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Frequent expression of neuroendocrine markers in mucinous tubular and spindle cell carcinoma of the kidney

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Summary. Mucinous tubular and spindle cell carcinoma (MTSCC) is a new tumorous entity which has been recently established. In this article, we examined the expression of neuroendocrine markers including neuron specific enolase (NSE), chromogranin A and synaptophysin in 16 cases of MTSCC using immunohistochemistry. The sex ratio (male: female) of the patients was 4:12. In normal kidney, distal tubules or collecting ducts were positive for NSE, but no structures were positive for chromogranin A or synaptophysin. All MTSCCs showed a positive reaction for NSE. Additionally, fifteen of sixteen neoplasms (93.8%) with MTSCC showed the expression of either chromogranin A or synaptophysin or both. Finally, it is possible that MTSCC may be one of renal neoplasms which frequently exhibit the neuroendocrine differentiation.

Key words: Mucinous tubular and spindle cell carcinoma, Immunohistochemistry, Neuroendocrine markers

Introduction

After Parwani et al. (2001), Hes et al. (2002) and Rakozy et al. (2002) reported detailed histological or genetic features of four, eleven and five renal tumors which were composed of cuboidal and spindle cells with mild cytologic atypia on the myxoid background, the new disease entity was introduced as mucinous tubular and spindle cell carcinoma (MTSCC) in the recent WHO classification (Srigley, 2004). We previously reported a neuroendocrine differentiation in such a case (Kuroda et al., 2004). However, whether neuroendocrine differentiation is a universal phenomenon in MTSCC or not remains uncertain. Therefore, we tried to examine the expression of neuroendocrine markers in 16 cases of MTSCC.

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Materials and methods

Archival tissues

We examined 16 cases of MTSCC. These specimens were retrieved from the files of the Departments of Pathology and Laboratory Medicine, Kochi Medical School, Kochi University, Japan (2 cases) and Department of Pathology, Charles University Hospital Pilzen, Czech Republic (14 cases) and their affiliated hospitals. Neoplams selected for the present study include some cases which have been previously reported (Hes et al., 2002; Kuroda et al., 2004). The mean age of the patients was 52.6 years (range 22 to 70 years). These numbers were calculated for 15 patients because the age of one patient (no.4) was uncertain. The sex ratio (male: female) of the patients was 4:12. The mean size of neoplasms was 7.8 cm (range 3.5 to 13 cm). These numbers were calculated for 15 neoplasms because the size of one neoplasm (no. 13) was unknown. Biopsy material was fixed in 10% neutral formalin and embedded in paraffin. Sections were cut (thickness, 3 μm) and routinely stained with hematoxylin-eosin.

Immunohistochemistry

Immunohistochemical staining of all renal tumors was performed on 3-μm-thick formalin-fixed and paraffin-embedded tissues using a Histofine simple stain MAX-PO (multi) kit (Nichirei, Tokyo, Japan). The tissues were deparaffinized in xylene (5 min, four times) and rehydrated in a graded ethanol series. After washing in PBS and treatment with 0.1% pronase E at 37 °C for 20 min, the sections were incubated with 0.3% hydrogen peroxide/methanol for 15 min, washed again in water for 5 min, and finally treated with antibodies against neuron

specific enolase (NSE) (clone: BBS/NC/VI-H14, dilution: 1:50, Dako Cytomation, Glostrup, Denmark), chromogranin A (polyclonal, dilution: 1:500, Dako Cytomation, Glostrup, Denmark) and synaptophysin (polyclonal, dulition: 150, Zymed, San Francisco, CA, USA) at 4 °C overnight. Sections were treated with 10mmol/L citrate, pH 6.0, in a 750-W microwave oven for three 5-minute cycles for antigen retrieval before all assays. Each incubation was followed by a rinse in PBS for 5 min, three times. Subsequently, the sections were incubated with anti-mouse IgG and anti-rabbit IgG conjugated with peroxidase, and for 1 hr at room temperature. After washing with Tris buffer for 5 min, DAB (Sigma Chemical, St Louis, MO) was employed to confirm the presence of immunocomplexes. Five normal renal tissue specimens located remotely from carcinomas resected by nephrectomy were used as appropriate positive and negative controls.

Results

Routine microscopic findings

Histologically, neoplasms were composed of cuboidal (Fig. 1a) and spindle (Fig. 1b) cells on the myxoid or edematous background. However, myxoid stroma was absent in some tumors. Cuboidal cells showed various growth patterns including tubular,

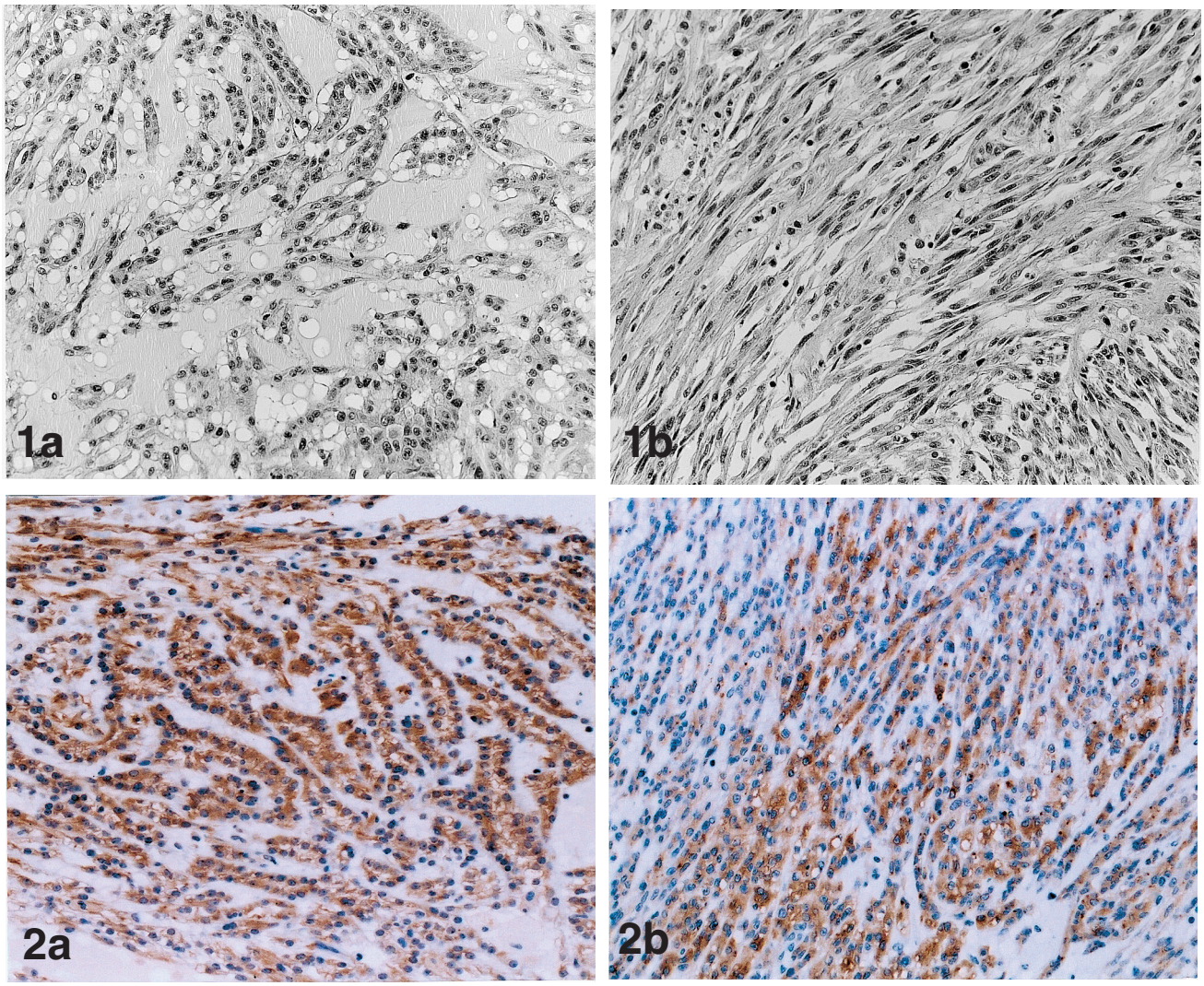

Fig. 1. Microscopic findings of mucinous tubular and spindle cell carcinoma (MTSCC). **a.** The proliferation of cuboidal neoplastic cells is observed. **b.** Spindle cell foci are evident. x 25

Fig. 2. Immunohistochemical results of chromogranin A and synaptophysin. **a.** Neoplastic cells are focally positive for chromogranin A. **b.** Neoplastic cells are strongly positive for synaptophysin. x 25

trabecular or cord-like, papillary and solid. Many neoplastic cells showed eosinophilic cytoplasm. Nuclei exhibited low-grade atypia, and nuclei were round and uniform in size without pleomorphism. Abnormal mitoses were absent. One tumor showed mixed subtype of MTSCC and conventional renal cell carcinoma.

Immunohistochemical findings

In normal kidney, the cytoplasm of collecting ducts or distal tubules were positive for NSE. However, no cells positive for chromogranin A or synaptophysin were identified.

Immunohistochemical results of MTSCC were summarized in Table 1. All neoplasms showed a positive reaction for NSE. Six neoplasms were diffusely positive for NSE and ten tumors were focally positive for NSE. Six neoplasms were intensively positive for NSE. Among them, three neoplasms were diffusely positive and the remaining three were focally positive. Fourteen neoplasms showed a positive reaction for chromogranin A and the remaining two neoplasms were negative. Among them, thirteen tumors were focally positive (Fig. 2a), whereas one tumor was diffusely and strongly positive. Thirteen neoplasms were focally reactive for synaptophysin and the remaining three neoplasms were negative. Among thirteen neoplasms showing positive reaction for synaptophysin, four neoplasms were intensively positive (Fig. 2b). In total, fifteen of sixteen MTSCCs showed the expression of either chromogranin A or synaptophysin or both. There were no significant differences between cuboidal and spindle cells on the positivity for NSE, chromogranin A and synaptophysin.

Discussion

Among unclassified renal cell carcinomas (RCCs), several renal tumors sharing common characteristic histological features have been reported (Ordonez et al, 1996; He et al., 1998; Lloreta et al., 1998; Otani et al., 2001). Parwani et al. (2001) reported four cases of low-grade myxoid renal epithelial tumors with distal nephron differentiation. Subsequently, Hes et al. (2002) reported 11 neoplasms designated as cuboidal and spindle cell carcinoma. Around the same time, Rakozy et al. (2002) reported five neoplasms designated as low-grade tubular-mucinous renal neoplasm. Additionally, they reported multiple losses of chromosomes 1, 4, 6, 8, 9, 13, 14, 15 and 22 in these neoplasms using comparative genomic hybridization. Srigley et al. (2002) also reported frequent losses of chromosomes 1, 4q, 6, 8p, 9q, 11q, 13, 14 and l5, and gains of chromosomes 11q, 12q, 16q, 17 and 20q. On the basis of the evidence presented, these neoplasms have been introduced as MTSCC in the recent WHO classification (Srigley, 2004).

Recently, we (Kuroda et al., 2004) elucidated a neuroendocrine differentiation in one case of MTSCC using immunohistochemistry and electron microscopy. Ultrastructurally, we found dense-core neurosecretory granules measuring 100-330 nm in the cytoplasm of neoplastic cells of MTSCC. However, whether the neuroendocrine differentiation is a universal phenomenon in MTSCC or not remained unknown. Therefore, we examined the expression of neuroendocrine markers in MTSCC in large series. In the present study, we found consistent (100%) positivity for NSE. However, as the specificity of NSE is not so good, Cohen et al. (1995) and Rasmuson et al. (1999) reported that cases with clear cell renal cell carcinoma (RCC) immunohistochemically showed the positivity of 78% and 100%, respectively. Additionally, NSE was observed in the normal kidney at the level of distal/medullary tubules, as was observed in the present study. On the other hand, we also confirmed the high frequency (93.8%) of neuroendocrine differentiation as typified by chromogranin A or synaptophysin positivity in MTSCC. Guy et al. (1999) found the minute paraganglion nests detected by chromogranin A within the renal hilum primitive stroma of two fetuses at 22 and 26 weeks, but completely absent in the kidney of infants, children and adults. Synaptophysin-positive cells were completely absent in all specimens of the kidney of fetuses, infants, children and adults. Kawabata (1999) reported that paraganglionic tissues were observed in fetal and adult materials in the kidney. Edgren et al. (1996) reported that 10 cases of clear cell RCC were completely negative for chromogranin A and synaptophysin. Rasmuson et al. (1999) also reported the low frequency (4%) of the positivity for chromogranin A in clear cell RCC. Therefore, our results suggest the possibility that MTSCC frequently may exhibit the neuroendocrine differentiation. The spindle cell morphology in MTSCC may explain the phenomenon of neuroendocrine differentiation, despite low nuclear

Table 1. Immunohistochemical results in MTSCC.

CASE NUMBER	NSE	CHROMOGRANIN A	SYNAPTOPHYSIN
1	d++	f+	f++
2	d+	f+	f+
3	f++	f+	f+
4	d+	f+	f+
5	d++	f+	f+
6	f+	f+	f+
7	f+	-	f+
8	f+	f+	f+
9	f+	f+	f+
10	f+	f+	-
11	f++	f+	f++
12	f+	f+	f++
13	d+	f+	f+
14	f+	-	-
15	f++	d++	-
16	d++	f+	f++

f, focal; d, diffuse; -, negative, +, positive; ++, strongly positive.

atypia. Although the discrepancies in the expression of neuroendocrine markers in kidney may depend on the difference of employed reagents or technical point of view, the expression of neuroendocrine markers in MTSCC may indicate the recapitulation of minute paraganglion nests in fetal kidney or the focal neuroendocrine transformation of neoplastic cells of distal nephron. The correlation between the prognosis and the expression of neuroendocrine markers remains unknown because of the small number of cases with MTSCC. Further examination will be required.

Among other renal neoplasms showing the neuroendocrine differentiation, carcinoid tumor and small cell carcinoma are generally well known (Stahl and Sighu, 1979; Tetu et al., 1987). Regarding the prognostic aspects, carcinoid tumor and small cell carcinoma in the kidney represent the low-grade and high-grade forms of neuroendocrine neoplasia. MTSCC generally pursue the favorable course, but some cases of MTSCC showing metastatic potential have been reported (Hes et al., 2002; Srigley, 2004). Considering results of our study, MTSCC should be distinguished from so called "neuroendocrine carcinoma of the kidney" (Guillow, 2004) in the differential diagnosis. In our opinion, further study has to improve the relationship between MTSCC and "neuroendocrine carcinoma of the kidney".

In summary, our study confirmed almost constant positivity of MTSCC for the neuroendocrine markers, ie NSE, chromogranin A and synaptophysin.

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References

Cohen C., McCue P.A. and DeRose P.B. (1995). Immunohistochemistry of renal adenomas and carcinomas. J. Urol. Pathol. 3, 61-71.

Edgren M., Stridsberg M., Kalknar K.M. and Nilsson S. (1996). Neuroendocrine markers; chromogranin A, pancreastatin and serotonin in the management of patients with advanced renal cell carcinoma. Anticancer Res. 16, 3871-3874.

Guillow L. (2004). Neuroendocine carcinoma of the kidney. In: Tumors of the urinary system and male genital organs. World Organization Classification of Tumours. 1st ed. Eble J.N., Sauter G., Epstein J.I. and Sesterhenn I.A. (eds). IARCPress. Lyon, France. pp 84.

Guy L., Begin L.R., Oligny L.L., Brock G.B., Chevalier S. and Aprikian A.G. (1999). Searching for an intrinsic neuroendocine cell in the kidney. Pathol. Res. Pract. 195, 25-30.

He Q., Ohsaki Y., Mori O., Asano G. and Tuboi N. (1998). A case of renal cell tumor in a 45-year-old female mimicking lower portion nephrogenesis. Pathol. Int. 48, 416-420.

Hes O., Hora M., Perez-Montiel D.M., Suster S., Curik R., Sokol L., Mikulastik J., Betlach J., Peychl L., Hrabal P., Kodet R., Straka L., Ferak L., Vrabec V. and Michal M. (2002). Spindle and cuboidal renal cell carcinoma, a tumour having frequent association with nephrolithiasis: report of 11 cases including a case with hybrid conventional renal cell carcinoma/ spindle and cuboidal renal cell carcinoma components. Histopathology 41, 549-555.

Kawabata K. (1999). Letter to the editor concerning searching for an intrinsic neuroendocrine cell in the kidney. Pathol. Res. Pract. 195, 865-866.

Kuroda N., Nakamura S., Miyazaki E., Hayashi Y., Taguchi T., Hiroi M., Yamasaki Y., Shuin T. and Enzan H. (2004). Low-grade tubular-mucinous renal neoplasm with neuroendocrine differentiation: A histological, immunohistochemical and ultrastructural study. Pathol. Int. 54, 201-207.

Lloreta J., Corominas J.M., Munne A., Dominguez D., Bielsa O., Gelabert A. and Serrano S. (1998). Low-grade spindle cell carcinoma of the kidney. Ultrastruct. Pathol. 22, 83-90.

Ordonez N.G., Mackay B. and Swanson D.A. (1996). Renal cell carcinoma with unusual differentiation. Ultrastruct. Pathol. 20, 27-30.

Otani M., Shimizu T., Serizawa H., Ebihara Y. and Nagashima Y. (2001). Low-grade renal cell carcinoma arising from the lower nephron: A case report with immunohistochemical, histochemical and ultrastructural studies. Pathol. Int. 51, 954-960.

Parwani A.V., Husain A.N., Epstein J.I., Beckwith J.B. and Argani P. (2001). Low-grade myxoid renal epithelial neoplasms with distal nephron differentiation. Hum. Pathol. 32, 506-512.

Rakozy C., Schmahl G.E., Bogner S. and Störkel S. (2002). Low-grade tubular mucinous renal neoplasms: Morphologic, immuno-histochemical, and genetic features. Mod. Pathol. 15, 1162-1171.

Rasmuson T., Grankvist K., Roos G. and Ljungberg B. (1999). Neuroendocrine differentiation in renal cell carcinoma. - evaluation of chromogranin A and neuron-specific enolase. Acta Oncol. 38, 623-628.

Srigley J.R., Kapusta L., Reuter V, Amin M., Grignon D., Eble J., Weber A. and Moch H. (2002). Phenotypic, molecular, and ultrastructural studies of a novel low-grade renal epithelial neoplasm possibly related to the loop of Henle. Mod. Pathol. 15, 182A.

Stahl R.E. and Sighu G.S. (1979). Primary carcinoid of the kidney. Light and eletron microscopic study. Cancer 44, 1345-1349.

Srigley J.R. (2004). Mucinous tubular and spindle cell carcinoma. In: Tumors of the urinary system and male genital organs. World Organization Classification of Tumours. 1st ed. Eble J.N., Sauter G., Epstein J.I. and Sesterhenn I.A. (eds). IARCPress. Lyon, France. pp 40.

Tetu B., Ro J.Y., Ayala A.G., Ordonez N.G. and Johnson D.E. (1987). Small cell carcinoma of the kidney. A clinicopathologic, immunohistochemical, and ultrastructural study. Cancer 60, 1809-1814.

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