약학석사학위논문

Increased serum level of GRS antigen as a novel diagnostic biomarker for renal cell carcinoma

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혈청 GRS 항원 수치의 증가

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ABSTRACT

Background: There is a lack of reliable and specific markers for the diagnosis of early stage renal cell carcinoma (RCC). In renal cell carcinoma, CDH 6 expression level is increased. Furthermore, in our previous study, we founded that CDH 6 is a functional receptor for GRS (Glycyl-tRNA synthetase). This study observed level of serum GRS antigen and antibody as detection biomarkers in renal cell carcinoma.

Methods: Serum samples from patients with renal cell carcinoma were examined for serum GRS antigen and antibody level by an enzyme-linked immunosorbent assay (ELISA). The receiver operating characteristic (ROC) curves were used to determine area under the curve (AUC), sensitivity, specificity, and cut-off value for prediction of RCC.

Results: The level of serum GRS antigen was significantly higher in RCC patients than in normal human serum (NHS) (RCC 34.11 ± 9.76 ug/ml; NHS 19.22 ± 6.06 ug/ml; $p < 0.0001$). The AUC for GRS antigen was 0.900 (95% CI = 0.840-0.960, $p < 0.0001$). The sensitivity and specificity for GRS antigen detection method were 91.0 % and 75.0 %, respectively, when cut-off value of GRS antigen was 22.86 ug/ml.

Conclusions: Serum antigen level of GRS was significantly associated with RCC. GRS antigen may be a good novel biomarker or drug target for renal cell carcinoma.

Key words: Glycyl-tRNA synthetase (GRS), Renal cell carcinoma (RCC), CDH 6 (K-cadherin), biomarker, Enzyme-linked immunosorbent assay (ELISA)

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INTRODUCTION

Kidney cancer consists of RCC (Renal Cell Carcinoma) and UCC (Urothelial Cell Carcinoma). RCC is a tumor which originates in the lining of the proximal convoluted tubule and accounts for nearly 80 % of kidney cancers. RCC is the most common type of kidney cancer. Kidney cancer is 64,770 new cases and 13,570 deaths in the United States in 2012. (1) RCC is divided into five types: clear cell RCC, papillary RCC, chromophobe RCC, collecting duct carcinoma, and unclassified which are based on cytologic appearance and cytogenetic aberrations. (2) RCC has no signs, symptoms and reliable RCC specific diagnostic methods, so it is difficult to find RCC in early stage. Indeed, many RCC patients are diagnosed RCC in the advanced metastatic stage. For these reasons, need of RCC biomarker discovery is increasingly emphasized.

The aminoacyl-tRNA synthetases (aaRSs) are ubiquitoulsy expressed and essential molecules for protein synthesis. The aaRSs attach each amino acid to their cognate tRNAs in the cytoplasm or mitochondria, then charged tRNAs is used for ribosomal protein synthesis in the cytoplasm. (3) The aaRSs are classified into two classes: class Ⅰ and class Ⅱ based on their structural characteristics. (4-6) Mammalian aaRSs contain additional domains and motifs such as glutathione S-transferase (GST), WHEP domains,

leucine zipper motifs, and α-helical appendices compared to prokaryotic counterparts. (7) These extra domains allow mammalian aaRSs to have noncanonical functions which include angiogenesis, immune response, and apoptosis. (8, 9) Various aaRSs are related to signaling pathways which are linked to the control of tumorigenesis. To be specific, lysyl-tRNA synthetase (KRS) plays a role for a signaling molecule in the cancer microenvironment. In tumor condition, TNF-α induces full-length KRS secretion, and secreted KRS activates macrophage and increases TNF-α secretion through ERK and p38 MAPK activation. Secreted TNF-α inhibits cell proliferation and increases cell death, so KRS-induced TNF-α secretion activates a cancer cell proliferative signal. (10) Several aaRSs also function as secreted cytokines and are linked to angiogenesis and immune response in cancer microenvironment. For example, secreted and truncated Tyrosyl- and Tryptophanyl-tRNA synthetase (YRS and WRS) are related to angiogenesis in tumor condition. (11, 12) Cleavaged YRS and WRS by alternative splicing or proteolysis bind to extracellular receptors. (13, 14) These observations suggest that there are some specific aaRSs in human serum. Anti-synthetase syndrome (ASS) is the disease producing autoantibodies to aminoacyl-tRNA synthetases (anti-aaRS autoantibodies) such as myositis, arthritis, interstitial lung disease (ILD), Raynaud's phenomenon, Mechanic's hands, fever, interstitial pneumonia and systemic lupus erythematosus (SLE). (15, 16)

There are eight anti-ARS autoantibodies: anti-HRS (anti-Jo-1) (17), anti-TRS (anti-PL-7) (18), anti-ARS (anti-PL-12) (19), anti-IRS (anti-OJ) (20), anti-GRS (anti-EJ) (20), anti-NRS (anti-KS) (15), anti-FRS (anti-Zo) (16), and anti-YRS autoantibody. These observations are able to explain that eight aaRSs circulate as antigens in human serum having specific ex-translational functions. (21, 22)

The glycyl-tRNA synthetase (GRS) is one of the 20 aaRSs. According to the recent research, in tumor condition, GRS is secreted from macrophage and binds to cadherin 6 (CDH 6, K-cadherin). CDH 6 bound GRS has an anti-tumor effect through ERK dephosphorylation. (23) CDH 6 also called K-cadherin is a member of transmembrane glycoprotein families. CDH 6 is a Ca^{2+}-dependent cell-cell adhesion molecule and a specific tissue marker for renal proximal tubule cancer. (24-27)

In this study, serum level of GRS and GRS autoantibody were measured in RCC patient serum and normal human serum (NHS). In addition, level of other aaRS antigens and autoantibodies in the serum of RCC patients and normal human subjects were also measured. According to the previous experiments, GRS is secreted from macrophage and secreted GRS has an anti-tumorigenic effect in tumor condition. These observations suggest that GRS has potential for a RCC biomarker or targeted drug therapy.

MATERIALS AND METHODS

Serum samples

RCC patient sera 100 cases and normal sera 20 cases were used in this study. RCC patient sera were obtained from the Samsung Medical Center. The normal sera were obtained from BioChemed Services. The sera were stored at -80℃ until use.

Gene Cloning

The full-length GRS gene was amplified by PCR with GRS specific primers (forward primer; 5'-GGAATTCCATATGATGGACGGCGCGGGGGCTGAG-3', reverse primer; 5'-CCCTCGAGTTCATTCCTCGATTGTCTCTTTTTTAC-3'). The amplified PCR product was digested with NdeI and XhoI and inserted into pET-28a vector. The constructed GRS plasmid was transformed into BL21 (DE3) *E.coli*, which produces 6x histidine tagged recombinant GRS protein at N-terminal. The recombinant plasmid was confirmed by double restriction enzyme digestion and DNA sequencing.

Expression and Purification of recombinant GRS protein

The day before a single colony was inoculated in 3 ml LB/kanamycin and

incubated at 37 ℃ in shaking incubator overnight. Next morning 3 ml LB/kanamycin *E.coli* was scaled up to 1 L LB/kanamycin and incubated at 37 ℃ in shaking incubator until OD_{600} value is 0.4~0.6. And *E.coli* was induced by 1 mM Isopropyl-β-D-Thiogalactopyranoside (IPTG) at 30 ℃ for 6 hour in shaking incubator. Overexpressed *E.coli* was harvested by centrifugation at 4000 rpm for 10 min and supernatant was discarded. The *E.coli* cell pellet was resuspended in pH 7.8 buffer (20 mM KH2PO4, 500 mM NaCl, 10% glycerol, 2 mM β-mercaptoethanol) and sonicated (SONICS & MATERIALS, INC.; VCX 750) at 30 % amplitude for 3 seconds on and 30 seconds rest 10 times on ice. The crude lysate was centrifuged at 10000 rpm for 30 min at 4 ℃ and supernatant was collected. The supernatana was filtered by 0.45 um PVDF membrane filter.

Overexpressed recombinant GRS protein was purified using open column (Bio-rad; 731-1550) and Ni-NTA Agarose resin (Invitrogen™; R901-15). 800 ul of Ni-NTA agarose resin was packed into open column and equilibrated with pH 7.8 buffer. After equilibration, E.coli cell lysate was poured into an open column to bind his-tagged recombinant GRS protein with Ni-NTA agarose resin. After column binding, column was washed by pH 7.8, pH 6.0, pH 5.2, and 20 mM Imidazole in pH 6.0 buffer. Recombinant GRS protein was eluted with 300 mM Imidazole in pH 6.0 buffer. The elution fractions

were analysed by a SDS-PAGE gel and coomassie staining.

The elution fractions were dialyzed with dialysis buffer (1X PBS, 15 % glycerol, 1 mM β-mercaptoethanol) using dialysis tubing for 4 hours. Then dialysis buffer was changed and protein sample was dialyzed for 8 hours. After dialysis, protein sample was analyzed concentration and purity using Bradford assay and SDS-PAGE gel. Recombinant GRS protein was purified >90 % purity for an ELISA.

Enzyme-linked immunosorbent Assays (ELISAs)

GRS antibody detection ELISA: Purified recombinant GRS protein was diluted in phosphate-buffered saline (PBS) to a final concentration of 4.0 ug/ml. Diluted GRS protein 100 ul was coated onto the 96 well plate (Nunc; 468667) and incubated overnight at room temperature. The plate was drained and tapped on a paper towel to remove excess liquid. The plate was blocked with 200 ul blocking solution (5 % BSA in PBS) for 2 hours at 37 ℃. Then GRS polyclonal antibody standard solution and diluted serum samples were incubated for 1 hour at 37 ℃. And the plate was washed by 200 ul PBST (PBS buffer containing 0.2 % Tween20) five times and incubated with diluted HRP-conjugated secondary antibody (anti-Rabbit; Thermo; 31460, anti-Human; Thermo; SA1-72039) for 1 hour at 37 ℃. And the plate was washed by 200 ul PBST five times and the

100 ul of 3,4,5-trimethoxy benzaldehyde (TMB) reagent was used to detect color change at room temperature. After 20 min incubation, 50 ul of 1 M Sulfuric acid (H_2SO_4) solution was used as stop solution. The OD value was read at 450 nm and at 620 nm reference wavelength.

GRS antigen detection ELISA: Purified GRS polyclonal IgG (rabbit) was diluted in phosphate-buffered saline (PBS) to a final concentration of 4.0 ug/ml. Diluted GRS polyclonal IgG 100 ul was coated onto the 96 well plate (Nunc; 468667) and incubated overnight at room temperature. The plate was drained and tapped on a paper towel to remove excess liquid. The plate was blocked with 200 ul blocking solution (5 % BSA in PBS) for 2 hours at 37 ℃. Then GRS antigen standard solution and diluted serum samples were incubated for 1 hour at 37 ℃. And the plate was washed by 200 ul PBST (PBS buffer containing 0.2% Tween20) four times and incubated with 100 ul of 20 pmol GRS aptamer in PBS per well for 1 hour at 37 ℃. The plate was washed by 200 ul PBST four times and incubated with diluted HRP-conjugated streptavidin (1:500) for 1 hour at 37 ℃. And the plate was washed by 200 ul PBST four times and the 100 ul of 3,4,5-trimethoxy benzaldehyde (TMB) reagent was used to detect color change at room temperature. After 20 min incubation, 50 ul of 1M Sulfuric acid (H_2SO_4) solution was used as stop solution. The OD value was read at 450 nm and at 620 nm reference

wavelength.

Statistical analysis

All statistical analysis was done with the IBM SPSS Statistics 19 software in this study. Statistical analysis was performed using the Mann-Whitney U test and Kruskal Wallis test. The level of aaRS antigens and antibodies between RCC and NHS group was compared using the nonparametric Mann-Whitney U test, because the sample size of NHS group was too small (n=20) to use a parametric statistical method. The clinical characteristics according to the stage of RCC were compared by performing Kruskal Wallis test. The level of aaRS antigens and antibodies according to sex in NHS and RCC group was compared by Mann-whitney U test. Receiver operating characteristic (ROC) curves were used to determine the area under the curve (AUC), sensitivity, specificity, and cut-off value for prediction of RCC. All *p*-values were two-sided and less than 0.05 were considered statistically significant in this study.

RESULTS

Patient's characteristics

In this study, total 120 sera (RCC patient serum 100 cases and normal serum 20 cases) were used to identify the level of aaRS antibodies and antigens. In 100 RCC patients, 73 patients were male and 27 patients were female. In 20 normal human subjects, male and female group were collected 10 cases each. The mean age ± S.D. of RCC group was 54.88 ± 11.71 years and of NHS group was 44.05 ± 11.71 years. The patients' tumor size was selected the largest tumor measurement. Average tumor size of RCC group was 4.95 ± 2.71 cm. RCC patients were categorized into five groups according to the Fuhrman stage: stage Ⅰ, Ⅱ, Ⅲ, Ⅳ, and uncategorized. In RCC patient, 2 patients were stage Ⅰ, 21 patients were stage Ⅱ, 62 patients were stage Ⅲ, 12 patients were stage Ⅳ, and 3 patients were uncategorized. RCC is classified five types based on the cytologic appearance and cytogenetic aberrations: papillary, clear cell, chromophobe, translocation, and uncategorized. In RCC group, 4 patients were papillary, 69 patients were clear cell, 8 patients were chromophobe, 1 patient was translocation, and 18 patients were uncategorized type (Table 1).

Prevalence of serum antibodies and antigens to aaRSs in Renal Cell Carcinoma by ELISA assay

The serum level of GRS antibody, GRS antigen, WRS antibody, and KRS antigen was measured by ELISA assay. Level of GRS antigen and WRS antibody in RCC patient serum was higher than in NHS (p<0.0001; p=0.0007). On the other hand, KRS antigen level of NHS was higher than that of RCC patient serum (p<0.0001). However, there were no significant differences in the level of GRS antibody (p=0.2844) between RCC and NHS group (Figure 2, Table 2).

To compare RCC patients' clinical characteristics according to the RCC stage, the Kruskal Wallis test was used. There were no significant differences in the level of GRS antibody, GRS antigen, WRS antibody, and KRS antigen according to the stage in RCC patients (p=0.3686; p=0.2247; p=0.9768; p=0.3555). On the contrary, there was a significant difference in the tumor size according to the stage in RCC group (p=0.0140) (Figure 3, Table 3). In the post-hoc analysis of the tumor size, there were only significant differences in the between stage Ⅱ and Ⅳ, and stage Ⅲ and Ⅳ (p=0.0047; p=0.0216) (data are not shown).

To identify whether gender is confounding factor in this study, level of aaRS antibodies and antigens was compared according to the sex in both RCC and NHS group

by Mann-Whitney U test. In NHS group, there were no significant differences in the level of GRS antibody (p=0.1655), GRS antigen (p=0.0630), WRS antibody (p=0.5787), and KRS antigen (p=0.0630) between male and female (Figure 4, Table 4). In RCC patient group, clinical characteristics such as tumor size, GRS antibody, GRS antigen, WRS antibody, and KRS antigen also have no significant differences between male and female (p=0.4324; p=0.8644; p=0.3091; p=0.0898; p=0.8052) (Figure 5, Table 5).

Evaluation of diagnostic values of aaRS antibodies and antigens in immunodiagnosis of Renal Cell Carcinoma

ROC curves were plotted to calculate an AUC and determine a cut-off value of each of aaRS antibodies and antigens. According to the ROC curve, the AUC for GRS antigen was 0.900 (95% CI = 0.840-0.960, $p < 0.0001$) (Figure 6B) and the AUC for WRS antibody was 0.740 (95% CI = 0.633-0.847, $p = 0.0007$) (Figure 6C). However, AUC value of GRS antibody and KRS antigen was less than 0.5 and not statistically significant (GRS antibody, AUC = 0.424, 95% CI = 0.282-0.566, $p < 0.2845$; KRS antigen, AUC = 0.211, 95% CI = 0.104-0.317, $p < 0.0001$) (Figure 6A; Figure 6D). Cut-off value of GRS antigen detection ELISA method was 22.86 ug/ml. Furthermore, sensitivity and specificity of GRS antigen detection ELISA method were 91.0 % and

75.0 % respectively. In addition, cut-off value of WRS antibody detection ELISA method was 7.19 U/ml. Sensitivity and specificity of WRS antibody detection ELISA method were 84.0 % and 45.0 % respectively.

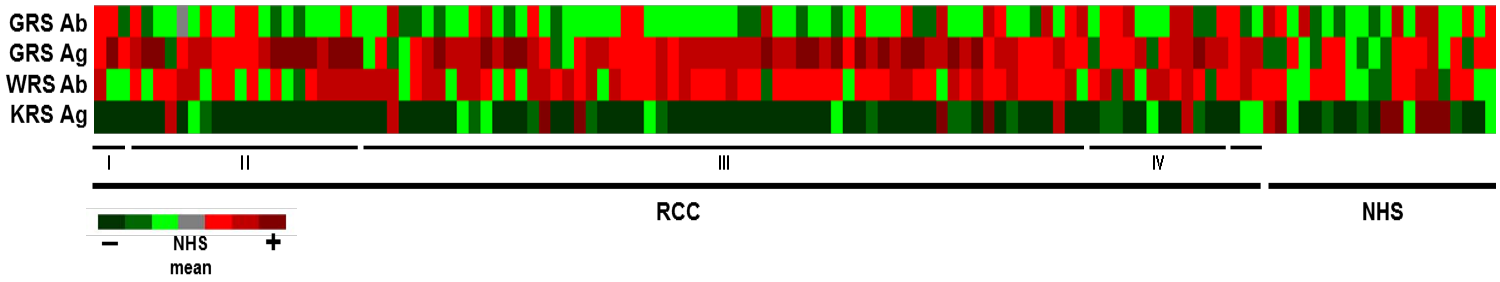

Figure 1. Cluster diagram of serum profiles of GRS antibody, GRS antigen, WRS antibody, and KRS antigen in RCC and NHS

Clustering of serum profile was performed for level of GRS antibody, GRS antigen, WRS antibody, and KRS antigen based on mean level of NHS. The green color means that level of RCC sera is lower than that of NHS average, and the red color means that level of RCC sera is higher than that of NHS average. The darker the color is the higher or lower the serum level is. The diagram depicts that the level of GRS antibody and KRS antigen is lower than normal serum level. On the other hand, the level of WRS antibody and GRS antigen is higher than normal level. RCC, Renal Cell Carcinoma; NHS, Normal Human Serum.

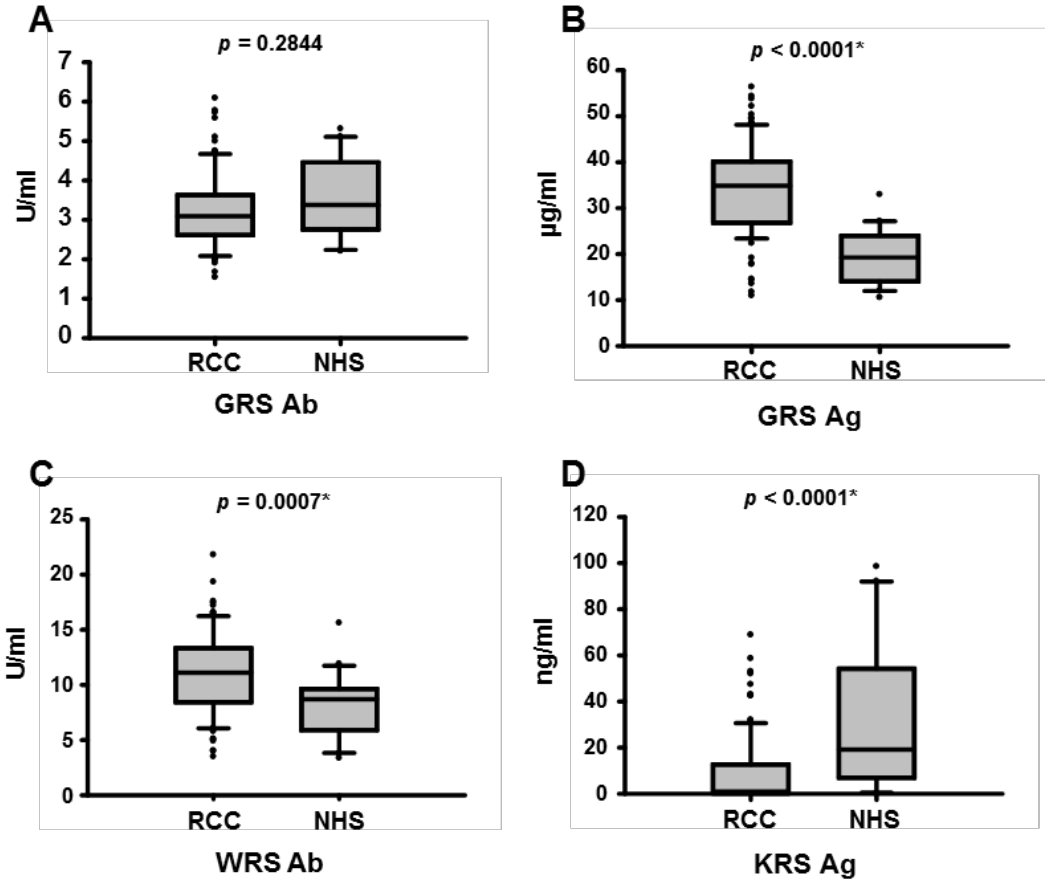

Figure 2. Comparison of level for aaRS antibodies and antigens between RCC and NHS

Level of GRS antibody, GRS antigen, WRS antibody, and KRS antigen in RCC patients (n=100) and NHS (n=20) is described by box-and-whiskers plot. The median, range (whiskers), 25th to 75th percentile (box) and outliers (circles) are shown. *p*-values were calculated using the Mann-Whitney U test and less than 0.05 were considered statistically significant. (A) GRS antibody. (B) GRS antigen. (C) WRS antibody. (D) KRS

antigen. 1 Unit is defined as GRS binding activity which corresponds to the binding activity of 100 µl maximum concentration of standard (GRS polyclonal antibody 1.6 ug/ml; WRS polyclonal antibody 1.6 ug/ml).

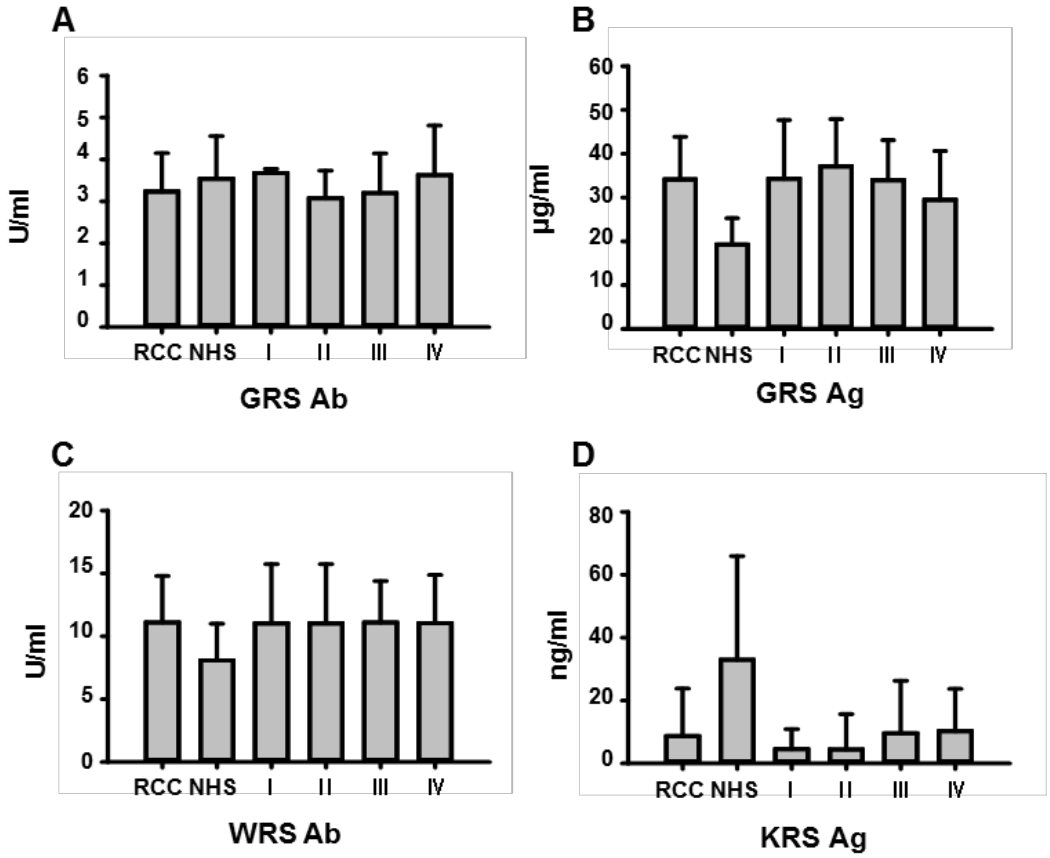

Figure 3. Comparison of level of aaRS antibodies and antigens according to the stage

Level of GRS antibody, GRS antigen, WRS antibody, and KRS antigen in RCC patients (n=100) and NHS (n=20) according to the stage is described by bar graph. Error bar shows standard deviation (SD). (A) GRS antibody. (B) GRS antigen. (C) WRS antibody. (D) KRS antigen.

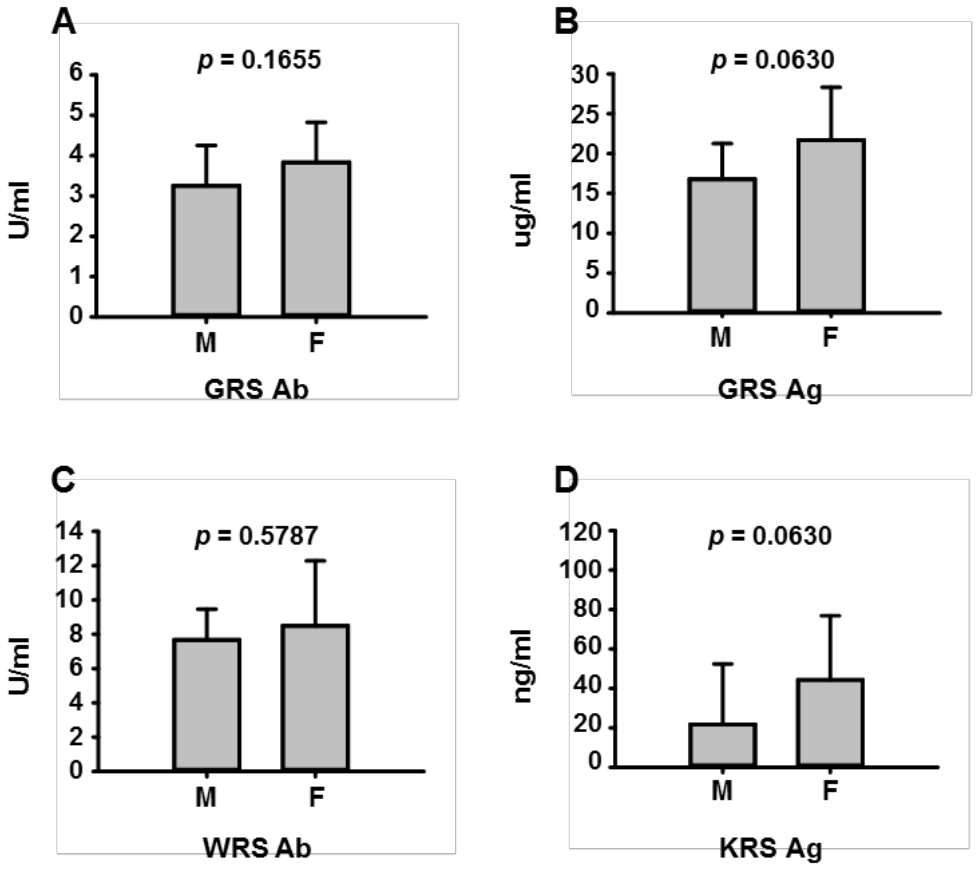

Figure 4. Level of aaRS antibodies and antigens between Male and Female in NHS

Level of GRS antibody, GRS antigen, WRS antibody, and KRS antigen in NHS samples (n=20) according to the sex (Male, n=10; Female, n=10) is described by bar graph. *p*-values were calculated using the Mann-Whitney U test and less than 0.05 were considered statistically significant. Error bar shows standard deviation (SD). (A) GRS antibody. (B) GRS antigen. (C) WRS antibody. (D) KRS antigen.

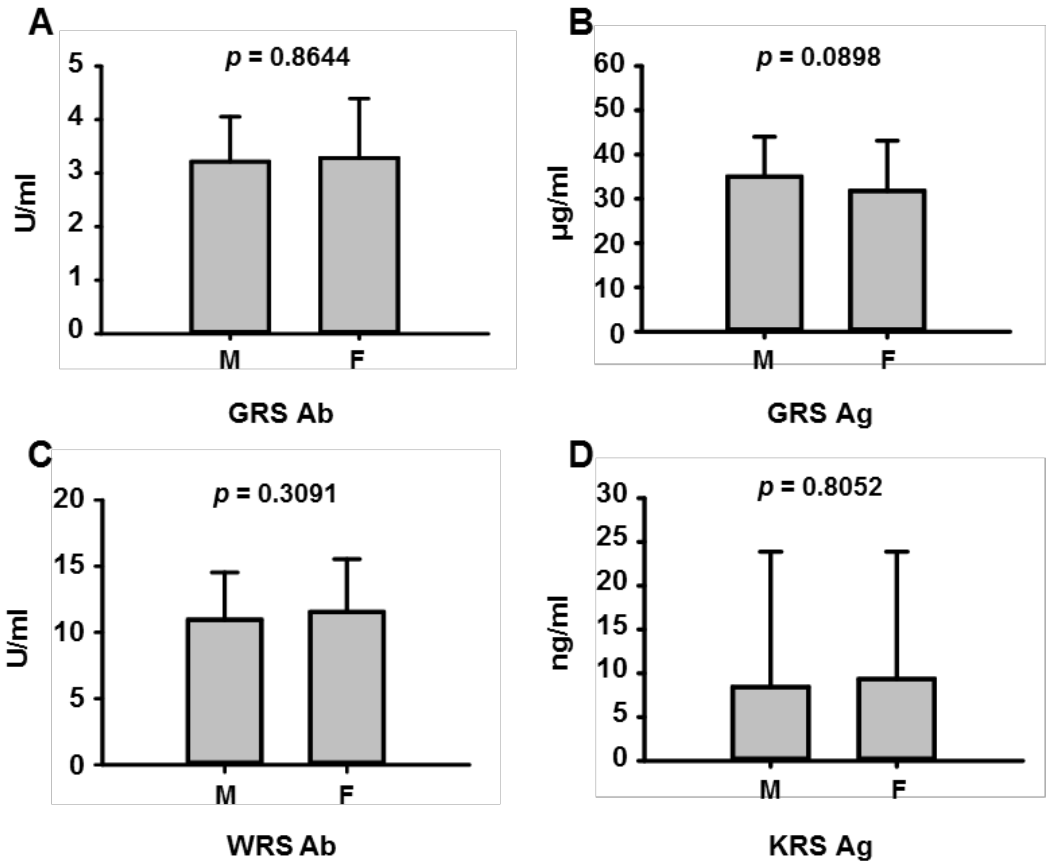

Figure 5. Comparison of level of aaRS antibodies and antigens between Male and Female in RCC

Level of GRS antibody, GRS antigen, WRS antibody, and KRS antigen in RCC patients (n=100) according to the sex (Male, n=73; Female, n=27) is described by bar graph. *p*-values were calculated using the Mann-Whitney U test and less than 0.05 was considered statistically significant. Error bar shows standard deviation (SD). (A) GRS antibody. (B) GRS antigen. (C) WRS antibody. (D) KRS antigen.

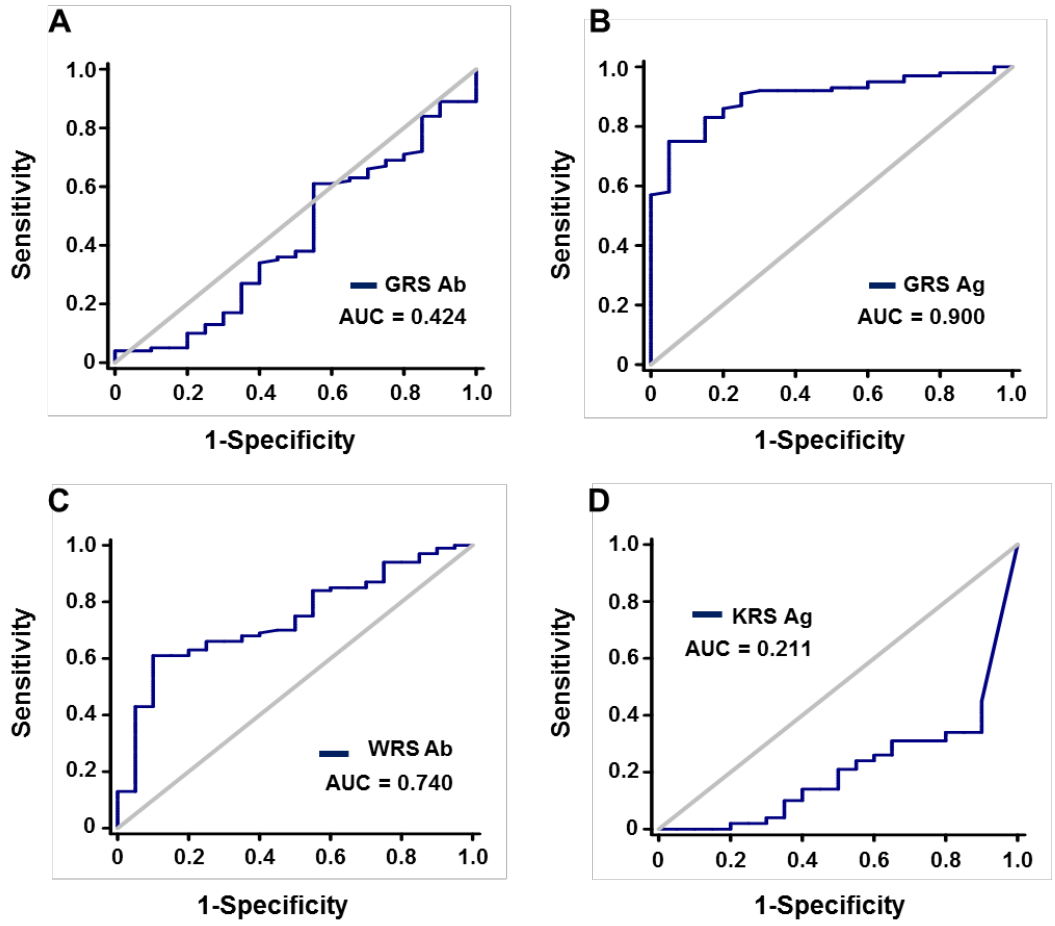

Figure 6. Receiver Operating Characteristic (ROC) curves

ROC curves were calculated for GRS antibody, GRS antigen, WRS antibody, and KRS antigen in RCC sera 100 cases and NHS 20 cases. AUC more than 0.5 and *p*-values less than 0.05 were considered statistically significant. (A) GRS antibody. (B) GRS antigen. (C) WRS antibody. (D) KRS antigen.

Table 1. Clinical and pathological information of RCC patients and NHS

		RCC	NHS
		No.	No.
Total		100	20
Sex	Male	73	10
	Female	27	10
Age (years)		54.88 ± 11.71	44.05 ± 11.71
Tumor size (cm)		4.95 ± 2.71	–
Fuhrman stage	Ⅰ	2	–
	Ⅱ	21	–
	Ⅲ	62	–
	Ⅳ	12	–
	uncategorized	3	–
Classification	papillary	4	–
	clear cell	69	–
	chromophobe	8	–
	translocation	1	–
	uncategorized	18	–

Values are presented mean±SD.
RCC, Renal cell carcinoma; NHS, Normal human serum

Table 1. Clinical and pathological information of RCC patients and NHS

Table 2. Comparison of level for aaRS antibodies and antigens between RCC and NHS

	RCC (n=100)	NHS (n=20)	*p*-value[1)]
GRS Ab (U/ml)	3.23 ± 0.91	3.54 ± 1.02	0.2844
GRS Ag (ug/ml)	34.11 ± 9.76	19.22 ± 6.06	<0.0001
WRS Ab (U/ml)	11.10 ± 3.69	8.07 ± 2.93	0.0007
KRS Ag (ng/ml)	8.66 ± 15.16	32.96 ± 32.96	<0.0001

Values are presented mean±SD.
1) *p* -values were calculated using the Mann-Whitney U test.

Table 2. Comparison of level for aaRS antibodies and antigens between RCC and NHS

Table 3. Comparison of clinical characteristics according to the stage of RCC

	Fuhrman Ⅰ (n=2)	Fuhrman Ⅱ (n=21)	Fuhrman Ⅲ (n=62)	Fuhrman Ⅳ (n=12)	Total (n=100)	*p*-value[1)]
Age (years)	55.50 ± 4.95	52.90 ± 12.10	56.13 ± 11.28	52.25 ± 11.27	54.88 ± 11.71	–
Male	1	17	46	8	73	–
Female	1	4	16	4	27	–
Tumor size (cm)	2.65 ± 0.21	3.92 ± 1.31	4.84 ± 2.66	7.00 ± 3.54	4.95 ± 2.71	0.0140
GRS Ab (U/ml)	3.68 ± 0.10	3.08 ± 0.66	3.20 ± 0.94	3.63 ± 1.18	3.23 ± 0.91	0.3686
GRS Ag (ug/ml)	34.26 ± 13.44	37.14 ± 10.77	33.99 ± 9.12	29.48 ± 11.14	34.11 ± 9.76	0.2247
WRS Ab (U/ml)	11.50 ± 8.07	11.03 ± 4.71	11.08 ± 3.30	11.03 ± 3.84	11.10 ± 3.69	0.9768
KRS Ag (ng/ml)	4.52 ± 6.39	4.47 ± 11.22	9.54 ± 16.75	10.23 ± 13.48	8.66 ± 15.16	0.3555

Values are presented mean±SD.
1) *p* -values were calculated using the Kruskal Wallis test.

Table 3. Comparison of clinical characteristics according to the stage of RCC

Table 4. Comparison of clinical characteristics according to the sex in NHS group

	Male (n=10)	Female (n=10)	*p*-value[1)]
Age (years)	43.00 ± 12.95	45.10 ± 10.92	–
GRS Ab (U/ml)	3.25 ± 1.01	3.83 ± 0.99	0.1655
GRS Ag (ug/ml)	16.76 ± 4.49	21.68 ± 6.63	0.0630
WRS Ab (U/ml)	7.66 ± 1.80	8.48 ± 3.80	0.5787
KRS Ag (ng/ml)	21.65 ± 30.77	44.27 ± 32.59	0.0630

Values are presented mean±SD.
1) *p* -values were calculated using the Mann-Whitney U test.

Table 4. Comparison of clinical characteristics according to the sex in NHS group

Table 5. Comparison of clinical characteristics according to the sex in RCC group

	Male (n=73)	Female (n=27)	*p*-value[1)]
Age (years)	55.01 ± 11.85	54.52 ± 11.54	–
Fuhrman Ⅰ	1	1	–
Fuhrman Ⅱ	17	4	–
Fuhrman Ⅲ	46	16	–
Fuhrman Ⅳ	8	4	–
Tumor size (cm)	5.05 ± 2.79	4.67 ± 2.52	0.4324
GRS Ab (U/ml)	3.22 ± 0.84	3.28 ± 1.11	0.8644
GRS Ag (ug/ml)	34.98 ± 9.03	31.76 ± 11.36	0.0898
WRS Ab (U/ml)	10.94 ± 3.58	11.54 ± 4.00	0.3091
KRS Ag (ng/ml)	8.41 ± 15.46	9.32 ± 14.57	0.8052

Values are presented mean±SD.
1) *p* -values were calculated using the Mann-Whitney U test.

Table 5. Comparison of clinical characteristics according to the sex in RCC group

DISCUSSION

GRS is an essential molecule for protein synthesis and additionally has noncanonical functions correlated with various diseases, including myositis, interstitial lung disease (ILD), (28) and Charcot-Marie-Tooth (CMT) disease. (29, 30) RCC has no early symptoms and clinically used sepcific biomarkers. Furthermore, RCC is resistant to both chemotherapy and hormonal therapy. (31, 32) For these reasons, the need for new RCC biomarker and treatment is increasing.

CDH 6 is an important molecule for mesenchyme-to-epithelial conversion in kidney development. (33, 34) In other words, CDH 6 is a useful marker to detect the circulating cancer cells in conventional RCC. (24) Aberrant expression of CDH 6 correlates with RCC metastasis and CDH 6 molecule plays a role for the tumor suppressor molecule in RCC. (35)

In this study, ELISA assays were used to compare the level of GRS antibody and antigen between RCC patients and normal human subjects. RCC patients exhibited higher serum GRS antigen level compared with normal human subjects. However, there was no correlation between serum GRS antigen level and RCC stage and tumor size in RCC patients.

The current study has two major limitations. First of all, patient number of each stage is small, especially, stage Ⅰ and Ⅳ to calculate parametric statistical analysis. Furthermore, the normal human subject number of male and female group is also small to calculate parametric statistical analysis. To achieve a more powerful statistical significance, sample size should be increased. In addition, CDH 6 expression level should be measured to identify correlation between serum GRS and CDH 6 expression level in RCC patients.

GRS is an essential enzyme for glycine synthesis. Moreover, in cancer microenvironment, GRS is secreted in extracellular and functions as anti-tumor effect through ERK dephosphorylation. However, GRS extracellular secretion mechanism is not known yet. Therefore, to find out the precise function of GRS in RCC, GRS secretion mechanism has to be identified.

In conclusion, this study suggests that GRS may provide a potential tool for a new biomarker and treatment of RCC.

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국문초록

배경 이론: 신세포암은 조기 진단에 임상적으로 사용될 수 있는 신세포암 특이적 진단 마커가 없다. 신세포암에서 CDH 6는 발현량이 증가되어 있고, 이전 연구 결과로 CDH 6가 GRS의 기능성 수용체라는 사실이 밝혀졌다. 이번 연구에서는 GRS의 신세포암 진단용 생체지표로서 여부를 알아보기 위해 신세포암 환자에서 혈청의 GRS 항원과 항체 수치를 측정하여 정상 대조군에서의 GRS 항원과 항체 수치와 비교하였다.

실험 방법: 신세포암 환자에서 혈청의 GRS 항원과 항체 수치 측정을 위해 효소 표식 면역 검사법 (ELISA)을 사용하였다. ELISA 측정 결과값을 토대로 ROC 곡선이 그린 후 GRS 항원과 항체 측정법에 대한 AUC, 민감도, 특이도, cut-off value 값을 계산하였다.

실험 결과: 신세포암 환자에서 혈청의 GRS 항원 수치가 정상 대조군 혈청의 GRS 항원 수치보다 높은 것으로 나타났다. (신세포암 환자 34.11 ± 9.76 ug/ml; 정상 대조군 19.22 ± 6.06 ug/ml; $p < 0.0001$) GRS 항원 측정법의 AUC 값은 0.900 (95% CI = 0.840-0.960, $p < 0.0001$) 로 측정되었다. GRS 항원 측정법의 민감도와 특이도는 각각 91.0 % 와 75.0 % 였고, cut-off value는 22.86 ug/ml 이었다.

결론: 실험 결과 혈청 GRS 항원 농도는 신세포암의 유무와 관련있는 것으로 나타났다. GRS 항원은 신세포암의 새로운 생체지표로써 뿐만 아니라 치료제 개발의 대상으로써 가능성을 지니고 있다.

주요어 : Glycyl-tRNA synthetase (GRS), 신세포암 (renal cell carcinoma), CDH 6 (K-cadherin), 생체지표 (biomarker), 효소 표식 면역 검사법 (ELISA)

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