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Role of adiponectin and leptin in non-diabetic, non-obese patients with endometrial cancer

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Summary

Objective: To clarify the roles of adiponectin and leptin in endometrial cancer, this study examined serum concentrations of adiponectin and leptin, intra-tumor expression of two adiponectin receptors (AdipoR-1 and AdipoR-2) and the leptin receptor (ObR), and analyzed their relationship to clinicopathological parameters in non-diabetic, non-obese patients with endometrial cancer. *Materials and Methods:* Samples were obtained from 50 patients with endometrial cancer with fasting blood glucose levels < 120 mg/dl and body mass indices < 30. Serum concentrations of adiponectin, high-molecular weight (HMW) adiponectin, and leptin were measured by immunoassays. Expression of AdipoR-1, AdipoR-2, and ObR in tumor tissue was examined by immunohistochemical staining. *Results:* In endometrial cancer, serum concentrations of adiponectin were higher in patients with G1 or G2 tumors and in patients without lymphovascular space involvement. In 32 patients with AdipoR-1-positive tumors, serum concentrations of adiponectin, HMW adiponectin, and leptin did not differ by any pathological parameter. In contrast, in 18 patients with AdipoR-1-negative tumors, serum adiponectin concentrations were higher in patients with G1 or G2 tumors and in patients without lymphovascular space involvement. High histological grade, positive peritoneal cytology, lymphovascular space involvement, negative AdipoR-1 expression, and lower serum adiponectin levels were associated with poor disease-free survival by univariate regression analysis. In patients with AdipoR-1-negative tumors, disease-free survival was shorter in cases with serum adiponectin concentrations < 11 μg/ml. *Conclusion:* Decreased serum adiponectin concentrations combined with the absence of AdipoR-1 expression in tumors are associated with high-grade tumor, lymphovascular space involvement, and poor prognosis in patients with endometrial cancer.

Key words: Endometrial cancer; Adiponectin; Leptin; Adiponectin receptor.

Introduction

The incidence of and mortality owing to endometrial cancer is rapidly increasing in Japan [1]. The link between obesity and endometrial cancer appears to be an excessive exposure to various factors produced by adipose tissue [2], such as estrogen, insulin, and insulin-like growth factors involved in endometrial tumorigenesis [3]. In addition to these factors, adipose tissue produces bioactive substances called adipokines, the most prominent of which are leptin and adiponectin. These may significantly influence tumor growth and invasion [4, 5].

Leptin, a product of the obese (*Ob*) gene, acts through the leptin receptor (*ObR*). Both leptin and its receptor are expressed more frequently in cancer tissue than in normal tissue [6]. Leptin has various functions, including appetite regulation, bone formation, reproduction, and angiogenesis [7], and may affect processes associated with cancer initiation and progression, resulting in metastasis [6, 8].

In contrast to leptin, adiponectin acts through the adiponectin receptor and may exert antineoplastic activity by suppressing tumor proliferation and neoangiogenesis, and inducing apoptosis [9]. Two adiponectin receptors (AdipoR-1 and AdipoR-2) have been identified to date [10], and their expression has been documented in several human cancer cell lines [11].

Patients with endometrial cancer have higher concentrations of leptin and lower concentrations of adiponectin in their sera compared to controls [12-17]. The balance of leptin and adiponectin levels, rather than their individual concentrations, may reflect physiological changes such as the development of endometrial cancer [17].

In a previous study [18], the present authors reported that AdipoR-1, AdipoR-2, and ObR are expressed in endometrial cancer tissue, and that AdipoR-1 expression inversely correlates with high histological grade, deep myometrial invasion, involvement of lymphovascular space, adnexal invasion, and lymph node metastasis, and is associated with improved progression-free and overall survival. However, that study was unable to address whether correlations exist between serum concentrations of adiponectin or leptin and AdipoR-1 expression in patients with endometrial cancer. Increased serum leptin or decreased serum adiponectin are considered markers for obesity and insulin resistance [19], so samples obtained from non-diabetic and non-obese individuals must be used to investigate the role of adiponectin and leptin in endometrial cancer.

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This study aimed to clarify the roles of adiponectin and leptin in relation to their receptors in endometrial cancer by examining their serum concentrations, along with intra-tumor expression of AdipoR-1, AdipoR-2, and ObR, and analyzing their relationship to clinicopathological parameters in non-diabetic, non-obese patients with endometrial cancer.

Materials and Methods

Between January 2006 and May 2013, the authors obtained formalin-fixed, paraffin-embedded tumor tissues and sera from 50 patients with endometrial cancer with fasting blood glucose levels < 120 mg/dl and body mass indices < 30 (endometrial cancer group). All patients visited the Gynecology Clinic at the Aichi Medical University Hospital (Nagakute, Japan) and were diagnosed with the International Federation of gynecology and Obstetrics (FIGO) Stage IA or IB endometrial endometrioid adenocarcinoma based on post-surgical assessment. Control serum samples were obtained from 49 patients with benign uterine disease (uterine fibroids, n=34; uterine prolapse, n=15), fasting blood glucose levels < 120 mg/dl, and body mass indices < 30 (control group). All study protocols were approved by the regional ethics committee of Aichi Medical University, School of Medicine (Nagakute, Japan). Informed consent was carried out in the form of an inclusion agreement. Study information was placed on the official Internet homepage of Aichi Medical University Hospital and written informed consent was obtained before therapy initiation from all patients prior to study enrollment.

Endometrial cancer tissues were analyzed by immunohistochemistry to examine the intra-tumor expression of AdipoR-1, AdipoR-2, and ObR. The detailed procedure using the Polymer Component was described in a previous report [18]. Primary antibodies used were rabbit anti-human AdipoR-1 antiserum (raised against amino acid residues 357-375), rabbit anti-human AdipoR-2 antiserum (raised against amino acid residues 374-386), and rabbit anti-human ObR antiserum (raised against amino acid residues 541-840, used at dilutions of 1:500, 1:200, and 1:200, respectively. Tissues were defined as having positive expression when > 50% of tumor cells demonstrated intense staining under microscopic examination (magnification, ×200).

Measurements of serum concentrations of adiponectin, high-molecular weight (HMW) adiponectin, and leptin were carried out at SRL, Inc. Serum concentrations of adiponectin were measured with a latex particle-enhanced turbidimetric immunoassay using the Human Adiponectin Latex Kit. Serum concentrations of HMW adiponectin were measured by chemiluminescent enzyme immunoassay (CLIA) using the Human High-Molecular Adiponectin Kit. Serum concentrations of leptin were measured by radio immunoassay using the Human Leptin RIA kit.

Statistical analyses were performed using StatView 5.0. Differences between assessed parameters were analyzed using the unpaired *t*-test and Fisher's exact test. Disease-free survival was analyzed by the Kaplan-Meier method with a log-rank test and regression analysis using the Cox proportional hazards model. $P < 0.05$ was considered statistically significant.

Results

Age and BMI did not differ between the endometrial cancer and control groups. Serum concentrations of adiponectin and HMW adiponectin were lower, while serum concentrations of leptin were higher, in the endometrial cancer group compared to the control group (Table 1).

Table 1. — *Age, body mass index (BMI), and serum concentrations of adiponectin, high-molecular weight adiponectin, and leptin in 50 non-diabetic, non-obese patients with endometrial cancer and 49 controls.*

	Endometrial cancer n=50 (M±SD)	Control n=49 (M±SD)	*p*-value
Age (years)	58.52±10.50	58.10±9.75	n.s.
BMI (kg/m^2)	22.85±3.25	22.25±3.18	n.s.
Adiponectin (μg/ml)	11.31±5.97	16.77±5.44	< 0.0001
HMW AdpN (μg/ml)	4.73±2.73	7.41±3.19	< 0.0001
Leptin (ng/ml)	16.22±5.89	9.98±4.351	< 0.0001

HMW AdpN: high-molecular weight adiponectin; n.s.: not significant.

Table 2. — *Serum concentrations of adiponectin, high-molecular weight adiponectin, and leptin compared by pathological parameters in 50 non-diabetic, non-obese patients with endometrial cancer.*

	n	Adiponectin (μg/ml) M±SD	HMW AdpN (μg/ml) M±SD	Leptin (ng/ml) M±SD
Grade				
G1/2	43	12.29±5.84	5.06±2.78	15.82±4.71
G3	7	5.29±1.68	2.66±0.85	18.63±10.97
		$p = 0.0030$	$p = 0.0293$	n.s.
Myometrial invasion				
< 1/2	38	11.48±5.58	4.73±2.56	16.64±5.87
≥ 1/2	12	10.77±7.32	4.72±3.33	14.88±6.01
		n.s.	n.s.	n.s.
Peritoneal cytology				
Negative	45	11.84±6.02	4.92±2.79	16.52±6.08
Positive	5	6.54±2.49	2.95±1.14	13.44±2.74
		n.s.	n.s.	n.s.
LVSI				
Negative	40	12.51±6.03	5.08±2.92	16.04±5.85
Positive	10	6.49±2.01	3.29±0.84	16.92±6.32
		$p = 0.0033$	n.s.	n.s.

HMW AdpN: high-molecular weight adiponectin;
LVSI: lymphovascular space involvement; n.s.: not significant.

Among the 50 patients in the endometrial cancer group, serum concentrations of adiponectin were higher in patients with G1 or G2 tumors compared to those with G3 tumors, and in patients without lymphovascular space involvement compared to those with involvement; however, there was no difference based on myometrial invasion or peritoneal cytology status. Serum concentrations of HMW adiponectin were also higher in patients with G1 or G2 tumors compared to those with G3 tumors, but did not differ by myometrial invasion, peritoneal cytology, or lymphovascular space involvement. Serum concentrations of leptin did not differ by any pathological parameter (Table 2). There were no differences in serum concentrations of adiponectin, HMW adiponectin, or leptin by the presence or absence of Adi-

Table 3. — *Serum concentrations of adiponectin, high-molecular weight adiponectin, and leptin compared by intratumor expression of AdipoR-1, AdipoR-2, and ObR in 50 non-diabetic, non-obese patients with endometrial cancer.*

	n	Adiponectin (μg/ml) M±SD	HMW AdpN (μg/ml) M±SD	Leptin (ng/ml) M±SD
AdipoR-1 expression				
Negative	18	10.29±5.85	4.42±2.20	16.44±7.96
Positive	32	11.88±6.05	4.90±3.00	16.09±4.49
	n.s.	n.s.	n.s.	
AdipoR-2 expression				
Negative	18	10.64±5.10	4.33±2.10	17.21±7.60
Positive	32	11.68±6.45	4.95±3.03	15.03±4.51
	n.s.	n.s.	n.s.	
ObR expression				
Negative	23	11.47±6.56	5.01±3.03	16.74±6.66
Positive	27	11.17±5.55	4.48±2.47	15.03±5.02
		n.s.	n.s.	n.s.

HMW AdpN: high-molecular weight adiponectin; n.s.: Not significant.

Table 4. — *Serum concentrations of adiponectin, high-molecular weight adiponectin, and leptin compared by clinicopathological characteristics in 32 endometrial cancer patients with AdipoR-1-positive tumors.*

		Positive AdipoR-1expression (n=32)		
	n	Adiponectin (μg/ml) M±SD	HMW AdpN (μg/ml) M±SD	Leptin (ng/ml) M±SD
Grade				
G1/2	31	11.98±6.12	4.94±3.04	16.19±4.52
G3	1	8.6	3.47	13
Myometrial invasion				
< 1/2	27	11.70±5.38	4.79±2.65	16.44±4.74
≥ 1/2	5	12.86±9.70	5.46±4.90	14.20±2.23
		n.s.	n.s.	n.s.
Peritoneal cytology				
Negative	30	12.06±6.21	4.98±3.08	16.10±4.61
Positive	2	9.10±1.41	3.62±1.09	15.90±2.69
		n.s.	n.s.	n.s.
LVSI				
Negative	28	12.44±6.26	5.17±3.11	16.11±4.71
Positive	4	7.93±1.26	2.97±0.88	15.93±2.84
		n.s.	n.s.	n.s.

HMW AdpN: high-molecular weight adiponectin;
LVSI: lymphovascular space involvement; n.s.: not significant.

poR-1, AdipoR-2, or ObR expression (Table 3).

In the 32 patients with AdipoR-1-positive tumors, there was no difference in serum concentrations of adiponectin, HMW adiponectin, or leptin by tumor grade, myometrial invasion, peritoneal cytology, or lymphovascular space involvement (Table 4). In contrast, in the 18 patients with AdipoR-1-negative tumors, serum concentrations of adiponectin were higher in patients with G1 or G2 tumors compared to G3 tumors, and in patients without lymphovascular space involvement, but there was no difference by myometrial invasion or peritoneal cytology status. Serum concentrations of HMW adiponectin were also higher in patients with G1 or G2 tumors compared to those with G3 tumors, although there was no difference by myometrial invasion, peritoneal cytology, or lymphovascular space involvement. Serum concentrations of leptin did not differ by any pathological parameter (Table 5).

Table 5. — *Serum concentrations of adiponectin, high-molecular weight adiponectin, and leptin compared by clinicopathological characteristics in 18 endometrial cancer patients with AdipoR-1-negative tumors.*

		Negative AdipoR-1expression (n=18)		
	n	Adiponectin (μg/ml) M±SD	HMW AdpN (μg/ml) M±SD	Leptin (ng/ml) M±SD
Grade				
G1/2	12	13.07±5.22	5.37±2.05	14.88±5.25
G3	6	4.73±0.92	2.53±0.842	19.57±11.71
		$p = 0.0015$	$p = 0.0052$	n.s.
Myometrial invasion				
< 1/2	11	10.94±6.28	4.57±2.45	17.14±8.30
≥ 1/2	7	9.27±5.41	4.20±1.89	15.36±7.88
		n.s.	n.s.	n.s.
Peritoneal cytology				
Negative	15	11.38±5.82	4.81±2.17	17.37±8.43
Positive	3	4.83±0.71	2.50±1.12	11.80±1.15
		n.s.	n.s.	n.s.
LVSI				
Negative	12	12.67±5.72	4.88±2.54	15.88±8.18
Positive	6	5.53±1.88	3.51±0.81	15.92±7.89
		$p = 0.0097$	n.s.	n.s.

HMW AdpN: high-molecular weight adiponectin;
LVSI: lymphovascular space involvement; n.s.: not significant.

Univariate regression analysis revealed high histological grade, positive peritoneal cytology, lymphovascular space involvement, negative AdipoR-1 expression, and decreased serum concentrations of adiponectin to be associated with poor disease-free survival. However, none of the variables were significantly associated with poor disease-free survival in multivariate regression analysis (Table 6). Kaplan-Meier analysis revealed shorter disease-free survival in the 18 patients with Adipo-R1-negative tumors compared to the 32 patients with Adipo-R1-positive tumors (Figure 1). There was no difference in disease-free survival by the presence or absence of AdipoR-2 or ObR expression. When the cut-off value of serum adiponectin was set at 11 μg/ml (calculated as the mean minus standard deviation from the 49 controls), there was no significant difference in disease-free survival between the 27 patients with serum concentrations of adiponectin < 11 μg/ml and the 23 patients with concentrations ≥ 11 μg/ml (Figure 2). In the 18 patients with AdipoR-1-negative tumors, disease-free survival was shorter in patients with serum concentrations of adiponectin

Table 6. — *Univariate and multivariate regression analyses of variables associated with disease-free survival of 50 patients with endometrial cancer using a Cox proportional hazards model.*

	Univariate analysis			Multivariate analysis		
Variables	HR	95% CI	*p*-value	HR	95% CI	*p*-value
Age (years)	1.04	0.968−1.117	n.s.			
Body mass index (kg/m^2)	1.086	0.842−1.402	n.s.			
Fasting blood glucose (mg/dl)	1.006	0.921−1.099	n.s.			
Grade (G3 *vs*. G1/G2)	0.057	0.010−0.322	0.0012	0.228	0.015−3.553	n.s.
Myometrial invasion (≥1/2 *vs*. <1/2)	0.56	0.102−3.067	n.s.			
Peritoneal cytology (positive *vs*. negative)	0.156	0.028−0.870	0.0341	0.632	0.085−4.696	n.s.
LVSI (positive *vs*. negative)	0.186	0.037−0.937	0.0414	1.018	0.133−7.819	n.s.
AdipoR-1 expression (positive *vs*. negative)	9.635	1.124−82.619	0.0388	3.642	0.289−45.852	n.s.
AdipoR-2 expression (positive *vs*. negative)	1.637	0.328−8.167	n.s.			
ObR expression (positive *vs*. negative)	1.914	0.221−16.539	n.s.			
Serum adiponectin level (μg/ml)	0.75	0.569−0.988	0.041	0.908	0.672−1.226	n.s.
Serum HMW AdpN (μg/ml)	0.688	0.422−1.122	n.s.			
Serum leptin level (ng/ml)	1.074	0.969−1.191	n.s.			

HR: hazard ratio; LVSI: lymphovascular space involvement; HMW AdpN: high-molecular weight adiponectin; n.s.: not significant.

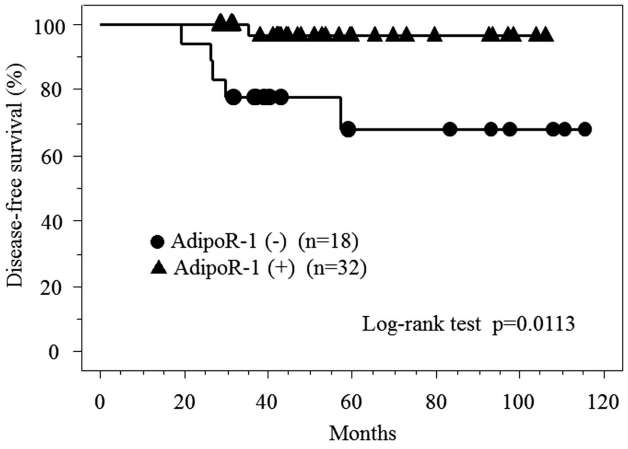

Figure 1. — Disease-free survival (DFS) by Kaplan-Meier analysis of 50 patients with endometrial cancer categorized by intratumor AdpoR-1 expression.

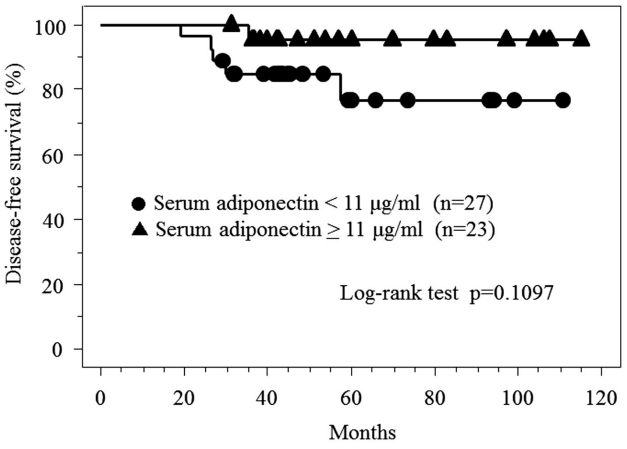

Figure 2. — Disease-free survival (DFS) by Kaplan-Meier analysis of 50 patients with endometrial cancer categorized by serum concentrations of adiponectin.

< 11 μg/ml. However, there was no difference in disease-free survival by serum concentrations of adiponectin in the 32 patients with AdipoR-1-positive tumors. Moreover, disease-free survival was significantly shorter in ten patients with AdipoR-1-negative tumors and serum concentrations of adiponectin < 11 μg/ml compared with 15 patients with AdipoR-1-positive tumors and serum concentrations of adiponectin ≥11 μg/ml (Figure 3).

Discussion

Both the decrease in adiponectin and increase in leptin observed in sera are thought to be associated with obesity or insulin resistance in patients with endometrial cancer [12-15]. The results of this study, which targeted patients with endometrial cancer who were not obese or diabetic, are consistent with previous reports that included obese and diabetic patients. This suggests that adiponectin and leptin are associated with the development and progression of endometrial cancer independent of obesity and diabetes mellitus.

As reported in a previous study [12], patients with endometrial cancer with high-grade tumors or tumors with lymphovascular space involvement showed decreased serum concentrations of adiponectin, although no association was found with concentrations of leptin. Serum concentrations of HMW adiponectin were lower in patients with high-grade tumors, but not in patients with tumors showing lymphovascular space involvement. Adiponectin in blood consists of three isoforms: HMW adiponectin, middle-molecular weight adiponectin, and low-molecular

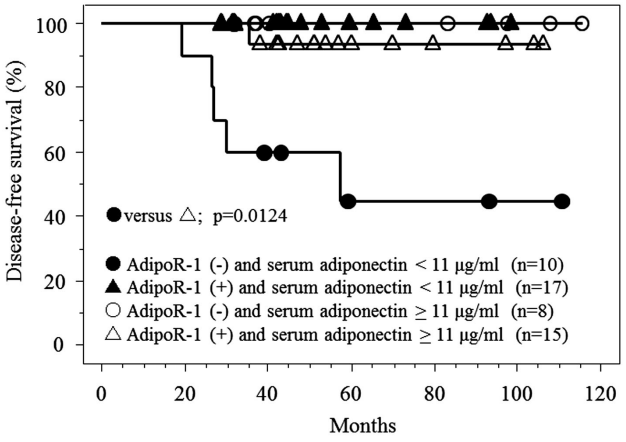

Figure 3. — Disease-free survival (DFS) by Kaplan-Meier analysis of 50 patients with endometrial cancer categorized by intra-tumor AdpoR-1 expression and serum concentrations of adiponectin.

weight adiponectin [20]. A decrease in serum concentrations of middle-molecular weight adiponectin is an independent risk factor for endometrial cancer development [21]. The results of this study suggest that a reduction in middle- or low-molecular weight adiponectin may be associated with lymphovascular space involvement in patients with endometrial cancer.

Consistent with the present authors' previous report [18], AdipoR-1, AdipoR-2, and ObR localized predominantly to the cell membrane and cytoplasm of tumor cells. Of the 50 patients with endometrial cancer, 32 (64%) had AdipoR-1-positive tumors, 32 (64%) had AdipoR-2-positive tumors, and 27 (54%) had ObR-positive tumors. G1 and G2 tumors were positive for AdipoR-1 more often than G3 tumors; however, there was no association with myometrial invasion, peritoneal cytology, or lymphovascular space involvement. AdipoR-2 and ObR expression appeared to be unrelated to any pathological parameter. Histological grade was only associated with AdipoR-1 expression in the present study, while myometrial invasion, lymphovascular space involvement, and histological grade were associated with AdipoR-1 expression in the present authors' previous study [18]. This discrepancy may reflect the inclusion of samples from patients with advanced endometrial cancer in the previous study.

Serum concentrations of adiponectin, HMW adiponectin, and leptin were not related to AdipoR-1 expression in endometrial cancer tissue. In patients with AdipoR-1-positive tumors, serum concentrations of these molecules were not related with any pathological parameter. In contrast, in patients with AdipoR-1-negative tumors, the decrease in serum concentrations of adiponectin was related to tumor grade and lymphovascular space involvement. These results suggest that the effects of adiponectin on tumor progression are not mediated by AdipoR-1 expressed on tumor cells.

Disease-free survival was shorter in patients with AdipoR-1-negative tumors compared to those with AdipoR-1-positive tumors. Disease-free survival was not related to serum concentrations of adiponectin in patients with AdiopR-1-positive tumors, but was shorter in patients with low serum concentrations of adiponectin and AdipoR-1-negative tumors. These data suggest that decreased serum concentrations of adiponectin, combined with a lack of AdipoR-1 expression on tumors, are associated with high tumor grade and lymphovascular space involvement, as well as poor prognosis in patients with endometrial cancer.

Adiponectin suppresses endometrial carcinoma cell proliferation through AdipoRs and upregulates the tumor suppressor gene liver kinase B1 (*LKB1*), which is required for the adiponectin-mediated activation of adenosine monophosphate-activated protein kinase (AMPK). Activation of intracellular signaling by adiponectin leads to a reduction of cell proliferation, colony formation, adhesion, and invasion of endometrial carcinoma cell lines *in vitro* [11, 22]. In addition, adiponectin potently inhibits endothelial cell proliferation and migration *in vitro*, and prevents new blood vessel growth in chick chorioallantoic membrane and mouse corneal angiogenesis assays. The antiendothelial mechanisms involve activation of caspases-8, -9, and -3, leading to endothelial cell apoptosis. Adiponectin significantly inhibits primary tumor growth in a mouse tumor model [9]. Impaired tumor growth appears to be associated with decreased neovascularization, leading to significantly increased tumor cell apoptosis. These data support endothelial cell apoptosis as a unique mechanism for adiponectin-mediated suppression of angiogenesis. As a direct endogenous angiogenesis inhibitor, adiponectin may have therapeutic implications in the treatment of angiogenesis-dependent diseases, such as endometrial cancer.

Recently, many studies have reported the effects of metformin, an antidiabetic drug, on cancer, such as endometrial cancer [23-25]. Pre-clinical endometrial cancer studies indicate that metformin induces cell cycle arrest and apoptosis. Studies investigating anti-cancer mechanisms of metformin focus on both systemic and direct effects on cancer cells. Systemically, metformin improves insulin sensitivity and increases insulin growth factor binding protein, leading to a net reduction in systemic glucose, insulin, and insulin growth factors, and inhibition of tumor growth. Metformin is hypothesized to directly affect cancer cells by acting as an mTOR inhibitor via AMPK activation, ultimately resulting in cell death. A clinical trial in preoperative non-diabetic patients with endometrial cancer found that metformin reduces the number of Ki-67-positive cells in tumors. A meta-analysis revealed that metformin treatment is associated with a significant increase in serum concentrations of adiponectin in patients with polycystic ovarian syndrome [26]. These studies suggest that metformin treatment may have anti-tumor effects on patients with endometrial cancer, particularly in those with decreased serum concentrations of adiponectin.

Several limitations must be considered when interpreting the results of the present study. The authors used hospital-based patients as control subjects. Discrepant characteristics were sometimes observed between the general population and hospital-based references. Secondly, the immunohistochemical expression of AdipoR-1, AdipoR-2, and ObR were analyzed, but gene and protein expression levels were not revealed. Thirdly, the significant correlation between the serum adiponectin concentrations and body mass indices (R^2=0.125, $p = 0.0116$) even in 50 patients with endometrial cancer with fasting blood glucose levels < 120 mg/ml and body mass indices < 30. Fourthly, the postsurgical follow-up periods of patients with endometrial cancer were 30 months to 115 months in the present study, and were less than 60 months in 17 patients. Despite these limitations, the authors considered the present observations to be meaningful, because they provide evidence that the decreased serum adiponectin levels and decreased intra-tumor expressions of AdipoR-1 are possibly a associated with disease-progression and poor prognosis in patients with endometrial cancer.

Conclusion

Decreased serum concentrations of adiponectin, combined with a lack of AdipoR-1 expression on tumors, are associated with high histological grade and lymphovascular space involvement, as well as poor prognosis, in patients with endometrial cancer.

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