Immunofluorescent Characterization of Rat Kidney Tumors According to the Distribution of Actin as Revealed by Specific Antiactin Antibody

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SUMMARY

A series of 15 mesenchymal and 10 cortical epithelial tumors induced in the rat kidney by dimethylnitrosamine was investigated for immunofluorescent reactivity with a human antiactin antibody. Cells of epithelial tumors showed staining restricted to peripheral sites, corresponding to the brush border region. All various neoplastic cell forms comprising renal mesenchymal tumors were characterized by cytoplasmic staining in patterns that varied with cell type. Epithelial profiles in the form of tubules and islands of epithelium showed staining patterns, or absence of them, consistent with their identity as sequestered segments of preexisting nephrons. It is suggested that the difference in actin distribution within the cytoplasm of cells of the two types of renal neoplasm, mesenchymal and epithelial, might reflect their difference in local invasive growth.

INTRODUCTION

Renal tumors may be induced in rats with a broad range of chemicals including nitroso compounds such as DMN and methylnitrosourea (for review, see Ref. 3). Most commonly, the induced neoplasms are either cortical epithelial tumors conforming to adenoma, adenocarcinoma, or carcinoma, or they are connective tissue mesenchymal tumors. The former display the usual spectrum of histological patterns seen in humans, ranging from papillary to lobular and solid, and take origin in the tubule elements, particularly proximal tubules of the cortex (4, 8, 9). On the other hand, the mesenchymal tumors have proved more difficult to interpret, and their characteristic cellular heterogeneity has resulted in such diverse classifications as renal sarcoma, hemangioendothelioma, and nephroblastoma or Wilms' tumor. Light and electron microscopic studies of these neoplasms (4, 7) and their genesis (5, 6) indicate that they are mesenchymal in nature, deriving from a mesenchymal cell-type resident in the interstitial space of the outer kidney zones. Histologically, the tumors consist of an admixture of fibroblast-like spindle cells, smooth muscle fibers, primitive or embryonic mesenchyme, and areas of neoplastic vascular tissue. On occasion, striated muscle, cartilage, and osseoid are present also (4, 7). Confusion with true nephroblastoma leading to error in nomenclature arises from 2 aspects (10). First, in proliferating between the tubules of the parenchyma, neoplastic mesenchymal cells progressively incorporate preexisting epithelial elements into the tumor tissue. Many of the engulfed tubules remain intact but show such pathological alteration as dilation to form cystic spaces, collapse with luminal obliteration, and hyperplastic proliferation of the lining cells. Where neoplastic mesenchyme invades the hilus, tongues, or islands of hyperplastic transitional epithelium derived from the urothelial lining of the pelvis become incorporated into the tumor mass. A 2nd source for misinterpretation lies in the predilection for mesenchymal tumor cells to condense around sequestered epithelial profiles in concentric layers, thus resembling the disposition of cells in nephroblastoma (10).

In a previous study (16), we have demonstrated that cells in renal mesenchymal tumors induced by a single injection of DMN showed strong cytoplasmic staining with a human AAA in contrast to the weak, nondescript staining of the resident interstitial cells from which the tumors are believed to be derived. This enhanced reactivity of tumor over normal cells with AAA has also been observed with tumors originating in skin (17), glial tissue (18), and liver (14).

In this study, we have examined the AAA staining patterns that characterize the common renal tumors of the rat, comparing, in particular, the difference between cortical epithelial and mesenchymal tumors and describing the reactivity of the heterogeneous cell forms which comprise the latter, including their epithelial profiles.

MATERIALS AND METHODS

Characterization of Human AAA. The AAA serum obtained from a patient with active chronic hepatitis was characterized by reactivity with the following tissues: smooth muscle (11), skeletal muscle (12), liver parenchymal cells (2), thymic medulla, renal glomeruli (19), brush border and peritubular fibrils of renal tubules (16), and central nervous system synaptic endings (15).

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**Immunohistology.** Six-μm cryostat sections of tumor tissue were stained with AAA by the standard "sandwich" immunofluorescence test (13), using sera at a dilution of 1:8. The conjugate for immunofluorescent tracing of bound immunoglobulin was a fluorescein isothiocyanate-labeled goat anti-human γ-globulin with a fluorescein:protein molar ratio of 4.0 and a protein content of 0.8 g/100 ml. Before use, the conjugate was absorbed with homogenates of rat liver, kidney, and gastrointestinal tract, and smooth muscle of pig stomach, until there was no staining reaction on test sections of normal kidney and tumor tissue. After immunofluorescent staining, the tissue preparations were examined by dark ground UV fluorescence microscopy using a condenser fitted with a colorless barrier filter and toric lens.

Specificity of the tests was established by failure to obtain staining with parallel control sections treated with normal human serum or AAA serum neutralized with actin derived from smooth (16) or skeletal muscle (1, 15).

**Induction of Renal Tumors.** A series of 15 mesenchymal tumors and 10 cortical epithelial tumors were characterized by their immunofluorescent reactivity with AAA. The neoplasms were induced in rats of Porter albino Wistar stock by the i.p. administration of a single dose of 60 mg/kg. The rats had been treated for 3 to 5 days previously with a diet of sugar and water, a regimen which leads to a 100% renal tumor incidence (4). Neoplasms were excised from anesthetized rats at intervals ranging from 6 to 12 months after carcinogen treatment. Tumor specimens were snap-frozen in an isopentane:liquid nitrogen slurry at −170°C for tests with AAA serum. Companion histological samples were prepared by staining cryostat and paraffin-embedded sections with hematoxylin and eosin.

**RESULTS**

The general morphology of DMN-induced renal mesenchymal and cortical epithelial tumors has been described and comprehensively depicted in earlier publications (4, 7, 8). To avoid unnecessary duplication of illustrative material, the reader is referred for details of histology and ultrastructure to those previous reports in this journal.

**Cortical Epithelial Tumors.** In each neoplasm examined, the cytoplasm of constituent tumor cells was consistently negative for AAA staining reactivity. Characteristically, however, there was a linear staining in epithelial cells conforming to the peripheral location and incidence of brush border. Additionally, the supporting stroma, consisting of fibrocytes and cells of blood vessel walls, stained positively. Thus, in papillary adenocarcinoma (Figs. 1 and 2), columns of epithelial cells supported by central spines of positively staining stroma showed bright linear staining restricted to the outer edge of their free surfaces. In tubular adenocarcinoma (Figs. 3 and 4), tumor cells displayed similar staining along the internal apical or luminal borders. In the lobular (Figs. 5 and 6) and solid (Fig. 7) forms of adenocarcinoma-carcinoma, a substantial proportion of cells showed a thin line of fluorescence around the entire periphery of individual cells or encircling small groups of 2 or more cells. Lobules and larger solid areas were themselves outlined by positively staining supporting stroma. In 1 solid carcinoma consisting of an intermixture of areas of clear and eosinophilic cells, the latter displayed the characteristic peripheral staining pattern while the clear cells were negative. The larger tumors contained frequent foci of necrosis which were always totally lacking in reactivity. Adenomas were generally typified by fluorescence patterns similar to the larger epithelial tumors but the staining could also be weak or mainly negative (Fig. 8).

**Mesenchymal Tumors.** In contrast to the epithelial neoplasms, the complete range of mesenchymal tumor cell types was characterized by positive cytoplasmic staining with AAA serum. Spindle or fibroblast-like cells forming the invading edge of the tumors (Figs. 9 and 10), swirling in concentric layers around engulfed tubules (Figs. 11 and 12) or forming fibrosarcoma-like sheets, showed brightly filamentous cytoplasmic staining. Areas consisting of smooth muscle (Fig. 13) were thrown into strong relief from the surrounding mesenchyme by intense staining of a compact, sheet-like nature. Rhabdomyoblasts and mature striated muscle were typified by an intense fibrillar or swirling pattern of staining (Fig. 14). Stellate cells forming areas that resembled embryonic mesenchyme (Figs. 15 and 16) reacted positively with a filamentous or stippled staining pattern. In islands of cartilage (Figs. 17 and 18), the chondrocytes showed a coarsely granular to diffuse cytoplasmic staining, with the matrix remaining negative. Likewise, neoplastic bone-forming cells stained positively but the osteoid was negative. Acellular myxoid zones and tufts and sheets of collagen did not react with the AAA serum.

At the invading edges of mesenchymal tumors, where neoplastic cells infiltrated between preexisting nephrons, the recently surrounded tubule profiles of the outer kidney zones usually retained bright staining restricted to the luminal surfaces, indicative of brush border reaction (Fig. 9), whereas those of the inner, medullary zones were lacking in reactivity (Fig. 10). With progressive engulfment, cortical tubules frequently became partially or entirely negative (Figs. 12 and 13), including some of those in which hyperplasia occurred. Where tumor cells invaded the hilus, long tongues of transitional epithelium from the urothelial lining were engulfed by the proliferating mesenchyme. These often appeared as isolated islands of epithelium surrounded by condensations of neoplastic spindle cells or primitive mesenchyme (Fig. 15). Such elements were characteristically negative, contrasting with the positive staining of the surrounding malignant mesenchyme (Fig. 16).

**DISCUSSION**

Comparative studies on the immunofluorescence reactivity of renal tumors with AAA show distinctive differences in the staining patterns between cortical epithelial tumors and mesenchymal tumors of the rat kidney. The cytoplasm of epithelial tumor cells is essentially negative, the reaction zone being restricted to peripheral borders or free edges, sites ultrastructurally associated with brush border formation (8). The localization of reactivity to sites indicative of brush border in almost all of the epithelial tumors examined...
is in agreement with the proposed derivation of many of these neoplasms from the proximal segment of the nephron (8, 9).

In contrast, all of the cell forms that comprise renal mesenchymal tumor, excluding epithelial profiles, are characterized by reactivity throughout their cytoplasm, in patterns that vary with the cell type. This is a predictable result in keeping with the belief that the neoplasms are essentially of connective tissue nature and derived from a resident intertubular, mesenchymal cell (4–7). The variations in staining pattern that occur within the heterogeneous range of cell forms enable various components of the tumors, namely smooth muscle, to be recognized easily. As has been pointed out in a previous study (16), the staining reactivity of mesenchymal tumor cells is enhanced beyond the weak reaction typical of the original, resident fibrocyte-like cells of normal interstitium. The staining characteristics of epithelial profiles incorporated within the mesenchymal tumor tissue are consistent with their origin from preexisting nephrons which have become sequestered by the infiltrating tumor cells. Staining restricted to the brush border of proximal tubules often becomes lost with progressive engulfment and pathological alteration.

The specificity of the AAA serum used in this study permits the conclusion that epithelial cells of rat kidney contain actin only at the cell periphery, presumably in microvillous structures, whereas all neoplastic cells in mesenchymal tumors possess actin distributed throughout the entire cytoplasm. This morphological difference in actin content may reflect a basic difference in the growth behavior of the 2 neoplasms. Mesenchymal tumor cells are locally very invasive, infiltrating and proliferating between the nephrons without a capsular reaction. On the other hand, growth of cortical epithelial tumors (with the exception of the infrequent anaplastic type not represented in this study) is largely a passive process occurring by slow outward expansion of the cell aggregate and stimulating a distinct capsule-like reaction. Thus, local invasiveness of mesenchymal tumor cells and the sessile nature of the epithelial cells may be functions of the cytoplasmic distribution of actin.

The observed differences noted in cytoplasmic distribution of actin may provide an additional basis for distinction between kidney tumor and cell types. Although there is usually little problem in distinguishing between mesenchymal and cortical epithelial tumors by conventional histological methods, some additional aid is necessary in those cases of mixed tumors where the epithelial cell components lose their cohesive growth properties. Furthermore, in cell culture, differentiation on morphological grounds between mesenchyme and epithelium can be difficult, and it is anticipated that the technique described here may be of value in establishing cell identity under such conditions. The inducing carcinogen used in this study is not associated with the production of nephroblastomas, the neoplasm with which renal mesenchymal tumor is most likely to be confused. Fresh tissue specimens of rat nephroblastoma were not available for comparison due to the infrequency of the neoplasm's occurrence (reviewed in Ref. 10). Consequently, differential staining reactivity with AAA of the mesenchymal and epithelial components of a range of human Wilms' tumors is presently under investigation.

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REFERENCES


Fig. 1. Adenocarcinoma with papillary form. H & E, × 150.
Fig. 2. Papillary adenocarcinoma. Staining by AAA is restricted to the free borders of the cords of epithelial cells. The central columns of supporting stroma are also positive. Indirect immunofluorescence, × 320.
Fig. 3. Adenocarcinoma with tubular differentiation. H & E, × 290.
Fig. 4. Tubular adenocarcinoma. Staining by AAA is restricted to the stroma at bottom left and the internal or lumenal borders of epithelial cells. Indirect immunofluorescence, × 320.
Fig. 5. Adenocarcinoma with lobular pattern. H & E, × 200.
Fig. 6. Lobular adenocarcinoma. Interlobular stroma shows bright immunofluorescence with AAA while small groups of epithelial cells within lobules are outlined by thin, peripheral staining. Indirect immunofluorescence, × 320.
Fig. 7. Adenocarcinoma of solid pattern. Individual epithelial cells or small groups of cells are clearly outlined by peripheral staining with AAA, but the main area of cytoplasm is negative. Indirect immunofluorescence, × 500.

Fig. 8. Small adenoma shows weak peripheral staining by AAA in a few epithelial cells but is largely negative. Indirect immunofluorescence, × 320.

Fig. 9. Mesenchymal tumor. Spindle cells swirling between proximal tubules at the invading edge show brightly filamentous staining of cytoplasm by AAA. The engulfed tubules show staining of the apical brush border region. Indirect immunofluorescence, × 320.

Fig. 10. Mesenchymal tumor. Invading edge of tumor in the inner kidney zones shows spindle cells with bright cytoplasmic staining by AAA, infiltrating between negative medullary tubules. Indirect immunofluorescence, × 160.

Fig. 11. Mesenchymal tumor. Characteristically, spindle cells become stratified in concentric layers around some sequestered tubules, an arrangement that mimics rat nephroblastoma. H & E, × 625.

Fig. 12. Mesenchymal tumor. Concentric layers of spindle cells stain brightly with AAA contrasting with the surrounded tubule which is negative but for partial staining of the brush border zone. Indirect immunofluorescence, × 260.
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Fig. 13. Mesenchymal tumor. Smooth muscle surrounding engulfed tubules shows compact, sheet-like staining of the cytoplasm by AAA. Some residual tubules show weak staining of the brush border zone; others are negative. The poorly cellular myxoid areas beyond the smooth muscle are also negative. Indirect immunofluorescence, × 320.

Fig. 14. Mesenchymal tumor. Rhabdomyoblasts show bright fibrillary staining by AAA. Indirect immunofluorescence, × 320.

Fig. 15. Mesenchymal tumor. Tumor cells in the pattern of embryonic mesenchyme condense around islands of engulfed urothelial cells where invasion of the pelvis has occurred. H × E, × 200.

Fig. 16. Mesenchymal tumor. Tumor cells in the pattern of embryonic mesenchyme show bright cytoplasmic staining by AAA which outlines the negative islands of transitional epithelium derived from the urothelial lining of the pelvis. Indirect immunofluorescence, × 200.

Fig. 17. Mesenchymal tumor. Islands of neoplastic cartilage within a spindle cell area. H × E, × 200.

Fig. 18. Mesenchymal tumor. The matrix of the cartilage is negative, while the chondrocytes show a coarsely granular or diffuse cytoplasmic staining by AAA. Indirect immunofluorescence, × 200.