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Immunohistochemical distribution of vitamin B_{12} R-binder in renal cell carcinoma

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Summary. Renal cell carcinomas and normal kidney tissues were examined for the expression of vitamin B_{12} R-binder by the indirect immunoperoxidase method. In normal kidney tissue, the presence of the vitamin B_{12} R-binder was shown to be confined to the straight portion (pars recta) of proximal tubules. Seven of the 38 cases of renal cell carcinoma (18%) expressed the vitamin B_{12} R-binder antigen. This provides a further evidence of the proximal tubular nature of renal cell carcinoma, and suggests that a small proportion of renal cell carcinomas originate from the straight portion of the renal proximal tubules.

Key words: Vitamin B_{12} R-binder – Renal cell carcinoma – Immunohistochemistry

Introduction

Vitamin B_{12} (cobalamin) binds to three specific binding proteins: intrinsic factor, transcobalamin II and vitamin B_{12} R-binder (transcobalamin I). Vitamin B_{12} R-binder (R-binder) is a family of immunologically identical glycoproteins found in plasma, granulocytes and various body fluids including urine (Retief 1969; Gibson et al. 1974; Wahlstedt and Gräsbeck 1985) and was recently demonstrated in various types of human epithelial tissue by immunohistochemical studies (Kudo et al. 1987a, b). The expression of this peculiar protein in human epithelial tumours is of interest from histogenetic and clinicopathologic standpoints. In the present study, we have examined the antigenicity of R-binder in renal cell carcinoma and normal kidney tissue with regard to the histogenetic aspects of renal cell carcinoma.

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Materials and methods

Tissue from 24 normal kidneys and 5 fetal kidneys (gestational age: 19, 24, 28, 31, and 34 weeks) were obtained at autopsy and from surgical specimens. From the surgical pathology file of Kyoto University Hospital 38 cases of renal cell carcinoma were selected; all the specimens were obtained by nephrectomy, fixed in formalin and embedded in paraffin. 4-μm thick sections were employed for the study. Additionally, each three samples of normal kidney and renal cell carcinoma were snap-frozen in OCT and cut on a cryostat. 4-μm frozen sections were then fixed for 5 min in 4° C acetone. The tumours were classified histologically according to the WHO-classification of kidney tumours (Mostofi et al. 1981).

Purification of vitamin B_{12} R-binder and production of rabbit anti R-binder antiserum were described previously (Kudo et al. 1987a). The specificity of the antiserum was confirmed by Ouchterlony's immunodiffusion method and immunoelectrophoresis.

Immunostaining was carried out using the indirect peroxidase-labelled antibody technique. Deparaffinized or frozen sections were treated with 0.3% hydrogen peroxide in absolute methanol for 30 min to block the endogenous peroxidase activity, were incubated with 10% normal goat serum for 30 min to block non-specific binding of immunoglobulins, and were then incubated with rabbit anti R-binder antiserum (diluted at 1:200) overnight at 4° C. After washing with phosphate-buffered saline, pH 7.2, the sections were reacted with peroxidase-conjugated goat anti-rabbit IgG (Cappel, PA, USA, diluted at 1:200) for 90 min at room temperature. Finally, the color was developed by a 5-min diaminobenzidine hydrochloride-hydrogen peroxide-reaction, and the sections were counter-

stained with methyl green. The specificity of R-binder staining was confirmed by replacing the primary antiserum with nonimmune rabbit serum.

Results

In normal kidney tissues, positive staining was confined to the straight portion (pars recta) of the proximal tubules (Fig. 1a). Intense staining was observed on the luminal aspects of the proximal tubules, especially along the brush border. Positive staining was also found, although weaker, in the adjacent part of the cytoplasm of the tubule cells (Fig. 1b). The reactivity on frozen sections was the same as on paraffin-embedded sections. Glomeruli, the convoluted portion (pars convoluta) of the proximal tubules, Henle's loops, distal and collecting tubules, transitional epithelium of the renal pelvis as well as renal interstitial tissues were unstained.

In fetal kidney tissues, positive staining was similarly demonstrated in the straight portion of proximal tubules. Reactivity was also found at luminal surface of the tubular structures scattered in the metanephrogenic zone present in the cases of earlier gestational age.

Of the 38 renal cell carcinomas 7 (18%) expressed the R-binder (Table 1). In two of the 16 tumours composed solely of clear cells, R-binder was detected along the cell surface and typically in the apical aspects of the carcinoma cells forming tubular structures (Fig. 2). Of the 8 mixed cell type tumours, 3 cases showed positive staining only in the granular cell portion. In 2 of these cases, there was cell-surface or luminal staining in linear form (Fig. 3), while in another case a faint diffuse reaction was seen in the cytoplasm of the tumour cells. Epithelial cysts observed in one case also revealed positive staining in the lining cells. Two of the 12 cases of granular cell type tumours showed distinct intracytoplasmic staining for the R-binder, while the others did not. R-binder was not detected in the 2 cases of sarcomatoid spindle cell variants. Three samples investigated on frozen sections showed negative staining for R-binder on both frozen and paraffin-embedded sections. No significant relationship was observed between the incidence of R-binder expression and the tumour cell type of carcinoma.

Table 1. Immunohistochemical localization of vitamin B_{12} R-binder in various types of renal cell carcinoma

Cell type	No. of positive cases/No. of cases studied
Clear cell type	2/16
Mixed cell type	3/ 8
Granular cell type	2/12
Spindle cell type	0/ 2
Total	7/38

Discussion

R-binder is commonly found in various body fluids, including serum and urine, and granulocytes. Bunge and Schilling (1957) also reported that kidney tissue extracts contained the vitamin B_{12} binding protein. We previously reported the

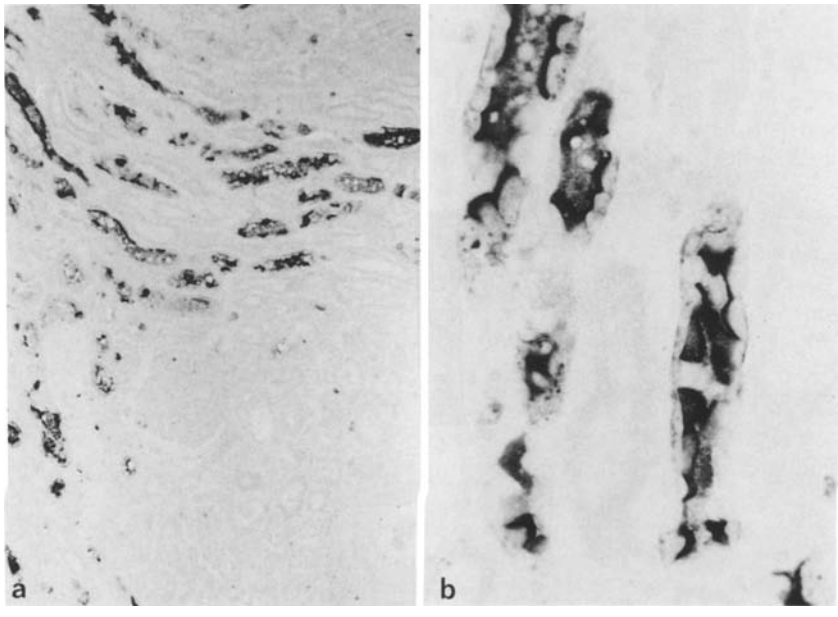

Fig. 1a, b. Normal kidney tissue. **a** R-binder is demonstrated exclusively in the straight portion (pars recta) of proximal tubules. (Immunoperoxidase, ×115); **b** Intense staining is observed along the brush border. Weak staining is also found in the adjacent cytoplasm of the tubular cells. (Immunoperoxidase, ×460)

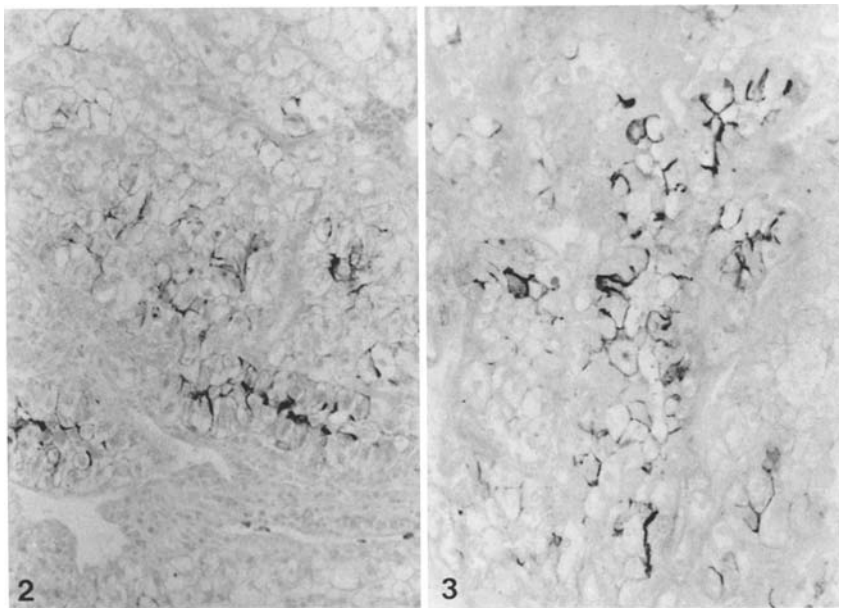

Fig. 2. Renal cell carcinoma; clear cell type. Cell-surface staining is distinctly observed especially at the luminal aspects. (Immunoperoxidase, ×230)

Fig. 3. Renal cell carcinoma; mixed cell type. Cell-surface staining is shown in linear form. (Immunoperoxidase, ×230)

distribution of R-binder in normal human tissues (Kudo et al. 1987b), and revealed that it is present in the following epithelia: all glands of the digestive system except for gastric mucosae, bronchial glands, straight portion of renal proximal tubules, prostate, uterine endometrial and cervical glands, Fallopian tube, mammary glands and sweat glands. Although Wahlstedt and Gräsbeck (1985) speculated that urinary R-binder originates from the leukocytes or transitional epithelium of the urinary tract, our previous study as well as the present study showed that R-binder is secreted from the straight portion of the renal proximal tubules.

The physiological function of the R-binder is not clear. However, it has been suggested that it eliminates cobalamin analogues and suppresses bacterial growth by inhibiting bacterial uptake of cobalamin (Gullberg 1974; Allen 1975; Donaldson 1981). R-binder may play a role in the antibacterial defense mechanisms of the urinary tract.

Brush border antigens were first isolated from the plasma membrane of the renal proximal tubules (Edgington et al. 1967). Using immunohistochemical methods, Miettinen and Linder (1976) reported that the brush border antigens were detected in various organs other than kidney tissue, including the epithelium of digestive systems, epididymal tubules, allantochorionic tissue, the lacrimal gland and salivary glands. It is striking that the tissue distribution of brush border antigens is quite similar to that of the R-binder. Two common features in the different epithelia, stained with both the anti-brush borders and anti-R-binder antisera, are a distinctive ultrastructure bearing microvilli and a highly specialized function (absorbing or secreting surfaces).

The brush border antigens have been regarded as a heterogenous group of renal proximal tubular antigens located towards the luminal part of the cells, and they appear to be immunological mediators, receptors, carrier proteins, structural proteins, or enzymes of the specialized surface membrane (Miettinen and Linder 1976; Ronco et al. 1984; Chatelet et al. 1986; Rodman et al. 1986). The straight portion of the renal proximal tubules bears the brush borders continuous with the convoluted portion (Fawcett 1986) and, supported by the similarity of the tissue distribution demonstrated between brush border antigens and R-binder, it seems probable that R-binder shows cross reacting antigenicity with brush border antigens or is a protein immunologically related to brush border antigens. The localization of R-binder in renal proximal tubules, however, is restricted to the straight portion and is not found in the convoluted portion.

Renal cell carcinomas have usually been considered to originate from the epithelial cells of proximal convoluted tubules. This concept has been partly supported by the ultrastructural observations that human renal carcinoma cells possess brush borders resembling those of proximal convoluted tubules (Oberling et al. 1960; Seljelid and Ericcson 1965; Tannenbaum 1971). More recently, some investigators, employing the anti-brush border antigens, have revealed that most renal cell carcinoma cells expressed the proximal tubular

brush border antigens (Wallace and Nairn 1972; Holthöfer et al. 1983; Weiss et al. 1985; Iizumi et al. 1986). In addition, Finstad et al. (1985), using a panel of monoclonal antibodies reacting with separate regions of the nephron, reported that most renal cell carcinomas in their study shared the antigenic phenotype of proximal tubular cells.

In the present study, the incidence of positive staining for R-binder in renal cell carcinomas (18%) was much lower than that of positive staining for brush border antigens in renal cell carcinomas reported in the previous reports. This discrepancy might be explained by the finding that brush border antigens are distributed along the whole proximal tubules, while the R-binder is localized restrictively in the straight portion of the proximal tubules. The present findings may reflect the various degrees of differentiation of renal cell carcinomas, or may indicate that a small proportion of renal cell carcinomas (about 20%) originates from the straight portion of the proximal tubules where the antigenicity of R-binder is exclusively found in the normal kidney.

Of the tumours positive for R-binder, some showed cell-surface or luminal surface staining, while the others showed intracytoplasmic staining. This difference in reactivity remains unexplained.

There is agreement that the different cell patterns constituting renal cell carcinomas have no prognostic significance (Böttiger 1970; Bennington and Beckwith 1975). In the present study, R-binder expression with the cell type of renal cell carcinomas showed no clear relationship. Whether the biological behavior of renal cell carcinoma is related to the R-binder expression remains to be studied.

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