

Elevated Expression of Pyrimidine Nucleoside Phosphorylase (PyNPase) in Renal Cell Carcinoma Tissue

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Background: Pyrimidine nucleoside phosphorylase (PyNPase) is an enzyme that converts 5'-deoxy-5-fluorouridine to 5-fluorouracil. PyNPase is identical to platelet-derived endothelial cell growth factor (PD-ECGF), and has angiogenic activity. In the present study of PyNPase activity in renal cell carcinoma, we tested for correlation between PyNPase activity and tumor extension, tumor proliferation and clinical characteristics.

Methods: Samples of tumor tissue and non-tumor tissue were obtained from 10 renal cell carcinoma patients who underwent radical nephrectomy. These samples were examined, and PyNPase activity of the tissues was assayed.

Results: PyNPase activity was significantly higher in renal cancer tissue than in normal tissue ($p < 0.01$), and in immunohistochemical assays PyNPase was strongly stained in the cytoplasm of renal cancer cells. However, there were no significant correlation between PyNPase activity and tumor extension, according to the results of histopathological examination and evaluation of vascularity of renal cancer tumor tissue.

Conclusion: In this study, we did not observe correlation between PyNPase activity and renal cancer extension and proliferation. However, the present data suggest that pyrimidine-class drugs may be useful against renal cell carcinoma, because PyNPase activity is significantly higher in renal cancer tissue than in normal tissue.

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Introduction

Pyrimidine nucleoside phosphorylase (PyNPase) is

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an enzyme that converts 5'-deoxy-5-fluorouridine (5'-DFUR), a pro-drug of 5-fluorouracil (5-FU), to 5-FU [1, 2]. Recently, it was reported that PyNPase expression is accelerated in various tumors [3]. Also, the higher the PyNPase activity in tumor tissue, the greater the anti-tumor effect of pyrimidine-class anti-tumor drugs [4, 5]. Many reports have communicated that pyrimidine-class anti-tumor drugs are useful in patients with cancer of the breast and digestive organs, especially colorectal cancer [6, 7], and are expected to be useful in treatment of other tumor types.

Although renal cell carcinoma (RCC) accounts for just 3% of all adult cancers, among urinary tract tumors it is one of the most frequently occurring [8]. Interferon (IFN), the only drug which has been found to be useful in the treatment of RCC, produces a clinical response corresponding to approximately 20% [9] after radical nephrectomy. Therefore, more effective therapy for RCC is needed.

In 1992, using cloning techniques, researchers determined that PyNPase and PD-ECGF are the same substance [10, 11]. PyNPase was found to be angiogenic, and to greatly enhance tumor growth in vivo [12]. Most RCCs are hypervascular, and overexpress angiogenic factors such as VEGF [13] and b-FGF [14]. Therefore, investigation into PyNPase activity in renal cancer tissue might aid in the evaluation of prognostic factors and treatments of RCC.

To determine whether pyrimidine-class anti-tumor drugs would be effective against RCC, we investigated PyNPase activity, histopathology and clinical characteristics of RCC patients who underwent radical nephrectomy. Furthermore, since PyNPase and PD-ECGF are the same substance, we also attempted to determine whether there is a relationship between PyNPase activity and tumor extension and proliferation.

Materials and Methods

Patients

Surgical specimens used in this study were obtained

from 10 RCC patients (6 men and 4 women; age range, 53-76 years; mean age, 64.3 years) who underwent radical nephrectomy at Nagasaki University Hospital. Patients had not received therapy prior to the operation.

PyNPase activity

We measured PyNPase activity in 1-cm² (1cm×1cm) samples of tumor tissue and surrounding normal tissue, obtained from resected fresh specimens according to a method of Eda et al. [15]. Activity levels were expressed as the amount of 5-FU generated from 1mg protein over a period of 60 minutes ($\mu\text{g FU}/\text{mg protein}/\text{hr}$).

Relationship between PyNPase activity and tumor volume, angiogenesis and histopathologic characteristics

We examined the results of preoperative angiography to determine the degree of vascularity, and investigated the relationship between vascularity and PyNPase activity. We used tumor diameter to estimate tumor volume, and examined the relationships between PyNPase activity and tumor volume and histopathologic characteristics.

Localization of PyNPase activity

Immunohistologic staining was done using specimens from resected renal tissue, fixed according to the AMEX method [16], and mouse anti-human thymidine phosphorylase monoclonal antibody (a gift from the Research Center, Nippon Roche K. K., Kamakura, Japan). PyNPase localization in RCC tissue was examined according to the procedure described by Sawase et al [17].

Results

High PyNPase activity in RCC tissue

As shown in Figure 1, PyNPase activity in surrounding normal renal tissue ranged from 2 to 28.5 $\mu\text{g FU}/\text{mg protein}/\text{hr}$ (mean, 16.6 $\mu\text{g FU}/\text{mg protein}/\text{hr}$), and in renal cancer tissue activity ranged from 13.1 to 260 $\mu\text{g FU}/\text{mg protein}/\text{hr}$ (mean, 159.7 $\mu\text{g FU}/\text{mg protein}/\text{hr}$). In each patient, PyNPase activity was significantly higher in renal cancer tissue than in normal tissue ($p < 0.01$), and these levels (within a single patient) differed on average by a factor of 5. In Patient 9, the cancer tissue was cystic rather than solid, and this was likely the reason for the observed low PyNPase activity.

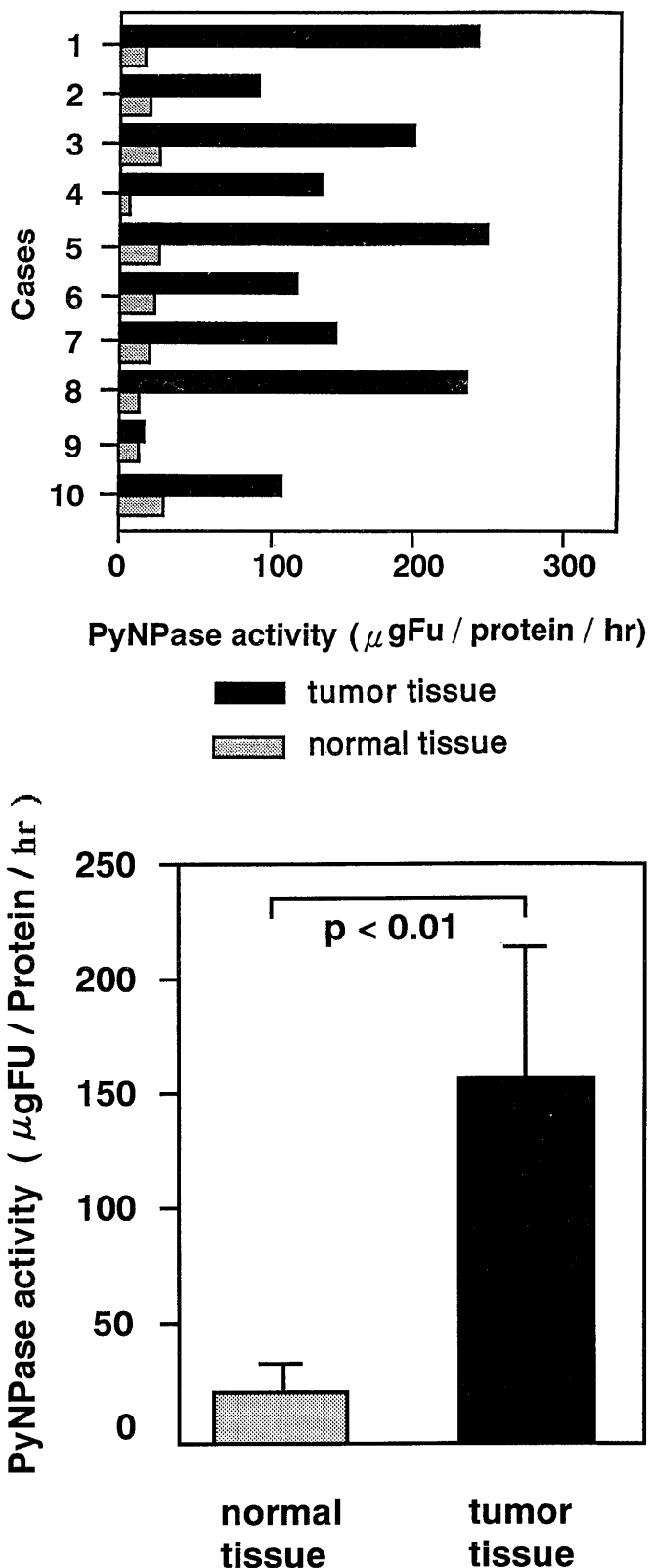


Fig. 1. PyNPase activity of renal cell carcinoma tissue and the surrounding normal renal tissue, in renal cell carcinoma patients. (A) PyNPase activity in each patient. (B) Mean of the PyNPase activity in all 10 RCC patients.

Tumor volume and PyNPase activity

Presumptive tumor volume was calculated from the operative specimens, in order to test for correlation with PyNPase activity in tumor tissue. Although PyNPase was observed to have angiogenic effects and to greatly enhance tumor growth, there was no significant correlation between PyNPase activity and tumor volume ($Y=0.08X148.4$, $X=148.4$, $R=0.134$) (Figure 2).

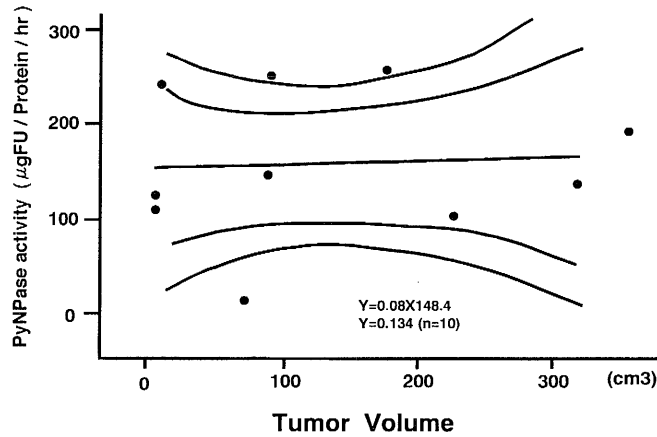


Fig. 2. Relationship between PyNPase activity in RCC tissue and tumor volume.

Gender and PyNPase activity

In general, the incidence of RCC is significantly higher in men than in women. In the present study, we found no correlation between gender and PyNPase activity in RCC patients (Table 1).

Table 1. Relationship between gender and PyNPase activity in RCC tissue.

Sex	N	Tissue PyNPase activity (µg FU/mg protein/hr)	T-test / correlation
male	6	171.5 ± 97.5	N.S.
female	4	141.9 ± 42.9	

Histopathologic characteristics and PyNPase activity

As show in Table 2, we observed no significant correlation between PyNPase activity and the following histopathologic characteristics: tumor stage (early stage vs advanced stage), cell type (clear cell subtype vs others), tumor grade (grade-1 vs grade-2), and infiltration (INF)

type (INF- α vs INF- β).

Table 2. Relationship between PyNPase activity in RCC tissue and histopathological findings.

	N	Tissue PyNPase activity (µg FU/mg protein/hr)	T-test/ correlation
Stage	I · II	6	177.7 ± 65.0
	III	4	132.6 ± 98.7
			N.S.
Cell type	Clear cell	7	181.9 ± 65.8
	Others	3	107.7 ± 94.0
Grade	G-1	4	158.4 ± 65.4
	G-2	6	160.5 ± 92.0
INF	α	7	163.2 ± 92.0
	β	3	151.3 ± 45.6
			N.S.

Vascularity and PyNPase activity

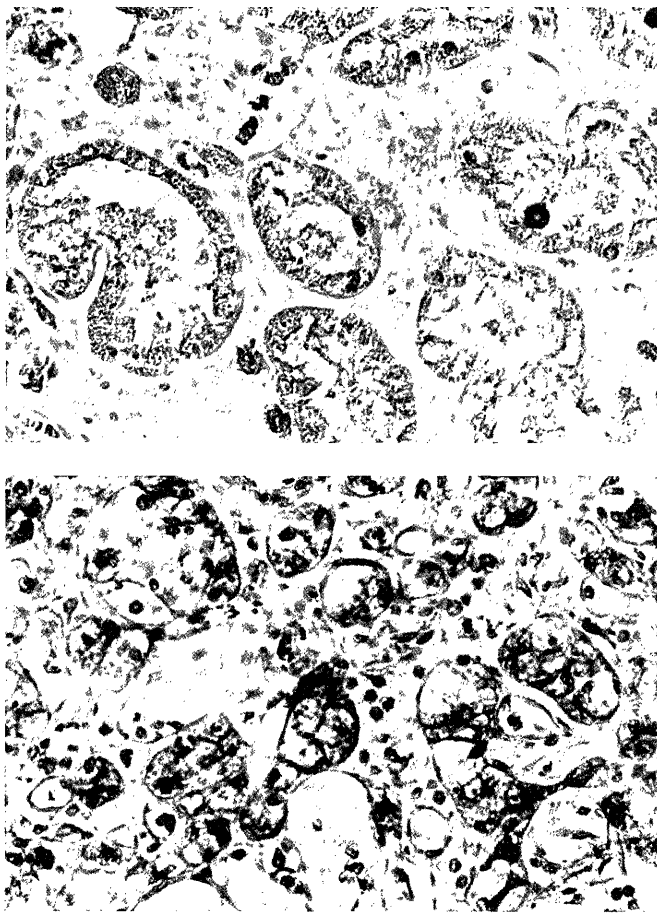
There was no correlation between results of preoperative angiography and PyNPase activity (Table 3). Even hypovascular RCC cells had high PyNPase activity levels.

Table 3. Relationship between PyNPase activity in RCC tissue and vascularity (as determined by angiography).

	N	Tissue PyNPase activity (µg FU/mg protein/hr)	T-test / correlation
Hypervascular	8	160.5 ± 85.5	N.S.
Hypovascular	2	156.3 ± 63.3	

Localization of PyNPase expression in RCC tissue and normal renal tissue

In normal kidney tubule cells, PyNPase expression was either weak or undetectable in the cytoplasm (Photograph A). In renal cancer cells, PyNPase was strongly expressed in the cytoplasm (Photograph B). Although there was no significant difference in localization of PyNPase expression between normal kidney tubule cells and renal cancer cells, higher levels of expression were observed in renal cancer cells.



Photograph. Immunohistochemical staining of PyNPase in renal tissue. (A) PyNPase was weakly expressed in normal renal tubular cells. (B) PyNPase was strongly stained in the cytoplasm of renal cell carcinoma cells. (A) and (B) are 400 \times field.

Discussion

In the present study, we found that PyNPase activity is significantly higher in RCC tissue than in surrounding normal tissues, and that it is localized in the cytoplasm of cancer cells; these results are similar to those of previous studies of PyNPase activity in colorectal and breast cancers. These data suggest that pyrimidine-class anti-tumor drugs would be effective

against RCC. Cytokines such as TNF- α , IL-1 α and INF- γ have been reported to increase PyNPase activity [18], and combined use of TNF- α and 5-FU has been reported to effectively inhibit tumor extension [19]. Based on these findings, it is reasonable to conclude that a therapy which combines IFN and 5'-DFUR might be particularly effective against RCC.

PyNPase and PD-ECGF (which has been determined to be an angiogenic factor) are the same substance. Some metabolites from nucleic acids that have been decomposed by thymidine phosphorylase induce angiogenic processes; in particular, chemotaxis and migration of bovine aortic endothelial (BAE) cells [20]. PD-ECGF has been reported to play a role in tumor extension/proliferation [21], and increased PD-ECGF expression has been reported to result in microvascular proliferation in tumor tissue [3, 22]. RCC is generally hypervascular, and the clinical features of this disease are influenced by angiogenic factors such as b-FGF [23] and VEGF [24]. These findings suggest a relationship between PyNPase activity and RCC extension, proliferation and tumor volume. However, our results did not provide evidence for a relationship between PyNPase activity in RCC cells and histopathologic characteristics such as tumor volume or angiography results. One possible reason for this finding is the small number of cases examined. Also, the present study did not include micro-level examination. Further study (involving a greater number of cases) will be needed in order to clarify the role of PyNPase in RCC.

In summary, there was strong staining of PyNPase in the cytoplasm of cancer cells, and PyNPase activity was significantly higher in RCC tissue than in normal tissue. This suggests that pyrimidine-class drugs may be effective against RCC.

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