Received: 20 January 2019 | Revised: 8 May 2019 | Accepted: 10 May 2019

DOI: 10.1111/cas.14066

ORIGINAL ARTICLE

Cancer Science WILEY

# Interleukin-13 and its signaling pathway is associated with obesity-related colorectal tumorigenesis

Shimpei Matsui[1] | Koji Okabayashi[1] | Masashi Tsuruta[1] | Kohei Shigeta[1] | Ryo Seishima[1] | Takashi Ishida[1] | Takayuki Kondo[1] | Yoshiyuki Suzuki[1] | Hirotoshi Hasegawa[2] | Masayuki Shimoda[3] | Shinya Sugimoto[4] | Toshiro Sato[4] | Yuko Kitagawa[1]

[1]Department of Surgery, Keio University School of Medicine, Tokyo, Japan

[2]Department of Surgery, Tokyo Dental College Ichikawa General Hospital, Chiba, Japan

[3]Department of Pathology, Keio University School of Medicine, Tokyo, Japan

[4]Department of Gastroenterology, Keio University School of Medicine, Tokyo, Japan

**Correspondence**
Koji Okabayashi, Department of Surgery, Keio University School of Medicine, Tokyo, Japan.
Email: okabayashikoji@gmail.com

## Abstract

The incidence of colorectal cancer (CRC) has been on the rise, which is linked to the increasing prevalence of obesity, based on global epidemiological evidence. Although chronic inflammation is implicated in tumor development, the mechanisms underlying obesity-associated CRC remain unknown. Here, we sought to identify the inflammatory cytokines and their roles in obesity-related colorectal tumorigenesis using cytokine array analyses in a mouse model. Colorectal tumorigenesis was induced through i.p. injection of azoxymethane once a week for 6 weeks in 6-week-old female WT C57Black/6J mice and the obesity diabetes model mouse KK/TaJcl, KK-Ay/TaJcl. The formation of aberrant crypt foci and colorectal tumors were more frequent in obese mice compared with WT mice, and both serum interleukin (IL)-13 and IL-13 receptor (R) expression in the normal intestinal mucosal epithelium were significantly increased in the obese mice. Furthermore, addition of IL-13 to a human CRC cell line and a human colon organoid culture altered the phenotype of intestinal epithelial cells. Knockdown experiments further revealed that IL-13Rα1 dominantly induced mucosal proliferation. Collectively, These results suggest an association between anti-inflammatory cytokines and colorectal carcinogenesis, and provide new research directions for cancer prevention strategies. In particular, inflammation provoked by obesity, notably by increased expression of the cytokine IL-13, could play an important role in the carcinogenesis of obesity-related CRC.

**KEYWORDS**

carcinogenesis, colorectal cancer, cytokine, IL-13, obesity

## 1 | INTRODUCTION

Obesity is an excessive accumulation of fat in the body and is associated with many serious health problems. Recent studies have reported that obesity increases the risk of developing various cancers.[1-3] In particular, global epidemiological evidence suggests that the increased incidence of colorectal cancer (CRC) is strongly associated with excessive intake of a high-fat diet, lack of physical activity, and obesity.[4-7] Obesity is strongly associated with adenoma, a precancerous condition[8], and a meta-analysis showed

that obese people had a 25% higher risk of colorectal adenoma formation compared to non-obese people. Thus, CRC can be considered to represent an obesity-associated form of cancer. However, the biological mechanisms of obesity-induced disease remain to be elucidated, which is critical for establishing effective preventive methods.

Experimental studies have also indicated an association between obesity and CRC tumorigenesis. In particular, chronic inflammation and cytokine secretion have been drawing attention as potential mechanisms of obesity-related tumorigenesis. The adipose tissue is considered to serve as a natural reservoir of macrophages and inflammatory cytokines, which suggests that obese people could be constantly exposed to chronic inflammation.[9] Leptin is an adipocytokine secreted from adipocytes that is known to act on the satiety center to inhibit food intake and enhance energy expenditure.[10] Interleukin (IL)-6 is another cytokine associated with obesity and carcinogenesis.[11] The serum IL-6 concentration was shown to be increased in obese individuals and in those with various types of cancers.[12] Moreover, IL-6 can trigger cell proliferation through phosphorylation of STAT3. Interleukin-6 knockout significantly suppressed the carcinogenesis of an obesity-related hepatocellular carcinoma mouse model.[12-14] Based on these results, inflammatory cytokines appear to be a likely candidate for determining the molecular mechanism linking obesity and colorectal carcinogenesis.

Previous studies addressing obesity-related colorectal tumorigenesis focused on the function of several cytokines, including IL-6 and tumor necrosis factor-α.[15,16] However, these previous studies provided information on the role of individual cytokines of interest, and identification of the dominant cytokines involved in CRC tumorigenesis and their dynamic effects on carcinogenesis under obese conditions remain to be elucidated. A high-throughput screening analysis of related cytokines and subsequent mechanistic analysis are needed to address this problem. To date, there has not been a comprehensive analysis for cytokine interactions in the context of obesity and colorectal tumorigenesis. Therefore, the objectives of this study were to identify causal cytokines involved in obesity-related colorectal tumorigenesis using cytokine array analyses, and to clarify the role of identified cytokines in tumorigenesis and cell proliferation.

Toward this end, we used a mouse model of CRC induced by azoxymethane (AOM) in WT and obese diabetic KK and KK-Ay mice. KK mice are type 2 diabetes mellitus model mice that possess intact leptin and leptin receptor.[17] The KK-Ay mice were established by cross-mating KK mice with C57BL/6J-Ay mice,[18] which carry the Agouti yellow (Ay) gene, and are characterized by severe hyperphagia, polydipsia, impaired glucose tolerance, hyperinsulinemia, and hyperlipidemia.[19] C57BL/6J mice are generally used as the nonobese, nondiabetic controls for KK mice.[20-22] Importantly, obese KK-Ay mice were shown to be highly susceptible to induction of colorectal premalignant lesions, known as aberrant crypt foci (ACF), and the development of CRC following AOM treatment.[23] Thus, we compared these 3 mouse strains, KK-Ay, KK, and C57BL/6J, with the aim of identifying the molecules and functions involved in the induction of obesity-associated cancer.

## 2 | MATERIALS AND METHODS

### 2.1 | Animals and AOM-induced colorectal tumor development

Female 5-week-old C57BL, KK, and KK-Ay mice were purchased from Clea Japan (Tokyo, Japan). Three to 4 mice were housed per plastic cage with sterilized softwood chips and fed a CE-2 basal diet (Clea Japan). All procedures were approved by the Institutional Animal Care and Use Committee of Keio University (Tokyo, Japan) in accordance with the guide for the care and use of laboratory animals. Each mouse was treated with 200 μg AOM (Wako Pure Chemical Industries, Tokyo, Japan) once per week for 6 weeks, beginning at 6 weeks of age. Negative control mice were given saline commensurately.

To count the number of ACF, half of the mice were killed at 12 weeks of age. The colon was opened longitudinally and fixed flat between sheets of filter paper in 10% buffered formalin for more than 24 hours. These sections were stained with 0.2% methylene blue and the mucosal surface was assessed for ACF using a stereoscopic microscope, as previously described.[24] Similarly, the remaining mice were killed at 26 weeks old to assess the number and size of colon tumors formed. Colon tumors and nontumorous tissue were fixed in 10% buffered formalin and embedded in paraffin blocks for histopathological evaluation. Blood samples from the abdominal aorta were also collected.

### 2.2 | Multiplex cytokine assay

The concentrations of obesity-related cytokines and chemokines were measured from the plasma of the killed mice using a Bio-Plex Pro Mouse Cytokine 23-Plex Panel (Bio-Rad, Hercules, CA, USA). The concentrations of cytokines and chemokines were calculated using Bio-Plex Manager 3.0 software (Bio-Rad) with a 5-parameter curve-fitting algorithm applied for standard curve calculations.

### 2.3 | Immunohistochemistry

For histopathological analysis, the entire colon was fixed in 4% paraformaldehyde followed by 70% ethanol, and then embedded in paraffin. Immunohistochemistry (IHC) was carried out to evaluate the proliferation of colon epithelial cells using the following Abs against: Ki-67 (ab16667, 1:1000 dilution; Abcam, Tokyo, Japan), p53 (ab31333, 1:100; Abcam), and BrdU (ab125306, 1:100; Abcam). The length of villi was measured from the base of the crypts to the top of the villi to evaluate proliferation, as previously reported.[25] Apoptosis in the villi was also evaluated using the TUNEL method using an ApopTag Peroxidase In Situ Apoptosis Detection Kit (Merck Millipore, Darmstadt, Germany). Immunohistochemistry against IL-13Rα1 (LS-C176640, 1:100; LifeSpan Biosciences, Seattle, WA, USA) and IL-13Rα2 (AF539, 1:100; R&D Systems, Minneapolis, MN, USA,) was also carried out to evaluate the involvement of the IL-13 cascade in obesity-related CRC carcinogenesis.

At the end of the experimental period, the colorectum was removed, opened longitudinally, and fixed flat between sheets of filter paper in 10% buffered formalin for more than 16 hours. The sections were divided into the proximal segment, rectum (approximately 15% in length), and the proximal (middle) and distal halves of the remainder. A script was counted in the distal and/or rectum area because no tumors developed in the proximal region. The percentages of positive cells were calculated in 100 crypts. The sum of the number of positive cells and negative cells in the crypt of each cross-section was defined as the total number of cells, and we calculated the ratio of the number of positive cells to the total number of cells. For assessment of IL-13 receptor expression, we showed the positive rate of cells counted from the bottom of the crypt because IL-13 receptor-positive cells were localized at the bottom of the crypt.

### 2.4 | Quantitative PCR

Total RNA was extracted from the colons of 26-week-old mice using an RNeasy Mini Kit (Qiagen, Hilden, Germany). Reverse transcription was carried out using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). Quantitative PCR (qPCR) was undertaken using a SYBR Fast qPCR Mix and ViiA 7 Real-Time PCR System (Applied Biosystems). Data were analyzed as a fold change in the relative expression level utilizing the ΔΔCt method for in vitro experiments. Samples were processed in duplicate, and results from 3 independent experiments were analyzed. The primer sequences used for qPCR are listed in Table S1.

### 2.5 | MTT reduction assay

The human CRC cell line HT-29 was obtained from ATCC (Manassas, VA, USA) and maintained in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin in the presence of 5% $CO_2$ in air at 37°C. Interleukin-13 was purchased from Cell Signaling Technology (Beverly, MA, USA) and dissolved in DMEM. After incubation, the cells were harvested to analyze proliferation. Cell proliferation was determined using the MTT assay method.[26] Briefly, HT-29 cells ($1 \times 10^4$ cells/well) were seeded into 96-well plates and incubated for 24 hours at 37°C in a 5% $CO_2$ incubator. Growth rates were assessed according to the doses of IL-13 after 24 hours and 48 hours treatment using the MTT assay.

### 2.6 | Small interfering RNA transfection

To assess the influence of IL-13 receptor expression on cell proliferation, HT-29 cells were transfected with siRNA against IL-13Rα1, IL-13Rα2, and a negative control (SI00446964, SI00013426, and SI03104066, respectively; Qiagen) at 10 nmol/L using a HiPerFect transfection reagent (Qiagen) according to the manufacturer's instructions. To minimize off-target effects, gene knockdown experiments on the 2 target genes, IL-13R1 and IL-13R2, were carried out using 3 corresponding siRNA oligonucleotides. Through this series of experiments, the most prominent siRNA oligonucleotide was selected and used for knockdown of each target gene. After transfection, cell proliferation following loading with IL-13 was evaluated using the MTT assay as described above.

### 2.7 | Western blot analysis

Total cell lysates were extracted as previously described using a lysis buffer (#9803; Cell Signaling Technology).[27] Following electrophoresis, the membrane with separated proteins was blocked with PBS containing 5% skimmed milk powder for 2 hours at room temperature, and then incubated at 4°C overnight with anti-human STAT6 rabbit polyclonal Ab (ab44718, 1:200; Abcam), anti-human phospho-STAT6 rabbit polyclonal Ab (ab28829, 1:100; Abcam), or anti-human β-actin mouse mAb ( #612656, 1:200; BD Biosciences, Tokyo, Japan). After overnight incubation, membranes were incubated for 30 minutes with an HRP-conjugated anti-mouse IgG (W4011, 1:2,500; Promega, Tokyo, Japan) or anti-rabbit IgG (NA934, 1:300; GE Healthcare, Tokyo, Japan). Each complex was detected using a Luminate Forte Western HRP Substrate (Merck Millipore) according to the manufacturer's instructions.

### 2.8 | Organoid culture

Human colonic tissues were obtained with written informed consent after approval by the ethical committee of Keio University School of Medicine. The organoids were established and maintained as previously described.[28,29] Human normal colon organoids were seeded on a 48-well plate and cultured with medium containing a gradient concentration of IL-13 (0-100 ng/mL) for 7 days after a single cell passage. Images of each well were captured over time and organoid areas were measured using image analysis software.

### 2.9 | Statistical analyses

Statistical analyses was undertaken using Stata/SE 12.1 (StataCorp LLC, Texas, USA) for Windows. A nonparametric Mann-Whitney test and ANOVA were used to compare 2 groups as appropriate. *P* values <.05 were considered significant.

## 3 | RESULTS

### 3.1 | Increased number of ACF and colorectal tumors in obese mice

Body weight data are presented in Figure 1A. All mice developed ACF in the colon and rectum after 4 weeks of AOM treatment (Figure 1B). As shown in Table 1, the total numbers of AOM-induced ACF significantly increased in proportion to body weight (WT, 6.2 ± 2.4 g/mouse; KK, 12.5 ± 7.3 g; KK-Ay, 40.2 ± 12.1 g). Conversely, saline-treated WT, KK, and KK-Ay mice did not develop any ACF. All KK-Ay mice treated with AOM developed colorectal tumors, with an incidence 10 times higher than that detected in WT mice (Table 1). KK-Ay mice developed a total of 25 visible tumors, which were mainly

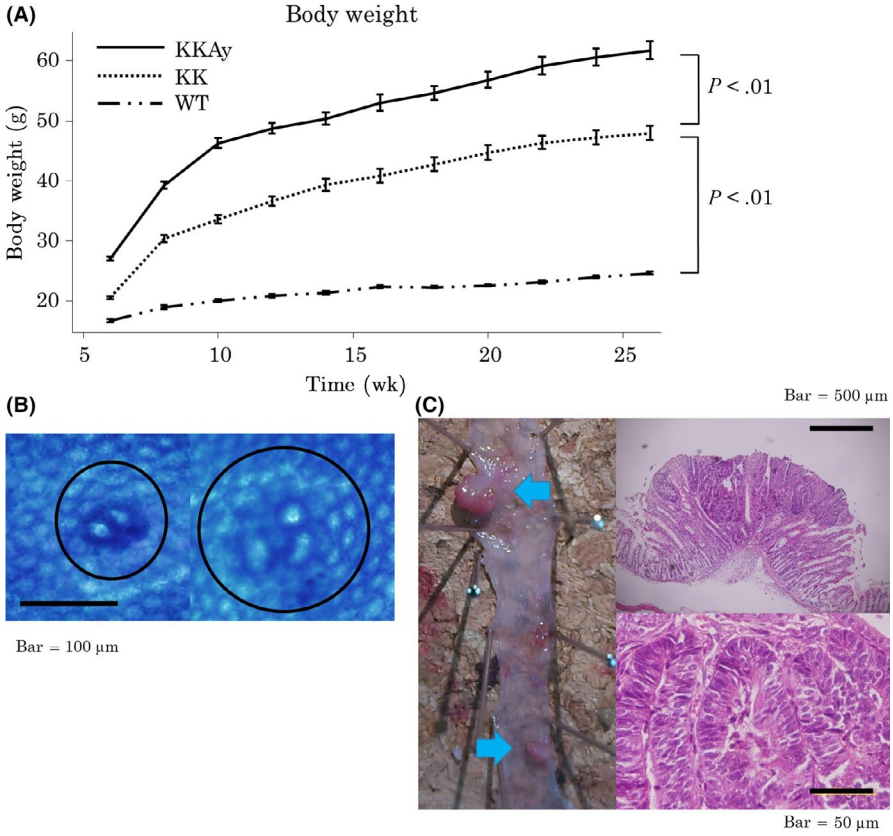


**FIGURE 1** A, Body weight changes during the experiment in KK-Ay, KK, and C57BL/6J mice. B, Each mouse was treated with 200 μg azoxymethane once a week from 6 wk to 11 wk, a total of 6 times. C, Appearance of aberrant crypt foci at the age of 12 wk in a KK-Ay mouse. C, Colon cancers (arrows) and their microscopic views with H&E staining at the age of 26 wk in a KK-Ay mouse

**TABLE 1** Incidence of colorectal aberrant crypt foci (ACF) and colorectal tumors in KK-Ay, KK, and C57BL/6J mice treated with azoxymethane

| | No. of mice with ACF | No. of ACF/mouse | *P* value | No. of mice with tumor | No. of tumor/mouse | *P* value |
|---|---|---|---|---|---|---|
| WT | 11/11 | 6.2 ± 2.4 | | 1/11 | 0.18 ± 0.40 | |
| KK | 11/11 | 12.5 ± 7.3 | <.01 | 3/11 | 0.36 ± 0.67 | <.01 |
| KK-Ay | 11/11 | 40.2 ± 12.1 | <.01 | 11/11 | 2.27 ± 2.05 | <.01 |

Number of ACF/mouse was expressed as mean ± SD. No. of ACF in KK-Ay and KK mice was significantly different from that in C57BL/6J mice.

located in the middle to distal portion of the colon. Furthermore, histopathological examination revealed that most AOM-induced tumors consisted of well-differentiated adenocarcinomas (Figure 1C). Thus, evident tumorigenesis was observed in obese mice.

## 3.2 | Increased mucosal and cellular proliferation in obese mice

As higher tumorigenesis in obese mice is based on abnormal mucosal proliferation, this was compared between WT and KK-Ay mice (Figure 2). Given the increased body size of KK-Ay mice, the length of the colon is also increased in these mice, thus the colon crypts were significantly longer in KK-Ay mice with AOM treatment compared with WT mice at 26 weeks. These results suggest opposing effects of an increase in cell proliferation at the bottom of the crypt and a decrease in apoptosis at the top of the crypt, respectively. However, there was no significant differences in p53 expression and TUNEL positivity between groups. Cellular proliferation was assessed by IHC analysis using Ki-67 and BrdU. Significant increases in Ki67 and BrdU labeling were detected in KK-Ay mice compared with WT counterparts. Most of the proliferating cells were observed at the bottom of the crypts.

## 3.3 | Serum inflammatory cytokines and chemokines

To evaluate the relationship between obesity-related systemic inflammation and tumorigenesis, serum inflammatory cytokines were quantified and visually expressed using a heat map (Figure 3A). The levels of the cytokines and chemokines IL-1α, IL-6, IL-10, IL-13, eotaxin, and macrophage inflammatory protein (MIP)-1α gradually increased with increased body weight. Conversely, IL-2 and glucagon

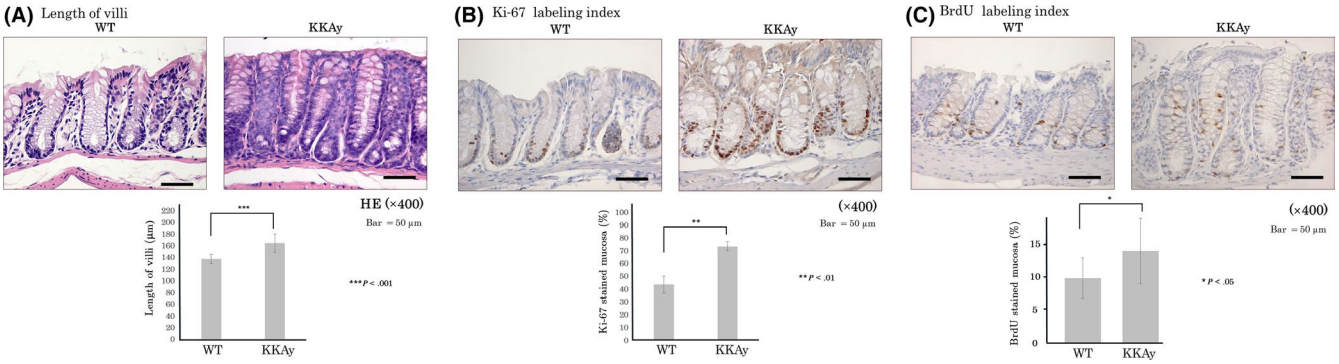


**FIGURE 2** Histological features of azoxymethane-treated normal colon mucosa between C57BL/6J and KK-Ay mice. Microscopic views of colon villi at the age of 26 wk are shown. A, Length of colon villi with H&E staining. B,C, Immunohistochemical staining of colon mucosa by Ki-67 (B) and BrdU (C)

levels decreased as body weight increased. Among the evaluated factors, IL-13, eotaxin, and MIP-1α significantly differed among the WT, KK, and KK-Ay mice (Figure 3B). Interleukin-13: WT 136.6 ± 27.1 ng/mL; KK 205.0 ± 9.8 ng/mL; KK-Ay 336.6 ± 85.1 ng/mL; WT vs. KK, $P = .02$; KK vs. KK-Ay, $P = .01$. Eotaxin: WT 124.2 ± 39.0 ng/mL; KK 284.6 ± 238.1 ng/mL; KK-Ay 346.8 ± 282.1 ng/mL; WT vs. KK-Ay, $P = .03$. Macrophage inflammatory protein-1α: WT 17.5 ± 10.7 ng/mL; KK 40.9 ± 30.2 ng/mL; KK-Ay 45.1 ± 13.5 ng/mL; WT vs. KK, $P = .03$; WT vs. KK-Ay, $P = .02$.

Among these cytokines/chemokines, IL-13 has been reported to play a central role in the pathogenesis of ulcerative colitis, a major type of in inflammatory bowel disease that is associated with a significantly increased risk of CRC.[30,31] Furthermore, elevated levels of IL-13 have been shown in patients with CRC.[32] Therefore, we further focused on the potential role of IL-13 in obesity-related colorectal tumorigenesis using CRC cells.

### 3.4 | Expression of IL-13 receptor in colon

The expression of 2 subtypes of IL-13 receptor (IL-13Rα1 and IL-13Rα2) was determined by RT-PCR and IHC (Figure 4A,B). The percentages of positive cells were significantly higher in KK-Ay mice compared with those of WT mice (Figure 4C). Most of the IL-13 receptor-positive cells were observed at the bottom of crypts, consistent with the expression of Ki-67 and BrdU. These findings suggested that IL-13 was involved in the induction of colonic mucosal proliferation.

### 3.5 | Interleukin-13 promotes CRC cell proliferation through IL-13Rα1

To examine the proliferative effect of IL-13, we used HT-29, which has high expression of both types of IL-13 receptors among several IL-13 receptor-expressing cell lines (Figure 5A). After treatment with IL-13, tumor proliferation was significantly induced in a dose-dependence manner (Figure 5B). Although this proliferative effect was strongly suppressed after knockdown of IL-13Rα1, it was not observed after knockdown of IL13-Rα2. These findings suggested that the signal transduction through IL-13Rα1 enhanced colonic mucosal proliferation. Previous studies reported that phosphorylation of STAT6 (pSTAT6) was key in the signaling pathway induced by IL-13Rα1.[33] Consistently, western blot analysis showed STAT6 was immediately phosphorylated after treatment with IL-13 (Figure 5C), whereas STAT6 was not phosphorylated by treatment with IL-13 after knockdown of IL-13Rα1. However, these findings were not observed after knockdown of IL-13Rα2 (Figure 5D). These results suggested that STAT6 phosphorylation was associated with colonic mucosal proliferation and tumor development.

### 3.6 | Morphological capacity changes induced by colonic IL-13 in organoid culture

Finally, we used an organoid culture to estimate the impact of IL-13 on normal mucosal epithelial cells in the colon. We observed morphological capacity changes in colon organoids following IL-13 treatment (Figure 6A). Accordingly, we assessed the change in morphology after IL-13 was added to the organoid culture by measuring the organoid area. The organoid area increased with time in a concentration-dependent manner after IL-13 exposure (Figure 6B).

## 4 | DISCUSSION

To our knowledge, this is the first study describing IL-13 as a potential factor involved in the development of obesity-related CRC. Colorectal premalignant lesions and colorectal tumors were observed more frequently in obese mice than in normal mice, and an increased proliferation potential was observed in normal intestinal epithelial cells from these animals. The levels of both serum IL-13 and IL-13 receptor expression in normal intestinal mucosal epithelium were significantly increased in obese mice. A key strength of the present study was that the proliferative effect of IL-13 was demonstrated in vitro using cancer cell lines. Furthermore, morphological alterations and increases in the quantitative organoid area positively correlated with the concentration of IL-13. In particular, IL-13Rα1

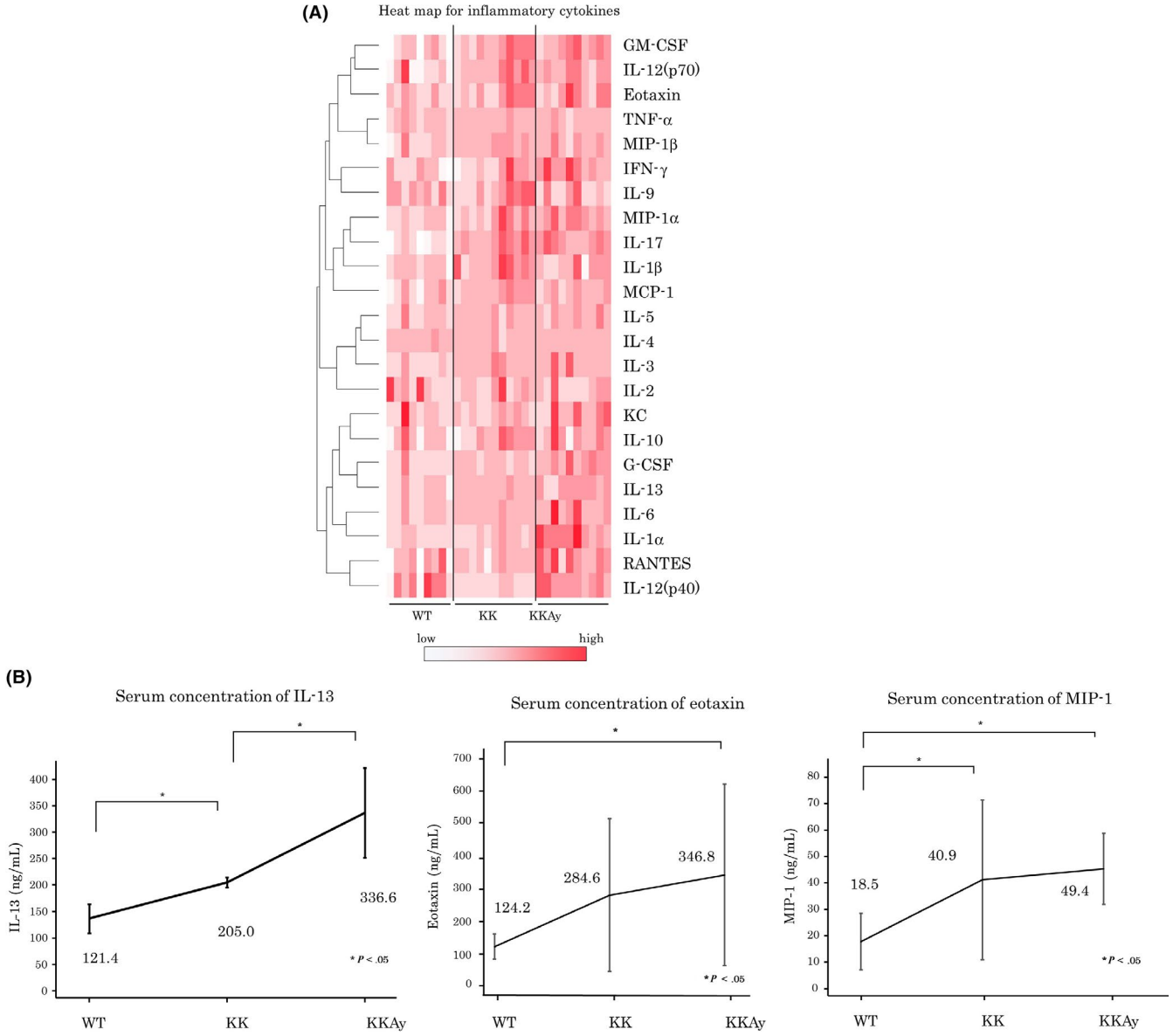


**FIGURE 3** Concentrations of (obesity-related) cytokines and chemokines were measured using a Bio-Plex Pro Mouse Cytokine 23-Plex Panel (Bio-Rad, Hercules, CA, USA). A, Serum inflammatory cytokines assay visually expressed using a heat map. B, Average of serum interleukin (IL)-13, eotaxin, and macrophage inflammatory protein (MIP)-1 levels are shown in each mice strain. Triplicate samples were analyzed in each experiment. GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN-γ, γ-interferon; KC, keratinocyte chemoattractant; MCP-1, monocyte chemotactic protein-1; TNF-α, tumor necrosis factor-α

dominantly induced mucosal proliferation. These results revealed an association between anti-inflammatory cytokines and colorectal carcinogenesis, and provide new research directions for drug discovery and cancer prevention strategies.

Using a cytokine array, differences between obese and WT mice were observed in the serum concentration of cytokines. Interestingly, anti-inflammatory factors, including IL-13, MIP-1α, and eotaxin, were significantly increased in obese mice. Several previous studies found an association between these cytokines and obesity but provided only limited evidence of their mechanistic role in gastrointestinal carcinogenesis. These factors generally stimulate CC and CXC chemokines and induce inflammation and tissue remodeling. Interleukin-13 is produced by T cells, B cells, mast cells, basophils, natural killer cells, and dendritic cells,[34] and plays a key role in inflammation and immune responses.[30] Additionally, IL-13 enhances the cellular turnover of gastrointestinal epithelial cells.[34] Macrophage inflammatory protein-1 also enhances the accumulation of fibroblasts.[35] These results indicate that the development of obesity-induced gastrointestinal carcinogenesis is associated with altered immune homeostasis.

A higher serum IL-13 concentration and higher expression of IL-13R in the mucosal colonic epithelium were observed in obese mice. Our experiments using cancer cell lines and colonic organoid culture further showed that treatment with IL-13 enhanced

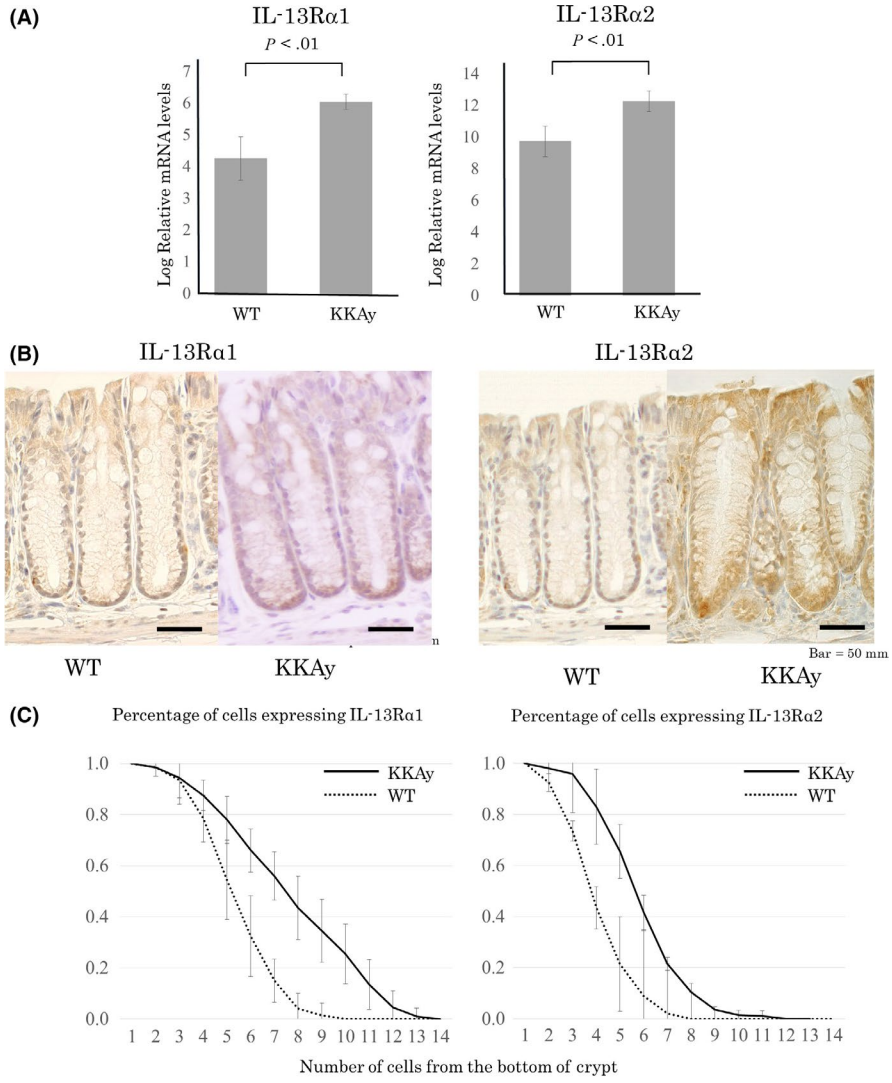


**FIGURE 4** Expression of interleukin-13 receptor (IL-13R) in colon mucosa. A,B, Expression of IL-13 receptors analyzed by RT-PCR (A) and by immunohistochemistry (B). C, Ratio of immunohistochemistry-positive cells from the bottom of crypts

cell proliferation and induced morphological changes in a dose-dependent manner. Although the influence of these morphological changes in organoids is not clear at present, a previous report indicated that morphological alterations in organoids represent possible distinct molecular and cellular differences in their tissue of origin.[36] Interleukin-13 regulates normal physiological processes, including inflammation and tissue reconstruction.[33,34] Interleukin-13 has also been closely linked with asthma and induces mucosal cell proliferation in the airway epithelium in asthmatic pathophysiology.[33] Furthermore, elevated levels of IL-13 have been detected in several cancers, suggesting that this cytokine could stimulate the proliferation of malignant cells in an autocrine fashion.[37,38] This IL-13-mediated proliferative effect on the gastrointestinal epithelium was first examined in this study and could be a key part of the mechanism of obesity-related CRC.

Interleukin-13R comprises IL-13Rα1 and IL-13Rα2, which are structurally and functionally different receptors. The structure of IL-13Rα1 is highly similar to that of the IL-4 receptor (IL-4R).[39] Both IL-13Rα1 and IL-4R can dimerize and mediate IL-13 signaling, which is transmitted downstream through phosphorylation of the transcription factor STAT6.[40] As a result of signal transduction through IL-13Rα1, pulmonary IL-13 has been reported to cause inflammation, mucus hypersecretion, subepithelial fibrosis, physiological abnormalities, and eotaxin production. Although IL-13Rα2 has a higher affinity for IL-13 than IL-13Rα1, its detailed function has not been elucidated. Our results showed that the proliferative effect of IL-13 was more completely suppressed by knockdown of IL-13Rα1 than by knockdown of IL-13Rα2. This result indicated that IL-13Rα1 plays a more important role in IL-13 signaling than IL-13Rα2. Based on these findings, the functional suppression of IL-13Rα1 could be a key therapeutic target to prevent the carcinogenesis of obesity-related CRC.

Epidemiological studies have revealed that polymorphic variations in the gene encoding IL-13 increased the risk of colorectal adenoma. Walczak et al[41] investigated IL-13 genetic variations with PCR RFLPs in a Polish population comprising 150 cancer patients and 170 healthy subjects. The study reported an association of the −1112 C/T polymorphism of the IL-13 gene with an increased the risk of CRC development. Sainz et al[42] reported that patients with diabetes have single nucleotide polymorphisms in the IL13_rs20541_T allele,

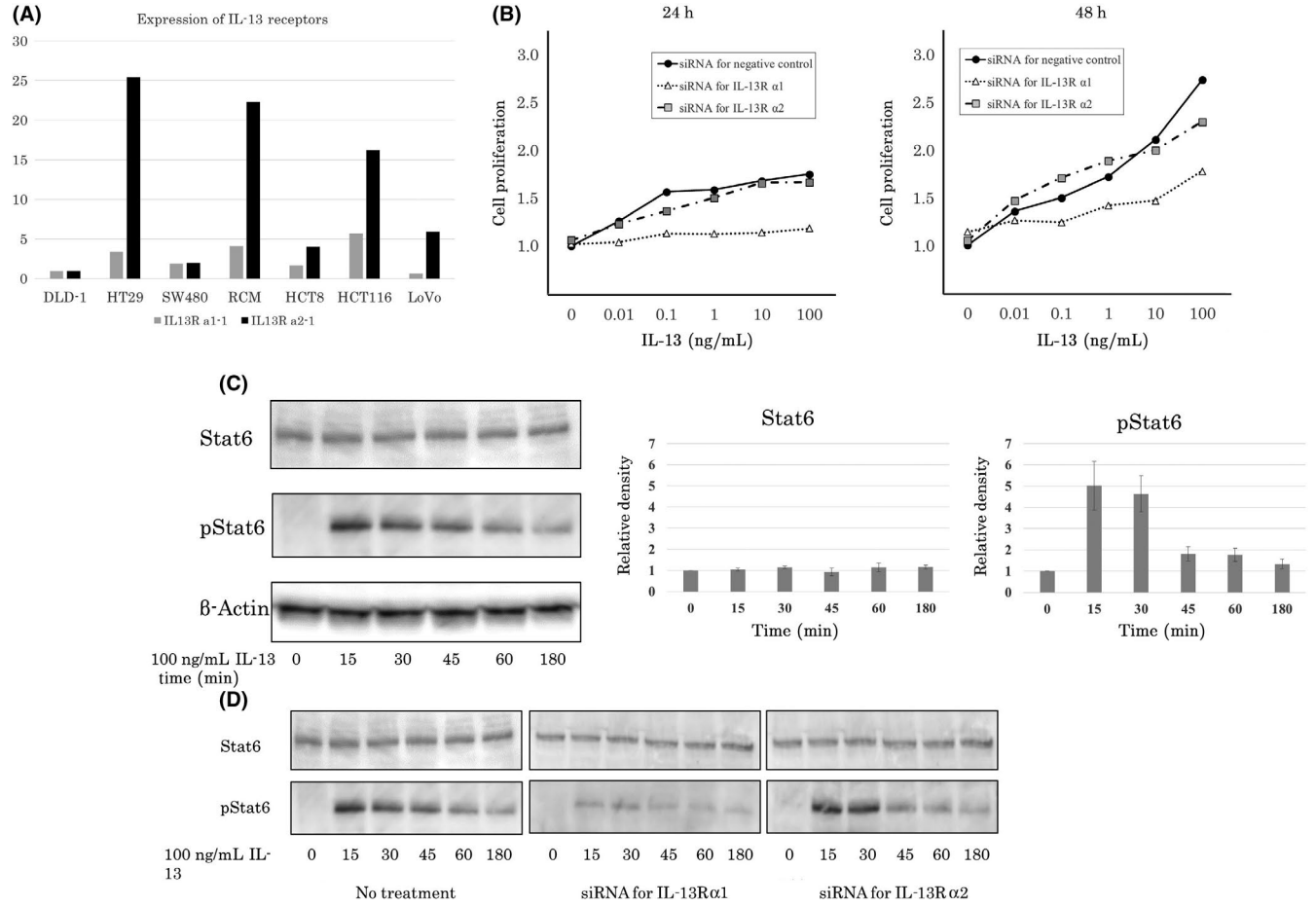


**FIGURE 5** Silencing interleukin-13 receptor (IL-13R)α1 inhibits IL-13-mediated proliferation in human colorectal cancer cell line HT29 through blocking STAT6 activation. A, Expression of IL-13 receptors in various cell lines. B, MTT analysis on HT29 cells. HT29 cells were treated with various concentration of IL-13 for 24 h (left) and 48 h (right). Phosphorylation levels of STAT6 were examined by immunoblotting. STAT6 and β-actin were used as sample loading controls. C, IL-13 dramatically (transiently) stimulated the phosphorylation of STAT6. D, Immunoblot analysis for the inhibitory effect of siRNA oligonucleotides on IL-13Rα1 (middle) and IL-13Rα2 (right)

and it was associated with CRC. As these various findings support that IL-13 and IL-13R could be therapeutic targets for CRC prevention, the potential therapeutic utility of IL-13 antagonists and Abs should be further explored.

An important question relating to the link between obesity and colorectal tumorigenesis is the potential effect of interventions directed against metabolic syndrome. A calorie-restricted diet significantly decreased the risk of AOM-induced colon cancer in a mouse model.[43] Furthermore, mice with diet-induced obesity displayed a specific increase in intestinal stem cells, which showed hyperproliferation.[44] A multicenter randomized control trial reported that an oral antidiabetic drug, metformin, reduced the prevalence and number of metachronous adenomas or polyps after polypectomy.[45] Although the serum levels of several cytokines were reported to be affected by these interventions,[43] the involvement of IL-13 has not been described to date. Thus, it will be interesting to investigate a potential role for IL-13 in the prevention of colorectal tumorigenesis and to ascertain the potential therapeutic targets of IL-13 and IL-13R.

This study has several limitations. First, the interaction between other obesity-related cytokines and IL-13 was not evaluated. Considering the complex cytokine network in obese subjects, it is difficult to fully understand the interactions of a complex cytokine network on colorectal carcinogenesis. Future epidemiologic studies could provide some important information on cytokine network dynamics in obese subjects. Second, the influence of obesity on other types of gastrointestinal cancers remains unclear, because there was no occurrence of other gastrointestinal cancers under IL-13 treatment. Finally, although we described a proliferative effect of IL-13 on CRC cells, our results require validation in animal models.

In conclusion, the present results provide new insight into the mechanism of carcinogenesis in obesity-related CRC. Obese mice, which had more colorectal premalignant lesions and colorectal tumors, displayed an increased IL-13 concentration in the blood. Furthermore, load experiments, in which IL-13 was applied to a human CRC cell line and a human colon organoid culture, revealed that IL-13 modified intestinal epithelial cells. Together, these findings

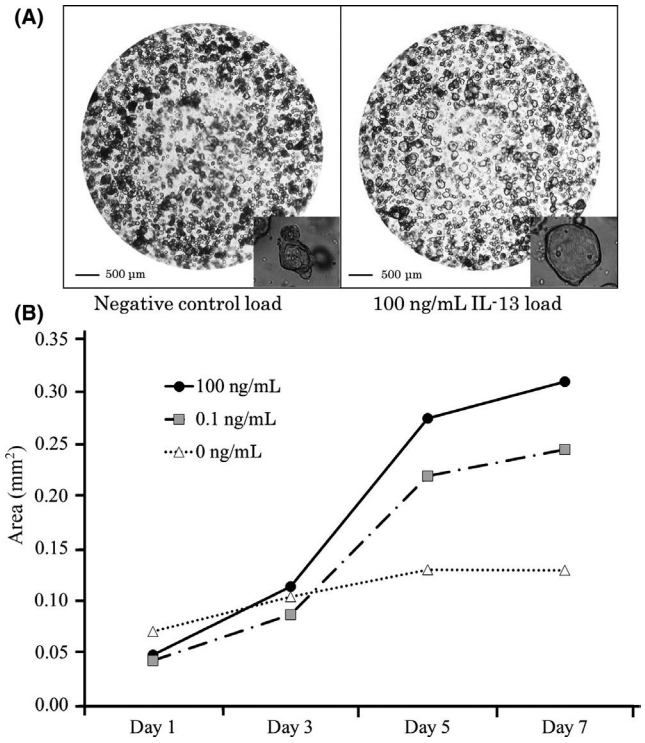


**FIGURE 6** Load experiment to organoid culture line by interleukin (IL)-13. A, Morphological change after negative control loading (left) and IL-13 loading (right) to organoid culture. B, Longitudinal change of area of organoid culture after loading IL-13

indicate that inflammation provoked by obesity, notably by increased IL-13 production, could play an important role in the carcinogenesis of obesity-related CRC.

## CONFLICT OF INTEREST

The authors have no conflict of interest.

## REFERENCES


1. Calle EE, Kaaks R. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. *Nat Rev Cancer*. 2004;4:579.
2. Lichtman MA. Obesity and the risk for a hematological malignancy: leukemia, lymphoma, or myeloma. *Oncologist*. 2010;15:1083-1101.
3. Makarem N, Lin Y, Bandera EV, et al. Concordance with World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR) guidelines for cancer prevention and obesity-related cancer risk in the Framingham Offspring cohort (1991-2008). *Cancer Causes Control*. 2015;26:277-286.
4. Otani T, Iwasaki M, Inoue M, et al. Body mass index, body height, and subsequent risk of colorectal cancer in middle-aged and elderly Japanese men and women: Japan public health center-based prospective study. *Cancer Causes Control*. 2005;16:839-850.
5. Bao Y, Nimptsch K, Chan AT, et al. Reported behavior of eating anything at anytime and risk of colorectal cancer in women. *Int J Cancer*. 2012;130:1395-1400.
6. Birmingham JM, Busik JV, Hansen-Smith FM, et al. Novel mechanism for obesity-induced colon cancer progression. *Carcinogenesis*. 2009;30:690-697.
7. Winkels RM, Heine-Broring RC, van Zutphen M, et al. The COLON study: Colorectal cancer: Longitudinal, Observational study on Nutritional and lifestyle factors that may influence colorectal tumour recurrence, survival and quality of life. *BMC Cancer*. 2014;14:374.
8. Okabayashi K, Ashrafian H, Hasegawa H, et al. Body mass index category as a risk factor for colorectal adenomas: a systematic review and meta-analysis. *Am J Gastroenterol*. 2012;107:1175-1185.
9. Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *J Clin Invest*. 2007;117:175-184.
10. Lin S, Thomas TC, Storlien LH, et al. Development of high fat diet-induced obesity and leptin resistance in C57Bl/6J mice. *Int J Obes Relat Metab Disord*. 2000;24:639-646.
11. Khandekar MJ, Cohen P, Spiegelman BM. Molecular mechanisms of cancer development in obesity. *Nat Rev Cancer*. 2011;11:886-895.
12. Park EJ, Lee JH, Yu GY, et al. Dietary and genetic obesity promote liver inflammation and tumorigenesis by enhancing IL-6 and TNF expression. *Cell*. 2010;140:197-208.
13. Fenton JI, Hursting SD, Perkins SN, et al. Interleukin-6 production induced by leptin treatment promotes cell proliferation in an Apc (Min/+) colon epithelial cell line. *Carcinogenesis*. 2006;27:1507-1515.
14. Matsuzawa Y. The metabolic syndrome and adipocytokines. *FEBS Lett*. 2006;580:2917-2921.
15. Zhang J, Wang C, Ha X, et al. DNA methylation of tumor necrosis factor-alpha, monocyte chemoattractant protein-1, and adiponectin genes in visceral adipose tissue is related to type 2 diabetes in the Xinjiang Uygur population. *J Diabetes*. 2016;9:699-706.
16. Hardwick JC, Van Den Brink GR, Offerhaus GJ, et al. Leptin is a growth factor for colonic epithelial cells. *Gastroenterology*. 2001;121:79-90.
17. Gohda T, Tanimoto M, Kaneko S, et al. Minor gene effect of leptin receptor variant on the body weight in KK/Ta mice. *Diabetes Obes Metab*. 2006;8:581-584.
18. Kondo K, Nozawa K, Romida T, et al. Inbred strains resulting from Japanese mice. *Bull Exp Anim*. 1957;6:107-112.
19. Nakamura M, Yamada K. Studies on diabetic KK strain of the mouse. *Diabetologia*. 1967;3:212-221.
20. Suto J, Matsuura S, Imamura K, et al. Genetic analysis of non-insulin-dependent diabetes mellitus in KK and KK-Ay mice. *Eur J Endocrinol*. 1998;139:654-661.
21. Kato H, Ohue M, Kato K, et al. Mechanism of amelioration of insulin resistance by beta3-adrenoceptor agonist AJ-9677 in the KK-Ay/Ta diabetic obese mouse mode. *Diabetes*. 2001;50:113-122.
22. Shiuchi T, Iwai M, Li HS, et al. Angiotensin II type-1 receptor blocker valsartan enhances insulin sensitivity in skeletal muscles of diabetic mice. *Hypertension*. 2004;43:1003-1010.
23. Teraoka N, Mutoh M, Takasu S, et al. High susceptibility to azoxymethane-induced colorectal carcinogenesis in obese KK-Ay mice. *Int J Cancer*. 2011;129:528-535.
24. Bird RP. Observation and quantification of aberrant crypts in the murine colon treated with a colon carcinogen: preliminary findings. *Cancer Lett*. 1987;37:147-151.
25. Sakatani T, Kaneda A, Iacobuzio-Donahue CA, et al. Loss of imprinting of Igf2 alters intestinal maturation and tumorigenesis in mice. *Science*. 2005;307:1976-1978.
26. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods*. 1983;65:55-63.
27. Matsunaga A, Ishii Y, Tsuruta M, et al. Inhibition of heat shock protein 27 phosphorylation promotes sensitivity to 5-fluorouracil in colorectal cancer cells. *Oncol Lett*. 2014;8:2496-2500.
28. Sato T, Stange DE, Ferrante M, et al. Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. *Gastroenterology*. 2011;141:1762-1772.

29. Sugimoto S, Sato T. Establishment of 3D intestinal organoid cultures from intestinal stem cells. *Methods Mol Biol*. 2017;1612:97-105.
30. Mannon P, Reinisch W. Interleukin 13 and its role in gut defence and inflammation. *Gut*. 2012;61:1765-1773.
31. Feagins LA, Souza RF, Spechler SJ. Carcinogenesis in IBD: potential targets for the prevention of colorectal cancer. *Nat Rev Gastroenterol Hepatol*. 2009;6:297-305.
32. Formentini A, Braun P, Fricke H, et al. Expression of interleukin-4 and interleukin-13 and their receptors in colorectal cancer. *Int J Colorectal Dis*. 2012;27:1369-1376.
33. Wynn TA. IL-13 effector functions. *Annu Rev Immunol*. 2003;21:425-456.
34. Cliffe LJ, Humphreys NE, Lane TE, et al. Accelerated intestinal epithelial cell turnover: a new mechanism of parasite expulsion. *Science*. 2005;308:1463-1465.
35. Sasaki S, Baba T, Shinagawa K, et al. Crucial involvement of the CCL3-CCR35 axis-mediated fibroblast accumulation in colitis-associated carcinogenesis in mice. *Int J Cancer*. 2014;135:1297-1306.
36. Han SH, Shim S, Kim MJ, et al. Long-term culture-induced phenotypic difference and efficient cryopreservation of small intestinal organoids by treatment timing of Rho kinase inhibitor. *World J Gastroenterol*. 2017;23:964-975.
37. Darkhal P, Gao M, Ma Y, et al. Blocking high-fat diet-induced obesity, insulin resistance and fatty liver by overexpression of Il-13 gene in mice. *Int J Obes*. 2015;39:1292-1299.
38. Srabovici N, Mujagic Z, Mujanovic-Mustedanagic J, et al. Interleukin 13 expression in the primary breast cancer tumour tissue. *Biochem Med*. 2011;21:131-138.
39. Minty A, Chalon P, Derocq JM, et al. Interleukin-13 is a new human lymphokine regulating inflammatory and immune responses. *Nature*. 1993;362:248-250.
40. Zhu Z, Lee CG, Zheng T, et al. Airway inflammation and remodeling in asthma. Lessons from interleukin 11 and interleukin 13 transgenic mice. *Am J Respir Crit Care Med* 2001;164:S67-S70.
41. Walczak A, Przybyłowska K, Trzciński R, et al. Association of -1112 c/t promoter region polymorphism of the interleukin 13 gene with occurrence of colorectal cancer. *Pol Przegl Chir*. 2011;83:27-31.
42. Sainz J, Rudolph A, Hoffmeister M, et al. Effect of type 2 diabetes predisposing genetic variants on colorectal cancer risk. *J Clin Endocrinol Metab*. 2012;97:E845-E851.
43. Olivo-Marston SE, Hursting SD, Perkins SN, et al. Effects of calorie restriction and diet-induced obesity on murine colon carcinogenesis, growth and inflammatory factors, and microRNA expression. *PLoS One*. 2014;9:e94765.
44. Mah AT, Van Landeghem L, Gavin HE, et al. Impact of diet-induced obesity on intestinal stem cells: hyperproliferation but impaired intrinsic function that requires insulin/IGF1. *Endocrinology*. 2014;155:3302-3314.
45. Higurashi T, Hosono K, Takahashi H, et al. Metformin for chemoprevention of metachronous colorectal adenoma or polyps in post-polypectomy patients without diabetes: a multicentre double-blind, placebo-controlled, randomised phase 3 trial. *Lancet Oncol*. 2016;17:475-483.

## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**How to cite this article:** Matsui S, Okabayashi K, Tsuruta M, et al. Interleukin-13 and its signaling pathway is associated with obesity-related colorectal tumorigenesis. *Cancer Sci*. 2019;110:2156–2165. https://doi.org/10.1111/cas.14066