Dear Sir,

Extensive multi-organ metastasis following orthotopic onplantation of histologically-intact human bladder carcinoma tissue in nude mice

Human transitional-cell bladder carcinoma can vary from low invasive potential to high metastatic activity. Until recently, there were no appropriate models to study the potential for metastasis and mechanisms involved in bladder carcinoma. A possible solution to these problems has emerged with the implementation of so-called “orthotopic” transplantation techniques (Fidler, 1990). Ibrahim et al. (1983) and Ahlering et al. (1987) found that orthotopic implantation of a bladder tumor-cell suspension into the rat bladder produced some tumor metastasis, whereas subcutaneous implantation did not. These results indicated that the orthotopic site of injection enabled the transitional-cell carcinoma cells to express part of their invasive potential.

Theodorescu et al. (1990) observed that the RT-4 human bladder carcinoma line is not invasive in nude mice, even after orthotopic injection. However, when a mutated human H-ras gene was transfected into RT-4 so that over-expression of the gene occurred in selected cell lines such as RT-4-mr-10 (RT-10), the selected cell line was able to locally invade the bladder after transurethral orthotopic inoculation of disaggregated cells. With regard to the orthotopically-implanted RT-10 in the nude mouse, broad areas of invasion of transitional-cell carcinoma deep into the muscularis propria of the bladder occurred which in some instances extended into the surrounding adipose tissue and vascular spaces. However, no contiguous or metastatic spread of RT-10 was found in other organs. The parental cell lines and the ras-transfectants all produced tumors when inoculated s.c. However, the tumors grew in the s.c. site as pseudo-encapsulated masses with no evidence of tissue invasion at the s.c. site.

We have developed an intact-tissue onplant method of orthotropic transplantation of human tumors in nude mice (Fu et al., 1991). This method, first developed with colon cancer, allows patient tissue to be directly onplanted on the scraped peritoneal colon or cecum, with resulting facilitation of local growth, extensive regional metastases, lymph-node involvement and liver metastases (Fu et al., 1991). In the present study, the onplant method was carried out using subcutaneously-grown tissue of RT-10 as a tissue source. The RT-10 xenograft was grown s.c. in a 4-week-old outbred female nude mouse, removed and cut into 2-mm³ pieces. Nine nude mice were anesthetized with isoflurane inhalation. The lower abdomen of the nude mouse was sterilized with iodine and alcohol swabs, then a small midline incision was made and the urinary bladder exposed. The surgical adhesive 2-cyanoacryllic acid ester was applied on one side of the 2-mm³ tumor xenograft tissue and the piece of tumor was subsequently glued on top of the urinary bladder. The abdominal incision was closed with 7-0 silk surgical sutures in one layer and the animals were then kept in a sterile environment. For s.c. implantation, 4 nude mice were anesthetized with isoflurane inhalation. One side of the flank was sterilized with iodine and alcohol swabs and, after a small incision was made, a 2-mm³ piece of RT-10 xenograft tissue was implanted s.c. The wound was then closed with 7-0 silk surgical sutures and the animals were kept in a sterile environment. When the mice were moribund or dead, full autopsies were performed. At autopsy all major organs were grossly examined. Each organ was then fixed in 10% formalin, dehydrated, embedded in paraffin, sectioned and stained with hematoxylin and eosin.

The results described below indicate extensive local growth and invasion of the bladder, with metastases occurring in the regional and distant lymph nodes, liver, pancreas, spleen, and tissue adjacent to the adrenal gland and ureter, as well as the lungs, after orthotopic onplantation of histologically-intact RT-10 bladder carcinoma (Table 1, Fig. 1). These results contrast with those seen when disaggregated RT-10 cells are injected transurethrally, in which case no metastases are formed (Theodorescu et al., 1990). They also contrast with results seen when RT-10 was implanted s.c., only encapsulated tumors being formed in this case. Therefore, it appears that the orthotopic onplant method using histologically-intact tissue makes for very extensive metastatic capability, possibly as a result of the maintenance of the native tissue architecture of the tumor and site of onplantation.

For bladder tumors and possibly others, it may be crucial for the tissue to remain histologically intact in the orthotopic xenografting process in order that the metastatic potential be fully expressed. Thus, it is quite possible that native cell-cell interactions are necessary for the full expression of metastatic potential in these tumors. The orthotopic onplant model described

<table>
<thead>
<tr>
<th>Table 1</th>
<th>ORTHOTOPIC ONPLANTATION OF HISTOLGICALLY-INTACT HUMAN BLADDER CARCINOMA RT-4-mr-10 TISSUE S.C. ORTHOTOPIC INJECTION OF DISAGGREGATED RT-4-mr-10 CELLS S.C. SUBCUTANEOUS GROWTH</th>
</tr>
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<tbody>
<tr>
<td>Implantation strategy</td>
<td>Mouse number</td>
</tr>
<tr>
<td>Orthotopic onplantation of intact tissue</td>
<td>Total of 9</td>
</tr>
<tr>
<td>Orthotopic injection of disaggregated cells</td>
<td>Total of 20</td>
</tr>
<tr>
<td>Subcutaneous implantation in flank</td>
<td>Total of 4</td>
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</tbody>
</table>

See text for experimental details.

1 Lymph nodes include iliac, inguinal and axillary.
2 Organs involved include liver, lung, pancreas spleen, diaphragm, adjacent tissue of adrenal gland and ureter.

"From Theodorescu et al. (1990)."
Figure 1 – Orthotopic growth and distant metastases of orthotopically-onplanted histologically-intact human bladder carcinoma in nude mice. Tumor was onplanted as described in the text. (a) Local growth of onplanted bladder tumor measuring 18 × 22 mm (black arrow). Original mouse bladder marked by blue arrow. (b) Metastatic spread of bladder tumor to the axillary lymph nodes (blue arrows). (c) Pathohistology of tumor invading nude mouse bladder. Blue arrow indicates the bladder wall. Black arrow indicates the remaining lumen of the bladder. Tumor invaded the majority of the bladder (the area below the hollow arrows). Bar = 14 μm. (d) Bladder tumor metastasized to the liver (arrows indicate the tumor deposits). (e) Bladder tumor metastasized to the pancreas. Arrow indicates the tumor mass. (f) Bladder tumor metastasized to the spleen. Arrow indicates the tumor mass. Pathohistology of all metastases is similar to that of primary tumor shown in (c).

It seems very realistic for metastatic bladder cancer in that, in the clinical situation, the tumor can invade the serosa transmurally and metastasize distantly, growing out from the serosa as in the model described here.

Yours sincerely,

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REFERENCES


