Original Article

http://dx.doi.org/10.3349/ymj.2014.55.4.861
pISSN: 0513-5796, eISSN: 1976-2437

Yonsei Med J 55(4):861-870, 2014

YMJ

# Insulin Receptor Expression in Clear Cell Renal Cell Carcinoma and Its Relation to Prognosis

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Received: January 29, 2014
Revised: March 22, 2014
Accepted: March 26, 2014
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· The authors have no financial conflicts of interest.

**Purpose:** Both insulin and insulin-like growth factor (IGF)-1 signaling are key regulators of energy metabolism, cellular growth, proliferation, and survival. The IGF-1 receptor (IGF-1R) is overexpressed in most types of human cancers including renal cell carcinoma (RCC) with poor prognosis. Insulin receptor (IR) shares downstream effectors with IGF-1R; however, the expression and function of IR in the tumorigenesis of renal cancer remains elusive. Therefore, we examined the expression of IR and its prognostic significance in clear cell RCC (CCRCC). **Materials and Methods:** Immunohistochemical staining for IR was performed on 126 formalin-fixed paraffin-embedded CCRCC tissue samples. Eight of these cases were utilized for western blot analysis. The results were compared with various clinico-pathologic parameters of CCRCC and patient survival. **Results:** IR was expressed in the nuclei of CCRCC tumor cells in 109 cases (87.9%). Higher IR expression was significantly correlated with the presence of cystic change, lower Fuhrman nuclear grade, lower pathologic T stage, and lower TNM stage, although it wasn't significantly related to diabetes status and patient survival. Western blot analyses supported the results of the immunohistochemistry studies. **Conclusion:** IR expression in CCRCC may be associated with favorable prognostic factors.

**Key Words:** Insulin receptor, clear cell renal carcinoma, diabetes mellitus, prognosis, immunohistochemistry

## INTRODUCTION

Renal cell carcinoma (RCC) comprises 2–3% of all malignant tumors in adults, accounting for about 209000 new cases and 102000 deaths per year worldwide.[1] The most common subtype of RCC is clear cell RCC (CCRCC), which represents 87.7%.[2] Most patients who have a localized tumor can be cured by surgery alone. However, one third of patients are diagnosed with metastatic RCC and an additional 20–40% of patients develop metastases after curative surgery.[3] Treatment of locally advanced and/or metastatic RCC is still complicated due to the lack of specific therapeutic targets and inadequate methods to assess certain drug efficacies.[4]

Obesity is a well-established risk factor for RCC and diabetes.[5,6] Diabetes is a serious health problem worldwide, characterized by failure of insulin production by the pancreatic β-cells and insulin resistance in peripheral tissues.[6] Patients with diabetes are at a higher risk of developing certain malignancies including kidney, liver, pancreas, bladder, breast, and colon compared with the general population.[7-13] Additionally, diabetes is a common cause of end stage renal disease and ~20% of diabetic patients develop an irreversible nephropathy after years of uncontrolled hyperglycemia.[14]

Insulin and insulin-like growth factor (IGF)-1 signaling regulate cellular growth, proliferation, metabolism, and survival.[15] The insulin/IGF signaling system is composed of three ligands, IGF-1, IGF-2, and insulin, and at least four receptors including IGF-1 receptor (IGF-1R), IGF-2 receptor, insulin receptor (IR), and the hybrid receptors of IGF and insulin.[16] IR is a heterotetrameric protein consisting of two extracellular α subunits and two transmembrane β subunits. The binding of ligand to the subunits of IR stimulates the intrinsic tyrosine kinase activity of the β subunits of the receptor.[17] IR is a close structural homolog of IGF-1R. Amino acid sequence similarities range from 40 to 85% in different domains, with the highest degree of homology being found in the tyrosine kinase domain.[18,19] The ligand-receptor interaction results in phosphorylation of several intracellular substrate proteins and activates multiple downstream signaling cascades including the phosphatidylinositol 3-kinase and mitogen activated protein kinase pathways, which mediate metabolic and mitogenic activities in cells.[20]

Overexpression of IGF-1R is associated with poor prognosis in various human malignancies including renal, breast, prostate and ovarian cancers.[21-23] However, the expression level of IR and its potential prognostic significance have not been elucidated in CCRCC. Therefore, we hypothesized that IR may be expressed in CCRCC which originates from proximal tubular epithelium and that IR expression may correlate with the clinico-pathological parameters of CCRCC. In this study, we examined the expression of IR in CCRCC and compared its expression with well-known prognostic factors of CCRCC and patient survival to validate the prognostic value of IR.

## MATERIALS AND METHODS

### Patients and tissue samples

Samples from 126 cases of CCRCC were collected from patients who underwent radical nephrectomy at the Yonsei University Wonju Severance Christian Hospital from 2001 to 2011. All tissue samples were formalin-fixed paraffin-embedded (FFPE) tissues with a pathological diagnosis of CCRCC. Eight fresh tissue samples were available for western blot analysis. Two expert pathologists reviewed all pathology slides along with the pathologic reports and clinical records. TNM stage and Fuhrman nuclear grading were reconfirmed.[24,25] Fuhrman nuclear grades were grouped into low (grade 1+2) and high (grade 3+4) grades. The institutional ethics committee of Yonsei University Wonju College of Medicine approved this study (YWMR-12-0-014).

### Immunohistochemistry (IHC)

*Tissue microarray (TMA) preparation*

A total of 126 FFPE samples were used for TMA. A representative tumor site without necrosis, hemorrhage, or artifact was marked in the paraffin blocks. The selected tumor area was harvested using a 5 mm Quick-ray tip-punch (Unitma, Seoul, Korea), placed on a 20 pore TMA mold (Unitma), and re-embedded with paraffin. 4 μm sections of TMA blocks were cut and attached onto coated slides.

*Staining*

Staining was performed using a Ventana Benchmark XT (Roche Diagnostics, Basel, Switzerland) automatic immunostaining machine. The sections were deparaffinized in xylene, rehydrated in graded alcohols, and subjected to pretreatment with CC1 (Roche Diagnostics). The sections were washed with reaction buffer followed by incubation with primary IR-β antibody (Abcam, Cambridge, MA, USA) at a 1:100 dilution for 60 min at 42°C. Bound antibody was detected with the Ultra View Universal DAB kit (Roche Diagnostics) and sections were counterstained with hematoxylin (Roche Diagnostics) according to the manufacturer's instructions. Positive and negative control stains were also performed.

*Quantification of IHC*

We used a modified Allred scoring system to evaluate positivity, with staining intensity and distribution being scored separately.[26,27] The staining intensity was scored as 0 points (negative), 1 point (weak), 2 points (intermediate), or 3 points (strong) and the distribution of positive-stained cells was assessed as 0 points (negative), 1 point (<1%), 2 points (1–10%), 3 points (11–33%), 4 points (34–67%), or 5 points

(>67%). The total staining score was calculated as the sum of two parameters. Total staining scores from 0 to 3 points were considered negative, while scores from 4 to 8 points were considered positive. To overcome the limitations of this quantification method, we also compared the mean staining score as continuous variables in each group.

### Western blot analysis

Western blot analysis was performed on eight cases of CCRCC with adjacent normal kidney parenchyma. The fresh tissues were lysed using 2 mL of PRO-PREP lysis buffer (iNtRon Biotechnology, Daejeon, Korea), and then ground for 15–20 sec on ice using a homogenizer (ProScience, Woburn, MA, USA). The lysates were centrifuged at 13000 rpm for 15 min; the supernatants were collected and protein concentration was measured using the Bradford method. 10 ug of protein was used for sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Proteins were transferred to immobilon-P membranes (Millipore, Billerica, MA, USA), using an electrophoretic transfer system (Bio-Rad, Hercules, CA, USA) at 100 V for 1.5 hours. The membranes were blocked with 5% skim milk in TBS-T buffer for an hour. The blocked membranes were washed with TBS-T buffer and incubated overnight at 4°C in primary IR-β (Abcam) and β-actin (Santa Cruz Biotechnology, Dallas, TX, USA) antibodies, diluted 1:1000 and 1:5000, respectively. The membranes were then incubated for 1 hour at room temperature in Anti-Mouse IgG-HRP secondary antibody (Santa Cruz Biotechnology) using dilutions of 1:2000 for IR-β and 1:5000 for β-actin. Bound antibodies were detected using Luminata TM Forte Western HRP Substrate (Millipore) and the Biospectrum Imaging System (UVP, Upland, CA, USA). Band densities on immunoblots were measured with ImageJ software (available at http://rsb.info.nih.gov/ij/index.html).

### Statistical analysis

Statistical analysis was performed using PASW, version 20.0 (SPSS Inc., Chicago, IL, USA). Student's t-test and $\chi^2$ test were used to compare the categorical and continuous variables. The period of overall survival was measured from the date of surgery to the date of death due to the tumor. Tumor recurrence was defined as the presence of clinically diagnosed or pathologically confirmed metastases. Survival analysis was performed using the Cox regression method. A value of $p<0.05$ was considered statistically significant.

## RESULTS

### General clinico-pathological characteristics

The patients were predominantly men with a mean age of 57.4±10.5 years. The mean tumor size was 5.3±2.7 cm. Twenty-two patients (17.5%) had a history of diabetes. Fifteen cases (11.9%) were Fuhrman nuclear grade 1, 57 cases (45.2%) were grade 2, 42 cases (33.4%) were grade 3, and 12 cases (9.5%) were grade 4. Eighty-seven cases (69.0%) were TNM stage I, 13 (10.3%) were stage II, 22 (17.5%) were stage III, and 4 (3.2%) were stage IV. Of the 126 patients, 123 had follow up information. The median follow up time was 45 months. Of the patients with follow up information, 9 (7.1%) had tumor recurrence. At the time of the last follow up, 12 patients (9.5%) had died due to the tumor. These statistics are detailed in Table 1.

### Expression pattern of IR in CCRCC and non-tumor renal tissues

The IGF-1 signaling plays a major role in cancer cell prolif-

**Table 1.** Summary of Clinical and Pathological Findings

| Characteristics | Number of cases (%) |
|---|---|
| Gender | |
| Male | 94 (74.6) |
| Female | 32 (25.4) |
| Age (yrs) | |
| Mean±SD | 57.4±10.5 |
| Diabetes | |
| Yes | 22 (17.5) |
| No | 104 (82.5) |
| Tumor size (cm) | |
| Mean±SD | 5.3±2.7 |
| Fuhrman nuclear grade | |
| 1 | 15 (11.9) |
| 2 | 57 (45.2) |
| 3 | 42 (33.4) |
| 4 | 12 (9.5) |
| TNM stage | |
| I | 87 (69.0) |
| II | 13 (10.3) |
| III | 22 (17.5) |
| IV | 4 (3.2) |
| Recurrence | |
| Present | 9 (7.1) |
| Absent | 103 (81.7) |
| Health status | |
| Alive | 111 (88.1) |
| Died | 12 (9.5) |

SD, standard deviation.

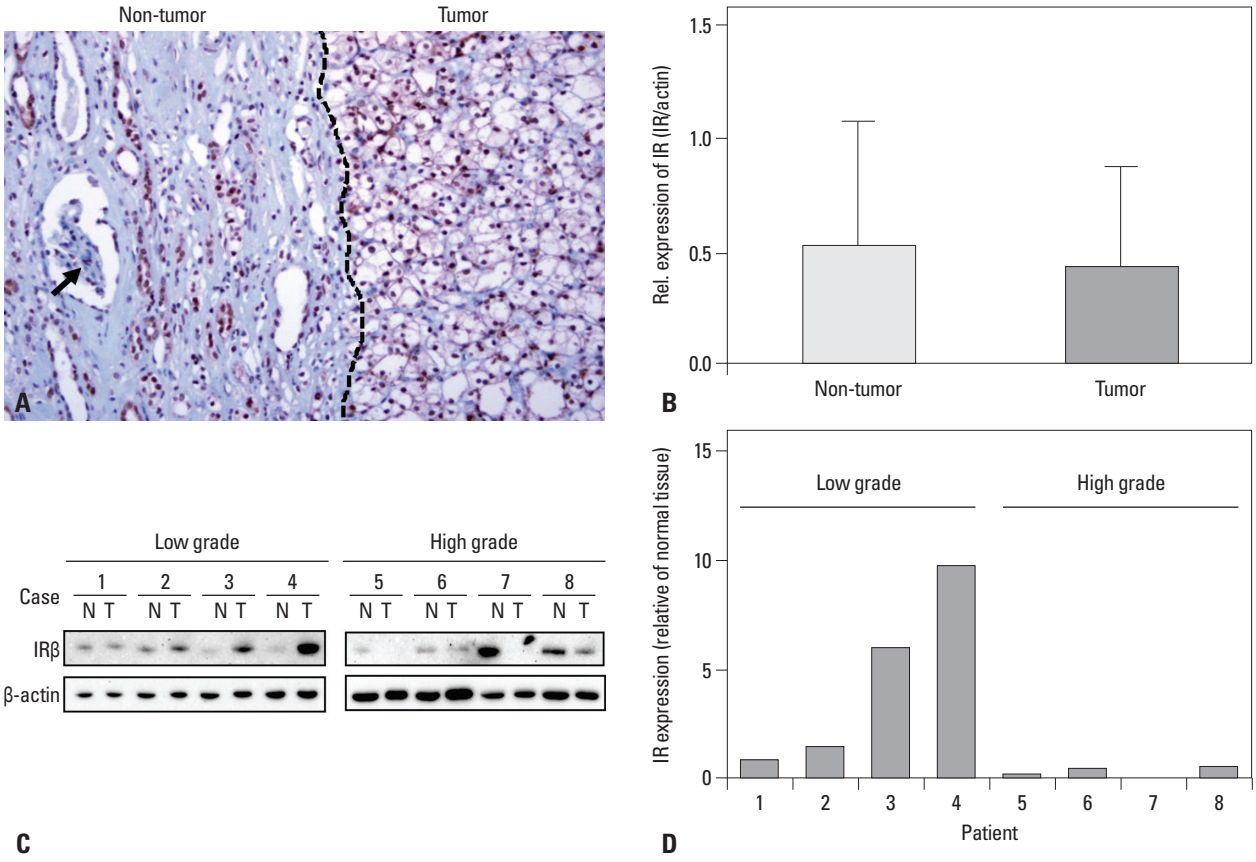


**Fig. 1.** Expression pattern of insulin receptor (IR) in clear cell renal cell carcinoma (CCRCC) and non-tumor tissues. (A) Immunohistochemical staining for IR in tumor and non-tumor tissue from same patient. Nuclear and/or cytoplasmic immunoreactivity of IR in podocytes (arrow), normal tubular epithelium, and lymphocytes in non-tumor tissues (left side) and nuclear positivity of IR in tumor cells (right side). (B) Relative expression of IR in non-tumor and tumor tissues was analyzed from fresh tissues using immunoblotting. β-actin served as a protein loading control. (C) Expression pattern of IR was examined in individual pair tissues from 8 cases of CCRCC (low and high nuclear grades). Expression level of IR in the pair tissues of carcinoma and adjacent non-tumor was analyzed with immunoblotting. (D) Quantitative analysis of IR immunoblotting. IR expression level of tumor tissues was normalized with those of normal renal parenchymal tissue. N, non-tumor tissues; T, tumor tissues.

eration and survival. Moreover, the IGF-1R is overexpressed in most types of cancer including CCRCC.[28] IR and IGF-1R share down-stream signaling cascades. Those observations prompted us to carry out the present study to examine the IR expression pattern and clinical significance in CCRCC. The IHC staining showed that IR was expressed in both carcinoma and normal renal parenchymal tissues (Fig. 1A). The immunoreactivity of IR was mostly observed nuclear in cancer tissues, whereas IR protein was detected in nuclear and/or cytoplasm in adjacent normal parenchymal tissues including podocytes of glomeruli, tubular epithelium, and endothelial cells of blood vessels (Fig. 1A). There was no difference of total protein amount of IR between non-tumor and tumor tissues in western blot analysis from 8 fresh samples (Fig. 1B). This observation leaded us to examine expression pattern of IR in individual pair tissues. Compared with that of non-tumor tissue, the expression level of IR was elevated in low-grade tumors but not in high-grade tumors (Fig. 1C and D).

### Expression level of IR is associated with Fuhrman nuclear grade and clinico-pathological parameters of CCRCC

Several clinico-pathological parameters are used to predict the prognosis of RCC. To determine if IR has any prognostic value, we carried out correlation analysis between IR expression and the well known prognostic factors for RCC. The analysis of IR expression in fresh tissue showed that the level of IR protein was inversely correlated with Fuhrman nuclear grade of CCRCC (Fig. 2A and B). We next examined the nuclear expression of IR in 126 FFPE samples from CCRCC cases using IHC. In a similar manner to immunoblot analysis, IHC staining showed that IR expression in FFPE samples was inversely correlated with Fuhrman nuclear grade (Fig. 2C and D). IR was positive in 76.5% of patients without diabetes and 68.2% of patients with diabetes. IR was positive in 74.8% of cases lacking sarcomatoid feature and 80.0% of cases with sarcomatoid feature. Furthermore, IR was positive in 77.8% of cases without tumor

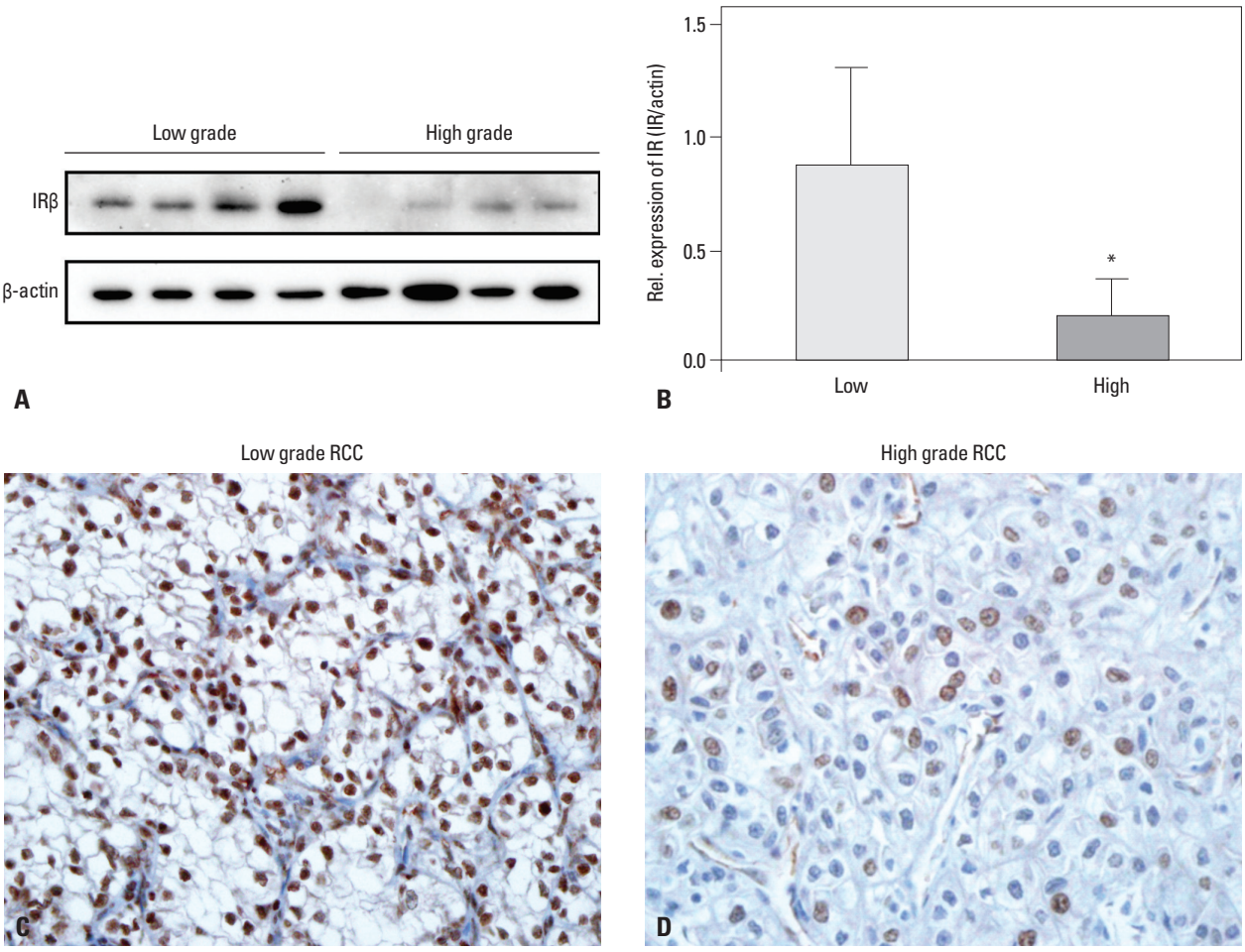


**Fig. 2.** Expression level of insulin receptor (IR) in clear cell renal cell carcinoma (CCRCC) is associated with tumor grade. (A) Representative immunoblotting of IR in the low and high grade CCRCC tissues. (B) Summary of results in (A) (mean±SEM, n=4). Asterisk denotes $p<0.05$ high versus low grade. (C and D) The IHC staining for IR from low (A) and high nuclear grade (B) CCRCC. IHC, immunohistochemistry; RCC, renal cell carcinoma; SEM, standard error of mean.

necrosis and 64% of cases with tumor necrosis. With respect to the rhabdoid component, IR positivity was seen in 76.3% of cases without rhabdoid feature and 60% of cases with rhabdoid feature (Table 2). IR was positive in 83.1% of cases with a low (1+2) Fuhrman nuclear grade and 64.2% of cases with a high (3+4) Fuhrman nuclear grade, which was statistically significant (Fig. 2C and D, Table 2). IR was positive in 77.1% of cases without perirenal fat invasion and 60% of cases with perirenal fat invasion. 74.8% of cases lacking renal pelvis invasion were IR positive, as were 77.8% of cases with renal pelvis invasion. IR was found to be positive in 77.5% of cases without vascular invasion and 53.8% of cases with vascular invasion (Table 2). Although IR expression seemed to be higher in cases without vascular invasion, this difference was not statistically significant. IR was also expressed in 74.8% of cases without renal sinus fat invasion and 80.0% of cases with renal sinus fat invasion. IR was positive in 87.9% of cases with cystic change and 70.3% of cases without cystic change. This difference in IR expression was statistically significant (Table 2). IR was expressed in 79.8% of cases with pathologic T stage 1 and 62.9% of stage 2–4 cases, which was again a statistically significant difference (Table 2). IR was expressed in 80.2% of cases of TNM stage I and 63.2% of cases with TNM stages II–IV. IR expression seemed be to higher in low TNM stage tumors as compared to high TNM stage tumors, although this was not a statistically significant finding.

We also compared the staining score of each group to overcome the potential limitations with respect to quantification in IHC assays. The mean staining score of IR was 5.45±1.79 in cases with cystic change and 4.52±1.90 in cases without cystic change, which was a statistically significant difference (Table 3). With respect to low and high Fuhrman nuclear grades, a statistically significant difference ($p$=0.002) was seen between the two groups with mean staining scores of 5.21±1.98 and 4.17±1.65 for low and high grades, respectively. For pathologic T stage, the mean staining score was 4.97±1.93 in cases with stage 1

**Table 2.** Correlation of Insulin Receptor Expression and Clinico-Pathological Parameters of Clear Cell Renal Cell Carcinoma

| Parameters | Insulin receptor expression | | *p* value |
|---|---|---|---|
| | No. of positive cases (%) | No. of negative cases (%) | |
| Diabetes | | | |
| Absent | 78 (76.5) | 24 (23.5) | 0.287 |
| Present | 15 (68.2) | 7 (31.8) | |
| Sarcomatoid feature | | | |
| Absent | 89 (74.8) | 30 (25.2) | 0.683 |
| Present | 4 (80.0) | 1 (20.0) | |
| Tumor necrosis | | | |
| Absent | 77 (77.8) | 22 (22.2) | 0.124 |
| Present | 16 (64.0) | 9 (36.0) | |
| Rhabdoid feature | | | |
| Absent | 87 (76.3) | 27 (23.7) | 0.217 |
| Present | 6 (60.0) | 4 (40.0) | |
| Fuhrman nuclear grade | | | |
| Low (1+2) | 59 (83.1) | 12 (16.9) | 0.014 |
| High (3+4) | 34 (64.2) | 19 (35.8) | |
| Perirenal fat invasion | | | |
| Absent | 84 (77.1) | 25 (22.9) | 0.134 |
| Present | 9 (60.0) | 6 (40.0) | |
| Renal pelvis invasion | | | |
| Absent | 86 (74.8) | 29 (25.2) | 0.601 |
| Present | 7 (77.8) | 2 (22.2) | |
| Vascular invasion | | | |
| Absent | 86 (77.5) | 25 (22.5) | 0.069 |
| Present | 7 (53.8) | 6 (46.2) | |
| Renal sinus fat invasion | | | |
| Absent | 89 (74.8) | 30 (25.2) | 0.633 |
| Present | 4 (80.0) | 1 (20.0) | |
| Cystic change | | | |
| Present | 29 (87.9) | 4 (12.1) | 0.035 |
| Absent | 64 (70.3) | 27 (29.7) | |
| Pathologic T stage | | | |
| 1 | 71 (79.8) | 18 (20.2) | 0.044 |
| 2–4 | 22 (62.9) | 13 (37.1) | |
| TNM stage | | | |
| I | 69 (80.2) | 17 (19.8) | 0.083 |
| II–IV | 24 (63.2) | 14 (36.8) | |

and 4.26±1.79 in cases with stages 2–4. Although higher IR expression was correlated with lower pathologic T stage, this was not a significant difference (Table 3). A statistically significant difference ($p$=0.042) was seen between the mean staining scores of TNM stage I and TNM stages II–IV (4.99±1.95 vs. 4.26±1.74). Survival analysis was performed after adjusting for sex, age, Fuhrman nuclear grade, cystic change status, and pathologic T stage. There was no significant difference between IR positive group and IR negative group in both survival ($p$=0.834) and recurrence rate ($p$=0.691) (Fig. 3).

## DISCUSSION

Prognosis of RCC can be predicted using several well-known prognostic factors. The TNM staging system is the most reliable prognostic factor and takes into account tumor size and the extent of the tumor.[24] The Fuhrman nuclear grading system, an independent prognostic factor based on nuclear and nucleolar morphology, is divided into grades 1 to 4.[25] Pathologic poor prognostic factors include the presence of tumor necrosis, sarcomatoid and rhabdoid fea-

tures, vascular, perirenal fat, renal pelvis, and renal sinus fat invasions.[29] By contrast, cystic change in RCC is a known favorable prognostic factor in predicting survival.[30] We found that IR expression was present in 109 cases (87.9%) of CCRCC. Higher IR expression was significantly related to the presence of cystic change, lower Fuhrman nuclear grade, lower pathologic T stage, and lower TNM stage. Additionally, the results of western blot analyses showed that the expression of IR was higher in low-grade CCRCC than in high-grade tumors. However, there was no difference in IR expression between CCRCC and adjacent normal tissues. Therefore, we suggest that high IR expression may be associated with the favorable prognostic factors of CCRCC. In this study, our survival analysis did not show significant results. This discrepancy may be explained by the fact that a few patients who underwent radical nephrectomy died, while the patients diagnosed with metastatic RCC did not undergo surgery.

IR exists in two isoforms, A and B, which are formed due to exclusion (isoform A) or inclusion (isoform B) of exon 11 of the IR gene.[17] IR-A is ubiquitously expressed, whereas IR-B is expressed largely in the classically insulin-sensitive tissues of liver, skeletal muscle, and adipose tissue. Interestingly, IR-B is also expressed abundantly in the kidney.[31] In the current study, we demonstrated diffuse IR staining in distal tubular epithelium as well as in podocytes of glomeruli. Therefore, we can conclude that the kidney is an insulin sensitive organ. In healthy individuals, blood glucose concentration is maintained by a state of balance between insulin production by specialized pancreatic β cells and insulin-mediated glucose uptake in target tissues, which is promoted by the glucose transporter type 4 (GLUT-4), to the cell surface.[32] Insulin resistance in classic insulin-target organs and the related hyperglycemia and hyperinsulinemia are pathologic hallmarks of metabolic disorders such as obesity and type 2 diabetes.[6] Several studies have reported an association between type 2 diabetes and an increase in risk of developing various human cancers, including liver, pancreas, bladder, breast, and colon.[7-12] However, the association between RCC, and type 2 diabetes and/or obesity remains controversial. In a meta-analysis of nine cohort studies by Larsson and Wolk[7] diabetes is associated with a 42% increased risk of kidney cancer. This association was found to be stronger in women than in men. In contrast, a study by Höfner, et al.[33] showed that obesity and type 2 diabetes have no

**Table 3.** Correlation of Mean Staining Score of Insulin Receptor Expression and the Major Clinico-Pathological Parameters of Clear Cell Renal Cell Carcinoma

| | Insulin receptor expression | |
|---|---|---|
| | Mean±SD | *p* value |
| Cystic change | | |
| Present | 5.45±1.79 | 0.014 |
| Absent | 4.52±1.90 | |
| Fuhrman nuclear grade | | |
| Low grade (1+2) | 5.21±1.98 | 0.002 |
| High grade (3+4) | 4.17±1.65 | |
| Pathologic T stage | | |
| 1 | 4.97±1.93 | 0.056 |
| 2–4 | 4.26±1.79 | |
| TNM stage | | |
| I | 4.99±1.95 | 0.042 |
| II–IV | 4.26±1.74 | |

SD, standard deviation.

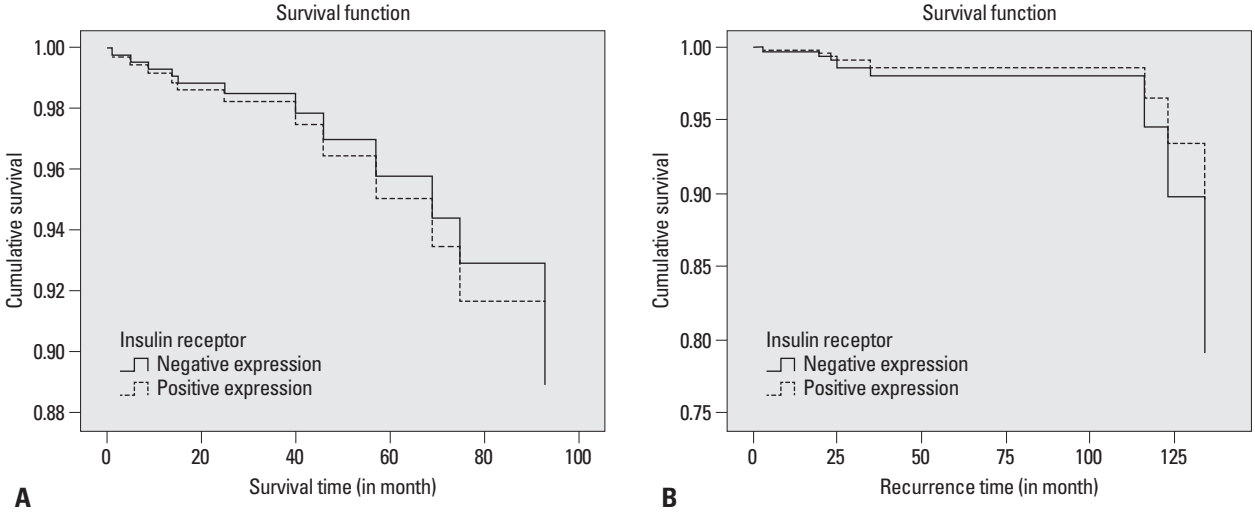


**Fig. 3.** Association between insulin receptor (IR) expression level and clinical outcome in clear cell renal cell carcinoma. Results of survival analysis shows no significant difference between the IR positive and IR negative groups in both survival (A) and recurrence rates (B). Survival analysis was determined using the Cox regression method after adjusting for sex, age, Fuhrman nuclear grade, cystic change status, and pathologic T stage.

significant effect on cancer-specific and recurrence-free survival in RCC patients who had undergone nephrectomy for localized RCC. In the present study, IR expression was not related to the diabetes status of patients with CCRCC supporting the notion that type 2 diabetes and/or obesity may not be associated with CCRCC development.

The IGF-1 pathway plays a crucial role in most cancer cell development and the overexpression of IGF-1R is one of the important factors of cancer hallmarks. Patients with IGF-1R positive CCRCC had poorer outcomes than patients with IGF-1R negative CCRCC.[21,34] However, high IR expression is correlated with more favorable prognostic factors of CCRCC in our study. Reasons for these differences between IGF-1R and IR are not well known. Indeed, although both IR and IGF-1R share major down-stream signaling pathways, there are several specific substrates for each receptor. For instance, pp120, a plasma membrane glycoprotein expressed by hepatocytes and a substrate of the IR tyrosine kinase, mediates the phosphorylation of IR but not the phosphorylation of IGF-1.[35] Similarly, mitotic arrest deficient 2, a cell cycle checkpoint regulator, binds to the C-terminal domain of IR but does not bind to the homologous region in IGF-1R.[36] It is also conceivable that IR and IGF-1R differently regulates downstream targets such as von Hippel-Lindau (VHL) acting on hypoxia-inducible factor-1α (HIF-1α). Hereditary RCC is commonly associated with mutational inactivation of *VHL* gene which plays an important role in tumor growth.[37] VHL protein is E3-ubiquitin ligase and functions as a tumor suppressor by inhibiting HIF-1α which is activated by hypoxia. VHL-mediated HIF-1α regulation is a major pathway involved in RCC biology and tumorigenesis.[38] IGF-1R activates HIF-1α independent of oxygen status by suppressing VHL that induces RCC development. Interestingly, IR signaling and hypoxia share common target genes, but HIF-1α is unique to hypoxia.[39] Whether the regulation of VHL-HIF-1α pathway by IR and IGF-1R has any role in the CCRCC biology and tumorigenesis awaits future investigation. Moreover, dissecting of IGF-1R and IR signaling cascades in RCC may provide clues for treatment or prognosis of CCRCC.

It has been reported that nuclear IGF-1R is detectable in primary RCC cultures, as well as in FFPE tissue from RCC and that this nuclear IGF-1R is associated with an adverse prognosis for CCRCC.[40] On the other hand, several studies reported that high IGF-1R expression was associated with better survival in malignancies of breast, lung and soft tissue.[41-43] Indeed, it has been shown that full-length IGF-1R translocates into the nucleus following activation by its ligands[40] and SUMOylation mediates this nuclear translocation of IGF-1R.[44] IR can be also translocated to the nucleus to regulate cell proliferation as well as IGF-1R.[45] Overexpression of IR is predictive of poor survival in patients with lung cancer.[46] In our IHC staining, IR was mainly expressed in the nuclei of tumor cell that was tumor grade-dependent manner supporting the notion that high IR expression was associated with the favorable prognostic factors of CCRCC.

In conclusion, IR expression was studied in CCRCC and its expression was compared with clinico-pathological parameters and survival data. We demonstrated that higher nuclear IR expression was significantly correlated with favorable prognostic factors of CCRCC. To the best of our knowledge, this is the first study to focus on IR expression in CCRCC patients with known diabetes status and prognosis. Caveats to our study include our small study sample size and the fact that we only performed analysis at the level of IR protein expression.

## ACKNOWLEDGEMENTS

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (NRF-2012R1A1A1004233 to M.E.) and the Ministry of Education (NRF-2010-0024789 to S.-K.C). We would like to thank Mr. Joong Seob Kim and Mr. Tae-Young Kang for their technical support.

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