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Distinctive patterns of renal neoplasms containing Tamm-Horsfall protein

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Abstract. Sections of 114 renal neoplasms from adults, and 2 renal rhabdoid tumours from children, were examined by an indirect immunoperoxidase method using two antibodies to Tamm-Horsfall protein. Forty-five of the adult neoplasms were also examined with an antibody to proximal tubular brush border. Tamm-Horsfall protein is normally only found in the cells of the thick limb of the loop of Henle, and there are widely divergent reports on its occurrence in renal neoplasms. In the present series, Tamm-Horsfall protein was detected in parts of 31 neoplasms. Four distinctive patterns of cell contained the protein: cells with a paranuclear inclusion typical of rhabdoid tumours; 'plasma rich' cells, which were large cells with cytoplasm that was centrally dense and peripherally clear; eosinophilic cells forming one type of papillary structure; and giant cells. The areas containing Tamm-Horsfall protein did not express markers of proximal tubular brush border, and appeared white to the naked eye, rather than the yellow of typical clear cell carcinomas. Tamm-Horsfall protein can therefore be found in renal neoplasms. The four distinctive patterns of positive cells appear to represent neoplastic phenotypes of thick limb cells. This has implications for the classification of renal neoplasms and for theories of their origin.

Key words: Tamm-Horsfall protein – Renal neoplasms – Proximal tubular brush border

Introduction

Tamm-Horsfall protein, the main protein in normal urine, is produced by the cells of the thick ascending limb of the loop of Henle (Hoyer and Seiler 1979; Kumar and Muchmore 1990; Howie and Johnson 1992). There have been divergent views on the frequency of occurrence of Tamm-Horsfall protein in renal cell carcinomas, ranging from those who have found it in no carcinomas (Holthöfer et al. 1983; Fowler et al. 1987; Ortmann et al. 1991) or in only a few (Wallace and Nairn 1972; Leuschner et al. 1991) to those who have found it in two-fifths or more (Costello et al. 1991; Gu et al. 1991). There was also a case report of Tamm-Horsfall protein in a renal cell carcinoma (Gaulier et al. 1990). The workers who reported a high rate of detection of Tamm-Horsfall protein in renal cell carcinomas found the protein in nearly all childhood rhabdoid tumours both of the kidney and in extrarenal sites (Kumar et al. 1987, 1988; Costello et al. 1991).

To try to resolve the question of whether or not Tamm-Horsfall protein occurs in renal cell carcinomas, and to see if immunoreactive cells are otherwise distinctive, we have studied immunohistologically a large series of renal neoplasms with two antibodies to the protein. We also studied 2 childhood renal rhabdoid tumours with the same antibodies, and a selection of the neoplasms with an antibody to the brush border of proximal tubules. Most renal cell carcinomas are said to express markers of proximal tubules (Wallace and Nairn 1972; Holthöfer et al. 1983; Yoshida et al. 1986).

Materials and methods

Formalin-fixed, paraffin-embedded material was obtained from 114 primary renal neoplasms resected from adult patients at the Queen Elizabeth Hospital, Birmingham, from 1982 to 1992. These were nearly consecutive except that transitional cell carcinomas of the renal pelvis were restricted to 2 cases. A detailed classification of renal neoplasms was used (Thoenes et al. 1986; Störkel and Jacobi 1989). Material was also obtained from 2 renal rhabdoid tumours, 1 surgical and 1 at necropsy, from the Children's Hospital, Birmingham.

Sections cut at 5 μm thick were dewaxed, washed in water, and treated sequentially with hydrogen peroxide, periodic acid and potassium borohydride to block endogenous peroxidase (Heyderman 1979). Sections were treated for 30 min at room temperature with either sheep polyclonal IgG anti-human Tamm-Horsfall protein (The Binding Site, Birmingham) at 1:600 in phosphate-buffered saline with 1% ovalbumin (OA-PBS), or mouse monoclonal

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IgG anti-human Tamm-Horsfall protein (Cedarlane Laboratories, Ontario, Canada) at 1:500 in OA-PBS. After a wash for 15 min in PBS containing 0.001% Brij 96 detergent (Sigma), sections were treated for 30 min as appropriate with either donkey anti-sheep IgG antibody, peroxidase conjugated, or sheep anti-mouse IgG antibody, peroxidase conjugated, both at 1:100 in OA-PBS (The Binding Site). After another wash, peroxidase was detected with 0.1% tetra-amino-biphenyl hydrochloride and 0.01% hydrogen peroxide in PBS. Sections were counterstained with Mayer's haemalum and mounted in Piccolyte (Eastman Kodak).

With each batch of neoplasms, a section of normal adult kidney was included as a positive control. For adsorption controls, Tamm-Horsfall protein was prepared from human urine using the original method, with repeated precipitation by 0.58 M sodium chloride and redissolving in water (Tamm and Horsfall 1952). Tamm-Horsfall protein (6 mg) was coupled to 1 g cyanogen bromide-activated Sepharose as instructed by the manufacturer (Pharmacia). The Tamm-Horsfall protein-Sepharose was used to adsorb the two antibodies, the sheep antibody by passage through a column packed with the coupled Sepharose, and the mouse antibody by incubation with the coupled Sepharose in a centrifuge tube. Adsorbed antibodies were used in the first stage of the immunohistological method.

On sections of 45 of the neoplasms, an indirect immunoperoxidase method similar to that described above was used with a sheep antibody to formalin-fixed human ileal brush border at 1:200. This was known to be specific for the brush border of proximal tubules in the kidney (Howie et al. 1990). A section of normal kidney was included with each batch of neoplasms as a positive control.

Results

Of the 116 renal neoplasms, 2 were the childhood rhabdoid tumours, 2 were transitional cell carcinomas of the renal pelvis, 1 was a recurrent nephroblastoma and 1 was an angiomyolipoma. The other 110 were epithelial neoplasms of renal parenchyma (Mostofi 1981) with the following types (Thoenes et al. 1986; Störkel and Jacobi 1989): clear, 70 (64%); chromophobe, 2 (2%); chromophilic, 9 (8%); mixed, 14 (13%); spindle-shaped/pleomorphic, 6 (5%); oncocytomas, 4 (4%); others including duct of Bellini carcinoma, 5 (4%). Amongst the 70 clear types, 45 were typical clear type, 12 were the variant called 'plasma rich', with dense cytoplasm next to the nucleus and a clear peripheral rim of cytoplasm in large cells with a distinct cell border, 7 were the 'clear cell – eosinophilic' type, and 6 had a mixture of these types. At least one of these types was also seen in 10 of the 14 neoplasms classified as 'mixed'.

The two antibodies to Tamm-Horsfall protein reacted with the thick limb of the loop of Henle in the control normal kidney and in many cases in normal kidney adjacent to neoplasms. They also marked extratubular deposits of Tamm-Horsfall protein in cases in which there had been obstruction of tubules (Howie and Brewer 1983; Howie 1987). Incubation with Tamm-Horsfall protein removed the reactivity of both antibodies.

There was immunoreactivity for Tamm-Horsfall protein in 31 neoplasms, including 29 (26%) of 110 epithelial neoplasms of renal parenchyma, and in these both antibodies showed reactivity. The patterns of neoplasms that contained Tamm-Horsfall protein were distinctive, and were as follows.

1. Rhabdoid (n=3). The 2 renal rhabdoid tumours from children, and a large part of a neoplasm in an adult classified as 'other', consisted of sheets of densely crowded, eosinophilic, small, round cells, many with a large, hyaline, paranuclear inclusion (Fig. 1). Immunoreactivity for Tamm-Horsfall protein was seen in the globular inclusion next to the nucleus (Fig. 2).

2. 'Plasma rich' (n=17). Overall, 24 neoplasms classified as 'clear' (18) and 'mixed' (6) contained areas consisting of such cells (Figs. 3, 4). Tamm-Horsfall protein was detected in the central part of cells (Fig. 5), in 17 of the 24 neoplasms. A few cells resembling this type were seen in the rhabdoid tumours.

3. Papillary (n=10). Small, angulated, lightly eosinophilic cells with a distinct border, sometimes with a little peripheral clearing beginning to resemble 'plasma rich' cells, usually forming papillary structures, were seen as the main stained component of 10 neoplasms, 7 of which

Fig. 1. Part of a renal neoplasm in an adult, stained by haematoxylin and eosin, resembling a renal rhabdoid tumour. ×150, bar = 40 μm

Fig. 2. Renal rhabdoid tumour in a child, stained immunohistologically with polyclonal anti-Tamm-Horsfall protein antibody. Most cells have an immunoreactive cytoplasmic inclusion. ×350, bar = 15 μm

Fig. 3. Renal neoplasm in an adult, stained by haematoxylin and eosin. The lighter zone consists of 'plasma rich' cells that on adjacent immunohistological sections contain Tamm-Horsfall protein. The other cells are more usual clear cells and do not contain the protein. ×35, bar = 150 μm

Fig. 4. Renal neoplasm in an adult stained by haematoxylin and eosin. The cells, as seen in the lighter area in Fig. 3, are large, with a clear peripheral zone, and have been called 'plasma rich'. ×350, bar = 15 μm

Fig. 5. Renal neoplasm in an adult, stained immunohistologically with polyclonal anti-Tamm-Horsfall protein antibody. The 'plasma rich' cells seen in Fig. 4 contain the protein. ×350, bar = 15 μm

Fig. 6. Renal neoplasm in an adult, stained by haematoxylin and eosin. Papillary structures are covered by small, angular cells, some with apical clearing. ×350, bar = 15 μm

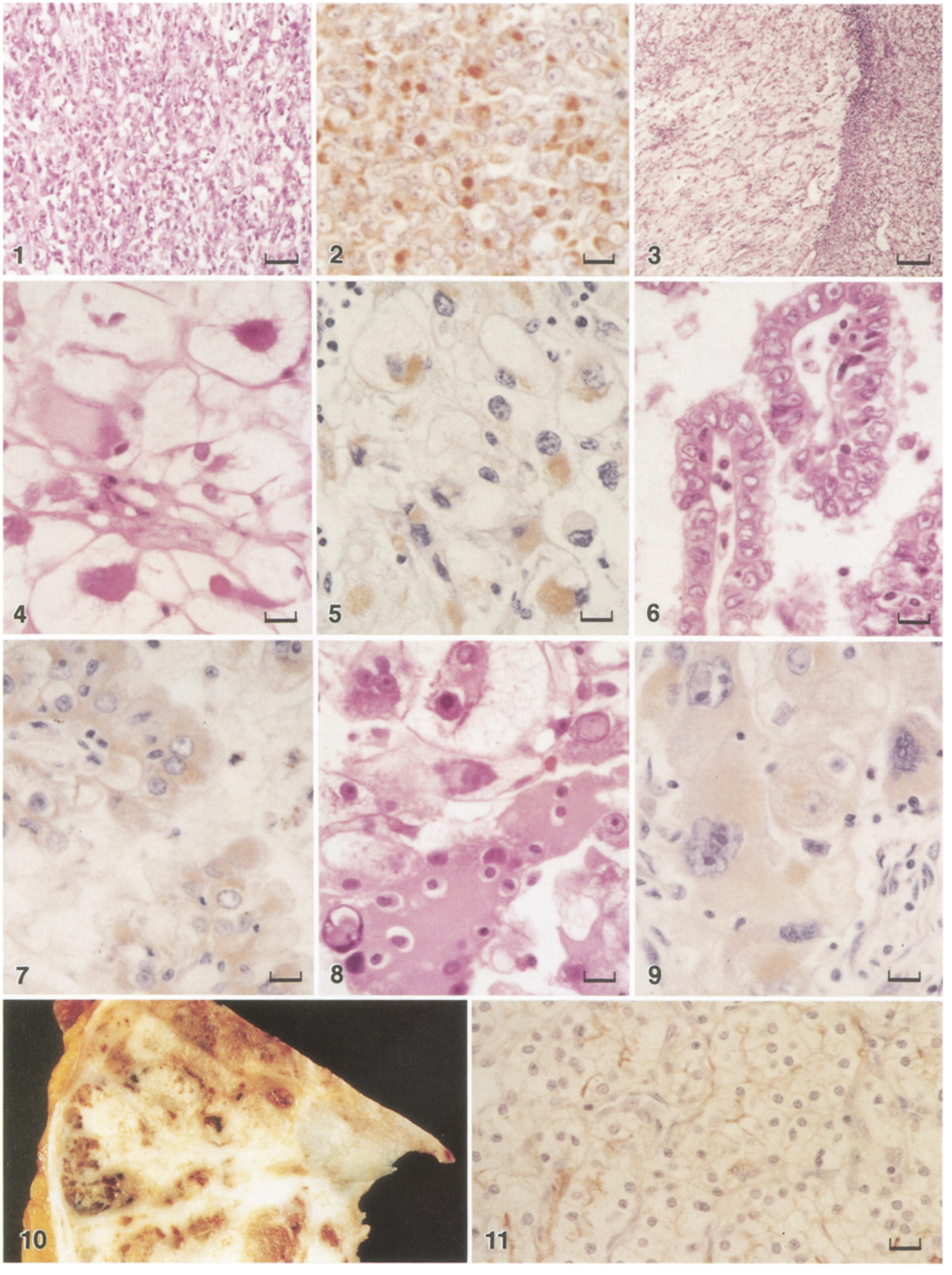
Fig. 7. Renal neoplasm in an adult, stained immunohistologically with monoclonal anti-Tamm-Horsfall protein antibody. Cells on the papillary structures as in Fig. 6 contain the protein. ×350, bar = 15 μm

Fig. 8. Renal neoplasm in an adult, stained by haematoxylin and eosin. There is a giant cell, with 'plasma rich' cells as seen in Fig. 4. ×350, bar = 15 μm

Fig. 9. Renal neoplasm in an adult, stained immunohistologically with monoclonal anti-Tamm-Horsfall protein antibody. Giant cells as seen in Fig. 8 contain the protein. ×350, bar = 15 μm

Fig. 10. Cross-section of a renal neoplasm in an adult, unstained. The neoplasm mostly appears white (compare the yellow fat). Microscopically, the neoplasm consists of a mixture of 'plasma rich' cells as in Fig. 4, and papillary eosinophilic tissue as in Fig. 6, ×3

Fig. 11. Renal neoplasm in an adult, stained immunohistologically with antibody to proximal tubular brush border. Clear cells are stained on their surface. ×350, bar = 15 μm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were classified as 'clear' of the 'clear cell-eosinophilic' type and 3 as 'mixed' (Fig. 6). Such cells were also seen with other areas immunoreactive for Tamm-Horsfall protein, especially 'plasma rich', in 6 other neoplasms. Foam cells, psammoma bodies and calcification were not seen in the papillary structures. The Tamm-Horsfall staining was throughout the cytoplasm or in the central part if the periphery appeared clear (Fig. 7).

4. Giant cell (n=1). Large, pleomorphic, angulated cells with multiple nuclei, with cytoplasmic reactivity for Tamm-Horsfall protein, were the main stained cell in only one neoplasm, classified as 'mixed'. These cells were mixed with 'plasma rich' and papillary areas in another neoplasm and were seen in small numbers in another 9 (Figs. 8, 9).

Areas of renal neoplasms containing Tamm-Horsfall protein were usually distinctive to the naked eye, appearing white rather than yellow, which was the appearance of usual clear cell renal carcinomas (Fig. 10).

Immunoreactivity for Tamm-Horsfall protein was not seen in other variants of renal cell neoplasms, in particular, the usual clear cell type without cytoplasmic condensation (Fig. 3); chromophobic cells; basophilic or densely eosinophilic chromophilic cells; oncocytomatous cells; spindle cells; duct of Bellini carcinomas; and papillary areas with psammoma bodies, calcification or foamy macrophages. Transitional cell neoplasms, the nephroblastoma and the angiomyolipoma were also not reactive.

The antibody to brush border of proximal tubules reacted with typical clear cells in 21 of the 24 neoplasms tested that contained such cells (Fig. 11). There was no reactivity with any of the 'plasma rich' cells or other cells that contained Tamm-Horsfall protein in the 10 such neoplasms tested. No reactivity was seen in the 11 other neoplasms tested, which were 4 spindly type, 3 oncocytomas, and 4 mixed and other types.

Discussion

Two antibodies to Tamm-Horsfall protein were used in this study to reduce the chances that immunoreactivity was not with Tamm-Horsfall protein but was with an epitope carried on another molecule, which is a possible drawback of monoclonal antibodies (Howie et al. 1984). Both antibodies were adsorbed by Tamm-Horsfall protein. This procedure is considered the best control for immunohistological staining (Heyderman 1979). The monoclonal antibody in our study had been used also by others, with a range of proportions of reactive neoplasms (Fowler et al. 1987; Gu et al. 1991; Leuschner et al. 1991). The polyclonal antibody in our study had been well characterised on normal human tissues (Howie 1987).

We confirm that renal rhabdoid tumours contain Tamm-Horsfall protein (Kumar et al. 1987, 1988; Costello et al. 1991) and show that some other renal neoplasms also contain it. One possible explanation of the divergent views on the prevalence of Tamm-Horsfall immunoreactivity in renal neoplasms lies in the fact that these neoplasms are so heterogeneous. The pattern usually considered characteristic of renal cell carcinoma, with uniform clear cells, does not react for the protein, and series dominated by this pattern would be expected to show little or no reactivity. We confirmed that the typical clear cells usually express markers of the brush border of proximal tubules (Wallace and Nairn 1972; Holthöfer et al. 1983; Yoshida et al. 1986).

A new finding in our series was that the Tamm-Horsfall positivity was in distinctive types of cells, which had a characteristic appearance to the naked eye (Fig. 10). The cells in a case of renal cell carcinoma that marked for Tamm-Horsfall protein (Gaulier et al. 1990) resembled those we have called 'plasma rich', which was the descriptive name used by Thoenes et al. (1986) who considered this to be a variant of the common clear cell type, and grouped 'plasma rich' cells, typical clear cells and 'clear cell-eosinophilic' cells in their classification. Otherwise the reports have not given enough details to be able to identify the reactive cells (Wallace and Nairn 1972; Costello et al. 1991; Gu et al. 1991; Leuschner et al. 1991). The four positive cell types we report are not independent, distinct entities, as most cases had a mixture of types, and even the rhabdoid tumours contained cells resembling the 'plasma rich' type.

We cannot be sure that the positive cells actually make Tamm-Horsfall protein since they could selectively absorb it. The assumption, though, that these cells make the protein implies that the cells are showing differentiation towards – not "are arising from" – cells of the thick limb of the loop of Henle, the only site in the normal kidney, or indeed normal body, that Tamm-Horsfall protein occurs (Howie 1987). That is, the four types we describe appear neoplastic phenotypes of the thick limb cells. Many workers favour the view that most renal cell carcinomas show differentiation towards proximal tubular cells (Wallace and Nairn 1972; Holthöfer et al. 1983; Yoshida et al. 1986; Farrow 1989; Störkel and Jacobi 1989). As it is common to see Tamm-Horsfall positive areas surrounded by negative areas, which express markers of proximal tubular cells (Figs. 3, 11), this suggests that renal cell carcinomas arise from cells that can differentiate both to proximal tubular cells and thick limb cells. Such cells could be derivatives of the original metanephric blastema (Droz et al. 1990; Bard and Woolf 1992). There is evidence that renal oncocytomas and chromophobe renal cell carcinomas may arise from cells with a different embryological origin, the collecting duct (Pitz et al. 1987; Zerban et al. 1987; Störkel and Jacobi 1989; Ortmann et al. 1991). Neither type of neoplasm expressed Tamm-Horsfall protein.

Classifications of renal neoplasms range from the simple (Mostofi 1981) through the slightly more complex (Farrow 1989) to the complicated (Thoenes et al. 1986; Störkel and Jacobi 1989). Such classifications are based on microscopic appearances and not on understanding of the cellular origins and patterns of differentiation of the neoplasms. We now show that evidence of differentiation towards the thick limb of the loop of Henle is a fairly common finding in renal cell carcinomas. This

may influence future classification of renal neoplasms and theories of their cellular origins.

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