Immunohistochemical Study on an Epithelial-Myoepithelial Intercalated Duct Carcinoma Transplanted to the Nude Mouse

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Abstract. Parotid gland carcinomas are rare. Among them, the epithelial-myoeipithelial carcinoma (EMC) is extremely rarely diagnosed. This case report was based on a female with a history of 17 years of recurrent EMC of the parotid and evidence of distant metastasis over a period of 2 years. During debulking procedures of her extensive facial tumour, small tumour samples were transplanted to nude mice. The tumours grew well on the mice and were characterized morphologically and immunohistochemically after explantation. Cellularity per mm² ranged between 3,470 and 7,410. The tumours were characterized by the typical bipolar pattern of tumour cells and broad stromal septae. All but one of 7 transplanted tumours were positive for pan-cytokeratin marker KL-1. The proliferation index in terms of MIB-1-stained nuclei increased from 2% to 20% and was correlated positively to the expression of EGFR. IGF-1R-, VEGF- and FLK1-stained cells were found in all cases. The increase in EGFR- and MIB-1-positive cells correlated with the clinical course of the patient who showed shorter periods of tumour recurrence prior to her death. These findings in EMC transplanted to the nude mouse demonstrate the feasibility of growing EMC in vivo.

Parotid gland carcinomas are rare. Among them, the epithelial-myoeipithelial carcinoma (EMC) is diagnosed extremely rarely (1). The Salivary Gland Registry (Hamburg University) recorded 21 cases collected during the first 15 years after establishment, with no patient dying from this cancer (2). However, the fatal outcome for patients with EMC is now well recognized, often after a long history of tumour recurrences and distant spread (3, 4, 5). This case report was based on a female with a history of 17 years of recurrent EMC of the parotid and evidence of distant metastasis over a period of 2 years. The clinical course of the patient is described in detail elsewhere (3).

Case Report

During debulking procedures, small samples of the tumour were transplanted to nude mice (Figures 1-4). The techniques are described in detail elsewhere (6, 7). These tumours grew well on the mice and were passaged on different animals for more than a year. The explanted tumours (Figure 5) were characterized morphologically and immunohistochemically (Table I). The nude mouse tumour was vital and showed characteristics of an adenocarcinoma. The tumour was characterized by the typical bipolar pattern of tumour cells and broad stromal septae. Cellularity per mm² ranged between 3,470 and 7,410. All but one of 7 transplanted tumours were positive for KL-1. The proliferation index in terms of MIB-1-stained nuclei increased from 2% to 20% and was positively correlated to the expression of EGFR. IGF-1R-, VEGF- and FLK1-stained cells were found in all cases (Table I). The increase in EGFR- and MIB-1-positive cells correlated with the clinical course of the patient who showed shorter periods of tumour recurrence prior to her death.

Discussion

This report described the successful transplantation of an EMC to the nude mouse and the maintenance of the adenoid pattern of the tumour even after several passages in this in vivo model. Transplantation of human salivary gland carcinomas to the nude mouse have been reported, e.g. for adenoid-cystic carcinomas (8, 9). Morphological changes of salivary gland carcinomas may occur in vivo (8).

In this case the dedifferentiation of EMC to an adenocarcinoma became evident. The histological investigation of the resected specimen was consistent with an EMC, despite

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obvious irradiation of the facial region (several years ago) and further local irradiation with $^{192}$iridium seeds as a palliation of the only seeing eye (3). Therefore, the dedifferentiation of EMC may be related to alterations of the xenograft in the nude mice.

On the other hand, the aggressive nature of EMC during the fatal course of the patient following ablative surgery and local irradiation may be indicated by the increase of the mitotic index in terms of Ki-67-positive xenotransplanted carcinoma cells in particular in tumour samples that were obtained after further debulking procedures, and in specimens of mice that were investigated at the latest time-points (Figure 6).

Figure 1. Computed tomography (CT) scan of the patient with recurrent EMC invading the orbit.

Figure 2. Resection specimen of EMC showing a pseudo-capsular definition of tumour borders and multi-lobulated surface.

Figure 3. Section of EMC showing the characteristic epithelial and myoepithelial components. HE, original magnification x50.

Figure 4. Immunoreactivity of luminal EMC cells to cytokeratin 8 (NCL5D3), APAAP, original magnification x40.

Figure 5. EMC explant from the nude mouse after 1 year.
Table 1. Immunohistochemical findings of EMC transplanted to nude mice.

<table>
<thead>
<tr>
<th>Xenotransplant no.</th>
<th>KL1</th>
<th>MIB-1 (%)</th>
<th>VEGF</th>
<th>FLK1</th>
<th>EGFR</th>
<th>IGF-1R</th>
<th>Cell density (mm&lt;sup&gt;2&lt;/sup&gt;)</th>
<th>Morphology</th>
</tr>
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<tr>
<td>RF109/92</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>5170</td>
<td>Broad stromal septae</td>
</tr>
<tr>
<td>RF109-92-2</td>
<td>2</td>
<td>6</td>
<td>2/3</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>3720</td>
<td>Broad stromal septae</td>
</tr>
<tr>
<td>RF003/93</td>
<td>0</td>
<td>3</td>
<td>0/3*</td>
<td>0/2*</td>
<td>0</td>
<td>1</td>
<td>7410</td>
<td>Broad stromal septae</td>
</tr>
<tr>
<td>RF051/93</td>
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<td>3</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>3470</td>
<td>Broad stromal septae</td>
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<tr>
<td>RF088/93</td>
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<td>13</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>4620</td>
<td>Broad stromal septae</td>
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<tr>
<td>RF094/93</td>
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<td>21</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>4940</td>
<td>Broad stromal septae</td>
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<tr>
<td>RF006/94</td>
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<td>20</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>4750</td>
<td>Breakdown of stromal septae, incohesive growth similar to cell culture</td>
</tr>
</tbody>
</table>

*Strong variations of staining intensity, possibly due to technical reasons.

Figure 6. Histopathological demonstration of tumor progression in successive xenotransplants: (a) lack of EGFR-1 expression in early xenotransplant (RF109-92); (b) labelling of proliferative cells in the tumour shown in (a) with Ki-67 antibodies; (c) late xenotransplant (RF94-93) showing EGFR-1 expression; (d) high proliferative activity of the tumour shown in (c) (Ki-67 labelling, visualization of bound antibodies with diaminobenzidine).
The transplantation of an EMC to the nude mouse may be a valuable tool for investigating new therapeutic concepts in this rare cancer. Interestingly, the inhibition of EGFR of a salivary gland carcinoma transplanted to the nude mouse was an effective measure to reduce local tumour growth and distant spread (10). In conclusion, this study on EMC transplanted to the nude mouse demonstrates the feasibility of growing EMC in vivo.

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References