Abundant expression of cytokeratin 7 in fibrolamellar carcinoma of the liver

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Two cases of fibrolamellar carcinoma of the liver, one with lymph node metastasis are reported. Using immuno- histochrometry as well as one- and two-dimensional gel electrophoresis and Western blotting, tumour cells of both primary lesions and of the metastasis were found to express cytokeratin polypeptides 8 and 18 and, surprisingly, cytokeratin 7. A small number of cells also expressed cytokeratin 19. This is the first detailed analysis of the cytokeratin expression of fibrolamellar carcinoma, and is also the first to present biochemical evidence that, contrary to what has been suggested, hepatocellular carcinomas do not always preserve the pattern of cytokeratin expression of normal hepatocytes.

Keywords: liver, fibrolamellar carcinoma, cytokeratins

Introduction

Fibrolamellar carcinoma of the liver is a recently recognized variant of hepatocellular carcinoma with distinctive clinicopathological features1–3. Ultrastructural and immunohistochemical studies suggest that this tumour originates from hepatocytes and is highly differentiated4. The expression of intermediate filaments of the cytokeratin type in fibrolamellar carcinoma has so far only been studied on paraffin sections using broadly cross-reactive monoclonal antibodies which interact with epitopes that are shared by several cytokeratin polypeptides5. Normal human hepatocytes express only cytokeratins 8 and 18 of the catalogue of Moll et al.6, whereas intrahepatic bile duct cells contain, in addition, cytokeratins 7 and 198–11. In a recent study of 34 'typical' hepatocellular carcinomas, we found a variable number of tumour cells immunoreactive for cytokeratin 7 or 19, or both, in 17 specimens12. We wanted to find out whether the supposedly 'highly differentiated' fibrolamellar carcinoma could also express 'bile duct type' cytokeratins.

Case reports

CASE 1
A 9-year-old boy presented with persistent high fever for one week and intermittent pain in the right hypochondrium. The right lobe of liver was painful on palpation, and a mass was apparent 4 cm below the costal margin. Laboratory data revealed: sedimentation rate (147 mm), SGOT: 40 U/l, SGPT: 47 U/l; normal values for alphafetoprotein and neuron-specific enolase. Ultrasonography and CT-scan revealed a mass in the right liver lobe.

After staging, a right hemihepatectomy was performed. The liver lobe was normal on inspection and palpation. Post-operative recovery was uneventful. As there are no clear data on either radiation sensitivity or chemotherapy responsiveness, the parents requested no further treatment. Four months after diagnosis, multiple pulmonary metastases were apparent. Two courses of cis-platin in combination with doxorubicin were given.
Table 1. Monoclonal antibodies used in this study

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Specificity*</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monoclonal anti-cytokeratin (glandular epithelia)</td>
<td>7</td>
<td>Amer sham, Buckinghamshire, UK</td>
</tr>
<tr>
<td>RCK 10510</td>
<td>7</td>
<td>Dr. F.C.S. Ramaekers, Nijmegen, The Netherlands</td>
</tr>
<tr>
<td>Monoclonal anti-cytokeratin 814</td>
<td>8</td>
<td>Amer sham</td>
</tr>
<tr>
<td>M 2015</td>
<td>8</td>
<td>Dr. F.C.S. Ramaekers</td>
</tr>
<tr>
<td>Monoclonal anti-cytokeratin (simple epithelia)</td>
<td>18</td>
<td>Amer sham</td>
</tr>
<tr>
<td>RGE 5318</td>
<td>18</td>
<td>Dr. F.C.S. Ramaekers</td>
</tr>
<tr>
<td>CK 18.216,17</td>
<td>18</td>
<td>Dr. F.C.S. Ramaekers</td>
</tr>
<tr>
<td>RCK 10610</td>
<td>18</td>
<td>Dr. F.C.S. Ramaekers</td>
</tr>
<tr>
<td>Monoclonal anti-cytokeratin 19 (LP2K)18</td>
<td>19</td>
<td>Amer sham</td>
</tr>
<tr>
<td>A53-B/A2519</td>
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<td>Dr. U. Karsten, Berlin-Buch, East Germany</td>
</tr>
<tr>
<td>RKCE 602011</td>
<td>10</td>
<td>Dr. F.C.S. Ramaekers</td>
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<td>Monoclonal anti-desmin22</td>
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<tr>
<td>Monoclonal anti-vimentin23</td>
<td>Vimentin</td>
<td>Labsystems, Oy, Finland</td>
</tr>
<tr>
<td>RV 20220</td>
<td>Vimentin</td>
<td>Dr. F.C.S. Ramaekers</td>
</tr>
</tbody>
</table>

* Specificity of antibodies directed against cytokeratin are given as numbers of the catalogue of human cytokeratins.

Figure 1. Case 1: paraffin sections of a the primary liver tumour and b the lymph node metastasis, showing a typical fibrolamellar carcinoma. Tumour cells are large, polygonal with an eosinophilic cytoplasm. The tumour trabeculae are separated by lamellae of fibrous stroma. Note the presence of some remaining lymphoid cells in b. H & E. × 175.
Although no regression of the lung metastases could be observed, the patient is relatively well 1 year after diagnosis.

**CASE 2**

A 10-year-old boy was referred with a palpable hard mass in the epigastrium. Sixteen months earlier, he had had an episode of fever, vomiting and abdominal pain. Hepatitis A and B serology was negative, but serum transaminase levels were raised to five times the normal values; they remained slightly raised throughout the following year. On admission a hard, craggy, painless epigastric mass was palpated, which measured 8 cm from the sternum. CT-scan and ultrasound revealed a solid mass in the left liver lobe. Chest X-ray and chest CT-scan were normal.

Laboratory data revealed: SCOT: 31 U/l, SGPT: 46 U/l, and an alpha-fetoprotein level of 23.6 µg/ml. A left hepatectomy was performed and the post-operative period was uneventful. Follow-up is now only 2 months.

**Materials and methods**

Both hemi-hepatectomy specimens and an enlarged abdominal lymph node from the first patient were received fresh. Part of the tumours and part of the lymph node were snap-frozen in liquid nitrogen-cooled isopentane and stored at −70°C until use. The remainder was fixed in formalin and processed to paraffin for routine histological examination.

On serially cut cryostat sections of the primary tumours and of the lymph node metastasis, immunohistochemical staining was performed using a panel of monoclonal antibodies in a three-step indirect immunoperoxidase procedure. The antibodies, their source and specificity are listed in Table 1. Controls which were consistently negative comprised omission of the primary antibody.

**GEL ELECTrophoresis and IMMUNOBLOTTING**

Part of the lymph node metastasis was used for biochemical analysis. For this purpose, 20 µm thick frozen sections were collected and extracted with a buffer containing 0.5% Triton X-100, 5 mm EDTA, 0.4 mm phenylmethylsulphonylfluoride and 10 mm Tris-HCl, pH 7.2 for 5 min at 4°C by homogenization of the sections in a Dounce potter. The pellet obtained by centrifugation for 5 min at 12000 g was washed with cold (4°C) buffer containing 5 mm EDTA, 0.4 mm phenylmethylsulphonylfluoride and 10 mm Tris-HCl, pH 7.2. After a second washing step in phosphate buffered saline (PBS) the cytoskeleton preparation was dissolved by boiling for 5 min in SDS-sample buffer.

One-dimensional gel electrophoresis was performed in 10% polyacrylamide slab gels containing 0.1% SDS. To compare the amounts of protein loaded on each lane, gels were stained with Coomassie brilliant blue R250 (Gurr, Hopkin & Williams, Chadwell Heath, Essex, UK) as described before. Two-dimensional gel electrophoresis was performed essentially as described by O’Farrell.

In the first dimension, isoelectric focusing was performed in 4% polyacrylamide rod gels (Biorad Laboratories, California, USA) containing 1% Bioyte, pH 3.5–10 (Biorad). For the second dimension, the rod gels were applied directly onto the stacking gel of SDS-polyacrylamide gels.

For immunoblotting experiments the electrophoretically separated polypeptides were transferred to a nitrocellulose sheet (Schleicher & Schüll Membrane Filters BA 85, Dassel, West Germany) by blotting for 1 h at 100V in

![Figure 2. Cryostat section of fibrolamellar carcinoma stained with monoclonal antibody M20, reactive with cytokeratin 8. Tumour cells are very strongly positive. Indirect immunoperoxidase; counterstained with Mayer's haemalum. × 170.](image-url)
a cold (4°C) buffer containing 25 mM Tris-HCl, 192 mM glycine, 0.02% SDS and 20% methanol (pH 8.3)\(^{16}\). The blots were incubated for 90 min with PBS containing 0.05% Tween 20 (Sigma). All reagents were diluted in this solution, which was also used for the washing steps. After incubation overnight with undiluted culture supernatants of the primary monoclonal antibodies, the blots were washed three times for 10 min and incubated for 1 h with peroxidase-conjugated rabbit anti-mouse IgG (Dakopatts, Glostrup, Denmark) diluted 1:400. Thereafter the blots were washed again three times for 10 min in 0.05% Tween 20/PBS and once in PBS alone for 10 min and stained with 4-chloro-1-naphthol (Merck, Darmstadt, West Germany) and 0.12% hydrogen peroxide (Merck). After staining, the blots were rinsed for 5 min with water.

Results

PATHOLOGICAL FINDINGS

Macroscopically, both tumours were well-delineated and contained a central scar-like area. Microscopically, they corresponded to typical fibrolamellar carcinomas (Figure 1). Sections of the lymph node showed metastatic fibrolamellar carcinoma almost completely replacing the nodal architecture (Figure 1b).

Figure 3. Cryostat section of fibrolamellar carcinoma of the liver and b the lymph node metastasis, stained with monoclonal antibody CK 105, specifically reactive with cytokeratin 7. Most tumour cells are strongly immunoreactive. Indirect immunoperoxidase; counterstained with Mayer’s haemalum. ×175.
IMMUNOHISTOCHEMICAL FINDINGS

Malignant cells of both primary tumours and of the lymph node metastasis showed identical immunohistochemical staining profiles. In frozen sections, all tumour cells were strongly positive with all the monoclonal antibodies directed against cytokeratins 8 and 18 (Figure 2). Most tumour cells were also intensely immunoreactive with monoclonal anti-cytokeratin antibodies (glandular epithelia) and RCK 105, both specifically directed against cytokeratin 7 (Figure 3). Only a small number of tumour cells were less strongly positive, a few being negative.

A small number of tumour cells also expressed cytokeratin 19, as assessed by staining with both monoclonal anti-cytokeratin 19 and A53-B/A2 (Figure 4). No immunoreactivity for cytokeratin 10, vimentin or desmin was detected in tumour cells.

IMMUNOBLOTTING ASSAYS

Cytoskeletal fractions were analysed by one- and two-dimensional gel electrophoresis. From the stained gels it
was apparent that cytoskeletal proteins migrating in the positions of cytokeratins 7, 8 and 18 were present in these preparations. One- and two-dimensional immunoblotting assays with these preparations and the chain-specific monoclonal anti-cytokeratin antibodies proved the cytokeratin nature of these polypeptides and characterized them as cytokeratins 7, 8 and 18 (Figure 5). Due to the low number of cells expressing cytokeratin 19, we were not able to convincingly prove the presence of this polypeptide in the cytoskeletal preparations. Vimentin was detected in the cytoskeletal preparations, probably because a number of lymphocytes (which contain vimentin) were still present in the metastatic lymph node.

Discussion

We present the first detailed analysis of cytokeratin expression in fibrolamellar carcinoma of the liver. Johnson et al. have previously reported staining of five paraffin-embedded fibrolamellar carcinomas with monoclonal antibody CAM 5.2 which reacts with cytokeratins 8, 18 and 19. The expression of the 'hepatocytic' cytokeratins 8 and 18 in tumour cells was an expected finding. Surprisingly, tumour cells also strongly expressed cytokeratin 7 which, in normal liver, occurs only in bile ducts. A small number of tumour cells even contained cytokeratin 19. Surprisingly, none of the five fibrolamellar carcinomas studied by Johnson et al. was found to be immunoreactive with monoclonal antibody AE-1 which, in the liver, reacts only with cytokeratin 19. Western blotting studies on a cytoskeletal preparation of the lymph node metastasis examined by us confirmed the immunohistochemical data for cytokeratins 7, 8 and 18, providing for the first time biochemical evidence that a hepatocellular tumour can express a 'bile duct type' cytokeratin. Both the primary tumour and the metastasis had an identical cytokeratin pattern. Studies on other tumours have suggested that metastatic lesions often retain the pattern of cytokeratin expression of the primary tumour.

Our findings clearly demonstrate that neoplasms, apparently originating from hepatocytes, do not always preserve the cytokeratin pattern of normal liver parenchymal cells. This has been reported previously in typical hepatocellular carcinomas, but no biochemical proof was provided. Our previous study and the present one clearly indicate that the presence of immunoreactivity for cytokeratins 7 and/or 19 in a liver tumour does not imply that the tumour is a cholangiocarcinoma. Cytokeratins should, thus, probably be used as markers for certain types of epithelial differentiation rather than as markers for histogenesis. Tumour cells originating from hepatocytes can apparently acquire phenotypic characteristics of bile duct cells, including bile duct type cytokeratins. In some cases, this phenotypic shift may even result in the appearance of mixed hepatocellular-cholangiocarcinoma as suggested by Fischer et al. Although we found abundant expression of cytokeratin 7 in the fibrolamellar carcinomas studied, the tumour cells did not have the light microscopic appearance of bile duct cells. The occurrence of bundles of tonofilaments has been reported in ultrastructural studies of fibrolamellar carcinoma. Such bundles are not a feature of normal liver parenchymal cells, but are present in bile duct cells. Unfortunately, material processed for electronmicroscopy was not available in our cases.

Finally, since we studied only two cases and one metastasis, it is uncertain whether abundant expression of cytokeratin 7 is a constant feature of fibrolamellar carcinoma. This will have to await analysis of additional cases.

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