria remains rare (16).

Electrophoretic aspects of G6PD and GPI are different in human and murine tissues. This provides a means of characterizing the human origin of the xenografts (10). Immunoneutralization techniques using monospecific antibodies directed against the isozymes make it possible to obtain quantitative data useful in following the stroma-tumor relationship in the serial xenografts.

In contrast with G6PD and GPI which are encoded by a single gene, PFK is encoded by 3 separate genes, the products of which are subunits predominant in the muscle, liver, and fibroblasts and abundant in platelets (13). These isozymic subunits were shown in a previous work to correspond to the differentiation level in 61 childhood tumors (2).

We describe here 7 embryonic tumor lines and one malignant histiocytic tumor line which were transplanted serially to nude mice. Morphological and isozymic criteria were used with a view to obtaining data on both the quantitative evaluation of host tissue participation and the maintenance of the differentiation level in serially heterotransplanted tumors.

MATERIALS AND METHODS

Tumor samples were obtained from the operating room or were removed from the nude mice. Tumor samples were subdivided into aliquots for histological controls, isozyme analysis, and transplantation to nude mice.

Light Microscopy. Tumor tissues were fixed in 15% formalin and embedded in paraffin. Five-μm-thick sections were stained with hematoxylin-eosin.

Isozymes. Human and mouse G6PD and GPI isozymes were separated by cellulose acetate electrophoresis in a 0.013 M Tris-0.0038 M citric acid buffer, pH 7.5 (5). Under these conditions, G6PD migrates towards the anode, and GPI, towards the cathode. In both cases, the isozymic mice exhibit the faster mobility. The enzyme activity was revealed on the cellulose acetate strip as described previously (1, 11). The ratio of human to mouse isozyme was evaluated by 2 means. (a) Evaluation of the relative intensity of the bands of the isozymes by scanning the cellulose acetate strips was made. (b) Specific immunoneutralization in selected conditions in which reactivity of the rabbit anti-human enzyme antisera against mouse enzymes was negligible (12).

PFK isozyme composition of the different tumors was analyzed by specific immunoneutralization using antisera directed against the 3 types of PFK subunits, i.e., muscle, liver, and fibroblast types (13).

Transplantation to Nude Mice. Male homozygous athymic nude mice of Swiss background were supplied from Iffa Credo (Lyon, France) and were inoculated s.c. when 4 to 6 weeks old. The mice were housed in plastic cages with air filter tops. Food, water, bedding, cages, and tops were autoclaved prior to use. Growing tumors (1 to 3 cm) were re inoculated s.c. into 4- to 6-week-old nude mice. The number of passages varied from 5 to 30; experiments were conducted over 2 years.

RESULTS

Growth Characteristics of the Serially Transplanted tumors

Rate of Tumor Growth. Macroscopic growth of tumors was evaluated for each tumor line (Chart 1) by noting the time period necessary for the grafts to reach a 0.8- to 1-cm mean diameter.-

Tumor growth rate was quite regular and varied from 1 to 2 weeks for the more aggressive ones (Tumors 4 and 8) to 1 to 2 months for the other cases. Tumor growth rate appears to be related directly to the clinical course of the initial tumor but not to the clinical state, such as primary tumor or recurrence. Only one tumor had an irregular growth curve (Tumor 3), growing very slowly for the first 2 passages (6 to 10 months) but changing its growth rate to reach a constant 1-week acceleration between 2 ulterior passages. Just before that acceleration in its growth rate,
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the tumor was found not in the s.c. locus of implantation but in the kidney of the mouse.

**Histological Appearance.** Tumors are usually well circumscribed by a thin capsule. Central necrosis may be noted in the largest tumors. Fine cellular characteristics of 2 tumor lines (Tumors 4 and 8) were demonstrated previously to be stable by both light and electron microscopy and by biological criteria (17, 18). Three other cases did not show noticeable modification. However, in all of these tumor lines, a monomorphic aspect was predominant, and cellular arrangement was more cohesive than in the primary tumor. Three cases showed some changes: decrease in large rhabdoid cells in the second and subsequent passages of a rhabdomyosarcoma (Tumor 5) (Fig. 1); disappearance of tubular structures in the third and subsequent passages of nephroblastoma (Tumor 1) (Fig. 2); and a complete morphological change in the second and subsequent passages of nephroblastoma (Tumor 3). In the last case, there was a sudden change in site and in rate of growth, and the tumor appeared no longer to be a nephroblastoma but a lymphoma. On the whole, apart from this last exceptional case, the histological aspect of the xenograft was stable after the second or the third passage.

### Table 2

**Immunological analysis of PFK in serially transplanted human tumors**

All results were obtained from at least 3 independent measures, the extreme values of which are given. When these values were not significantly different, only one result is given.

<table>
<thead>
<tr>
<th>Tumors</th>
<th>Passage</th>
<th>% of residual activity after neutralization by excess</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Anti-muscle</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>30-50</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>38-58</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>46-60</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>25-33</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>20-30</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>32-35</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>18-27</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>13-23</td>
</tr>
</tbody>
</table>

### Table 1

**Quantification of human G6PD and GPI in serially transplanted tumors**

The results of 2 independent measures are given. Measurement of the percentage of human enzyme was carried out by scanning the electrophoretograms and, in Tumors 1, 2, and 8, by immunoneutralization.

<table>
<thead>
<tr>
<th>Tumor</th>
<th>% of total enzyme activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GPI</td>
</tr>
<tr>
<td>1. Nephroblastoma</td>
<td>70-80</td>
</tr>
<tr>
<td>2. Nephroblastoma (metastasis)</td>
<td>75-85</td>
</tr>
<tr>
<td>3. Nephroblastoma</td>
<td>&gt;90</td>
</tr>
<tr>
<td>5. Rhabdomyosarcoma</td>
<td>&gt;90</td>
</tr>
<tr>
<td>6. Rhabdomyosarcoma (relapse)</td>
<td>&gt;90</td>
</tr>
<tr>
<td>7. Rhabdomyosarcoma (relapse)</td>
<td>&gt;90</td>
</tr>
<tr>
<td>8. Malignant histiocytosis</td>
<td>70-80</td>
</tr>
</tbody>
</table>
Isozymic Pattern

Host Component. G6PD and GPI immunoneutralization studies of the tumor lines made it possible to show that the human and/or mouse percentage was stable in the serial passages for a particular tumor (Table 1). Only one case (Tumor 8) showed after the sixth passage a slight increase in the murine constituent. G6PD data were quite similar to those of GPI, and electrophoretic patterns corresponded with immunoneutralization results with a maximum divergency of 10%. The percentage of murine component is clearly dependent on the type of tumor studied. Rhabdomyosarcomas would appear to interact less with the stroma than do other tumor types, since the murine component never exceeded 10% in any sample studied. The nephroblastoma tumor line which changed into a lymphoma (Tumor 3) showed only murine subunits G6PD and GPI.

Differentiation. PFK activities were grossly stable in serial passages except for the fibroblastic subunit, which increased in every case after the second or fourth passage (Table 2). In addition to this major variation, one rhabdomyosarcoma (Tumor 7) showed a slight increase in the liver subunit form during the passages.

DISCUSSION

Growth rate of childhood tumors transplanted serially to nude mice corresponded grossly to tumor growth in the patient and appeared stable for any one particular tumor line. The 2 tumors which were the most aggressive in the children, since their deaths occurred a few months after excision, grew more rapidly in the nude mice.

For 7 tumor lines, the time interval for the first tumor growth in the nude mouse varied from case to case but was most often longer than the mean time between any 2 ulcerating consecutive passages, which required a minimum of 1 week and a maximum of 2 months. The appearance of a murine lymphoma in a slowly growing tumor line was detected by an acceleration in the growth rate and a complete change in the histological and isozymic patterns. Other histological changes were slight and concerned dedifferentiation. Stability of growth rate is described for most human tumors xenotransplanted to nude mice (16). The data on histological stability are more heterogeneous. The histological aspect of xenotransplanted tumors was most often described as being similar to that of the patient's tumor, but an increase or decrease in differentiation was noted in 37% of the 52 cases studied by Sharkey and Fogh (20). Morphological changes reported for soft tissue sarcoma (7) or for bronchial tumors (14) show primarily a tendency to dedifferentiation. Among other minor histological changes, an increase in cell density or in cellular cohesion is often noted (9,14) as well as, to a lesser extent, progressive fibrosis (4). Finding a murine lymphoma or a spontaneous tumor is not rare in either grafted nude mice or control nude mice (21).

Results using 2 different isozymes, G6PD and GPI, allowed the quantification of the stroma reaction in the xenografts. The murine component of the grafts shows the stroma to be made up of endothelial and blood cells, lymphocytes, macrophages, and connective tissue. Stroma appears quantitatively variable from one tumor type to another but constant for a same tumor type in the early serial passages. However, after the sixth passage, grafts from the malignant histiocytosis showed an increase in the murine constituent without any consequent change in morphology or growth time.

Few studies have been done which relate to the stroma of tumors. Morphological characterization and quantification of the stromal infiltrate from suspensions of tumor cells were done in mammary adenocarcinoma experimental tumors and showed that 20 to 30% of the cells belonged to the stroma cells (3). Murine stroma was demonstrated in carcinoma of the colon (22) by anti-species antibody reactivity in immunofluorescence as closely related to the successful take of a particular xenograft. In spite of the paucity of data on the exact role of the stroma-tumor interaction, it appears to be a stimulating factor in tumor growth (6).

PFK isozyme pattern confirms the low level of differentiation of the tumors before transplantation. The expression of PFK by the subunits was relatively stable in the successive passages except for the fibroblast form, which increased in every tumor line, whatever the initial tumor type. This variability cannot be related to an increase in stroma participation in at least 7 tumor lines, since this was shown to be stable by morphological and G6PD and GPI data. Nevertheless, an increase in stroma participation in the last passages of the malignant histiocytosis case cannot be excluded. In the 7 other tumor lines, the changes in the fibroblastic form may be linked to a progressive dedifferentiation (15), which agrees with our morphological data.

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REFERENCES


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Fig. 1. Case 5. The large rhabdoid cells seen in the initial rhabdomyosarcoma (A; × 250) are less numerous in the transplanted tumor (B; × 250). Paraffin-embedded sections (5 μm) stained with H & E.

Fig. 2. Case 1. The well-differentiated nephroblastoma (A; × 250) shows rare tubular structures after the second passage in the nude mice (B; × 250). Paraffin-embedded sections (5 μm) stained with H & E.
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