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Interaction between PD-L1 and EGFR, ERK1/2 in clear cell renal cell carcinoma

투명세포 신세포암의 진행에서 PD-L1 과 EGFR, ERK1/2 의 상호작용에 관한 연구

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ABSTRACT

Introduction: The interaction between the programmed cell death protein 1 (PD-1) and programmed death-ligand 1 (PD-L1) is known to suppress T cell function. The overexpressed PD-L1 in tumor cells inhibits the antitumor effect of the cytotoxic T cells by binding PD-1 on the tumor-infiltrating cytotoxic T cells, and then creates a favorable environment for the survival of tumor cells. Although the pathways involved in the expression of PD-L1 remain unclear, immunotherapy against PD-1 and PD-L1 has been tried in various malignant tumors, including renal cell carcinomas. Recent studies reported that activation of epidermal growth factor receptor (EGFR), and thereby activation of extracellular signal-regulated kinase (ERK), resulted in expression of PD-L1 in non-small cell lung cancer. Previous studies showed EGFR is overexpressed in clear cell renal cell carcinomas (CCRCC) and other studies showed phosphorylated extracellular signal-regulated kinase 1/2 (p-ERK1/2) and PD-L1 are also expressed in CCRCC. It is uncertain however what are roles of these key molecules in the growth of CCRCC and how they are regulated. In this study, I evaluated EGFR-ERK1/2-PD-L1 pathway in CCRCC to elucidate the significance of expression of PD-L1 in the activation of EGFR and ERK1/2.

Methods: To study signaling pathways involved in PD-L1 regulation mediated by p-ERK1/2, I performed western blot analysis for two human CCRCC cell lines, Caki-1 and Caki-2 after treatment with

stimulant of ERK1/2 pathway, recombinant human epidermal growth factor or inhibitor of ERK1/2 pathway, U0126. I performed immunohistochemical staining for PD-L1, EGFR and p-ERK1/2 for 368 CCRCC surgical samples and analyzed the correlation between each degree of staining and clinicopathologic factors. To validate the immunohistochemical results of formalin-fixed paraffin-embedded (FFPE) samples, I examined 16 fresh frozen CCRCC samples and analyzed the relationship of PD-L1 and p-ERK1/2 protein expression level by western blot analysis.

Results: I identified a change in PD-L1 expression upon artificial stimulation and inhibition of the ERK1/2 pathway in the Caki-1 CCRCC cell line by western blot analysis, although this was not observed in Caki-2 cell line. I also found a positive correlation between p-ERK1/2 expression and PD-L1 expression by immunohistochemical staining of FFPE samples. (Pearson $r=0.324$, $p<0.001$) I validated that relationship by western blot analysis of fresh frozen surgical samples. (Pearson $r=0.748$, $p=0.001$)

Conclusions: In this study, the ERK1/2-PD-L1 pathway was confirmed by in vitro and in vivo assay as a new mechanism of PD-L1 upregulation in CCRCC that has not been known until now. This would contribute to presenting a new mechanism of the pathogenesis of CCRCC by identifying the pathway involved in PD-L1 expression, and the combination of p-ERK1/2 and PD-L1 immunohistochemical staining could be used as a therapeutic response predictor for anti-PD-1/PD-L1 therapy in the future.

Keywords: Clear Cell Renal Cell Carcinoma; Programmed Cell Death 1 Ligand 1; B7-H1 Antigen; ERK Pathway; Receptor, Epidermal Growth Factor; Blotting, Western; Immunohistochemistry

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CONTENTS

LIST OF ABBREVIATIONS

PD-1: Programmed cell death protein 1

PD-L1: Programmed death-ligand 1

RCC: Renal cell carcinoma

NSCLC: Non-small cell lung cancer

CCRCC: Clear cell renal cell carcinoma

EGFR: Epidermal growth factor receptor

IHC: Immunohistochemistry

ERK: Extracellular signal-regulated kinase

MAPK: Mitogen-activated protein kinase

p-ERK: Phosphorylated extracellular signal-regulated kinase

FFPE: Formalin-fixed paraffin-embedded

EGF: Recombinant human epidermal growth factor

TMA: Tissue microarray

OS: Overall survival time

PFS: Progression-free survival time

AUC: Area under the curve

HR: Hazard ratio

CI: Confidence interval

TKI: Tyrosine kinase inhibitor

INTRODUCTION

It is known that programmed cell death protein 1 (PD-1), also known as CD279 (cluster of differentiation 279), is a T cell-inhibitory receptor that binds with its ligand to inhibit T cell apoptosis, cytokine secretion, and T cell clonal expansion, and as a result, it suppresses T cell function. PD-L1 (Programmed death-ligand 1, also known as B7-H1), which is one of the ligands of PD-1, is aberrantly expressed in various cancers, including renal cell carcinoma (RCC), melanoma, non-small cell lung cancer (NSCLC), urothelial carcinoma, breast carcinoma and ovarian carcinoma, and its high expression has been reported to be associated with poor prognosis in various cancers. (1-19) The overexpressed PD-L1 in tumor cells inhibits the antitumor effect of the cytotoxic T cells by binding PD-1 on the tumor-infiltrating cytotoxic T cells, and then creates a favorable environment for the survival of tumor cells. (20)

Recently, treatment using monoclonal antibodies against PD-1 and PD-L1 has been actively attempted as a new cancer treatment that targets the immune system rather than directly attacking tumor cells. (21-29) With inhibition of the interaction between the two by PD-1 and PD-L1 inhibitors, the function of cytotoxic T cells against the tumor cells is enhanced to treat the tumor. (20)

RCC is the most common carcinoma originating from the kidney, and clear cell renal cell carcinoma (CCRCC) is the most common subtype. Since recurrence and metastasis are common, a variety of targeted

therapies for advanced RCC have recently been tried. (30, 31) Immunotherapy against PD-1 and PD-L1 has been tried in various malignant tumors, including RCCs, and positive results are being reported. (32)

In 2006, it was reported that PD-L1 was overexpressed in many RCCs associated with poor prognosis. (13) Although its prognostic effect is still controversial, it has been reported in association with poor prognosis of RCC patients in most published results. (6, 10, 11, 16, 17) The pathways involved in the expression of PD-L1 remain unclear, but studies are underway. (33, 34)

Epidermal growth factor receptor (EGFR) has been reported to be associated with carcinogenesis in many epithelial tumors. In CCRCC, genetic alterations of EGFR has been rarely reported despite wide investigations (35-37) while EGFR expression via immunohistochemistry (IHC) has been reported as a common finding. (38, 39)

The extracellular signal-regulated kinase (ERK) is a part of the mitogen-activated protein kinase (MAPK) cascades and includes ERK1 and ERK2, also known as p44 and p42 MAPK. (40, 41) The ERK pathway is activated by phosphorylation and is known to be associated with cell signaling pathways controlling embryogenesis, cell differentiation, proliferation and death. (40) ERK1 and ERK2 are often referred to as ERK1/2 due to their high structural and functional similarities, although the coding genes are different. (42) Phosphorylated extracellular signal-regulated kinase 1/2 (p-ERK1/2)

has been demonstrated to be associated with carcinogenesis in many human cancers. Previous studies showed that the MAPK cascade was activated in a significant number of RCCs via western blot analysis, in vitro kinase assays using fresh tissue (43) and IHC using formalin-fixed paraffin-embedded (FFPE) CCRCC samples. (42, 44)

Previous studies on NSCLC surgical specimens and cell lines have suggested that PD-L1 expression was associated with the EGFR pathway.(45-47) A recent study suggested that PD-L1 expression was induced by EGFR activation through the ERK pathway but not through AKT pathway in NSCLC cell lines. (48)

Previous studies showed EGFR is overexpressed in clear cell renal cell carcinomas (CCRCC) and other studies showed phosphorylated extracellular signal-regulated kinase 1/2 (p-ERK1/2) and PD-L1 are also expressed in CCRCC. It is uncertain however what are roles of these key molecules in the growth of CCRCC and how they are regulated. In this study, I evaluated EGFR-ERK1/2-PD-L1 pathway in CCRCC to elucidate the significance of expression of PD-L1 in the activation of EGFR and ERK1/2 in CCRCC cell lines and human CCRCC tissues.

MATERIALS AND METHODS

Cell culture

Two human CCRCC cell lines, Caki-1 and Caki-2, were purchased from the Korean Cell Line Bank (KCLB, Seoul, Korea) and maintained in a humidified atmosphere at 37°C with 5% CO2 in media supplemented with 10% fetal bovine serum.

Stimulant and inhibitor treatment assays

To study signaling pathways involved in PD-L1 regulation mediated by p-ERK1/2, the following stimulants and inhibitors of ERK1/2 pathway were used: Caki-1, 2 cells were serum-starved for 2 hours, then treated with 50 ng/ml EGF (recombinant human epidermal growth factor; Invitrogen, CA, United States) or treated with 30 mM U0126 (1,4-diamino-2,3-dicyano-1,4-bis[2- aminophenylthio] butadiene; Cell Signaling Technology, Beverly, MA, USA). Cells were harvested for western blot analysis. After treatment with 30 mM U0126 for 3 or 4 hours, cells treated with 50 ng/ml EGF were also harvested.

Western blot analysis

Cell lysates were resolved using a 10% polyacrylamide gel in a sodium dodecyl sulfate buffer by electrophoresis and then transferred onto nitrocellulose membranes. After transfer onto a nitrocellulose membrane, the blots were incubated with rabbit monoclonal antibody

for human p-ERK1/2 (Cell Signaling Technology, Beverly, MA, USA) or rabbit polyclonal antibody for human PD-L1 (Novus Biologicals, Littleton, CO, USA).

Patients and tissue microarray

I examined 368 CCRCC samples from patients who underwent radical or partial nephrectomy at Seoul National University Hospital between 2005 and 2008. Tissue microarray (TMA) blocks were made from representative tumor core sections (2 mm in diameter) from each formalin-fixed paraffin block (SuperBioChips Laboratories, Seoul, Korea). I reviewed the hematoxylin and eosin-stained slides for all samples in order to confirm the adequacy of diagnosis and regraded tumors as grade 1 to 4 according to the WHO/ISUP grading system. (49) I collected clinical and pathological information from electronic medical records and pathologic reports. The follow-up period of the patients ranged from 0 to 135 months, and the median follow-up period was 67.5 months. This study was approved by the Institutional Review Board of Seoul National University Hospital.

Immunohistochemistry

Immunohistochemical staining for PD-L1, p-ERK1/2 and EGFR expression was performed on 4-μm-thick sections taken from the TMA. IHC was performed using the Ventana Benchmark XT automated staining system (Ventana Medical Systems, Tucson, AZ, USA). Rabbit polyclonal antibody for human PD-L1 (ab58810, Abcam,

Cambridge, UK) was diluted 1:100, rabbit monoclonal antibody for human p-ERK1/2 (Cell Signaling Technology, Beverly, MA, USA) was diluted 1:100, and mouse monoclonal antibody for the extracellular domain of human EGFR protein (Ventana Medical Systems, Tucson, AZ, USA) was not diluted. Immunohistochemically stained TMA slides were reviewed separately by two pathologists who were blinded to the clinicopathologic parameters.

Fresh frozen sample study

To validate the immunohistochemical results of FFPE samples, I examined 16 fresh frozen CCRCC samples from patients who underwent radical or partial nephrectomy at Seoul National University Hospital in 2011. These samples were collected at Seoul National University Hospital Human Biobank and stored at -180°C in liquid nitrogen until use. I analyzed the EGFR and p-ERK1/2 protein expression level of each tumor by western blot analysis using the procedure described above.

Statistical Analysis

Statistical analysis was performed with the statistics program SPSS ver. 25.0 (IBM Co., Armonk, NY, USA). The Chi-square test was used to analyze the relationship between the PD-L1 expression level and treatment with stimulants or inhibitors and between PD-L1, p-ERK1/2, EGFR immunohistochemical staining and clinicopathologic parameters. Correlations were determined by the Pearson correlation

analysis. Receiver operating characteristic (ROC) curves were used to obtain optimal cut-off values of each immunohistochemical staining. Optimal cut-off values with improved sensitivity and specificity were obtained based on the highest Youden index. (50) Survival curves were plotted using the Kaplan-Meier method, and differences in survival were compared using the log-rank test. The importance of various variables in predicting survival was analyzed using a multivariate Cox proportional hazard model. A p-value of less than 0.05 was considered statistically significant.

RESULTS

PD-L1 and p-ERK1/2 expression following treatment with stimulants and inhibitors

Western blot analysis showed that PD-L1 expression did not change with EGF treatment in both Caki-1 and Caki-2 CCRCC cell lines. (Figure 1) Additionally, PD-L1 expression was suppressed by U0126 treatment in the Caki-1 CCRCC cell line but not in Caki-2. (Figure 2A, 2D) After 3 hours of treatment with U0126, Caki-1 cells treated with EGF for 2 hours showed initially reduced PD-L1 expression, which then increased again. (Figure 2B) After 4 hours of treatment with U0126, Caki-1 cells treated with EGF also showed initially reduced PD-L1 expression, which then increased again. (Figure 2C)

Basic clinicopathologic characteristics

Of the 368 patients in this study, 277 (75.3%) were males and 91 (24.7%) were females. The age at diagnosis was between 20 and 81 years old. The mean age (SD) and median age were 56.5 (11.8) years and 58 years, respectively. I classified the patients into two groups: younger than 57 years or older than 57 years. Six cases (1.6%) were WHO/ISUP grade 1, 174 cases (47.3%) were grade 2, 170 cases (46.2%) were grade 3 and 18 cases (4.9%) were grade 4. The tumor size ranged from 0.5 to 15.0 cm, and the mean size (SD) was 4.3 (3.0) cm. According to the prognostic classification of the American

Joint Committee on Cancer 8th edition, the T categorization was as follows: 289 cases were T1 (78.5%), 22 cases were T2 (6.0%), 47 cases were T3 (12.8%) and 10 cases were T4 (2.7%). Additionally, the prognostic stages were as follows: 282 cases were stage I (76.6%), 18 cases were stage II (4.9%), 37 cases were stage III (10.1%) and 31 cases were stage IV (8.4%). (51) (Table 1)

Immunohistochemical analysis of EGFR and p-ERK1/2 and their relationship with PD-L1 expression

I analyzed the percentage of tumor cells showing more than moderate intensity of membranous staining in EGFR and PD-L1 IHC, and more than moderate intensity of nuclear staining in p-ERK1/2 IHC among all tumor cells. I analyzed the correlation between the percentage in EGFR, p-ERK1/2 and PD-L1, respectively. There was a statistically significant positive correlation between p-ERK1/2 and PD-L1 (Pearson $r=0.324$, $p<0.001$), while there was no correlation between EGFR and PD-L1 (Pearson $r=0.069$, $p=0.184$) or EGFR and p-ERK1/2 (Pearson $r=-0.055$, $p=0.296$). (Figure 3) When analyzed according to the patients' stage, there was a positive correlation between p-ERK1/2 and PD-L1 (Pearson $r=0.350$, $p<0.001$) in 282 patients with stage I, while there was no correlation between EGFR and PD-L1 (Pearson $r=-0.054$, $p=0.366$) or EGFR and p-ERK1/2 (Pearson $r=-0.044$, $p=0.460$). When 86 patients with stage II-IV were included,

there was a still positive correlation between p-ERK1/2 and PD-L1 (Pearson r=0.306, p=0.004), also EGFR and PD-L1 (Pearson r=0.318, p=0.003) but EGFR and p-ERK1/2 were not associated (Pearson r=0.107, p=0.327). Similar results were observed when analyzed for 68 patients with only stage III and IV (Pearson r=0.331, p=0.006, Pearson r=0.292, p=0.016 and Pearson r=0.070, p=0.570 respectively).

Immunohistochemical analysis of EGFR, p-ERK1/2, PD-L1 and its relationship with clinicopathologic characteristics

I performed ROC curve analysis for each immunohistochemical staining results. For grouping by EGFR staining, the cut-off value of overall survival time (OS) and progression-free survival time (PFS) were all 22.5%. The area under the curve (AUC) were 0.694 and 0.697 for OS and PFS (all $p<0.001$). (Figure 4A, 4B) Among 368 cases, 122 cases (33.2%) showed high expression of EGFR and 246 cases (66.8%) showed low expression. (Figure 5)

For grouping by p-ERK1/2 staining, the cut-off value of OS and PFS were all 16.25%. The AUC were 0.585 and 0.571 for OS and PFS (p=0.083 and 0.089 respectively). Although not statistically significant, the cut-off value was determined to maximize the Youden index, since the cut-off value for grouping by p-ERK1/2 staining was not known. Among 368 cases, 146 cases (39.7%) showed high expression of p-ERK1/2 and 222 cases (60.3%) showed low

expression. (Figure 6)

For grouping by PD-L1 staining, the cut-off value of OS and PFS were all 17.5%. The AUC were 0.500 and 0.526 for OS and PFS (p=0.998 and 0.541 respectively). Although this value was not statistically significant, I did not have established criteria for PD-L1 expression, so I categorized cases according to this criterion. Among 368 cases, 204 cases (55.4%) showed high expression of PD-L1 and 164 cases (44.6%) showed low expression. (Figure 7)

PD-L1, EGFR and p-ERK1/2 expression by IHC and its relationship with clinicopathologic parameters is as follows. (Table 1) PD-L1 expression was statistically correlated with young age group (<57) (p=0.043), male patients (p<0.001), a smaller tumor size (<5 cm) (p=0.002) and a lower T category (p=0.029), but the presence of node metastasis (p=0.010). It was also associated with better prognostic stage, but it was not statistically significant (p=0.060). EGFR expression was correlated with old age group (≧57) (p<0.001), a higher WHO/ISUP grade (p<0.001), a larger tumor size (≧5 cm) (p<0.001), a higher T category (p<0.001), the presence of distant metastasis (p=0.002) and worse prognostic stage (p<0.001). P-ERK1/2 expression was also correlated with a lower WHO/ISUP grade (p<0.001), a smaller tumor size (<5 cm) (p<0.001), a lower T category (p<0.001), the absence of distant metastasis (p=0.029) and better prognostic stage (p=0.001).

Survival analysis of CCRCC patients according to

clinicopathologic parameters

I analyzed the OS and PFS of CCRCC patients according to each clinicopathologic parameter via the Kaplan-Meier method. Significantly poor OS was observed for old age group (≧57) (p=0.026), a higher WHO/ISUP grade (p<0.001), a larger tumor size (≧5 cm) (p<0.001), a higher T category (p<0.001), the presence of node metastasis (p<0.001), the presence of distant metastasis (p<0.001), and worse prognostic stage (p<0.001), while there was no difference according to sex (p=0.946). Additionally, significantly poor PFS was observed in a higher WHO/ISUP grade (p<0.001), a larger tumor size (≧5 cm) (p<0.001), a higher T category (p<0.001), the presence of node metastasis (p<0.001), the presence of distant metastasis (p<0.001) and worse prognostic stage (p<0.001), while there was no difference according to age (p=0.081) and sex (p=0.960). (Figure 8)

Survival analysis of CCRCC patients according to EGFR, p-ERK1/2 and PD-L1 expression

Survival analysis was performed according to EGFR, p-ERK1/2 and PD-L1 expression status. Higher EGFR expression was associated with poor OS (p<0.001) and PFS (p<0.001) of the patients. Higher p-ERK1/2 expression was associated with favorable survival of the patients, although not statistically related to PFS (OS: p=0.024, PFS: 0.061). PD-L1 expression was not associated with patients' survival (OS: p=0.342, PFS: 0.238). (Figure 9)

When analyzed according to the patients' stage, p-ERK1/2 and PD-L1 expression was not associated with the survival of stage I patients (OS: p=0.704, PFS: 0.746 and OS: 0.818, PFS: 0.505, respectively). Also p-ERK1/2 and PD-L1 expression was not associated with the survival of stage II-IV patients (OS: p=0.775, PFS: 0.907 and OS: 0.587, PFS: 0.414, respectively). Similar results were observed when analyzed for the patients with only stage III and IV (OS: p=0.309, PFS: 0.599 and OS: 0.691, PFS: 0.358, respectively).

Cox regression analysis of patient survival

Univariate cox regression analysis showed significant correlation with age (<57 or not) (p=0.030), WHO/ISUP grade (p<0.001), tumor size (<5cm or not) (p<0.001), T category (p<0.001), node metastasis (p<0.001), distant metastasis (p<0.001), stage (p<0.001), EGFR expression (p<0.001), p-ERK1/2 expression (p=0.028) and OS. Also it showed significant correlation with WHO/ISUP grade (p<0.001), tumor size (<5cm or not), T category (p<0.001), node metastasis (p<0.001), distant metastasis (p<0.001), stage (p<0.001), EGFR expression (p<0.001) and PFS. (Table 2) However, adjusted multivariate analysis of age, WHO/ISUP grade and stage revealed that EGFR and p-ERK1/2 expression was not independently correlated with OS of the patients (p=0.630 and 0.274, respectively). Also adjusted multivariate analysis of WHO/ISUP grade and stage revealed that EGFR expression was not independently correlated with PFS (p=0.051). (Table 2)

When analyzed according to the patients' stage, univariate cox regression analysis showed no correlation with age (<57 or not) (p=0.458), sex (p=0.140), WHO/ISUP grade (p=0.354), EGFR expression (p=0.135), p-ERK1/2 expression (p=0.707), PD-L1 expression (p=0.819) and OS in 282 patients with stage I. Also it showed no correlation with age (<57 or not) (p=0.759), sex (p=0.190), WHO/ISUP grade (p=0.630), p-ERK1/2 expression (p=0.746), PD-L1 expression (p=0.508) and PFS in patients with stage I, except EGFR expression (Hazard ratio (HR) 6.057, Confidence interval (CI) 1.821 to 20.149, p=0.003). When 86 patients with stage II-IV were included, there was no correlation with age (<57 or not) (p=0.391), sex (p=0.604), EGFR expression (p=0.231), p-ERK1/2 expression (p=0.776), PD-L1 expression (p=0.589) and OS, except WHO/ISUP grade (HR 4.068, CI 1.245 to 13.289, p=0.020). Also it showed no correlation with age (<57 or not) (p=0.621), sex (p=0.464), EGFR expression (p=0.094), p-ERK1/2 expression (p=0.909), PD-L1 expression (p=0.421) and PFS in patients with stage II-IV, except WHO/ISUP grade (HR 5.285, CI 1.630 to 17.136, p=0.006). Similar results were observed when analyzed for 68 patients with only stage III and IV. It showed no correlation with age (<57 or not) (p=0.186), sex (p=0.965), WHO/ISUP grade (p=0.062), EGFR expression (p=0.800), p-ERK1/2 expression (p=0.315), PD-L1 expression (p=0.693) and OS in patients with stage III and IV. Also it showed no correlation with age (<57 or not) (p=0.377), sex (p=0.893), EGFR expression (p=0.314), p-ERK1/2 expression (p=0.605), PD-L1 expression (p=0.367) and PFS in patients with stage III and

IV, except WHO/ISUP grade (HR 4.873, CI 1.172 to 20.261, p=0.029).

The relationship between p-ERK1/2 and PD-L1 expression by western blot analysis using fresh CCRCC surgical specimens

I analyzed p-ERK1/2 and PD-L1 expression by western blot analysis to validate the relationship observed in FFPE samples by IHC. I used 16 fresh-frozen CCRCC surgical specimens, and there was a statistically significant positive correlation between p-ERK1/2 expression levels and PD-L1 expression levels (Pearson r=0.748, p=0.001). (Figure 10)

DISCUSSION

The interaction of PD-1 and its ligand PD-L1 inhibits T cell apoptosis, cytokine secretion, and T cell clonal expansion, and as a result, it suppresses T cell function. The overexpressed PD-L1 in tumor cells inhibits the antitumor effect of the cytotoxic T cell by binding to PD-1 on the tumor-infiltrating cytotoxic T cell, and then creates a favorable environment for the survival of tumor cells. (20) As mentioned earlier, PD-L1 is aberrantly expressed in various cancers including RCC and its high expression has been reported to be associated with poor prognosis in various cancers due to reducing the antitumor effect of immune cells. (1-19) Although the pathways involved in the expression of PD-L1 remain unclear, immunotherapy has been attempted with favorable results in a variety of malignant tumors including RCC in a way that treats tumors by blocking the interaction of PD-1 and PD-L1 to enhance the function of cytotoxic T cells on the tumor. (20, 32) In 2006, it was reported that PD-L1 was overexpressed in many RCCs associated with poor prognosis. (13) Although its prognostic effect is still controversial and my data did not show a significant correlation between PD-L1 expression and clinicopathologic factors, it has been reported in association with poor prognosis of RCC patients in most published results. (6, 10, 11, 16, 17)

The EGFR belongs to the ErbB family of receptor tyrosine kinases. (52) These trans-membrane proteins are activated following binding

with peptide growth factors of the EGF-family of proteins. (52) The physiological function of EGFR is to regulate epithelial tissue development and homeostasis. (53) EGFR is commonly upregulated in many carcinomas including NSCLC, metastatic colorectal cancer, glioblastoma, head and neck cancer, pancreatic cancer, and breast cancer. (54) Various mechanisms mediate the upregulation of EGFR activity and these EGFR alterations activate downstream oncogenic pathways, including the RAS-RAF-MEK-ERK-MAPK and AKT-PI3K-mTOR pathways. (54) This pathway activates many biological processes involved in tumor formation and progression. (54) Anti-EGFR therapies, including tyrosine kinase inhibitors (TKIs), have been performed in a variety of carcinomas including NSCLCs, colorectal carcinomas and head and neck squamous cell carcinomas. (55) Anti-EGFR therapy has also been tried in RCC patients, but the effectiveness of the treatment has not been proven. (56, 57) Some studies have explained this based on the rarity of genetic alteration of EGFR in RCCs. (39) Although the genetic alteration has been unproven, EGFR immunoreactivity has been reported in 50-90% of RCCs compared to non-neoplastic renal tissues in the literature. (35, 39) The mechanism of EGFR expression without specific genetic alteration is not fully known; some studies have suggested that the function of von Hippel-Lindau (VHL) tumor suppressor gene, which is a major genetic alteration of CCRCC, is associated with EGFR signaling. (58, 59) Moreover, immunohistochemically high expression of EGFR is known to be an unfavorable prognostic factor in CCRCC

despite its unclear pathogenesis. (38, 39, 60) My result showed that EGFR was expressed in many CCRCCs, and its expression levels were correlated with adverse prognostic factors, such as a higher WHO/ISUP grade, a larger tumor size, a higher stage and poor clinical outcome by OS and PFS.

As mentioned earlier, ERK1/2 is a part of MAPK cascades and activated ERK pathway by phosphorylation is known to play an important role in cell proliferation in mature differentiated eukaryotic cells (61) and tumorigenesis in many human cancers. (62) A previous study showed that the ERK pathway was activated in many RCCs via western blot analysis, in vitro kinase assays using fresh tissue. (43) Other study showed that ERK1/2 activation through p-ERK1/2 IHC was found to be overexpressed in 33% of CCRCC surgical specimens and was associated with a favorable prognosis in patients. (42) Conversely, in another study, p-ERK expression was observed in 36% of RCCs and high expression was associated with poor prognostic factors such as increased tumor size and stage. (44) Although the clinical implications of ERK1/2 activation are controversial, my data showed that overexpressed p-ERK1/2 in CCRCC samples was correlated with favorable prognostic factors, such as a lower WHO/ISUP grade, a smaller tumor size, a lower stage and good clinical outcome by OS and PFS.

The molecular mechanism of PD-L1 expression in tumor cells and its associated factors have been widely studied; however, it remains largely unknown. (33, 34) A previous study suggested that PD-L1

expression was associated with the EGFR pathway on the basis that PD-L1 expression was elevated in NSCLC surgical specimens and cell lines with EGFR mutations compared to those with wild-type EGFR. (45) Similarly, it was reported that the expression of PD-L1 was increased in NSCLC cell lines with EGFR mutations and was reduced by EGFR inhibitors. (46) It was also reported that the response rate in NSCLC patients treated with EGFR tyrosine kinase inhibitors was correlated with the PD-L1 expression of cancer cells. (47) From this point of view, several clinical trials are in progress to assess the effectiveness of adding PD-1/PD-L1 blockade therapy in EGFR-mutated NSCLC cancer patients. (63, 64)

As a downstream pathway of EGFR signaling that is associated with PD-L1 expression, a recent study suggested that PD-L1 expression was induced by EGFR activation through the ERK pathway in NSCLC cell lines. (48) They also explained that EGFR-TKI not only directly inhibited the viability of tumor cells but also indirectly enhanced antitumor immunity through down-regulation of PD-L1. (48) Similarly, the expression of PD-L1 was increased in BRAF inhibitor-resistant melanoma cell lines, and the ERK pathway was involved in the expression of PD-L1, which was confirmed by observing that the expression of PD-L1 decreased upon treatment with the MEK inhibitor U0126, which inhibited the ERK pathway. (65) In addition, various signaling pathways, including IFN-γ, NF-κB, PI3K/AKT, and mTOR, have been reported in association with PD-L1 expression in various human cancers. (33, 34, 66-82)

Therefore,

PD-L1 expression was associated with many different pathways in various human cancers. Unlike other cancers, the pathways associated with PD-L1 expression have rarely been studied in CCRCC.

In this study, I identified a change in PD-L1 expression upon artificial stimulation and inhibition of the ERK1/2 pathway in the Caki-1 CCRCC cell line by western blot analysis, although this was not observed in Caki-2. I also found a relationship between p-ERK1/2 expression and PD-L1 expression by immunohistochemical staining of FFPE samples from a number of CCRCC surgical specimens. I validated that relationship by western blot analysis of fresh frozen surgical samples. Through a comparative analysis of the results, I ensured the reliability of the immunohistochemical results in FFPE samples. I analyzed the interrelation of these expressions and their relation to the clinicopathologic parameters for a number of CCRCC surgical specimens. Although my data did not show a significant correlation between PD-L1 expression and clinicopathologic factors, the prognosis of CCRCC patients and EGFR expression level, I found that the ERK pathway might be one of the complex pathways associated with PD-L1 expression in CCRCC, as in NSCLC or melanoma, although the role of EGFR mutations was reported to be different. In addition, I suggested the clinical significance and function of immunohistochemical staining of PD-L1 and p-ERK1/2 in FFPE samples, and the combination of p-ERK1/2 and PD-L1 immunohistochemical results instead of PD-L1 alone would help to

develop more accurate therapeutic response prediction for anti-PD-1/PD-L1 therapy.

Anti-PD-1/PD-L1 therapy, which is currently being tried for medical therapy, is expected to be effective in patients with high expression of PD-L1, but there are exceptional cases so it is difficult to predict the precise therapeutic response compared to other targeted therapy. Since the expression level of PD-L1 is assessed by IHC, the accuracy of this result is important. Several different PD-L1 IHC assays have been approved by the FDA as companion diagnostics or complementary diagnostics in NSCLC. Some studies comparing the equivalence of these tests have been carried out and have produced satisfactory results. (83-85) Although studies on PD-L1 testing as a biomarker in RCC are underway, there is no standard test yet. (86) I tested several PD-L1 antibodies, including clone E1L3N (Cell Signaling Technologies, Danvers, MA), SP263 (Ventana Medical Systems, Tucson, AZ), 22C3 (Dako, Carpinteria, CA), and ab58810 (Abcam, Cambridge, MA). PD-L1 expression in CCRCC was not found to be as high as in NSCLC under the conditions and equipment used in NSCLC using clone E1L3N, SP263 and 22C3. I could achieve comparable expression levels under-modified conditions using the clone ab58810. Previous studies have shown the desired results using clone ab58810 in various human cancers, including NSCLC. (47, 87) These differences of PD-L1 immunohistochemical results using several antibodies in CCRCC compared to NSCLC suggested the differences of quantified expression level according to

the difference of associated pathways, such as EGFR mutations. It will become more apparent in the process of standardizing the assay along with trying anti-PD-1/PD-L1 therapy in CCRCC patients.

In this study, the ERK1/2-PD-L1 pathway was confirmed by in vitro and in vivo assay as a new mechanism of PD-L1 upregulation in CCRCC that has not been known until now. This would contribute to presenting a new mechanism of the pathogenesis of CCRCC by identifying the pathway involved in PD-L1 expression, and the combination of p-ERK1/2 and PD-L1 immunohistochemical staining could be used as a therapeutic response predictor for anti-PD-1/PD-L1 therapy in the future.

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Table 1. Immunohistochemical results of PD-L1, EGFR, p-ERK1/2 and its relationship with clinicopathologic characteristics

Characteristics	Cases (n=368)	PD-L1			EGFR			p-ERK1/2		
		High (n=204, 55.4%)	Low (n=164, 44.6%)		High(n=122, 33.2%)	Low(n=246, 66.8%)		High(n=146, 39.7%)	Low(n=222, 60.3%)	
Age				**P=0.043**			**P<0.001**			P=0.050
<57	176 (47.8%)	108	68		40	136		79	97	
≧57	192 (52.2%)	96	96		82	110		67	125	
Sex				**P<0.001**			P=0.186			P=0.825
Male	277 (75.3%)	168	109		97	180		109	168	
Female	91 (24.7%)	36	55		25	66		37	54	
WHO/ISUP grade				P=0.626			**P<0.001**			**P<0.001**
1	6 (1.6%)	5	1		2	4		5	1	
2	174 (47.3%)	96	78		34	140		83	91	
3	170 (46.2%)	93	77		75	95		53	117	
4	18 (4.9%)	10	8		11	7		5	13	
Tumor size				**P=0.002**			**P<0.001**			**P<0.001**
<5cm	251 (68.2%)	153	98		63	188		125	126	
≧5cm	117 (31.8%)	51	66		59	58		21	96	
T category				**P=0.029**			**P<0.001**			**P<0.001**

1a	214 (58.2%)	170	119		80	209		129	160
1b	75 (20.4%)								
2a	18 (4.9%)	9	13		9	13		5	17
2b	4 (1.1%)								
3a	36 (9.8%)								
3b	11 (3.0%)	20	27		28	19		11	36
3c	0 (0%)								
4	10 (2.7%)	5	5		5	5		1	9
N category				**P=0.010**			P=0.060		P=0.292
0	356 (96.7%)	193	163		115	241		143	213
1	12 (3.3%)	11	1		7	5		3	9
M category				P=0.976			**P=0.002**		**P=0.029**
0	339 (92.1%)	188	151		105	234		140	199
1	29 (10.6%)	16	13		17	12		6	23
Prognostic stage				P=0.060			**P<0.001**		**P=0.001**
I	282 (76.6%)	166	116		76	206		126	156
II	18 (4.9%)	7	11		7	11		3	15
III	37 (10.1%)	15	22		22	15		10	27
IV	31 (8.4%)	16	15		17	14		7	24

Table 2. Univariate and multivariate Cox regression analysis of OS and PFS

	Parameter	Univariate analysis			Multivariate analysis		
		HR	95% CI	*p*	HR	95% CI	*p*
OS	Age <57 *vs.* 57	2.095	1.076-4.079	**0.030**	1.679	0.858-3.288	0.131
	Sex Male *vs.* Female	1.025	0.500-2.104	0.946			
	WHO/ISUP grade 1, 2 *vs.* 3, 4	12.257	3.774-39.807	**<0.001**	3.788	1.105-12.983	**0.034**
	Tumor size <5cm *vs.* ≧5cm	21.937	7.794-61.746	**<0.001**			
	T category 1, 2 *vs.* 3, 4	11.233	5.884-21.445	**<0.001**			
	Node mets No *vs.* Yes	10.313	4.520-23.531	**<0.001**			
	Distant mets No *vs.* Yes	54.850	27.227-110.498	**<0.001**			
	Stage I, II *vs.* III, IV	41.434	16.167-106.189	**<0.001**	26.334	9.933-69.816	**<0.001**
	PD-L1 expression Low *vs.* High	0.739	0.394-1.384	0.345			
	EGFR expression Low *vs.* High	3.592	1.883-6.852	**<0.001**	1.179	0.604-2.299	0.630
	p-ERK1/2 expression Low *vs.* High	0.435	0.206-0.915	**0.028**	0.659	0.312-1.392	0.274
PFS	Age <57 *vs.* 57	1.604	0.938-2.743	0.084			
	Sex Male *vs.* Female	1.016	0.555-1.860	0.960			
	WHO/ISUP grade 1, 2 *vs.* 3, 4	5.514	2.701-11.256	**<0.001**	2.102	0.965-4.581	0.062
	Tumor size <5cm *vs.* ≧5cm	12.325	6.215-24.442	**<0.001**			
	T category 1, 2 *vs.* 3, 4	8.835	5.208-14.991	**<0.001**			
	Node mets No *vs.* Yes	10.577	5.112-21.883	**<0.001**			
	Distant mets No *vs.* Yes	32.177	18.012-57.484	**<0.001**			
	Stage I, II *vs.* III, IV	18.610	10.250-33.786	**<0.001**	12.797	6.773-24.180	**<0.001**
	PD-L1 expression Low *vs.* High	0.731	0.433-1.234	0.241			
	EGFR expression Low *vs.* High	3.900	2.268-6.705	**<0.001**	1.777	0.996-3.168	0.051
	p-ERK1/2 expression Low *vs.* High	0.580	0.325-1.035	0.065			

HR, hazard ratio; CI, confidence interval; Mets, metastasis

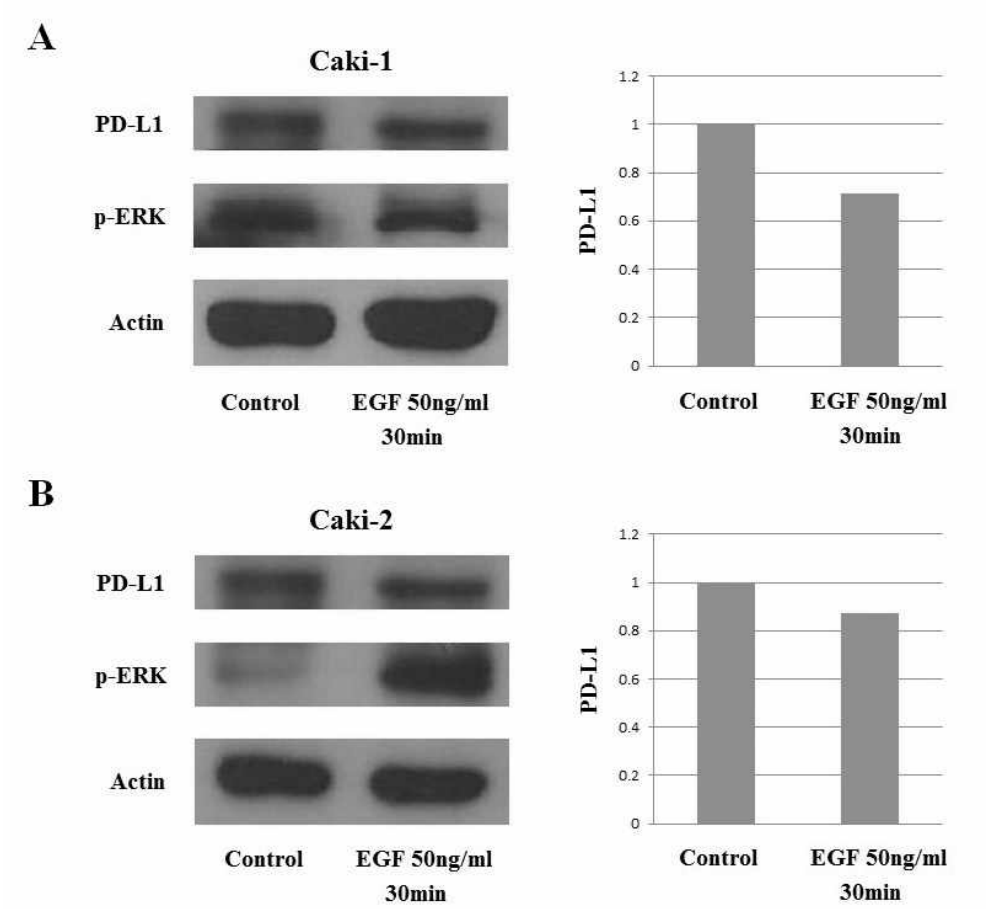
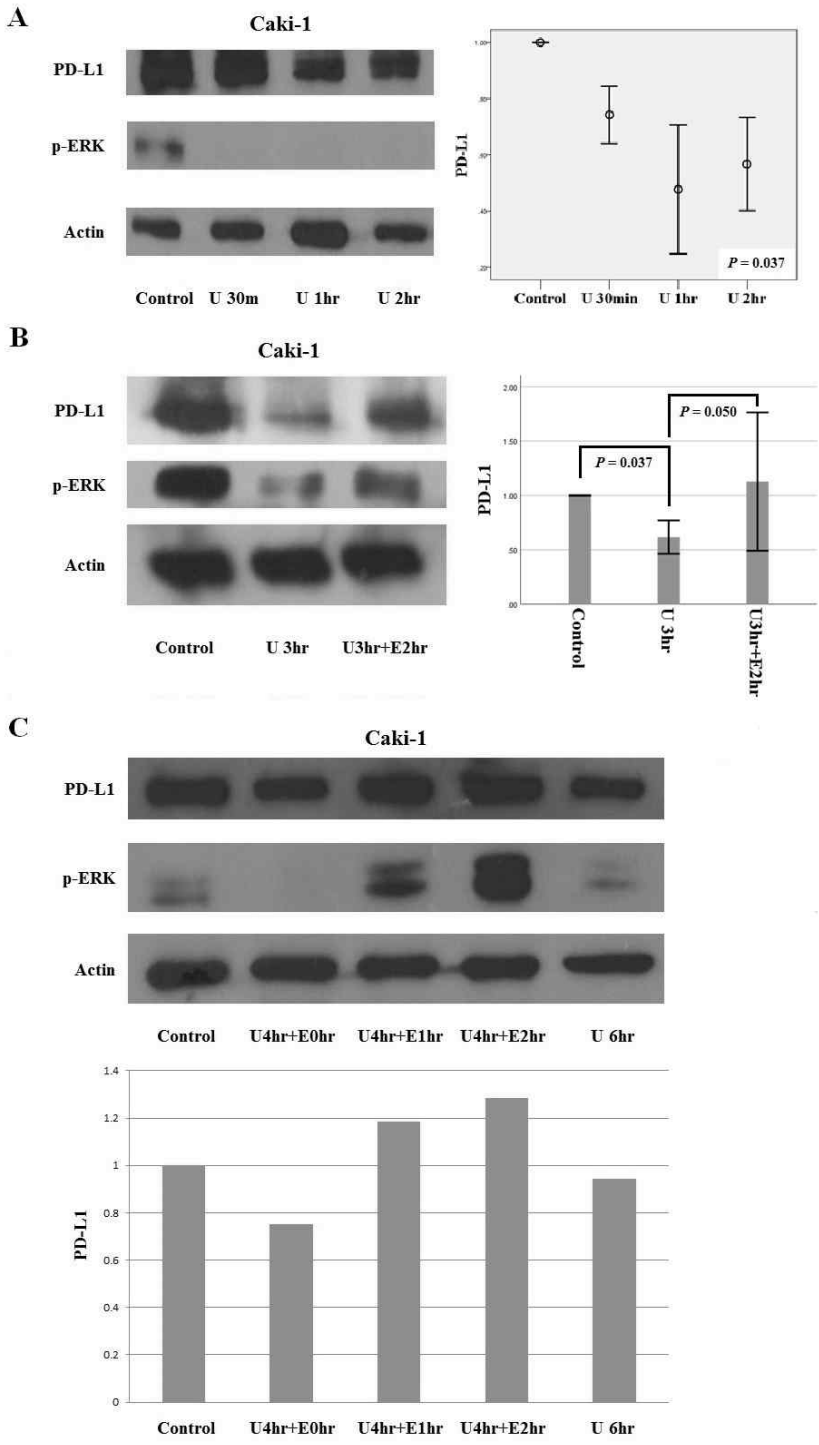


Figure 1. Western blot analysis of PD-L1 and p-ERK1/2 following stimulants treatment. PD-L1 expression did not change with EGF 50ng/ml treatment for 30min in both Caki-1 (A) and Caki-2 (B) CCRCC cell lines.


A
Caki-1
PD-L1
p-ERK
Actin
Control
U 30m
U 1hr
U 2hr
PD-L1
P = 0.037
Control
U 30min
U 1hr
U 2hr
B
Caki-1
PD-L1
p-ERK
Actin
Control
U 3hr
U3hr+E2hr
PD-L1
P = 0.037
P = 0.050
Control
U 3hr
U3hr+E2hr
C
Caki-1
PD-L1
p-ERK
Actin
Control
U4hr+E0hr
U4hr+E1hr
U4hr+E2hr
U 6hr
PD-L1
Control
U4hr+E0hr
U4hr+E1hr
U4hr+E2hr
U 6hr

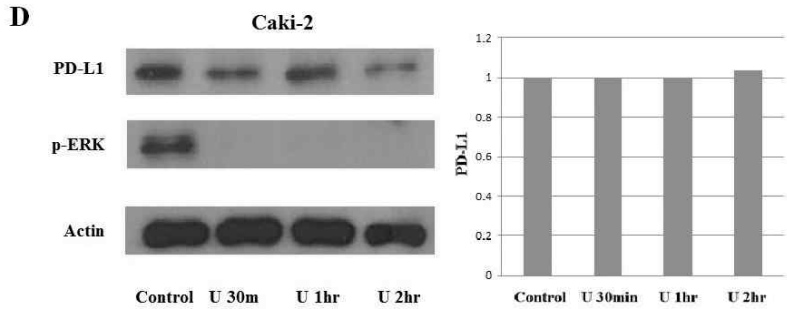


Figure 2. Western blot analysis of PD-L1 and p-ERK1/2 following treatment with stimulants and inhibitors. PD-L1 expression was suppressed by U0126 treatment with U0126 30mM in the Caki-1 CCRCC cell line but not in Caki-2. (A, D) After 3 hours of treatment with U0126 30mM, Caki-1 cells treated with EGF 50ng/ml for 2 hours showed initially reduced PD-L1 expression, which then increased again. (B) After 4 hours of treatment with U0126 30mM, Caki-1 cells treated with EGF 50ng/ml also showed initially reduced PD-L1 expression, which then increased again. (C)

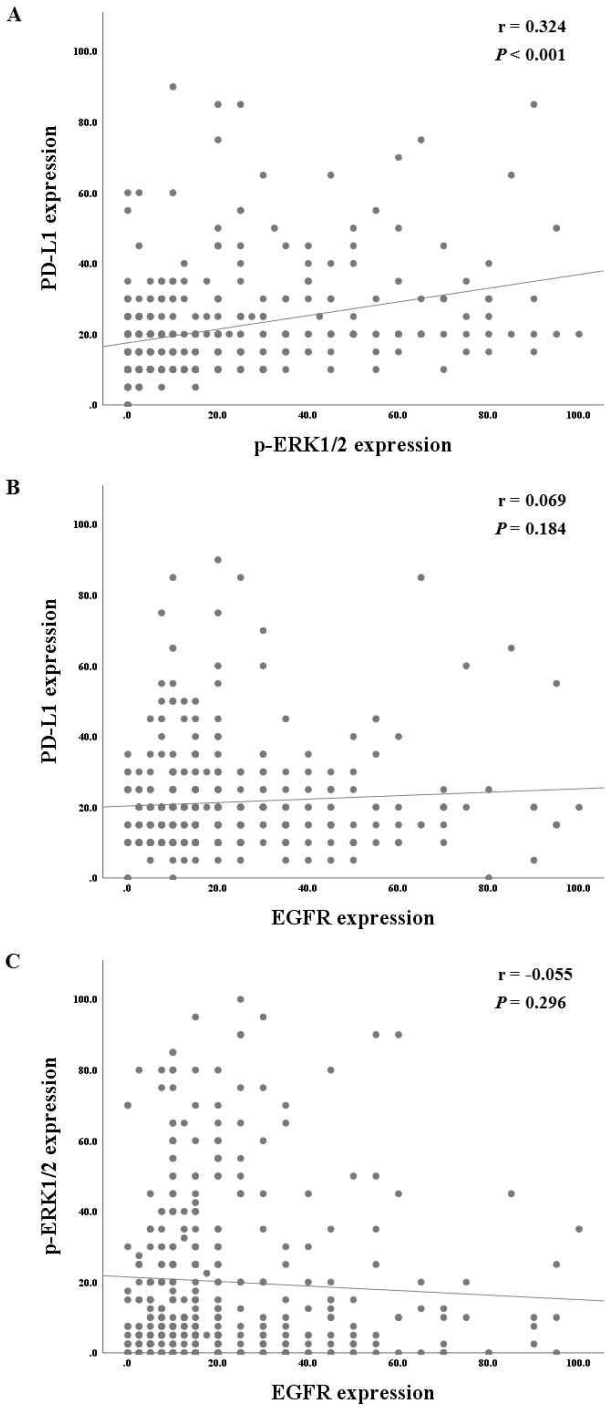

A
r = 0.324
P < 0.001
PD-L1 expression
p-ERK1/2 expression
B
r = 0.069
P = 0.184
PD-L1 expression
EGFR expression
C
r = -0.055
P = 0.296
p-ERK1/2 expression
EGFR expression

Figure 3. Immunohistochemical analysis of EGFR and p-ERK1/2 and their relationship with PD-L1 expression. There was a positive correlation between p-ERK1/2 and PD-L1 (Pearson r=0.324, p<0.001) (A), while there was no correlation between EGFR and PD-L1 (Pearson r=0.069, p=0.184) (B) or EGFR and p-ERK1/2 (Pearson r=-0.055, p=0.296) (C).

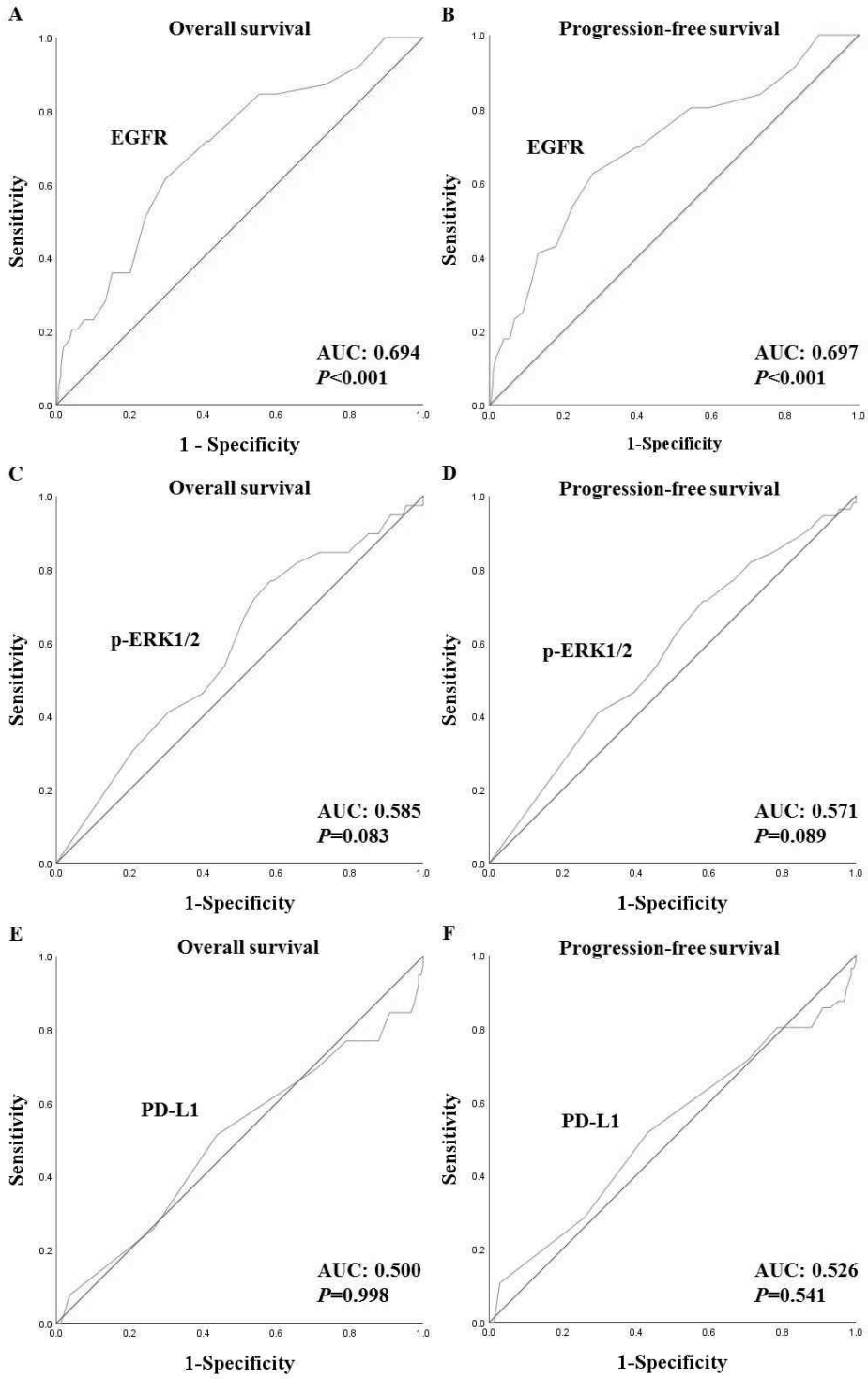

A
Overall survival
EGFR
Sensitivity
1 - Specificity
AUC: 0.694
P<0.001
B
Progression-free survival
EGFR
Sensitivity
1-Specificity
AUC: 0.697
P<0.001
C
Overall survival
p-ERK1/2
Sensitivity
1-Specificity
AUC: 0.585
P=0.083
D
Progression-free survival
p-ERK1/2
Sensitivity
1-Specificity
AUC: 0.571
P=0.089
E
Overall survival
PD-L1
Sensitivity
1-Specificity
AUC: 0.500
P=0.998
F
Progression-free survival
PD-L1
Sensitivity
1-Specificity
AUC: 0.526
P=0.541

Figure 4. ROC curve for EGFR, p-ERK1/2 and PD-L1 staining. For grouping by EGFR staining, the AUC were 0.694 and 0.697 for OS and PFS (all $p<0.001$). (A, B) For grouping by p-ERK1/2 staining, the AUC were 0.585 and 0.571 for OS and PFS ($p=0.083$ and 0.089 respectively). (C, D) For grouping by PD-L1 staining, the AUC were 0.500 and 0.526 for OS and PFS ($p=0.998$ and 0.541 respectively). (E, F)

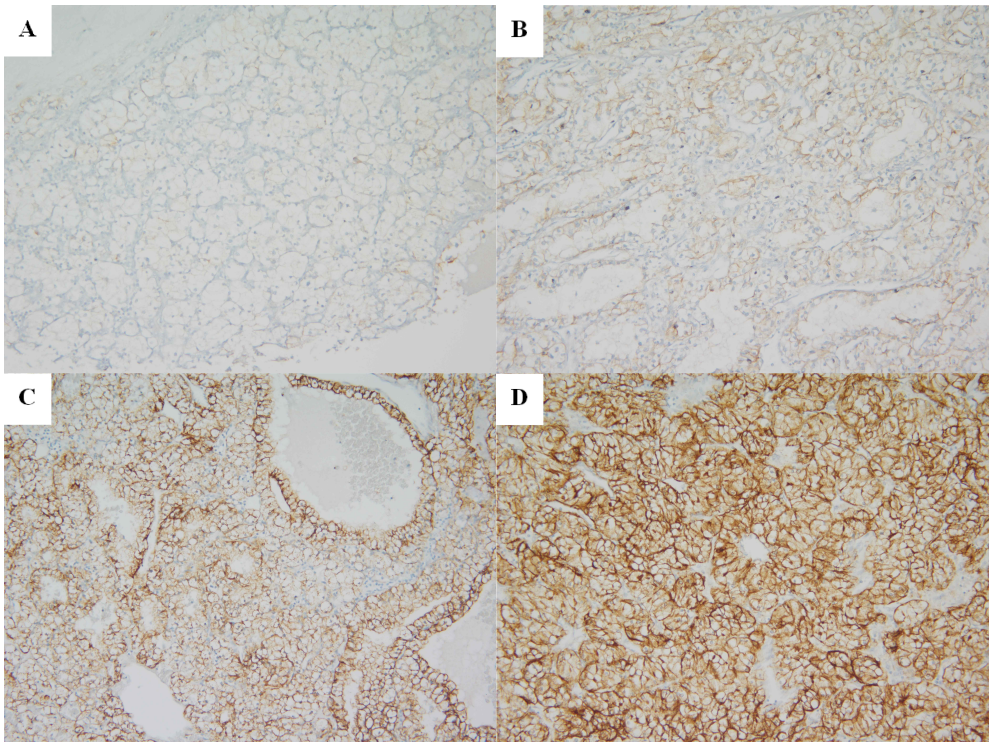


Figure 5. EGFR expression in CCRCC. Among 368 cases, 246 cases (66.8%) showed low expression (A, B) and 122 cases (33.2%) showed high expression. (C, D)

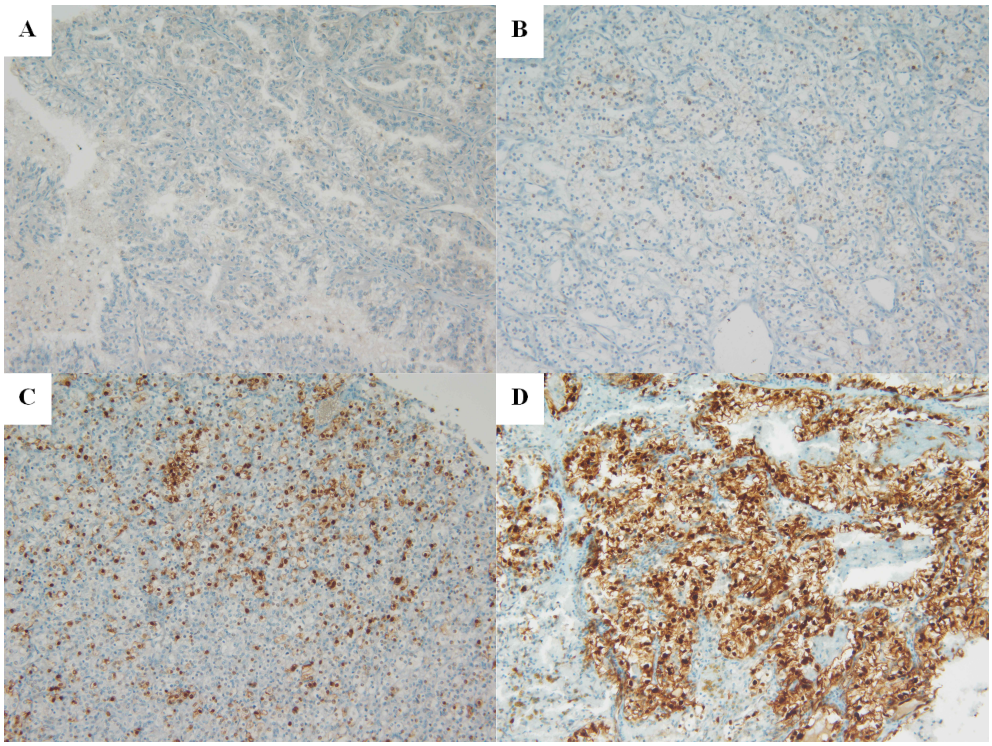


Figure 6. P-ERK1/2 expression in CCRCC. Among 368 cases, 222 cases (60.3%) showed low expression (A, B) and 146 cases (39.7%) showed high expression. (C, D)

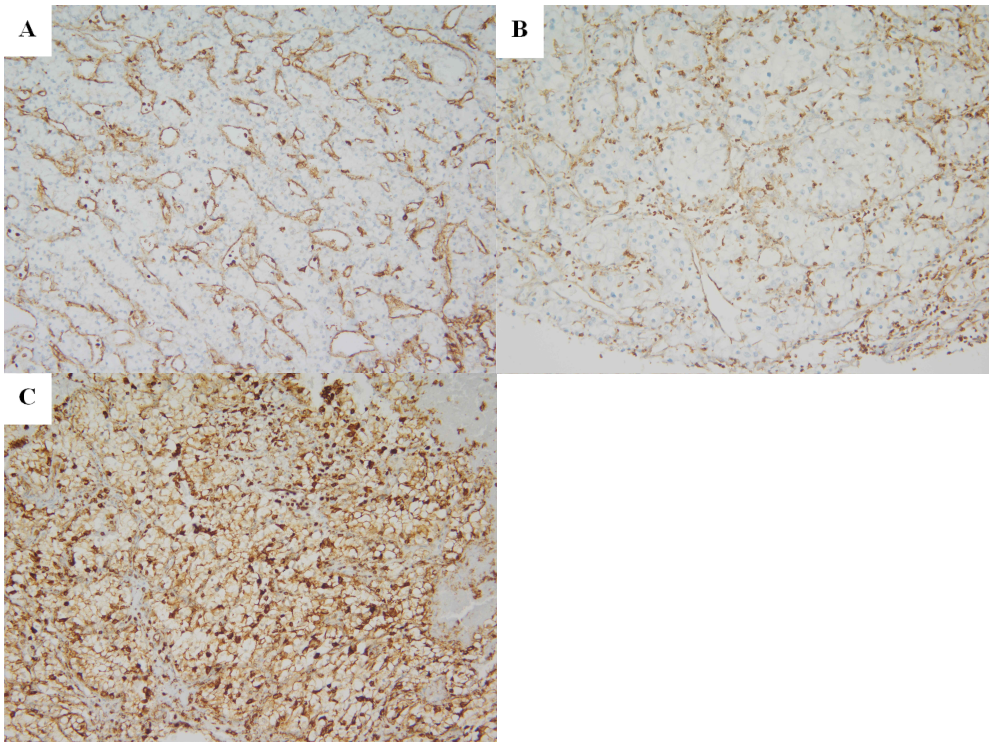
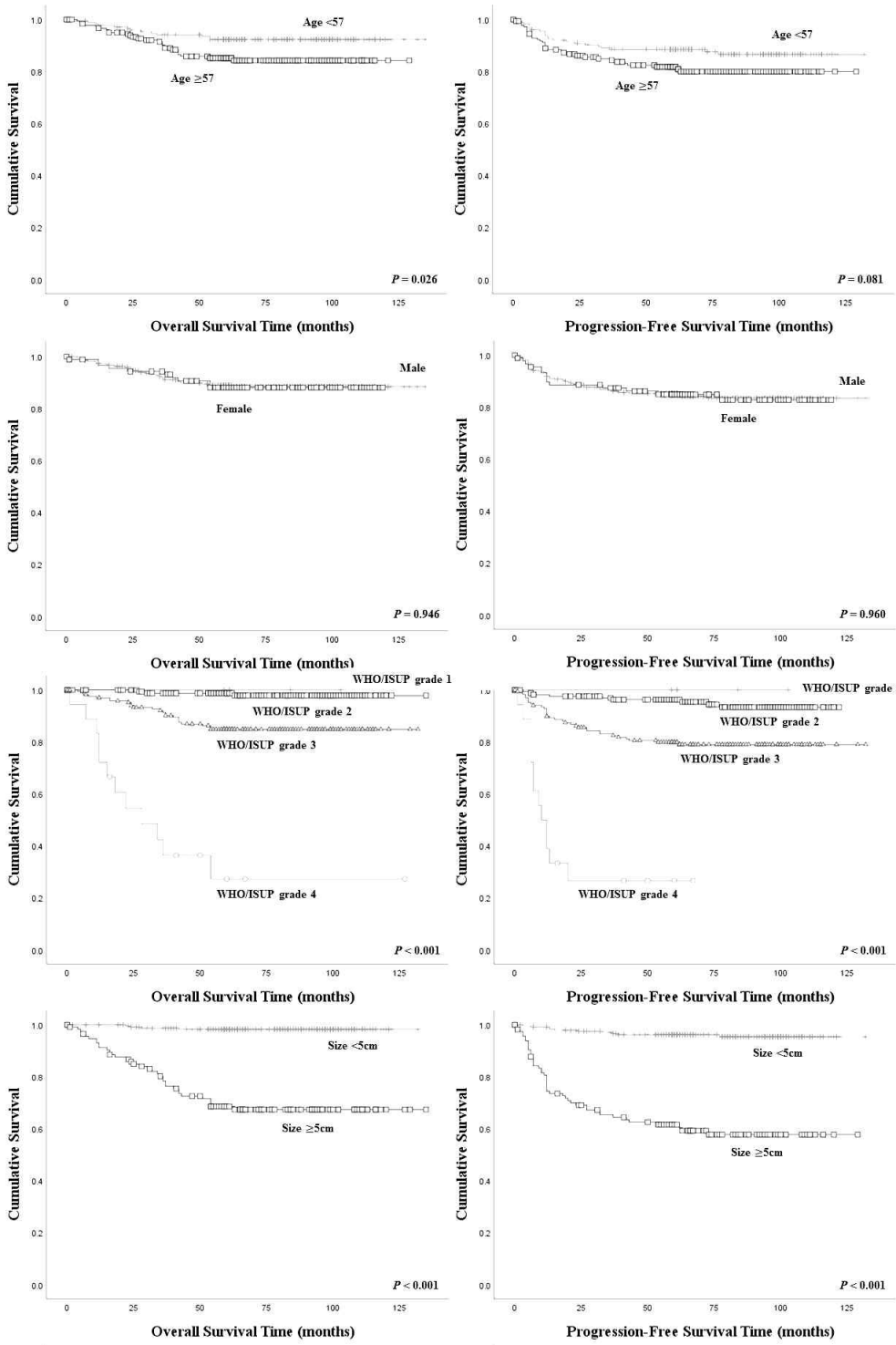
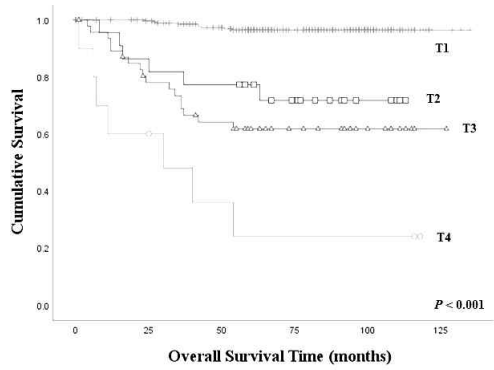
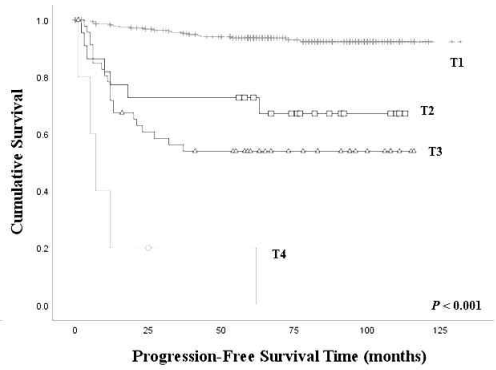
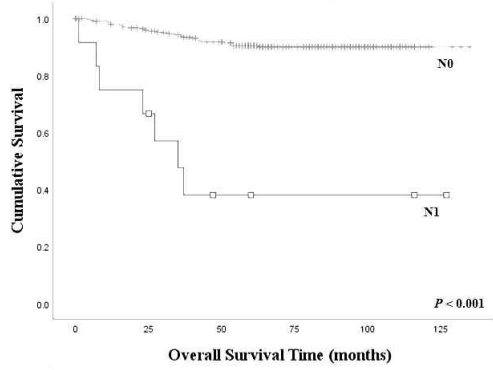


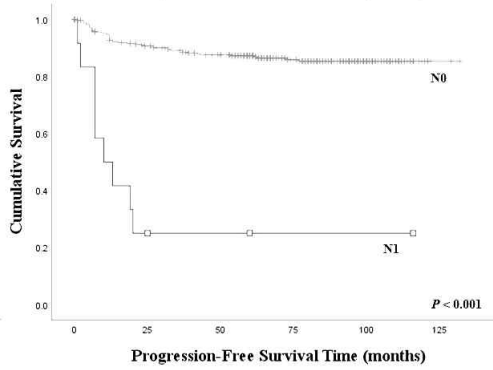
Figure 7. PD-L1 expression in CCRCC. Among 368 cases, 164 cases (44.6%) showed low expression (A, B) and 204 cases (55.4%) showed high expression. (C, D)

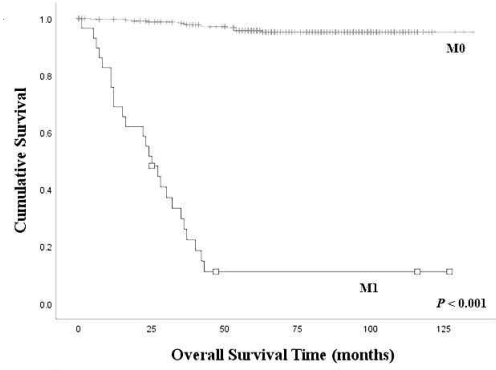

Cumulative Survival
Age <57
Age ≥57
P = 0.026
Overall Survival Time (months)
Cumulative Survival
Age <57
Age ≥57
P = 0.081
Progression-Free Survival Time (months)
Cumulative Survival
Male
Female
P = 0.946
Overall Survival Time (months)
Cumulative Survival
Male
Female
P = 0.960
Progression-Free Survival Time (months)
Cumulative Survival
WHO/ISUP grade 1
WHO/ISUP grade 2
WHO/ISUP grade 3
WHO/ISUP grade 4
P < 0.001
Overall Survival Time (months)
Cumulative Survival
WHO/ISUP grade 1
WHO/ISUP grade 2
WHO/ISUP grade 3
WHO/ISUP grade 4
P < 0.001
Progression-Free Survival Time (months)
Cumulative Survival
Size <5cm
Size ≥5cm
P < 0.001
Overall Survival Time (months)
Cumulative Survival
Size <5cm
Size ≥5cm
P < 0.001
Progression-Free Survival Time (months)

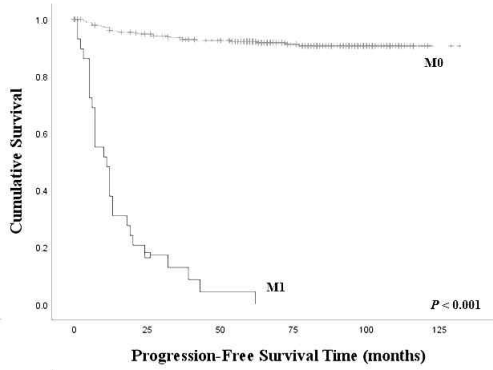

Cumulative Survival
T1
T2
T3
T4
P < 0.001
Overall Survival Time (months)

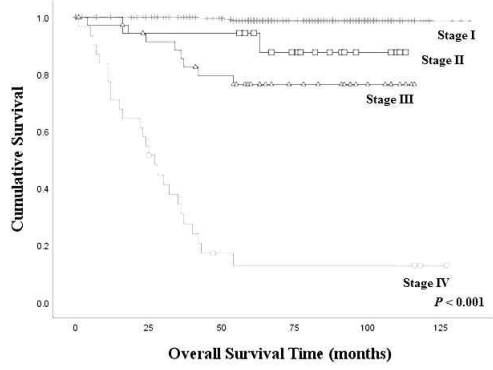

Cumulative Survival
T1
T2
T3
T4
P < 0.001
Progression-Free Survival Time (months)


Cumulative Survival
N0
N1
P < 0.001
Overall Survival Time (months)


Cumulative Survival
N0
N1
P < 0.001
Progression-Free Survival Time (months)


Cumulative Survival
M0
M1
P < 0.001
Overall Survival Time (months)


Cumulative Survival
M0
M1
P < 0.001
Progression-Free Survival Time (months)


Cumulative Survival
Stage I
Stage II
Stage III
Stage IV
P < 0.001
Overall Survival Time (months)

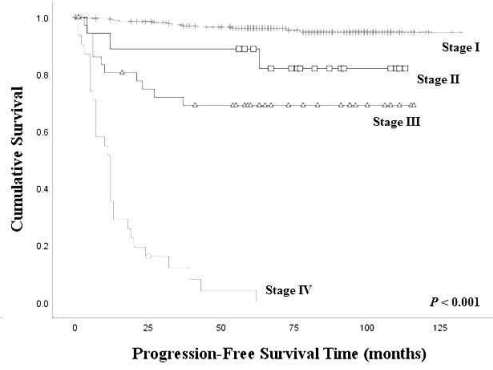

Cumulative Survival
Stage I
Stage II
Stage III
Stage IV
P < 0.001
Progression-Free Survival Time (months)

Figure 8. Kaplan-Meier curves. OS and PFS according to clinicopathologic parameters. Significantly poor OS was observed for old age group (≧57) ($p=0.026$), a higher WHO/ISUP grade ($p<0.001$), a larger tumor size (≧5 cm) ($p<0.001$), a higher T category ($p<0.001$), the presence of node metastasis ($p<0.001$), the presence of distant metastasis ($p<0.001$), and worse prognostic stage ($p<0.001$), while there was no difference according to sex ($p=0.946$). Additionally, significantly poor PFS was observed in a higher WHO/ISUP grade ($p<0.001$), a larger tumor size (≧5 cm) ($p<0.001$), a higher T category ($p<0.001$), the presence of node metastasis ($p<0.001$), the presence of distant metastasis ($p<0.001$) and worse prognostic stage ($p<0.001$), while there was no difference according to age ($p=0.081$) and sex ($p=0.960$).

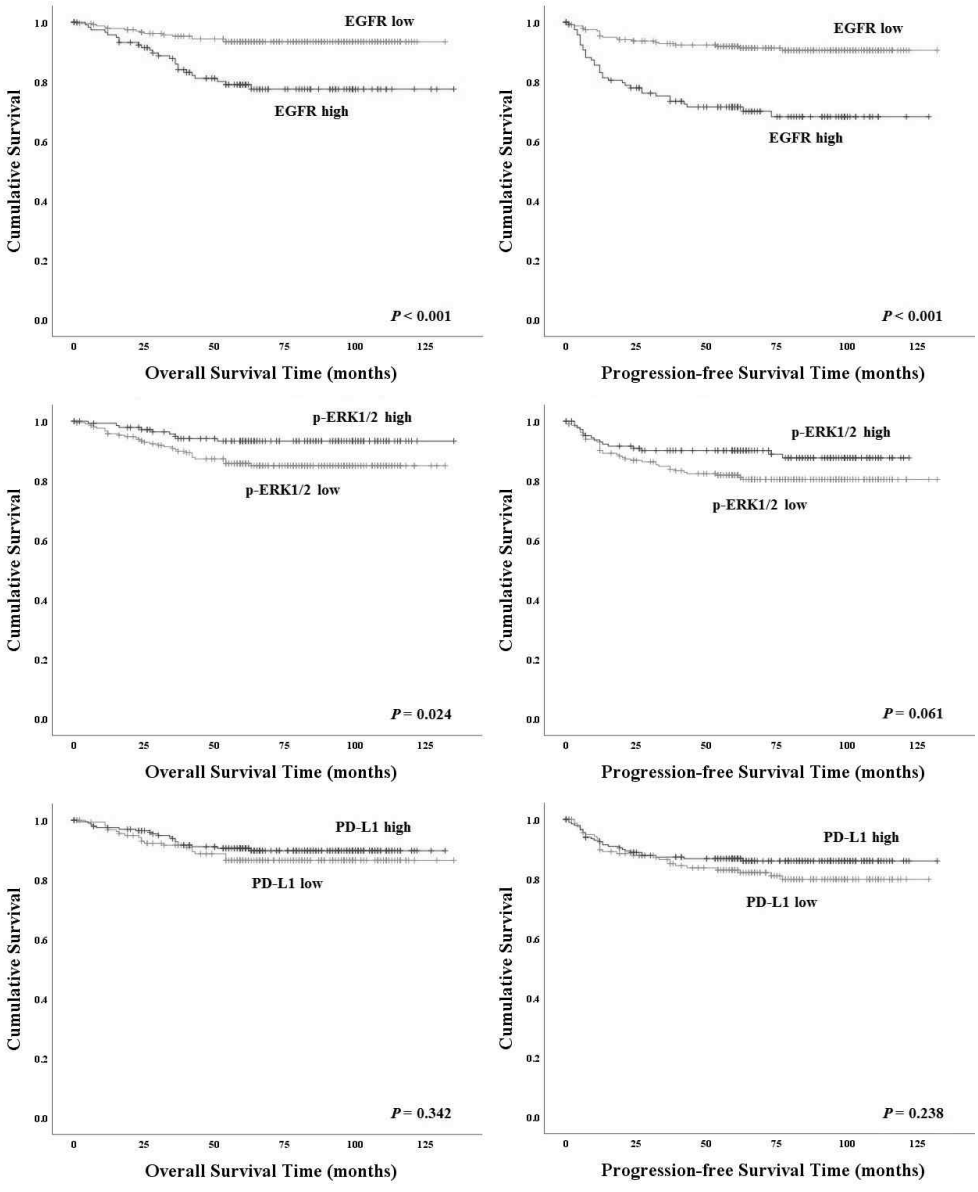

EGFR low
EGFR high
Cumulative Survival
P < 0.001
Overall Survival Time (months)
EGFR low
EGFR high
Cumulative Survival
P < 0.001
Progression-free Survival Time (months)
p-ERK1/2 high
p-ERK1/2 low
Cumulative Survival
P = 0.024
Overall Survival Time (months)
p-ERK1/2 high
p-ERK1/2 low
Cumulative Survival
P = 0.061
Progression-free Survival Time (months)
PD-L1 high
PD-L1 low
Cumulative Survival
P = 0.342
Overall Survival Time (months)
PD-L1 high
PD-L1 low
Cumulative Survival
P = 0.238
Progression-free Survival Time (months)

Figure 9. Kaplan-Meier curves. OS and PFS according to EGFR, p-ERK1/2 and PD-L1 expression. Higher EGFR expression was associated with poor OS ($p<0.001$) and PFS ($p<0.001$) of the patients. Higher p-ERK1/2 expression was associated with favorable survival of the patients, although not statistically related to PFS (OS: p=0.024, PFS: 0.061). PD-L1 expression was not associated with patients' survival (OS: p=0.342, PFS: 0.238).

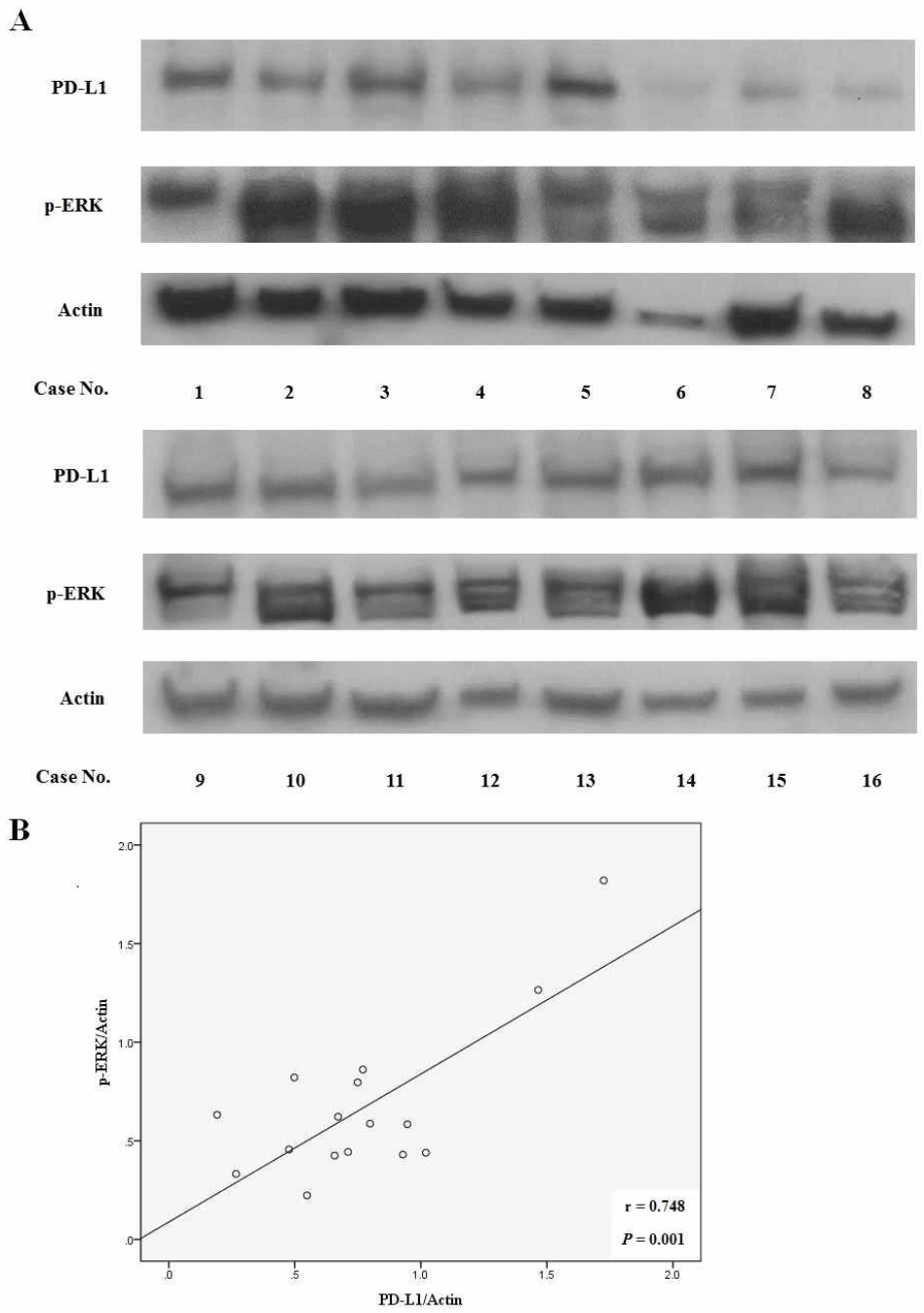

A
PD-L1
p-ERK
Actin
Case No.
1 2 3 4 5 6 7 8
PD-L1
p-ERK
Actin
Case No.
9 10 11 12 13 14 15 16
B
p-ERK/Actin
PD-L1/Actin
r = 0.748
P = 0.001

Figure 10. P-ERK1/2 and PD-L1 expression using fresh CCRCC surgical specimens. There was a positive correlation between p-ERK1/2 expression levels and PD-L1 expression levels (Pearson r=0.748, p=0.001). (A) Western blot analysis (B) Scatter plot

국문초록

서론: Programmed cell death protein 1 (PD-1)과 그 리간드인 programmed death-ligand 1 (PD-L1)의 상호작용은 T세포 기능을 억제하는 것으로 알려져 있다. 종양세포에서 과발현된 PD-L1은 종양 침윤성 세포 독성 T세포의 PD-1과 결합하여 세포 독성 T 세포의 항종양 효과를 억제하고 종양세포의 생존에 유리한 환경을 조성한다. PD-L1의 발현에 관여하는 경로는 아직 불분명하지만, 신세포암을 포함한 다양한 악성 종양에서 PD-1과 PD-L1에 대한 면역요법이 효과를 보이고 있다. 한편 대표적인 종양 성장 촉진물질인 표피 성장 인자 수용체 (EGFR)의 활성화 및 그로 인한 세포 외 신호 조절 인산화효소 (ERK)의 활성화가 비소세포폐암에서 PD-L1 발현 증가와 연관됨이 보고되었다. 투명세포 신세포암 (CCRCC)에서도 EGFR의 과발현이 50-90%에서 나타남이 알려져 있고, 인산화된 ERK1/2 (p-ERK1/2) 및 PD-L1 발현 역시 각각 알려져 있다. 그러나 이들 물질이 CCRCC에서 어떤 기전으로 상호 작용을 하고 조절되는지는 알려져 있지 않다. 본 연구는 CCRCC에서 EGFR-ERK1/2가 PD-L1에 미치는 영향과 조절경로를 조사하여 PD-L1의 발현이 EGFR과 ERK1/2의 활성화에 의해 유도되는지를 밝히고자 하였다.

방법: P-ERK1/2가 매개하는 PD-L1 조절에 관여하는 신호 전달 경로를 연구하기 위해 인간 CCRCC 세포주인 Caki-1 및 Caki-2를 대상으로 ERK1/2 경로의 자극제인 재조합 인간 상피 세포 성장 인자 (EGF)와 억제제인 U0126을 처리한 후 western blot 분석을 시행하였다. 또한 368례의 CCRCC 수술검체를 이용하여 PD-L1, EGFR, p-ERK1/2 면역 조직화학 염색을 시행하였고 각각의 염색 정도와 임상 병리학적 인자들과의 상관관계를 분석하였다. 파라핀 샘플의 면역 조직 화학 염색 결과를 검

증하기 위해 16개의 신선 냉동 CCRCC 샘플을 이용하여 western blot 분석을 통해 PD-L1과 p-ERK1/2 단백질 발현의 상관관계를 분석했다.

결과: 본 연구에서는 Caki-1 CCRCC 세포주를 대상으로 ERK1/2 경로의 자극 및 억제에 대한 PD-L1 발현의 변화를 western blot 분석으로 확인하였다. 이 변화는 Caki-2 세포주에서는 확인되지 않았다. 또한 파라핀 샘플의 면역 조직 화학 염색을 통해 p-ERK1/2 발현과 PD-L1 발현 사이의 양의 상관관계를 발견하였으며 (Pearson r=0.324, $p<0.001$) 신선 동결 수술 조직의 western blot 분석으로 그 상관관계를 재확인했다. (Pearson r=0.748, p=0.001)

결론: 이 연구를 통해 지금까지 알려지지 않았던 CCRCC에서의 PD-L1 발현의 새로운 기전으로 ERK1/2-PD-L1경로를 세포실험 및 생체조직 분석을 통해 확인하였다. 본 연구에서 확인한 CCRCC의 PD-L1 발현에 관여하는 ERK1/2 경로는 향후 CCRCC의 발병 기전의 새로운 과정을 밝히는데 이용될 것이며, p-ERK1/2 및 PD-L1 면역 조직 화학 염색 결과의 조합은 추후 항 PD-1/PD-L1 치료에 대한 반응 예측에 이용될 것이다.

주요어: 투명세포 신세포암; Programmed Cell Death 1 Ligand 1; B7-H1 Antigen; ERK Pathway; 표피 성장 인자 수용체; Blotting, Western; 면역조직화학염색

학번: 2011-21898