Renin in renal cell carcinoma—an immunocytochemical study using an antibody to pure human renin

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SUMMARY We have studied a series of 19 primary renal cell carcinomas using an antibody to pure human renin and the indirect PAP technique. Seven tumours contained immunoreactive renin. No renin was identified in seven cases of metastatic tumour within the kidney, but immunoreactive renin was present in two out of seven metastatic renal cell carcinomas in other organs. None of the subjects had clinical, biochemical or histological evidence of excess renin secretion. We suggest that renal cell carcinoma may commonly secrete renin and that the hormone may be biologically inactive.

It is now well known that malignant tumours can secrete many hormones. Renal cell carcinomas have been shown to be associated with a growing list of secretory products. There are three isolated case reports of secretion of renin by a renal cell carcinoma. We have studied one of these cases and successfully stained the renin-containing cells within the tumour with an antibody to pure human renin and the peroxidase-antiperoxidase (PAP) method. The renin secreted by this tumour was mainly biologically inactive. Since the presence in the plasma of a biologically inactive hormone may not be clinically apparent, renal cell carcinoma may secrete renin more often than is realised. To investigate this possibility, we have examined a series of renal cell carcinomas using the renin antiserum and the PAP technique. We report here the immunocytochemical study of primary and metastatic renal tumours and of secondary tumours within the renal cortex.

Material and methods

To obtain optimally fixed material we confined our study to surgically excised tumours. These represent an unselected consecutive series of renal tumours from the files of the Pathology Department, Western Infirmary, Glasgow.

All tissue was fixed in 10% neutral buffered formalin and embedded in paraffin wax. From each case we selected two blocks which contained both tumour and a piece of adjacent kidney in which the renin-containing juxtaglomerular cells provided a known positive staining reaction. Sections (4 μm) were cut for routine histological stains and adjacent sections used for immunocytochemistry.

Immunocytochemistry

Renin antibody

The antibody to human renin was raised in rabbits from human renin completely purified from a juxtaglomerular cell tumour. The rabbit antiserum has been tested for in vitro specificity and its use in staining renin with the PAP technique in the normal and pathological human kidney has been published. We have found it to reliably stain renin in paraffin and Araldite-embedded tissue examined both by light and electron microscopy.

Staining techniques

We used a modification of the peroxidase-antiperoxidase (PAP) method of Sternberger and Cucullis together with appropriate controls as previously published. Each section was examined independently by two observers and only those classified as positive by both were included. The tumours in which there was an appreciable amount of iron pigment were counterstained with Perls' reaction to distinguish haemosiderin from the brown peroxidase reaction product.

To identify macrophages, the tumour sections were stained with antibodies to muramidase and α1-antichymotrypsin (Dako Labs, UK) as before.
CLINICAL MEASUREMENTS
Pre and postoperative measurements of blood pressure and serum potassium were obtained from the clinical case records where available. Where more than one measurement was made the mean was used.

RESULTS

IMMUNOCYTOCHEMISTRY
The PAP preparations always showed stainable renin in the juxtaglomerular apparatus. Indeed, the compressed kidney adjacent to the tumour often showed hyperplasia of the renin-containing juxtaglomerular cells (Fig. 1).

Primary renal cell carcinoma
In this unselected consecutive series of renal cell carcinomas, we found that seven of 19 tumours contained immunoreactive renin. The histological typing of these tumours is shown in Table 1. The majority of the tumours which contained immunostainable renin were clear cell carcinomas. In five of the seven tumours the cells containing immunoreactive renin were small perivascular cells located immediately external to the endothelium of the blood vessels in the tumour (Figs. 2, 3 and 4). These cells were sometimes very scanty in number and usually present singly. They were constant in position but variable in morphology. Their nuclei were most commonly flattened, sometimes notched, and usually smaller than the cells which were clearly tumour cells. They had inconspicuous nucleoli and when closely applied to a blood vessel, the cells often had long cytoplasmic processes containing PAP-positive granules (Fig. 4). When the cells were in stroma and apparently not compressed by surrounding tumour cells they were sometimes larger. They occasionally approached the tumour cells in size (Fig. 2) and their nucleus was often as large (Fig. 3). These renin-containing cells did not resemble the normal juxtaglomerular apparatus granular cells, nor could it be established by histology whether or not they were neoplastic cells. There was no histological or immunocytochemical evidence that they were macrophages.

In two cases, one a clear cell carcinoma and the other a more granular cell variant, cells which had the histological appearance of tumour cells contained granular deposits of immunoreactive renin (Fig. 5). These cells were indistinguishable from the other tumour cells and were present focally throughout the tumour. The clear cell carcinoma also contained occasional perivascular cells which contained immunostainable renin as described above. These

Table 1

<table>
<thead>
<tr>
<th>Primary renal tumours</th>
<th>No examined</th>
<th>Tumours containing immunoreactive renin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clear cell carcinoma</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>Oncocytic tumour</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Papillary adenocarcinoma</td>
<td>3</td>
<td>–</td>
</tr>
<tr>
<td>Spindle cell carcinoma</td>
<td>2</td>
<td>–</td>
</tr>
<tr>
<td>Anaplastic carcinoma</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td>7</td>
</tr>
</tbody>
</table>

Fig. 1 Renin PAP preparation of the kidney adjacent to a renal cell carcinoma which contained immunostainable renin. It shows hyperplasia of the renin containing cells in the afferent arteriole. ×396.

Fig. 2 Renin PAP preparation of a clear cell carcinoma showing two cells containing immunostainable renin (arrows). Both positive cells are situated just external to the endothelium of the sinusoidal blood vessels. The larger cell stains as strongly as the renin containing cells in the juxtaglomerular apparatus of the adjacent kidney. ×504.
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**Fig. 3** Renin PAP preparation of an oncocytic renal tumour. The cells containing immunostainable renin (arrows) are situated in the interstitium external to the capillary. Some of the positive staining is granular (short arrows). ×1250.

**Fig. 4** Renin PAP preparation of the same tumour as Fig 3. The cell which contains immunostainable renin has a flattened nucleus and a long cytoplasmic process which contains positive granules (arrows). ×1250.

**Fig. 5** Renin PAP preparation showing a clear cell carcinoma with areas of granular tumour cells, many of which contain immunostainable renin in granular form. ×396.

did not resemble the positively stained tumour cells and transitional forms could not be identified.

Granular staining could be demonstrated with PAS after diastase and with Wilson's and Bowie's methods in the same areas of the tumours which contained immunostainable renin.

**Metastatic tumours in the kidney**
Kidneys are rarely removed for metastatic tumours so most of these tumours arose in the renal pelvis; their histological type is shown in Table 2. Only those tumours which had invaded the renal cortex were selected for study. In no case was renin detectable in
the tumour cells or in the vessels within the tumour deposits. Small amounts of renin were occasionally seen in areas of renal cortex invaded by these tumours. These were always located in relation to a pre-existing structure in the invaded renal cortex, either a glomerulus or artery, and which was surrounded by infiltrating tumour.

Metastatic renal cell carcinomas

Seven secondary renal cell carcinomas were studied. The sites of metastasis were liver, bone, lung and lymph nodes. In two cases there was faint granular positive staining in tumour cells. One was a metastasis in bone and the other in liver. These were both clear cell carcinomas and the positive staining was present focally in granular tumour cells similar to those illustrated in Fig. 5. The primary tumours from these cases were not available for study.

CLINICAL MEASUREMENTS

Blood pressure and serum potassium levels

Renin-secreting renal tumours are usually associated with hypertension and hypokalaemia, both of which usually resolve after nephrectomy. When the patients whose tumours contained immunostainable renin were compared to those whose tumours were negative there was no significant difference in mean blood pressure levels (140/90 and 110/80 mmHg respectively) nor in mean serum potassium concentrations (4.48 mmol/l and 4.40 mmol/l respectively) between the two groups. Furthermore nephrectomy made no significant difference to blood pressure or serum potassium concentrations. This applied both to the mean levels of the two groups and to individual cases within the two groups. Three patients were under treatment for mild hypertension. Two had tumours containing immunoreactive renin. Nephrectomy made no significant difference to their blood pressures.

Discussion

Renal cell carcinoma has been associated with many hormone-like secretions, but there are only three published cases in which there is evidence of renin secretion by renal cell carcinoma. Of these three cases, one was an oncocytic tumour in which no granules could be identified and one was a typical clear cell carcinoma in which the tumour cells contained granules whose ultrastructural appearance was typical of renin granules. The third case (which we have studied), showed immunostainable renin in small perivascular granular cells in a clear cell carcinoma, the tumour cells being uniformly negative. In this present study we have detected immunostainable renin in seven of 19 primary renal cell carcinomas and in five of these cases the cells containing immunostainable renin were morphologically similar and had the same perivascular distribution as our previously reported case.

Renin in cells within a tumour could either have been taken up from the plasma or synthesised by the cells. The positive cells contained granules and in the single case which we have previously studied by immunocytochemistry and electron microscopy, there was strong clinical and morphological evidence that these perivascular cells in the tumour were secreting renin. We are currently studying the renin content of tumour tissue and pre- and postoperative plasma renin concentrations in a prospective series of renal tumours.

There are two possible explanations for the presence of renin secreting cells within a tumour in the renal cortex: they may have entered the tumour along with blood vessels which have grown in from the surrounding kidney or they may be tumour cells. Two of the tumours showed positive staining of cells which were histologically indistinguishable from tumour cells. One of the three published cases also showed the presence of renin-like granules in tumour cells. It is therefore possible that the perivascular renin containing cells, although appearing histologically different from the typical renal carcinoma cells, may be tumour cells. These may have undergone a different pathway of differentiation, perhaps analogous to the endocrine differentiation found in many epithelial tumours of other organs such as the gut, cervix, prostate, pancreas, etc. Indeed other endocrine cells have been described in nephroblastoma. If these renin secreting cells are tumour cells this finding has important implications for the histogenesis of renal cell carcinoma which is currently believed to be derived from renal tubular epithelium.

Renal cell carcinoma exhibits multiple different appearances. In this small series, most of the tumours with renin-containing cells were clear cell or oncocytic tumours. The study of a larger series is clearly required to establish whether other morphological types contain renin.

In the study of metastatic tumours in the renal cortex we could find no renin-containing cells associated with the blood vessels of the tumours.
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However, these tumours had a more infiltrative mode of spread and a different vascular architecture to renal cell carcinoma.

Most renin secreting renal tumours have been juxtaglomerular cell tumours. These are associated with clinical manifestations of renin excess, most commonly hypertension and hypokalaemia. These usually revert to normal after nephrectomy. Histological studies have usually failed to demonstrate granules and immunostainable renin in the juxtaglomerular cells of the surrounding kidney, due to suppression of physiological renin secretion. In the cases we have studied there was no clinical evidence of excess renin secretion as judged by plasma electrolyte and blood pressure levels before and after nephrectomy. Moreover, the compressed kidney adjacent to the tumours often showed hyperplasia of the renin-containing cells. These findings would suggest that either the cells within the tumour which contain renin did not release it in amounts sufficient to raise plasma angiotensin II (and therefore suppress physiological renin secretion in the kidney) or that any renin secreted was biologically inactive. The case which we have already reported showed hyperplasia of the renin containing cells of the juxtaglomerular apparatus in the adjacent kidney. The secreted renin by this tumour was mainly biologically inactive, had a higher molecular weight, and was possibly a prohormone.

The antibody used in this study binds to both active and inactive renin in vitro, therefore the nature of the renin which we have shown to be present in renal cell carcinoma remains uncertain and will have to be studied biochemically. This is at present underway; however, it is well known that in other situations tumours may secrete biologically inactive hormones. Thus we suggest that while secretion of active renin by renal cell carcinoma appears to be rare, our preliminary study indicates that secretion of inactive renin may be common.

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References


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