Is renal cell (Grawitz) tumor a carcinosarcoma?

Evidence from analysis of intermediate filament types*

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Summary. The expression of intermediate filament type was determined in 13 renal cell (Grawitz) tumors (10 primary renal tumors and 3 lymph node metastases). All of the tumors except one lymph node metastasis contained cells expressing vimentin intermediate filaments, generally a marker of mesodermally-derived tissues and their tumors, the sarcomas. In addition, the 10 primary renal tumors and two lymph node metastases contained cells expressing keratin proteins. Using a monoclonal antibody to keratins, specific for glandular epithelial cells, it has been shown that some of the tumor cells resemble adenocarcinomas, at least in this respect. Double immunofluorescence labeling demonstrated that some of the vimentin-containing cells contained keratin while others did not. Only occasional cells were found to contain keratin but not vimentin. However, one of the lymph node metastases was positive only for vimentin. Thus Grawitz tumor cells express intermediate filament types which are generally biological markers of both sarcomatous and carcinomatous tumors.

Key words: Intermediate filaments – Cytoskeleton – Keratin – Vimentin – Renal cell adenocarcinoma

Introduction

The origin and nature of renal cell (Grawitz) tumor has been controversial since the original description of this tumor (for review see Bennington and Beckwith 1975). The morphologic and histochemical features of the tumor have gradually led pathologists to accept that it is an adenocarcinoma,

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* Supported in part by a grant from the Netherlands Cancer Society, Queen Wilhelmina Fund. A preliminary report of this work was presented at the International Academy of Pathology, US – Canadian division meeting, Atlanta, Ga, USA, March, 1983 (Herman et al. 1983)

Materials and methods

Tumor tissue from twelve patients with a histologic diagnosis of Grawitz tumor was utilized in the present study. Table 1 lists the pertinent clinical and pathologic data. Between one and seven blocks of tumor were taken from fresh surgical specimens and frozen immediately in liquid nitrogen. The blocks were chosen to include all macroscopically identifiable patterns of tumor, including the border of tumor with normal kidney, but excluding necrotic and hemorrhagic areas. Six um frozen sections were cut and one section stained with hematoxylin and eosin. Further sections from the same blocks were stained by the immunofluorescence technique using rabbit antisera to keratin and vimentin (for detailed description of immunohistochemical methods, see Ramaekers et al. 1981, 1983c) and a monoclonal antibody to keratin, specific for glandular epithelium. In our experience and that of other investigators, keratin proteins are specific for cells of epithelial origin (Sun et al. 1979), while vimentin is in general a marker for cells of mesodermal derivation (Franke et al. 1978; see also Discussion). The production and specificity of the polyclonal antibodies to keratin and vimentin used in the present study have been described in detail elsewhere (Ramaekers et al. 1981, 1982, 1983a, b, d). Because of the recent demonstration of keratin-reactivity with anti-vimentin sera (Moll et al. 1982), we have used anti-vimentin antisera pre-absorbed with either human callus keratin or purified bovine lens vimentin. Furthermore, the anti-vimentin antisera was analyzed for its specificity by one-dimensional immunoblotting following SDS-polyacrylamide gel electrophoresis (Towbin et al. 1979). Figure 1 shows that the anti-vimentin antibodies interact only with a 57 Kd protein band in cytoskeletal preparations from HeLa cells and human hepatocellular carcinoma cells (PLC/PRF/S). This protein was identified further by 2-dimensional gel electrophoresis (O'Farrell 1975) as vimentin.

In addition, a monoclonal antibody (RGE 53) which reacts specifically with a keratin protein of glandular epithelium (Ramaekers et al. 1983e, f) was used in the present study. Briefly, this antibody was prepared as follows: Balb/C mice were immunized intraperitoneally with approximately 50 μg of HeLa-cell cytoskeleton, prepared by extracting a cell pellet with Triton X-100 and 1.5 M KCl, and 100 μg of human callus keratins. After two weeks the mice were boosted twice (intravenously four and two days prior to spleen harvesting). Splenic lymphocytes were isolated and fused with mouse myeloma Sp 2/0 Ag 14 cells in PEG-4000 and hybrids grown in 24-well clusters in RPMI 1640 (Dutch modification) containing 15% fetal calf serum. The cells were incubated 24 h before adding HAT-medium and hybridomas were tested for antibody production two weeks later. Cultures showing fibrillar staining when tested on HeLa-cell monolayers and a negative reaction on human skin epidermis by the indirect immunofluorescence technique were cloned, tested again for fibrillar staining in HeLa cells, subcloned and grown according to standard techniques (Fazekas de St Groth and Scheidegger 1980). Undiluted culture medium or 1:500 to 1:1,000-diluted ascitic fluid from Balb/C mice injected with RGE 53 hybrids were used for tests on frozen sections of human tissues by the indirect immunofluorescence technique. FITC-conjugated rabbit antimouse IgG (Nordic, Netherlands) was used as a second antibody. As a control, growth medium from non-producing hybridomas or hybridomas producing antibodies specific for human epidermis were used. The double labeling immunofluorescence technique using RGE 53 and
Table 1. Clinical and pathologic data of 12 patients with renal cell (Grawitz) tumor, including intermediate filament types

<table>
<thead>
<tr>
<th>Patient age/sex</th>
<th>Tumor location</th>
<th>Histologic type</th>
<th>Vimentin</th>
<th>Keratin</th>
<th>RGE 53</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–53 M</td>
<td>Kidney</td>
<td>Granular cell, solid</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Clear cell, tubular</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2–73 F</td>
<td>Kidney</td>
<td>Clear and granular cell, solid and tubular</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3–49 M</td>
<td>Kidney</td>
<td>Clear cell, solid</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4–72 M</td>
<td>Kidney</td>
<td>Granular cell, tubular</td>
<td>+</td>
<td>+</td>
<td>NDb</td>
</tr>
<tr>
<td>5–57 F</td>
<td>Kidney</td>
<td>Clear cell, solid and tubular</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6–39 F</td>
<td>Kidney</td>
<td>Clear and granular cell, solid and tubular</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7–57 M</td>
<td>Kidney</td>
<td>Clear cell, solid and tubular</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8–65 F</td>
<td>Kidney</td>
<td>Clear cell, tubular</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9–67 M</td>
<td>Kidney</td>
<td>Clear cell, solid and tubular</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<tr>
<td>10–42 M</td>
<td>Kidney</td>
<td>Clear cell, solid and tubular</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Lymph node</td>
<td></td>
<td>Clear cell, solid and tubular</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11–23 M</td>
<td>Lymph node</td>
<td>Granular cell, tubular and papillary</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12–46 M</td>
<td>Lymph node</td>
<td>Clear and granular cell, solid and papillary</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*RGE 53: Monoclonal antibody which reacts specifically with glandular epithelium. See text
*ND: Not done

Fig. 1a–c. One-dimensional SDS-polyacrylamide gel electrophoresis of cytoskeletal proteins from cultured HeLa cells (a), human hepatocellular carcinoma cells (b) and human callus keratin (c). This figure also shows results of the immunoblotting assay of the anti-vimentin antiserum when tested on these gel lanes after electrophoretic transfer to nitrocellulose sheets (d, e, f respectively). Note reaction of the serum only with a polypeptide band in the 57 kd molecular weight region and no reaction with keratins of diverse sources (V, vimentin; A, actin).

The anti-vimentin or anti-keratin antisera was performed as described previously (Ramaekers et al. 1983a).

RGE 53 has been tested for its reaction on cultured cells and in the immunoblotting assay. These experiments have shown that the RGE 53 antibody produces fibrillar staining patterns in cultured HeLa cells, in cultured human hepatocellular carcinoma cells (PLC/PRF/5), in differentiated F9 mouse teratocarcinoma cells and in a human colon carcinoma cell line (WiDr-218). The fluorescence obtained in these cells demonstrates a typical intermediate filament cytoskeleton organization, virtually identical to the filament patterns visualized by rabbit
antiserum to human skin keratin. No reaction was seen in cultured bovine lens cells. Furthermore, immunoblotting has shown that RGE 53 antibodies interact with a cytoskeleton-associated polypeptide with a molecular weight of approximately 45,000 (cytokeratin 18; Moll et al. 1982b) in both HeLa cells and cultured human hepatoma cells (PLC/PRF/5). RGE 53 is an IgG1 with kappa light chains. For further details on reactions of RGE 53 on human tissues, both normal and tumorous, see Ramackers et al. (1983a, b). Two-dimensional gel electrophoretic analysis of cytoskeletal preparations of Grawitz tumors was performed as described by O'Farrell (1975). Cytoskeletal proteins from the tumors were prepared by extracting tissue with Triton X-100 and high salt buffers essentially as described by Franke et al. (1981b).

Results

Of the thirteen tumors examined, ten were sections of kidney containing the primary tumor and three were of lymph node metastases. To ensure that staining artefacts did not produce either false-positive or false-negative results, immunofluorescent staining of tumor cells was compared with the reactions of other, non-neoplastic cells of known reactivity in the same tissue sections. For example, transitional epithelial cells of the renal pelvic epithelium contain keratin but not vimentin while vascular endothelial cells show the opposite reaction by immunofluorescence staining.

As can be seen in Table 1, the tumors investigated contained both clear and granular cell types and tubular, papillary and solid areas. All of these histologic patterns demonstrated tumor cells which contained vimentin. In the cases examined, most of the tumor cells were vimentin-positive (Fig. 2a, b). These vimentin-containing tumor cells were demonstrated in both primary and metastatic tumors, and thus are not a metastasis-dependent phenomenon. Ten of the thirteen tumors also contained cells staining positively for keratin (Fig. 2c). It is noteworthy that two lymph node metastases contained cells expressing only vimentin (Case 11) or only keratin (Case 12).

The immunofluorescence pattern produced by staining with the monoclonal antibody to glandular epithelium (RGE 53) was qualitatively similar to that produced by the heterologous keratin antiserum (Table 1). However, the staining reaction with RGE 53 was generally stronger than with the anti-keratin serum. Thus double labeling was carried out with the RGE 53 antibody in combination with the anti-vimentin antiserum (Fig. 3a, c). Using this technique, some vimentin-positive cells were also positive with RGE 53 (Fig. 3b), while other vimentin-containing cells were negative with the RGE 53 antibody (Fig. 3d). Only rarely were cells encountered which stained positively for keratin but were negative for vimentin.

As additional controls, anti-vimentin antiserum that was absorbed either with a purified calf lens vimentin preparation or with human callus keratin, and anti-keratin antiserum that was absorbed with keratin were used. Fluorescence was completely eliminated by absorption with the homologous antigen (Fig. 2d), while absorption of vimentin antiserum with keratin did not affect staining. Antiserum to desmin (the muscle-specific type of intermediate filament) or glial fibrillary acidic protein (specific for glial tissues) did not stain the Grawitz tumor cells (results not shown). Two-dimensional gel electrophoretic analysis of cytoskeletal preparations from primary
Fig. 2a-d. Frozen sections from metastatic (a, b) and primary (c, d) Grawitz tumors incubated with an anti-vimentin antiserum (a, b), a polyclonal anti-keratin antiserum (c) and an antiserum to vimentin which has been preabsorbed with purified calf lens vimentin prior to incubation (d). a, × 200; b-d, × 400
Fig. 3a–f. Double labeling immunofluorescence of primary Grawitz tumor frozen sections incubated with rabbit antibodies to vimentin (a, c) or keratin (e) and a monoclonal anti-keratin antiserum (b, d, f). a and b, c and d, and e and f represent identical areas viewed either in the fluorescein (a, c, e) or rhodamine (b, d, f) channels. Note the positive reaction in the blood vessel (BV) endothelium and smooth muscle cells with anti-vimentin (a) but not with the monoclonal anti-keratin antibody (b). a–f, × 400
Grawitz tumors shows that the vimentin spot, detected in these gels (Fig. 4a, b), behaves identically to vimentin from other sources, for example, human thecofibroma (Fig. 4c) and bovine lens (not shown).

Discussion

The present study demonstrates that twelve of thirteen primary and metastatic Grawitz tumors contain tumor cells expressing vimentin intermediate filament protein. In addition, eleven Grawitz tumors present either as primary tumor in the kidney or as metastases in lymph nodes also contained cells expressing keratin. This keratin is shown to contain determinants which are immunochemically identical to those from a keratin protein expressed by glandular but not by stratified squamous epithelial cells. Double labeling experiments have shown that some vimentin-containing Grawitz tumor cells also express keratin while others do not. Only occasional cells express keratin but not vimentin. A striking observation in this respect is the finding of a metastatic Grawitz tumor which is only positive for keratin (Table 1, Case 12).

Interpretation of the significance of these findings is made more difficult by the nature of the vimentin protein. As has been pointed out (Damjanov 1982), cellular expression of vimentin behaves in at least some cases like a differentiation marker. For example, immature muscle cells (Bennett et al. 1979) and glial cells express vimentin which is later replaced by the mature tissue-specific intermediate filament protein type, desmin and glial fibrillary acidic protein respectively (see also Franke et al. 1981a; Osborn et al. 1981). A more serious problem in interpreting the results of the present study is the fact that carcinoma cells can, under somewhat abnormal circumstances
of growth, express vimentin in addition to keratin. These circumstances include in vitro growth (Franke et al. 1979) and growth in malignant effusions in animals and man (Ramaekers et al. 1983a). Thus, interpretation of the present study in terms of histogenesis of Grawitz tumor would be unjustified. Another complicating factor has been described recently by Moll and associates (1982a). These authors have shown that anti-vimentin antisera may, in exceptional cases, show cross-reactivity with keratin polypeptides. We have excluded this possibility in our material by preabsorption of the anti-vimentin antiserum with both purified keratin and vimentin.

The tumors studied in the present series were all solid tumors, the majori-
ty of them being the renal primary. Under these circumstances all carcinomas studied in our laboratory have expressed only keratin (Ramaekers et al. 1983a-f). The expression of vimentin by Grawitz tumor may therefore be a signal of its biologic potential and, in these terms, a marker that Grawitz tumor should be considered a carcinosarcoma rather than a pure adenocarcinoma. This interpretation of the present study correlates well with some clinical features of Grawitz tumor, especially its marked resistance to adjuvant therapy and its tendency to early blood vessel invasion and hematogenous dissemination in addition to lymph node metastasis. By analogy, the recent report of intermediate filaments of the keratin type in the epithelioid cells of synovial sarcoma (Miettinen et al. 1982) may provide an explanation for this tumor's tendency to lymph node metastasis (Hajdu 1979) if it is also considered biologically a carcinosarcoma.

Even prediction of biologic behavior with the help of intermediate filament analysis requires considerable caution, however. It is interesting to note that in the present series the three lymph node metastases available for study contained tumor cells expressing either vimentin or keratin alone or both. Clearly a larger number of primary and metastatic Grawitz tumors must be analyzed and the intermediate filament expression by the tumor cells compared both qualitatively and quantitatively with the clinical course of the patient. These results may also shed some light on cell biological aspects of the process of metastasis of this tumor.

The use of immunopathological techniques to investigate the histogenesis of tumors will certainly continue to suggest new answers to old questions. In addition, the expression of intermediate filament protein type(s) may eventually prove to be a valuable adjunct in predicting a tumor's biologic potential, independent of its histogenesis. Whether this prediction of behavior can be translated into more effective management of patients with cancer remains to be demonstrated in retrospective and prospective studies.

References


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Received May 2 / Accepted June 6, 1983