Urol Res (2001) 29:7–12

ORIGINAL PAPER

K. Suzuki · T. Morita · S. Hashimoto · A. Tokue

Thymidine phosphorylase/platelet-derived endothelial cell growth factor (PD-ECGF) associated with prognosis in renal cell carcinoma

Received: 26 June 2000 / Accepted: 11 October 2000

Abstract We investigated the correlation between thymidine phosphorylase (TP)/platelet-derived endothelial cell growth factor (PD-ECGF) expression, angiogenesis, and prognosis in renal cell carcinoma (RCC) patients. We prepared paraffin block specimens from 56 postradical nephrectomy RCC patients. The preparations were immunohistochemically stained using anti-CD34 antibody and anti-TP antibody. Angiogenic findings were evaluated based on both microvessel density (MVD) and renal arteriography findings as classified by Roosen et al. TP expression showed heterogeneity in 56 patients: 11 (19.6%) were negative, 28 (50.0%) weak, and 17 (30.4%) positive. There was no correlation between TP expression, MVD, and renal arteriography. There was no TP expression in chromophobe types. Univariate analysis showed a significant correlation between survival and TP expression, patient age, tumor infiltration type, pathologic T- and N-stages, venous involvement, distant metastasis, and tumor grade. There was no correlation between survival and MVD or renal arteriography. Multivariate analysis showed a significant correlation between survival and pathologic T-stage, distant metastasis, tumor infiltration type, and TP expression. TP expression in RCC may be an independent prognostic factor rather than just an index for angiogenesis.

Key words Thymidine phosphorylase · Microvessel density · Renal arteriography · Renal cell carcinoma · Prognosis

K. Suzuki (✉) · T. Morita · S. Hashimoto · A. Tokue
Department of Urology, Jichi Medical School,
3311-1, Yakushiji, Minamikawachi-machi,
Kawachi-gun, Tochigi, 329-0498, Japan
Tel.: +81-285-58-7379; Fax: +81-285-40-6595

Introduction

Angiogenesis is essential for tumor growth and metastasis [6], and there is a significant correlation between angiogenesis and tumor progression, metastasis, and prognosis in various cancers, including renal cell carcinoma (RCC) [13, 24, 29, 36, 38, 39]. Angiogenesis is induced by various angiogenic factors [6]. For example, platelet-derived endothelial cell growth factor (PD-ECGF) has a molecular weight of 45 000 [19] and is related to the growth and chemotaxis of endothelial cells in vitro and to angiogenesis in vivo [7, 9]. Recent reports show TP is identical to PD-ECGF [21, 31]. TP expression is higher in tumor tissue in the esophagus, stomach, colon, rectum, lung, gallbladder, pancreas, kidney, and breast than in the surrounding normal tissue [11, 33, 35]. Furthermore, there is a significant correlation between TP expression and the recurrence rate in bladder cancer [20], and between TP expression and survival in gastric and pulmonary non-small cell carcinoma [14, 16]. Imazono et al. reported TP expression is an independent prognostic factor in RCC [11]. The purpose of the present study was to clarify the clinicopathologic significance of TP expression in RCC. We investigated: (1) TP expression in RCC, (2) angiogenesis based on microvessel density (MVD) and renal arteriography, and (3) the correlation between survival and clinicopathologic factors, including angiogenesis and TP expression.

Materials and methods

Patients and materials

Fifty-six patients with primary RCC who underwent radical nephrectomy at Jichi Medical School between 1977 and 1996 were included in this study. The patients included 46 men and 10 women with a mean age of 57.4 ± 11.7 years (mean ± SD). The average follow-up period was 62.9 ± 48.2 months. Adjuvant immunotherapy with interferon-alpha (IFN-α) was performed in 28 cases (50%). The response rate (including complete, partial and minor response) of the patients treated with IFN-α was 14.2% (4 of 28

cases). Pathological evaluation in the present study was based on a method reported by Imazono et al. [11] and conducted according to the criteria designated by the Japanese Urological Association [34]. Tumor grade was classified into two groups (grade 1 and grade 2 + 3) and, in cases when different grades coincided in either of the groups, the classification was based on the higher grade in the groups. Tumor infiltration type (INF) was divided into two groups: INFα and INFβ + INFγ. Patients were divided into two groups for venous involvement: no involvement (pV0) and involvement (pV1 + 2). Lymph node metastasis (pN) was divided into two groups: pN1 + 2, showing renal cell carcinoma cells in lymph nodes of the renal hilus, and pN0, no lymph node metastasis. Distant metastasis was classified into two groups: metastasis (M1) confirmed by preoperative imaging including chest X-ray, abdominal CT scan, and bone scintigraphy, and no metastasis (M0).

Immunohistochemical staining

Resected specimens were fixed in 4% paraformaldehyde solution, and embedded in paraffin. The preparation was deparaffinized, sliced into 4 μm slices, put on slide glasses, and treated in a microwave oven. The endogenous biotin was blocked using H_2O_2 followed by incubation with PBS containing 3% skim milk. The preparations were incubated overnight at 4 °C using either anti-TP antibody [1, 25] provided by Nippon Roche K.K. (Kamakura, Japan) or anti-CD34 antibody (My 10, CD34, Becton Dickinson Inc., Tokyo, Japan). Slices of the preparations, regarded as secondary antibody, were incubated with biotin-labeled anti-mouse IgG antiserum. Finally, the specimens were stained using an avidin-biotin complex method.

Evaluation of TP expression

TP expression in tumor cells was classified into three stages, based on Koukourakis et al. [14]: (1) negative (0%–20% cancer cells stained), (2) weak (weak and diffuse staining or strong staining in the cells <70%), and (3) positive (strong staining in the cells ≥70%). Staining with an irrelevant mouse IgG was performed routinely as a negative control and normal liver sections were used as a positive control because Kupffer cells express a high level of TP [28].

Evaluation of angiogenesis

Findings of renal arteriography were classified into two groups based on the classification described by Roosen et al. [27]: (1) hypervascular group, strongly stained in the tumor equal to or greater than 2/3, and (2) hypovascular group, stained in the tumor less than 1/3. MVD stained immunohistochemically by anti-CD34 antibody was calculated by a light microscope under a 200× field (ocular lens, 10×; object lens, 20×; and in a 0.7386 mm^2 field). MVD of the tumor was classified into three grades: (1) grade 1, 0–50 microvessels per field, (2) grade 2, 51–100 microvessels per field, and (3) grade 3, more than 100 microvessels per field. The staining of vessels in the stroma outside the tumor was used as a positive control and a staining with an irrelevant mouse IgG was performed routinely as a negative control. MVD measurement was conducted by a physician who had no information on the patients.

Statistics

For statistical analysis, we used statistical software, StatView-J 4.1 (Abacus Concepts, Inc., Berkeley, CA, USA). Fisher's exact probability test and chi-square test were used to assess correlation, and Cox's proportional hazard model was used for multivariate analysis. The Kaplan-Meier test was used to calculate the survival rate and we also conducted a significance test based on the log-rank test. Survival periods were defined based on postoperative periods. Statistical significance was designated at $P < 0.05$.

Results

Correlation between TP expression and clinicopathologic factors

TP expression was observed in both RCC cytoplasm and nucleus (Fig. 1). TP expression revealed heterogeneity in all 56 patients: 11 (19.6%) negative, 28 (50.0%) weak, and 17 (30.4%) positive (Table 1). Regarding the tumor growth pattern, there was no staining or negative results in the chromophobe type (two patients) which had been

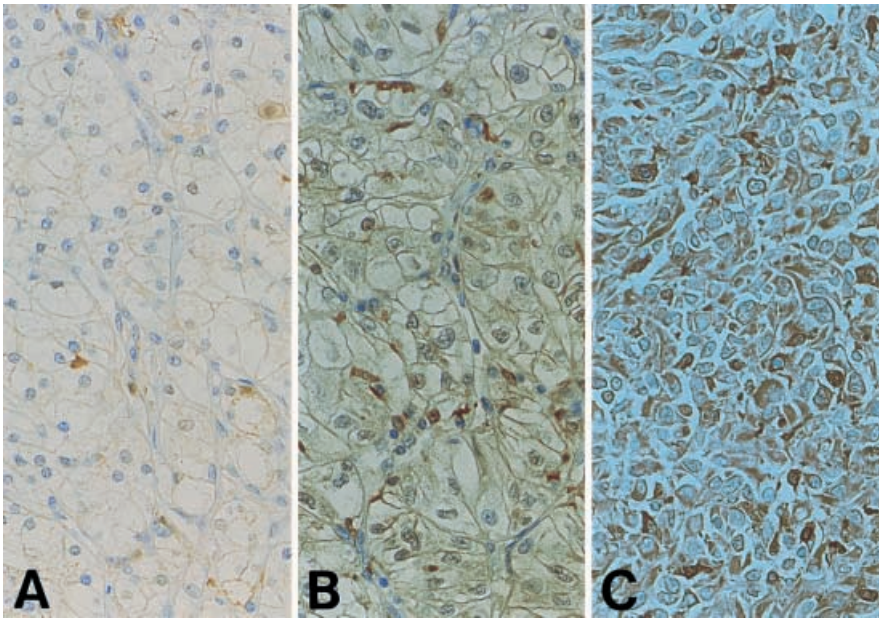

Fig. 1A–C Immunohistochemical staining of renal cell carcinoma (RCC). Thymidine phosphorylase (TP) expression was observed in both cytoplasm and nucleus of RCC. (**A**: TP negative, **B**: TP weak, **C**: TP positive) (×200)

Table 1 Relationships between thymidine phosphorylase (*TP*) expression and clinicopathological factors (*INF* infiltration, *NS* not significant)

Factor	Patients (*n*)	TP expression						*P* value[a]
		Negative ($n = 11$)		Weak ($n = 28$)		Positive ($n = 17$)		
Sex								
Male	46	8[b]	(17.4[c])	23	(50.0)	15	(32.6)	NS
Female	10	3	(30.0)	5	(50.0)	2	(20.0)	
Age								
≤65 years	39	10	(25.6)	21	(53.8)	8	(20.5)	<0.05
>65 years	17	1	(0.6)	7	(41.2)	9	(52.9)	
Arteriography[d]								
Hypovascular	14	3	(21.4)	4	(28.6)	7	(50.0)	NS
Hypervascular	40	8	(20.0)	22	(55.0)	10	(25.0)	
Unknown	2	0	(0.0)	2	(100.0)	0	(0.0)	
Growth pattern								
Alveolar	16	4	(25.0)	6	(37.5)	6	(37.5)	NS
Papillary	14	1	(7.0)	10	(71.4)	3	(21.4)	
Tubular	5	2	(40.0)	2	(40.0)	1	(20.0)	
Others	21	4	(19.0)	10	(47.6)	7	(33.3)	
Cell type								
Clear cell	30	7	(23.3)	19	(63.3)	4	(13.3)	<0.05
Mixed	9	2	(22.2)	3	(33.3)	4	(44.4)	
Granular	8	0	(0.0)	5	(62.5)	3	(37.5)	
Others	9	2	(22.2)	1	(11.1)	6	(66.7)	
Tumor grade								
1	17	4	(23.5)	13	(76.5)	0	(0.0)	<0.05
2 + 3	39	7	(17.9)	15	(38.5)	17	(43.6)	
INF								
INFα	34	7	(20.6)	20	(58.8)	7	(20.6)	NS
INFβ + INFγ	22	4	(18.2)	8	(36.4)	10	(45.4)	
pT								
pT1 + pT2	35	8	(22.9)	8	(51.4)	9	(25.7)	NS
pT3 + pT4	21	3	(14.3)	10	(47.6)	8	(38.1)	
pN								
pN0	41	9	(22.0)	19	(46.3)	13	(31.7)	NS
pN1 + pN2	3	0	(0.0)	2	(66.7)	1	(33.3)	
pN_x	12	2	(18.1)	7	(63.6)	3	(27.2)	
Distant metastasis								
M0	42	10	(23.8)	23	(54.8)	9	(21.4)	<0.05
M1	14	1	(7.0)	5	(35.7)	8	(57.1)	
Venous involvement								
pV0	36	10	(27.7)	19	(52.8)	7	(19.4)	<0.05
pV1 + pV2	20	1	(5.0)	9	(45.0)	10	(50.0)	
MVD								
Grade 1	32	4	(12.5)	18	(56.3)	10	(31.3)	NS
Grade 2	17	6	(35.3)	6	(35.3)	5	(29.4)	
Grade 3	7	1	(14.3)	4	(57.1)	2	(28.6)	

[a] Chi-square test
[b] Number of patients
[c] Percentage
[d] Classified according to Roosen's classification

classified into the other group (Fig. 2). Factors showing significant correlation with TP expression were patient age, tumor cell type, tumor grade, distant metastasis, and venous involvement. There was no correlation between TP expression, angiogenesis evaluated by renal arteriography findings, or MVD. We also examined the correlation between MVD and renal arteriography findings and other clinicopathologic factors, but none showed a correlation. (data not shown).

Correlation between survival and clinicopathologic factors

We used univariate analysis to examine correlation between survival and various clinicopathologic factors in RCC. Figure 3 shows survival curves with three groups classified according to TP expression, indicating 5-year survival rates of 100% for the TP negative group ($n = 11$), 83% for the TP weak group ($n = 28$), and 43%

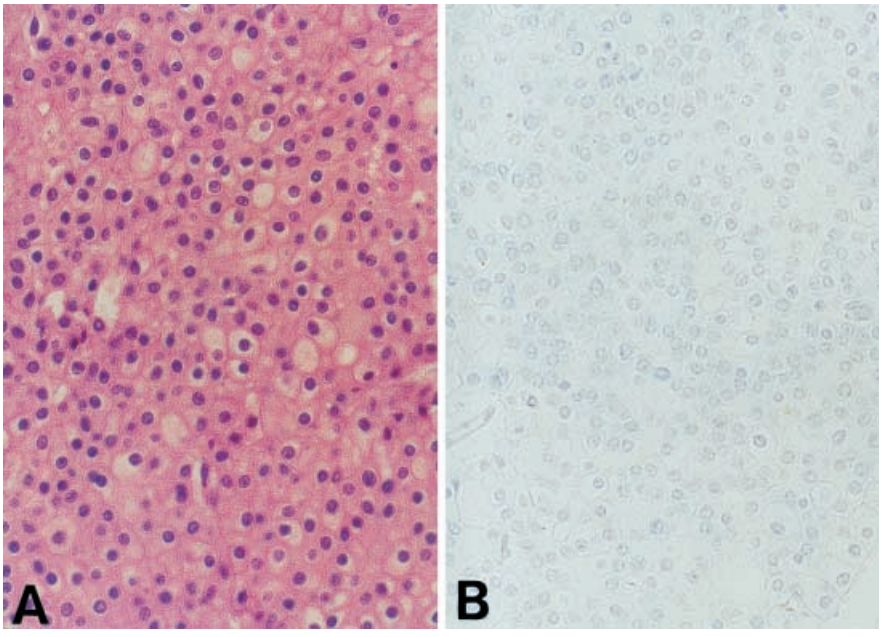

Fig. 2 Hematoxylin and eosin staining (**A**) and immunohistochemical staining for TP (**B**) of chromophobe renal cell carcinoma (RCC) (×200) No thymidine phosphorylase expression was shown in chromophobe RCC

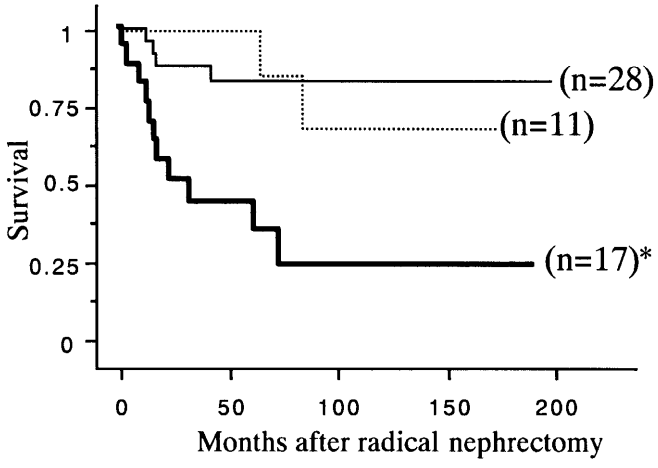

Fig. 3 Overall survival curves according to thymidine phosphorylase (TP) expression level in renal cell carcinoma (RCC) patients. Survival was estimated by the Kaplan-Meier method and the log-rank test was used to compare the survival between patients with negative TP expression (*dotted line*), those with weak TP expression (*thin line*), and those with positive TP expression (*thick line*). $*P < 0.05$ as compared to patients with negative TP expression

($n = 17$) for the TP positive patients. Survival was correlated closely with TP expression, patient age, INF, pathologic T- and N-stages, distant metastasis, venous involvement, and tumor grade (Table 2). There was no significant correlation between survival and factors including angiogenesis evaluated by renal arteriography findings and MVD, IFN therapy, and tumor growth pattern.

We conducted multivariate analysis using Cox's proportional hazard model to assess TP expression, MVD, and other clinicopathologic factors such as prognostic factors. For the analysis of the data, TP expression in tumor cells was divided into two groups: one was negative and weak, and the other was the positive group described above. Results showed that five factors – pathologic T-stage, distant metastasis, tumor grade, INF, and TP expression – were prognostic factors (Table 3). Chromophobe-type patients in our study with no TP expression are still alive without recurrence.

Discussion

The present study, investigating TP expression and clinicopathologic factors including angiogenesis, found: (1) a correlation between TP expression and tumor grade, distant metastasis, venous involvement, and patient age, but (2) no correlation between TP expression, MVD, and renal arteriography. However, Imazono et al., studying 133 RCC patients, reported TP expression had nine times higher activity in the tumor than in normal tissue, indicating a significant correlation between TP expression and MVD as well as tumor grade [11]. The discrepancy may result from different surface markers for the vascular endothelium used to count the microvessels. Imazono et al. [11] used the Factor-VIII-related antigen (von Willebrand factor) marker for MVD count, while we used a CD34 marker. CD34 is reportedly a better surface marker for vascular endothelium, qualitatively and quantitatively, than Factor-VIII-related antigen [37]. For example, when CD34 gives positive results, Factor-VIII-related antigen can give false weak positive or negative results. Thus, results from these markers will not always coincide [37]. Other factors which might contribute to the discrepancy include anti-TP antibody used and the number of patients examined. Furthermore, angiogenic factors in RCC reportedly include basic fibroblast growth factor (bFGF), placenta growth factor (PlGF), and vascular endothelial

Table 2 Prognostic factors in univariate analysis (*IFN* interferon, *INF* tumor infiltration type, *TP* thymidine phosphorylase, *MVD* microvessel density)

Factor	Category	*P* value
Age	(≤65 years vs. >65 years)	0.0019
Sex	(Male vs. female)	0.8386
IFN therapy	(Yes vs. no)	0.3479
INF	(INFα vs. INFβ + INFγ)	<0.0001
pT	(pT1 + pT2 vs. pT3 + pT4)	<0.0001
pN	(pN0 vs. pN1 + pN2)	0.0047
Distant metastasis	(M0 vs. M1)	<0.0001
pV	(pVo vs. pV1 + pV2)	<0.0001
Tumor grade	(Grade 1 vs. grade 2 + 3)	0.0051
TP expression	(Negative vs. weak vs. positive)	0.0011
Arteriography	(Hypovascular vs. hypervascular)	0.8302
Growth pattern	(Alveolar vs. papillary vs. tubular vs. others)	0.4429
Cell type	(Clear cell vs. mixed vs. granular vs. others)	0.0432
MVD	(Grade 1 vs. grade 2 vs. grade 3)	0.1122

Table 3 Prognostic factors in multivariate analysis (*HR* hazard ratio, *CI* confidence interval)

Factor	Category	*P* value	HR (95% CI)
Age	(≤65 years vs. >65 years)	0.5669	0.682 (0.184–2.531)
INF	(INFα vs. INFβ + INFγ)	0.0109	11.099 (1.739–70.8409)
pT	(pT1 + pT2 vs. pT3 + pT4)	0.0173	98.839 (1.468–53.209)
pN	(pN0 vs. pN1 + pN2)	0.337	2.382 (0.405–14.015)
Distant metastasis	(M0 vs. M1)	0.0007	19.325 (3.461–107.912)
pV	(pVo vs. pV1 + pV2)	0.9292	1.093 (0.154–7.734)
Tumor grade	(Grade 1 vs. grade 2 + 3)	0.0216	84.085 (1.919–3684.5769)
TP expression	(Negative + weak vs. positive)	0.0295	0.224 (0.054–0.929)

growth factor (VEGF) [4, 23, 32]. The present result of no correlation between TP expression and MVD suggests that factors other than TP are more closely related to angiogenesis in RCC. This needs further investigation.

Clinicopathologic factors possibly related to RCC prognosis reportedly include tumor size [8, 17], tumor growth pattern type, tumor cell type [17], tumor grade [17, 26, 27, 30], pathologic N-stage, as well as distant metastasis [8, 30], pathologic stage [8, 17, 30], and erythrocyte sedimentation rate (ESR)/alkaline phosphatase (ALP) [27].

In the present study, there was a significant correlation between survival and pathologic T-stage, distant metastasis, tumor grade, tumor infiltration type, and TP expression, but no correlation between survival, MVD, and angiogenesis on renal arteriography. Imazono et al. reported TP expression is an independent prognostic factor in RCC [11], which has been supported by our results.

Regarding prognosis and angiogenesis in RCC, reports show that MVD is an independent prognostic factor [24], MVD is a risk factor for distant metastasis [40], no correlation between MVD and survival [11], a vascular supply, and extension of the tumor on arteriography is significantly correlated with survival [15], whereas an increase in tumor vascularity (renal arteriography) is not a risk factor for prognosis [27] and not correlated with survival [2]. In the present study, there was no correlation between angiogenesis and survival, and we believe that the correlation between survival and angiogenesis by histologic examination or renal arteriography is still unclear. Chromophobe-type RCC patients predominantly show low stages and grades with good prognosis [3]; two patients in our study are alive without a postoperative recurrence. These two patients, with no TP expression, support our results that TP expression is an independent prognostic factor in RCC.

In conclusion, TP expression in RCC tumor tissue is thought to have clinical significance as an independent prognostic factor rather than just an index for angiogenesis. Besides being an angiogenic factor, TP reportedly plays an important role as a metabolic enzyme for fluoropyrimidine, mainly 5-fluorouracil (5-FU), doxifluridine (5′-DFUR), and capecitabine [5, 12, 18]. Recently, a therapeutically improved, effective agent for RCC is combined chemotherapy with 5-FU, IFN-α, and/or interleukin-2 [10]. We also reported IFN-enhanced TP expression results in increased sensitivity to 5-FU, a result of so-called biomodulation which was more frequently observed in patients with higher TP expression [22]. This suggests evaluation of TP expression may be useful for selecting those RCC patients expected to benefit most from biomodulation with chemoimmunotherapy.

References

1. Alcalde RE, Terakado N, Otsuki K, Matsumura T (1997) Angiogenesis and expression of platelet-derived endothelial cell growth factor in oral squamous cell carcinoma. Oncology 54: 324
2. Blath RA, Mancilla-Jimenez R, Stanley RJ (1976) Clinical comparison between vascular and avascular renal cell carcinoma. J Urol 115: 514

3. Crotty TB, Farrow GM, Lieber MM (1995) Chromophobe cell renal carcinoma: clinicopathological features of 50 cases. J Urol 154: 964
4. Dosquet C, Coudert MC, Lepage E, Cabane J, Richard F (1997) Are angiogenic factors, cytokines and soluble adhesion molecules prognostic factors in patients with renal cell carcinoma? Clin Cancer Res 3: 2451
5. Evrard A, Cuq P, Robert B, Vian L, Pelegrin A, Cano JP (1999) Enhancement of 5-fluorouracil cytotoxicity by human thymidine-phosphorylase expression in cancer cells: in vitro and in vivo study. Int J Cancer 80: 465
6. Folkman J (1990) What is the evidence that tumors are angiogenesis dependent? J Natl Cancer Inst 82: 4
7. Furukawa T, Yoshimura A, Sumizawa T, Haraguchi M, Akiyama S, Fukui K, Ishizawa M, Yamada Y (1992) Angiogenic factor. Nature 356: 668
8. Guinan PD, Vogelzang NJ, Fremgen AM, Chmiel JS, Sylvester JL, Sener SF, Imperato JP, Members of the Cancer Incidence and End Results Committee (1995) Renal cell carcinoma: tumor size, stage and survival. J Urol 153: 901
9. Haraguchi M, Miyadera K, Uemura K, Sumizawa T, Furukawa T, Yamada K, Akiyama S, Yamada Y (1994) Angiogenic activity of enzymes. Nature 368: 198
10. Hofmockel G, Theiss M, Gruss A, Langer W, Frohmuller H (1997) Immunochemotherapy of metastatic renal cell carcinoma with interleukin-2, interferon-alpha and 5-fluorouracil. Urologie A 36: 45
11. Imazono Y, Takebayashi Y, Nishiyama K, Akiba S, Miyadera K, Yamada Y, Akiyama S, Ohi Y (1997) Correlation between thymidine phosphorylase expression and prognosis in human renal cell carcinoma. J Clin Oncol 15: 2570
12. Ishikawa T, Sekiguchi F, Fukase Y, Sawada N, Ishitsuka H (1998) Positive correlation between the efficacy of capecitabine and doxifluridine and the ratio of thymidine phosphorylase to dihydropyrimidine dehydrogenase activities in tumors in human cancer xenografts. Cancer Res 58: 685
13. Jaeger TM, Weinder N, Chew K, Moore DH, Kerschmann RL, Waldman FM, Carroll PR (1995) Tumor angiogenesis correlates with lymph node metastases in invasive bladder cancer. J Urol 154: 69
14. Koukourakis MI, Giatromanolaki A, O'Byrne KJ, Comley M, Whitehouse RM, Talbot DC, Gatter KC, Harris AL (1997) Platelet-derived endothelial cell growth factor expression correlates with tumor angiogenesis and prognosis in non-small-cell lung cancer. Br J Cancer 75: 477
15. Lang EK (1971) Arteriographic assessment and staging of renal-cell carcinoma. Analysis of a series of 120 patients. Radiology 101: 17
16. Maeda K, Chung Y-S, Ogawa Y, Takatsuka S, Kang S-M, Ogawa M, Sawada T, Onoda N, Kato Y, Sowa M (1996) Thymidine phosphorylase/platelet-derived endothelial cell growth factor expression associated with hepatic metastasis in gastric carcinoma. Br J Cancer 73: 884
17. Medeiros LJ, Gelb AB, Weiss LM (1988) Renal cell carcinoma: prognostic significance of morphologic parameters in 121 cases. Cancer 61: 1639
18. Miwa M, Ura M, Nishida M, Sawada N, Ishikawa T, Mori K, Shimma N, Umeda I, Ishitsuka H (1998) Design of a novel oral fluoropyrimidine carbamate, capecitabine, which generates 5-fluorouracil selectively in tumours by enzymes concentrated in human liver and cancer tissue. Eur J Cancer 34: 1274
19. Miyazono K, Okabe T, Urabe A, Takaku F, Heldin C-H (1987) Purification and properties of an endothelial cell growth factor from human platelets. J Biol Chem 262: 4098
20. Mizutani Y, Okada Y, Yoshida O (1997) Expression of platelet-derived endothelial cell growth factor in bladder carcinoma. Cancer 79: 1190
21. Moghaddam A, Bicknell R (1992) Expression of platelet-derived endothelial cell growth factor in *Escherichia coli* and confirmation of its thymidine phosphorylase activity. Biochemistry 31: 12141
22. Morita T, Tokue A (1999) Biomodulation of 5-fluorouracil by interferon-ain human renal cell carcinoma cells: relationship to the expression of thymidine phosphorylase. Cancer Chemother Pharmacol 44: 91
23. Nanus DM, Schmitz DBJ, Motzer RJ, Lee AC, Vlamis V, Cordon CC, Albino AP, Reuter VE (1993) Expression of basic fibroblast growth factor in primary human renal tumors: correlation with poor survival. J Natl Cancer Inst 85: 1597
24. Native O, Sabo E, Reiss A, Wald M, Madjar S, Moskovitz B (1998) Clinical significance of tumor angiogenesis in patients with localized renal cell carcinoma. Urology 51: 693
25. Nishida M, Hino A, Mori K, Matsumoto T, Yoshikubo T, Ishitsuka H (1996) Preparation of anti-human thymidine phosphorylase monoclonal antibodies useful for detecting the enzyme levels in tumor tissues. Biol Pharm Bull 19: 1407
26. Nurmi MJ (1984) Prognostic factors in renal carcinoma. An evaluation of operative findings. Br J Urol 56: 270
27. Roosen JU, Engel U, Jensen RH, Kvist E, Schou G (1994) Renal cell carcinoma: prognostic factors. Br J Urol 74: 160
28. Saito S, Tsuno N, Nagawa H, Sunami E, Zhengxi J, Osada T, Kitayama J, Shibata Y, Tsuruo T, Muto T (2000) Expression of platelet-derived endothelial cell growth factor correlates with good prognosis in patients with colorectal carcinoma. Cancer 88: 42
29. Schoell WMJ, Pieber D, Reich O, Lahousen M, Janicek M, Guecer F, Winter R (1997) Tumor angiogenesis as a prognostic factor in ovarian carcinoma. Quantification of endothelial immunoreactivity by image analysis. Cancer 80: 2257
30. Strohmeyer T, Ackermann R (1991) Classic and modern prognostic indicators in renal cell carcinoma. Review of the literature. Urol Int 47: 203
31. Sumizawa T, Furukawa T, Haraguchi M, Yoshimura A, Takeyasu A, Ishizawa M, Yamada Y, Akiyama S (1993) Thymidine phosphorylase activity associated with platelet-derived endothelial cell growth factor. J Biochem 114: 9
32. Takahashi A, Sasaki H, Kim SJ, Tobisu K, Kakizoe T, Tsukamoto T, Kumamoto Y, Sugimura T, Terada M (1994) Markedly increased amounts of messenger RNAs for vascular endothelial growth factor and placenta growth factor in renal cell carcinoma associated with angiogenesis. Cancer Res 54: 4233
33. Takebayashi Y, Yamada K, Miyadera K, Sumizawa T, Furukawa T, Kinoshita F, Aoki D, Okumura H, Yamada Y, Akiyama S, Aikou T (1996) The activity and expression of thymidine phosphorylase in human solid tumours. Eur J Cancer 32: 1227
34. The Japanese Urological Association, The Japanese Pathological Society and Japanese Radiological Society (1992) General rule for clinical and pathological studies on renal cell carcinoma (in Japanese), 2nd edn. Kanehara, Tokyo
35. Toi M, Hoshina S, Taniguchi T, Yamamoto Y, Ishitsuka H, Tominaga T (1995) Expression of platelet-derived endothelial cell growth factor/thymidine phosphorylase in human breast cancer. Int J Cancer 64: 79
36. Tomisaki S, Ohno S, Ichiyoshi Y, Kuwano H, Maehara Y, Sugimachi K (1996) Microvessel quantification and its possible relation with liver metastasis in colorectal cancer. Cancer 77: 1722
37. Traweek ST, Kandalaft PL, Mehta P, Battifora H (1991) The human hematopoietic progenitor cell antigen (CD34) in vascular neoplasia. Am J Clin Pathol 96: 25
38. Weinder N, Caroll PR, Flax J, Blumenfeld W, Folkman J (1993) Tumor angiogenesis correlates with metastasis in invasive prostate carcinoma. Am J Pathol 143: 401
39. Weinder N, Semple JP, Welch WR, Folkman J (1991) Tumor angiogenesis and metastasis – correlation in invasive breast carcinoma. N Engl J Med 324: 1
40. Yoshino S, Kato M, Okada K (1995) Prognostic significance of microvessel count in low stage renal cell carcinoma. Int J Urol 2: 156