The Korean Journal of Pathology
2008; 42: 215-22

The Prognostic Implications of the Histologic Subtype and the Expression of Phosphorylated ERK 1/2 in Papillary Renal Cell Carcinoma

**Bo Sung Kim · Dong Il Kim
Tae Hoon Kang · Eun Shin
Kyung Chul Moon[1]**

Department of Pathology, Seoul National University College of Medicine; [1]Department of Pathology and Kidney Research Institute, Medical Research Center, Seoul National University College of Medicine, Seoul, Korea

Received : June 16, 2008
Accepted : July 31, 2008

Corresponding Author
Kyung Chul Moon, M.D.
Department of Pathology and Kidney Research Institute, Medical Research Center, Seoul National University College of Medicine, 28 Yeongeon-dong, Jongno-gu, Seoul 110-799, Korea
Tel: 02-2072-1767
Fax: 02-743-5530
E-mail: blue7270@snu.ac.kr

*This work was supported by grant No. 04-2007-014 from the SNUH Research Fund.

Background : The authors of this study wanted to confirm the prognostic implication of the histologic subtype; further, we wanted to explore the expression of phosphorylated extracellular signal-regulated kinase 1/2 (pERK) in papillary renal cell carcinoma (PRCC) and determine its clinicopathologic and prognostic significance. **Methods :** A total of 45 patients who underwent nephrectomy for PRCC were enrolled in this study. The hematoxylin and eosin slides were reviewed and pERK immunohistochemistry was performed. **Results :** Type 2 PRCC was significantly correlated with a larger tumor size (p=0.030), a higher nuclear grade (p<0.001), a more advanced tumor stage (p=0.041) and more frequent distant metastasis (p=0.019). The tumors were pERK-low (0 and 1+) in 30 cases (66.7%) and pERK-high (2+) in 15 cases (33.3%). The pERK-high PRCC was significantly associated with a smaller tumor size (p=0.001) and an earlier tumor stage (p=0.004). On the univariate analysis, the histologic subtype, the TNM stage and the pERK status were significantly associated with progression-free survival (PFS). Multivariate analysis showed that the histologic subtype (hazard ratio 22.81, p=0.042) and the TNM stage (hazard ratio 23.48, p=0.009) were independent prognostic factors for PFS. **Conclusions :** Type 2 PRCC, together with the TNM stage, was identified as one of independent poor prognostic factors for PFS. The pERK status was a prognostic factor for PFS on the univariate analysis, but not on the multivariate analysis.

Key Words : Papillary renal cell carcinoma; ERK; Immunohistochemistry; Prognosis

Papillary renal cell carcinoma (PRCC) is a well-defined disease entity with specific histologic, immunohistochemical and genetic features, comprising approximately 10% of all RCCs.[1] It is debatable whether PRCC shows a better prognosis compared to the other types of RCC including clear cell RCC (CCRCC), although some studies have reported PRCC has a more favorable outcome when detected at an early stage.[2] Other studies have reported that no survival benefit for patients with PRCC.[3]

The classical prognostic factors used for PRCC have been the TNM stage, the Fuhrman grade and some other histologic features, including necrosis, the presence of foam cells, angiolymphatic invasion and so on. Since Delahunt and Eble[4] first proposed two subtypes of PRCC (type 1 and type 2) in 1997, a series of reports have also compared the clinicopathologic features and prognosis between type 1 and type 2 PRCC, and several molecular markers have recently been explored to predict the clinical outcome in PRCC.[5] However, it remains controversial whether these clinicopathologic features are, in fact, independent prognostic factors,[6-8] and the prognostic role of the variables for PRCC still needs to be confirmed.

The extracellular signal-related kinase (ERK) 1/2 mitogen-activated protein kinases (MAPKs) are protein serine/threonine kinases that are located within protein kinase cascades and activated by various stimuli, and these kinases play a crucial role in the control of cell proliferation via induction of G1- to S-phase progression by phosphorylating various nuclear substrates.[9] The ERK signaling pathway is also known to play a crucial role in tumorigenesis, tumor invasion and metastasis *in vitro*.[10] ERK activation and its prognostic relevance have also been studied in a variety of human tumors.

With regard to RCC, ERK 1/2 has been reported to be constitutively activated in several tumor cell lines and tissues from kidney, and to be linked to tumor cell invasion, growth and angiogenesis in a variety of RCC cell lines and *in vivo* systems.[11,12]

Although the number of cases was limited and the histologic type of the enrolled RCC was mostly clear cell type, Oka *et al*.[13] reported constitutive activation of the MAPK cascade in human RCC tissues and there was a relationship between MAPK activation and a high nuclear grade. It has recently been reported that the genes involved in G1-S checkpoint regulation (cyclin D2, cyclin-dependent kinase 6, retinoblastoma-like 2 and $p21^{Cip1}$), which are closely associated with ERK 1/2 activation, showed a relatively increased expression in type 1 PRCC,[14] suggesting the possibility of ERK activation in PRCC. However, the degree of ERK activation in PRCC and its clinicopathologic and prognostic significance still remain unclear.

Thus, the aims of this study were to confirm the prognostic utility of the clinicopathologic features, particularly the histologic subtype. We also wanted to explore the expression of pERK in PRCC by immunohistochemistry, and to determine its clinicopathologic and prognostic significance.

MATERIALS AND METHODS

Patients and the clinicopathologic evaluation

A total of 45 patients who underwent partial or radical nephrectomy for PRCC from January 1995 to December 2004 at Seoul National University Hospital were included in this study. The hematoxylin and eosin (H & E) stained slides were reviewed by a single pathologist for determining the histologic type of RCC and the subtype, according to the WHO 2004 classification (Fig. 1).[1] The tumor was graded according to the Fuhrman grade system[15] and staged using the 2002 TNM classification system.[16] The clinical data, including cancer-specific death or disease progression, was obtained from the patients' medical records.

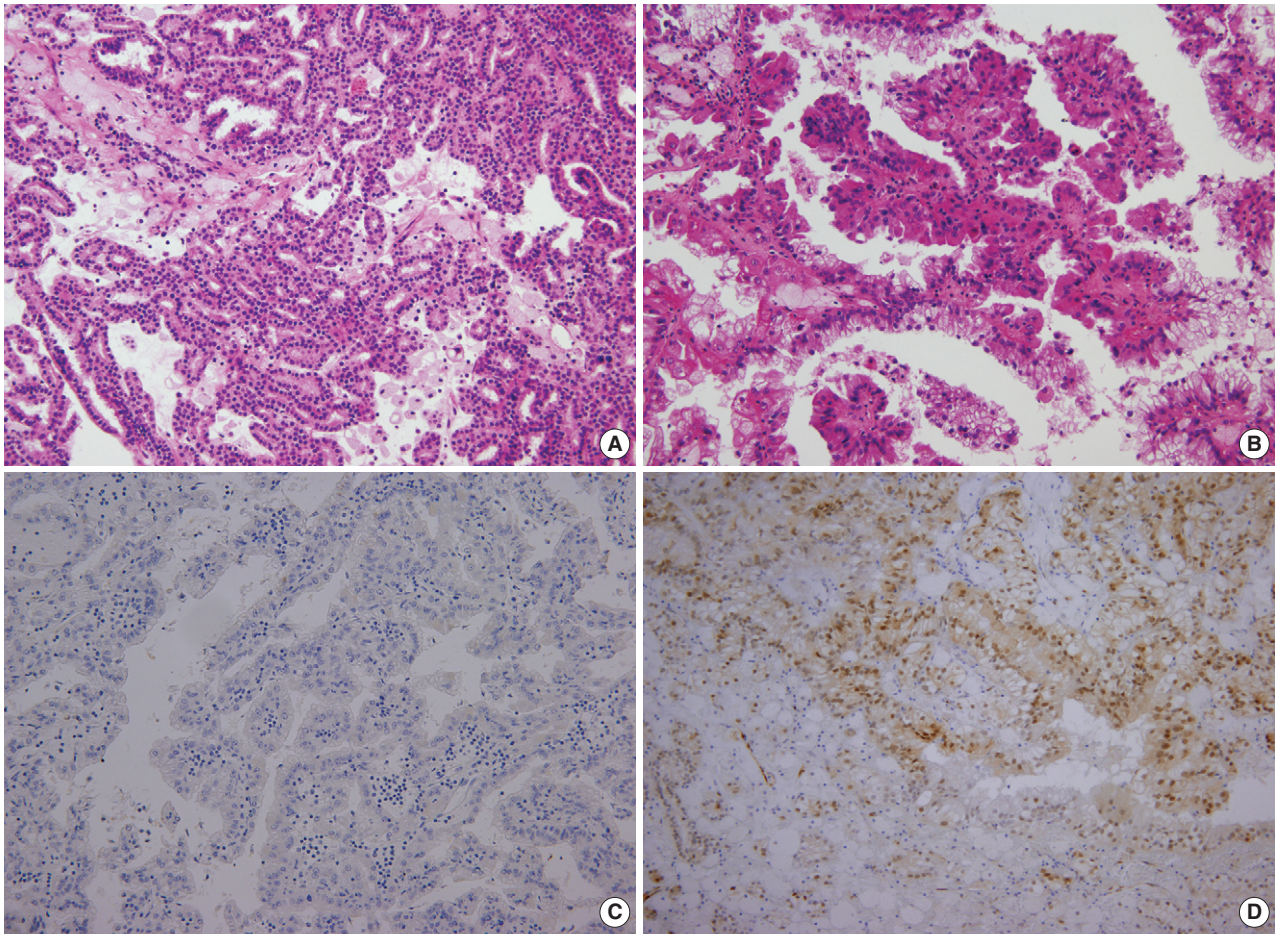


Fig. 1. The PRCC histology and the immunohistochemical staining of pERK: (A) Type 1 PRCC exhibits papillae covered by small cells with scanty cytoplasm, arranged in a single layer. (B) Type 2 PRCC exhibits cells with eosinophilic cytoplasm and pseudostratified nuclei on papillary cores (Hematoxylin-eosin stain). (C) The pERK-low and (D) pERK-high expression in the nucleus and cytoplasm of tumor cells is shown (×200, respectively).

Construction of the tissue microarray

The most representative tumor area was carefully marked on the H & E stained slide to sample tissue cores (0.2 cm in diameter) from the formalin fixed, paraffin embedded tissue blocks. The representative cores were arranged in a new tissue microarray block. One core from each PRCC specimen was obtained for TMA construction and the block also contained nonneoplastic renal parenchyme. One section from the block was stained with H & E for tissue confirmation.

Immunohistochemistry

Immunohistochemical staining was performed on the formalin-fixed, paraffin-embedded tissue microarray slides with using a mouse monoclonal antibody specific for phosphor-p44/p42 MAPK (Thr202/Tyr204) (Cell Signaling Technology, Beverly, MA, USA). Briefly, after deparaffinization, the sections were rehydrated, washed, and subjected to microwave antigen retrieval in citrate buffer. The sections were then immersed in 3% H_2O_2 for 15 min to block the endogenous peroxidase activity and next they were incubated overnight at 4℃ with the primary antibody for pERK at a 1:100 dilution. The expressions were detected using a LSAB+ kit and 3,3'-diaminobenzidine (Dako, Carpinteria, CA, USA). The sections were counterstained with Mayer's hematoxylin.

The proportion of tumor cells showing positive staining was semiquantitatively evaluated as negative (0%), 1+ (1% to 10%) and 2+ (more than 10%) without the reviewer having any knowledge of the clinical outcome.

Statistical analysis

The SPSS 12.0K® program for Microsoft Windows® (SPSS Inc, Chicago, IL, USA) was used for statistical analysis. The clinicopathologic characteristics according to the histologic subtype or the pERK status were compared using χ^2 tests (or Fisher's exact test) and Student's t test. The cancer-specific survival (CSS) was determined from the date of surgery to death by disease or the last follow-up date, and progression-free survival (PFS) was calculated from the date of surgery to the first relapse or metastasis, or the last follow-up date. The survival rates were calculated by the Kaplan-Meier method. Univariate and multivariate analyses were performed using the log-rank test and the Cox proportional hazards model, respectively. p values <0.05 were considered to be statistically significant.

RESULTS

Clinicopathologic characteristics of PRCC

The patients with PRCC included 33 men and 12 women with an age at presentation ranging from 22 to 76 (mean age: 51.2) years. The PRCC was type 1 in 27 patients (60%) and type 2 in 18 patients (40%) with a predominance of type 1. The mean tumor size was 4.5 cm. The tumors were grades I-II in 25 patients and grades III-IV in 20 patients. There were 33 patients with pT1 PRCC and 12 patients with pT2-4 PRCC. Lymph node (LN) metastases and distant metastases were found in 3 and 9 patients, respectively.

Correlation between the histologic subtype and the clinicopathologic characteristics

The clinicopathologic features according to the histologic subtype of tumor are shown in Table 1. There was no significant difference in age or gender distribution between the type 1 and type 2 PRCC, but the type 2 PRCC had a significantly larger tumor size (p=0.030), a higher nuclear grade (p<0.001), a more advanced tumor stage (p=0.041) and more frequent dis-

Table 1. The comparison of clinicopathological characteristics between type 1 and type 2 papillary renal cell carcinomas

Characteristic	Type 1 (%)	Type 2 (%)	p value
Number of cases	27 (60)	18 (40)	
Mean age±SD (years)	51.15±11.06	51.22±15.95	0.985
Sex			
Male	21 (77.8)	12 (66.7)	0.499
Female	6 (22.2)	6 (33.3)	
Tumor side			
Right	14 (51.9)	8 (44.4)	0.763
Left	13 (48.1)	10 (55.6)	
Mean tumor size±SD (cm)	3.77±2.20	5.46±2.82	0.030
Fuhrman nuclear grade			
Low (I-II)	22 (81.5)	3 (16.7)	0.000
High (III-IV)	5 (18.5)	15 (83.3)	
pT category			
pT1	23 (85.2)	10 (55.6)	0.041
pT2-4	4 (14.8)	8 (44.8)	
pN category			
pN0	27 (100)	15 (83.3)	0.058
pN1-2	0 (0)	3 (16.7)	
Distant metastasis			
M0	25 (92.6)	11 (61.1)	0.019
M1	2 (7.4)	7 (38.9)	
TNM stage			
I-II	24 (88.9)	9 (50.0)	0.006
III-IV	3 (11.1)	9 (50.5)	

tant metastasis (p=0.019), as compared to the type 1 PRCC. Type 2 PRCC had a tendency of more frequent LN metastasis, but this was not statistically significant (p=0.058).

The pERK expression and its correlation with the clinicopathologic characteristics

Phospho-ERK immunohistochemistry was available for all the 45 enrolled cases. The tumor tissue showed nuclear and/or cytoplasmic staining (Fig. 1), whereas the nonneoplastic renal tissue exhibited none or, if any, minimal cytoplasmic staining in the tubular epithelial cells (figures not shown). In total, the immunohistochemical staining for pERK in the tumor was negative in 20 cases (44.4%), 1+ in 10 cases (22.3%) and 2+ in 15 cases (33.3%); a pERK expression in the PRCC was detected in 25 out of the 45 cases (55.6%). For the statistical analysis, the cases were subdivided into 2 groups: the pERK-low (0 and 1+) status group and the pERK-high (2+) status group. The tumors were pERK-low in 30 cases (66.7%) and pERK-high in 15 cases (33.3%). Among the 15 pERK-high PRCCs, 7 tumors exhibited both nuclear and cytoplasmic staining, while 7 tumors only displayed cytoplasmic staining, and a single tumor demonstrated only nuclear staining.

The clinicopathologic features of the patients with pERK-low and pERK-high PRCC are shown in Table 2. There was no significant difference in the age, the gender distribution, the histologic subtype or the nuclear grade between the pERK-low and pERK-high PRCC, but the pERK-high PRCC was significantly associated with a smaller tumor size (p=0.001) and an earlier tumor stage (p=0.004). In fact, all of the 15 pERK-high PRCCs were pT1 and there were no pT2-4 tumors. The difference in LN or distant metastasis between the 2 groups was not

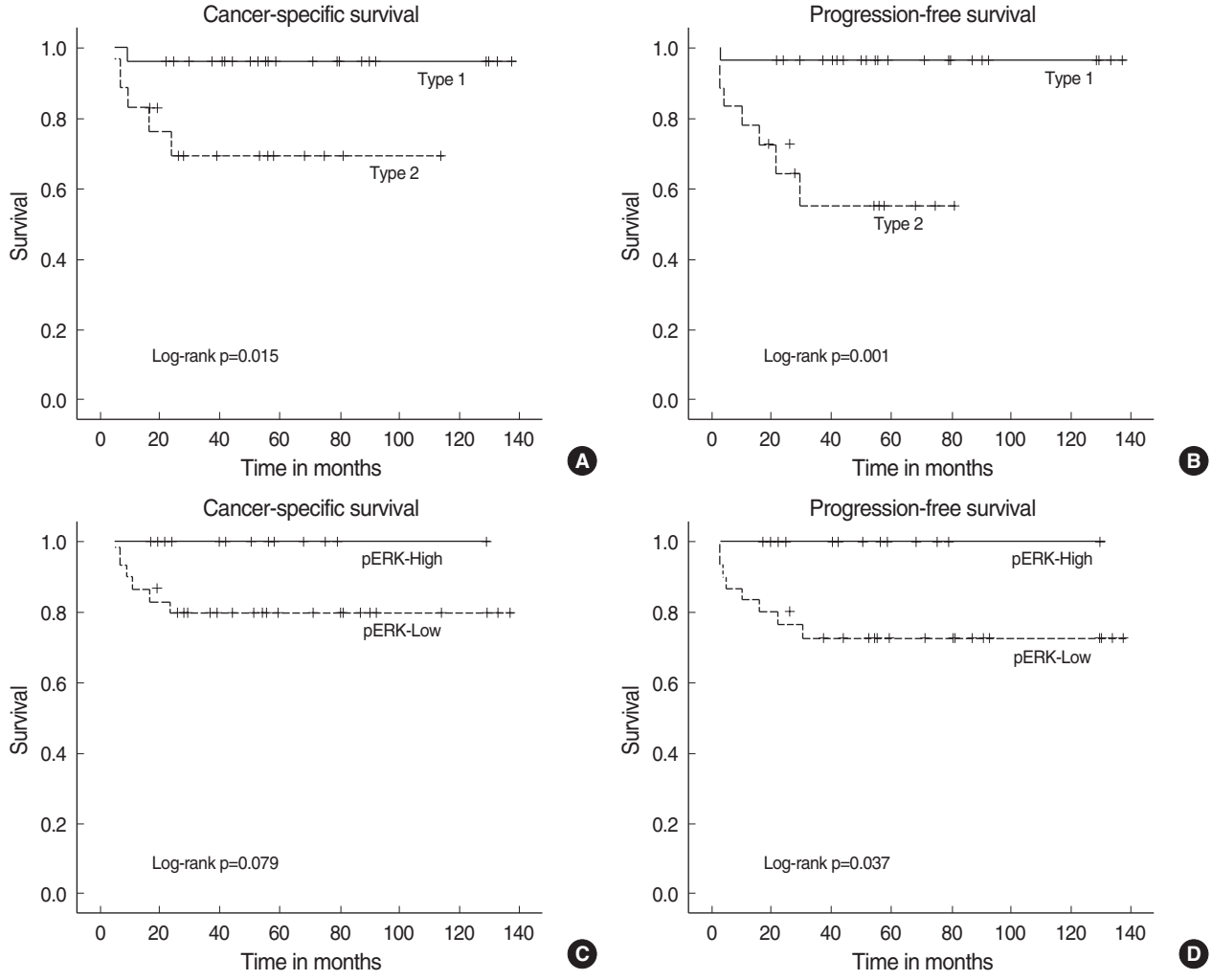


Fig. 2. Kaplan-Meyer survival curves: (A) The cancer-specific survival and (B) progression-free survival according to the histologic type is shown. (C) The cancer-specific survival and (D) progression-free survival according to the pERK status is shown.

Table 2. The comparison of clinicopathological characteristics according to pERK status of papillary renal cell carcinomas

Characteristic	pERK-Low (%)	pERK-High (%)	p value
Number of cases	30 (66.7)	15 (33.3)	
Mean age±SD (years)	50.27±12.48	53.00±14.42	0.514
Sex			
Male	23 (60.0)	10 (26.7)	0.496
Female	7 (40.0)	5 (73.3)	
Tumor side			
Right	18 (51.9)	4 (44.4)	0.057
Left	12 (48.1)	11 (55.6)	
Histologic subtype			
Type 1	18 (60.0)	9 (60.0)	1.000
Type 2	12 (40.0)	6 (40.0)	
Mean tumor size±SD (cm)	5.28±2.62	2.89±1.65	0.001
Fuhrman nuclear grade			
Low (I-II)	17 (56.7)	8 (53.3)	1.000
High (III-IV)	13 (43.3)	7 (46.7)	
pT category			
pT1	18 (60.0)	15 (100)	0.004
pT2-4	12 (40.0)	0 (0)	
pN category			
pN0	28 (93.3)	14 (93.3)	1.000
pN1-2	2 (6.7)	1 (6.7)	
Distant metastasis			
M0	27 (90.0)	15 (100)	0.540
M1	3 (10.0)	0 (0)	
TNM stage			
I-II	19 (63.3)	14 (93.3)	
III-IV	11 (36.7)	1 (6.7)	0.038

pERK, phosphorylated extracellular signal-regulated kinase 1/2.

significant.

Survival analysis

The median follow-up was 52 months (range: 5 to 137 months). Cancer-specific deaths occurred in 8 patients and disease progression occurred in 6 patients. One patient died of an unrelated cause. The 5-year cancer-specific and disease-free survival rates were 86.7% and 82.2%, respectively. The Kaplan-Meier survival curves are presented in Fig. 2. The 5-year CSS & PFS rates were both 96.3% for type 1 and they were 72.2% and 61.1%, respectively, for type 2. Meanwhile, the 5-year CSS & PFS rates were 80% and 73.3%, respectively, for the pERK-low PRCC, while they were both 100% for the pERK-high PRCC.

Univariate analysis showed the histologic subtype, the tumor size and the TNM stage were significantly associated with CSS and PFS. Phospho-ERK had a significant effect on PFS, but not on CSS (Table 3). Multivariate analysis showed that the histologic subtype (hazard ratio 22.81, p=0.042) and TNM stage (hazard ratio 23.48, p=0.009) were independent prognostic factors for PFS (Table 4).

Table 3. Univariate analysis (Kaplan-Meier survival analysis, log-rank test) of progression-free survival in 45 cases with papillary renal cell carcinoma

Prognostic factor	Log-rank p-value	
	CSS	PFS
Age	0.738	0.731
Sex	0.693	0.907
Histologic subtype	0.015	0.001
Tumor size	<0.001	<0.001
Grade	0.245	0.051
TMN stage	<0.001	<0.001
pERK	0.079	0.037

CSS, cancer-specific survival; pERK, phosphorylated extracellular signal-regulated kinase 1/2; PFS, progression-free survival.

Table 4. Multivariate analysis (Cox proportional hazards model) to determine the independent prognostic factors in relation to progression-free survival (Phospho-ERK did not fit the model and was omitted)

Prognostic factor	p value	HR (95% CI)
Age	0.323	0.463 (0.101-2.132)
Histologic subtype	0.042	22.81 (1.117-465.8)
Grade	0.170	0.166 (0.013-2.159)
TNM Stage	0.009	23.48 (2.213-249.1)

CI, confiden interval; HR, hazard ratio; Phospho-ERK, phosphorylated extracellular signal-regulated kinase 1/2.

DISCUSSION

In this study, we demonstrated that the histologic subtype, together with the stage, was a useful prognostic factor for PFS, and type 2 PRCC had a more unfavorable outcome. Since Delahunt and Eble[4] first proposed two subtypes of PRCC (type 1 and type 2) in 1997, a series of reports have compared the clinicopathologic features and prognosis between type 1 and type 2 PRCC, and these studies have shown conflicting results regarding the prognostic significance of subtyping. However, there are an increasing number of reports that the two subtypes are different entities in their clnicopathologic and prognostic aspects, with type 2 being associated with a poorer prognosis. Mejean *et al*.[6] and Yamashita *et al*.[7] reported that type 2 PRCC had a significantly higher grade and stage, and this was significantly associated with prolonged survival on univariate analysis, although the histologic subtype was not retained on multivariate analysis. On the other hand, Pignot *et al*.[8] have shown that the tumor type and TNM stage were the independent prognostic factors

affecting disease-free survival on multivariate analysis, with overall and disease-free survival rates of 89% and 92%, respectively, for type 1 PRCC and 55% and 44%, respectively, for type 2 PRCC. Therefore, our findings that the histologic subtype and the TNM stage independently have a significant prognostic effect on survival concur with the other recent studies.

This explains the conflicting results when comparing the prognosis between PRCC and CCRCC in previous different studies,[2,3] and most of these studies did not classify the subtype of PRCC. However, type 1 and type 2 are indeed disparate entities with different biologic behaviors, and it is not logical that the PRCC group as a whole can be compared with the CCRCC group without considering the heterogeneity between the two types within the PRCC group. With regard to the survival of RCC patients in South Korea, Kim *et al*.[17] have reported that the overall survival was not significantly related to the histological type (clear cell vs papillary, p=0.8651), but they did not divide the PRCC into subtypes either. As far as we know, there have been no large-scale studies that have fully taken into account of the heterogeneity of PRCC when comparing the prognostic relevance among the histologic types of RCC. Therefore, it is likely that exact comparison is possible only when the appropriate subtyping of PRCC has been performed.

The reason for the poorer prognosis for type 2 PRCC is partly because type 2 PRCC is significantly associated with poor prognostic factors such as a larger tumor size, a higher nuclear grade, a more advanced tumor stage and more frequent distant metastasis, but these factors alone do not explain the entire picture; the histologic subtype was still an independent prognostic factor even after adjusting for the confounding factors, which included the nuclear grade and stage, in the multivariate analysis. Kyle *et al*.[18] have recently revealed that MYC activation was specifically correlated with high-grade type 2 PRCC, and inhibition of MYC signaling disrupted the growth of a cell line model of type 2 PRCC. Accordingly, they showed that the activation of MYC was associated with the aggressiveness or progression of type 2 PRCC. Delahunt and Eble[19] have also reported that the rates of cellular proliferation, based on the Ki-67 indices, were significantly higher for type 2 PRCC. It appears that the increased cytogenetic changes,[20] including the MYC activation in type 2 PRCC compared with type 1 PRCC, is related to a poorer prognosis for type 2 PRCC.

The ERK signaling pathway plays an important role in the tumorigenesis, invasion and metastasis of various tumor cells *in vitro*,[10] and several reports have explored the effects of ERK activation on the prognosis of human tumors, yet these reports showed inconsistent results according to the tumor's origin. While strong pERK staining was, as expected, related to aggressive tumor behaviors or short overall survival for carcinomas from colon[21] and liver,[22] and salivary gland mucoepidermoid carcinoma,[23] it was associated with a low grade, an early stage, a negative nodal status and long relapse-free survival or better overall survival in carcinomas from breast[24] and endometrium,[25] and serous ovarian carcinoma.[26] The ERK 1/2 staining did not significantly predict survival for cervical cancer on univariate or multivariate analysis.[27] Thus, the pathogenetic role and prognostic effect of ERK activation seem to be different for tumors of different origins.

With regard to human RCC, Oka *et al*.[13] reported constitutive activation of the ERK cascade in 48% of human RCC tissues and there was a relationship between ERK activation and a high nuclear grade; those results stand in contrast to our findings. This might be explained by the fact that the number of cases in the previous study was limited (n=25) and the histologic type of the enrolled RCC was mostly confined to the clear cell type. Furthermore, Oka *et al*. used western blots and immunoprecipitation to investigate the constitutive activation of the ERK pathway; these are methods that require a large amount of tumor tissue, and contamination by the adjacent nonneoplastic tissue cannot be excluded. In our study, we utilized immunohistochemistry, which has been used in recent studies to evaluate the expression of pERK in tumor.[21-27]

To the best of our knowledge, this is the first study that examined the expression of pERK 1/2 and its prognostic significance in human PRCC. We demonstrated that the expression of pERK was obvious in PRCC, and it was higher in tumor than in the adjacent nonneoplastic tissue. Unexpectedly, the pERK-high status was significantly correlated with a small tumor size and an early stage. It was also significantly correlated with prolonged progression-free survival on univariate analysis, but this could not be identified on Cox multivariate regression analysis due to failure to fit the model. These results suggest that activation of the ERK signaling pathway plays an important role in tumorigenesis in the early stage of PRCC, but loss of ERK activation is required for disease progression as a pathogenetic aspect; at the same time, the results suggest that the pERK status might be clinically useful as a prognostic factor.

It remains unclear how the loss of pERK is related to disease progression, and ERK activation predicts a better outcome for PRCC. These might be partly explained by the studies that have shown that hyperactivation of the ERK1/2 pathway elicits cell cycle arrest by inducing the accumulation of cyclin-dependent

kinase inhibitors (e.g., p21, $p16^{Ink4a}$ and/or $p15^{Ink4b}$) in not only cell lines, but also in non-immortalized primary cells.[28] However, this interpretation has its limits in that the nuclear staining in this study was found only in 8 out of 15 pERK-high PRCCs, and cell cycle arrest mediated by hyperactive ERK 1/2 signaling in tumor cells has not yet been proven. Another possible explanation for the correlation is that cytoplasmic retention of active ERK has no activity in DNA replication and cell transformation.[29] Tohgo *et al*.[30] have demonstrated that β-arrestins inhibit ERK-dependent transcription by binding to pERK, leading to its retention in the cytosol. In this study, cytoplasmic staining alone was found in 7 out of 15 pERK-high PRCCs. However, this interpretation is not deemed complete because the action of β-arrestins in renal cell carcinoma has not yet been shown. Further studies including RCC cell lines and *in vivo* systems are needed to elucidate the pathogenetic role and the prognostic significance of ERK activation in PRCC.

In conclusion, this study confirms that the histologic subtype, together with the TNM stage, is an independent prognostic factor for PFS and that type 2 PRCC has a poorer prognosis that that of type 1 PRCC. Phospho-ERK 1/2, known to be expressed in various human tumors, obviously more highly expressed in PRCC than in the adjacent nonneoplastic tissue, and the pERK-high status is unexpectedly correlated with a small tumor size and an early stage. A high expression of pERK is also significantly correlated with prolonged progression-free survival on univariate analysis, but this cannot be identified on Cox multivariate regression analysis due to failure to fit the model. It is unclear how loss of pERK is related to disease progression, and ERK activation predicts a better outcome in PRCC. Further studies including RCC cell lines and *in vivo* systems are needed to elucidate the pathogenetic role and the prognostic significance of ERK activation in PRCC.

REFERENCES

1. Delahunt B, Eble JN. Papillary renal cell carcinoma. In: Eble JN, Sauter G, Epstein JI, Sesterhenn IA, eds. World Health Organization classification of tumours: pathology and genetics of tumours of the urinary system and male genital organs. Lyon: IARC press, 2004; 27-9.
2. Patard JJ, Leray E, Rioux-Leclercq N, *et al*. Prognostic value of histologic subtypes in renal cell carcinoma: a multicenter experience. J Clin Oncol 2005; 23: 2763-71.
3. Beck SD, Patel MI, Snyder ME, *et al*. Effect of papillary and chromophobe cell type on disease-free survival after nephrectomy for renal cell carcinoma. Ann Surg Oncol 2004; 11: 71-7.
4. Delahunt B, Eble JN. Papillary renal cell carcinoma: a clinicopathologic and immunohistochemical study of 105 tumors. Mod Pathol 1997; 10: 537-44.
5. Shuch BM, Lam JS, Belldegrun AS, Figlin RA. Prognostic factors in renal cell carcinoma. Semin Oncol 2006; 33: 563-75.
6. Mejean A, Hopirtean V, Bazin JP, *et al*. Prognostic factors for the survival of patients with papillary renal cell carcinoma: meaning of histological typing and multifocality. J Urol 2003; 170: 764-7.
7. Yamashita S, Ioritani N, Oikawa K, Aizawa M, Endoh M, Arai Y. Morphological subtyping of papillary renal cell carcinoma: clinicopathological characteristics and prognosis. Int J Urol 2007; 14: 679-83.
8. Pignot G, Elie C, Conquy S, *et al*. Survival analysis of 130 patients with papillary renal cell carcinoma: prognostic utility of type 1 and type 2 subclassification. Urology 2007; 69: 230-5.
9. Pearson G, Robinson F, Beers Gibson T, *et al*. Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions. Endocr Rev 2001; 22: 153-83.
10. Hoshino R, Chatani Y, Yamori T, *et al*. Constitutive activation of the 41-/43-kDa mitogen-activated protein kinase signaling pathway in human tumors. Oncogene 1999; 18: 813-22.
11. Inamoto T, Azuma H, Sakamoto T, *et al*. Invasive ability of human renal cell carcinoma cell line Caki-2 is accelerated by gamma-aminobutyric acid, via sustained activation of ERK1/2 inducible matrix metalloproteinases. Cancer Invest 2007; 25: 574-83.
12. Huang D, Ding Y, Luo WM, *et al*. Inhibition of MAPK kinase signaling pathways suppressed renal cell carcinoma growth and angiogenesis in vivo. Cancer Res 2008; 68: 81-8.
13. Oka H, Chatani Y, Hoshino R, *et al*. Constitutive activation of mitogen-activated protein (MAP) kinases in human renal cell carcinoma. Cancer Res 1995; 55: 4182-7.
14. Yang XJ, Tan MH, Kim HL, *et al*. A molecular classification of papillary renal cell carcinoma. Cancer Res 2005; 65: 5628-37.
15. Fuhrman SA, Lasky LC, Limas C. Prognostic significance of morphologic parameters in renal cell carcinoma. Am J Surg Pathol 1982; 6: 655-63.
16. Greene FL, Page DL, Fleming ID, *et al*. AJCC cancer staging manual. 6th ed. New York: Springer, 2002; 323-8.
17. Kim H, Cho NH, Kim DS, *et al*. Renal cell carcinoma in South Korea: a multicenter study. Hum Pathol 2004; 35: 1556-63.
18. Furge KA, Chen J, Koeman J, *et al*. Detection of DNA copy number changes and oncogenic signaling abnormalities from gene expression data reveals MYC activation in high-grade papillary renal cell carcinoma. Cancer Res 2007; 67: 3171-6.
19. Delahunt B, Eble JN, McCredie MR, Bethwaite PB, Stewart JH, Bilous

AM. Morphologic typing of papillary renal cell carcinoma: comparison of growth kinetics and patient survival in 66 cases. Hum Pathol 2001; 32: 590-5.

20. Gunawan B, von Heydebreck A, Fritsch T, *et al*. Cytogenetic and morphologic typing of 58 papillary renal cell carcinomas: evidence for a cytogenetic evolution of type 2 from type 1 tumors. Cancer Res 2003; 63: 6200-5.
21. Schmitz K, Wohlschlaeger J, Alakus H, *et al*. Activation of extracellular regulated kinases (ERK1/2) but not AKT predicts poor prognosis in colorectal carcinoma and is associated with k-ras mutations. Virchows Arch 2007; 450: 151-9.
22. Schmitz KJ, Wohlschlaeger J, Lang H, *et al*. Activation of the ERK and AKT signalling pathway predicts poor prognosis in hepatocellular carcinoma and ERK activation in cancer tissue is associated with hepatitis C virus infection. J Hepatol 2008; 48: 83-90.
23. Handra-Luca A, Bilal H, Bertrand JC, Fouret P. Extra-cellular signal-regulated ERK-1/ERK-2 pathway activation in human Sslivary gland mucoepidermoid carcinoma: Association to Aggressive Tumor Behavior and Tumor Cell Proliferation. Am J Pathol 2003; 163: 957-67.
24. Milde-Langosch K, Bamberger AM, Rieck G, *et al*. Expression and prognostic relevance of activated extracellular-regulated kinases (ERK1/2) in breast cancer. Br J Cancer 2005; 92: 2206-15.
25. Mizumoto Y, Kyo S, Mori N, *et al*. Activation of ERK1/2 occurs independently of KRAS or BRAF status in endometrial cancer and is associated with favorable prognosis. Cancer Sci 2007; 98: 652-8.
26. Hsu CY, Bristow R, Cha MS, *et al*. Characterization of active mitogen-activated protein kinase in ovarian serous carcinomas. Clin Cancer Res 2004; 10: 6432-6.
27. Branca M, Ciotti M, Santini D, *et al*. Activation of the ERK/MAP kinase pathway in cervical intraepithelial neoplasia is related to grade of the lesion but not to high-risk human papillomavirus, virus clearance, or prognosis in cervical cancer. Am J Clin Pathol 2004; 122: 902-11.
28. Meloche S, Pouyssegur J. The ERK1/2 mitogen-activated protein kinase pathway as a master regulator of the G1- to S-phase transition. Oncogene 2007; 26: 3227-39.
29. Robinson MJ, Stippec SA, Goldsmith E, White MA, Cobb MH. A constitutively active and nuclear form of the MAP kinase ERK2 is sufficient for neurite outgrowth and cell transformation. Curr Biol 1998; 8: 1141-50.
30. Tohgo A, Pierce KL, Choy EW, Lefkowitz RJ, Luttrell LM. Beta-arrestin scaffolding of the ERK cascade enhances cytosolic ERK activity but inhibits ERK-mediated transcription following angiotensin AT1a receptor stimulation. J Biol Chem 2002; 277: 9429-36.