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Detection of renin in chromophobe renal cell carcinomas

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A B S T R A C T

Aim: To evaluate frequency of renin detection in chromophobe renal cell carcinoma, and if this expression was associated to systemic high blood pressure.
Material and methods: A descriptive retrospective study. All the cases with confirmed diagnosis of chromophobe carcinoma and resected between 1990 and 2004 were included in our study: 31 cases from 31 patients. Immunohistochemistry was carried out on sections from the paraffin-embedded tissue using a monoclonal antiserum. Patient blood pressure before and after neoplasm resection was registered from clinical histories. We compared frequencies of hypertension in cases with and without expression of renin (Fisher's text or χ^2 as appropriate) and evolution of HTA after tumour resection.
Results: We found that 10 of 31 tumors (32.3%) contained immunoreactivity for renin; this staining was diffuse in 6 cases and focal in the other 4. Systemic hypertension was detected in 6 of 10 (60.0%) patients with renin expression and in 6 of 21 (28.6%) patients without renin immunolabeling (p=0.13). After tumor resection no patient with renin expression and high blood pressure showed remission of the hypertension.
Conclusion: Renin is frequently expressed in chromophobe renal cell carcinoma, but this renin appears clinically inactive. More studies will be necessary to know implications of this feature on clinical presentation, diagnosis or pathogenesis.

Detección de renina en carcinomas cromófobos de células renales

Palabras clave:
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Tumores renales
Renina

R E S U M E N

Objetivo: Evaluar la expresión de renina en carcinomas cromófobos de células renales y la posible asociación de esta expresión con hipertensión arterial (HTA) sistémica.
Material y métodos: Estudio descriptivo retrospectivo. Se incluyeron todos los casos con diagnóstico confirmado de carcinoma cromófobo entre 1990-2004: 31 casos provenientes de 31 pacientes. De los bloques de tejido tumoral incluidos en parafina se hizo inmunohistoquímica para detectar renina usando un anticuerpo monoclonal. De los archivos de historias clínicas obtuvimos información completa con respecto a la presión arterial sistémica antes y después de la resección tumoral. Comparamos fre-

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cuencias de HTA en casos con y sin expresión de renina (prueba de Fisher o χ^2, según lo adecuado) y evolución de la HTA posresección.

Resultados: En 10 de los 31 tumores (32,3%) hubo inmunotinción para renina; esta tinción fue difusa en 6 casos y focal en los 4 restantes. Se detectó HTA en 6 de los 10 pacientes con expresión de renina (60,0%) y en 6 de los 21 pacientes sin expresión de renina (28,6%) (p = 0,13). Después de la resección tumoral, ningún paciente con expresión de renina e HTA mostró remisión de la hipertensión.

Conclusión: En el carcinoma cromófobo de células renales es frecuente la expresión de renina, pero esta renina parece clínicamente inactiva. Serán necesarios más estudios para conocer las implicaciones en el diagnóstico, la patogénesis y la presentación clínica de esta expresión.

Introduction

Many benign and malignant neoplasms may secrete hormones, some of them active and others with no evidence of clinical activity. Several renal tumors have been shown to secrete these products.[1,2] There are small case series and isolated case reports of renal epithelial tumors expressing renin.[3-8] Renin is synthesized at the juxtaglomerular complex cells as a 406-amino acid proenzyme, prorenin, which is enzymatically inactive but is converted by proteolysis into the active enzyme (renin), which contains 340 amino acids.[9-11] The enzyme responsible for conversion of prorenin into renin has not been identified yet.[11] In many renin-expressing tumors, the enzyme has been shown to be inactive (with no associated systemic high blood pressure [HBP]). The hormone may be secreted into plasma in an inactive form or may be stored in cell cytoplasm, but not secreted.[12] Most of these studies and cases were reported before the 90s, and some of them before 1985, when chromophobe carcinoma and renal oncocytoma had not been recognized and characterized. Thus, such reports do not usually state the specific type of renal epithelial tumor, at least according to our current classification.[13] Renin expression in Wilms tumor (nephroblastoma) has been reported more frequently in medical literature,[14-17] and has also been reported in tumors originating in other organs.[12,18-23]

A comprehensive review of medical literature reveals, based on the microscopic description of neoplasms and/or microscopic images published, that many of these renin-producing renal tumors have cells with an eosinophilic granular appearance of their cytoplasms, suggesting that they could be chromophobe carcinomas, one of the renal tumors most frequently showing this cytological appearance.[13] This led us to wonder whether renin expression is common in this histological type of carcinoma. In a retrospective review of the file of one of the authors, 31 cases of chromophobe carcinoma were found. These tumors were microscopically reassessed and included in this study.

The purpose of this study was to investigate by immunohistochemistry (IHC) whether chromophobe carcinomas express renin and, if so, with what frequency, what are the microscopic characteristics of immunolabeling, and if renin expression is associated to systemic HBP.

Materials and methods

This was a descriptive, retrospective study including cases of chromophobe carcinoma diagnosed from January 1990 to May 2004. The reason for including cases diagnosed from 1990 only was the improved quality of tissue processing from that date (fixation and paraffin embedding), which made tissues more adequate for IHC. All renal tumors were reviewed, and only fully documented cases of chromophobe carcinoma were selected. Cases where diagnosis was not fully documented were excluded (these were mainly cases with a presumptive diagnosis of chromophobe carcinoma, eosinophilic variant versus oncocytoma). Based on these criteria, 31 tumors (from 31 patients) were found and included for renin detection by IHC. Basic clinical data and information about systemic blood pressure before and after tumor resection were available for all patients. All patients had undergone surgical resection of their lesions (by total or partial nephrectomy). No cases had been diagnosed by incisional or needle biopsy. Variables considered included age at diagnosis and sex, tumor size, systemic HBP diagnosis before tumor resection, persistence or not of HBP after resection, and renin immunolabeling and its extent (see below). The study materials consisted of histological sections, paraffin-embedded tissue blocks, and clinical information available at the respective records.

Immunohistochemistry. Sections 4 µm-thick of the tumor tissue blocks selected were placed into loaded plates adequate for IHC. Sections were deparaffined with xylol for 30 min and rehydrated using ethanol. IHC was performed using a monoclonal antibody directed against human renin. This antibody has been tested and shown to be specific.[24] It is not commercially available, and requires a 1:1,000 dilution and incubation for 40 min. Endogenous peroxidase activity was blocked with oxygen peroxide. Antigen recovery was performed with phosphate buffer in a vaporizer with ethylenediaminetetraacetic acid, pH 9.0, for 30 min. Diaminobenzidine was used as chromogen. Sections of a juxtaglomerular cell renal tumor (reninoma) were used

as positive controls. Renin staining was considered to be positive if expressed in the cytoplasm of neoplastic cells. The extent of staining was semiquantitated as minimal if 5% or less tumor cells were positive; as focal if 5%-50% of tumor cells were positive; or as diffuse if more than 50% of tumor cells were positive.

Statistical analysis. Results are given as median, minimum and maximum or as percentages. To compare percentages, a Fisher's test or a Chi-square test was used as appropriate. SPSS®, Chicago, Il, version 17 was used for analysis.

Results

Median patient age at diagnosis was 60 years (32-80). Eighteen patients (58.1%) were females. Tumor size ranged from 2.5-23.0 cm (median, 7.0 cm). Tumor staging at resection was: stage I, 15 patients (48.4%); stage II, 11 patients (35.5%); stage III, 3 patients (9.7%); and stage IV, 2 patients (6.5%). Twelve patients (38.7%) had been diagnosed with mild to moderate HBP (stage I or II) before tumor resection.[25] No patient had organ damage attributable to HBP. No other renal lesions or tumor lesions in other organs were found in any patient.

Renin immunostaining. Renin was detected in 10 out of the 31 carcinomas (32.3%). Immunolabeling was granular and diffuse (in more than 50% of tumor cells) in 6 cases (19.4%) and focal (>5% to 50%) in 4 cases (12.9%) (figs. 1 and 2). In two cases, labeling was shown in 100% of tumor cells. Strikingly, granular positivity was shown in two cases in the apical portion of cytoplasm, and immunostaining was diffuse (>50% of cells) in both. In the remaining 8 cases, labeling was evident throughout the cytoplasm (figs. 1 and 2). In the 10 positive cases, cells expressing positivity for renin were neoplastic cells.

Of the total 31 cases, 7 corresponded to the eosinophilic variant of chromophobe carcinoma. In another 5 cases, areas with cells whose cytoplasms had an eosinophilic granular appearance were included in sections selected for immunolabeling. Among these 12 cases with eosinophilic cells, 4 showed renin expression (33.3%), as compared to 6 out of 19 cases with the usual (non-eosinophilic) cytoplasm appearance (31.6%) (p=1.00).

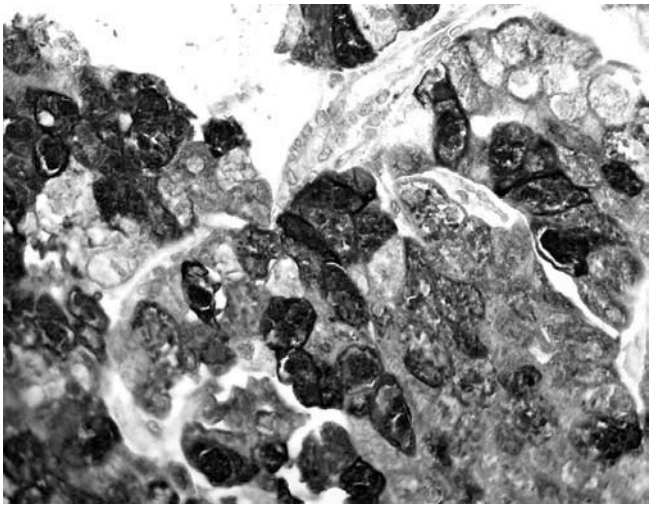

Figure 1 – Chromophobe carcinoma with strong diffuse immunolabeling, expressed throughout the cytoplasm of neoplastic cells. Immunostaining for renin, original magnification ×400.

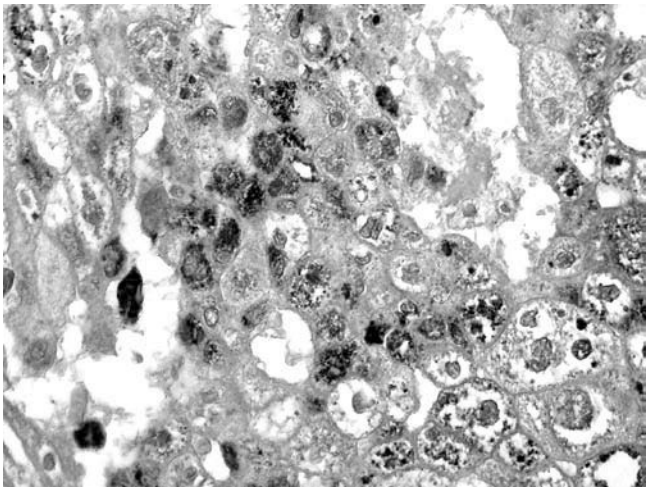

Figure 2 – In 4 out of the 10 renin-positive cases, immunolabeling was focal, occurring in less than 50% of neoplastic cells, as in this case, where cells expressing the hormone are mixed with other renin-negative cells. Immunostaining for renin, original magnification ×400.

Renin expression and systemic high blood pressure. Six of the 10 patients (60%) with renin expression and 6 of the 21 patients (28.6%) with no renin expression in their carcinomas had HBP before tumor resection. However, this greater HBP rate in patients with renin expression was not statistically significant (p=0.13; Fisher's test). After tumor resection, all 6 patients with HBP and renin expression in neoplastic cells continued to have HBP after a median follow-up of 20 months (12-65), and they all received drugs for HBP control some time during the 12 months following resection.

Discussion

Many cases of hormone-producing renal cell tumors have been reported in medical literature, including some small case series of renin-secreting epithelial tumors. There is however no study reporting the frequency of renin expression in each histological type of renal tumor. Our study was intended to determine the frequency of renin expression in a tumor which is not very common (accounts for approximately 5% of renal epithelial tumors[13,26]) but is seen with some frequency in our daily practice. The objective of this study was to detect expression of the enzyme and its potential association to systemic manifestations such as HBP.

Our results show that approximately one third of chromophobe carcinomas express renin in the cytoplasm of their cells. However, we do not know yet the significance, origin, and clinical implications of this finding. Although HBP

was found somewhat more frequently in patients positive for renin (60% vs 28.6%), no statistically significant association was shown for this greater frequency, and 4 of the 10 renin-positive patients had no HBP. More relevant than these data is the fact that in all 6 patients with renin expression and HBP, the latter did not disappear after tumor resection and patients continued to have systemic blood pressure figures similar to those found before resection and continued to receive antihypertensive drugs. This finding suggests that renin detected in tissue is not active (or is structurally abnormal) or is not released into the systemic circulation. Another interesting possibility, suggested by other authors, is that the renin detected could be taken up from plasma by tumor cells.[5]

It would be useful to know whether renin is synthesized or taken up by neoplastic cells. Knowledge of the precise location of renin granules in cytoplasm could help determine this: if renin (or prorenin) is synthesized by cells, at least some renin granules would be located in the smooth endoplasmic reticulum. The exact location of granules cannot be determined with IHC. In reninomas, granules appear throughout the cytoplasm, as occurred in 8 of our cases. However, granule location was apical in two of them, which suggests the possibility that granules are in a cytoplasmic organelle, such as endocytotic vesicles.

The granular appearance of cytoplasm in the eosinophilic variant of the chromophobe renal cell carcinoma is due to the presence of abundant mitochondria, as shown in ultrastructural studies;[27,28] however, such granular appearance is common in other renin-positive renal neoplasms (including conventional carcinomas and oncocytomas),[3-8] which may suggest that renin granules may also impart this granular, eosinophilic appearance to the cytoplasm of renin-expressing tumors. This study found renin expression in both eosinophilic granular cells and cells with the clear granular appearance of the usual chromophobe carcinoma. There was no difference in enzyme expression frequency between cases with eosinophilic tumors (33.3%) and those with the usual cytological appearance (31.6%). Thus, cytoplasm appearance was not related in our series to renin expression.

This study was intended to detect renin by IHC in a renal tumor which is not the most common, and has provided interesting results suggesting the need for further studies to ascertain the source of this renin, whether it is a structurally modified protein, if it is released into blood or not, why it is not clinically active, and whether enzyme expression frequency is different in this type of renal epithelial tumor as compared to other types. Although no ultrastructural studies were available in the reported cases and plasma renin and prorenin levels are unknown, our results are very important to address other projects to better understand the clinical, diagnostic and/or therapeutic implications of this aberrant expression.

On the other hand, we do not know yet whether this renin expression may have diagnostic implications for histological classification or "typing" of renal tumors. Our study, focused on a single type of tumor, does not allow for determining whether it may be helpful for differential diagnosis. As regards implications of this renin expression for understanding of histogenesis, it may help to better understand it, because chromophobe carcinomas have traditionally been thought to be derived from renal tubular epithelium, a site where no renin production has been reported.

In conclusion, a series of chromophobe renal cell carcinomas were studied, and cytoplasmic renin expression was shown in one third of them. This renin was shown to be inactive, as it was not associated to HBP. Chromophobe carcinomas may express renin in their cells much more frequently than previously thought. This finding raises questions that should be answered in future projects aimed at investigating the relevance of renin expression with regard to clinical characteristics, diagnosis, histogenesis, or treatment.

Conflict of interest

Authors state that they have no conflicts of interest.

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