Histochemical Analysis of the Development of Estradiol-induced Kidney Tumors in Male Syrian Hamsters

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ABSTRACT

Chronic administration of the estrogen 17β-estradiol induces kidney tumors in male Syrian hamsters within 6 months of initial exposure. Although these tumors have previously been studied histologically and histochemically and have been postulated to be derived from proximal tubular and/or interstitial cells, there exists no unambiguous evidence for an epithelial or mesenchymal origin. To elucidate the histogenesis of these neoplasms, kidney sections of hamsters treated with estradiol for 4, 5, and 6 months and age-matched untreated controls were investigated histologically and histochemically. Proliferating foci were observed in kidneys exposed to estradiol for 5 and 6 months. They consisted of clusters of spindle-shaped cells forming solid blocks, cords, or branches located between tubules. These foci were judged to be precursors of larger tumors identified in the latter treatment group. The histological and histochemical profile of foci and tumors matched closely. These lesions were marked by very high activities of alkaline phosphatase, adenylyl cyclase, and glucose 6-phosphate dehydrogenase. In contrast, glycogen content and activities of glucose 6-phosphatase, succinate dehydrogenase, and γ-glutamyl transpeptidase were low or absent. Immunofluorescence studies revealed that foci and tumors solely expressed vimentin and desmin but not cytokeratin.

The morphology, enzyme histochemical pattern, and immunofluorescence strongly support a mesenchymal origin of the estradiol-induced hamster kidney tumors studied. The neoplasms were probably derived from vascular smooth muscle cells of a cell subtype particularly sensitive to hormonal stimulation and transformation.

INTRODUCTION

Natural and synthetic estrogens induce kidney tumors in male Syrian hamsters (1). The mechanistic details and origins of tumor development are still unknown, although these neoplasms have been investigated extensively (reviewed in Refs. 2 and 3) as a model system of hormone-associated cancer. In early histological studies (4–6), an epithelial origin of these tumors arising from proximal or distal convoluted tubules has been postulated. Some authors (4, 7, 8) discussed both epithelial and mesenchymal origins for these tumors. In subsequent histological characterizations by Liombart-Bosch and Peydro (9, 10), 3 types of tumor structure were worked out: a solid blastemal structure consisting of cords of anastomosing cells. Less common were solid tubules or papillary-cystic formations. The 3 histological types were described to be distributed at random within the same kidney tumor displaying a continuity between epithelial and blastemal-mesenchymal cells. According to these authors (9), the histochemical characterization of the tumor revealed an enzymatic activity resembling that of normal proximal convoluted tubule. They further concluded from their experiments that the neoplasms may have an interstitial cell origin and may develop into a solid-blastemal and a tubular-epithelial neoplasm. Although elevated incorporation of [3H]-estradiol as well as [3H]thymidine and [3H]uridine in proximal tubule cells of estrogen-treated hamster kidneys (11, 12) has been shown, direct evidence for an epithelial and/or mesenchymal origin is still missing.

This continuing uncertainty with respect to origin and cell type(s) of the tumors led to investigations using novel histochemical and immunocytochemical techniques. The results of these tests provide evidence for a mesenchymal origin of the estrogen-induced kidney tumors studied.

MATERIALS AND METHODS

Induction of Tumors. Male Syrian hamsters (4–6 weeks of age, from Harlan Sprague-Dawley, Houston, TX) were divided into 2 groups of 15 animals each. On day 0 and after 3 months, the first group was treated each with one pellet of 17β-estradiol (25 mg estrogen plus 10% cholesterol) implanted s.c. as described previously (1, 13, 14). The other group was left untreated. Throughout the experiment, Purina rodent chow and water were available ad libitum. After 4, 5, and 6 months, 5 hamsters of each group were killed by decapitation and their kidneys were immediately excised. One kidney of each animal was rapidly frozen in isopentane at −140 to −150°C and stored in a −75°C freezer. The other kidney was fixed in Carnoy's solution for 4–5 h and subsequently embedded in paraffin for light microscopy as described previously (15).

Histology. Hematoxylin/eosin and PAS3 (16) stains were used for histological examinations.

Histochemistry. Native kidney cryostat sections of all treatment groups and controls were analyzed for activities of the following enzymes using methods developed or adapted in one of our laboratories (17–20): SYN, PHO, G6Pase, G6PDH, GAPDH, SDH, ALP and ADC. In addition, the sections were also analyzed for the presence of glycogen by PAS, for acidic mucosubstances by cationic colloidal iron (21), and neutral lipids by fat red B (22).

Immunohistochemistry. For the demonstration of intermediate filaments in tissues, monoclonal anti-cytokeratin (RPN. 1160, directed to cytokeratins of simple epithelia) antibodies purchased from Amersham Laboratories (Buckinghamshire, England), antivimentin (katal. no. 814318; Boehringer, Mannheim, Federal Republic of Germany) and antidesmin (katal. no. 814377; Boehringer) were used in combination with Texas Red conjugated goat anti-mouse antibodies (code no. 1157568, dianova; Hamburg, Federal Republic of Germany). Sections of frozen tissue were processed for immunofluorescence microscopy as described by Franke et al. (23).

In addition, paraffin sections of Carnoy-fixed kidneys from experimental and control animals sacrificed at 4, 5, and 6 months were tested for the presence of renin using specific antibodies (kindly provided by Prof. C. Taugner, Physiology II, University of Heidelberg). For visualization the peroxydase-antiperoxydase procedure was applied (24).

RESULTS

Tumor Induction. Using the procedure of Kirkman (1), renal tumors were induced in male Syrian hamsters by chronic treat-

1 The abbreviations used are: PAS, periodic acid/Schiff's reaction; SYN, glycogen synthetase, EC 2.4.1.11; PHO, glycogen phosphorylase, EC 2.4.1.1; G6Pase, glucose 6-phosphatase, EC 3.1.3.2; G6PDH, glucose 6-phosphate dehydrogenase, EC 1.1.1.49; GAPDH, glyceraldehyde 3-phosphate dehydrogenase, EC 1.2.1.12; SDH, succinate dehydrogenase, EC 1.3.99.1; ALP, alkaline phosphatase, EC 3.1.3.1; γ-GT, γ-glutamyl transpeptidase, EC 2.3.2.2; ADC, adenylyl cyclase, EC 4.6.1.1.

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2 To whom requests for reprints should be addressed.

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HISTOCHEMISTRY OF HAMSTER KIDNEY TUMORS

Fig. 1. a, stained paraffin section of a kidney tumor after treatment with 17β-estradiol for 6 months. Note also small nests of highly basophilic tumor cells between intact tubules (arrows) of kidney remnant adjacent to the tumor. H & E, × 41. b, very high ALP activity in kidney tumor of a male Syrian hamster treated with 17β-estradiol for 6 months. The dark area (arrow) indicates a region with tumor cells containing glycogen. PAS, × 26. c and d, serial sections. d, decreased PHO activity in the region of glycogen-containing tumor cells (arrow) shown in c. High PHO activity in tumor cells surrounding this glycogen-containing region. × 26. e, very high ADC activity in kidney tumor of a male Syrian hamster treated with 17β-estradiol for 6 months. × 6. b, e, and f, serial cryostat sections. f, very high G6PDH activity in kidney tumor of a male Syrian hamster treated with 17β-estradiol for 6 months. × 6.

Histology. Hamster kidneys exposed to 17β-estradiol for 4 months did not show any proliferating lesions or tumors and were indistinguishable from age-matched controls. Small proliferating foci were detected in hamster kidneys exposed to estrogen for 5 and 6 months. In addition, kidneys of the latter treatment group also contained large tumors of 1–5 mm di-

ment with 17β-estradiol. By gross examination, 3 of 5 hamsters carrying 17β-estradiol implants for 5 months had kidney tumors. In the 6-months’-treatment group, all animals presented neoplastic lesions. Kidneys of the 3 treatment groups and age-matched controls were analyzed histologically and histochemically.
ameter. It was noted that most of the small proliferating foci were localized close to renal blood vessels, the arteriae arcuatae. The foci consisted of clusters of spindle-shaped cells forming solid nests, cords, or branches intercalating between intact tubules. Both foci and tumor cells were highly basophilic when stained with hematoxylin/eosin or toluidine blue and showed similar morphology (Figs. 1a and 2a). In contrast to previous findings of others (8, 9) but in accordance with Arcadi (25)
some areas of the tumors contained small amounts of glycogen as demonstrated by PAS stains (Fig. 1c). Small droplets of neutral lipids were regularly found in the small proliferating foci. In the larger tumors, lipids were not uniformly distributed but were present only in the vicinity of necrotic areas. In intact tumors, neutral lipids could not be detected. Also, tumor cells stained positively with colloidal iron (21) possibly indicating the presence of acidic mucosubstances or other highly negatively charged compounds.

Histochemistry. Histochemical analyses were carried out to shed light on the origin of the tumors and to evaluate any differences in biochemical characteristics between tumor cells and proliferating foci. According to Table 1 no differences in enzyme activities were detected between proliferating foci and kidney tumors. As mentioned above, some of the tumors contained small amounts of glycogen (Fig. 1c). In contrast, the proliferating foci were free of any detectable glycogen. The close match in morphological and in biochemical characteristics between proliferating foci and kidney tumors identify the foci as early stages of these neoplasms. High ALP (Figs. 1b and 2b) and lacking G6Pase were observed in tumors and proliferating foci (Table 1). However, SDH activity could not be detected in proliferating foci and in tumors (Table 1), while decreased activity of this enzyme was described previously (9). Moreover, G6PDH activity in foci (Fig. 2d) and tumors (Fig. 1f) was markedly increased (Table 1), while no such activity could be found by previous authors (9, 26) using histochemical methods. Our findings agree with the results of electrophoretic studies of Dodge (27, 28) on G6PDH isozymes in estrogen-induced hamster kidney tumors.

Since glycogen was identified in some areas of the tumors, the distributions of SYN, PHO, and ADC, which previously had not been studied, were also investigated (Table 1). Activity of SYN was detected only in those cells which also contained glycogen (Table 1). They were identified in these areas by a reddish-brown colored iodine/glycogen complex. This color is typical of glycogen synthesized by SYN or PHO in concert with branching enzyme (29). PHO activity (Fig. 1d) was also demonstrated in these areas showing the same coloring with increased intensity. In glycogen-free regions of the tumors, however, PHO activity was characterized by a black-blue stain. This color strongly points to a lack of the branching enzyme yielding an amylose-like polysaccharide with little branching, which stains with iodine in a black-blue color (29). Since PHO is regulated by a cascade of activating processes in which ADC plays a key role (30), the latter enzyme was also studied (20). In proliferating foci and in tumors, this enzyme was uniformly distributed with very high activity (Figs. 1e and 2c).

GAPDH, an important glycolytic enzyme which had frequently been found to be increased in kidney tumors (31), was either unchanged or even slightly decreased when compared to untreated or 17β-estradiol-exposed kidneys (Table 1). In many human or experimental tumors in laboratory animals, γ-GT was demonstrated to have decreased activity when compared to normal tubules (31). In 17β-estradiol-induced proliferating foci and renal tumors, this enzyme consistently was absent (Table 1).

Immunohistochemistry. Intermediate filament typing of tissues yields information relevant to their histogenetic or cytogenetic origin. Hamster kidney tumors were therefore examined using specific antibodies to cytokeratin, vimentin, and desmin. Untreated hamster kidneys showed the same distribution of cytokeratin, vimentin, and desmin (data not shown) as had previously been demonstrated for rat kidneys (32). All proliferating foci and tumors in 17β-estradiol-exposed hamster kidneys were devoid of cytokeratin but were rich in vimentin and desmin (Fig. 3). Control reactions without specific antibodies against cytokeratin, vimentin, or desmin were devoid of immunofluorescence (figs. not shown). The absence of cytokeratin and the presence of vimentin and desmin in cells of tumors and proliferating foci demonstrate their mesenchymal origin.

Renin could be demonstrated in the juxtaglomerular granular cells of vas afferens in kidneys of controls and 17β-estradiol-treated animals. It was, however, lacking in foci as well as in tumors.

**DISCUSSION**

Kidney tumors induced by chemical carcinogens have been studied in detail (reviewed in Refs. 31 and 33) and have been compared to different types of human renal tumors (31). The experimental epithelial tumors of renal parenchyma closely resemble those of man in morphology and histochemical profile (31, 33). In particular, G6Pase, ALP, γ-GT, and acid phosphatase were decreased, whereas enzymes of the glycolytic, e.g., GAPDH, and pentosephosphate pathway, e.g., G6PDH, were increased (31). Enzymes directly involved in glycogen metabolism, PHO and SYN, did not show consistent patterns.

The series of histochemical tests used previously (31) was now applied to 17β-estradiol-induced hamster kidney tumors to identify their origin. Although these hamster neoplasms have been suggested to be derived from proximal tubule (4, 6), an interstitial cell origin has also been discussed (4, 7). The histochemical pattern of proliferating foci and tumors in 17β-estradiol-exposed hamster kidney (Table 1) did not provide unambiguous evidence for an epithelial or mesenchymal origin: G6PDH activities in hamster kidney tumors (Table 1) closely matched the elevated enzyme activities in rat epithelial tumors (31). In contrast, the following differences in enzyme activities were found: G6Pase, SDH, and γ-GT activities, decreased in chemically induced rat kidney tumors (31), were completely absent in 17β-estradiol-induced hamster kidney tumors or proliferating foci (Table 1). Furthermore, ALP activities, also decreased in rat kidney tumors (31), were markedly increased in hamster lesions (Figs. 1b and 2b; Table 1). The activities of this enzyme and also that of ADC (Figs. 1e and 2c) were so elevated that these histochemical tests could be used as marker reactions for the identification of 17β-estradiol-induced tumors and proliferating foci in hamster kidney. It should be noted, however, that activities of ALP (34), G6PDH (35), or other enzymes may have been elevated by the direct action of administered hormone and may not be linked to cell transformation. As to Kirkman’s (7) observation of high alkaline phosphatase

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Table 1  Histochemistry of proliferating foci and tumors in 17β-estradiol-treated hamster kidney

<table>
<thead>
<tr>
<th>Enzymatic activity</th>
<th>Foci</th>
<th>Tumors</th>
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<tr>
<td>Glycogen</td>
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<td>SYN</td>
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<td>ADC</td>
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<td>G6Pase</td>
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<td>G6PDH</td>
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<td>GAPDH</td>
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<td>SDH</td>
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<td>ALP</td>
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<td>γ-GT</td>
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* - none; +, low activity; ± none in some, low activity in other cells; ++, moderate activity; ++++, high activity.

* H. J. Hacker and P. Bannasch, unpublished data.
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Fig. 3. Immunofluorescence microscopy of tumor-bearing kidney of a hamster treated with 17β-estradiol for 6 months. In a, kidney tissue is reacted with anticytokeratin. Note intense immunofluorescence (bright areas) in adjacent tubular remnant compressed by totally dark tumor tissue. b, reaction for vimentin. Note intense fluorescence in tumor tissue as well as in the interstitium between the dark-appearing kidney tubules. In c, tumor cells are highly positive for desmin which is also found in the interstitium of the adjacent dark kidney tubules in the form of short bright rods (arrow). Serial cryostat sections. ×160.

activities in certain fibroblasts and his suggestion that these might be a site of origin of the estrogen-induced hamster kidney tumors we could not detect such cells in the nonneoplastic cortical renal tissue.

The difficulties experienced in deriving an epithelial origin for the 17β-estradiol-induced hamster lesions from comparisons with histochemical patterns of epithelial tumors in rats or humans required the application of immunohistochemical methods. Intermediate filament typing allows a positive classification of normal cells and tumors as to whether they are of epithelial, mesenchymal, muscle, glial, or neuronal origin (36). The absence of cytokeratin and the presence of vimentin and desmin has previously been found to be expressed in a hamster cell line, BHK-21, probably derived from mesangial or vascular smooth muscle cells (37). An identical intermediate filament typing has now been demonstrated in 17β-estradiol-induced
hamster kidney tumors (Fig. 3) thus excluding an epithelial origin. The morphology, enzyme histochemical profile, and intermediate filament typing demonstrate a mesenchymal origin of 17β-estradiol-induced hamster kidney tumors. The neoplasms are probably derived from vascular smooth muscle cells. A particular subtype of vascular smooth muscle cells are the juxtaglomerular granular epithelioid cells well known for their renin production (38). Dodge and Kirkman (39) described a secretory stilbestrol-induced renal tumor of Syrian hamster and postulated an origin of this tumor from juxtaglomerular cells. Because we could not detect renin in either foci or tumors, we think that the granular epithelioid cells of the vas afferens most probably are not the site of origin of the kidney tumors. Further studies are necessary to identify the mesenchymal cell sensitive to hormonal stimulation and neoplastic transformation.

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