Acid, basic, and neutral peptidases present different profiles in chromophobe renal cell carcinoma and in oncocytoma

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RENAL CELL CARCINOMAS (RCCs) are neoplasias with high prevalence and mortality. We previously reported that several peptidases may be involved in the pathophysiology of clear cell renal cell carcinoma (CCRCC). Now, to gain insight into the reasons that lead the various RCC types to behave very differently with regard to aggressiveness and response to anticancer treatments, we analyzed subsets of chromophobe renal cell carcinoma (ChRCC), and renal oncocytoma (RO), a benign tumor, as well as different grades and stages of CCRCCs. Particulate APN, APB, and APA activities were decreased in both ChRCC and RO (tumor vs. nontumor tissues). Interestingly, activities were downregulated in a tumor-type specific way and the intensities of the decreases were stronger in the benign tumor than in the malignant type. Moreover, when two key histopathological parameters for tumor prognosis (high vs. low stage and grade) were analyzed, increases of activity were also observed in several of these cell surface peptidases (APN, APB). Some soluble activities (APB, Asp-AP) were also downregulated in the RCCs. With respect to genetic expression, PSA and APN were in a positive correlation related to their activities in both ChRCC and RO; but not APB, Asp-AP, APA, and PGI. These results may suggest an involvement of several peptidases in the pathophysiology of renal cancer, since they presented different patterns of activity and expression in tumors with different behaviors.

peptide signaling; renal tumor behavior; chromophobe carcinoma; renal oncocytoma

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phenotypes, a physiological approaching to the question in the kidney by establishing first peptidase profiles of alteration throughout different RCCs could be helpful in understanding the clinic of this disease and may be of diagnostic and prognostic interest.

These facts, taken together, make the comparative study of peptidase profiles in CCRCC, ChRCC, and RO an interesting model to test how the multiple and complex factors interacting in the tumor cells lead to a disease that behaves differently with regard to aggressiveness in the patient and response to treatment.

To implement these goals in the present study, we investigated the soluble and particulate activities and their mRNA levels of acid (Asp-AP, APA), basic (APB), neutral (PSA, APN), and omega (PGI) peptidases in ChRCCs and ROs (comparing tumor vs. nontumor tissue). Since a high or low tumor grade and stage of the kidney by establishing first peptidase profiles of alterations throughout different RCCs could be helpful in understanding the clinic of this disease and may be of diagnostic and prognostic interest.

The same peptidase profile as in the aforementioned study by our group was selected for a better comparison of the results with those in a CCRCC series, and to cover the complete spectrum of peptide-converting activity.

**MATERIALS AND METHODS**

The authors declare that all the experiments carried out in this study comply with current Spanish and European Union laws.

**Materials.** All the chemicals used were obtained from Sigma (St. Louis, MO).

**Renal tissue specimens and sample storage.** We analyzed renal tissue in a series from 10 patients with ChRCC (5 males, 5 females, mean age 64 yr, range 46–79), in a series from 8 patients with RO (6 males, 2 females, mean age 67 yr, range 44–84), and in a series of staged and graded CCRCCs from 73 patients (59 males, 14 females, mean age 62 yr, range 37–84). Profile of grading: G1–G2 = 37, G3–G4 = 36. Profile of staging: T1–T2 = 44, T3–T4 = 29. Patient consent and Hospital Ethics Committee approval were obtained in advance. Fresh tissue samples were obtained from surgical specimens from renal tumor patients. Tumor and nontumor (surrounding unin- volved tissue) areas were obtained for the study in all cases. Tissue samples were embedded in OCT, frozen in isopentane, and stored at −80°C until the enzyme assays were performed. In addition, selected tissue samples were formalin-fixed and paraffin-embedded for histopathological diagnosis following routine methods at the pathology lab. The 2002 TNM Edition was used for tumor staging (33), and the Furman’s method for grading (15).

**Sample preparation.** Explanted tissue samples were homogenized in 10 mM Tris-HCl buffer, pH 7.4, for 30 s at 800 rpm using a Heidelberg PZR 50 Selecta homogenizer and ultracentrifuged in a Centrifugon T-2070 Kontron Instruments apparatus at 100,000 g for 35 min. The resulting supernatants were used to measure soluble enzyme activities and protein concentrations. To avoid contamination with soluble enzymes, the resulting pellets were washed three times by incubation with the specific PSA inhibitor puromycin (40 μM) were performed in parallel. APB and acid aminopeptidase activities (Asp-AP, APA) were quantified with Arg-β-naphthylamide and Asp-β-naphthylamide substrates, respectively. Omega peptidase (PGI) activity was measured fluorimetrically using pGlu-β-naphthylamide as a substrate. These assays are based on the fluorescence of β-naphthylamine generated from the hydrolysis of the substrate by the enzyme. Reactions were initiated by adding 30–50 μl of sample to 1 ml of appropriate incubation mixture, depending on the enzyme, and substrate was analyzed as follows: PSA, APN, APB, and PGI activities (50 mM phosphate buffer, pH 7.4 for PSA, APN, and PGI activities, pH 6.5 for APB and 0.125 mM aminoacyl-β-naphthylamide); Asp-AP/APA activities (50 mM Tris-HCl buffer, pH 7.4, 1 mM MnCl2, and 0.125 mmol/l aminoacyl-β-naphthylamide). After 30-min incubation at 37°C, 1 ml of 0.1 M sodium acetate buffer (pH 4.2) was added to the mixture to terminate the reaction. The released β-naphthylamine was determined by measuring the fluorescent intensity at 412 nm with excitation at 345 nm, in a Shimadzu RF-540 Spectrofluorometer. Blanks were used to determine background fluorescence. Relative fluorescence was converted into picomoles of product using a standard curve, constructed with increasing concentrations of β-naphthylamine.

**Protein determination.** Protein concentration was measured in triplicate by the method described by Bradford (8), using 1 mg/ml BSA as the calibrator. The results were recorded as units of peptidase (UP) per milligram of protein. One unit of peptidase activity is the amount of enzyme necessary to release 1 pmol of β-naphthylamide per minute. Fluorogenic assays were linear with respect to hydrolysis time and protein content.

**Real-time quantitative PCR analysis.** Quantitative RT-PCR for detecting PSA, APN, APB, Asp-AP, APA, and PGI mRNA was performed to determine the transcription levels of these peptidases. The RNA of tumor and nontumor tissue samples from six ChRCC and five RO patients was isolated with the RNaseasy Protect kit (Qiagen), including a DNase digestion step using an RNase-free DNase kit (Qiagen) to exclude possible contamination by genomic DNA.

The Taqman real-time PCR system is based on a three-primer method. Two of the primers are designed following the traditional PCR concept (upper and lower primers). The third primer has a fluorescent group which, when included in the amplification, increases fluorescence emission. This system enhances amplification specificity (3 primers are used) and avoids nonspecific double-strand DNA detection, as the fluorescence is bound to one of the primers. cDNA was obtained using a Transcripter reverse transcriptase kit (Roche). Intron spanning Taqman probes were designed following the Roche Universal Probe Library method (world.roche-applied-science.com). Amplifications were run in a 7900 Real-Time PCR System (Applied Biosystems). Each value was adjusted by using 18S RNA levels as reference.

The following primers were used: Human PSA: probe human 19: 5′-CAGTGTAGGGAGTTCCTGTTG-3′ (upper primer), 5′-TGAAGGAGCCTGGGTGACTCT-3′ (lower primer). Human APN: probe human 18: 5′-CATCCATCAGAGATGGCAGAC-3′ (upper primer), 5′-TGCTGAAGAGATCGTTCTGG-3′ (lower primer). Human APB: probe human 22: 5′-ACCATGTACGCCAGAGG-3′ (upper primer), 5′-CAGGCCCTCAAGCAGGTTA-3′ (lower primer). Human Asp-AP: probe human 64: 5′-AGTCCCCCTCTCTCTCAGTCG-3′ (upper primer), 5′-TTCCTATTTCCTAGCTCTTGAG-3′ (lower primer). Human APA: probe human 72: 5′-GCCGATCGACCTGCACGAGG-3′ (upper primer), 5′-CAGCCTCTAGGAGCTGTA-3′ (lower primer). Human Asp-AP: probe human 46: 5′-GCTGAGGAAGCATCTGTCG-3′ (lower primer). Human APB: probe human 33: 5′-CATCCATCAGAGATGGCAGAC-3′ (upper primer), 5′-TGCTGAAGAGATCGTTCTGG-3′ (lower primer). Human APA: probe human 72; 5′-CGGAGACGCAGGACGT-3′ (upper primer), 5′-GGGCTCATCIAAAAACAGGGA-3′ (lower primer). Human PGI: probe human 47, 5′-GGGAAAGGACAGGACGTCCACAG-3′ (upper primer), 5′-TGTAACCCCTTTTGTTGTTCTCCA-3′ (lower primer).
Quantitative data for the mRNA of each peptidase were expressed as relative values with respect to “1”; “1” being the total amount of mRNA for a specific peptidase detected in the nontumor area of the sample labeled as n = 1.

Statistical analyses. Data were analyzed statistically using SPSS, version 10. Unpaired Student’s t-test was performed to detect differences between uninvolved tissues and tumors, as well as among low and high grades and stages. Statistically significant differences were considered at P < 0.05.

RESULTS

Figure 1 shows the peptidase activities measured in the soluble and particulate fractions of ChRCC patients. Activity is recorded as picomoles of product per minute per milligram of protein (UP/mg protein) and presented as means ± SE for n = 10.

Soluble basic and acid activities (Fig. 1A) decreased significantly in the ChRCC. Thus, APB activity decreased 1.6-fold when the tumor compared with nontumor tissue (tumor (t) = 6,382 ± 823 vs. nontumor (n) = 10,618 ± 502 UP/mg prot.; Student’s t-test, P < 0.01), and Asp-AP decreased 4-fold in the same way (t = 85 ± 17 vs. n = 368 ± 28 UP/mg prot.; Student’s t-test, P < 0.001). In contrast, neutral (PSA) and omega (PGI) activities in the soluble fraction of the ChRCC were not significantly altered (PSA: t = 10,269 ± 198 vs. n = 12,569 ± 262 UP/mg prot.; PGI: t = 130 ± 22 vs. n = 205 ± 29 UP/mg prot.).

On the other hand, all the cell surface peptidase activities of the ChRCC (Fig. 1B) decreased significantly, with the exception of the PGI, which did not vary significantly (t = 369 ± 36 vs. n = 578 ± 66 UP/mg prot.). APN/CD13 activity decreased 1.5-fold in the tumor compared with surrounding uninvolved tissue (t = 2,694 ± 1,183 vs. n = 45,637 ± 2,432 UP/mg prot.; Student’s t-test, P < 0.001). In the case of APB and APA/gp160 activities, these decreased 12-fold (t = 1,591 ± 356 vs. n = 19,306 ± 1,324 UP/mg prot.; Student’s t-test, P < 0.001) and 5-fold (t = 158 ± 98 vs. n = 813 ± 65 UP/mg prot.; Student’s t-test, P < 0.01), respectively.

In Fig. 2, we represent soluble and membrane-bound peptidase activities in tumor and unaffected human tissue from human kidney with RO. Activity is recorded as picomoles of product per minute per milligram of protein (UP/mg protein) and presented as means ± SE for n = 8.

As shown in Fig. 2A, only the acid activity significantly decreased (1.5-fold) in the soluble fraction of RO (Asp-AP: t = 163 ± 17 vs. n = 320 ± 21 UP/mg prot.; Student’s t-test, P < 0.05). No significant differences were found in soluble PSA, APB, and PGI activities of the human oncocytoma (PSA: t = 12,819 ± 1,183 vs. n = 8,515 ± 853 UP/mg prot.; APB: t = 6,039 ± 743 vs. n = 7,757 ± 679 UP/mg prot.; PGI: t = 262 ± 34 vs. n = 334 ± 26 UP/mg prot.).

However, as in the case of the ChRCC, most of the membrane-bound activities (Fig. 2B) decreased significantly in the RO when compared with the nonpathological surrounding tissue. Again, particulate PGI activity of the RO was the only exception (t = 111 ± 42 vs. n = 345 ± 73 UP/mg prot.). Moreover, we observed that decreases for each peptidase activity in the RO were higher than those for the chromophobe.
tumor. Thus, particulate APN/CD13 activity decreased more than 30-fold in RO when compared with healthy cells (t = 1,265 ± 811 vs. n = 38,510 ± 2,232 UP/mg prot.; Student's t-test, P < 0.001), APB activity in the membrane-bound fraction of the tumor decreased 16-fold (t = 1,325 ± 379 vs. n = 21,319 ± 1,582 UP/mg prot.; Student's t-test, P < 0.001), and cell surface APA/gp160 activity decreased 7.5-fold (t = 128 ± 53 vs. n = 966 ± 78 UP/mg prot.; Student's t-test, P < 0.01).

The relative expressions of PSA, APN, APB, Asp-AP, APA, and PGI in the tumor and nontumor tissue from human ChRCC (n = 6) and RO kidney samples (n = 5) are shown in Figs. 3 and 4, respectively.

As shown in Fig. 3, in the ChRCC only APN/CD13 (Fig. 3B) and PSA (Fig. 3A) expression correlated positively with the results in enzyme activity. In this sense, the relative expression of PSA was slightly but not significantly upregulated (1.5-fold) in the tumor, whereas the corresponding catalytic activity did not vary significantly. The mRNA for the APN decreased twofold in the ChRCC, in parallel with the significant downregulation observed in its enzymatic activity. In contrast, Asp-AP, APA, and PGI expression in the ChRCC were not in correspondence with these activities. Thus, Asp-AP expression increased 25% (Fig. 3C), and mRNA of PGI was 40% downexpressed.

The expression pattern for peptidases in RO (Fig. 4) was similar to that in the chromophobe subtype of renal carcinoma. The mRNA of PSA (Fig. 4A) increased 10-fold in the tumor, whereas a slight nonsignificant upregulation in its activity was observed. APN expression decreased 1.4-fold in the RO when compared with the surrounding unaffected tissue, which correlated with the results in enzyme activity (Fig. 4B). In contrast to the observed decrease in activity, mRNA for Asp-AP in oncocytic cells was sixfold higher than mRNA for Asp-AP in nontumor cells (Fig. 4C). APA expression (Fig. 4D), whose activity was also decreased in RO, did not change in a significant way (20% upexpression) with respect to nontumor expression. Finally, the expression levels for APB (Fig. 4E) and PGI (Fig. 4F) were highly upregulated in RO (APB = 3-fold; PGI = 4.6-fold tumor vs. nontumor).

Table 1 shows the peptidase activity profile throughout low and high grades and stages in CCRCCs. Low grades (LG) correspond to G1 and G2 phenotypes of the Furhman's scale, being G3 and G4 the high grades (HG) in that scale. With respect to the tumor staging, low stages (LS) were T1 and T2 of the 2002 TNM Classification, corresponding T3 and T4 to the high stages (HS).

All the activities were decreased in the low grade samples when compared with tumors presenting a high grade. Statistically significant decreases were found in PSA (LG vs. HG activity decreases 2-fold; Student's t-test, P < 0.001), APN (1.52-fold; Student's t-test, P < 0.05), APB (1.44-fold; Student's t-test, P < 0.05), and membrane-bound PGI (1.43-fold; Student's t-test, P < 0.05).

Fig. 2. Soluble (A) and particulate (B) peptidase activity profiles in kidney samples from 8 patients with renal oncocytoma (RCO). Columns compare tumor tissue (RCO) with nontumor surrounding tissue. Values represent means ± SE of peptidase activities recorded as pmol of aminoacyl-b-naphthylamide hydrolyzed per min (UP) per mg of protein.
Fig. 3. Relative PSA (A), APN (B), Asp-AP (C), APA (D), APB (E), and PGI (F) expressions in kidney samples from 6 ChRCC patients. Columns compare tumor tissue with surrounding nontumor tissue. Scattered diagrams represent mean (black line) ± SE of quantified mRNA recorded as values related to “1”; “1” being the total amount of mRNA for a specific peptidase detected in the nontumor area of the sample labeled as $n = 1$. 
In contrast, when CCRCC low and high stages were compared, only particulate APB activity decreased significantly (LS vs. HS activity decreases 1.45-fold; Student’s t-test, *P < 0.05*). Slight, but not significant, decreases of activity were found in APN (1.3-fold LS vs. HS) and APA (1.23-fold).

**DISCUSSION**

According to our results, peptidase-mediated proteolysis in renal tumors appears to tend toward activity decreases. We analyzed peptidase activities and expression and found that they were selectively altered by the tumoral process in affected tissue; moreover, we obtained profiles of activity and relative expression that were different with regard to the tumor subtype.

The major roles of peptidases in the pathobiology of ChRCC and RO seem to be mediated by cell surface forms, since, as we previously reported in CCRCC, once again, APN, particulate APB, and APA activities were strongly decreased in the tumor tissue. The main differences between the profiles of cell surface activities in ChRCC and those in RO were with regard to the intensity of the decrease they suffered. Thus, the intensity of APN, particulate APB, and APA activity inhibition was much higher in RO than in ChRCC.

When peptidase activities were compared throughout grade and stage in the CCRCC, the aforementioned tumor aggressiveness-related profile of the cell surface peptidases appears to maintain, since APN, APA, and membrane-bound APB and PGI activities were higher in high grades or stages than in the low grades and stages of the CCRCC samples.

Our results agree with those in the specialized literature, since peptidases are generally reported to be decreased in RCCs and in different intensities with regard to the subtype. In this sense, Göhring and co-workers (17) also found medium-to-strong downregulation in APN and APA activities when they compared several RCC subtypes. Moreover, immunohistochemistry assays reported that APA is practically absent from the cell surface in oncocytic cells, whereas, although there is a decrease in staining with respect to normal renal cells, this enzyme is strongly expressed in CCRCC (28). As cell surface APN, APB, and APA were found to be altered in a tumor type-specific way, our results are consistent with other studies that involve the aforementioned particulate forms in specific roles in renal cancer (9, 10, 20, 29).

In contrast with the consensus about the nature of the alterations of peptidase activity in the pathobiology of RCCs, results in the genetic expression are controversial. Both loss and gain of expression have been reported for most peptidases (29). This fact may be partially due to the functional complexity of carcinogenic mechanisms. In this sense, our results are not an exception. Thus, in the ChRCC as well as in the RO, only the relative expression of APN and PSA correlated positively with their activities. However, the mRNA levels of Asp-AP, APA, APB, and PGI were not in concordance with our data on peptidase activity. We reported a similar pattern of expression in our previous study on CCRCC (37).

Despite their uncertain results, these data could be helpful in elucidating whether changes in peptidase activities were due to real alterations in protein transcription or whether other epigenetic factors may be involved. The positive correlation between alterations of activity and mRNA of PSA and APN that were found in both ChRCC and RO tumor subtypes, and previously in CCRCC, points to real upregulation of PSA and real downregulation of APN protein transcription in RCCs. Our results for the relative expression of Asp-AP, APA, APB, and PGI in ChRCC and RO also agree with those we obtained in CCRCC and may suggest the involvement of genetic muta-
with our results, inhibition of soluble Asp-AP activity was also certain soluble peptidases induces accelerated growth and re-
example, it has recently been reported that upregulation of soluble activities may be taking a part in renal cancer. For
ogy. Thus, not only membrane-bound peptidases but also interplay with intracellular circuits, and so both cell surface and
tumor phenotype is malignant (ChRCC), decreases of cell
activities throughout the sequence of bad prognostic (low: G1–G2; high: G3–G4) and stages (low: T1–T2; high: T3–T4) in the
evolution of the clear cell renal cell carcinoma (CCRCC). n.s., Not significant.

tions or epigenetic cellular factors, different from the regul-
ation of gene expression, in the alterations of catalytic activity. Many proteases have functions that are not limited to proteol-
ysis (2), while each of the individual peptidases is able to perform several overlapping functions (10). As a result, the expression of each peptidase must be precisely regulated in a tissue-
and cell-specific manner, and mRNA levels may not always reflect the final status of the enzymatic activity in the disease (19, 21, 36).

On the other hand, our data extend the role of aminopepti-
dase activity in renal cancer to include cytosolic forms, since several soluble peptidases (Asp-AP, soluble APB) were found to be altered in a tumor-specific way, and with regard to both histological subtype and stage/grade. During carcinogenesis, many of the essential alterations which occur in cell physiology are mediated through extracellular cell signaling pathways that interact with intracellular circuits, and so both cell surface and intracellular molecules play pivotal roles in cancer pathobiology. Thus, not only membrane-bound peptidases but also soluble activities may be taking a part in renal cancer. For example, it has recently been reported that upregulation of certain soluble peptidases induces accelerated growth and re-
sistance to apoptosis in the human kidney (34). In agreement with our results, inhibition of soluble Asp-AP activity was also detected in rats with breast cancer induced by N-methyl-
nitrosourea (11).

The involvement of peptidases, mainly cell surface forms, in the pathophysiology of cancer has been widely documented. Most evidences are related to APN (9, 12, 14, 16, 18, 23, 30, 31). Although not as frequent as for APN, there is also evidence of the involvement of APA (18, 35, 25) and APB (31, 32) in cancer pathobiology. Despite the rich variability of function that peptidases appear to have in cancer, most of the studies point to an anti-tumor effect, and thus a protective role, when these enzymes are inhibited or downexpressed (31).

Although functions in the pathophysiology of renal cancer have been described for many cell surface peptidases (10), the ultimate mechanisms of action and targets for all these func-
tions remain uncertain and in many cases controversial. That controversy is due, in part, to the multifunctional properties and the tissue- or tumor-specific expression of peptidases (2, 10). Since peptidases appear to be down- or upregulated in the cancer depending on the affected tissue and the specific type, grade, and stage of the tumor, it is very difficult to reach a consensus about the specific mechanism of action for a con-
crete enzyme. In addition, several peptidases, such as APN, APB, and APA, may be playing overlapping roles in different renal tumors (6, 10). In this sense, the link of the results we obtained in CCRCC, ChRCC, and RO provides a model to study how the different and complex cellular and tissue factors in renal tumors are leading to a disease that behaves quite differently with regard to aggressiveness in the patient and response to treatment (1, 3, 5). Thus, ROs are benign neo-
plasms (5, 26), whereas CCRCCs are strongly aggressive and have poor prognosis (3, 15). ChRCCs appear to behave more indolently than the clear cell phenotype (5, 13), and thus, although malignant, they usually manifest a better prognosis than CCRCCs.

Interestingly, we obtained profiles of APN, APB, and APA activities in CCRCC (data from Ref. 37), ChRCC, and RO that were decreased with respect to normal renal parenchyma in a tumor type-dependent manner (Table 2); and in a different way with regard to aggressiveness due to the different specific behavior of each type. Thus, when tumor type is benign (RO), the cell surface peptidase activities apparently involved in the renal cancer are found strongly decreased (up to 30-fold with respect to the nontumor tissue in the case of APN). The tumor phenotype is malignant (ChRCC), decreases of cell surface peptidase activities (tumor vs. nontumor tissue) are much lower than the aforementioned in the RO (17-fold for APN). This tumor type-dependent behavior repeats for several peptidase activities whose decreases are well-established in the renal cancer (APN, APB, APA). CCRCC is reported to

### Table 1. Peptidase activities in CCRCC in different grades and stages

<table>
<thead>
<tr>
<th>Peptidase Type</th>
<th>Recommended Name</th>
<th>EC Number</th>
<th>Grade</th>
<th>Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutral</td>
<td>Puromycin-sensitive aminopeptidase (EC 3.4.11.14)</td>
<td>7,980 ± 922</td>
<td>14,378 ± 1,423</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Aminopeptidase N (EC 3.4.11.2)</td>
<td>4,756 ± 720</td>
<td>2,728 ± 978</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Basic</td>
<td>Soluble aminopeptidase B (EC 3.4.11.6)</td>
<td>5,078 ± 630</td>
<td>6,713 ± 717</td>
<td>n.s</td>
</tr>
<tr>
<td></td>
<td>Particulate aminopeptidase B (EC 3.4.11.6)</td>
<td>1,888 ± 259</td>
<td>2,443 ± 243</td>
<td>n.s</td>
</tr>
<tr>
<td>Acid</td>
<td>Aspartyl aminopeptidase (EC 3.4.11.21)</td>
<td>139 ± 14</td>
<td>200 ± 25</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Aminopeptidase A (EC 3.4.11.7)</td>
<td>513 ± 67</td>
<td>656 ± 68</td>
<td>n.s</td>
</tr>
<tr>
<td>Omega</td>
<td>Soluble pyroglutamyl peptidase I (EC 3.4.19.3)</td>
<td>95 ± 18</td>
<td>188 ± 41</td>
<td>n.s</td>
</tr>
<tr>
<td></td>
<td>Particulate pyroglutamyl peptidase I (EC 3.4.19.3)</td>
<td>105 ± 12</td>
<td>151 ± 15</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Values are means ± SE of peptidase activities recorded as pmol of aminoacyl-naphthylamide hydrolyzed per min (UP) per mg of protein. Profiles of soluble and particulate peptidase activity throughout different histopathological grades (low: G1–G2; high: G3–G4) and stages (low: T1–T2; high: T3–T4) in the evolution of the clear cell renal cell carcinoma (CCRCC).

### Table 2. Comparative pattern of APN, APB, and APA activities throughout the sequence of bad prognostic (CCRCC>ChRCC>RO) in the renal cancer

<table>
<thead>
<tr>
<th>Intensity of the Activity Decrease (Tumor vs. Normal Parenchyma)</th>
<th>CCRCC</th>
<th>ChRCC</th>
<th>RO</th>
</tr>
</thead>
<tbody>
<tr>
<td>APN</td>
<td>↓ 5-fold</td>
<td>↓ 17-fold</td>
<td>↓ 30-fold</td>
</tr>
<tr>
<td>APB</td>
<td>↓ 5-fold</td>
<td>↓ 12-fold</td>
<td>↓ 16-fold</td>
</tr>
<tr>
<td>APA</td>
<td>+++1.6-fold</td>
<td>↓ 5-fold</td>
<td>↓ 7.5-fold</td>
</tr>
</tbody>
</table>

Profiles of APN, APB, and APA activity throughout tumors of different aggressiveness: CCRCC, an aggressive carcinoma; chromophobe cell carcinoma (ChRCC), a malignant neoplasm; renal cell oncocytoma (RO), a benign tumor. Values in the rows represent, for each enzyme and throughout the 3 tumor types, how many folds the activity in the tumor tissue were decreased with respect to the nontumor renal parenchyma. Data for CCRCC are taken from our previous study (Ref. 37).

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behave with a worse prognosis than the ChRCC, and so to be more malignant, and the decreases of the cell surface peptidase activities we recorded on it (again comparing CCRCC tumor vs. nontumor) were lower than those we obtained in the ChRCC and in the RO (only 5-fold for APN). Thus, when the three tumor types are compared, it is seen how the peptidase activities of the tumor samples behave in an antiparallel way with regard to the specific malignancy of the tumor (minor decreases when the tumor is more malignant). Moreover, that antiparallel behavior of the peptidase activity also maintains along grading and staging in the CCRCC.

A peptidase-mediated antitumor mechanism in renal cancer concurs with data in the scientific bibliography. For example, inhibitors of the APN and APB, such as bestatin and puromycin, have been reported to induce apoptosis onset in solid tumors (12, 30, 31). Moreover, decreases of mRNA for APN cause inhibition of angiogenesis (16), and APA appears to promote tumor angiogenesis (25). As a result, its inhibition may be an antitumor mechanism.

In summary, our data may suggest an involvement of cell surface peptidases in the pathophysiology of renal cancer. When several peptidases downregulate, it appears to be beneficial with respect to tumor evolution. The nature of the mechanism still remains speculative. Further studies on other peptidases and a new approach to the profile of these enzymes may be an antitumor mechanism.

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