Screening for Identification of Personalized Food to Promote Adiponectin Secretion in Patients with Cancer

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Abstract. *Background/Aim: Adiponectin is secreted specifically from adipose tissue. Low serum adiponectin levels may cause metabolic syndrome, which is also a risk factor for carcinogenesis. Several studies have suggested a negative correlation between adiponectin and risk of cancers. This study examined the adiponectin secretion-promoting effect of food ingredients in adipose-derived stem cells (ADSCs) obtained from patients with cancer. Patients and Methods: ADSCs from 7 lifestyle disease cancer patients were differentiated into adipocytes. Subsequently, the adipocytes were treated with 49 food constituents. The adiponectin levels in cell culture supernatants were measured after 48 and 96 h. Results: Soy genistein extract, lychee low-molecular-weight polyphenol, olive extract and turmeric promoted adiponectin secretion. Conclusion: Food constituents that promoted adiponectin secretion were identified using ADSCs derived from patients. This study suggested the possibility of a new treatment approach to prevent cancer recurrence.*

Obesity is increasing around the world. It is a serious global problem. According to a global (188 countries) survey, in 2013, the proportion of adults with obesity (body mass index (BMI) >25) was 37% in men and 38% in women (1), which is about 10% more than that in 1980. The relationship between obesity and metabolic syndrome is one of the risk factors for cancer, as well as cardiovascular diseases and diabetes. Obesity is associated with the development of esophageal, pancreatic, rectal, breast and prostate cancers (2). Adiponectin is an adipocytokine secreted by adipocytes (3, 4). Several studies have suggested a negative correlation between adiponectin and risk of cancer (5, 6). Decreased serum adiponectin is a risk factor for the development of cancers, such as breast (7, 8, 9), endometrial (10), prostate (11), colon (12) and stomach cancer (13). These cancers are known as "lifestyle disease-type cancers". Inflammatory cytokines, such as human tumor necrosis factor (TNF)-α and interleukin (IL)-6, are secreted by matured adipocytes and induce insulin resistance and atherosclerosis (14-16). Interestingly, serum adiponectin levels are decreased by accumulation of visceral fat in patients with metabolic syndrome (17). Adiponectin has anti-inflammatory effects that improve glucose intolerance and dyslipidemia. Furthermore, there is also a negative correlation between the blood adiponectin concentration and development of cardiovascular diseases. Therefore, an increase in the blood adiponectin concentration may prevent the lifestyle disease associated with obesity.

Recently, dietary supplements have been widely used as one of the preventive medical approaches. However, the safety and efficacy of some supplements remains unknown.

Some food constituents may inhibit the metabolic syndrome. Yasueda *et al*. investigated the dietary anti-obesity supplements used in the Japanese market. They found clinical evidence of anti-obesity efficacy only for 11 dietary supplements (18).

Recently, we developed a screening system based on adipose-derived stem cells (ADSCs) (19) with enhanced adiponectin selection capability. In the present study, the ADSCs screening system was used to identify the drug and dietary supplement ingredients that promote adiponectin secretion.

Patients and Methods

Adipose tissue was obtained from 7 cancer patients with lifestyle diseases during prostate, colon and pancreatic surgery (Table I). ADSCs were isolated as reported previously (20). Briefly, tissues were washed several times in phosphate buffered saline (PBS). After removal of other tissues, such as blood vessels, the adipose tissue was minced. They were digested with type 2 collagenase (0.1%, Sigma-Aldrich, St. Louis, MO, USA) solution for 1 h in a 37˚C

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Table I. *Sources of the adipose tissue used to isolate adipose-derived stem cells (ADSCs).*

	Gender	Age	BMI (kg/m^2)	Cancer type
Case 1	Male	59	25.2	Prostate
Case 2	Female	42	31.4	Colon
Case 3	Male	78	24.5	Colon
Case 4	Male	64	24.9	Colon
Case 5	Male	65	25.6	Colon
Case 6	Male	63	25.4	Prostate
Case 7	Male	64	25.6	Pancreatic

water bath under constant shaking. Subsequently, the digested tissues were filtered using a mesh filter (100 μm, 70 μm and 40 μm; BD Falcon, MA, USA) and centrifuged to isolate ADSCs. The isolated ADSCs were placed in a 100-mm culture dish and incubated at 37˚C in a humidified 5% CO_2 atmosphere. They were cultured in a pre-adipocyte medium (PM-1®, Zen-bio, Inc, NC, USA). For assay, ADSCs were placed in 96-well plates and grown to confluence. For differentiation into adipocytes, ADSCs were cultured in an adipocyte differentiation medium (DM-2, Zen-bio®). After culturing for 8 days, cells were placed in another medium (DMEM/Ham's F-12 with L-Gln, sodium pyruvate and HEPES; Nacalai Tesque Inc., Kyoto, Japan) and treated with food constituents for 48 and 96 h (Figure 1). Adiponectin levels of cell supernatants after 48 and 96 h were measured using an ELISA kit specific for human adiponectin (Otsuka Pharmaceuticals, Tokushima, Japan). In order to avoid the maturity effects of fat cells, evaluation was made by calculating a ratio of adiponectin production after 48 and 96 h (adiponectin production ratio (APR)=adiponectin after 96 h/48 h; n=49). The ethical committee of the Osaka University Hospital approved this study.

Results

The results of screening of 49 samples are shown in Table II. APR is referred to as 0.5<+≤1, 1.0<++. According to the APR volumes, soy genistein extract (no. 9), lychee low-molecular-weight polyphenol (no. 13), olive extract (no. 31) and turmeric (no. 45) promoted adiponectin secretion. Soy genistein promoted adiponectin secretion in 4 patients. Lychee low-molecular-weight polyphenol promoted secretion in five patients. Olive extract promoted secretion in five patients. Turmeric promoted four patients. Because of the protocol's regulations, other samples cannot be disclosed.

Discussion

This study screened and identified food constituents that affect adiponectin production using ADSCs obtained from cancer patients with lifestyle diseases. We have previously used the same system for screening of drugs that affect adiponectin production (19). Some drugs, such as pioglitazone hydrochloride, telmisartan, bezafibrate, sodium salt, glibenclamide, glimepiride, gliclazide, metformin and verapamil, increased adiponectin levels in the cell culture supernatants. To the best of our knowledge, this is the first

Table II. *Results of screening 49 samples. Items from the above list that can be disclosed due to protocol's regulations are: soy genistein extract (no. 9), lychee low-molecular-weight polyphenol (no. 13), olive extract (no. 31) and turmeric (no. 45).*

Sample no.	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
1							
2							
3				++		++	
4							
5							
6						+	+
7						+	
8							
9		+		++	+	++	
10							
11							+
12							+
13			+	+	+	+	++
14				+	+	+	
15						+	
16							
17				+	+		
18							
19				+			
20							
21						+	+
22	+	++	++		++		
23				+			
24							
25							
26							
27							
28					+		+
29							
30							+
31			+	+	++	++	++
32						+	+
33						+	
34							
35	+					+	++
36							+
37							
38							
39	+						+
40							
41				+			
42							+
43					+		
44							
45	+	++	++		++		
46	+			+	+		+
47							
48			+		+	+	+
49					+		

0.5<+≤1, 1.0<++

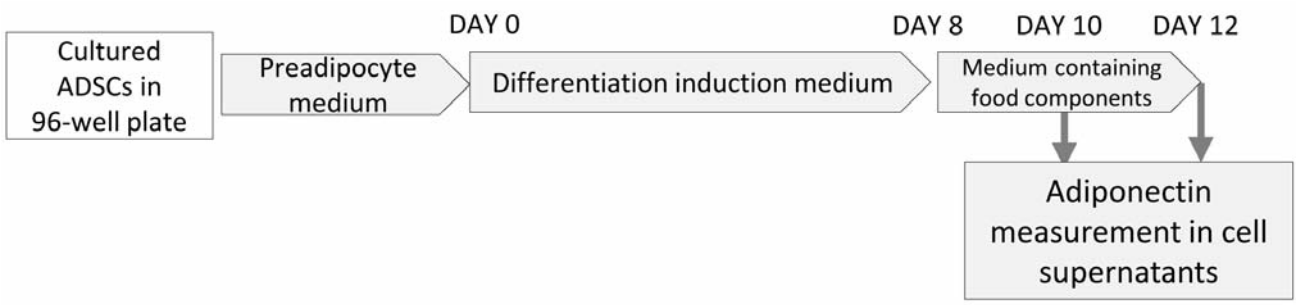

Figure 1. *Study's protocol*.

report of screening for food constituents exhibiting an adiponectin-promoting effect.

Results showed that soy genistein extract, lychee low-molecular-weight polyphenol, olive extract and turmeric had anti-obesity effects and enhanced adiponectin levels. Lychee low-molecular-weight polyphenol has potent antioxidant (23-26), anti-obesity (27) and anti-cancerous (28) effects. It has been suggested that treatment of high-fat-fed mice with lychee polyphenol could regulate adipocytokines' genes in adipocytes (26). It also increases the expression level of the gene encoding adiponectin. Furthermore, anticancer effect(s) and increased adiponectin have been reported after intake of olive extract (29-31). Similarly, turmeric has shown anti-obesity (32-34) and anti-cancer effects (35-38), while increasing adiponectin levels (39). The mechanism by which adiponectin exerts its anti-cancer effects has not yet been elucidated. However, the AMP-activated protein kinase signaling pathway has been reported to be important (40).

In this study, adipose tissue specimens from cancer patients with lifestyle diseases were screened. It has been reported that lower levels of adiponectin and cancer are related, particularly in patients with colon and pancreatic cancer (41-44).

Some food components screened in this study showed adiponectin-enhancing effect. The results of this study indicated that adiponectin secretion-promoting effects of food constituents vary from patient to patient. However, with the screening system used in this study, it is possible to select tailor-made food components to suit individual patients. Lastly, our screening system based on ADSCs may serve as a new therapeutic approach in the lifestyle disease-related cancer patients.

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