

Original Article

Dysplastic (“in-situ”) Lesions in multifocal renal oncocytomas (oncocytosis)

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Abstract: Preneoplastic lesions for renal oncocytosis have not been well defined. We have attempted to identify the putative in-situ or dysplastic change in nephrectomy specimens with oncocytosis. Cases of multiple oncocytoma previously identified in radical nephrectomy specimens (n = 5) were reviewed for early lesions of renal oncocytosis by light microscopic analysis and by immunohistochemical studies for p53, bcl2 and MIB-1. Microscopic analysis showed that the renal cortical regions in all cases contain isolated groups of tubules partially or completely replaced by oncocytic cells with morphologic features resembling tumor cells in oncocytosis. The oncocytic cells within these tubules are increased in number and are arranged either as solid groups or as single layers in cystically dilated tubules, and may assume a hobnail appearance. They can be distinguished from small foci of oncocytosis as they do not form a coalescent group but are separated in part by intervening normal-appearing tubules. Cytologically, the cells have abundant eosinophilic, granular cytoplasm with a low nuclear/cytoplasmic ratio and demonstrate distinct cell borders. A very characteristic feature of these cells is the retraction space (“windows”) between the oncocytic cells. Nuclear features of these cells are not distinctive from normal tubules. Immunostaining with Bcl-2, p53 and MIB-1 antibodies also does not differentiate the putative preneoplastic lesions from normal tubules. Thus, recognition of a putative dysplastic lesion for oncocytosis is possible by routine microscopic analysis. Identification of this lesion in a biopsy or partial nephrectomy specimen should raise the possibility of the existence of renal oncocytosis (multifocality), leading to adequate clinical management.

Key Words: oncocytoma, oncocytomatosis, dysplasia, kidney, in situ tumor, precursor.

Introduction

Preneoplastic lesions have been studied extensively for many epithelial tumors such as carcinomas of the breast, esophagus, colon, lung and prostate. Finding these lesions is very important in determining if an invasive cancer is primary to the organ. In addition, the presence of these lesions suggests the likelihood of developing a true neoplasm and calls for preventive and surveillance modalities. In the kidney, however, preneoplastic changes for renal epithelial neoplasms have not been well defined. A putative precursor change in tubules adjacent to renal cell carcinoma termed “intratubular epithelial dysplasia” was described by Mourad et al. [1]. This “dysplastic” tubular epithelial change consists of foci of crowded tubular epithelium with large, vesicular nuclei 2-3

times larger than those of benign tubular cells, and with eosinophilic macronucleoli. Subsequently, p53 accumulation in tubules showing intratubular epithelial dysplasia has been reported [2] and proposed as supporting evidence that this morphologic change represents a precursor lesion. Similar changes, however, have not been evaluated in renal oncocytosis.

Renal oncocytoma in its usual solitary form (95% of cases) is a benign neoplasm of the kidney, which differs from renal cell carcinoma in both nuclear and cytoplasmic features [3, 4]. The tumor cells of oncocytoma have round and regular nuclei with evenly dispersed chromatin and occasionally prominent central nucleoli, but are distinctive in having an abundant granular eosinophilic cytoplasm.

Table 1 Clinical and pathologic data of cases of renal oncocytosis

Case	Age	Sex	# of oncocytosis	# of intratubular lesions/low power field (4x objective)	Location of intratubular lesions	Associated lesions
1	81	M	4	2	cortical	urothelial carcinoma of renal pelvis
2	60	M	5	4	cortical	papillary RCC and contralateral clear cell RCC
3	70	M	10	5	cortical	none
4	68	M	8	1	cortical	clear cell RCC
5	78	M	4	2	cortical	papillary RCC, oncocytic variant

Oncocytomas occur only rarely as multiple tumors in the same kidney, and in these few reported cases have been termed “renal oncocytosis” [5, 6]. In these cases, oncocytomas not only occur as tumors but also as multiple microscopic lesions (possibly a field effect) offering an opportunity for the identification of a preneoplastic lesion. We have studied tubular changes in uninvolved renal parenchyma in cases of renal oncocytosis by light microscopy and immunohistochemistry in an attempt to identify an early intratubular lesion.

Materials and Methods

Selection of cases

Renal tumors diagnosed at New York University Medical Center were retrieved for the study. From this group, 5 cases of renal oncocytosis were identified, based on the presence of 3 or more oncocytomas in the nephrectomy specimen (**Table 1**). In four of the five cases, renal cell carcinoma coexisted in the same kidney. In addition, five cases each of solitary renal oncocytoma, solitary renal cell carcinoma and non-neoplastic kidneys from cases without renal epithelial neoplasms (angiomyolipoma, retroperitoneal sarcoma and perirenal hematoma) were selected as control groups.

Immunohistochemistry

Immunohistochemical staining for p53 (1:50, Santa Cruz, CA), MIB-1 (Ki-67, 1:20, Zymed laboratories, San Francisco, CA) and Bcl-2 (1:20, Dako Corporation, Carpinteria, CA) was performed using a standard avidin-biotin peroxidase complex method on a NEXES automated immunohistochemical instrument (Ventana Medical Systems, Tuscon, AZ) with

protocols as suggested by the manufacturers of the antibodies.

Results

Morphologic features of putative early intratubular lesions of oncocytosis

Sections from 5 cases each of oncocytosis, solitary oncocytoma, renal cell carcinoma and non-neoplastic kidney were carefully examined to search for tubules containing features that may represent preneoplastic (dysplastic) changes for oncocytosis. In cases of renal oncocytosis, we identified with high frequency tubular changes that may represent such changes in the uninvolved renal parenchyma. The architectural and cytologic features of the putative dysplastic tubular changes are summarized as follows:

Architectural features: The tubules were partially or entirely replaced by oncocytic cells (**Figure 1A and B**), and showed a cystic, solid or hobnail appearance. These tubules showed some overlapping features with small oncocytomas. However such tubules could be distinguished from true oncocytosis as they did not form a coalescent group and were separated by normal-appearing tubules.

Cytological features: The tubular epithelial cells showed characteristic oncocytic changes including dense eosinophilia of cytoplasm, increased cytoplasmic granularity and increased amount of cytoplasm as compared with lining epithelium of proximal convoluted tubules (**Figure 2A-D**). As a result, there was usually a significant increase in cell size and a corresponding decrease in Nuclear/Cytoplasmic ratio. Cell borders were very distinct with the appearance of “windows” or separation of cells (**Figure 2A-D**), probably due

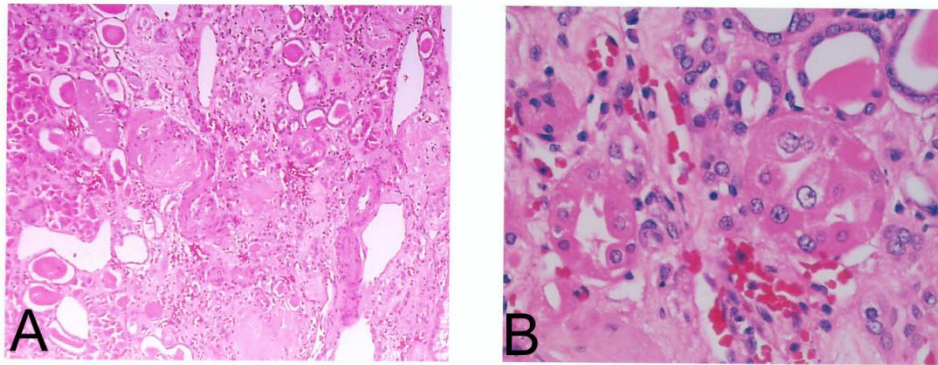


Figure 1. Dysplastic tubules at low (A, 100x) and high magnifications (B, 400x).

to artifactual retraction during processing, which we found to be a very useful clue for the identification of these cells. These changes differed from proximal convoluted tubular cells, which have a less dense eosinophilic cytoplasm, indistinct cell membrane, and a preserved apical villous border.

Only few oncocytic cells showed binucleation (Figure 2D) or multinucleation. Therefore, in general, nuclear features were not characteristic enough for separation of these dysplastic cells from normal cells in proximal convoluted tubules.

We required a combination of architectural and cytologic features to diagnose a putative intratubular lesion. The putative intratubular lesions were identified to varying degree in multiple scattered cortical tubules in all cases

of renal oncocytosis. In 2 of 5 cases the changes were easily identified (as probable “in-situ oncocytosis”). In the remaining 3 cases, changes were more subtle and considered to represent oncocytic epithelial hyperplasia. In solitary renal oncocytoma, early intratubular lesions were not observed although individual features could be seen in occasional tubules adjacent to the tumor. In the cases of renal cell carcinoma and non-neoplastic kidneys, these changes were not seen.

Immunohistochemical studies with p53, Bcl-2 and ki-67 antibodies do not identify putative intratubular lesions of renal oncocytosis

It has been reported previously that some renal cell carcinomas and their precursor lesions show increased expression of p53 by

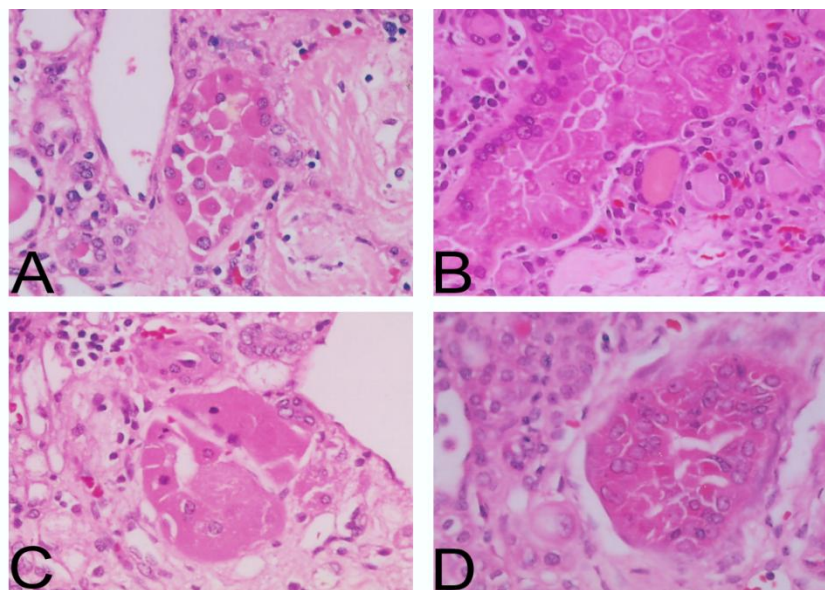


Figure 2 Dysplastic foci with typical “window” formation at high magnification (A-D, x400).

immunohistochemistry [2, 7]. In addition, decreased expression of Bcl-2 has been observed for some renal oncocytomas [8]. We therefore stained our cases with anti-p53 and anti-Bcl-2 antibodies as well as MIB-1 antibody to determine the proliferative activity. In 2 of 5 cases of renal cell carcinoma, there was an increased p53 staining in proximal tubular epithelium. Similarly, 2 of 5 cases of renal oncocytosis also showed occasional tubular epithelial cells with increased p53 staining. This staining did not correspond with the putative intratubular lesions. The remaining 3 cases were negative for p53.

Bcl-2 and MIB-1 staining showed no significant difference among the study groups. The results are summarized in **Table 2**.

Discussion

We have identified early or putative preneoplastic (dysplastic) lesions for renal oncocytoma and oncocytosis. These lesions represent tubules partially or completely replaced by oncocytic cells which do not coalesce to form tumors (oncocytosis). The morphologic similarity to oncocytosis and the fact that these tubular lesions are frequently identified in oncocytosis but infrequently and incompletely seen in kidneys with solitary oncocytomas and not seen in kidneys without oncocytosis suggest that they may represent true preneoplastic lesions for renal oncocytosis. In fact, these lesions has been recognized as various entities from “oncocytic changes in the renal tubules” [9, 10] to “microscopic oncocytic nodules” [11].

Renal oncocytoma is a relatively rare neoplasm of the kidney. It is usually slow-growing and not considered to possess any malignant potential. It constitutes approximately 6% to 7% of all primary renal cortical epithelial tumors. About 4% to 5% are bilateral and as many as 13% are multifocal [3, 4]. Since its first description [12], many studies have addressed the histogenesis and

cytogenetics of this tumor. There is biochemical and enzymologic evidence that oncocytosis may originate from collecting ducts [13-16] although different views exist [17, 18]. Cytogenetic studies have shown translocations between chromosome 11q13 and other chromosomes, or the loss of chromosome Y, 1 and 14 [19-22]. Although to date the preneoplastic change for this neoplasm in human has not been well described, Ahn et al (10) have found that oral administration of N-nitrosomorpholine (NNM) for 7 weeks induced renal oncocytosis in male Sprague-Dawley rats. Oncocytic tubules, similar to what we observed in renal oncocytosis of humans, appeared before the occurrence of oncocytosis. Protein expression and enzymology were very similar between the oncocytic tubules and oncocytosis. This animal model, therefore, is consistent with our proposal that the oncocytic tubules may represent true preneoplastic lesions for renal oncocytic neoplasia.

Immunohistochemical studies have not proven to be of value in assisting with the identification of the dysplastic change. Intranuclear accumulation of p53 was shown to be increased in some renal carcinomas and their precursor lesions (2,7). However, we did not find such increases in renal oncocytosis or the putative precursor lesions. In two of the cases of oncocytosis, the positivity was limited to proximal tubules, but not found in any of the oncocytic cells. A similar increase was observed in two cases of renal cell carcinoma. Therefore, we believe that the increase in the oncocytosis cases was likely related to the co-existing renal cell carcinoma in these cases. The same holds true for MIB-1, which shows a low proliferation rate in all groups of cases studied. Bcl-2 has been reported to be decreased in renal oncocytosis (8). However, we were not able to reproduce this finding in our study and the putative preneoplastic tubules did not show any difference from the neighboring tubules in Bcl-2 expression.

Table 2 Immunohistochemical characterization of renal oncocytosis and other renal lesions

Case type	p53 in renal tubules	Bcl-2 in renal tubules	Ki-67 in renal tubules
Oncocytosis	positive in 2 of 5 cases	negative	rarely positive
Solitary oncocytosis	all negative	negative	rarely positive
Renal cell Ca	positive in 2 of 5 cases	negative	rarely positive
Non-neoplastic kidney	all negative	negative	rarely positive

Current management of small renal lesions favors partial nephrectomy where possible. This approach is warranted for solitary small lesions, especially when they prove to be benign tumors such as oncocytoma. On the other hand, the predisposition to multifocality, if it can be predicted by the presence of dysplastic lesions may in some instances be clinically useful. After examination of a partial nephrectomy specimen with oncocytosis, the identification of dysplastic change in the specimen may suggest a predisposition to tumor multifocality, and influence future patient management.

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