Monosodium glutamate-induced diabetic mice are susceptible to azoxymethane-induced colon tumorigenesis

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Obese people and diabetic patients are known to be high risk of colorectal cancer (CRC), suggesting need of a new preclinical animal model, by which to extensively study the diverse mechanisms, therapy and prevention. The present study aimed to determine whether experimental obese and diabetic mice produced by monosodium glutamate (MSG) treatment are susceptible to azoxymethane (AOM)-induced colon tumorigenesis using early biomarkers, aberrant crypts foci (ACF) and β-catenin-accumulated crypts (BCACs), of colorectal carcinogenesis. Male Crj:CD-1 (ICR) newborns were daily given four subcutaneous injections of MSG (2 mg/g body wt) to induce diabetes and obesity. They were then given four intraperitoneal injections of AOM (15 mg/kg body wt) or saline (0.1 ml saline/10 g body wt). Ten weeks after the last injection of AOM, the MSG-AOM mice had a significant increase in the multiplicity of BCAC (13.83 ± 7.44, P < 0.002), but not ACF (7.80 ± 11.20, when compare to the Saline-AOM mice (5.45 ± 1.86 of BCAC and 69.27 ± 8.06 of ACF). Serum biochemical profile of the MSG-treated mice with or without AOM showed hyperinsulinemia, hypercholesterolemia and hyperglycemia. The mRNA expression of insulin-like growth factor-1 receptor (IGF-1R, P<0.01) was increased in the MSG-AOM mice, when compared with the mice given AOM alone. IGF-1R was immunohistochemically expressed in the BCAC, but not ACF, in the AOM-treated mice. Our findings suggest that the MSG mice are highly susceptible to AOM-induced colorectal carcinogenesis, suggesting potential utility of our MSG-AOM mice for further investigation of the possible underlying events that affect the positive association between obesity/diabetes and CRC.

Materials and methods

Animals and chemicals

The pregnant Crj:CD-1 (ICR) mice were purchased from Charles River Japan, Inc. (Kanagawa, Japan) and their newborns were used in the study. MSG was obtained from Wako Pure Chemical Industries, Ltd (Tokyo, Japan) and AOM from Sigma Chemical Co. (St Louis, MO). Mice used for the experiment were maintained in the well-controlled room with a high-efficiency particulate air filter, a 12 h lighting (7:00–19:00), 25 ± 2°C room temperature and 55 ± 15% humidity. Mice (3–6 mice/cage) were housed in polycarbonate cages measuring W225 × D338 × H140 mm (Japan CLEA, Inc., Tokyo, Japan) with the floor covered with a sheet of roll paper (Japan SLC). MF (Oriental Yeast Co., Ltd, Tokyo, Japan) was used as a basal diet throughout the study. Groundwater that was chlorine-treated and subjected to ultraviolet disinfection was used as drinking water in a bottle. We fully complied with the ‘Guidelines Concerning Experimental Animals’ issued by the Japanese Association for Laboratory Animal Science and exercised due consideration so as not to cause any ethical problem.

Experimental procedure

The newborns were divided into two groups according to the treatments. The birth date was the beginning of four daily subcutaneous injections of MSG (2 mg/g body wt, MSG mice) and physiological saline (Saline mice). Among these mice, males were subjected to the study. They were divided into four groups at 4 weeks of age: groups 1 (12 males) and 2 (6 males) of the MSG mice received four weekly intraperitoneal injections of AOM (15 mg/kg body wt, other tissue (27.28) and the axis is a good target for cancer chemoprevention (25–28). However, the underlying mechanisms of how these chronic diseases promote colon carcinogenesis still remain unknown (19). On this context, new research animal models are needed to investigate the diverse aspects of the mechanisms.

We have previously reported that development of AOM-induced precursor lesions is enhanced in C57BL/KsJ-db/db mice with hyperperleptinemia and hyperinsulinemia (29). Such an animal model may give important implications for further exploration of the possible underlying events that affect the positive association between CRC and obesity and/or diabetes (30–32). A number of animal models for diabetes and/or obesity have been reported. One such model is produced by injection of monosodium glutamate (MSG). When MSG is applied to Crj:CD-1 (ICR) newborn mice (MSG mice), they develop diabetic condition (hyperinsulinemia, hyperglycemia and hyperplastic islets) without polyphagia (33,34).

It is believed that colorectal carcinogenesis is a representative multistep tumorigenesis with events of genetic alterations. Several small lesions, including aberrant crypt foci (ACF) (35,36), mucin-depleted foci (37) and β-catenin-accumulated crypts (BCACs) (38) are proposed as early-appearing preneoplastic lesions (37). While ACF and mucin-depleted foci are recognized on the surface of cancer-predisposed colons of rodents and human (37), BCAC are identified in colon mucosa at the early stages of colon carcinogenesis (39). Accumulating evidence suggests that BCAC are independent small dysplastic lesions and/or microadenomas and progressed precancerous lesions (40) in colon carcinogenesis when compared with ACF and mucin-depleted foci (39). These early lesions are widely used for investigating pathobiology of colorectal carcinogenesis (37).

In the current study, new born Crj:CD-1 (ICR) mice were treated with MSG to produce diabetes and obesity and, subsequently, they received a colonic carcinogen, azoxymethane (AOM). Our results indicated that the MSG mice are highly susceptible to AOM-induced colorectal carcinogenesis by counting the number of BCAC, but not ACF, and possible involvement of the IGF/IGF-1R axis in colorectal tumorigenesis of diabetic and obese mice induced by MSG and AOM. Our main goal is to assess the involvement of obesity/diabetes-associated events, such as hyperinsulinemia, in colorectal carcinogenesis in vivo.

Introduction

Epidemiological studies have shown that obesity and diabetes mellitus may be one of the risk factors for colorectal cancer (CRC) development (1–7). At present, hyperinsulinemia (8,9), hypercholesterolemia (10,11), hyperglycemia (9,12) and hyperlipidemia (7) are considered to be the possible risk factors of CRC. In addition, insulin-like growth factor (IGF) pathway is involved in colorectal carcinogenesis (13–16) and the signaling pathway is reported to be a potential target of CRC treatment (17–19) and CRC chemoprevention (20,21). Thus, importance of the growth hormone/IGF-1 axis (22) and IGF/IGF-1 receptor (IGF-1R) axis (15,23,24) is postulated in carcinogenesis in CRC development. In fact, our experimental studies indicated that the IGF/IGF-1R axis is altered during carcinogenesis in colorectum (25,26) and other tissue (27,28) and the axis is a good target for cancer chemoprevention (25–28). However, the underlying mechanisms of how these chronic diseases promote colon carcinogenesis still remain unknown (19). On this context, new research animal models are needed to investigate the diverse aspects of the mechanisms.

We have previously reported that development of AOM-induced precursor lesions is enhanced in C57BL/KsJ-db/db mice with hyperperleptinemia and hyperinsulinemia (29). Such an animal model may give important implications for further exploration of the possible underlying events that affect the positive association between CRC and obesity and/or diabetes (30–32). A number of animal models for diabetes and/or obesity have been reported. One such model is produced by injection of monosodium glutamate (MSG). When MSG is applied to Crj:CD-1 (ICR) newborn mice (MSG mice), they develop diabetic condition (hyperinsulinemia, hyperglycemia and hyperplastic islets) without polyphagia (33,34).

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Abbreviations: ACF, aberrant crypts foci; AOM, azoxymethane; BCAC, β-catenin-accumulated crypt; CRC, colorectal cancer; IGF-1R, insulin-like growth factor-1 receptor; MSG, monosodium glutamate.
the MSG-AOM mice) and physiological saline (0.1 ml/10 g body wt, the MSG-Saline mice), respectively. Similarly, two groups of the ICR-Saline mice were given AOM or saline, belonging to groups 3 (11 males, the Saline-AOM mice) and 4 (5 males, the Saline-Saline). At the termination of the experiment (10 weeks after the last injection of AOM and 17 weeks of age of mice), all animals were killed to analyze the number of colonic ACF and BCAC, clinical serum chemistry and mRNA expression of IGF1, IGF2 and IGF-1R in the colonic mucosa.

Counting the numbers of ACF and BCAC

The ACF and BCAC were determined according to the standard procedures described previously (30,31,41). ACF are defined as single or multiple crypts that have altered luminal openings, exhibit thickened epithelia and are larger than adjacent normal crypts (35). BCAC, which have high-frequency mutations in β-catenin gene, demonstrate histological dysplasia with a disruption of the cellular morphology (Figure 2A) and an accumulation of this protein (Figure 2B) (39). BCAC do not have a typical ACF-like appearance because the lesion is not recognized on the mucosal surface like ACF and is only identified in the histological sections of ‘en face’ preparations. Both of these lesions are utilized as biomarkers to evaluate a number of agents for their potential chemopreventive (42–44) and tumor promotion (45) properties.

After the colons were fixed flat in 10% buffered formalin for 24 h, the mucosal surface of the colons were stained with methylene blue (0.5% in distilled water) and then the number of ACF were counted under a light microscope. Theretofore, the distal parts (5 cm from anus) of the colon were cut into transverse. To identify BCAC intramuscosal lesions, the distal part of the colon (mean area: 0.7 cm²/colon) was embedded in paraffin and then a total of 20 serial sections (4 µm thick each) per colon were made by an en face preparation (30,31,41). For each case, two serial sections were used to analyze BCAC.

Histopathology and immunohistochemical analyses for β-catenin and IGF-1R

Three serial sections were made from paraffin-embedded tissue blocks. Two sections were subjected to hematoxylin and eosin (H and E) staining for histopathology and β-catenin immunohistochemistry to count the number of BCAC. Immunohistochemistry for β-catenin and IGF-1R was performed using the labeled streptavidin-biotin method (LSAB kit; DAKO, Glostrup, Denmark), as described previously (30,31). Primary antibodies of anti-β-catenin antibody (1:1000 final dilution) and anti-IGF-1R antibody (1:100 final dilution) obtained from Transduction Laboratories (catalog no. 610154; San Jose, CA) and Santa Cruz Biotechnology, Inc. (sc-7907; Santa Cruz, CA), respectively, were applied (1:1000 final dilution) and anti-IGF-1R antibody (1:100 final dilution) obtained from Transduction Laboratories (catalog no. 610154; San Jose, CA) and Santa Cruz Biotechnology, Inc. (sc-7907; Santa Cruz, CA), respectively, were applied on the sections. Negative control sections were immunostained without the primary antibody.

Blood chemistry

At 17 weeks of age, blood samples (0.5–1.0 ml/mouse) were collected for determination of total cholesterol, triglyceride and glucose by a simple measurement kit (DRICHIM Fujifilm Medical Co., Ltd, Tokyo). The concentration of blood insulin was measured by an LBIS insulin measuring kit for mouse (Shibayagi Co., Ltd, Gunma).

RNA extraction and quantitative real-time reverse transcription–polymerase chain reaction analysis

A quantitative real-time reverse transcription–polymerase chain reaction analysis was carried out in the scraped colonic mucosa of the MSG-AOM mice and the Salin-AOM mice. Total RNA was isolated from the scraped colon mucosa of the mice using the RNAqueous-4PCR kit (Ambion Applied Biosystems, Austin, TX). The cDNA was synthesized from 0.2 µg total RNA using the SuperScript III First-Strand Synthesis System (Invitrogen, Carlsbad, CA). The primers used for the amplification of IGF-1-, IGF-2- and IGF-1R-specific genes were as follows: IGF-1 forward, 5′-CTGAGACAGACCTTCTTGG-3′ and reverse, 5′-GACGGGAGCTTCTGAGTCTT-3′; IGF-2 forward, 5′-GTGCCTGTCTGCTGTTAC-3′ and reverse, 5′-ACTGCCTTCTCAGGACTGTTG-3′; and IGF-1R forward, 5′-GTCGGGCTCGTTTCTC-3′ and reverse, 5′-CATCCACTGTGGTTTCTCCA-3′. Real-time PCR was done in a LightCycler (Roche Diagnostics Co., Indianapolis, IN) with SYBR Premix Ex Taq (Takara Bio, Shiga, Japan). The expression levels of the IGF-1, IGF-2 and IGF-1R genes were normalized to the β-actin gene expression level.

Statistical analysis

Measurements are expressed as mean ± SD, and differences if present were compared by one-way analysis of analysis of variance (Tukey–Kramer’s multiple comparison’s test) or two-tailed unpaired t-test. The incidences of intestinal tumors were compared by Fisher’s exact probability test. The results were considered statistically significant if the P values were <0.05.

Results

General observations

As shown in Figure 1, the mean body weight of MSG–Saline mice (group 2) was much greater than Saline–Saline mice (group 4) during the study. The average body weights of the AOM-injected groups belonging to groups 1 (MSG–AOM) and 3 (Saline–AOM) were smaller than that of saline-injected groups, groups 2 (MSG-Saline) and 4 (Saline–Saline), during the study. At the termination of the experiment, the mean body weights of groups 1 and 3 were significantly lower than groups 2 and 4, respectively (P < 0.001), as listed in Table I.

The numbers of ACF and BCAC

ACF and BCAC (Figure 2A and B) developed in all the mice belonging to group 1 (the MSG-AOM mice) and 3 (the Saline-AOM mice) but not in the mice of groups 2 (the MSG-Saline) and 4 (the Saline-Salin mice) that did not receive AOM. Table I summarizes the total numbers of ACF and BCAC in all groups. The number of BCAC of group 1 was significantly greater than group 3 (P < 0.001), whereas the numbers of ACF developed in groups 1 and 3 were comparable.

Serum levels of glucose, total cholesterol, triglyceride and insulin

The serum concentrations of glucose, total cholesterol, triglyceride and insulin at 17 weeks of age are shown in Table II. MSG treatment significantly elevated all measures regardless of the AOM exposure (group 1 versus group 3, P < 0.001; and group 2 versus group 4, P < 0.001). However, the AOM administration did not affect all the measurements (group 1 versus group 2; and group 3 versus group 4).

β-Catenin and IGF-IR immunohistochemistry

Immunohistochemical expression of β-catenin revealed the presence of BCACs, where β-catenin was accumulated in the nucleus and/or cytoplasm (Figure 2B). Immunohistochemical expression of IGF-1R in the cytoplasm of BCAC that develop in the MSG-AOM mice was intensive when compared with the surrounding crypts (Figure 2C). Inflammatory cells infiltrated into the surrounding stroma of BCAC also showed positive reaction against IGF-1R.

mRNA expression levels of IGF-1, IGF-2 and IGF-1R in the colonic mucosa

The expression levels of IGF-1, IGF-2 and IGF-1R mRNAs of the colonic mucosa from the MSG-AOM mice (group 1) and the Saline-AOM mice (group 3) were determined. As illustrated in Figure 3, the MSG + AOM treatment mice showed significantly increased mRNA levels of IGF-1 (1.81-fold increase) and IRF-1R (2.43-fold increase), when compared with the Saline-AOM mice. The increase of IGF-1R was statistically significant (P < 0.01). mRNA levels of IGF-2 of the two groups were comparable.

Fig. 1. Body weight gains of all groups during the study.

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As expected, the findings described suggest that development of AOM-induced precancerous lesions, BCAC, of the colon in the MSG mice with hyperinsulinemia, hypercholesterolemia, hyperglycemia and hyperlipidemia was increased, when compared with the Salin-AOM mice. Our findings are in accordance with our previous findings that AOM-induced colon carcinogenesis is enhanced in another obese model using C57BL/KsJ-db/db mice (29–31). In the current study, the number of ACF was not different between the

### Table I. Body weights and numbers of ACF and BCAC per colon of mice treated with AOM and/or MSG at the end of study (17 weeks of age)

<table>
<thead>
<tr>
<th>Group number</th>
<th>Number of mice examined</th>
<th>Treatments</th>
<th>Body weight (g)</th>
<th>Total number of ACFs/colon</th>
<th>Total number of BCACs/colon</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (MSG-AOM)</td>
<td>12</td>
<td>+ (4 times daily) + (4 times weekly)</td>
<td>44.82 ± 2.22&lt;sup&gt;a,b,c&lt;/sup&gt;</td>
<td>78.00 ± 11.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.83 ± 7.44&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>2 (MSG-Saline)</td>
<td>6</td>
<td>+ (4 times daily) − (saline)</td>
<td>52.29 ± 2.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3 (Salin-AOM)</td>
<td>11</td>
<td>− (saline) + (4 times daily)</td>
<td>36.62 ± 4.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>69.27 ± 8.06&lt;sup&gt;f&lt;/sup&gt;</td>
<td>5.45 ± 1.86&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>4 (Saline–Saline)</td>
<td>5</td>
<td>− (saline) − (saline)</td>
<td>42.42 ± 0.78</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean ± SD.
<sup>b</sup>Significantly different from group 2 (<i>P</i> < 0.001).
<sup>c</sup>Significantly different from group 3 (<i>P</i> < 0.001).
<sup>d</sup>Significantly different from group 4 (<i>P</i> < 0.001).
<sup>e</sup>Significantly different from group 4 (<i>P</i> < 0.01).
<sup>f</sup>Significantly different from group 4 (<i>P</i> < 0.001).

### Table II. Serum levels of total cholesterol, triglycerides, glucose and insulin of mice treated with AOM and/or MSG at the end of study (17 weeks of age)

<table>
<thead>
<tr>
<th>Group number</th>
<th>Number of mice examined</th>
<th>Treatments</th>
<th>MSG (2 mg/kg body wt)</th>
<th>AOM (15 mg/kg body wt)</th>
<th>Total cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>Glucose (mg/dl)</th>
<th>Insulin (ng/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (MSG-AOM)</td>
<td>12</td>
<td>+ (4 times daily) + (4 times weekly)</td>
<td>167.92 ± 19.96&lt;sup&gt;b&lt;/sup&gt;</td>
<td>96.25 ± 14.38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>196.67 ± 34.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.66 ± 1.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 (MSG-Saline)</td>
<td>6</td>
<td>+ (4 times daily) − (saline)</td>
<td>177.50 ± 23.09&lt;sup&gt;c&lt;/sup&gt;</td>
<td>93.17 ± 12.64&lt;sup&gt;c&lt;/sup&gt;</td>
<td>202.67 ± 15.24&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.18 ± 1.41&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 (Salin-AOM)</td>
<td>11</td>
<td>− (saline) + (4 times daily)</td>
<td>107.64 ± 18.65</td>
<td>49.45 ± 13.87</td>
<td>110.36 ± 10.48</td>
<td>0.49 ± 0.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 (Saline–Saline)</td>
<td>5</td>
<td>− (saline) − (saline)</td>
<td>104.60 ± 13.69</td>
<td>45.20 ± 9.98</td>
<td>123.60 ± 11.30</td>
<td>0.50 ± 0.07</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean ± SD.
<sup>b</sup>Significantly different from group 3 (<i>P</i> < 0.001).
<sup>c</sup>Significantly different from group 4 (<i>P</i> < 0.001).

**Discussion**

As expected, the findings described suggest that development of AOM-induced precancerous lesions, BCAC, of the colon in the MSG mice with hyperinsulinemia, hypercholesterolemia, hyperglycemia and hyperlipidemia was increased, when compared with the Salin-AOM mice. Our findings are in accordance with our previous findings that AOM-induced colon carcinogenesis is enhanced in another obese model using C57BL/KsJ-db/db mice (29–31). In the current study, the number of ACF was not different between the
MSG-AOM mice and Saline-AOM mice, suggesting that BCAC rather than ACF has potential to progress to malignancies (39,42). This may be explained by the differences of pathobiological characteristics between two lesions: BCAC is dysplastic and microadenomas and ACF is consisted of hyperplastic and dysplastic lesions.

ACF have attracted attention as putative precancerous lesions in the colon in both experimental models and in humans (36). A number of molecular abnormalities, including increased expression of K-ras and APC gene mutations, are demonstrated in human ACF (46–48). BCAC, which accumulate β-catenin protein in the nucleus and cytoplasm, are also regarded as putative precursors to colorectal adenomas (37). Several rodent studies have shown that both of these lesions are useful as biomarkers to evaluate the chemopreventive properties of specific agents (49,50). In human colorectum, increased plasma IGF-1 levels are associated with the number of dysplastic ACF (51), suggesting that IGF-1 may be a promoter of the growth of dysplastic ACF and an independent risk factor of CRC. It may be possible that the number of dysplastic ACF, but not hyperplastic ACF, may be increased in the MSG-AOM mice, although we did not analyze two types of ACF.

Neonatal injections of MSG to mice or rats cause hyperthalamic damage (52,53), and as a consequence, these animals present several neuroendocrine and metabolic alterations, which lead central obesity, type 2 diabetes, insulin resistance, hyperinsulinemia, hypertriglyceridemia and hyperlipidemia (33). These abnormalities are risks for the development of CRC (9). The pancreatic islets in the mice subcutaneously injected MSG in neonatal period are hyperplastic up to 54 weeks of age (33). Therefore, our model described here may be suitable to study the pathobiology of diabetes- and obesity-associated colorectal carcinogenesis.

In this study, blood insulin level of the MSG-Saline mice (group 2) was the highest among the groups. There is accumulating evidence suggesting that hyperinsulinemia is involved in colon carcinogenesis, obesity and diabetes (3,8,9,12,54,55). Several epidemiological studies indicate that type 2 diabetic patients with hyperinsulinemia increases risk for CRC (3,12). Additionally, continuous injections of insulin promote AOM-induced colon carcinogenesis in rats (56,57). Hence, it seems likely that hyperinsulinemia in the MSG-AOM mice enhanced the development of AOM-induced lesions in the present study. Hyperglycemia and hypercholesterolemia observed in the MSG-AOM mice also contribute the development of BCAC and ACF in the colorectum because these conditions are positively associated with CRC occurrence (9,11,12). Hyperinsulinemia, hyperglycemia and hypercholesterolemia may singly or synergistically promote the development of preneoplastic and neoplastic colonic lesions. Although insulin resistance in colorectal carcinogenesis of obese people and/or type 2 diabetic patients is reported (7,55,58,59), we did not investigate presence or absence of insulin resistance in this study. Corpet et al. (60) reported that diet that increase some indirect insulin resistance markers does not promote colon carcinogenesis in female rats when ACF are used as a biomarker.

Regarding the mode of action, the current consensus assumes that the IGF-1 pathway plays a role in insulin-related tumor promotion in the colon (20,61). IGF-1 binds to the IGF-1R, activates a signal cascade and triggers cell proliferation in several tissues, including colon (62). Insulin at supra-physiological levels also binds to and activates the IGF-1R because of its homology with the insulin receptor (62). Furthermore, hyperinsulinemia was shown to indirectly increase bioavailability of IGF-1 by regulating levels of IGF-binding proteins (63). In this study, IGF-1R immunohistochemical expression of IGF-1 was strongly positive in the cytoplasm of BCAC developed in the MSG-AOM mice. Indeed, overexpression of IGF-1R was also reported in human CRC (64). Accordingly, it may be possible that hyperinsulinemia in MSG-AOM mice activates the signaling cascades involving the IGF-1R, resulting in a proliferative response (19,59,61). Another interesting findings regarding IGF-1R immunohistochemistry is that inflammatory cells infiltrated around BCAC were positively reacted against the IGF-1R antibody. Significance of the findings is not known, but similar findings have been reported in human Crohn’s disease (65). IGF-1 and IGF2 are potentially relevant mediators in the chronic inflammation (27) and mediate the majority of their biological action through IGF-1R (66). Thus, our findings on the IGF-1R immunohistochemical positivity in inflammatory cells suggest that inflammation in the microenvironment of precancerous lesions for CRC may contribute to the growth of the lesions (67,68).

In conclusion, our data indicate that the MSG-AOM mice with hyperinsulinemia, hypercholesterolemia, hyperglycemia and hypercholesterolemia are highly susceptible to colorectal carcinogenesis and the MSG-AOM mouse model could be useful for investigating the mechanisms of obesity/diabetes-associated events involving in colorectal carcinogenesis and the therapeutic and chemopreventive strategies of CRC in obese people and/or type 2 diabetic patients.

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