Association between visceral adiposity and DDX11 as a predictor of aggressiveness of small clear-cell renal-cell carcinoma: a prospective clinical trial

Jee Soo Park

Yonsei University College of Medicine

Won Sik Jang

Yonsei University College of Medicine

Jongchan Kim

Yonsei University College of Medicine

Seung Hwan Lee

Yonsei University College of Medicine

Koon Ho Rha

Yonsei University College of Medicine

Won Sik Ham (✉ uroham@yuhs.ac)

Yonsei University College of Medicine <https://orcid.org/0000-0003-2246-8838>

Research

Keywords: Adipose tissue, Clear-cell renal-cell carcinoma, Intra-abdominal fat, Obesity, Small renal mass

Posted Date: July 21st, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-44973/v1>

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Version of Record: A version of this preprint was published at Cancer & Metabolism on April 6th, 2021.
See the published version at <https://doi.org/10.1186/s40170-021-00251-y>.

Abstract

Background

Visceral fat produces several hormones and cytokines associated with carcinogenesis and tumor progression. Herein, we investigated the association between visceral adiposity and target-gene mRNA expression in patients with localized small clear-cell renal-cell carcinoma (ccRCC).

Methods

We included 105 patients with localized clinical T1a stage ccRCC who had undergone nephrectomy from November 2018 to November 2019 in a prospective clinical trial (NCT03694912). Visceral, subcutaneous, and total adipose tissue in these patients was measured via preoperative computerized tomography of the mid-third lumbar vertebra region. We then examined the association between adiposity and the mRNA levels of *PBRM1*, *BAP1*, *SETD2*, *KDM5C*, *FOXC2*, *CLIP4*, *AQP1*, *DDX11*, *BAIAP2L1*, and *TMEM38B* in matched frozen tumor tissues and plasma samples.

Results

Upon the stratification of patients into quartiles according to their relative visceral adiposity, high visceral adiposity was found to be significantly associated with low ISUP grade ($P = 0.004$). Multivariate logistic regression analysis revealed a significant association between visceral adiposity and body mass index [odds ratio (OR) 1.462, 95% confidence interval (CI) 1.175–1.820, $P = 0.017$], plasma levels of *DDX11* mRNA (OR 0.928, 95% CI 0.870–0.989, $P = 0.022$), and ISUP nuclear grade (OR 0.258, 95% CI 0.069–0.965, $P = 0.044$). The plasma mRNA expression of *DDX11* was identified as a biomarker for visceral adiposity.

Conclusions

The results obtained herein will aid in inferring the aggressiveness of small ccRCCs, represented by ISUP nuclear grade, in clinical practice. Our findings indicated that *DDX11* and visceral fat play active roles in small ccRCC. These roles should be examined in future studies for the possible use of *DDX11* and visceral fat as prognostic biomarkers in the treatment of patients with ccRCC.

Trial Registration

ClinicalTrials.gov, NCT03694912, Registered 3 October 2018,
<https://clinicaltrials.gov/ct2/show/NCT03694912>

1. Background

Renal-cell carcinoma (RCC) is the sixth and tenth most common type of cancer in men and women worldwide, respectively [1]. Clear-cell RCC (ccRCC) represents the most prevalent type of RCC, and it is

characterized by mutations in genes governing the hypoxia signaling pathway. Several studies have identified specific genetic variances, and possible biomarkers for ccRCC, leading to therapeutic innovations [2]. However, the tumor biology of most commonly identified masses, which are ≤ 4 cm in diameter and classified as small renal masses (SRMs), is poorly understood [3, 4]. A wide variety of therapeutic modalities is used against SRMs, including surgical tumor resection, ablation, as well as active surveillance in some instances. Therefore, understanding the molecular characteristics and pathogenesis of SRMs is important for identifying specific biomarkers and selecting the optimal therapeutic options in clinical settings [4].

Although obesity represents a well-established risk factor regarding RCC [5, 6], the detailed association between obesity and the prognosis of RCC patients is still controversial and unclear. Some studies have reported improved prognoses in overweight patients [6–12], whereas other studies have reported no association between the clinical course of RCC and body weight [13, 14]. Regarding SRMs, the prognosis of RCC is inversely proportional to the body mass index (BMI), indicating that the tumor biology of SRMs is distinct from that of non-SRMs.

Previous studies have mostly employed BMI as a measure of obesity; however, BMI cannot be used to distinguish between fat, muscle, and bone. Furthermore, BMI does not provide any information on fat distribution. The measurement of visceral fat can be used to assess true obesity because visceral fat is the largest endocrine organ in the body, producing several hormones and cytokines related to carcinogenesis and tumor progression [15].

Previously, we found that several potential prognostic biomarkers are considerably up- or downregulated in ccRCC, and can be used to identify the aggressive clinical T1-stage [16, 17]. In this study, we aimed to determine the association between visceral adiposity and the mRNA expression of potential biomarkers using frozen tumor tissues and preoperative plasma of patients with small ccRCC. The present study presents information regarding the development of rapid and straightforward techniques for the evaluation of the aggressiveness of small ccRCCs in clinical practice.

2. Materials And Methods

2.1. Patients and tissues

All procedures involving human participants were performed in accordance with the ethical standards of the institutional and/or national research committee as well as the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the Institutional Review Board of the Yonsei University Health System (project no: 4-2018-0753). Written informed consent was obtained from all patients, and the manuscript does not contain any person's data in any form.

The present study included 105 patients with small localized ccRCC (≤ 4 cm, pT1aN0M0), who were treated via nephrectomy only, and for whom frozen tumor tissues and matching preoperative plasma

samples were available from a prospective study (ClinicalTrials.gov Identifier: NCT03694912) conducted between November 2018 and November 2019.

The inclusion criteria used were as follows: 1) localized small ccRCC (≤ 4 cm, pT1aN0M0); 2) availability of information on preoperative height and weight; 3) availability of preoperative computed tomography (CT) data and follow-up for more than 1 year; and 4) no neoadjuvant or adjuvant systemic therapy. Clinicopathological data, including age, sex, BMI, and tumor size, were recorded for each patient. All tumors were reviewed according to the 2016 World Health Organization (WHO) classification [18] and International Society of Urologic Pathologists (ISUP) grading [19]. Grades 1 and 2 were considered as low-grade disease, while grades 3 and 4 were considered as high-grade disease. Minimum four slides were reviewed for each case in order to minimize bias from intratumoral heterogeneity (ITH). Diameters of the primary tumors were obtained via CT imaging. We assessed the expression levels of six genes (*PBRM1*, *BAP1*, *SETD2*, *KDM5C*, *FOXC2*, and *CLIP4*) reportedly associated with ccRCC, and four genes (*AQP1*, *DDX11*, *BAIAP2L1*, and *TMEM38B*) examined in our previous study involving RNA-seq analysis of aggressive ccRCC in clinical T1 stage [16, 17].

2.2. Adiposity measurement

As shown in Fig. 1, the contents of visceral adipose tissue (VAT), subcutaneous adipose tissue, and total adipose tissue were measured via preoperative CT of the mid-third lumbar vertebra region using Aquarius iNtuition Viewer, version 4.4.12 (TeraRecon, Foster City, CA, USA) with patients in a supine position. Preoperative CT was performed within 1 month before the surgery in order to minimize the change in body fat tissue over time. Previous studies have consistently used mid-third lumbar level for CT-defined image analysis [20]. Different body compositions were evaluated using predefined Hounsfield unit (HU) thresholds: -190 to -30 HU for subcutaneous adipose tissue, and -150 to -50 HU for VAT [20]. VAT% was calculated using the formula $\text{VAT\%} = [\text{VAT} / \text{total adipose tissue}] \times 100$ [20].

2.3. Blood sample processing

Peripheral blood was collected from each participant, aliquoted into ethylenediaminetetraacetic acid (EDTA)-coated tubes, and centrifuged at $1,600\ g$ for 10 min at $4\ ^\circ\text{C}$. The plasma was then carefully transferred into new tubes and further centrifuged at $4,000\ g$ for 10 min at $4\ ^\circ\text{C}$ and stored at $-80\ ^\circ\text{C}$ until further analysis.

2.4. RNA extraction and reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted from frozen tissues and plasma samples using TRIzol (Ambion, Life Technologies, USA). RNA isolated from 1 mL of plasma was dissolved in 20 μL of DEPC-treated water. The quantity and quality of RNA were assessed using a Nanodrop spectrophotometer (NanoDrop ND-1000, Thermo Scientific, Wilmington, DE, USA). Precisely 1 μg per sample was reverse-transcribed into first-strand cDNA using an iNtRon Maxime RT PreMix (Cat No. 25082; Intronbi, Seongnam, South Korea),

following the manufacturer's protocol. qPCR was performed using Power SYBR® Green Master Mix (Thermo Fisher, Cat No. A25742, USA) in a 10-μL reaction volume consisting of 5 μL of SYBR Green master PCR mix, 1 μL each of the forward and reverse primers (10 pmol), 1 μL of the diluted cDNA template, and UltraPure™ distilled water (Invitrogen, NY, USA). The conditions for amplification are listed as follows: Initial denaturation at 95 °C for 10 min; 40 cycles of denaturation at 95 °C for 15 s, annealing at 58 °C for 60 s, and elongation at 72 °C for 60 s; and final elongation at 72 °C for 5 min. qPCR was performed on an ABI StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). All measurements were conducted using *GAPDH* as the reference gene to normalize the relative expression levels of the target genes. PCR primer sequences are shown in Table S1. The relative gene expression was analyzed using the $2^{-\Delta\Delta CT}$ method, and the results are expressed as percentage change compared to the control values. At least three replicates of RT-qPCR experiments were performed, and the results were analyzed by a blinded investigator.

2.5. Statistical analyses

Data are presented as the means $\pm$ standard deviations or median (interquartile ranges) for continuous variables, and as percentage for categorical variables. Locally weighted scatterplot smoothing curves and Pearson's correlation coefficient were used to assess the relationship between BMI and total adipose tissue, subcutaneous adipose tissue, VAT, or VAT%. High visceral adiposity was defined as a VAT level higher than the median VAT level. For univariate analysis, Wilcoxon test, Student's *t*-test, or one-way analysis of variance was used to compare continuous variables, and chi-square test or Fisher's exact test was used to compare categorical variables. Multivariate logistic regression analyses were performed to identify predictors of high visceral adiposity, and odds ratios (ORs) with 95% confidence intervals (CIs) were calculated. SPSS software version 23.0 (IBM Corp., Armonk, NY, USA) was used for all statistical analyses. All statistical tests were two-tailed. *P*-values less than 0.05 were considered statistically significant.

3. Results

3.1. Baseline demographics

The clinicopathological features of the study population (105 patients) are listed in Table 1. The median patient age was 54.0 years, and 72.4% of the patients were men. The median BMI was 24.7 kg/m², and one (1.0%) patient was classified as underweight (less than 18.5 kg/m²), 55 (52.4%) as normal weight (18.5 to 24.9 kg/m²), 42 (40.0%) as overweight (25 to 29.9 kg/m²), and seven (6.7%) as obese (30 kg/m² or greater) according to the WHO BMI cutoff points [21]. The median tumor size was 2.3 cm, and 57 (54.3%) tumors were classified as high-grade.

The prevalence of hypertension, diabetes, and hypercholesterolemia in the patient population was 44.8%, 15.2%, and 3.8%, respectively (Table 1). As shown in Fig. 2, the total adipose tissue content [Pearson's

correlation coefficient ($r = 0.448$, $P < 0.001$)] and the subcutaneous adipose tissue content ($r = 0.686$, $P < 0.001$) were significantly correlated with BMI. The VAT content ($P = 0.075$) and VAT% ($P = 0.895$) did not demonstrate strong correlations with BMI. Age, diabetes status, hypercholesterolemia, smoking status, and alcohol consumption differed significantly between men and women enrolled in the study (Table 1). Apart from subcutaneous adipose tissue, the adiposity variables, BMI, total adipose tissue, VAT, and VAT% were all significantly higher in men than in women.

Table 1
Clinicopathological characteristics of the study population according to gender

	Total (n = 105)	Men (n = 76; 72.4%)	Women (n = 29; 27.6%)	P
Median age (range)	54.0 (46.0–65.5)	51.5 (43.0–59.8)	62.0 (58.5–70.0)	< 0.001
Median tumor size (cm) (range)	2.3 (1.5–3.0)	2.3 (1.6–3.0)	2.1 (1.3–3.0)	0.892
Median BMI (kg/m ²) (range)	24.7 (23.0–27.3)	25.9 (23.9–28.3)	23.0 (21.9–24.4)	0.001
Median TAT (mm ²) (range)	28,380 (22,144–35,764)	29,817 (24,793–37,647)	26,400 (19,826–28,088)	0.003
Median SAT (mm ²) (range)	13,302 (10,365–17,033)	13,362 (10,312–16,976)	13,268 (11,542–18,437)	0.952
Median VAT (mm ²) (range)	14,048 (10,170–18,486)	15,740 (11,634–20,844)	9,508 (7,220–14,048)	< 0.001
Median VAT% (range)	51.3 (41.9–58.0)	54.0 (46.3–62.5)	38.5 (32.2–49.7)	0.001
No. comorbid conditions, n (%)				
Hypertension	47 (44.8%)	31 (40.8%)	16 (55.2%)	0.185
Hypercholesterolemia	4 (3.8%)	0 (0.0%)	4 (13.8%)	0.005
Diabetes	16 (15.2%)	7 (9.2%)	9 (31.0%)	0.012
No. smoking status, n (%)				
Never	49 (46.7%)	20 (26.3%)	29 (100.0%)	< 0.001
Former	35 (33.3%)	35 (46.1%)	0 (0.0%)	
Current	21 (20.0%)	21 (27.6%)	0 (0.0%)	
No. alcohol status, n (%)				
Never	43 (41.0%)	18 (23.7%)	25 (86.2%)	< 0.001
Former	18 (17.1%)	18 (23.7%)	0 (0.0%)	
Current	44 (41.9%)	40 (52.6%)	4 (13.8%)	
No. ISUP grade, n (%)				

	Total (n = 105)	Men (n = 76; 72.4%)	Women (n = 29; 27.6%)	<i>P</i>
Low-grade (1–2)	48 (45.7%)	37 (48.7%)	11 (37.9%)	0.323
High-grade (3–4)	57 (54.3%)	39 (51.3%)	18 (62.1%)	
Data are presented as medians (interquartile ranges) for continuous variables and as percentage for categorical variables. <i>P</i> -values from the chi-square test or Fisher's exact test were used to calculate mean differences for categorical variables and those from Wilcoxon test were used to calculate mean differences for continuous variables. BMI: body mass index, SAT: subcutaneous adipose tissue, TAT: total adipose tissue, VAT: visceral adipose tissue, VAT%: percentage of visceral adipose tissue.				

3.2. Clinicopathological characteristics according to VAT

Patients were stratified into quartiles (Q1 to Q4) according to the relative VAT contents (Table 2). High VAT content at diagnosis was associated with the male sex, an increased BMI, an increased prevalence of former and current smoking status, as well as a low ISUP grade. Although the prevalence of hypercholesterolemia differed significantly among the VAT quartiles ($P = 0.007$), it was only reported in Q2. The VAT quartiles did not differ in terms of age, tumor size, the prevalence of hypertension and diabetes, or alcohol consumption. Furthermore, subcutaneous adipose tissue quartiles were not associated with ISUP grades ($P = 0.104$).

Table 2
Clinicopathological characteristics according to visceral adipose tissue content

	Q1	Q2	Q3	Q4	<i>P</i>
Median age (range)	59.0 (49.0– 67.0)	52.0 (46.0– 68.0)	58.5 (44.8– 65.0)	51.0 (43.0– 58.0)	0.660
No. gender, n (%)					
Male	11 (42.3%)	17 (63.0%)	22 (84.6%)	26 (100.0%)	< 0.001
Female	15 (57.7%)	10 (37.0%)	4 (15.4%)	0 (0.0%)	
Median tumor size (cm) (range)	2.1 (1.4– 2.8)	2.7 (2.0– 3.5)	1.9 (1.5– 3.5)	2.3 (1.5– 3.0)	0.257
Median BMI (kg/m ²) (range)	22.7 (21.7– 24.4)	24.1 (23.0– 26.3)	26.5 (24.0– 28.7)	26.9 (24.8– 29.6)	< 0.001
No. comorbid conditions, n (%)					
Hypertension	12 (46.2%)	13 (48.1%)	10 (38.5%)	12 (46.2%)	0.900
Hypercholesterolemia	0 (0.0%)	4 (14.8%)	0 (0.0%)	0 (0.0%)	0.007
Diabetes	7 (26.9%)	4 (14.8%)	2 (7.7%)	3 (11.5%)	0.243
No. smoking status, n (%)					0.016
Never	19 (73.1%)	15 (55.6%)	8 (30.8%)	7 (26.9%)	
Former	5 (18.2%)	8 (29.6%)	10 (38.5%)	12 (46.2%)	
Current	2 (7.7%)	4 (14.8%)	8 (30.8%)	7 (26.9%)	
No. alcohol status, n (%)					
Never	15 (57.7%)	13 (48.1%)	8 (30.8%)	7 (26.9%)	0.143
Former	2 (7.7%)	3 (11.1%)	5 (19.2%)	8 (30.8%)	

	Q1	Q2	Q3	Q4	<i>P</i>
Current	9 (34.6%)	11 (40.7%)	13 (50.0%)	11 (42.3%)	
No. ISUP grade, n (%)					
Low-grade (1–2)	7 (26.9%)	8 (29.6%)	17 (65.4%)	16 (61.5%)	0.004
High-grade (3–4)	19 (73.1%)	19 (70.4%)	9 (34.6%)	10 (38.5%)	
Data are presented as medians (interquartile ranges) for continuous variables and as percentage for categorical variables. <i>P</i> -values from one-way analysis of variance and chi-square test or Fisher's exact test were used to determine mean differences for continuous and categorical variables, respectively, based on VAT quartiles. BMI: body mass index, SAT: subcutaneous adipose tissue, TAT: total adipose tissue, VAT: visceral adipose tissue, VAT%: percentage of visceral adipose tissue.					

3.3. Expression of target genes in frozen tissues and plasma according to high visceral adiposity

The relative mRNA levels of *DDX11* in the frozen tissue and plasma were significantly lower in patients with high visceral adiposity, revealed using univariate analysis ($P = 0.009$ and $P = 0.037$, respectively) (Table 3). When we included the clinicopathological parameters and the mRNA expression of target genes that were significantly associated with VAT contents in the univariate analysis, the univariate logistic regression analysis revealed that sex, BMI, the prevalence of former and current smoking status, *DDX11* expression in frozen tissue, and FG score were significantly associated with high visceral adiposity (Table 4). Multivariate logistic regression analysis revealed that BMI (OR 1.400, 95% CI 1.122–1.748, $P = 0.003$), plasma *DDX11* levels (OR 0.928, 95% CI 0.868–0.991, $P = 0.026$), and FG (OR 0.258, 95% CI 0.069–0.965, $P = 0.044$) were significantly associated with high visceral adiposity (Table 4).

Table 3

Expression of target genes in patients with clear-cell renal-cell carcinoma characterized by high/low visceral adiposity

	Low visceral adiposity	High visceral adiposity	<i>P</i>
mRNA expression (× 100)	(N = 53)	(N = 52)	
<i>Frozen tissue</i>			
<i>FOXC2</i>	9.409 ± 14.805	13.930 ± 28.954	0.318
<i>CLIP4</i>	22.536 ± 39.736	15.907 ± 23.464	0.301
<i>PBRM1</i>	10.709 ± 12.296	9.949 ± 12.777	0.757
<i>SETD2</i>	1.134 ± 1.661	1.600 ± 1.939	0.189
<i>BAP1</i>	10.705 ± 43.113	6.922 ± 17.568	0.559
<i>KDM5C</i>	1.252 ± 2.504	1.713 ± 3.882	0.470
<i>AQP1</i>	1.659 ± 1.694	1.799 ± 1.417	0.646
<i>DDX11</i>	7.635 ± 8.734	4.089 ± 3.841	0.009
<i>BAIAP2L1</i>	2.125 ± 3.162	2.763 ± 3.605	0.337
<i>TMEM38B</i>	5.929 ± 9.697	6.073 ± 8.668	0.936
<i>Plasma</i>			
<i>FOXC2</i>	15.447 ± 13.299	16.318 ± 9.170	0.697
<i>CLIP4</i>	20.658 ± 27.442	20.255 ± 23.180	0.935
<i>PBRM1</i>	2.320 ± 2.271	9.960 ± 58.052	0.341
<i>SETD2</i>	1.611 ± 1.889	2.018 ± 1.622	0.240
<i>BAP1</i>	153.828 ± 481.825	77.992 ± 336.844	0.353
<i>KDM5C</i>	13.094 ± 19.729	7.462 ± 11.179	0.076
<i>AQP1</i>	11.689 ± 17.340	7.793 ± 18.914	0.274
<i>DDX11</i>	6.954 ± 14.027	2.671 ± 4.030	0.037
<i>BAIAP2L1</i>	10.998 ± 18.225	7.082 ± 16.326	0.249
<i>TMEM38B</i>	20.291 ± 23.112	23.359 ± 32.876	0.581
Data are shown as the means ± standard deviations.			
<i>P</i> -values were determined using Student's <i>t</i> -test.			

Table 4
Predictors of high visceral adiposity in clinical T1a clear-cell renal-cell carcinoma

	Univariate		Multivariate	
	OR (95% CI)	p ^a	OR (95% CI)	p ^b
Clinical parameters				
Gender (reference male)	0.093 (0.029–0.296)	< 0.001	0.276 (0.050–1.510)	0.138
BMI (kg/m ²)	1.494 (1.247–1.789)	< 0.001	1.400 (1.122–1.748)	0.003
Hypercholesterolemia (vs. none)	—	0.999	—	0.999
Smoking (vs. never)		0.002		0.270
Former	3.836 (1.535–9.588)	0.004	2.368 (0.640–8.771)	0.197
Current	5.667 (1.840–17.453)	0.003	3.211 (0.714–14.445)	0.128
mRNA expression levels				
<i>DDX11</i> (frozen tissue)	0.909 (0.840–0.983)	0.017	1.028 (0.883–1.197)	0.725
<i>DDX11</i> (plasma)	0.947 (0.893–1.004)	0.068	0.928 (0.868–0.991)	0.026
ISUP nuclear grade (reference low-grade)	0.227 (0.100–0.517)	< 0.001	0.258 (0.069–0.965)	0.044
^a P-values determined using logistic regression for univariate analysis.				
^b P-values determined using logistic regression for multivariate analysis. BMI: body mass index.				

4. Discussion

In the present study, we identified the plasma mRNA levels of *DDX11*, which is involved in cellular growth and division, as an independent prognostic factor for high visceral adiposity even after adjusting for clinicopathological parameters that are significantly associated with adiposity. To the best of our knowledge, this is the first study to investigate the association between the expression of genes potentially involved in ccRCC and adiposity, as well as to use visceral adiposity as a marker to infer the aggressiveness of small ccRCC.

BMI increases the relative risk for RCC [22]. However, the association between BMI and RCC prognosis remains controversial. Prior studies have mainly reported the proportional associations between BMI and RCC prognosis, whereas inverse, flat, or null associations have also been reported [23]. The inconsistencies among the results reported by these studies may be attributed to the use of BMI as a surrogate marker for obesity. Among various types of adipose tissue, VAT is the largest endocrine organ and produces hormones and cytokines that are related to carcinogenesis and tumor progression [15]. Subcutaneous adipose tissue and VAT share scant functional similarities other than their efficiency in energy storage [24]. VAT releases high levels of adipokines that are involved in inflammation and angiogenesis, including interleukin-6, vascular endothelial growth factor, and plasminogen activator inhibitor 1 [24]. In our study, BMI was not significantly correlated with VAT, indicating that the use of BMI would not provide consistent results in assessing the association between obesity and RCC prognosis. Among anthropometric measurements, the VAT content measured via CT has recently been examined for its utility in predicting the risk of cancer.

Five studies have examined the VAT content in patients with localized and/or advanced RCC [25]. Among these, three reported that low VAT contents are associated with poor prognosis in patients with RCC [25], and one study reported no association between VAT contents and overall mortality [26]. Park and colleagues reported that the lowest and the highest vs. the second quartiles of the VAT% are associated with a higher risk of recurrence [23]. Most studies have reported better prognosis in patients with high obesity, especially in those with localized SRMs [11, 23]. Moreover, Parker et al. reported that, in terms of aggressiveness, high BMI is associated with the presentation of a less aggressive form of RCC [9].

Among 28 studies that analyzed the body composition regarding the clinical outcomes of RCC in October 2016, nine studies used fat index, which is the fat area divided by the height of the patients [25]. Since 19 studies used the fat area, we used it as well. However, there could be some bias in using only the fat area, not adjusted to the height of the patients, although our analysis of VAT index showed a non significant association with ISUP grades ($P = 0.073$).

Although subcutaneous adipose tissue is not associated with perioperative outcomes and survival in RCC, for other cancers, subcutaneous adipose tissue is reportedly associated with cancer-survival outcomes. Takamasa et al. reported that high subcutaneous adipose tissue volume in hepatocellular carcinoma is associated with better survival outcomes when treated with transcatheter intra-arterial therapies [27]. Moreover, leptin and adiponectin, which play a role in cancer biology, are both influenced by VAT and the subcutaneous adipose tissue [28, 29]. Therefore, although subcutaneous adipose tissue is not significantly associated with aggressive RCC, we should not overlook the unidentified importance of the subcutaneous adipose tissue.

DDX11 expression, which is involved in cell-cycle progression, is used to predict tumor aggressiveness in clinically localized T1-stage ccRCC [16, 17]. Additionally, the inhibition of *DDX11* expression decreases the proliferation rate of melanoma cells and induces apoptosis [30]. In patients with lung adenocarcinoma, upregulated *DDX11* expression is associated with poor prognosis [31].

Consistent with the results of previous studies, our present study showed an inverse relationship between high visceral adiposity and the expression of *DDX11* mRNA in the frozen tissues and plasma. Previously, we showed that aggressive ccRCC, such as that associated with synchronous metastasis, recurrence, and/or cancer-specific death, is also associated with the upregulated expression of *DDX11* mRNA in both plasma and frozen tissues. Most studies showed that high VAT contents are associated with improved prognosis; thus, we suggest that less aggressive ccRCC is likely associated with high VAT contents. The results obtained in our present study indicated that non-aggressive ccRCC is associated with high VAT contents and low expression of *DDX11*. Based on these results, conservative therapeutic options, such as ablation and active surveillance, would be prudent strategies for treatment of patients with small ccRCC, a high VAT, and decreased *DDX11* expression.

The underlying mechanism that links visceral adiposity to the upregulation of *DDX11* expression is unknown. *DDX11*, a DNA-dependent ATPase and helicase, plays an important role in the cohesion of chromosome arms and centromeres [32]. The depletion of *DDX11* results in mitotic failure because the replicated chromosomes fail to segregate after prometaphase [32]. *DDX11* expression may be associated with the G1–S phase of the cell-cycle and the pathways involved in DNA replication [32]. Recent studies suggest that adipocyte differentiation, lipogenesis, and lipolysis are strongly modulated by cell-cycle regulators, which control the checkpoints for cell duplication [33]. Our results suggest that *DDX11*, which is involved in cell-cycle regulation, may be associated with VAT generation. Because the exact pathways connecting cell-cycle regulation and adiposity remain unknown, future studies should elucidate the mechanisms underlying visceral adiposity and *DDX11* expression.

Our study was the first to evaluate the association between visceral adiposity and mRNA expression of target genes. Moreover, our results enabled us to easily infer the aggressiveness of ccRCCs using visceral adiposity calculated from preoperative CT without any invasive diagnostic modalities.

Our study has a few limitations. First, the ITH of primary tumors is a considerable problem, even in SRMs [25]. ITH causes sampling bias in conventional needle biopsies. Clinical trials are currently examining the use of circulating tumor DNA in plasma to overcome the limitations imposed by ITH. Second, we could not use visceral adiposity and target-gene expression to predict prognostic indexes, such as cancer-specific or progression-free survival, in patients with ccRCC, owing to the short follow-up period. Third, the study population was relatively small. Future studies should investigate the correlations among visceral adiposity, target-gene expression, and prognosis in large populations of patients with ccRCC.

5. Conclusions

In the present study, we investigated the association between visceral adiposity and target-gene mRNA expression in patients with localized small ccRCC. *DDX11* mRNA levels in the frozen tissues and plasma are significantly associated with high visceral adiposity. Quantifying the VAT contents in preoperative CT scans will enable us to infer the aggressiveness of small ccRCCs in clinical practice. The role of *DDX11* in the regulation of VAT warrants further investigation in future studies.

List Of Abbreviations

CI Confidence intervals

CT Computed tomography

HU Hounsfield unit

ISUP International Society of Urologic Pathologists

ITH Intratumoral Heterogeneity

KHIDI Korea Health Industry Development Institute

OR Odds ratios

RCC Renal-cell carcinoma

SAT Subcutaneous adipose tissue

SRM Small renal masses

TAT Total adipose tissue

VAT Visceral adipose tissue

WHO World Health Organization

Declarations

Ethics approval and consent to participate: All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the Yonsei University Health System (project no: 4-2018-0753).

Consent for Publication: All authors agree for publication.

Availability of Data and Materials: Data are not publically available to other researchers and only be permitted after the approval of IRB.

Competing Interests: The authors declare that they have no competing interests.

Funding: This study was supported by a grant from the Korea Health Technology R&D Project through the Korea Health Industry Development Institute funded by the Ministry of Health & Welfare, Republic of Korea (grant number: HI17C1095).

Author Contributions: JSP: literature search, figure preparation, study design, data collection, data analysis, data interpretation, and writing; WSJ: data collection; JK: data collection; SHL: data collection; KHR: data collection; WSH: study design, data collection, data analysis, data interpretation, and writing.

Acknowledgments: None.

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Figures

Figure 1

Computed tomography (CT) analysis of visceral and subcutaneous adipose tissue. Measurement of (a) visceral adipose tissue (VAT), (b) subcutaneous adipose tissue (SAT), and (c) total adipose tissue (TAT) by CT analysis software for a representative patient.

Figure 2

Correlation between body mass index (BMI) and adipose tissue parameters. Correlation between body mass index (BMI) and (a) total adipose tissue (TAT), (b) subcutaneous adipose tissue (SAT), (c) visceral adipose tissue (VAT), and (d) VAT%. Locally weighted scatterplot smoothing curves (blue line) were fitted in plots.