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Clinical Study

PPARγ Ligand as a Promising Candidate for Colorectal Cancer Chemoprevention: A Pilot Study

Hirokazu Takahashi,[1] Kunihiro Hosono,[1] Takashi Uchiyama,[1] Michiko Sugiyama,[1] Eiji Sakai,[1] Hiroki Endo,[1] Shin Maeda,[1] Katherine L. Schaefer,[2] Hitoshi Nakagama,[3] and Atsushi Nakajima[1]

[1] *Gastroenterology Division, Graduate School of Medicine, Yokohama City University, 3-9 Fuku-ura, Kanazawa-ku, Yokohama, 236-0004, Japan*
[2] *Gastroenterology and Hepatology Division, University of Rochester, Rochester, NY 14627, USA*
[3] *Biochemistry Division, National Cancer Center Research Institute, Chuo-Ku, Tokyo 104-0045, Japan*

Correspondence should be addressed to Hirokazu Takahashi, hirokazu@med.yokohama-cu.ac.jp

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Activating synthetic ligands for peroxisome proliferator-activated receptor gamma (PPARγ), such as pioglitazone, are commonly used to treat persons with diabetes mellitus with improvement of insulin resistance. Several reports have clearly demonstrated that PPARγ ligands could inhibit colorectal cancer cell growth and induce apoptosis. Meanwhile, aberrant crypt foci (ACF) have come to be established as a biomarker of the risk of CRC in azoxymethane-treated mice and rats. In humans, ACF can be detected using magnifying colonoscopy. Previously, CRC and adenoma were used as a target for chemopreventive agents, but it needs a long time to evaluate, however, ACF can be a surrogate marker of CRC even for a brief period. In this clinical study, we investigated the chemopreventive effect of pioglitazone on the development of human ACF as a surrogate marker of CRC. Twenty-nine patients were divided into two groups, 20 were in the endoscopically normal control group and 9 were in the pioglitazone (15 mg/day) group, and ACF and adenoma were examined before and after 1-month treatment. The number of ACF was significantly decreased (5.8 ± 1.1 to 3.3 ± 2.3) after 1 month of pioglitazone treatment, however, there was no significant change in the number of crypts/ACF or in the number and size of adenomas. Pioglitazone may have a clinical application as a cancer-preventive drug. This investigation is just a pilot study, therefore, further clinical studies are needed to show that the PPARγ ligand may be a promising candidate as a chemopreventive agent for colorectal carcinogenesis.

1. Introduction

Peroxisome proliferator-activated receptor gamma (PPARγ) is expressed in adipose tissue and plays a central role in adipocyte differentiation and insulin sensitivity. Activated synthetic ligands for PPARγ are widely used as treatment for type 2 diabetes mellitus (DM) in order to improve insulin resistance. PPARγ is also overexpressed in many tumors [1–5]. Several studies have reported that treatment of cancer cells with PPARγ ligands induces cell differentiation and apoptosis, suggesting their potential application as chemopreventive agents against carcinogenesis [4, 6, 7]. Recent studies have suggested that PPARγ has an inhibitory effect on cancer cell growth [8–10] and might inhibit cell growth and induce apoptosis in adenocarcinomas [9, 11–13], as well as affect tubulin formation *in vitro* [14]. Initial efforts have focused on activation with PPARγ ligands, as these have been shown to induce G1 cell cycle arrest in a variety of tumor cell lines [15, 16]. Su et al. reported that PPARγ agonist inhibits both initiation and progression of colon tumors in the AOM-mouse model study [17]. We have reported previously that PPARγ ligands suppress colonic epithelial cell turnover and colon carcinogenesis through inhibition of the beta-catenin/T cell factor pathway [18], and PPARγ ligands may be potential chemopreventive agents in an azoxymethane-induced colorectal carcinogenesis model and $Apc^{Min/+}$ mice

TABLE 1: Clinical characteristics of study participants.

	Treatment group		
	Control	Pioglitazone	P value
N	20	9	
Age (years)	63.9 ± 10.0	61.8 ± 5.7	> .05
Waist Circumference (cm)	96.5 ± 13.6	91.8 ± 4.0	> .05
BMI (kg/m^2)	24.4 ± 3.7	23.4 ± 3.1	> .05
VFA (cm^2)	130.0 ± 61.2	146.1 ± 40.9	> .05
SFA (cm^2)	151.5 ± 60.5	141.8 ± 53.5	> .05

Data are expressed as mean ± SD.

model [19, 20]. Colorectal cancer (CRC) is potentially one of the most preventable malignancies [21, 22]. However, the results of clinical trials with PPARγ ligands in CRC have shown only modest results. This implies that focusing on PPARγ as a specific antitumoral target is not likely to be successful, because PPARγ ligands are not an active agent for the treatment of metastatic CRC or liposarcoma [23, 24]. Therefore, we have evaluated chemopreventive effects of PPARγ ligand on the formation of the human aberrant crypt foci (ACF), which is an early stage of colorectal carcinogenesis. ACF were first discovered in mice treated with azoxymethane [25] and have become established as a biomarker of the risk of CRC in azoxymethane-treated mice and rats [26]. In humans, ACF can be detected using magnifying colonoscopy [27]. Previously, CRC and adenoma were used as a target for assessing the efficacy of potential chemopreventive agents; however, this model can only be evaluated over a long period of time. In contrast, the therapeutic efficacy of a product can be evaluated in ACF within a comparatively brief period. We report hire on the results of a study that evaluated the chemopreventive effect of PPARγ ligand by using ACF as a surrogate marker of CRC.

2. Methods

2.1. Magnifying Colonoscopy for Identification of ACF. Bowel preparation for the colonoscopy was carried out using polyethylene glycol solution. A Fujinon EC-490ZW5/M colonoscope was used to perform the magnifying colonoscopy (Fujinon Toshiba ES Systems Co., Ltd, Tokyo, Japan). Total colonoscopy was performed before imaging of rectal ACF. The exclusion criteria included: presence of contraindications to colonoscopy; current or past nonsteroidal anti-inflammatory drug use including aspirin; or family history of CRC; or history of adenoma, carcinoma, familial adenomatous polyposis, inflammatory bowel disease, or radiation colitis. Subjects with a history of colectomy, gastrectomy, or colorectal polypectomy were also excluded. Colorectal adenomas were diagnosed from pathological findings. Subsequently, 0.25% methylene blue was applied to the mucosa of the lower rectal region extending from the middle Houston's valve to the dentate line using spray catheter. ACF were described as lesions consisting of large, thick crypts in methylene blue-stained specimens of the colon (Figure 1). All ACF were recorded photographically and evaluated by two independent endoscopists who were unaware of the subjects' clinical histories. All the patients were divided into two groups, 20 were in the endoscopically normal control group and 9 were in the pioglitazone (PPARγ ligand) group, (15 mg/day); ACF and adenoma were examined before and after 1 month of treatment.

2.2. Measurement of the Visceral and Subcutaneous Fat Areas. Body mass index (BMI) was calculated using the following equation: body weight (kg)/[height (m)^2]. Intra-abdominal adipose tissue was assessed, as previously described, by measuring the visceral fat area (VFA), subcutaneous fat area (SFA), and waist circumference from computed tomographic (CT) images at the level of the umbilicus. All CT scans were carried out with the subjects in the supine position. The borders of the intra-abdominal cavity were outlined on the CT images, and the VFA was quantified using Fat Scan software (N2 System Corporation, Kobe, Japan).

2.3. Statistical Analysis. Data are expressed as mean ± standard deviation (SD), unless otherwise indicated. The relationships between the number of ACF and relevant covariates were examined by univariate regression analysis and determined using the Stat View software (SAS Institute Inc., Cary, NC, USA).

3. Results and Discussion

The clinical characteristics of the study participants are shown in Table 1. There were no significant differences between the groups in terms of their mean age, waist circumference, BMI, VFA, and SFA. The typical colonoscopic features of ACF are shown in Figure 1. The number of ACF was significantly decreased after 1 month's treatment with PPARγ ligand compared with the controls who received no treatment (P = .0226), however, there was no change in the number of crypts/ACF (Table 2) and in ACF size (data not shown). Similarly, after one month of treatment there was no change in the number and size of adenoma (Table 2).

In the present study, pioglitazone treatment decreased the number of ACF, however, the number of crypts/ACF remained unchanged. These results suggest that pioglitazone affects ACF incidence rather than growth. The lack of change in the number and size of the adenomas may have been because the duration of pioglitazone administration was too short to be effective in this respect.

The limitations of this pilot study were its small size, its short duration, and the absence of histological evaluation. Additional research in a large number of subjects is needed to elucidate the clinical effect and benefits of pioglitazone in colorectal carcinogenesis. Chemopreventive trials, the use of medications to prevent disease, have now been carried out extensively in colorectal tumors, for example, supplemental fibers [28], calcium supplementation [29], aspirin [30], nonsteroidal anti-inflammatory drugs (NSAIDs), and selective cyclooxygenase (COX)-2 inhibitors [31, 32], have all been evaluated. Higher doses and longer durations of use of

(a) (b) (c)

FIGURE 1: Typical features of ACF on magnifying colonoscopy with methylene blue staining.

TABLE 2: Effect of PPARγ ligand for human ACF and adenoma.

			Pre-treatment	Post-treatment	*P* value
Number of ACF	Control		5.4 ± 4.0	5.6 ± 5.8	> .05
	Pioglitazone		5.8 ± 1.1	3.3 ± 2.3	.0226
Number of Crypts/ACF	Control		16.8 ± 5.2	17.8 ± 6.4	> .05
	Pioglitazone		14.3 ± 5.9	13.6 ± 6.7	> .05
Number of adenoma	Control		2.0 ± 1.0	1.8 ± 0.8	> .05
	Pioglitazone		2.2 ± 1.5	2.3 ± 2.1	> .05
Mean size of adenoma (mm)	Control		6.9 ± 3.2	7.0 ± 3.3	> .05
	Pioglitazone		5.7 ± 2.8	5.8 ± 2.8	> .05
Location of maximum adenoma	Control	Right side	8	8	
		Left side	12	12	
	Pioglitazone	Right side	4	4	
		Left side	5	5	

Data are expressed as mean ± SD.

NSAIDs and COX-2 inhibitors seem to be associated with greater protection from CRC and adenoma. However, these agents are associated with significant cardiovascular events and/or gastrointestinal harms [33]. Thus, the balance of benefits to risk does not favor chemoprevention by these agents in average-risk individuals. In conclusions, our preliminary results from this pilot study suggest that pioglitazone may have a preventive potential for human ACF and have a good safety profile in this patient population. Further clinical study is required to demonstrate that the PPARγ ligand may be a promising candidate as a chemopreventive agent for colorectal carcinogenesis.

Abbreviations

ACF: Aberrant crypt foci
CRC: Colorectal cancer
PPARγ: Peroxisome proliferator-activated receptor gamma.

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Leptin receptor is involved in STAT3 activation in human colorectal adenoma

Takashi Uchiyama,[1] Hirokazu Takahashi,[1] Michiko Sugiyama,[1] Eiji Sakai,[1] Hiroki Endo,[1] Kunihiro Hosono,[1] Kyoko Yoneda,[1] Masato Yoneda,[1] Masahiko Inamori,[1] Yoji Nagashima,[2] Yoshiaki Inayama,[2] Koichiro Wada[3] and Atsushi Nakajima[1,4]

[1]Gastroenterology Division, [2]Pathology Division, School of Medicine, Yokohama City University, Yokohama, Kanagawa; [3]Department of Pharmacology, School of Dentistry, Osaka University, Osaka, Japan

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The possible role of leptin in colorectal tumors has been investigated in previous studies; however, to date, the conclusions remain under debate. Therefore, we investigated the serum leptin levels in colorectal adenoma patients. In addition, expression of the leptin receptor, and the leptin receptor-mediated signaling pathways were investigated in biopsy specimens collected from human patients with colorectal adenoma. No significant difference in the mean serum leptin level was observed between the colorectal adenoma patients and the control subjects; however, increased expression and activation of the leptin receptor, as indicated by findings such as the phosphorylation of Tyr 1141, was observed in the colorectal adenoma tissues. In addition, activation of the JAK/STAT signaling pathway mediated by the leptin receptor and increased transcriptional regulation of downstream target molecules were observed in colorectal adenomas compared with the non-adenoma tissues. These results indicate STAT3-mediated leptin receptor signaling pathways may be activated in human colorectal adenomas. (*Cancer Sci* 2011; 102: 367–372)

Colorectal cancer is a major cause of mortality and morbidity worldwide;[1] however, the mechanism of colorectal carcinogenesis remains unclear. Recently, the existence of an association between obesity or metabolic abnormalities and an elevated risk of colorectal cancer was reported.[2,3] Adipose tissue was reported to be not only an energy storage organ, but also an active endocrine organ that secretes important adipocytokines such as adiponectin, leptin, tumor necrosis factor-α (TNF-α), free fatty acid and resistin.[4,5]

Leptin is a 167-amino acid peptide that plays a central role in the hypothalamus in relation to mammalian feeding behavior and energy expenditure.[6] Plasma leptin levels have been reported to be strongly correlated with the body mass index (BMI) in humans[7–9] and also to be elevated in obese subjects. Leptin exerts its activity thorough its specific membrane receptor, the leptin receptor (ObR), belonging to the class 1 cytokine receptor family.[5] Two isoforms, the long and short variants of ObR, namely, ObRL and ObRS, have been identified, and only the long isoform of ObR has been shown to have full signaling potential, with the short isoform showing diminished or abolished capacity for signaling.[5,10]

Several studies have reported the association between serum leptin levels and the presence of several cancers such as prostate[11–13] and breast[14,15] cancer. Similarly, previous studies have also shown an association between serum leptin levels and the presence of colorectal cancer.[16–23] However, the results of these previous studies are contradictory and difficult to interpret. While some studies have shown a decrease in the serum leptin levels in colorectal cancer patients,[16–20] others have reported elevated serum leptin levels in colorectal cancer patients.[21–23] Thus, the association between leptin and the presence of colorectal cancer has not yet been clarified. In addition, previous studies have shown that leptin stimulated cell proliferation in several types of carcinoma cell lines *in vitro*.[24–26] However, the molecular mechanisms underlying the promotion of human colorectal carcinogenesis by leptin remain unclear.

In the present study we investigated the association between plasma leptin levels/leptin receptor-mediated signaling and the development of colorectal adenoma.

Materials and Methods

Study population. One hundred and forty-four patients who underwent endoscopic mucosal resection for colorectal adenoma between June 2006 and April 2009 at Yokohama City University Hospital, and 64 control subjects who were detected to have no colorectal polyps on colonoscopy were recruited for this study. The exclusion criteria were subjects with colorectal carcinoma, familial adenomatous polyposis, inflammatory bowel disease, radiation colitis or any malignant disease, and also subjects with a previous history of colectomy, gastrectomy or colorectal polypectomy. Written informed consent was obtained from all subjects prior to their participation in the study. The study protocol was approved by the Yokohama City University Hospital Ethics Committee.

Collection and analysis of blood samples for determination of the leptin levels. Blood samples were obtained in the morning on the day of colonoscopy after the subjects had fasted overnight. Serum leptin levels were measured by enzyme-linked immunosorbent assay of human leptin (SRL Co., Tokyo, Japan).

Immunohistochemical analyses. The expressions of ObR and phospho-STAT3 (p-STAT3) were investigated in the colorectal adenoma and normal colorectal tissues. A total of 61 adenoma tissue samples were obtained endoscopically from the study subjects. Formalin-fixed and paraffin-embedded samples were deparaffinized and rehydrated. The sections were incubated with antibodies for ObR (1:50; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and p-STAT3 (Tyr 705) (1:50; Cell Signaling Technology, Danvers, MA, USA) as the primary antibodies, using an LSAB2 kit (Dako Cytomation, Carpinteria, CA, USA). They were then incubated with biotinylated immunoglobulin as the secondary antibody and treated with peroxidase-conjugated streptavidin. The antibody complex was visualized with 3,3′-diaminobenzidine, tetrahydrochloride (Dojindo Laboratories, Kumamoto, Japan). The expressions of ObR and p-STAT3 were analyzed by light microscopy in 10 different fields of each section, and the mean percentage of adenoma cells that showed positive staining was scored by two pathologists. The ObR and p-STAT3 expressions were classified into two categories depending on the percentage of cells showing positive staining:

[4]To whom correspondence should be addressed.
E-mail: nakajima-tky@umin.ac.jp

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negative, 0–15% of all the tumor cells showing positive staining; and positive, >15% of all tumor cells showing positive staining, as previously described.[27]

Western blot analysis. Twenty-five colorectal adenoma patients were randomly selected, and biopsy samples obtained from the colorectal adenomas and normal areas were isolated. The extracted protein was separated using sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) and the separated proteins were transferred to a polyvinylidene difluoride (PVDF) membrane (Amersham, London, UK). The membranes were probed with primary antibodies specific for phospho-ObR (p-ObR) (Tyr 1141), p-ObR (Tyr 985), ObR (Santa Cruz Biotechnology), phospho-JAK2 (p-JAK2), JAK2, p-STAT3 (Tyr 705) STAT3 (Cell Signaling Technology) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Trevigen, Gaithersburg, MD, USA). Horseradish-peroxidase-conjugated secondary antibodies and the electrochemi-luminescence (ECL) detection kit (Amersham) were used for the detection of specific proteins.

Real-time RT-PCR. Twelve colorectal adenoma patients were randomly selected and biopsy samples of the adenoma and adjacent normal tissues obtained from the colorectal adenoma and normal areas were isolated. Total RNA from the colorectal adenoma and normal tissue biopsy specimens was extracted using the RNeasy mini kit (Qiagen, Hilden, Germany). For the real-time reverse-transcriptase polymerase chain reaction, total RNA was reverse-transcribed into cDNA and amplified using the real-time quantitative polymerase chain reaction using the Step One Plus Real Time PCR System (Applied Biosystems, Foster City, CA, USA). Probes and primer pairs specific for ObRL, ObRS, BclX, c-Myc, cyclin D1, cdc2, cyclin B1, VEGF and 18S were purchased from Applied Biosystems. The concentrations of the target genes were determined using the competitive computed tomography method and the values were normalized to the internal control.

Statistical analysis. Statistical analyses were performed using the Mann–Whitney *U*-test and chi-square test. All analyses were performed using the Stat View software (SAS Institute, Cary, NC, USA). $P < 0.05$ was regarded as denoting statistical significance.

Table 1. Characteristics of the study patients

	Normal	Adenoma	*P* value
N	64	144	
Age (years)	62.1 ± 13.8	64.7 ± 10.2	0.12
Sex (M/F)	33/31	100/44	0.18
Waist circumference (cm)	84.4 ± 10.3	86.5 ± 10.5	0.28
BMI (kg/m^2)	22.7 ± 3.5	23.3 ± 3.2	0.20
VFA (cm^2)	75.7 ± 50.8	93.5 ± 53.5	0.08
FBS (mg/dL)	112.6 ± 26.9	108.7 ± 31.1	0.44
HbA1c (%)	5.7 ± 1.2	5.6 ± 1.0	0.43
Leptin (ng/mL)	5.6 ± 4.3	5.4 ± 4.2	0.70

Data are shown as mean ± standard deviation. Statistical analysis was performed using the Mann–Whitney *U*-test. *$P < 0.05$. **$P < 0.01$. BMI, body mass index; VFA, visceral fat area; FBS, fasting blood sugar.

Results

Serum leptin levels and colorectal tumors. The clinical characteristics of the colorectal adenoma patients and control subjects without colorectal polyps are shown in Table 1. No significant difference in the mean serum leptin level was observed between the two groups. There were also no significant differences in age, BMI or other obesity-related factors between the two groups. A good correlation was observed between the BMI and serum leptin levels ($R = 0.533$, $P < 0.01$) (Fig. 1a), as previously reported.[7–9] We also investigated the differences in the serum leptin levels depending on the tumor size and pathological grade; however, no correlations were observed (Fig. 1b,c).

Leptin receptor, ObR, expression in the colorectal adenoma and normal colorectal tissues. To examine the ObR expression in colorectal adenoma and normal colorectal tissues, immunohistochemical staining and gene expression analyses were performed. ObR was clearly expressed in the cytoplasm of the colorectal adenoma gland cells, but only slightly in the normal colorectal gland cells in the vicinity of the adenomas (Fig. 2a–f). The frequency of detection of ObR in the colorectal adenomas was 67.2% (41/61). For the present study, no isoform-specific antibodies for ObRL and ObRS were available. Therefore, we

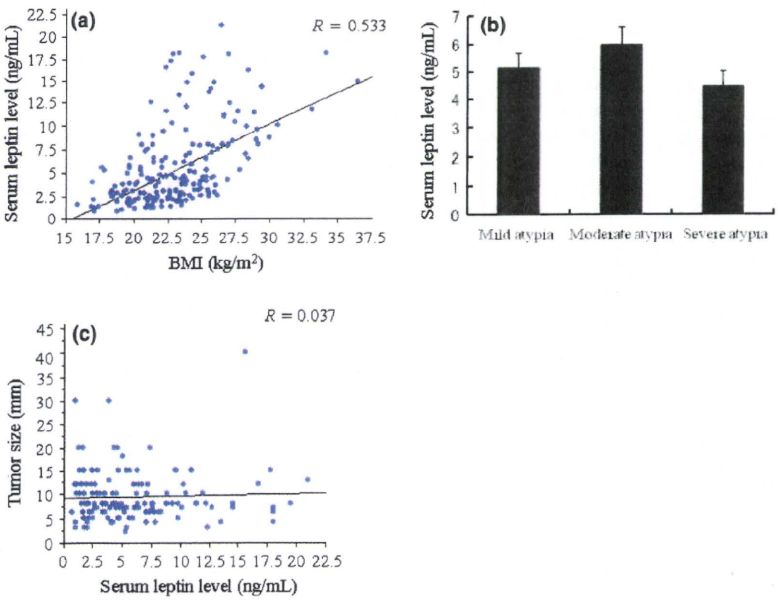

Fig. 1. Correlation between serum leptin levels and body mass index (BMI), tumor pathology and size. (a) Correlation between serum leptin levels and BMI. Each point represents each individual patient ($P < 0.01$, $R = 0.533$). (b) Correlation between serum leptin levels and pathological grade (mild, moderate and severe atypia). Each column represents the mean ± SEM from 23 to 65 patients. (c) Correlation between serum leptin levels and tumor size. Each point represents each individual patient ($P = 0.664$, $R = 0.037$).

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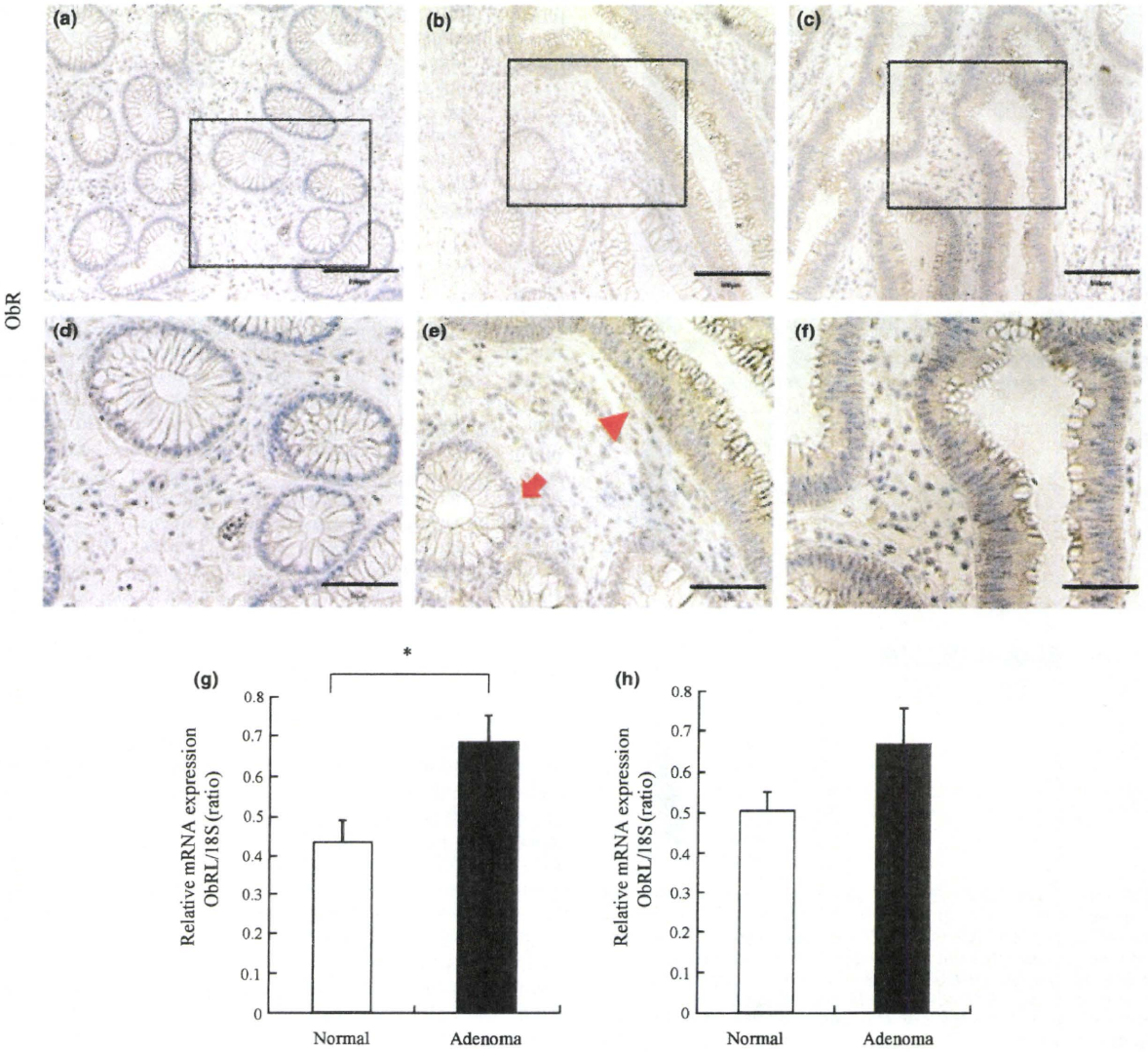

Fig. 2. Immunohistochemical staining for ObR and mRNA level of the leptin receptor. (a) Normal colorectal tissue. (b,c) Colorectal adenoma tissues. (d–f) magnified view of (a–c), respectively. The red arrowhead represents a normal gland and the red arrow points to an adenoma gland. The relative mRNA expressions of (g) ObRL and (h) ObRS in colorectal adenoma and normal colorectal tissues were expressed as the ratio relative to the expression of 18S. Each column represents the mean ± SEM from 12 patients. Statistical analysis was performed using the Mann–Whitney *U*-test. $*P < 0.05$. $**P < 0.01$. ObR, leptin receptor; ObRL, leptin receptor long variant; ObRS, leptin receptor short variant.

conducted gene expression analyses specific for ObRL and ObRS. The mRNA expression levels of ObRL and ObRS in the colorectal adenomas and normal colorectal tissues were investigated. The mRNA expression level of ObRL was significantly higher in the colorectal adenomas than in the normal colorectal tissues. In contrast, the expression of ObRS was slightly but not significantly higher in the colorectal adenomas than in the normal colorectal tissues (Fig. 2g,h). Furthermore, western blot analysis was performed to analyze the phosphorylation level of the cytoplastic domain of ObR to investigate the signaling pathway of the leptin receptor. Western blot analysis showed significant increase of ObR expression in the colorectal adenomas than in the normal colorectal tissues (Fig. 3a). Moreover, the Tyr 1141 phosphorylation level of ObR that is required for leptin-induced activation of STAT3[28] was significantly higher in the colorectal adenomas than that in the normal colorectal tissues. In contrast, no difference was observed in the Tyr 985 phosphorylation level of ObR that is required for activation of the extracellular-signal-regulated kinase (ERK) signaling pathway (Fig. 3b,c).[29] These results suggest that the phosphorylation of ObRL in adenomas might activate the JAK/STAT signaling pathway.

Phosphorylated STAT3 in colorectal adenoma. To investigate the activation of STAT3, immunohistochemical staining and western blot analysis of STAT3 phosphorylation (p-STAT3) status were performed. Expression of p-STAT3 was predominantly observed in the nuclei of the adenoma gland cells, but only faint expression was observed in the normal gland cells (Fig. 4). The percentage of cells showing positive staining for p-STAT3 in the examined tissue specimens of colorectal adenoma was 49.1% (30/61). The expression level of p-STAT3 was significantly higher in ObR-positive adenomas than in ObR-negative adenomas, as shown in Table 2. The western blot analysis showed that the levels of p-JAK2 and p-STAT3 were significantly higher in the adenomas than in the normal colorectal tissues (Fig. 5). In addition, the mRNA levels of the genes

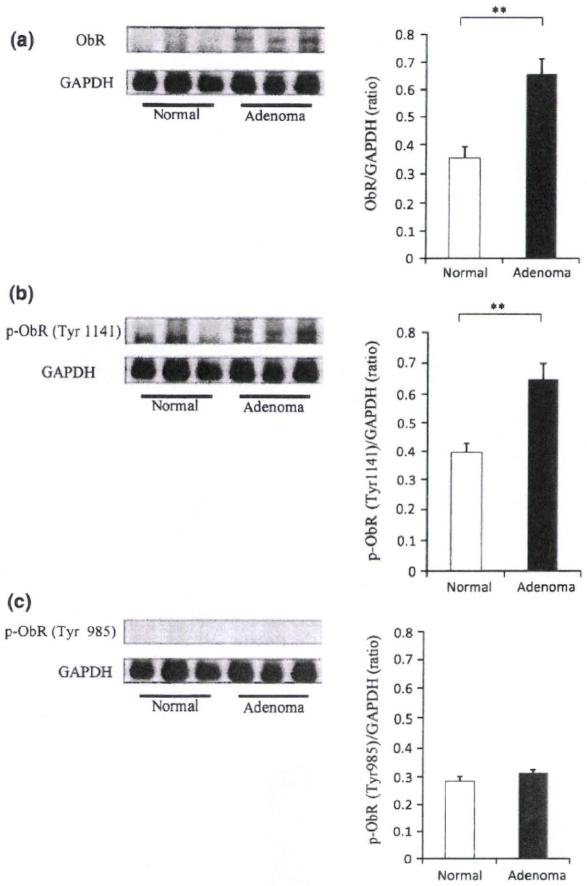

Fig. 3. Western blot analysis for leptin receptor (ObR) and phosphorylated ObR (Tyr 1141 or Tyr 985). (a) ObR, (b) Tyr 1141-phosphorylated and (c) Tyr 985-phosphorylated ObR. Left panels: representative western blot images for ObR, Tyr 1141-phosphorylated and Tyr 985-phosphorylated ObR. Lanes 1, 2 and 3, normal colorectal tissues; lanes 4, 5 and 6, colorectal adenoma tissues. Right panels: ratios of ObR, Tyr 1141-phosphorylated and Tyr 985-phosphorylated ObR expressions to the expression level of GAPDH are shown. Each column represents the mean with the SEM from 25 patients. Statistical analysis was performed using the Mann–Whitney *U*-test. $*P < 0.05$. $**P < 0.01$.

encoded by STAT3 were analyzed by real-time RT-PCR. The expression levels of the apoptosis-suppressing protein BclX, the late G1 to G1/S phase proteins cyclin D1 and c-Myc, the G2/M phase proteins cdc2 and cyclin B1[30] and the genes encoding the angiogenesis protein VEGF[31] were significantly higher in the adenomas than in the normal colorectal tissues (Fig. 6). These results suggest that the JAK/STAT signaling pathway is activated in colorectal adenomas. As shown in Table 3, the mRNA expression levels of BclX, c-Myc, cdc2 and cyclinB1 were significantly higher in ObR-positive colorectal adenomas than in ObR-negative colorectal adenomas, as evaluated by immunohistochemistry.

Discussion

Although recent studies have shown an association between serum leptin levels and the presence/absence of colorectal adenoma, the relationship still remains controversial.[16–23] Serum leptin levels have been shown to be strongly correlated with the BMI.[7–9] Therefore, as bodyweight might influence this correlation, the bodyweight differences should be carefully analyzed while interpreting the above correlation. Patients with cachexia were included in the cancer group in several studies.[18,19] We therefore suspect that this might have influenced the results and caused the conflicts in the results of the previous studies. To reduce the differences in the serum leptin levels caused by the effect of cancer on bodyweight, we investigated the serum leptin levels in patients with colorectal adenoma, which is regarded as a precancerous lesion,[32] not associated with bodyweight loss. Also in the present study, we observed a significant correlation between serum leptin levels and the BMI, consistent with previous reports;[7–9] we consider that this reflects the high reliability of our data. We observed no statistically significant differences in the serum leptin levels between patients with colorectal adenoma and normal control subjects in the present study. This result suggests the possibility of colorectal adenoma being associated with leptin receptor expression or activation, but not with serum leptin concentrations, assuming that leptin plays some role in colorectal adenoma growth. To elucidate this hypothesis, we investigated the expression and signal transduction mediated by leptin receptors in colorectal adenomas.

Several earlier studies have demonstrated, by immunohistochemical analysis, the expression of ObR in colorectal cancer and normal colorectal tissues.[33] In addition, recent studies have also confirmed the expression of ObR in colorectal adenomas and cancers.[34] However, none of the previous studies considered the expression of ObR isofoms, namely, ObRL and ObRS, in the colorectal tissues. In the present study, we showed, by immunohistochemical analysis, that ObR was clearly expressed in colorectal adenomas, but only weakly expressed in normal colorectal tissues. In addition to the immunohistochemical data, colorectal adenomas were also found to show significantly

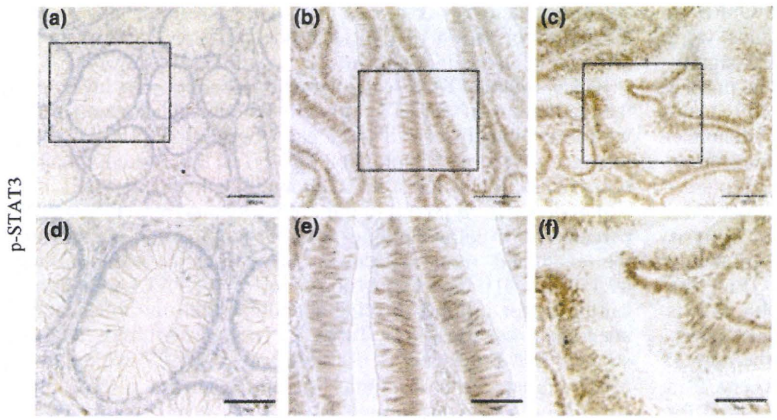

Fig. 4. Immunohistochemical staining for phosphorylated STAT3 in colorectal tissues. (a) Normal colorectal tissue. (b,c) Colorectal adenoma tissues. (d–f) Magnified view of (a–c), respectively.

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Table 2. Correlation between the expressions of ObR and p-STAT3 in colorectal adenomas by immunohistochemical analysis

	ObR-positive adenoma	ObR-negative adenoma	*P* value
p-STAT3-positive adenoma	58.8% (24/41)	30% (6/20)	<0.05*

Data are shown as the percentage and number of phospho-STAT3 (p-STAT3)-positive colorectal adenoma samples in ObR-positive and ObR-negative colorectal adenoma. Statistical analysis was performed using the chi-square test. *$P < 0.05$. **$P < 0.01$. ObR, leptin receptor.

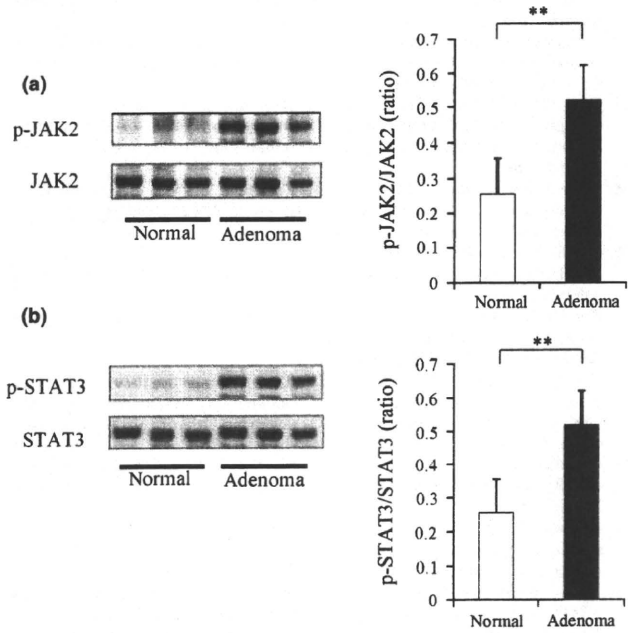

Fig. 5. Western blot analysis for phosphorylated JAK2 and STAT3. (a) Phosphorylated JAK2 and (b) phosphorylated STAT3 in normal colorectal and adenoma tissues. Left panels: representative western blot images for phosphorylated and total levels of JAK2 and STAT3. Lanes 1, 2 and 3, normal colorectal tissues; lanes 4, 5 and 6, colorectal adenoma tissues, respectively. Right panels: ratios of the phosphorylated protein levels compared with the total protein level. Each column represents the mean with the SEM from 25 patients. Statistical analysis was performed using the Mann–Whitney *U*-test. *$P < 0.05$. **$P < 0.01$.

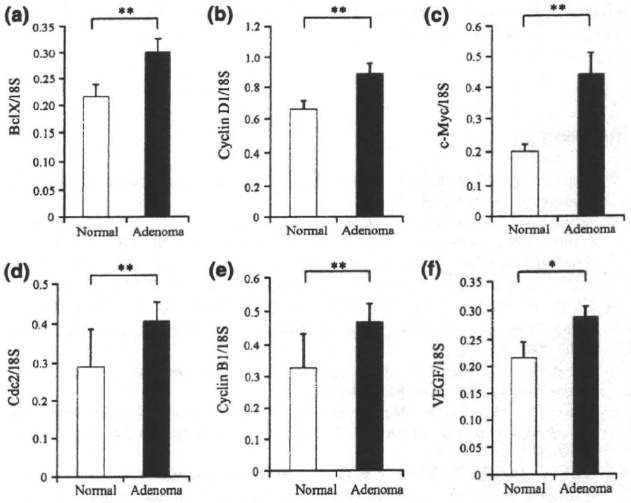

Fig. 6. The expression of downstream genes encoded by STAT3 transcriptional regulation in normal colorectal and adenoma tissues. The relative mRNA expressions of (a) BclX, (b) cyclinD1, (c) c-Myc, (d) cdc2, (e) cyclin B1 and (f) VEGF in colorectal adenoma and normal colorectal tissues were expressed as the ratios relative to the expression of 18S. Each column represents the mean with the SEM from 12 patients. Statistical analysis was performed using the Mann–Whitney *U*-test. *$P < 0.05$, **$P < 0.01$.

Table 3. The expression of downstream genes encoded by STAT3 transcriptional regulation in ObR-positive adenomas and ObR-negative adenomas

	ObR-positive adenoma ($n = 7$)	ObR-negative adenoma ($n = 5$)	*P* value
BclX	0.34 ± 0.09	0.24 ± 0.07	<0.05*
cdc2	0.50 ± 0.12	0.24 ± 0.11	<0.01**
Cyclin D1	0.96 ± 0.17	0.76 ± 0.32	0.21
Cyclin B1	0.58 ± 0.17	0.28 ± 0.07	<0.01**
c-Myc	0.57 ± 0.23	0.21 ± 0.09	<0.01**
VEGF	0.29 ± 0.06	0.28 ± 0.08	0.46

Data are shown as mean ± standard deviation. Statistical analysis was performed using the Mann–Whitney *U*-test. *$P < 0.05$. **$P < 0.01$. ObR, leptin receptor; VEGF, vascular endothelial growth factor.

higher expression levels of the gene for ObRL, but not for ObRS, than normal colorectal tissues. These results suggest that the expression of ObRL rather than ObRS might be important for the downstream signal transduction in colorectal adenomas. Therefore, we investigated the ObRL-mediated signaling pathways in colorectal adenomas. It is known that phosphorylation of Tyr 1141 of ObRL by leptin activates the JAK/STAT signaling pathway.[28] We demonstrated significantly increased phosphorylation of Tyr 1141, but not Tyr 985, in colorectal adenomas than in the normal colorectal tissues. Taken together, these results suggest that induction of ObRL gene expression in colorectal adenomas might augment phosphorylation of Tyr 1141 of ObRL by leptin, which might result in activation of the JAK/STAT signaling pathway. In fact, we showed enhanced activation of the JAK/STAT signaling pathway and higher gene expressions downstream of the STAT3 signaling pathway in colorectal adenomas than in the normal colorectal tissues.

Although we could not show direct evidence of this signaling in human colorectal adenomas, our results provided evidence to suggest that leptin-mediated STAT3 signaling through activation of ObRL in colorectal adenoma might control the expression of genes involved in the cell cycle and apoptosis. Further investigations are required to clarify the growth mechanism of colorectal adenoma.

In conclusion, STAT3-mediated leptin signaling through the activation of ObRL in colorectal adenoma directly controls the expressions of genes involved in the cell cycle and apoptosis, resulting in the growth of adenoma cells.

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Disclosure Statement

The authors declare no conflict of interest.

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特集 メタボリックシンドロームと消化器疾患

Gastrointestinal Research

メタボリックシンドロームと大腸癌

内山 崇* 高橋宏和* 中島 淳*

Summary
わが国では近年，食生活の欧米化に伴い大腸癌罹患率，死亡率が急増している．しかし，肥満による大腸発癌のメカニズムは依然不明な点が多い．これまでに生活習慣，高血糖や脂質異常症以外にも，アディポサイトカイン，インスリンやIGF-1といった肥満関連因子の関与が徐々に明らかになってきている．メタボリックシンドロームを改善するアプローチが，肥満関連大腸癌予防において必要であり，今後更なるメカニズムの解析が望まれる．

Key words
大腸癌 メタボリックシンドローム 肥満 アディポサイトカイン インスリン IGF-1

はじめに

わが国では近年，大腸癌罹患率，死亡率が急増している．女性では乳癌についで癌死の原因第2位，男性では肺癌，胃癌についで第3位となっており，その対策・予防が急務とされている．これまでおこなわれてきた多くの疫学調査から大腸癌増加の背景には糖尿病の増加，肥満・運動不足や肉食・高脂肪食を中心とした欧米型の食事がきわめて重要な危険因子であることが明らかにされているが[1]，これら危険因子による大腸発癌メカニズムは依然不明な点が多い．本稿では，肥満と大腸発癌とのかかわりを概説するとともに最新の知見を提示する．

1 ▌肥満と大腸癌の関連について

肥満，脂肪蓄積が大腸発癌を亢進させるメカニズムは内臓脂肪蓄積の原因としての運動不足・頻回の食事・高脂肪食・肉類の多量摂取などが大腸癌の危険因子である，もしくは脂肪，とくに内臓脂肪蓄積の結果として耐糖能異常・脂質異常や内臓脂肪から分泌されるアディポサイトカインが細胞増殖に作用する可能性が推測される．

2 ▌生活習慣と大腸癌

いわゆるメタボリックシンドロームは肥満に起因しており，食事に関しては赤身肉・高脂肪食やアルコールの摂取などの食生活が大腸発癌の危険因子である一方[2]，身体活動はコホート研究において，大腸癌に対する予防効果が認められている[3][4]．従来，野菜と果物を十分に摂取することは大腸癌予防に効果的であると考えられていたが，最近では，関連がないとする報告がつづいている．わが国の大規模コホート研究の結果でも野菜と果

*UCHIYAMA Takashi, TAKAHASHI Hirokazu, NAKAJIMA Atsushi/横浜市立大学附属病院消化器内科

物を十分摂取しても，大腸癌に対する予防効果はないとしている[5]．

3 ▌高血糖，高インスリン血症と大腸癌

大腸発癌と血糖に関しては多くの研究報告がある．ノルウェーやイタリアの大規模研究では高血糖と大腸癌はおおむね相対危険度1.8～2.0程度の関連を示している[6]．インスリン抵抗性はメタボリックシンドロームの根幹となるが，メタボリックシンドロームの診断基準には含まれていない[6]．インスリンの代謝産物であるC-peptideや糖負荷後2時間値のインスリンで測定したインスリン抵抗性は大腸癌のリスクをおおむね2～4倍亢進させており，高インスリン血症はほぼ大腸癌を促進すると考えてよさそうである[6]．

4 ▌脂質異常と大腸癌

わが国におけるケースコントロール研究では，高中性脂肪血症の大腸腺腫の相対リスクは1.5であり[7]，わが国を含むアジア人種を対象とした研究において高中性脂肪血症と大腸癌は有意に相関する報告が多い．しかし欧米での大規模前向き試験では高中性脂肪血症と大腸癌との相関は有意でなく否定的であり[8]，人種差の存在が示唆されている[7]．家族性大腸腺腫症のモデルマウスである$Apc^{Min/+}$マウスは脂質異常を呈すが，脂質異常症治療薬のベザフィブラートによりポリープ数が減少するという報告があり[9]，ヒトにおける検討が待たれる．

5 ▌アディポサイトカインと大腸癌

内臓脂肪はアディポサイトカインを分泌する生体最大の内分泌臓器であり[10]，アデポネクチン，レプチン，レジスチン，腫瘍壊死因子（tumor necrosis factor：TNF）-αおよび遊離脂肪酸（free fatty acid：FFA）などがある．アディポサイトカインのうちレジスチン，TNF-α，FFAなどは骨格筋や肝臓でインスリンの情報伝達を阻害し，インスリン抵抗性を惹起する[11]．一方，アディポネクチンとレプチンは抗炎症作用を有し腫瘍形成に対し抑制的にはたらく．

1）アディポネクチンと大腸癌

アディポネクチンは1995年にわが国から当初脂肪細胞で最も強く発現する遺伝子として同定され，adipose most abundant gene transcript 1（apM1）として報告された[12]．アディポネクチンは受容体（Adipo R1，2）を介してperoxisomal proliferator-activated receptor-α（PPAR-α），AMP-activated protein kinase（AMPK）を活性化し，骨格筋での脂肪酸代謝，糖取り込み，糖利用の促進，肝臓での糖新生の抑制にはたらく．また*in vivo*において急性投与により血糖値低下作用，脂質蓄積の低減とTNF-αなどの発現抑制により抗動脈硬化作用を示す．アディポネクチンの血清値はレプチンなどほかのアディポサイトカインとは逆に内臓脂肪の増加により低下する．AdipoR1はおもに骨格筋に，AdipoR2はおもに肝臓に多く発現する．アディポネクチンはこれらの受容体を介して標的臓器でAMPKやmitogen-activated protein kinase（MAPK），PPARγなどを活性化させ，mammalian target of rapamycin（mTOR）シグナル経路を抑制することにより大腸癌発癌が抑制する[13]．内臓脂肪型肥満では低アディポネクチン血症が大腸発癌を促進することから，大腸癌予防においてはアディポネクチンを減少させない，すなわち内臓脂肪を増加させないことが重要である．

2）レプチンと大腸癌

レプチンは167のアミノ酸より構成され，視床下部に作用し，エネルギーバランスや節食調整をする[14][15]．肥満者においては血清レプチン値が上昇しているが，レプチン抵抗性のため，生理作用は低下する．レプチン刺激は受容体（ObR）を介して細胞内に伝達される．視床下部以外でも，乳

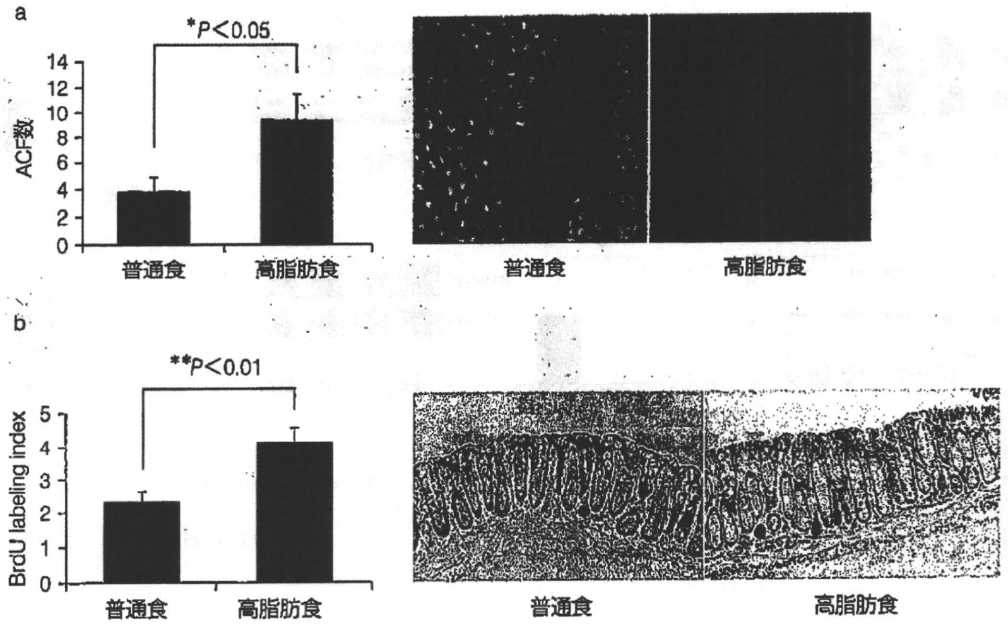

図 1. 高脂肪食負荷における ACF 数および BrdU labeling index の変化
a：マウス 1 匹あたりの ACF 数．*P<0.05．ACF 顕微鏡像（右）．
b：BrdU labeling index の比較．**P<0.01．BrdU 免疫染色像（右）．
高脂肪食群では，ACF 数，BrdU labeling index が有意に増加していた．
ACF：aberrant crypt foci（異常腺窩巣）
（Endo H *et al*, 2009[25] より改変引用）

癌，前立腺癌などでは ObR の発現が強く，レプチンとこれらの癌の関連が示唆されている[16][17]．大腸癌において血清レプチンは減少する[18][19]，変わりない[20]，さらに高レプチン血症が発癌の危険因子である[21][22]など血清レプチン値や，ObR 発現に関して統一した見解は得られていない．ObR に関しては，大腸癌で発現が認められており[23]，レプチンが大腸発癌にも作用する可能性がある．

3）その他アディポサイトカインと大腸癌

血清レジスチン値と大腸癌に関して報告が散見されるが，相関や作用機序は明らかにされていない．TNFα，FFA，plasminogen activator inhibitor type-1（PAI-1）に関しての報告はほとんどなく，今後更なる検討が必要であると考えられる．

6 ▌インスリン，IGF-1 と大腸癌

高インスリン血症の大腸発癌メカニズムはインスリンそのものの細胞の増殖促進作用や[24]，成長ホルモンの刺激により肝臓で産生される insulin like growth factor-1（IGF-1）結合蛋白質の転写を抑制して IGF-1 活性を亢進させ，インスリンと同じく上皮細胞の増殖促進，アポトーシス抑制を介して，発癌を促進させる[25]．

7 ▌今後の研究課題

肥満が大腸癌を促進するメカニズムとして *in vivo* では，モデルマウスを用いた解析が進んでいる．われわれはアゾキシメタン誘発大腸腫瘍モデルマウスにおいて，高脂肪食群では普通食群よりも細胞増殖が有意に上昇しており（図 1），血清インスリン値は高脂肪食群で有意に高いことから，

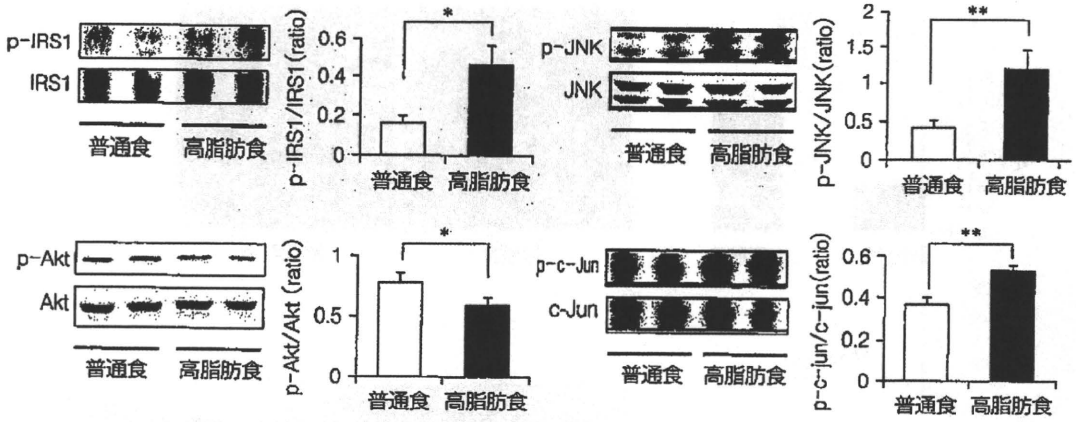

図 2. 大腸上皮における各蛋白の発現量
IRS1：insulin receptor substrate 1, JNK：c-Jun N-terminal kinase
$^{*}P<0.05$ $^{**}P<0.01$

(Endo H *et al*, 2009[26]より改変引用)

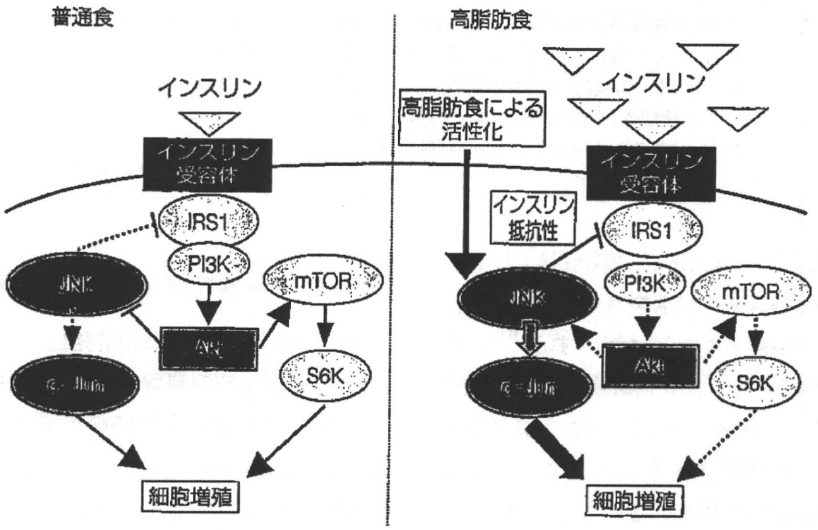

図 3. 高脂肪食条件における，細胞増殖作用仮説
高脂肪食摂取時，インスリンは高値であるにもかかわらずAkt活性は抑制されており，細胞増殖はJNK経路により促されている．
IRS1：insulin receptor substrate 1, JNK：c-Jun N-terminal kinase, PI3K：Phosphoinositide 3-kinase, S6K：S6 kinase

インスリンシグナルの発癌への関与を報告した[26]．大腸上皮の蛋白質解析では，高脂肪食群においてinsulin receptor substrate-1 (IRS-1) のリン酸化は亢進しているものの，Aktのリン酸化はむしろ低下しており，肝臓などインスリン標的臓器におけるインスリン抵抗性と類似した状態が示唆された (図2)．高脂肪食群において細胞増殖シグナル経路のうち，JNK経路の活性が亢進しており，MAPKやmTORを介する経路には差がなかったことから，インスリンによる発癌促進は，

古典的なPI3K/Akt経路ではなく，JNKを介した経路が示唆された（図3）．

おわりに

適切な食事摂取や体重コントロール，運動はメタボリックシンドロームから脱却し，肥満関連大腸癌のリスクを減らす最も確実な道であると考えられる．過剰エネルギーは，メタボリックシンドロームを引き起こし，糖尿病や動脈硬化などを惹起する．さらに余剰エネルギーは行き場をなくし，大腸上皮などの増殖促進に使われ，ひいては発癌促進に至る．今後，肥満患者における大腸発癌予防において生活習慣の改善が浸透し，これらの知見が応用されることを期待する．

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特集Ⅰ 性差からみた消化器疾患の病態と予後

内臓脂肪およびアディポネクチンと大腸発癌の性差*

高橋宏和**
内山　崇**
中島　淳**

Key Words: colorectal cancer, visceral fat, adiponectin, gender difference

はじめに

大腸癌は増加の著しい癌の一つであり，その原因としてライフスタイルの欧米化が考えられている．平成20年厚生労働省人口動態統計によると，全癌における人口10万対死亡率は男性では3位，女性では2位となっている．大腸発癌の原因は遺伝的要因や環境因子などが考えられこれらに男女差はないが，大腸癌罹患率は男性のほうが女性より高く，これにはエストロゲンなどの性ステロイドホルモンの影響が考えられている．また，近年大腸発癌に影響する環境因子として男性において顕著である内臓脂肪型肥満などのメタボリックシンドロームの関与が指摘されており，病態や予後に性差が存在する可能性が示唆されている．本稿では，内臓脂肪およびそれより分泌されるアディポネクチンと大腸発癌の性差について概説する．

肥満，内臓脂肪と大腸癌の疫学的エビデンス

メタアナリシスなどから，肥満は大腸癌のリスクを増加させ[1][2]，body mass index(BMI)が2増えると大腸癌は7％増加し，ウエストが2cm増えると大腸癌は4％増加することが明らかにされている[3]．世界がん研究基金(WCRF)と米国がん研究協会(AICR)の2007年の報告によると，大腸癌のリスクを上げる確実なものとして肥満や内臓脂肪型肥満が挙げられている(表1)[4]．この莫大な疫学的データは男女差を指摘しており，肥満に伴う大腸癌相対リスクは，男性1.5～2.0，女性1.2～1.5であり男性がやや高く，メタアナリシスでは，肥満に伴う大腸癌相対リスクは男性1.6，女性1.3であった．このメカニズムとして，インスリン，インスリン様成長因子-I(insulin-like growth factor-I；IGF-I)，アディポネクチン，レプチン，慢性炎症，閉経後女性におけるエストロゲンの減少などの関与が示唆されている．欧州での37万人規模の6.1年間の前向きスタディー

表1　食事，栄養，運動と大腸癌

	抑制因子	促進因子
確実	身体活動	赤身肉 加工肉 飲酒(男性) 肥満 内臓脂肪型肥満 成人での高身長
ほぼ確実	食物繊維 にんにく 牛乳 カルシウム	飲酒(女性)

(文献[4]より一部引用改変)

* Gender differences between colorectal carcinogenesis and visceral fat or adiponectin.

** Hirokazu TAKAHASHI, M.D., Takashi UCHIYAMA, M.D. & Atsushi NAKAJIMA, M.D.: 横浜市立大学附属病院消化器内科〔〒236-0004 横浜市金沢区福浦3-9〕; Division of Gastroenterology, Yokohama City University Hospital, Yokohama 236-0004, JAPAN

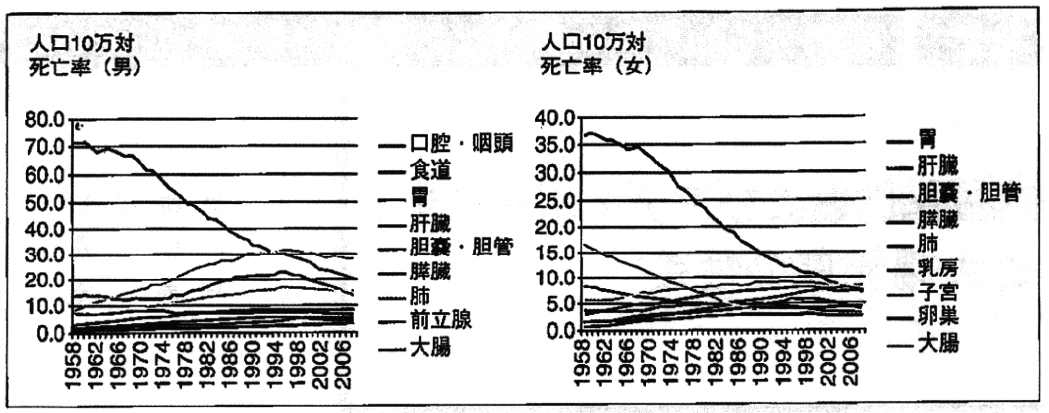

図1　わが国における男女別癌死亡率の年次推移(文献[7]より引用改変)

では，結腸癌とBMIのリスク相関は男性にのみ認められ，女性では認められなかった．サブアナリシスでは身体計測でウエスト周囲径とウエストヒップ比(WHR)が男女ともに結腸癌の危険因子であった[5]．このように非常に信頼性の高い研究において，大腸癌はBMIよりも腹部脂肪型肥満すなわち内臓脂肪型肥満と強く相関することが示されている．

わが国における中高年期の生活習慣と，癌あるいは循環器疾患や全死亡との関連を観察しているJapan Collaborative Cohort Study(JACC Study)では，40歳から79歳の約10万人を1988年から約10年間追跡し結腸癌死亡と，BMIを＜20，20～22，22～24，24～26，26～28，＞28の6群に分け，結腸癌による死亡との関連を検討した．これによると男性では試験開始時のBMIと結腸癌による死亡との関連は認められなかったが，女性では，エントリー時のBMIが大きくなる群ほど結腸癌により死亡するリスクが高くなった[6]．また，この研究では聞き取り調査により20歳ごろの体重も検討しているが，女性では20歳ごろのBMIが大きい人ほど結腸癌によって死亡するリスクが高くなった．男性では20歳ごろの体重との相関は認めなかった．大腸癌における肥満の関与は，欧米と異なり日本人ではBMI＜25からすでにリスクが高くなること，皮下脂肪よりも内臓脂肪の相関が強いことが発癌初期においては示されている．また，内臓脂肪は多くのホルモンを分泌する最大の内分泌臓器であり，内臓脂肪から分泌されるアディポネクチン等の発癌への作用が重要である．

大腸癌死亡率，罹患率と性差

2009年の人口動態統計によると，2008年の大腸癌の人口10万対死亡率は，男性では肺癌，胃癌に次いで3位，女性では乳癌に次いで2位であり，胃癌，肝癌などが減少するなか，最近50年間で男性は2倍近く，女性は1.5倍程度増加しており，危惧すべき状況が続いている(図1)[7]．2004年の人口10万対罹患率では男性は胃癌に次いで2位，女性では乳癌，子宮癌(体癌，頸癌)に次いで3位であり，罹患率は男性は3倍程度，女性は2倍程度増加していることから，今後も効果的な早期診断が求められる癌の一つである(図2)[8]．大腸癌罹患率の男女差に関しては，性ステロイドホルモンの関与が指摘されており，エストロゲンが結腸癌に対し予防的に作用する報告がされている[9]．

アディポネクチンと大腸癌

アディポネクチンは，1996年にわが国から脂肪細胞よりcDNAがクローニングされた[10]．アディポネクチンはその受容体(Adipo R)1，2を介してPPARα，AMPキナーゼを活性化し，骨格筋での脂肪酸燃焼，糖利用の促進，肝臓での糖新生の抑制に関与し，*in vivo*では血糖値の低下[11)～13)]，スカベンジャー受容体の発現抑制を介する脂質蓄積の低減，TNF-αなど炎症にかかわる分子の発現抑制による抗動脈硬化作用などが明らかにされている[11)14)15)]．血清アディポネクチン濃度は，レプチンなどほかのアディポサイトカインとは対照的に

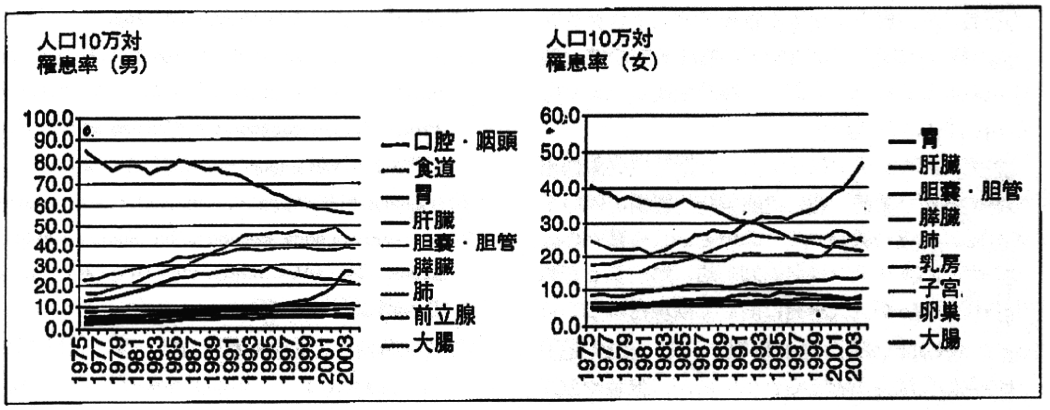

図２　わが国における男女別癌罹患率の年次推移（文献[8]より引用改変）

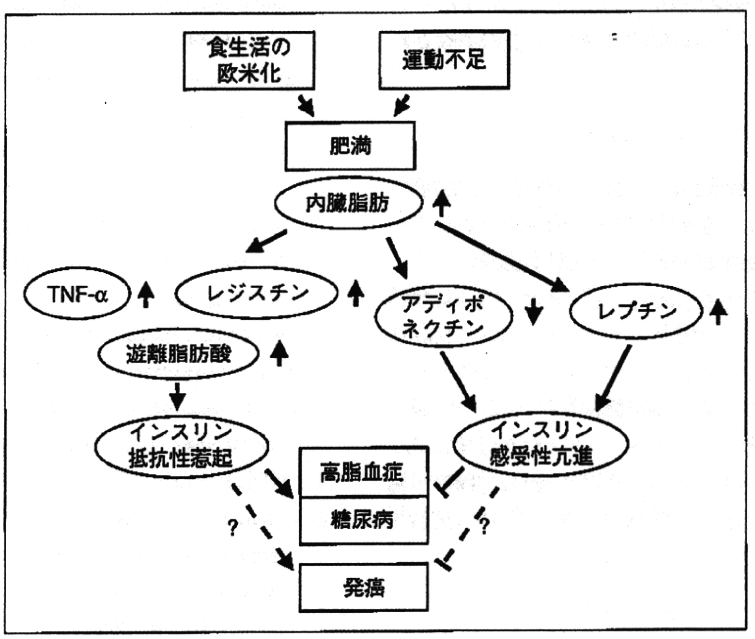

図３　肥満とアディポサイトカインの関連

内臓脂肪の増加により低下する（図３）[16][17]．われわれは日本人94人を対象に大腸癌のサロゲートマーカーであるaberrant crypt foci（ACF）の数と生活習慣因子の相関を単変量解析し，年齢，ウエスト周囲径，内臓脂肪面積（CTスキャンによる計測），低アディポネクチン血症において有意な相関を認めている[18]．

大腸発癌におけるアディポサイトカインの関与および性差

男性は皮下脂肪に比べ内臓脂肪が蓄積しやすいが，女性ではエストロゲンの作用により，内臓脂肪より皮下脂肪が蓄積されやすい．女性においても閉経後は男性同様に内臓脂肪が蓄積されやすくなる．血清アディポネクチン濃度はヒトの成長過程において大きく変化する．臍帯血中のアディポネクチン濃度は胎生22週から40週の間に20倍増加する[19]．小児期には性差は認められず[20]，性成熟期では血清アディポネクチン濃度は男児のほうが女児よりも低値である[21]．血清アディポネクチン濃度は血清テストステロン濃度と逆相関するが，一方で血清エストラジオール濃度に相関しないと