TECHNIQUE REPORT

An Optimized Protocol of Azoxymethane-Dextran Sodium Sulfate Induced Colorectal Tumor Model in Mice

Xi Liang[#], Jingnan Hu[#], Jianming He^{*}

Department of Radiotherapy, Hebei Province Hospital of Chinese Medicine,
Hebei University of Chinese Medicine, Shijiazhuang 050011, China

Key words: animal model, colorectal cancer, inflammation, protocol

Abstract Since azoxymethane (AOM)-dextran sulfate sodium (DSS) induced tumorigenesis was used to explore inflammation-associated carcinogenesis of sporadic colorectal cancer (CRC), different administration modes of AOM or DSS have been reported. In this article we optimized the protocol of the AOM-DSS modeling using C57BL/6 mice for study on sporadic CRC by intraperitoneal injecting AOM solution at a proper concentration with a 100 µl sterile syringe once, feeding DSS solution for 7 days in a row and change DSS solution every day. More than 100 C57BL/6 mice had been treated with the optimized protocol, and all mice were demonstrated to suffer from colorectal tumors when sacrificed in 8 to 20 weeks after AOM injection. These tumors mainly occurred in distal segment of colorectum with an increase in tumor density, which is similar to CRC in human beings. Our results also suggested that tumor per mouse was high and variation of tumor number per mouse was low. In the AOM-DSS model, the histology of tumor developed through defined stages ranged from precursor lesions, adenomatous lesions, adenomas to adenocarcinomas. The modified protocol of AOM-DSS model is easy, cheap, with high tumor formation rate of colorectal tumors.

INTRODUCTION

Background

Colorectal cancer (CRC) is the third most common cancer in males and the second in females.¹ The incidence is increasing in most of developing countries, partly attributing to lipid metabolism.¹⁻³ According to its inci-

dence, CRC is categorized in three forms: family, hereditary, and sporadic (about 80% of CRC).^{4, 5}

Many *in vitro* models and *in vivo* models have been developed and used in understanding CRC.^{2, 6} *In vivo* models include inoculated CRC models and tumors induced by "diet" (high fat, low calcium, etc), by chemical, by gene mutation or genetic modification (APC lines, Kras, Msh2, P53, Rb, and so on).⁷⁻⁹ Subcutaneous inoculation models are widely used to study responses of CRC to chemotherapy, radiotherapy and other types of therapy^{10, 11}. Inoculated CRC models (the orthotopic model, the intrasplenic inoculation model, the intraportal inoculation model, etc) are options to study metastasis of CRC.^{9, 12} "Diet" (high fat, low calci-

Received August 9, 2018; Accepted October 10, 2018. Published online December 4, 2019.

*Corresponding author Email: hjmlovelx@hotmail.com

[#] Authors contributed equally to this work.

Supported by the Hebei Provincial research foundation of Health and Family Planning Commission (No. 20180688)

um, etc) alone can hardly induce tumors and they are commonly employed as a factor of promoting tumors¹³. Gene mutant or genetically modified colorectal tumor models are primarily used for studies addressing hereditary aspects of CRC (family, hereditary).^{8,9} These models are confounded by development of tumors outside colorectum.^{8,9,13} Furthermore, these models rarely develop malignant tumors, let alone metastases.^{8,9,13}

Chemically induced colorectal tumor models are optimal to study sporadic CRC.^{2,7,9,14} Carcinogens used in these models include dimethylhydrazine (DMH), azoxymethane (AOM, a downstream metabolite of DMH), dextran sulfate sodium (DSS), 2-amino-1-methyl-6-phenylimidazol (4,5-b) pyridine (PhIP), N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), methylnitrosourea (MNU), 3,2'-dimethyl-4-aminobiphenyl (DMBA).^{8,9,13,14} AOM can initiate cancer by alkylation of DNA.^{2,7,14} DSS is a pro-inflammatory reagent and using DSS alone can hardly induce colorectal tumor.^{2,7,14} But, the combination of AOM and DSS model (the AOM-DSS model) has been proved to be an easy, cheap, rapid and reproducible model with high incidence of colorectal tumors.^{2,7,14} AOM-DSS induces much higher tumor incidence, more tumors per mouse at earlier time point than most of other carcinogens.^{2,8,13} All tumors located in colorectum, with more distal tumors increasing in density, which is similar to CRC in human beings.^{2,14} Tumors develop through defined stages ranging from precursor lesions (abberant crypt foci formation or mucin-depleted foci), adenomatous lesions, adenoma to adenocarcinomas.^{2,14} Tumors induced by AOM-DSS resemble human CRC at the molecular level. Mutations of β -catenin play a key role in CRC carcinogenesis, progression, chemoresistance and so on. AOM-DSS induced colorectal tumors often have gene mutations at codons 32 to 34 in mice and this is similar to human CRC.^{9,15} The protein level of β -catenin increases in almost all tumors in the AOM-DSS model and in most of human CRC.^{2,7} Other genes and proteins, for example, Kras, cyclin D1, also share the same feature in both the AOM-DSS model and human CRC⁷. Consequently, the AOM-DSS model is well accepted as an excellent model to analyze sporadic and inflammation-driven human CRC.

Several AOM-DSS models (1 cycle AOM followed by 1-5 cycles of DSS) have been reported.^{7,14,16,17} The 1 AOM followed by 3 cycles of DSS model and the 1 AOM followed by 1 cycle of DSS model are widely used.^{2,14} The prior model mimics inflammatory bow-

el disease (IBD) course of human beings, including Crohn's disease and ulcerative colitis. IBD is strongly associated with inherited factors and is undoubtedly a precancerous disease.^{7,8} It develops both chronic colitis and colitis activity, and deep inflammatory lesions, active colitis, UI-III ulcer and even more serious ulcer commonly coexists.^{18,19} Therefore, it is widely used to study the role of inflammation in carcinogenesis of CRC. Most of sporadic CRC are not, at least not strongly, correlated with inflammation. To study sporadic CRC, the 1 AOM followed by 1 cycle of DSS model may be more ideal because it develops neither chronic colitis nor flares of colitis activity, at the same time, it has lower mortality rate (below 10%) than the 1 AOM followed by 3 cycles of DSS model.^{2,7,9,14} In the 1 AOM followed by 1 cycle of DSS model, inflammation in colorectum gradually decrease after DSS treatment, and UI-II ulcer or serious ulcer are not found in the colorectum in 6 week.²⁰ Several researchers mentioned that 1 AOM followed by 1 cycle of DSS induces tumors was slower than 1 AOM followed by 3 cycles of DSS, but, to our knowledge, no head-to-head study has been reported.⁷ Here, we studied the 1 AOM followed by 1 cycle of DSS model, addressing spontaneous colorectal tumor⁷.

APPLICATION AND VALUE OF THIS PROTOCOL

We modified a few technicalities of the 1 AOM followed by 1 cycle of DSS models (Figure 1) and described the whole procedure of the model in detail in this article.

Unprecise AOM intraperitoneal injection will definitely cause high variability. Some researchers injected intraperitoneally AOM working solution of 1 mg/ml, 10 μ l/g body weight with a 1ml syringe. The body weight of the C57BL/6 mouse is about 20 to 25 gram. That means, a mouse will be injected 200 to 250 μ l AOM solution and it is not easy to inject accurate volume of solution with a 1ml syringe. We proposed to inject intraperitoneally AOM working solution at a concentration of 2.5 mg/ml, volume of 4 μ l/g body weight using a 100 μ l sterile syringe for C57BL/6 mice. Under this condition, a mouse will be injected about 80 to 100 μ l AOM solution and the injection is easy to be performed accurately.

Different modes of DSS administration were reported.^{2,7} Most researchers fed mice with DSS-containing water at day 7 and replaced the water at day 10

and day 12.⁷ Because DSS is not stable in solution under room temperature in light, we replaced DSS-containing water every day to avoid the influence of DSS degradation.

This protocol with technicality modifications is expected to provide researchers an easy, cheap, rapid and reproducible model with high incidence to understand CRC, including, but not limited to, carcinogenesis, progression, prevention, biomarkers, the identification and pre-clinical validation of therapeutic targets^{2, 7}. However, as an AOM-DSS models, it still shares the same limitations such as coexistence of benign tumors and malignant tumors in an experimental mouse, more low-grade cancers than high-grade cancer, as well as rare metastases.^{2, 8}

EXPERIMENTAL DESIGN

Mice selection

The study was approved by the Ethics Committee of Hebei Province Hospital of Chinese Medicine. This protocol has been reported in previous study with mice and rats.^{2, 21} Different mouse strains show different susceptibility to AOM or DSS.^{2, 7, 9, 14} Researchers may adjust concentration and volume of AOM solution, concentration and duration of DSS according to the employed strain. We have successfully used this protocol without any adjustment on 6 to 7 week-old male C57BL/6 mice in our previous study.²

As in human beings lots of factors increase CRC risk, such as heredity, gender, age, obesity, smoking, consumption of red meat, alcohol, gut microbiota,^{1, 2, 13} in mice, heredity, gender, age, body weight, gut microbiota, diet etc. also influence colorectal tumorigenesis.^{2, 7, 22} Therefore, taking these factors into account, we suggest that both the control mice and the experimental mice be maintained in one cage to exclude potential effects from uncertain factors, such as gut microbiota, diet, and etc.

AOM treatment

Different mouse strains show different susceptibility

to AOM. Some mouse strains, such as Crj, A/J, SWR/J or FVB/N are considered to have high to moderate susceptibility to AOM, while C57BL/6, Balb/C, ICR, AKR/J, 129SvJ and DBA/2J are considered to have low susceptibility to AOM.^{2, 7, 9, 14, 18} A dose of 10 ± 2.5 mg AOM per kg body weight was reported in studies of different mouse sublines.^{7, 23} Some researchers injected intraperitoneally AOM working solution of 1 mg/ml, 10 μ l/g body weight. For C57BL/6 mice, we proposed to inject intraperitoneally AOM working solution of 2.5 mg/ml, 4 μ l/g body weight with a 100 μ l sterile syringe once. It is easy to handle and easy to inject AOM accurately, because each mouse receives about 80 to 100 μ l AOM solution. If used in other stains mice, adjusting concentration of AOM working solution may be needed.

DSS treatment

Different mouse strains show different susceptibility to DSS. C57BL/6 and ICR showed moderate to high susceptibility to DSS, while FVB/N mice showed low to moderately susceptible to DSS.^{2, 7, 9} Variant dose (1–4% weight/volume), apply time (5–7 days) and administration method of DSS have been reported.^{7, 9, 24} Dr. Neufert suggested to feed mice with DSS-containing water at day 7 and replaced the water at day 10 and day 12.⁷ Because DSS is not stable enough in solution at room temperature under the light, degradation will accumulate with time. To avoid the effect from DSS degeneration, we empty and refill DSS-containing water every day in our protocol.

Waiting time period

The progress of tumor also depends on the duration of induction. AOM/DSS treatment in mice offers a powerful model ranged from precursor lesions to adenocarcinoma^{2, 9, 25}. Precursor lesions represent early end points and in this case, they should be scored several weeks after DSS treatment. According to literature, 3 to 13 weeks of waiting period were adopted.^{7, 26, 27} To score spontaneous tumor progression, mice are commonly sacrificed at 10 to 20 weeks after DSS treat-

Figure 1. The schematic of the protocol.

ment, rarely at 29 weeks after AOM treatment^{2, 7, 25}.

Monitoring body weight

Since both AOM and DSS are toxic, the body weight of mice should be monitored. AOM rarely causes decrease in body weight but almost all mice decrease their body weights at the beginning or after DSS treatment because of enteritis caused by DSS. If body weight of a mouse decrease by more than 20% within 1 week during this period, euthanasia is suggested. The mortality of mice is usually kept under 5%; if the mortality reaches 10% or higher, 0.5% to 1% concentration reduction is required. Colorectal tumors cause weight loss too, but this is expected to happen a few months after DSS treatment. Prolapse of rectum and hematochezia are common symptoms at 10 weeks after DSS treatment.

MATERIALS

Reagents

AOM (Sigma-aldrich; Cat.: A5486)

DSS (MP Biomedicals; Cat.: 0218055880-100g)

PBS (beyotime; Cat.: C0221A)

Equipments

N95 mask (Medicom; Cat.: MED0002321-0080020),

100 μ l Syringes (Hamilton Company; Cat.: 80601)

0.22 μ m Filters (Millipore; Cat.: SLGP033RB)

60 ml plastic water bottles with a steel ball in the steel tube (Alex; Cat.: AL142)

Biological Safety Cabinets (Thermo Scientific; Cat.: 51025411)

PROCEDURES

Mouse preparations for AOM-DSS induced colorectal tumorigenesis

1. Sex- and age-matched C57BL/6 mice are housed in cages in a specific pathogen-free animal facility under standard conditions of 22 ± 2 °C with a daylight cycle from 6 AM to 6 PM.

2. Label mice. Divide mice into groups, maintaining both the control mice and the experimental mice in one cage if the experiment has the control group and the experimental group. Allow no less than one week of acclimation before any treatment.

Note: a. Use animals of the same gender, male mice are preferred to avoid influence from menstrual periods. b. We suggest that the age gap in one experiment be less than 1 week. c. Operate the procedure 2 at the mouse age of three- to five-week old to reduce fighting among mice, and the earlier the better. d. We suggest that mice in control group and experimental group are littermates. This could reduce effects by differences in origin/genetic backgrounds. e. If possible, separate mice in different cages and one cage contains both control mice and experimental mice.

AOM stock solution preparation

3. When mice reach six- to eight-week-old, weigh them. Calculate the amount of AOM needed (10 mg/kg body weight). Dissolve AOM in saline at the concentration of 10 mg/ml, and filtrate AOM solution with a sterile 0.22 μ m filter.

4. Aliquot and store at -20 °C in sterile glass tubes.

Note: a, AOM is more stable in saline than in PBS. b, AOM solution should be stored in glassware rather than in plastic tubes. c, aliquot to avoid multiple freeze/thaw cycles of stock solution. d, mice with body weight below 18 g are not used.

AOM treatment

5. Thaw stock solution in amount as needed; dilute the AOM stock solution 1:4 in sterile saline to obtain working solution at a concentration of 2.5 mg/ml.

6. Weigh mouse and calculate the volume of AOM working solution needed by weight (4 μ l/g body weight).

7. Inject AOM working solution intraperitoneally with a 100 μ l sterile syringe.

8. After AOM injection, weight mice and record for day 0.

Note: a, when treated with AOM, the body weight gap of mice in one experiment should be less than 5 g. b, As AOM is a strong carcinogen and flammable, AOM stock solution and working solution should be prepared in a laminar airflow biosafety cabinet, and intraperitoneal injection in a fume hood. c, we suggest the administration of AOM working solution be a one-time intraperitoneal injection at a proper concentration using a 100 μ l sterile syringe. d, in case that body weight of a mouse is over 25g, the concentration of AOM working solution should be calculated to meet the maximum volume of a 100 μ l sterile syringe.

The treatment interval

9. House mice for 6 days and weight mice every days.

DSS treatment

10. On day 7, prepare 2% DSS solution of the amount as needed (6ml per mouse per day for 7 days).

11. Fill the 60 ml plastic water bottle of the mouse cage with 2% DSS solution and record the volume (A mouse drinks less than 5 ml DSS solution per day). Store the left DSS solution at 4 °C.

12. On the next day, record the volume of DSS solution left in the bottle. Then, empty and refill the water bottle with DSS solution stored.

13. Repeat step 11 and 12 every day from day 9 to day 13.

14. On the day 14, write down the left DSS solution in the bottle, replace the water bottle and fill it with normal water.

Note: a, DSS is toxic. Preparation of DSS solution should be in a laminar airflow biosafety cabinet. For procedure 10-14, the operator should wear a N95 mask or a better one. b, molecular weight of DSS should be 36,000–50,000 Da. c, we suggest DSS solution be replaced every day.

Waiting period

15. House mice for 5 to 20 weeks.

Colon collection

16. At the time point of choice, fast mice for 6 to 12 hours.

17. Euthanize mice. Immobilize the dead mouse in a supine position. Spray the abdomen with 70% ethanol.

18. Open the abdominal cavity using forceps and scissors. Cut the pelvic bone to dissect the distal part of the colon completely.

19. Remove the entire colon (including the rectum and the anus) using forceps and scissors.

20. Immediately sink the colon in a Petri dish filled with ice cold PBS.

21. Open the colon longitudinally, rinse the colon twice with ice cold PBS to clean up feces, and be ready for subsequent studies.

TIMING

Steps 1–2, mouse preparations: 1 week

Steps 3–4, AOM stock solution preparation:

1–2 hours

Steps 5–8, AOM treatment: 1–4 hours (depending on the number of mice)

Steps 9, time interval between AOM and DSS treatment: 6 days

Steps 10–14, DSS treatment: every day for 7 days

Step 15, waiting period for collection: 5 to 20 weeks (depending on the objective of the study)

Steps 16–21, colon collection: 1 d

ANTICIPATE RESULTS

The AOM-DSS model in this protocol is optimized to induce colorectal tumors for the studies of sporadic CRC in mice. All C57BL/6 mice treated using this protocol had colorectal tumors when sacrificed 8 to 20 weeks after AOM injection. The incidence of tumors in C57BL/6 mice after AOM treatment was 100%, and the multiplicity at 8 weeks, 10 weeks and 20 weeks were 5.17 ± 0.95 , 6.83 ± 0.79 , and 11.83 ± 1.58 respectively (**Table 1**). All tumors located in colorectum, with higher tumors density in distal end (**Figure 2**), which is similar to CRC in human beings. After AOM-DSS treatment, colorectal tumors increased in number and size along with time. Tumors develop through defined stages ranging from precursor lesions (aberrant crypt foci formation or mucin-depleted foci), adenomatous lesions, adenoma to adenocarcinomas (**Figure 3**). The progression of tumors also depends on the time duration, which means, colorectal tumors increased in number, size and ratio of malignancies along with time.

This protocol offers an easy, rapid, reproducible and powerful model for studies on CRC. It produces high tumor incidence, more tumors per mouse, and low variation of tumor number per mouse. Meanwhile, although after DSS treatment, the body weights of almost all mice decreased due to enteritis caused by DSS, most of these mice regained weight to the pretreatment level in two weeks after termination of DSS treatment (**Figure 4**).

Table 1. The colorectal tumor in C57BL/6 mice induced by AOM-DSS after AOM injection

Time after AOM injection	Number of mice	Number of mice with tumors	Tumor number per mouse [§]
8 W	6	6	5.17 ± 0.95
10 W	6	6	6.83 ± 0.79
20 W	6	6	11.83 ± 1.58

§ mean $\pm$ standard error

Figure 2. Gross specimen photographs of colorectums in mice with colorectal tumors induced by AOM-DSS. A. mouse sacrificed 10 weeks after AOM injection. Colon was excised (up) and longitudinally opened (down), showing tumors were mainly distributed at the distal part of colorectum. B. mouse sacrificed 20 weeks after AOM injection. Colon was excised (up) and longitudinally opened (down), showing increased tumor number and size, with higher tumor density found in distal end.

Figure 3. Histopathological findings of colorectal adenoma (up) and colorectal adenocarcinoma (down) of mice (sacrificed 20 weeks after AOM injection) induced by AOM-DSS treatment (HE staining.).

CONFLICT OF INTEREST

The authors disclosed no potential conflicts of interest.

REFERENCES

1. Torre LA, Bray F, Siegel RL, et al. Global cancer statistics, 2012. *CA Cancer J Clin* 2015; 65(2): 87-108. doi: 10.3322/caac.21262.
2. He J, Shin H, Wei X, et al. NPC1L1 knockout protects

Figure 4. Body weight changes of the mice treated with AOM-DSS along with time (n=10).

The body weight of mice decreased at the beginning of and early after DSS treatment (the second week), regained to the level before DSS treatment in about two weeks after the termination of DSS treatment. The data shown are the mean $\pm$ standard error.

against colitis-associated tumorigenesis in mice. *BMC Cancer* 2015; 15: 189. doi: 10.1186/s12885-015-1230-0

3. Jain R, Austin Pickens C, Fenton JI. The role of the lipidome in obesity-mediated colon cancer risk. *J Nutr Biochem* 2018; 59: 1-9. doi: 10.1016/j.jnutbio.2018.02.015.
4. Dudley-Brown S, Freivogel M. Hereditary colorectal cancer in the gastroenterology clinic: how common are at-risk patients and how do we find them? *Gastroenterol Nurs* 2009; 32(1): 8-16. doi: 10.1097/SGA.0b013e3181965d04.
5. Zelga P, Przybylowska-Sygut K, Zelga M, et al. Polymorphism of Gly39Glu (c.116G>A) hMSH6 is associated with sporadic colorectal cancer development in the Polish population: Preliminary results. *Adv Clin Exp Med* 2017; 26(9): 1425-29. doi: 10.17219/acem/64877.
6. He J, Liang X, Luo F, et al. P53 Is Involved in a Three-Dimensional Architecture-Mediated Decrease in Chemosensitivity in Colon Cancer. *J Cancer* 2016; 7(8): 900-9. doi: 10.7150/jca.14506.
7. Neufert C, Becker C, Neurath MF. An inducible mouse model of colon carcinogenesis for the analysis of sporadic and inflammation-driven tumor progression. *Nat Protoc* 2007; 2(8): 1998-2004. doi: nprot.2007.279.
8. Taketo MM, Edelmann W. Mouse models of colon cancer. *Gastroenterology* 2009; 136(3): 780-98. doi: 10.1053/j.gastro.2008.12.049.

TROUBLE SHOOTING

Problem	Possible reasons	Solutions	Step
High death rate of mice during AOM and DSS treatment	Mouse strain highly susceptible to AOM	Switch to a strain of lower susceptibility	1
		Decrease AOM dose	6
	Injury of blood vessels and/or abdominal viscera	Ensure precise injection technique	7
		Injection AOM into vein	7
High death rate of mice after DSS treatment	Mouse strain highly susceptible to DSS	Switch to a mouse strain of lower susceptibility	1
		Decrease DSS concentration and/or reduce the period of administration	10-14
		Decrease AOM dose	6
	Too many and/or too large colorectal tumorous lesions	Decrease DSS concentration and/or reduce the period of administration	10-14
		Reduce the waiting period	15
Low tumor incidence and/or no or few tumors per mouse	Mouse strain lowly susceptible to AOM/DSS	Switch to a mouse strain of highly susceptibility	1
		Increase AOM dose	6
		Increase DSS concentration and/or prolong the period of administration	10-15
	AOM dose too low	Increase AOM dose	10
	DSS concentration too low	Increase DSS concentration	10-14
High variability within experimental groups	Waiting period too short	Prolong waiting period	15
	Genetic backgrounds not pure	Purify genetic background	1
	Differences in origin	Use mice from the same offspring	1
	Differences in gender	Do not use female mice	1
	Affect by menstrual periods	Do not use female mice	1
	Imprecise injection	Ensure precise injection technique	7

9. Tong Y, Yang W, Koeffler HP. Mouse models of colorectal cancer. *Chin J Cancer* 2011; 30(7): 450-62. doi: 10.5732/cjc.011.10041.
10. He JM, Wang FC, Qi HB, et al. Down-regulation of alphav integrin by retroviral delivery of small interfering RNA reduces multicellular resistance of HT29. *Cancer Lett* 2009; 284(2): 182-8. doi: 10.1016/j.canlet.2009.04.023.
11. Mikalsen SG, Mikalsen LTG, Sandvik JA, et al. Low dose-rate irradiation with [(3)H]-labelled valine to selectively target hypoxic cells in a human colorectal cancer xenograft model. *Acta Oncol* 2018; 57(9): 1216-24. doi: 10.1080/0284186X.2018.1457223.
12. O'Rourke KP, Loizou E, Livshits G, et al. Transplantation of engineered organoids enables rapid generation of metastatic mouse models of colorectal cancer. *Nat Biotechnol* 2017; 35(6): 577-82. doi: 10.1038/nbt.3837.
13. Fleet JC. Animal models of gastrointestinal and liver diseases. New mouse models for studying dietary prevention of colorectal cancer. *Am J Physiol Gastrointest Liver Physiol* 2014; 307(3): G249-59. doi: 10.1152/ajpgi.00019.2014.
14. Tanaka T, Kohno H, Suzuki R, et al. A novel inflammation-related mouse colon carcinogenesis model induced by azoxymethane and dextran sodium sulfate. *Cancer Sci* 2003; 94(11): 965-73. doi: 10.1111/j.1349-7006.2003.tb01386.x.
15. Kim IJ, Kang HC, Park JH, et al. Development and applications of a beta-catenin oligonucleotide microarray: beta-catenin mutations are dominantly found in the proximal colon cancers with microsatellite instability. *Clin Cancer Res* 2003; 9(8): 2920-5.
16. Turpin B, Miller W, Rosenfeldt L, et al. Thrombin drives tumorigenesis in colitis-associated colon cancer. *Cancer Res* 2014; 74(11): 3020-30. doi: 10.1158/0008-5472.CAN-13-3276.
17. Bugni JM, Meira LB, Samson LD. Alkylation-induced colon tumorigenesis in mice deficient in the Mgmt and Msh6 proteins. *Oncogene* 2009; 28(5): 734-41. doi: 10.1038/onc.2008.426.
18. Chen Y, Zhang P, Xu SC, et al. Enhanced colonic tumorigenesis in alkaline sphingomyelinase (NPP7) knockout mice. *Mol Cancer Ther* 2015; 14(1): 259-67. doi: 10.1158/1538-7445.AM2015-00019.

- 10.1158/1535-7163.MCT-14-0468-T.
19. Lavi I, Nimri L, Levinson D, et al. Glucans from the edible mushroom *Pleurotus pulmonarius* inhibit colitis-associated colon carcinogenesis in mice. *J Gastroenterol* 2012; 47(5): 504-18. doi: 10.1007/s00535-011-0514-7.
20. Suzuki R, Kohno H, Sugie S, et al. Sequential observations on the occurrence of preneoplastic and neoplastic lesions in mouse colon treated with azoxymethane and dextran sodium sulfate. *Cancer Sci* 2004; 95(9): 721-7. doi: 10.1111/j.1349-7006.2004.tb03252.x.
21. Shirakami Y, Kochi T, Kubota M, et al. Inhibitory effects of pentoxifylline on inflammation-related tumorigenesis in rat colon. *Oncotarget* 2018; 9(74): 33972-81. doi: 10.18632/oncotarget.26119.
22. Chen L, Jiang B, Zhong C, et al. Chemoprevention of colorectal cancer by black raspberry anthocyanins involved the modulation of gut microbiota and SFRP2 demethylation. *Carcinogenesis* 2018; 39(3): 471-81. doi: 10.1093/carcin/bgy009.
23. Yao J, Zhao L, Zhao Q, et al. NF-kappaB and Nrf2 signaling pathways contribute to wogonin-mediated inhibition of inflammation-associated colorectal carcinogenesis. *Cell Death Dis* 2014; 5: e1283. doi: 10.1038/cddis.2014.221.
24. Clapper ML, Cooper HS, Chang WC. Dextran sulfate sodium-induced colitis-associated neoplasia: a promising model for the development of chemopreventive interventions. *Acta Pharmacol Sin* 2007; 28(9): 1450-9. doi: 10.1111/j.1745-7254.2007.00695.x.
25. Femia AP, Dolara P, Luceri C, et al. Mucin-depleted foci show strong activation of inflammatory markers in 1,2-dimethylhydrazine-induced carcinogenesis and are promoted by the inflammatory agent sodium dextran sulfate. *Int J Cancer* 2009; 125(3): 541-7. doi: 10.1002/ijc.24417.
26. Boddicker RL, Whitley EM, Davis JE, et al. Low-dose dietary resveratrol has differential effects on colorectal tumorigenesis in adiponectin knockout and wild-type mice. *Nutr Cancer* 2011; 63(8): 1328-38. doi: 10.1080/01635581.2011.607538.
27. Osburn WO, Karim B, Dolan PM, et al. Increased colonic inflammatory injury and formation of aberrant crypt foci in Nrf2-deficient mice upon dextran sulfate treatment. *Int J Cancer* 2007; 121(9): 1883-91. doi: 10.1002/ijc.22943.