Two human melanoma xenografts with different metastatic capacity and glycosaminoglycan pattern

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Summary. Two human melanoma xenografts were compared with respect to their in vivo growth and metastatic potentials as well as glycosaminoglycan patterns. The less differentiated HT 168 tumor showed faster growth at primary sites and a more pronounced capacity for metastasis into the liver. Although chondroitin sulfate was the dominant glycosaminoglycan subtype in both tumors, the more invasive xenograft had a higher heparan sulfate/chondroitin sulfate (HS/CS) ratio. We suggest that tumor progression is influenced by this ratio in this human melanoma system.

Key words: Human melanoma – Metastasis – Glycosaminoglycan – Xenograft

Introduction

Human tumor xenografts, including melanomas, metastasizing in immunosuppressed mice provide a new system for addressing the problems of metastatic phenotype in human tumor cells (Kerbel et al. 1984; Fidler 1986; Ganderup et al. 1986; Doré et al. 1987). In such experiments the principles are similar to those followed in studies with rodent tumors, i.e. to reveal differences in phenotype between tumors (selected, primary vs metastatic, common anatomical origin or histology) with different metastatic capacity. Metastatic phenotype is frequently associated with altered cell-cell or cell-matrix interactions that are influenced by extracellular matrix components (Liotta 1986; Nicolson 1987), among which are the glycosaminoglycans (GAGs) (Iozzo 1988). This relationship has been extensively studied in rodent but not in human tumors.

Materials and methods

Human melanoma xenografts. Human amelanotic melanoma (HT 18 – code used in our institute) was established as a xenograft from the recurrence of a skin melanoma and was maintained in immunosuppressed (IS) CBA mice by the inoculation of 20–30 mm^3 tumor tissue as previously described (Kopper and Steel 1975). HT 168 melanoma xenograft was developed from the A2058 human amelanotic melanoma cell line (kindly provided by Prof. L.A. Liotta, National Cancer Institute, NIH, Bethesda). Both tumors appeared to be non-metastatic after s.c. transplantation, even when judged by histology. Tumor-volume doubling time (TD) was estimated from serial caliper measurements of tumor diameters. The human origin of the xenograft tumors was proved by chromosomal analysis and by the presence of human HLA antigens on the cell surface. The melanoma character was supported by the binding of monoclonal antibodies recognizing the high-molecular-weight melanoma-associated antigen (MAA) and nerve-growth-factor receptor (kindly provided by Prof. M. Herlyn, Wistar Institute, USA).

Metastasis model. Single-cell suspensions were prepared mechanically from the solid s.c. tumors by crossed scalpels, followed by filtering of the cells through four-fold gauze. In all, 10^6–3 x 10^6 viable tumor cells were inoculated into the spleen of IS CBA mice in a volume of 30 μl according to the method of Kopper et al. (1982). The animals were killed after 4–6 weeks and the spleen and liver were examined under a stereomicroscope and by histology.

GAG biochemistry. For isolation of GAGs, 1–2 g tumor tissue obtained from three different passages was homogenized in a Teflon-glass homogenizer in 0.1 M TRIS-HCl buffer (pH 7.9). Lipids were extracted five times with 5 vol. acetone and materials were dried in N₂ gas. Proteins were digested by 1 mg/ml pronase (Serva) three times for 24 h in 0.1 M TRIS-HCl buffer (pH 7.9) at 50 °C; then the remaining peptides were eliminated by alkali treatment as described
by Montreuil (1984). After neutralization, the residual macromolecules were precipitated with 10% (w/v) trichloroacetic acid (TCA). The supernates were dialysed against running tap water for 48 h and distilled water for 24 h. GAGs were precipitated by 1% cetylpyridinium chloride (Sigma) at room temperature and were then centrifuged. The sediment was washed in 2 M NaCl:96% EtOH (100:1, v/v) solution, re-precipitated by 96% EtOH containing 1% potassium acetate, washed twice with acetone and air-dried. The GAG content was determined by carboxyl reaction (Bitter and Muir 1962) on uronic acid content.

For cellulose acetate electrophoresis, 1.5-2 µg GAG from each sample, diluted in 5 µl distilled water, was run for 3 h on cellulose acetate membrane (Sartorius 11200) in 0.2 M calcium-acetate buffer (pH 6.1) in an OE211 ELFO apparatus (LaborMIM, Hungary) at 100 V/mA. The strips were stained with alcan blue 8GX (Sigma) in 0.025 M sodium-acetate buffer (pH 5.8) and were differentiated in methanol:water:acetic acid (50:45:5, by vol.).

For enzyme degradation of GAGs, aliquots of the latter were treated with Streptomyces hyaluronidase (type IX, Miles), testicular hyaluronidase (Sigma) or chondroitinase ABC (Miles) to degrade hyaluronic acid, hyaluronic acid and chondroitin sulfate (CS), or CS and dermatan sulfate (DS), respectively. Nitrous acid was used to oxidate heparan sulfate (HS) (Timfir et al. 1987). For scanning densitometry, black/white negatives were made from cellulose acetate strips and analysed with a Helena Laboratories-Parascan QED apparatus attached to a Tantung Einstein PC.

Tyrosinase assay. Tyrosine (Sigma) at a concentration of 10^{-6} M was applied to the cells in vitro for 48 h in complete culture medium. The cell layer was then washed, fixed in 4% formaldehyde/PBS (pH 7.2) for 30 min at room temperature, washed and stained for melanin using the argentaffin silver reaction of Sevier and Munger (1965). A positive reaction was judged on the presence of black stain in the cytoplasm. The tyrosinase activity was assessed by comparing the tyrosine-treated samples with the untreated ones.

**Results**

**Growth and metastatic capacity of human melanoma xenografts**

The human melanoma xenografts maintained as solid s.c. tumors showed local growth and invasion of surrounding tissues but no metastases. HT 18 and HT 168 cells showed the characteristics of the intermediate/late and early/intermediate stages of melanocyte differentiation, respectively. The in vivo growth rate of HT 18 was much lower than that of HT 168 (Table 1), and a similar difference was found in the primary cultures as well. HT 168 injected intrasplenically showed a higher take rate and grew more rapidly than HT 18. Moreover, HT 168 had a higher capacity to form liver metastases (Table 2).

**GAGs in s.c. growing melanomas**

The two melanomas contained similar amounts of GAGs as measured by their uronic acid content (Table 3). The cellulose acetate electrophoresis of GAGs demonstrated two major bands (I and II), with a minor one between them (III) (Fig. 1). Band I proved to be CS due to its sensitivity to chondroitinase ABC and testicular hyaluronidase. Band II was sensitive only to nitrous acid degradation; therefore, it was considered to be HS. The minor band (III) was identified as DS because of its exclusive sensitivity to chondroitinase ABC. In three separate experiments the scanning densitometric analysis of cellulose acetate phoretograms showed a much higher HS/CS ratio for HT 168 than for HT 18 (Table 3, Fig. 2).

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**Table 1. Characteristics of s.c. growing HT 18 and HT 168 human melanomes**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HT 18</th>
<th>HT 168</th>
</tr>
</thead>
<tbody>
<tr>
<td>TD (days)</td>
<td>In vivo 7.5-11.0</td>
<td>3.5-5.0</td>
</tr>
<tr>
<td>Morphology</td>
<td>Spindle-dendritic</td>
<td>Epithelioid/spindle</td>
</tr>
<tr>
<td>Tyrosinase</td>
<td>In vivo Naevoid</td>
<td>Epithelioid/spindle</td>
</tr>
<tr>
<td>Melanosomes (TEM)</td>
<td>Rare</td>
<td>Rare</td>
</tr>
<tr>
<td>Premelanosomes</td>
<td>Rare</td>
<td>Rare</td>
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* Criteria for differentiation: according to Delcroix et al. (1968) and Bomirski et al. (1988)
TD, tumor volume doubling time; TEM, transmission electron microscopy

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**Fig. 1. Cellulose acetate electrophoresis of melanoma GAGs identified as being (I) CS, (II) HS and (III) DS. Lane 1, control; lane 2, Streptomyces hyaluronidase; lane 3, testicular hyaluronidase; lane 4, nitrous acid degradation; lane 5, chondroitinase ABC**

**Fig. 2. Densitometric analysis of electrophoresed GAGs (experiment 1). Scanning was carried out on lanes 1a and 1b in Fig. 1 and revealed that HT 168 had a higher HS/CS ratio than did HT 18**