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RESEARCH

Adiponectin Receptor Expression Predicts Favorable Prognosis in Cases of Hepatocellular Carcinoma

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Abstract Obesity influences risk, progression and prognosis of various cancers including hepatocellular carcinoma (HCC). Adipose-tissue-derived adipokines has been considered to be involved in tumorigenesis and adiponecin, one such adipokine, has antiproliferative effect on obesity-related malignancies, though variable signal pathway mediated by adiponectin receptors-AdipoR1 and AdipoR2. In this study, we investigated expression of adiponectin and adiponectin receptors in tumor and non-tumorous hepatic tissues of HCC patients and its clinicopathological significance. We collected 75 HCC tissues and 70 non-tumorous hepatic tissues from HCC patients who underwent surgical resection. The tissue microarrays were constructed and immunohistochemical study for adiponectin, AdipoR1 and AipoR2 was performed. Adiponectin and AdipoR1 expression rates were significantly lower in HCC than non-neoplastic hepatic tissues (82.7 % vs. 97.1 % and 24.0 % vs. 90 %, P=0.005 and <0.001, respectively). Immunopositivity for adiponectin was associated with small tumor size, low Edmonson-Steiner grade and absence of other organ invasion (P=0.015, 0.021 and 0.028, respectively). AdipoR1 expression had association with absence of vascular invasion (P=0.028) and AdipoR2 expression was correlated with lower histologic grade and low pathologic T-stage (P=0.003 and 0.008, respectively). Cox regression analysis revealed that low expression of AdipoR1 and AdipoR2 were associated with increased risk of recurrence and death, respectively (hazard ration = 3.222 and 14.797, respectively). These findings suggest that loss of adiponectin, and adiponectin receptors expression is associated with aggressive clinicopathological features of HCC and AdipoR1 and AdipoR2 might serve as the independent prognostic factors for HCC patients.

Keywords Hepatocellular carcinoma · Adiponectin · Immunohistochemistry · Prognosis

Introduction

Large epidemiologic studies have indicated significant associations between obesity and a variety of cancer types, including colorectal, postmenopausal breast, endometrial, esophageal, pancreatic, biliary, and renal cancers [1–3]. Moreover, recent studies have further established obesity as a new risk factor for hepatocellular carcinoma (HCC) [3, 4]. Several candidate biological factors underpin the association between obesity and malignancy, including steroid hormones, insulin, insulin-like growth factor systems, obesity-related inflammatory markers, and the nuclear factor kappa beta [1]. Recently, adipokines, which are bioactive substances secreted by adipocytes, have been increasingly studied and are considered as an additional mechanism through which obesity is associated with carcinogenesis. Adiponectin, one such adipokine, is a key hormone that is related to the pathogenesis of diabetes through the modulation of glucose and fatty acid metabolism and insulin sensitivity in various stromal or epithelial cells [5]. Recent studies reported lower serum adiponectin levels in patients with various malignancies, including colorectal, breast, and prostate cancers, than in control individuals [6–8], and some in vitro studies have shown that adiponectin

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repressed the growth of colorectal, breast, and prostate cancer cells by inhibiting cell proliferation and inducing apoptosis [9–14]. These findings suggest that adiponectin has a potential role in the development of malignancies; thus, mechanisms that link adiponectin with carcinogenesis have been actively sought. Two adiponectin receptor forms (AdipoR1 and AdipoR2) have been cloned and identified as mediators between adiponectin and downstream signaling cascades such as the 5′-AMP-activated protein kinase (AMPK), inducing the anti-angiogenic and anti-proliferative effects of adiponectin [15, 16]. Recently, the expression of adiponectin receptors has been reported in cancers in various organs, including the kidney, lung, breast, and pancreas, among others [17–20]. However, only a few studies have investigated the expression of adiponectin or adiponectin receptors in HCC [17, 21, 22], and thus, little is currently known about the clinicopathological implications of the expression of these markers in HCC.

Herein, we sought to analyze the expression and clinicopathological implications of adiponectin and the adiponectin receptors in human HCC tissues using immunohistochemical and tissue microarray methods. Non-neoplastic hepatic tissues were used as controls, and the evaluated clinicopathological features included prognostic outcomes.

Materials and Methods

Patients and Tissue Samples

Tissue specimens from 75 patients with surgically resected HCC between 1996 and 2011, for whom paraffin blocks and follow-up information were available, were collected from the files of the Department of Pathology at Korea University Anam Hospital. Patients who underwent total hepatectomy for subsequent liver transplantation were not included in the study group. Histopathological features were retrospectively evaluated from hematoxylin and eosin–stained slides, and clinical and follow-up data were collected from the medical records. Histologic grades were scored according to the Edmonson-Steiner grading system, and pathologic staging (pT stage) was determined according to the American Joint Committee on Cancer grading system (seventh edition). The main clinical and pathological variables evaluated in this study are shown in Table 1. The study population comprised 66 men (85.7 %) and 9 women (11.7 %). The median age of the individuals was 57.8 years (range, 36–80 years). Moreover, 75.3 % of patients had hepatitis B virus infection, 8.2 % had hepatitis C virus infection, 1.4 % had concurrent hepatitis B virus and hepatitis C virus infections, and 1.4 % had alcoholic liver disease. The disease etiology was unknown in 13.7 % of the cases. Of the 74 patients with follow-up data, 37 (50.0 %) had local recurrence, 8 (10.8 %) had distant metastasis, and 29 (39.2 %) had no recurrence or metastasis.

Table 1 Clinical characteristics of HCC patients

Age		57.8±9.1 years
Sex	Male	66 (85.7 %)
	Female	9 (11.7 %)
Size of tumor		4.5±3.1 cm
Number of tumor	1	66 (85.7 %)
	2	5 (6.5 %)
	More than 2	4 (5.3 %)
Associated liver disease	HBV-associated	55 (75.3 %)
	HCV-associated	6 (8.2 %)
	HBV + HCV co-associated	1 (1.4 %)
	Alcoholic liver disease	1 (1.4 %)
	Unknown	10 (13.7 %)

HBV hepatitis B virus; *HCV* hepatitis C virus

The control group included 70 non-neoplastic hepatic tissue samples. These were retrieved from the non-neoplastic portions of livers resected because of HCC, and 59 of the 70 non-neoplastic hepatic tissue samples were taken from the same patients with HCC who were enrolled in the present study.

Tissue Microarray Construction

Previously stained hematoxylin and eosin slides were retrospectively reviewed, and one representative formalin-fixed, paraffin-embedded archival block was selected for each case. The arrays were assembled by taking core tissue biopsies (5 mm in diameter) from specific locations in the preexisting formalin-fixed, paraffin-embedded blocks (donor blocks) and re-embedding them in recipient paraffin blocks (tissue array blocks) using a trephine apparatus.

Immunohistochemical Staining

Immunohistochemical staining for anti-human adiponectin (ab62551, 1:100; Abcam, Cambridge, UK), AdipoR1 (H-001-44, 1:500; Phoenix Pharmaceuticals, Burlingame, CA, USA), and AdipoR2 (H-001-23, 1:500; Phoenix Pharmaceuticals) was performed on tissue array slides. The detailed procedure was as follows: 4-μm-thick sections from the tissue array blocks were dewaxed, and endogenous peroxidases were blocked by immersing the slides in a 3 % hydrogen peroxidase-methanol solution for 10 min. This was followed by an antigen retrieval step. The slides were immersed in a 10-mmol/l citrate buffer solution (pH 6.0) and placed in a microwave oven for 25 min. After a wash in 0.01 mol/L phosphate-buffered saline (PBS; pH 7.4), the sections were covered with normal goat serum in a humidity chamber for 1 h at room temperature. Excess serum was rinsed with 0.01 mol/L PBS, and the slides were incubated with the primary antibodies in a

humidity chamber for 45 min at room temperature. A biotinylated anti-rabbit antibody at a 1:400 dilution was used as the secondary antibody and was applied for 30 min at 37 °C. After rinsing with PBS, a streptavidin-peroxidase complex reagent (StrepABComplex/HRP Duet; Dako, Glostrup, Denmark) was added. The slides were incubated for 45 min at room temperature, washed in 0.01 mol/L PBS, and covered with 3,3′-diaminobenzidine tetrahydrochloride solution for 15 min under a microscope. The sections were then immersed in running tap water and counterstained with hematoxylin for 1 min. This was followed by immersion in a tap water bath, a series of increasingly concentrated alcohol baths, and xylene. The slides were then covered with coverslips.

Analysis of Immunohistochemical Staining

Two pathologists (E Shin and NH Won) who were blinded to the follow-up data in the patients' medical records independently evaluated the immunostained slides. After the staining results were interpreted, equivocal evaluations were reevaluated and a consensus was reached. Immunopositivity was determined on the basis of the presence of brown staining within the cytoplasm. All staining intensity grades were considered. Cases that did not show any staining were considered to be negative for adiponectin or adiponectin receptor expression. Adipose tissue sections were used as positive controls for adiponectin immunostaining, and skeletal muscle and normal hepatic tissue samples were used as positive controls for AdipoR1 and AdipoR2 immunostaining.

Statistical Analysis

Statistical analyses were performed using the SPSS version 12.0 software package (SPSS Inc., Chicago, IL, USA). Correlations between altered adiponectin, AdipoR1, and AdipoR2 expression and clinicopathological features were analyzed using Pearson's χ^2 test for categorical variables and the Student's *t*-test for descriptive characteristics. Cases and controls were compared with regard to adiponectin or adiponectin receptor expression using the McNemar test. The results were considered statistically significant when P was ≤ 0.05. For the survival data analysis, Kaplan-Meier survival curves were constructed and the differences between the survival curves were determined using the log-rank test. Multivariate analysis was performed according to Cox's proportional hazards regression modeling, and $P \leq 0.05$ was considered statistically significant. The analyzed variables included overall survival (OS), which was calculated from the date of surgery until death or the last follow-up appointment, and disease-free survival (DFS), which was calculated from the date of surgery until the date when recurrence or metastasis was detected.

Results

Adiponectin and Adiponectin Receptor Expression in Hepatocellular Carcinomas and Non-neoplastic Hepatic Tissues

Immunoreactivity against adiponectin was observed in the cytoplasm and expressions of the adiponectin receptors were detected in the cytoplasm and/or cytoplasmic membrane of tumor cells from patients with HCC and hepatocytes from the non-neoplastic hepatic parenchyma. Figure 1 shows representative micrographs of immunohistochemical staining of adiponectin and adiponectin receptors. Of the 75 HCC cases, adiponectin expression was observed in 62 cases (82.7 %) and AdipoR1 and AdipoR2 expression was observed in 18 cases (24.0 %) and 63 cases (90.0 %), respectively. Of the 70 non-neoplastic hepatic tissue specimens, adiponectin was expressed in 68 cases (97.1 %) and AdipoR1 and AdipoR2 expression was observed in 63 cases (90.0 %) and 69 cases (98.6 %), respectively. The adiponectin and AdipoR1 expression rates differed significantly between the HCC and non-neoplastic hepatic tissues ($P=0.005$ and $P<0.001$, respectively; Table 2). The frequencies of adiponectin and adiponectin receptor expression were analyzed in the 59 pairs of matched HCC and non-neoplastic hepatic tissues. We found that adiponectin and AdipoR1 expression was significantly lower in the HCC tissues than in the corresponding non-neoplastic hepatic tissues (McNemar test: $P=0.012$ and $P<0.001$ for adiponectin and AdipoR1, respectively)

Correlations Between Adiponectin and Adiponectin Receptor Expression Status and Clinicopathological Variables

The Student's *t*-test and Pearson's χ^2 test were used to evaluate correlations between altered adiponectin and adiponectin receptor expression and available tumor characteristics, including the presence of vascular invasion, other organ invasion, stage, and histologic grade. The P values are presented in Table 3.

Analysis of tissue microarray slides according to the Edmondson-Steiner grading system showed that the expression frequencies of adiponectin and AdipoR2 decreased as the Edmondson-Steiner grades increased ($P=0.021$ and $P=0.003$, respectively). Tumor size was significantly smaller in the adiponectin-positive HCC samples than in the adiponectin-negative HCC samples ($P=0.015$). Glisson capsule involvement was correlated with decreased AdipoR2 expression ($P=0.015$). Direct involvement of other organs was significantly associated with decreased adiponectin expression ($P=0.028$).

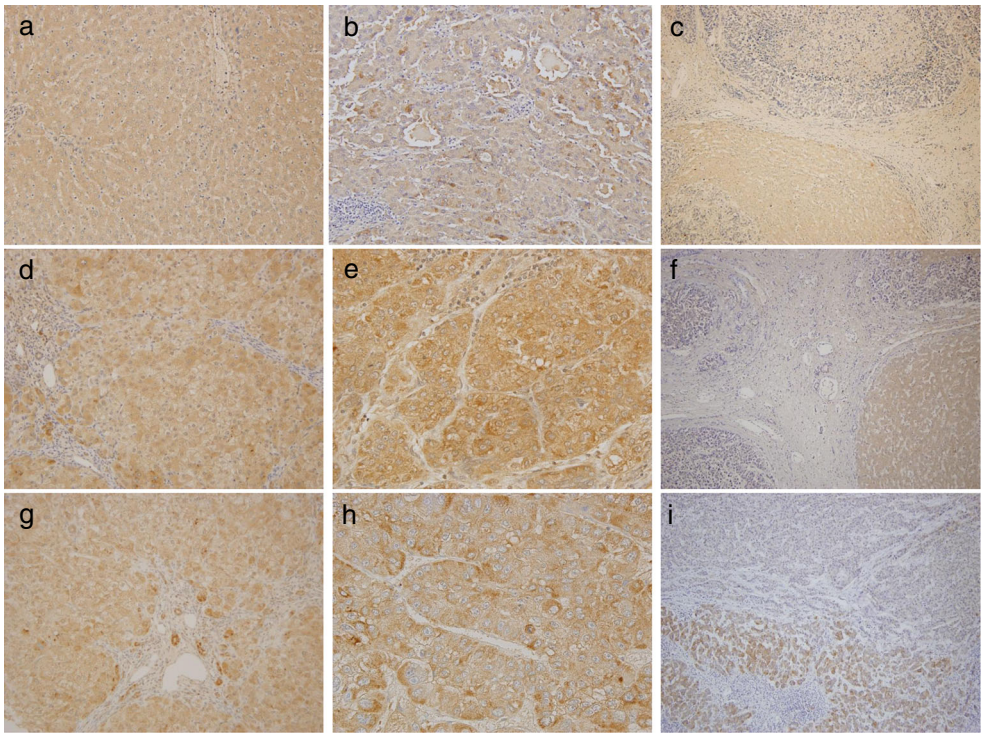

Fig. 1 Representative examples of immunohistochemical expression of adiponectin (**a**–**c**), AdipoR1 (**d**–**f**), and AdipoR2 (**g**–**h**) in non-neoplastic hepatic parenchyma (*left column*) hepatocellular carcinoma (*middle column*) and hepatocellular carcinoma with adjacent non-neoplastic hepatic parenchyme (*right column*) **a**, **b** Positive staining for adiponectin. **c** Negative staining in hepatocellular carcinoma (*upper part*) and positive staining in adjacent non-neoplastic hepatic parenchyme (*lower part*) for adiponectin. **d**, **e** Positive staining for AdipoR1. **f** Negative staining in hepatocellular carcinoma (*right upper* and *left part*) and positive staining in adjacent non-neoplastic hepatic parenchyme (*right lower part*) for AdippoR1. **g**, **h** Positive staining for AdipoR2. **i** Negative staining in hepatocellular carcinoma (*upper part*) and positive staining in adjacent non-neoplastic hepatic parenchyme (*lower part*) for AdippoR2. (**a**, **b**, **d**, **g** ×200. **e**, **h** ×400. **c**, **f**, **i** ×100 magnification)

Our results also indicated that AdipoR1 expression correlated inversely with microscopic vascular invasion (P=0.028).

Correlation Between Adiponectin and Adiponectin Receptor Expression and Disease-Free Survival

The median and mean DFS at the last follow-up appointment were 10.4 and 28.7 months, respectively. The mean DFS of patients with HCC with and without AdipoR1 expression was 75.7 and 21.8 months, respectively, and this difference was statistically significant (P=0.022; Fig. 2b). Multivariate analyses identified AdipoR1 overexpression as an independent positive prognostic indicator of DFS (P=0.033). Other clinicopathological variables, including tumor size (P=0.008), microscopic vascular invasion (P=0.046), and tumor multiplicity (P<0.000), also had significant effects on DFS (Table 4). Adiponectin and AdipoR2 expression was not correlated with DFS in univariate analysis (P=0.632 and 0.558, respectively; Fig. 2a and c).

Correlation Between Adiponectin and Adiponectin Receptor Expression and Overall Survival

The median and mean OS at the last follow-up appointment were 18.6 and 82.5 months, respectively. Univariate

Table 2 Expression frequencies of adiponectin and adiponectin receptors

		Number of cases (%)		
		Negative	Positive	
Adiponectin	Hepatocellular carcinoma	13/75 (17.3 %)	62/75 (82.7 %)	P=0.005*
	Non-tumorous hepatic tissue	2/70 (2.9 %)	68/70 (97.1 %)	
Adiponectin receptor 1	Hepatocellular carcinoma	57/75 (76.0 %)	18/75 (24.0 %)	P=0.000*
	Non-tumorous hepatic tissue	7/70 (10.0 %)	63/70 (90 %)	
Adiponectin receptor 2	Hepatocellular carcinoma	7/75 (9.3 %)	68/75 (90.7 %)	P=0.064
	Non-tumorous hepatic tissue	1/70 (1.4 %)	69/70 (98.6 %)	

*P<0.05

Table 3 Correlation analysis of clinicopathologic parameters and adiponectin/adiponectin receptor expression

	Adiponectin		AdipoR1		AdipoR2	
	Negative (n=13)	Positive (n=62)	Negative (n=57)	Positive (n=18)	Negative (n=7)	Positive (n=68)
Age (year)	0.369		0.443		0.479	
Mean ± SD	59.9±8.9	57.4±8.8	58.3±8.0	56.4±12.1	59.9±7.3	57.7±9.3
Size (cm)	0.015*		0.987		0.896	
Mean ± SD	7.1±3.8	4.0±2.9	4.5±3.2	4.5±3.1	4.6±1.2	4.5±3.2
Sex	0.650		0.678		0.196	
Male (n=66)	11/66 (16.7 %)	55/66 (83.3 %)	49/66 (74.2 %)	17/66 (25.8 %)	5/66 (7.6 %)	61/66 (92.4 %)
Female (n=9)	2/9 (22.2 %)	7/9 (77.8 %)	8/9 (88.9 %)	1/9 (11.1 %)	2/9 (22.2 %)	7/9 (77.8 %)
Number of lesion	1.000		1.000		1.000	
1 (n=59)	11/59 (18.6 %)	48/59 (81.4 %)	45/59 (76.3 %)	14/59 (23.7 %)	6/59 (10.2 %)	53/59 (89.8 %)
More than 1 (n=15)	2/15 (13.3 %)	13/15 (86.7 %)	11/15 (73.3 %)	4/15 (26.7 %)	1/15 (6.7 %)	14/15 (93.3 %)
ES grade	0.021*		0.795		0.003*	
I (n=2)	0/2 (0 %)	2/2 (100 %)	1/2 (50 %)	1/2 (50 %)	0/3 (0 %)	3/3 (100 %)
II (n=33)	2/33 (6.1 %)	31/33 (93.9 %)	26/33 (78.8 %)	7/33 (21.2 %)	1/33 (3.0 %)	32/33 (97.0 %)
III (n=37)	9/37 (24.3 %)	28/42 (75.7 %)	28/37 (75.7 %)	9/37 (24.3 %)	4/37 (10.8 %)	33/37 (89.2 %)
IV (n=3)	2/3 (66.7 %)	1/3 (33.3 %)	2/3 (66.7 %)	1/3 (33.3 %)	2/3 (66.7 %)	1/3 (33.3 %)
PV thrombus	0.059		0.496		0.606	
Present (n=14)	5/14 (35.7 %)	9/14 (64.3 %)	12/14 (85.7 %)	2/14 (14.3 %)	2/14 (14.3 %)	12/14 (85.7 %)
Absent (n=61)	8/61 (13.1 %)	53/61 (86.9 %)	45/61 (73.8 %)	16/61 (26.2 %)	5/62 (8.1 %)	57/62 (91.9 %)
Microscopic VI	0.115		0.028*		0.098	
Present (n=29)	8/29 (27.6 %)	21/29 (72.4 %)	26/29 (89.7 %)	3/29 (10.3 %)	5/29 (17.2 %)	24/29 (82.8 %)
Absent (n=46)	5/46 (10.9 %)	41/46 (89.1 %)	31/46 (67.4 %)	15/46 (32.6 %)	2/47 (4.3 %)	45/47 (95.7 %)
Capsule invasion	0.095		0.186		0.015*	
Invasion or perforation (n=7)	3/7 (42.9 %)	4/7 (57.1 %)	7/7 (100.0 %)	0/7 (0.0 %)	3/7 (42.9 %)	4/7 (57.1 %)
Absent (n=68)	10/68 (14.7 %)	58/68 (85.3 %)	50/68 (73.5 %)	18/68 (26.5 %)	4/68 (5.9 %)	64/68 (94.1 %)
Other organ involvement	0.028*		0.575		0.177	
Present (n=2)	2/2 (100.0 %)	0/2 (0.0 %)	2/2 (100.0 %)	0/2 (0.0 %)	1/2 (50.0 %)	1/2 (50.0 %)
Absent (n=73)	11/73 (15.1 %)	62/73 (84.9 %)	55/73 (75.3 %)	18/73 (24.7 %)	6/74 (8.1 %)	68/74 (91.9 %)
pT stage	0.214		0.222		0.008*	
I (n=42)	6/42 (14.3 %)	36/42 (85.7 %)	28/42 (66.7 %)	14/42 (33.3 %)	2/43 (4.7 %)	41/43 (95.3 %)
II (n=23)	5/23 (21.7 %)	18/23 (78.3 %)	19/23 (82.6 %)	4/23 (17.4 %)	4/23 (17.4 %)	19/23 (82.6 %)
III (n=1)	0/1 (0 %)	1/1 (100.0 %)	1/1 (100.0 %)	0/1 (0.0 %)	0/1 (0.0 %)	1/1 (100.0 %)
IV (n=8)	1/8 (12.5 %)	7/8 (87.5 %)	8/8 (100.0 %)	0/8 (0.0 %)	0/8 (0.0 %)	8/8 (100.0 %)

*P<0.05; *SD* standard deviation; *NS* not significant; *ES* Edmonson-Steiner; *PV* portal vein; *VI* vascular invasion

analysis indicated that adiponectin and AdipoR2 expression was correlated with OS (P<0.001 for both). The mean OS of patients with HCC with and without adiponectin expression was 90.5 and 19.2 months, respectively, and that of patients with HCC with and without AdipoR2 expression was 89.8 and 18.4 months, respectively (Fig. 3a and c). AdipoR1 expression was not associated with OS (P=0.493; Fig. 3b). Multivariate analyses identified AdipoR2 overexpression as an independent positive prognostic indicator of OS (P=0.006). Other clinicopathological variables, including tumor size (P=0.004) and background liver disease (P=0.005), also had a significant effect on OS (Table 5).

Discussion

A significant proportion of obesity-associated HCC develops in patients with cryptogenic cirrhosis, which is largely associated with the progression of nonalcoholic fatty liver disease; thus, the close relationship between obesity and the risk for HCC has been thought to result from the progression of underlying nonalcoholic fatty liver disease to cirrhosis [23, 24]. However, since epidemiologic studies in hepatitis-endemic areas also indicated a relationship between overweight status and HCC progression in hepatitis virus-positive populations [25, 26], obesity has unequivocally been considered a risk factor for HCC.

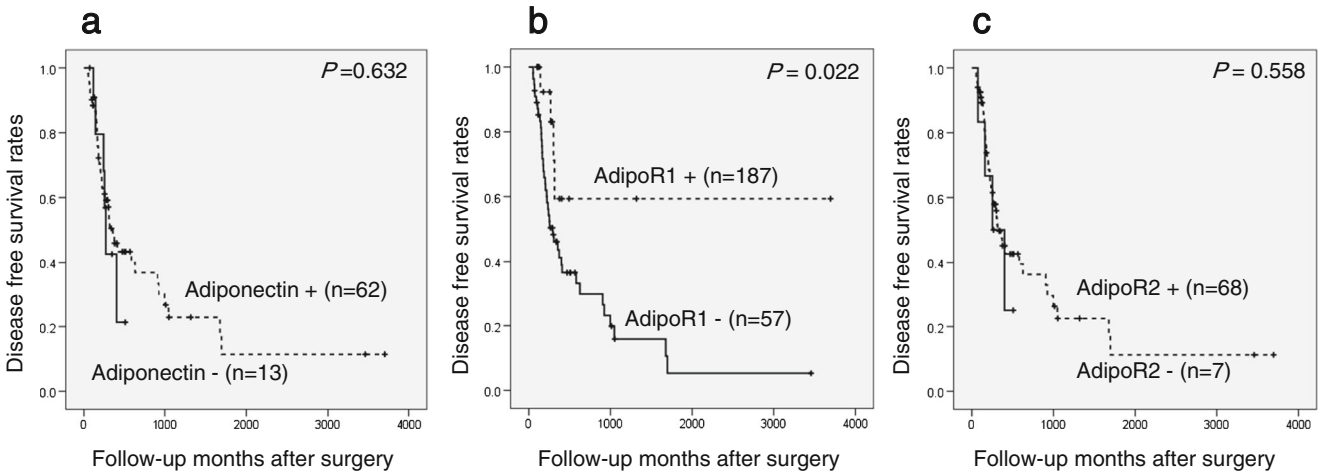

Fig. 2 Correlation between disease-free survival rates and the expression of adiponectin and adiponectin receptors. **a** Kaplan-Meier survival curves for patients with HCC according to adiponectin expression (P=0.632). **b** Kaplan-Meier survival curves for patients with HCC according to AdipoR1 expression (P=0.022). **c** Kaplan-Meier survival curves for patients with HCC according to AdipoR2 expression (P=0.558)

Adiponectin, a relatively abundant adipokine, has attracted attention as a target for versatile treatment strategies for metabolic syndrome because it possesses insulin-sensitizing, anti-inflammatory, and anti-atherogenic activities. In addition, many epidemiologic studies have indicated that plasma adiponectin levels were lower in obese individuals than in lean individuals [5]. However, recent studies have suggested that adiponectin and its receptors are closely related to the progression and development of obesity-related malignancies, and the effect of adiponectin has been reported even in non-obesity-related malignancies. Two adiponectin receptors, AdipoR1 and AdipoR2, are uniquely distributed. AdipoR1 is abundantly expressed in the skeletal muscle and is moderately expressed in endothelial cells and other tissues. AdipoR2 is predominantly expressed in the liver [15].

Although several in vitro studies have shown that adiponectin influences HCC development, and epidemiologic studies have described the serum adiponectin levels in HCC patients, investigations into adiponectin or adiponectin receptor expression and its clinical implications in HCC tissues are very limited and these did not include control tissues [17, 21, 22]. Herein, we evaluated the expression of adiponectin and adiponectin receptors in HCC tissues using immunohistochemistry and found that adiponectin and AdipoR1 were expressed at significantly lower rates in HCC tissues than in corresponding non-neoplastic hepatic tissues and that the AdipoR2 expression rate was lower in HCC tissues, although the latter difference was not significant. In the previous studies, gastric and prostatic cancers were shown to have lower adiponectin receptor expression than the normal controls [27, 28] and many studies reported that the circulating adiponectin levels were lower in patients with various malignancies than in healthy people [7, 8, 29, 30]. These findings suggest that downregulation of the adiponectin/adiponectin receptor axis might be associated with tumorigenesis in at least some cancer types.

We also found a significant relationship between adiponectin/adiponectin receptor expression and several clinicopathological parameters in cases of HCC. First, adiponectin immunopositivity was associated with a small tumor size. This finding is consistent with those of previous studies by Saxena et al. and Sharma et al. [21, 22]. The anti-proliferative effects of adiponectin have been demonstrated in many types of malignancies, including HCC, in in vitro and in vivo studies [9, 11, 21, 31], and various mechanisms to link adiponectin with tumor proliferation have been proposed. Adiponectin improves insulin resistance in obese individuals, thereby decreasing the circulating levels of insulin and insulin-like growth factors that promote cellular proliferation. Adiponectin regulates cell proliferation and apoptosis

Table 4 Cox regression analysis of prognostic factors for disease-free survival

Variables	HR	95 % CI	P value
AdipoR1 expression (positive vs. negative)	3.222	1.098–9.457	0.033*
Tumor size (<4.5 cm vs. >4.5 cm)	2.508	1.587–32.436	0.008*
Number of tumors (single vs. multiple)	4.040	2.252–483.805	0.000*
Microscopic vascular invasion (absent vs. present)	2.196	0.091–4.302	0.046*
Capsule involvement (absent vs. present)	1.266	0.080–1.153	0.635

*P<0.05

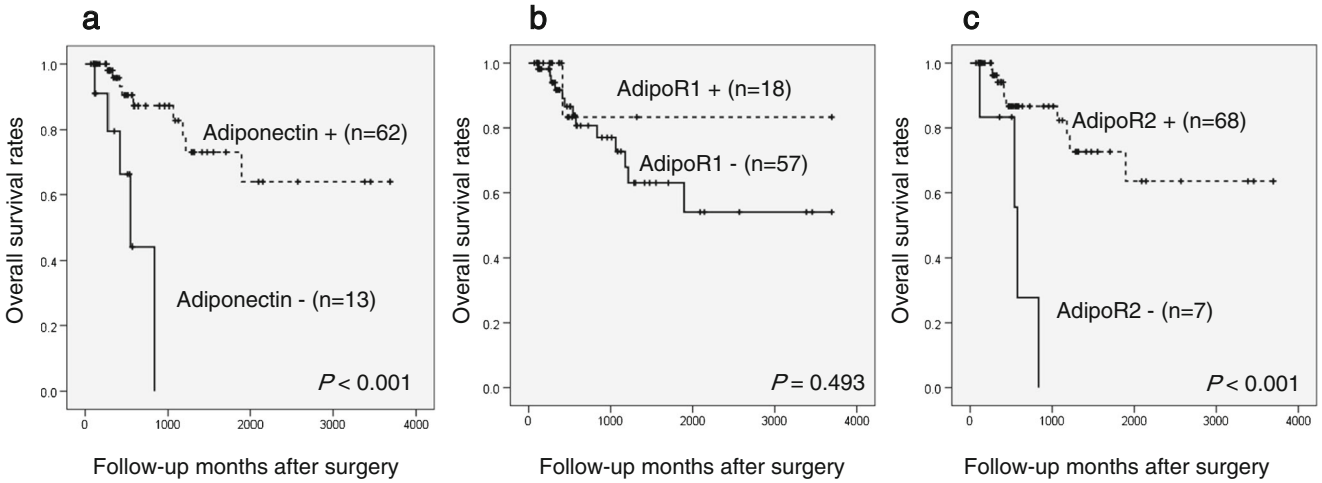

Fig. 3 Correlation between overall survival rates and the expression of adiponectin and adiponectin receptors. **a** Kaplan-Meier survival curves for patients with HCC according to adiponectin expression ($P<0.001$). **b** Kaplan-Meier survival curves for patients with HCC according to AdipoR1 expression ($P=0.493$). **c** Kaplan-Meier survival curves for patients with HCC according to AdipoR2 expression ($P<0.001$)

indirectly through adiponectin receptors and a number of interrelated signaling pathways, including AMPK/mammalian target of rapamycin and c-Jun NH2-terminal kinase/STAT3 (JNK-STAT3) pathways. It selectively binds to and regulates several mitogenic growth factors [32]. In a HCC cell line. adiponectin could inhibit the oncogenic actions of leptin, another important adipokine [22].

In the present study, the pathological parameters that indicate tumor invasiveness were also found to be associated with adiponectin/adiponectin receptor expression in HCC. Adiponectin expression was negatively correlated with other organ involvement. AdipoR1 expression was significantly associated with the absence of microscopic vascular invasion, while immunonegativity for AdipoR2 had association with Glisson capsule invasion. Several studies have elucidated the relationship between adiponectin receptor expression, particularly AdipoR1, and cancer invasiveness. Tsukada et al. observed a negative relationship between AdipoR1 expression and the presence of lymphatic invasion and peritoneal dissemination in human gastric cancer tissues [33]. In addition, low AdipoR1 mRNA expression was associated with lymph node metastasis of non-small cell lung cancer [34]. A negative correlation between low AdipoR1 mRNA expression and venous invasion was reported in colorectal cancer [35], and AdipoR1 and AdipoR2 immunopositivity levels were inversely related to tumor stage in colonic cancer [36]. Also some in vivo studies investigated the relationship between adiponectin and tumor invasiveness. Ishikawa et al. showed that treatment with adiponectin suppressed peritoneal metastasis of gastric cancer in a mouse model [37]. Man et al. sought the underlying mechanism by which adiponectin reduced tumor cell invasiveness in a HCC mouse model. Adiponectin treatment was found to reduce microvessel density and repress hepatic stellate cell activation and macrophage infiltration, which redued tumor invasion and angiogenesis. The authors also observed the suppressive effects of adiponectin on some of the cell signaling pathways associated with invasion, migration, and angiogenesis, such as Rho kinase, interferon-inducible protein 10, matrix metalloproteinase 9, and vascular endothelial growth factor [38].

We also observed an inverse relationship between adiponectin and AdipoR2 expression and the HCC histologic grade. Also in colorectal cancer, the absence of AdipoR1 and AdipoR2 expression was correlated with poor tumor differentiation [36], suggesting that adiponectin/adiponectin receptor expression might disappear during tumor dedifferentiation.

Most importantly, in the present study, patient survival rates were associated with the expression of adiponectin and adiponectin receptors, and AdipoR1 and AdipoR2 were identified as independent favorable prognostic factors for DFS and

Table 5 Cox regression analysis of prognostic factors for overall survival

Variables	HR	95 % CI	*P* value
Adiponectin expression (positive vs. negative)	1.446	0.300–6.974	0.646
AdipoR2 expression (positive vs. negative)	14.797	2.180–100.451	0.006*
Tumor size (<4.5 cm vs. >4.5 cm)	7.437	1.895–29.189	0.004*
Other organ invasion (absent vs. present)	1.462	0.175–12.234	0.726
Background liver disease (cryptogenic liver cirrhosis vs. others)	10.677	2.031–56.131	0.005*

*$P<0.05$

OS, respectively. In gastric cancer, AdipoR1 expression was associated with a good patient prognosis [33, 39] and lung cancer patients with AdipoR1 expression showed longer overall survival [34]. These results suggest that adiponectin receptors could be used as important prognostic factors and therapeutic candidates in some types of cancer, including HCC. In fact, adiponectin per se, adiponectin analogues, and adiponectin receptor agonists have been considered potentially effective anticancer agents with important therapeutic implications. However, several contradictory results have been reported in human clinical studies and in vitro studies with regard to the relationship between adiponectin and tumor progression. Tang et al. showed that adiponectin could direct the migration of chondrosarcoma and prostate cancer cells via adiponectin receptors [40, 41]. AdipoR2 expression was found to be correlated with nodal metastasis and vascular invasion in esophageal and breast cancers [42, 43]. This discrepancy might result from the different methodologies used by the investigators, but could also reflect the complex influence of adiponectin on tumor biology and tissue-type or cell-type dependency [44, 45]. Therefore, the complexity surrounding the biological functions of adiponectin should be fully considered, and further studies are needed to determine its clinical applications.

In conclusion, adiponectin and adiponectin receptor expression is related to HCC development and progression. Adiponectin receptors are suggested to be prognostic markers in HCC patients. Further intensive studies will be indispensable in identifying the exact role of adiponectin and the adiponectin receptors in HCC carcinogenesis and progression. A detailed characterization of the functions of adiponectin will provide insight into novel therapeutic approaches that could modulate this system and ameliorate the heightened risk of HCC.

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