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Renal oncocytoma: immuno- and carbohydrate histochemical characterization

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Summary. Samples from renal and thyroid oncocytomas were studied with antibodies against intermediate filament proteins, nephron site specific antigens and nephron segment specific lectins to gather information on the immunohistological and carbohydrate histochemical features of these tumours. The results show a surprising failure of most antibodies and lectins used to react with the oncocytomas, although readily staining the surrounding normal tissue areas. No immunohistological evidence for derivation of oncocytomas from proximal tubular epithelial cells could thus be found. Instead, *Triticum vulgaris* (wheat germ agglutinin; WGA) and *Concanavalin A* (ConA) lectins were seen to stain the oncocytes specifically, suggesting that these lectins are useful to further characterize oncocytomas.

Key words: Lectin histochemistry – Intermediate filaments – Kidney – Oncocytoma

Introduction

Oncocytes, large epithelial cells with granular, eosinophilic cytoplasm characterized ultrastructurally by packing of the cells with mitochondria, can be found among the normal cells of salivary glands, thyroid, parathyroids, the adrenals, kidney and some other epithelial organs (Hamperl 1962; Yu et al. 1980; Barnes and Beckman 1983). These cells are thought to be altered, degenerative forms of the respective epithelial cells, as oncocytes are particularly numerous in tissues of elderly and cachectic individuals (Hamperl 1962; Yu et al. 1980).

Kidney tumours composed entirely of oncocytes (oncocytomas), are found with increasing frequency (Klein and Valensi 1976; Morales et al. 1980; Eble and Hull 1984). This is considered to be a real increase rather than due to better diagnostic alertness, although obviously some renal oncocytomas are still being misdiagnosed as adenocarcinomas. Clinically most renal oncocytomas are benign, the main symptoms arising from the sometimes enormous size of the tumour.

In spite of difficulties in distinguishing oncocytoma from renal adenocarcinomas, little effort has been made to characterize further the enzyme- and immunocytochemical features of oncocytomas.

As the derivation of oncocytomas is still a matter of dispute, the present study was undertaken in order to study the immunocytochemical and carbohydrate histochemical characteristics of oncocytomas, using antibodies against intermediate filaments, nephron site specific antigens and lectins.

Table 1. Immuno- and carbohydrate histochemical features of oncocytomas and of the surrounding normal kidney tissue

Antibody or lectin[1]	Reactivity with normal tissue	Reactivity with oncocytomas
MDCK cytokeratin	PT, DT, CD	–
PKK1 cytokeratin	PT, DT, CD	–
vimentin	GLOM, PERIT, ENDOT	interstitium
desmin	SM	–
Brush border	PT	–
Tamm-Horsfall	DT	–
HPA	DT, CD	interstitium
PNA	DT, CD	interstitium
UEA I	ENDOT	ENDOT
DBA	CD	–
MPA	PT	–
WGA	GLOM, PT, DT, CD	+++
ConA	GLOM, PT, DT, CD	+++

[1] For antibody characterization and lectin names, see materials and methods. Abbreviations used: PT = proximal tubulus; DT = distal tubulus; CD = collecting duct, GLOM = glomerules; PERIT = peritubular areas; ENDOT = endothelial cells; SM = smooth muscle of vessels; – = no reactivity; +++ = strong reactivity of tumor cells

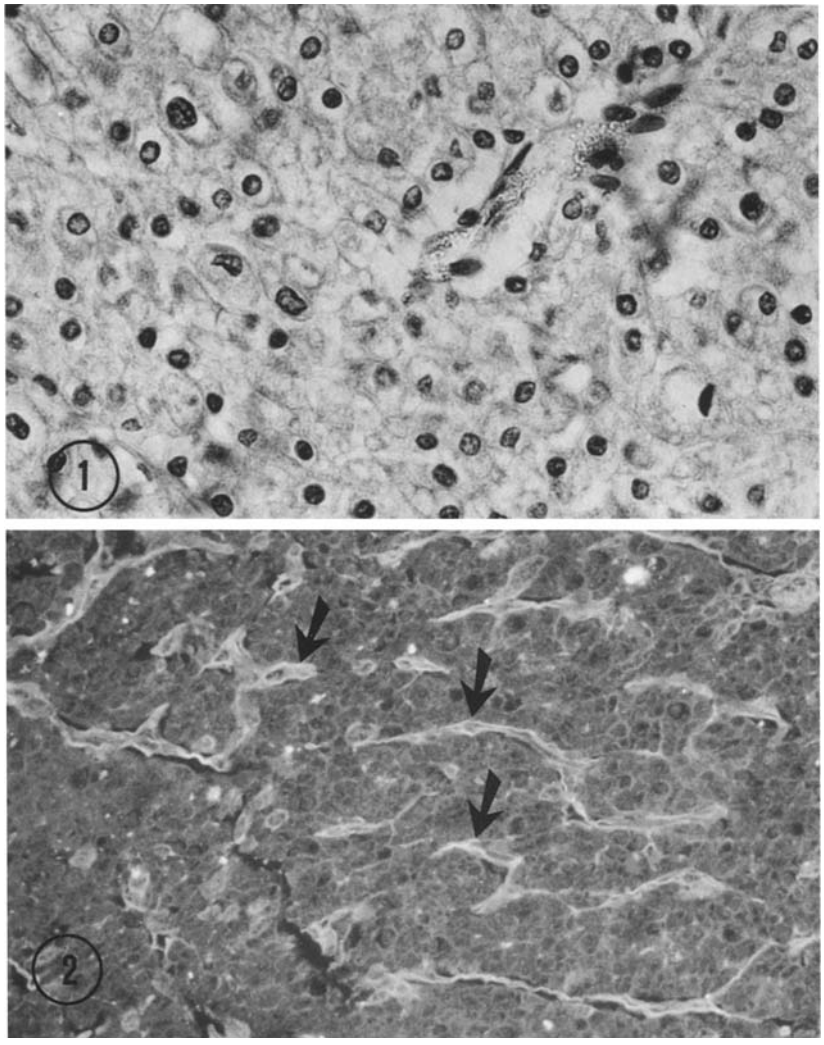

Fig. 1. Paraffin section of a renal tumour showing a typical appearance of oncocytoma. × 306

Fig. 2. Sample of renal oncocytoma stained with antivimentin antibodies. Only the interstitial areas (arrows) are positive. × 253

Materials and methods

Tissue samples. Paraffin embedded tissue samples from five renal tumours fulfilling the criteria of oncocytoma (Klein and Valensi 1976), found during the years 1980–1984 were collected from the files of the Department of Pathology (University of Helsinki, Helsinki, Finland). In addition to samples from renal oncocytomas, one specimen of thyroid oncocytoma was included.

Rabbit and mouse anticytokeratin and rabbit antivimentin and antidesmin antibodies (available at Labsystems, Inc., Helsinki, Finland), their characterization and reactivity on normal kidney tissue have been reported earlier (Holthöfer et al. 1983). Anti brush border (anti BB) and anti Tamm-Horsfall glycoprotein (anti TH) antibodies, reacting with proximal and distal tubules, respectively, have been characterized earlier (Holthöfer et al. 1981). Fluorochrome coupled anti mouse and anti rabbit IgG (Cappel Laboratories, Cochranville Pennsylvania, USA) were used as second antibodies.

Commercially available, fluorochrome labeled *Concanavalin A* (Con A), *Triticum vulgaris* (wheat germ, WGA), *Arachis hypogaea* (PNA), *Dolichos biflorus* (DBA), *Helix pomatia* (HPA), *Ulex europaeus* (UEA I) and *Maclura pomifera* (MPA) lectins used for detection of various carbohydrate moieties, were obtained from E – Y laboratories (San Mateo, California).

Tissue samples were routinely fixed in 3.8% buffered formalin and embedded in paraffin. After deparaffination and rehydration, pretreatment with 0.2% trypsin (Sigma Chemical Company, St. Louis, Mo, USA) in 37 C for 30 min was used to recover antibody and lectin reactivity. Thereafter, tissue sections were incubated with appropriate dilutions of the primary antisera or lectins (30 min, 20° C), followed by thorough washing in phosphate buffered saline (PBS) and mounting in nicetamide or, for the antibodies, further incubated with the second antibodies before mounting.

For specificity testing of lectin binding, prior incubation of the lectin solution with the respective inhibitory saccharides at 0.2 M was used as described earlier (Holthöfer et al. 1981).

A Zeiss Standard microscope equipped with epi-illuminator and a filter system for FITC-fluorescence was used for microscopy.

Results

The results of this study show a limited reactivity of antibodies and lectins with the oncocytomas. Thus, all the antiintermediate filament antibodies, nephron site-specific antibodies and all but ConA

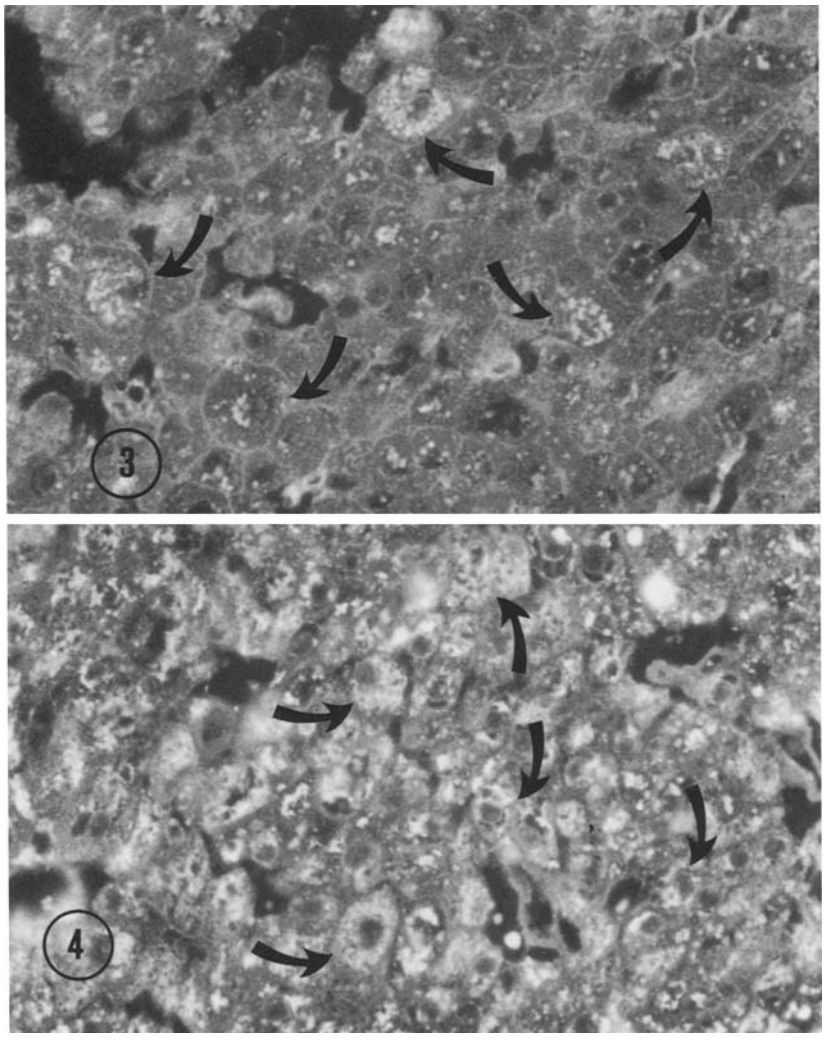

Fig. 3. FITC-WGA gives a granular staining (arrows) of the tumor cells in renal oncocytomas, but also reacts with the plasmalemmae. Note the variation in staining intensity of individual cells. × 306

Fig. 4. FITC-ConA gives a strong granular staining of all the tumour cells of all the tumour cells in oncocytomas. × 306

and WGA lectins failed to stain the oncocytes, whilst readily reacting with the normal tissue surrounding the tumours (for details, see Table 1), thus serving as positive controls. The sample from a thyroid oncocytoma gave similar staining results for renal oncocytomas.

In light microscopy, all the tumour samples studied (Fig. 1) fulfilled the criteria of renal oncocytoma. In addition, specificity testing of lectin binding by prior incubation of ConA and WGA, the lectins giving a positive staining reaction with the tumor cells with their respective nominal specific sugars (α-methylmannoside and N-acetylglucosamine, respectively), resulted in no tissue fluorescence.

All the tumour samples failed to react with the antibodies against cytokeratin, vimentin and desmin types of intermediate filaments. These antibodies, however, gave their typical binding pattern in the surrounding normal tissue areas in the same sections. Anti-vimentin antibodies stained some interstitial tissue areas of the oncocytomas (Fig. 2), but there was no reaction with the oncocytes.

Anti brush border and anti Tamm-Horsfall antibodies failed to stain any structures in the oncocytomas, although these antibodies gave their typical staining reactions in proximal and distal tubules at the normal kidney pole of the samples.

HPA, PNA, UEA I, DBA and MPA lectins failed to react with tumour cells but reacted with the interstitial elements. UEA I gave a strong positive fluorescence for vascular walls in the tumour tissue. In the same way as the antibodies used, the lectins also gave their typical staining in the normal kidney areas.

WGA (Fig. 3) and especially ConA (Fig. 4) reacted brightly with the tumour cells, giving a lumpy granular staining. This binding pattern was observed throughout the tumour tissue, although considerable variation between the positivity of in-

dividual cells was observed with WGA. The typical staining pattern of ConA and WGA of oncocytes could be seen in all the tumour samples studied, including the sample from the thyroid oncocytoma.

The results are summarized in Table 1.

Discussion

The present results show the failure of antibodies and most nephron segment specific lectins to react with oncocytoma tissue, although readily detecting their respective antigenic sites in the surrounding normal kidney areas in the same tissue sections.

Based on gross morphological and some cytoplasmic similarities, renal oncocytomas have classically been supposed to originate from the senescent epithelial cells of proximal tubules (Hamperl 1962). Recently, α-chymotrypsin has been found to be shared both in proximal tubular epithelia and in oncocytes (Alanen et al. 1984), which was taken to indicate a proximal tubular derivation for oncocytes. However, lysozyme, although present in proximal tubular cells could not be found in oncocytes (Merino and Livolsi 1981; Alanen et al. 1984). There are thus few enzymatic similarities between oncocytes and proximal tubular cells thus far identified.

In this study, both of the anti-cytokeratin antibodies used failed to react with the tumour cells. As the presence of the cytokeratin type of intermediate filament polypeptides is characteristic for epithelial cells (Altmannsberger et al. 1982; Osborn et al. 1982) the current results would suggest a non-epithelial derivation for the oncocytes. However, masking of cellular cytokeratins induced by tissue processing, may explain the observed lack of staining in epithelial tissue. However, as reported by Pinkus et al. (1984) trypsin pretreatment of the tissue sections, used also in this study, should recover tissue reactivity. Oncocytomas might alternatively express a combination of cytokeratin polypeptides not detectable with either of the antibodies used. Anti-vimentin and anti-desmin antibodies also failed to stain the tumour cells, while giving their typical staining pattern in the normal kidney areas (Holthöfer et al. 1983).

Anti-BB antibodies, normally reacting with the proximal tubular epithelial cells (Miettinen and Linder 1976) also failed to stain the oncocytes of the tumour samples studied. This suggests that the previously observed morphological and enzymatic similarities between proximal tubular cells and oncocytes may be incidental and not indicative of a common origin. However, the possibility that the tumour cells of oncocytomas are not sufficiently differentiated to express brush border antigens cannot be excluded, although during embryonic development the brush border antigens are known to appear very early (Ekblom et al. 1980).

There are several reports of the strict compartmentalisation of saccharide moieties in various nephron elements, allowing lectins to be used as markers for the respective nephron segments (Holthöfer et al. 1981; Faraggiana et al. 1982; Hennigar et al. 1984). Most lectins used in this study gave no staining in the tumour cells, while reacting with the basement membrane structures, or vascular endothelia (UEA I) of the tumours. Binding of ConA and WGA do not show a strict segment specificity on the normal kidney, but rather react with most cell types of the nephron (Holthöfer et al. 1981), due perhaps to their rather wide nominal sugar specificity (Goldstein and Hayes 1978). In oncocytomas, however, these lectins gave a typical granular staining in the tumour cells, perhaps identifying some structural glycoproteins in the numerous mitochondria. ConA and WGA also gave similar staining reaction to thyroid oncocytomas, suggesting that oncocytomas at different tissue loci may rise from a common cell type. Thus, ConA and WGA lectins would seem to provide useful tools to identify oncocytes but also to study these cells further.

The finding that a wide variety of antibodies failed to react with oncocytoma is surprising, although some lectins still reacted with epitopes of the tumour cells. This suggests an excessive and selective denaturation of antigens expressed by the oncocytes during tissue processing. This interpretation is further substantiated by the fact that several of the probes used reacted also with the interstitial structures of tumour areas. The present results call for further caution in interpreting some results obtained with some immunostainings on paraffin sections, even when cytochemical controls give a normal reaction pattern. However, the intriguing possibility that oncocytes may represent a completely different cell type with presently unknown origin and functions remains to be studied further.

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