

Original Article

Immunohistochemical phenotype and molecular pathological characteristics of metanephric adenoma

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Abstract: To assess the clinicopathological, immunohistochemical and molecular features of metanephric adenoma (MA). Clinicopathologic data were obtained for 5 cases of MA with follow-up information. Specimens from these patients were stained by HE and immunohistochemistry for the detection of WT1, vimentin, S-100 protein, CK7, P504s, CD10 and renal cell carcinoma marker (RCC). Fluorescence *in situ* hybridization (FISH) was performed on 4 tumors. The patients included 1 male and 4 females, aged from 30 to 49 (mean=39) years. Tumor diameters ranged from 3 to 5.5 cm. Histologically, the tumors had tubular, papillary, or glomeruloid architectures, and were composed of cells with uniform and round nuclei, inconspicuous nucleoli, and high ratio of nucleus to cytoplasm. Nuclear polymorphism and mitotic figures were not observed. Immunohistochemically, they expressed WT1 (5/5), vimentin (5/5), S-100 (4/5), CK7 (2/5), P504s (2/5), and CD10 (1/5) and not RCC. FISH study was carried out on 4 metanephric adenoma cases, and no abnormalities were observed in chromosomes 3, 7, 17, and P16 gene of chromosomes 9. MA is an uncommon renal tumor. Its diagnosis depends on morphological, immunohistochemical and molecular features.

Keywords: Metanephric adenoma, immunohistochemistry, molecular pathology, FISH

Introduction

Metanephric adenoma (MA) is an uncommon renal benign tumor, derived from the renal residual organization during embryonic development [1]. Named by Brisigotti since 1992 [2], few cases are found in the literature, because of the lack of specific clinical characteristics: they are frequently histologically misdiagnosed as malignant tumors of kidney. Indeed, their pathological and immunohistochemical characteristics, biological and genetic changes are not fully understood. Here, we assessed 5 cases of MA at our hospital's outpatient care, to study the clinicopathological, immunohistochemical and molecular features of this disease. In addition, our data were combined with the literature for discussing the research progress and differential diagnosis of MA.

Materials and methods

Cases and specimen source

From November 2005 to January 2014, 383 specimens were obtained by surgical treatment

of renal tumor cases. Diagnosis of renal adenoma was made for 4 cases by a number of experienced pathology doctors, according to WHO 2004 tumors of the urinary system and male reproductive organs pathology and genetics standards; the remaining 1 case was diagnosed upon consultation. The 5 patients included 1 man and 4 women, aged from 30 to 49 years, averaging 39; all were single, with the left kidney affected in 3 individuals, and the right kidney in 2. A total of 4 cases showed no discomfort or clinical symptoms. Only 1 case had right flank, intermittent episodes of discomfort, and pronounced tiredness. There was no urgency, frequent urination, urinary pain and other symptoms. The 5 cases underwent CT scanning to strengthen the inspection, which confirmed the tumors to be kidney single solid space-occupying lesion (**Figure 1**).

Methods

Pathological examination: Kidney specimens were obtained by resection, fixed with 10% formalin, and paraffin-embedded; sections were

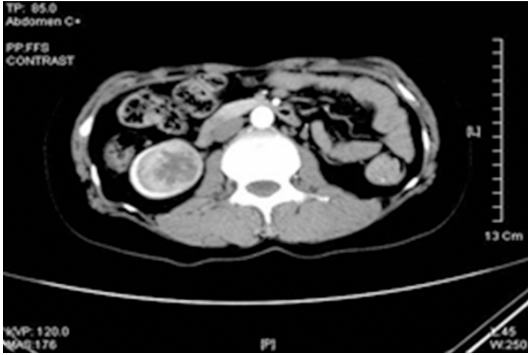


Figure 1. CT showed a substantial portion of arterial phase mass, obviously irregular annular patchy enhancement.

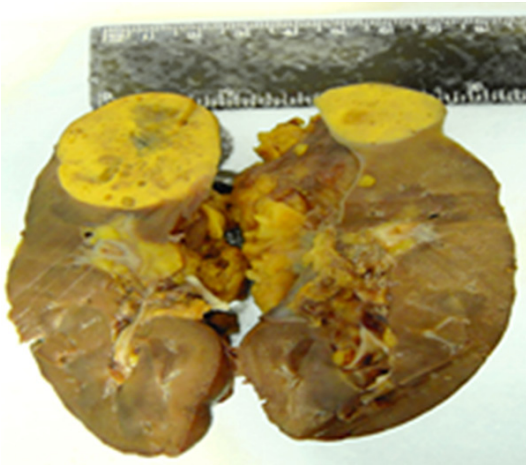


Figure 2. MA giant check: round mass with surrounding grayish yellow, clear boundaries.

HE stained, followed by light microscopy observation.

Immunohistochemistry: Samples were dewaxed, and the EnVision was used for immunohistochemical staining, with the following antibodies: WT1 (ready-to-use), S-100 (1:2000 DAKO), Vimentin (1:400, Zymed), CK7 (1:150, Zymed), RCC (1:100, Zymed), P504S (1:60, ZETA), and CD10 (1:60, NoVocastra). Staining was carried out according to manufacturer's instructions with the DAB chromogenic kit. The samples were assessed as follows: at high magnification ($\times 400$), 10 representative fields were randomly selected, and the rate of positive cells was computed as (positive tumor cell number/total tumor cells) $\times 100$. The following scaling was adopted: negative, $\leq 10\%$ positive cells; positive, $> 10\%$. Meanwhile, 20 cases of renal tumor (WT) and papillary renal cell carcinoma

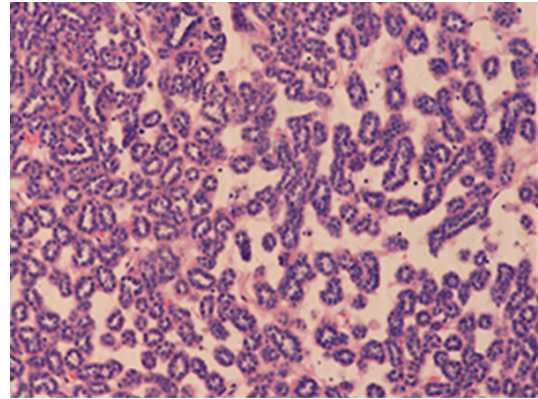


Figure 3. Metanephric adenoma tumor cells are smaller, with similar size, forming a small tubular or acinar structure HE $\times 100$.

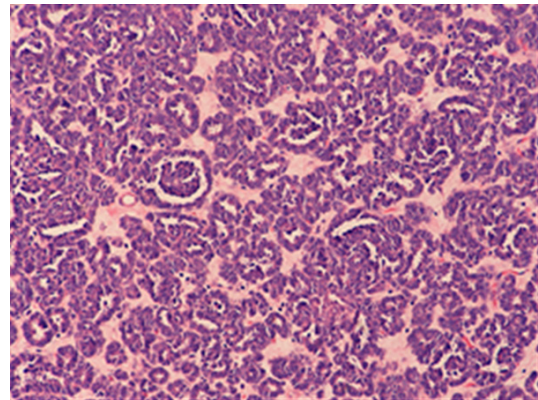


Figure 4. Metanephric adenoma is seen in the glomerular structure of tumor tissue HE $\times 100$.

(PRCC) were selected as controls for immunohistochemistry.

Fluorescence in situ hybridization (FISH): The FISH detection kit for cell chromosomes and genetic abnormalities was purchased from Beijing Gold Pujia Medical Technology Co. LTD. It contains green fluorescence labeling probe on chromosomes 3 and 7 centromeres (CSP3, CSP17), red fluorescence labeling probe on chromosome 7 centromere (CSP7), hybrid to the chromosome 9 long arm 9 p21 gene, and p16 red fluorescence labeling probe (GLP p16). Paraffin-embedded tissue sections (4 microns) were conventionally dewaxed with xylene, rehydrated by alcohol gradient in water, after 56°C baking, pepsin digestion, 2 \times SSC buffer rinsing and fixation with formaldehyde.

For modified hybridization, 10 μ l hybrid probe degeneration drops were added to the glass

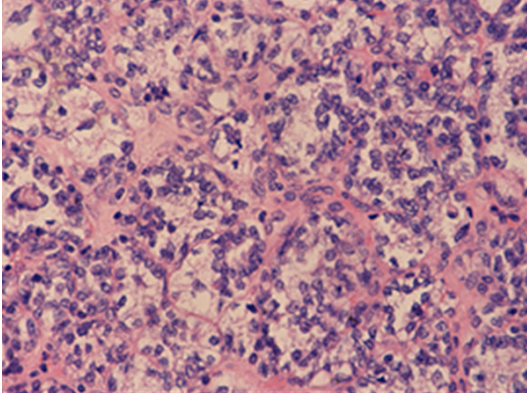


Figure 5. Metanephric adenoma associated with atypical composition: cell empty bright, nuclear in the center or to the side HE×200.

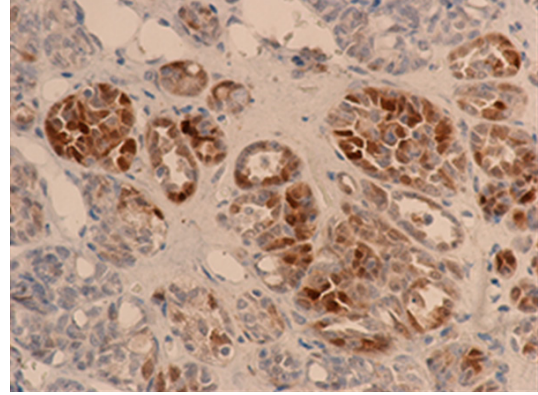


Figure 7. Metanephric adenoma tumor cells were S-100 positive (EnVision method ×400).

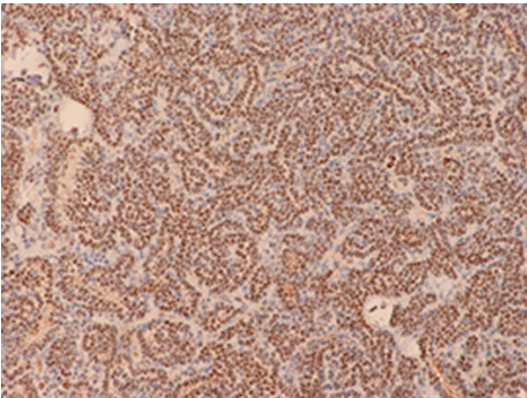


Figure 6. Metanephric adenoma tumor cells were WT1 positive (EnVision method ×100).

containing tumor cells, incubated at 78°C for 6 min, and hybridized in a wet box at 42°C overnight. After washing, the results were obtained by counterstaining with 15 µl DAPI under a fluorescence microscope.

Results

Gross and histological examination

The 5 cases were analyzed after bilateral renal tumor resection; tumors were 3-5.5 cm in diameter, averaging about 4 cm; nodular, grey and grayish sections, with 1 case showing tangent plane of focal hemorrhages; the 5 tumor cases were thin layer coated, with clear surrounding tissue boundaries (**Figure 2**).

As for histopathological characteristics, MA tumor cells were of uniform size, formed a small tubular or acinar structure (**Figure 3**), with less

nuclear matter; the nuclei were round or oval, with inconspicuous nucleoli; no atypia and mitosis, interstitial fiber separation were observed. Among 2 cases with dense cell populations, a tubular arrangement, in part pseudopapillary or glomeruloid structures were observed (**Figure 4**); cells were cubic or ovoid, with no to little mitosis, interstitial edema. In another example, in addition to the above structure, empty bright visible part of the cytoplasm was observed, with the nucleus in the middle or partially to one side (**Figure 5**). The 5 cases showed tumor tissues with a clear demarcation from the surrounding kidney tissues.

Immunohistochemical staining data

MA tumor cells from all 5 cases showed consistent expression of Vimentin and WT1, which were diffuse and strongly positive (**Figure 6**); 4 cases displayed scattered or diffuse expression of S-100 (**Figure 7**); 2 cases each showed expression of CK7 (focal +) and P504S (focal +~+++); 1 case showed expression of CD10 (++) and all 5 were negative for RCC. The 20 cases of renal tumor (WT) samples displayed WT1 and Vimentin expression. In addition, the 20 PRCC cases showed different degrees of CD10, RCC, Vimentin, P504S and CK7 expression (**Table 1**).

Fluorescence in situ hybridization

The fluorescence labeled probes NO. 3, NO. 7 and NO. 17 of chromosome centromeres were detected by FISH in 4 MA cases, confirming that NO. 3, NO. 7 and NO. 17 chromosome abnormality; the P16 gene of chromosome 9

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Table 1. Positive expression of MA, WT and PRCC by immunohistochemistry [positive cases (positive rate)]

	cases	WT1	Vimentin	CD10	P504S	RCC	S-100	CK7
MA	5	5 (100%)	5 (100%)	1 (20%)	2 (40%)	0 (0%)	4 (80%)	2 (40%)
WT	20	18 (90%)	20 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
PRCC	20	0 (0%)	20 (100%)	12 (60%)	18 (90%)	16 (80%)	0 (0%)	16 (80%)

Note: MA: metanephric adenoma; WT: renal tumor; PRCC: corpora mammillaria renal cell carcinoma.

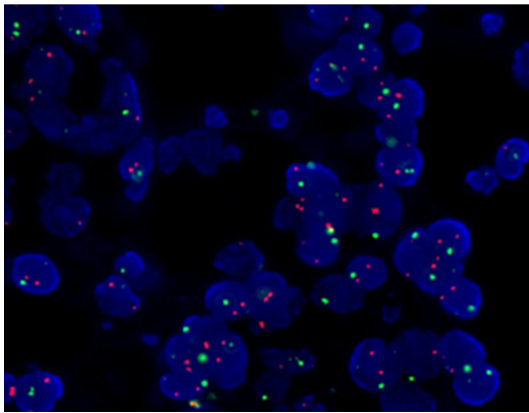


Figure 8. Metanephric adenoma cells' P16 (red), 17 (green) normal diploid signals.

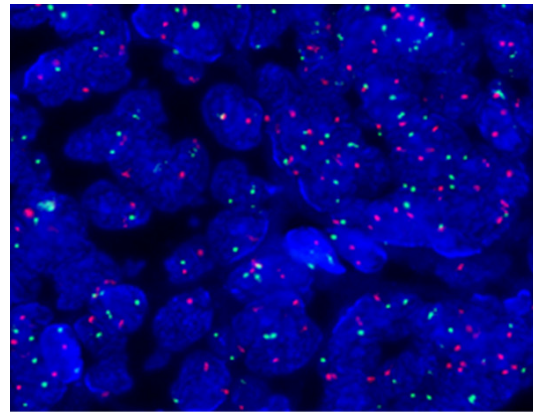


Figure 9. Metanephric adenoma: NO. 3 (green) and NO. 7 (red) normal diploid signals.

had no abnormality (**Figures 8, 9**). However, in positive controls (1 case of clear cell renal carcinoma and 1 case of papillary renal cell carcinoma) NO. 3, NO. 7 and NO. 17 chromosome showed abnormally amplified signals (**Figures 10, 11**).

Discussion

MA is an uncommon renal tumor. Its histological origin is usually ascribed to renal residual tissue tumor during embryonic development. Muir [1] suggested that MA occurrence is associated with NRS and WT1 originated in nephrogenic primitive cells, with both MA and WT belonging to the same lineage. However, other studies revealed that the occurrence of MA is related to the Bowman epithelial primitiveness [3].

MA affects people of any age, preferentially women, with a predilection age between 50 and 60 (average, 41 years). Arroyo [4] reported a minimum age of 5 months for this disease. With no specific clinical manifestations, the majority of cases are accidentally discovered during examination or CT. According to previous reports, polycythemia can be seen in 12% of

patients, with MA cells producing and secreting erythropoietin. and a variety of other factors; the 5 cases studied here did not show symptoms. CT, MRI and other imaging techniques showed solid renal parenchyma in the placeholder, but which is not be specific to disease diagnosis; no difference between benign and malignant lesions was obtained.

MA is unilateral, more located in the cortex; tumor sizes range from 0.3 and 20 cm, with more having 3-6 cm; the tumors are thin layer coated, with clear surrounding kidney tissues; they are gray or yellowish gray, homogeneous, solid section, and can also be associated with cystic change, hemorrhage, necrosis and secondary calcification change.

Histologically, MA tumor cells have similar size, small volume, no obvious atypia, no mitotic activity or occasional mitotic figures; the following forms of common arrangement are found: small gland bubble and glomerular and tubular structures, papillary and bud sample structures. These histological characteristics often overlap with PRCC and WT, identification is particularly important especially when the tumor cell morphology is not typical. Wt is a common

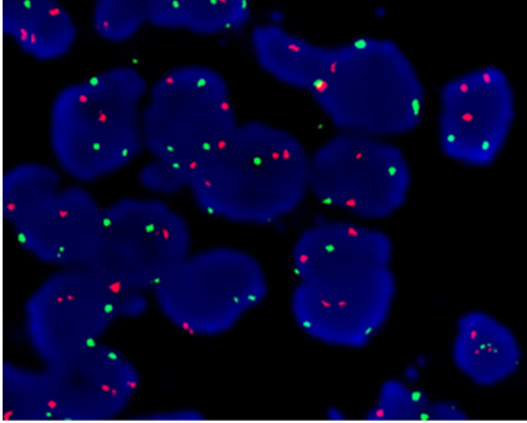


Figure 10. Clear cell renal carcinoma: NO. 3 (green), NO. 7 (red) abnormal amplification signals.

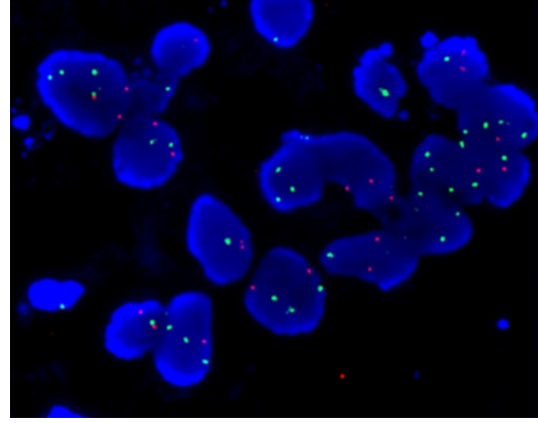


Figure 11. Clear cell renal cell carcinoma: NO. P16 (red), and NO. 7 (green) abnormal amplification signal.

malignant embryonal kidney tumor in children, defined by the cells and degree of differentiation of embryonic epithelial and mesenchymal components; two or more ingredients of differentiation can be often observed: tumor cell pleomorphism and with nuclear division. PRCC is more of a cortex tumor, with clear boundaries, volume changes and multifocal structures. Microscopic cancer tissue are papillary structures, containing the fiber axis of blood vessels; cells are cubic or columnar, undergo mitosis, foam cells is frequent to see, with partly visible hemosiderosis. When the three forms and structures are difficult to diagnose, immunohistochemistry and FISH can be helpful for differential diagnosis. The known immune phenotypes of MA include vimentin, WT1 and S-100 expression, and no CK7 and EMA detection [5]. The group of 5 cases expressed vimentin and WT1, 4 cases expressed S-100; only 2 cases expressed CK7 and P504S; RCC and CD10 were not detected. In controls, WT immunolabeling showed WT1 and vimentin expression, while S-100, CK7, P504S, CD10, and RCC were negative [6, 7]. PRCC mainly expressed CD10, RCC, vimentin, P504S and CK7, with WT1 and S-100 negative; thus, combined application of WT1, S-100, P504S, CD10, RCC and CK7 antibodies has certain value in the differential diagnosis of the three tumor types.

MA FISH data reported in the literature are not consistent: most studies confirmed normal chromosome copy numbers for no. 7 and 17, e.g. Kato H [8] et al; however, flow cytometry analysis of the tumor cells showed that they have a diploid karyotype.

Only few reports [9] indicated that 7 and 17 chromosomes are triploid. The group FISH was detected in 4 MA cases, confirming that chromosomes no. 7 and 17 have normal diploid karyotypes.

Patients with good prognosis, the 5 cases were followed up for 3 months to 6 years without signs of local tumor recurrence and metastasis. But in recent years, MA mixed with malignant spindle cell sarcoma in composition has been described: gland renal sarcoma [10-12]; in addition, MA containing papillary carcinoma and regional lymph node metastasis has been reported. Therefore, with the atypical behavior of MA, patients affected by this disease still need to be paid attention to, with long-term and close follow up.

Disclosure of conflict of interest

None.

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