Effect of Cold Exposure and Capsaicin on the Expression of Histone Acetylation and Toll-like Receptors in 1,2-dimethylhydrazine-induced Colon Carcinogenesis

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Research Article

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Abstract

Previous studies have indicated that capsaicin-rich diet and cold weather have showed strong association with tumor incidence. Thus, we investigated the effects of capsaicin and cold exposure in 1,2-dimethylhydrazine-induced colorectal cancer as well as the mechanisms underlying capsaicin and cold induced CRC. Rats were randomly divided into four groups and received cold still water and capsaicin via intragastric gavage until the end of the experiment. The rat's body weight, thymus weight, and food intakes were assessed. Global levels of histone H3K9, H3K18, H3K27, H4K16 acetylation and, histone deacetylase (HDACs) in colon mucosa were assessed by western blot. Expression levels of Toll-like receptors 2 (TLR2) and Toll-like receptors 4 (TLR4) were measured by western-blot and reverse-transcriptase quantitative polymerase chain reaction (qPCR). We found that cold and low-does capsaicin increased tumor numbers and multiplicity, although there were no differences in tumor incidence. Additionally, rat exposure of cold water and capsaicin display further higher levels of histone H3 lysine 9 (H3K9AC), histone H3 lysine 18 (H3K18AC), histone H3 lysine 27 (H3K27AC) and HDACs compared with the DMH and normal rats. In contrast, a considerable decrease of histone H4 lysine 16 (H4K16AC) was detected in the colon mucosa. Cold and low-dose capsaicin exposure group were also increased TLR2 and TLR4 proteins levels and mRNA levels. These results suggest that chronic cold exposure and capsaicin at a low-does intervention exacerbate ectopic expression of global histone acetylation and TLR level, which are crucial mechanisms responsible for the progression of colorectal cancer in rat.

1. Introduction

Colorectal cancer (CRC) has been recognized as a multi-factorial malignant disorder and remains its status as the most common cause of cancer-related death in men and women (Siegel, Miller, & Jemal, 2019). Colon cancer is caused by complex interactions of the immune system with non-modifiable (eg, genetic and epigenetic alterations) and modifiable (eg, environmental, diet and lifestyle) risk factors (Grazioso, Brandt, & Djouder, 2019; Lao & Grady, 2011). The fact that known high penetrance gene and epigenetic alterations that are related to colorectal cancer risk explain fewer than 5% of the observed case, suggested that environmental factors including diet, alcohol consumption, and ambient environment play a pivotal role in risk (Lichtenstein et al., 2000). Moreover, it is important to note that these modifications of known factors are most associated with cancer death in China (Chen et al., 2019).

Identify the epigenetic mechanisms and their interaction with diet and Toll-like Receptors is crucial to reveal the regulation of molecular pathways leading to colorectal carcinogenesis. Colorectal cancer (CRC) is characterized by the accumulation of genetic and epigenetic alterations (Okugawa, Grady, & Goel, 2015). Epigenetic modifications have been implicated in the regulation of gene expression, without affecting the DNA sequence (Strahl BD, 2000). Among these modifications, histone acetylation on lysine residues is the most common histone modifications. The expression level of histone acetylation is important regarding chromatin states and gene expression. Overall, lysine acetylation generally opens the chromatin and activates gene expression, whereas deacetylated is related to transcriptional repression (Berger, 2007). It has previously been reported that aberrant histone acetylation was implicated in the

initiation and progression of various solid tumors including CRC (Kaypee et al., 2016). Dynamic levels of reversible acetylation are mediated by histone acetyltransferases (HATs) and histone deacetylases (HDACs). They are classified into four classes based on cellular localization and functional regulation (Annemieke J. M. DE RUIJTER & KUILENBURG, 2003). More and more research indicated that germline mutations of HDACs increase the risk of breast and lung cancers, and abnormal HDAC overexpression has also been observed in a wide range of malignancies, thereby serving as therapeutic targets for CRC (Glozak & Seto, 2007; Singh, Bishayee, & Pandey, 2018). Previous study indicated that animal housing at sub-thermoneutral (22–26°C) has a profound effect on obesity, inflammation, cancer, and other metabolic disorders (Ganeshan & Chawla, 2017; Hylander & Repasky, 2016). For example, studies in broilers revealed that long-term cold stimulation aggravated oxidative stress, ultrastructural cardiac damage, immune dysregulation, and inflammation (Wei et al., 2018). It was also found that the cold environment substantially potentiates the development of malignancy resulting from subthermoneutral laboratory housing temperature cause mild chronic cold stress, which triggers suppression of the antitumor immune response (Kokolusa et al., 2013). Some published reports have described an anti-cancer role for capsaicin, while others have argued that capsaicin implied as a promoting factor by inducing the growth and progression of preexisting neoplasms (Bode & Dong, 2011). In vitro and vivo experimental of CRC indicated that capsaicin exerts anti-tumor activity through induces cytotoxicity and apoptosis (Lu et al., 2010). On the other hand, Yang et al. reported that human colon carcinoma promotion involved in the Akt/mTOR/STAT-3 signaling pathway (Yang et al., 2013). Toll-like receptors (TLRs) have an important role in the activation of both innate and adaptive immunity, and aberrant activation of the TLR signal can result in disturbance of intestinal immune homeostasis, chronic inflammatory, and therefore an important modulator in the pathogenesis of CRC (Lavelle, Murphy, O'Neill, & Creagh, 2009). It has been also demonstrated that TLR agonists have immune regulatory applications as vaccine adjuvants in cancer therapy (Moradi-Marjaneh et al., 2018). Furthermore, epigenetics has recently been proven to be a new regulator of TLR expression (Xie et al., 2018). However, much less known regarding the mechanism underlying the putative effect of potential cold and capsaicin exposure contribution on colon carcinoma in rat models.

Therefore, the purpose of this research was to evaluate the expression of aberrant histone modification via the modulation of HDACs as well as the TLR signaling pathway on colon carcinogenesis.

2. Material And Method

2.1. Chemicals and preparation

1,2-dimethylhydrazine (1,2-DMH) was purchased from Sigma-Aldrich (St.Louis, MO, USA). DMH was weighed and dissolved in 1Mm EDTA-saline to ensure the stability of the chemical (Gungor, Ilhan, & Eroksuz, 2018).And the pH was adjusted to 7.0 each time with 1 M NaOH solution. 95% purity of Capsaicin provided by Xian Ruilin Biotechnology Co (Xian, China).

2.2. Animals and treatment

Six week old male Wistar rats weighed between 200-250g and were kept in SPF laboratory animal room generated from Experimental Animal Center in Guangzhou University of Chinese Medicine. The animals were given ad libitum access to water and food and housed in plastic cages in a temperature-controlled (24°C) room with a 12h light-dark cycle. All the experimental protocols were permitted by the Institutional Animal Ethics Committee of the Guangzhou University of Chinese Medicine (No.20130001). Based on the previously described protocol [23, 24], we made some minor revisions to established colon carcinoma model induced by 1, 2-dimethylhydrazine (DMH) in rats. Briefly, after a period as long as 3d for adapting the environment, rats were randomly divided into 4 groups with 10 animals each (normal control group, DMH group,capsaicin exposure group, and cold exposure group). Capsaicin exposure group was given capsaicin at 10mg/kg b.wt. everyday. Cold exposure group was treated with cold distilled water (0°C) at 10mg/kg b.wt. until the end of the experiment.

Group A (normal control): Rats received basal diet, drink water, and gavage with the equivalent volume of saline once a day.

Group B (DMH): Rat was administered with subcutaneously injected with DMH (25mg/kg b.wt.) once a week for 12 weeks and also received saline once a day.

Group C (capsaicin combined with DMH): Rats were administered with DMH as in GP B and also given capsaicin (10mg/kg b.wt.) every day throughout the experiment. DMH was weighed and dissolved in normal saline to ensure the stability of the chemical before use with a final concentration of 2 %. And the pH was adjusted to 6.5 each time with 1 M NaOH solution.

Group D (DMH + Cold distilled water): Rats were administered with DMH as in GP B and also given cold distilled water (10mg/kg b.wt.) via intragastric gavage continued till the end of different phases of experiments.

The Appearance change of rats was monitored daily. Bodyweight and food intake of rats were recorded weekly throughout the experiment. For food consumption measurement, the model rats were provided with 500g food in the morning. Food weight was detected at a fixed time to determine daily food intake with the last 24 hours. All the rats were anesthetized with chloral hydrate and then sacrificed 30 weeks after the end of DMH administration. The experiment lasted 30 weeks. The experimental protocol is exhibited in Fig. 1.

2.3. Morphological study of colon tissues

Colons were longitudinally dissected from the anal to the cecum and then washed it with PBS. The colons were spread out on cleaning tissue paper. The number of colon tumors was recorded for tumor incidence and the size of the tumors was measured using a caliper paper. Tumor volume was caculated using the fumula: tumor volume (mm^3) = length (mm) × $width^2$ (mm^2)/2 as reported by Isha Rani (Isha Rani, 2014).

2.4. Histopathological observation

For histology, the colon sections were placed in a 15ml conical tube filled with a 4% paraformaldehyde solution over 24 h. Colon paraffin sections were prepared by the pathology department at Guangzhou University of Chinese Medicine. H&E staining sections were visualized through an Olympus TH4-200 (Tokyo Japan) under 400× magnifications. Microscope was classified as tumors in accordance with specific pathological criteria described by Nolte. Colonic neoplasms was classified as adenomas or adenocarcinomas (tubular or mucinous) (Nolte et al., 2016). Tumor incidence is the percentage of rat bearing the indicated type of tumor. Incidence values are represented as percentage.

2.5. Histone extraction

We extracted the total histones from colon colon tumor samples (n = 3/group) using the EpiQuik total histone extraction kit (Epigentek, Farmingdale, NY, U.S.A.). We prepared histone following the manufacturer's protocol. Briefly, 100mg colon tissues were homogenized in a mortar with liquid nitrogen. Adding lysis buffer, then incubated on ice for 30 min. The homogenized mixture was transferred into a 2ml vial and centrifuged at 3000g for 5 min at 4℃, and then remove supernatant. Balance buffer was added and incubated on ice for 30 min, centrifuged at 3000g for 5 min at 4℃and transferred the supernatant fraction into a new vial, and adding Balance-DTT buffer immediately. Protein concentrations were quantified by Bradford protein assay kit (KeyGEN, China) and the extracts were stored at -80℃.

2.6. Western bolts

For western blotting, we fractionated 30ug of extracted protein on a 12% SDS-PAGE gel and then transferred to a polyvinylidene fluoride (PVDF) membrane with a 22-mm pore size (Millipore, MA, USA). The membrane was blocked in 5% milk PBS-T (phosphate-buffered saline with 0.1% Tween-20) for 2 hours at room temperature and probed with antibodies acetylated H3-Lys9 (1:2000), acetylated H3-Lys18 (1:2000), acetylated H3-Lys27 (1:2000), acetylated H4-Lys16 (1:4000), TLR2 (1:1000), and TLR4 (1:1000) at 4℃ overnight. We used horseradish peroxides(HRP)-conjugated goat anti-rabbit as the secondary antibody and histone H3, H4, and β-actin (1:5000) were used as a loading control. All antibodies were purchased from Abcam (Cambridge, U.K). Band intensity was visualized by ChemiDoc ™XRS^{+} (Bio-RAD, USA)

2.7. Real-time quantitative RT-PCR (RT-qPCR)

Total RNA was extracted from colon tumor tissues(n = 6/group) using Trizol reagent (Takara, Japan). The concentration and quality of RNA samples were evaluated with a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). Reverse transcription was carried out to obtain cDNA using the Master Mix kit (Takara, Japan) following standard protocols. The mRNA levels of TLR2 and TLR4 in colon mucosa were assessed using a Step One Plus real-time PCR system (Thermo Fisher Scientific, CFX384TM Real-time System). β-Actin were used as housekeeping genes to normalize mRNA expression. The relative levels of gene expression were enumerated using the comparative formula $2^{-\Delta\Delta Ct}$ (C. Liu et al., 2018). Detailed information of the primer sequences was as follows:

5'-AGCCATGTACGTAGCCATCC-3'/3'-ACCCTCATAGATGGGCACAG-5' for β-Actin.

5’-GCTCCTGTGAACTCCTGTCC-3’/3’-GACACTCCAAGACTGAGGGC-5’

for TLR2.

5’-CCAGAGCCGTTGGTGTATCT-3’/3’-GGCGATACAATTCGACCTGC-5’

for TLR4.

2.8. Statistical analysis

All the data were summarized as mean ± standard deviation (SD) and analyzed by SPSS 23.0. To determine if there were differences between groups, we performed the data with a one-way analysis of variance (ANOVA). The Fisher’s exact test was performed to compare the tumor incidence between the different groups. We considered differences significant at $P < 0.05$.

3. Result

3.1. General observation

As shown in Fig. 2A, before 20 weeks, weekly monitor throughout the experimental period showed no noticeable body weight loss among groups. After 20 weeks, DMH-treated rats showed a decrease in body weight as compared to control rats. Compared with the DMH-treated group, the weight of cold and capsaicin exposure groups was reduced, but the statistical difference among groups was not significant. No difference in food intake was found among groups by the end of weeks 2–6. The food intakes of DMH-treated rats were decreased at the rest of the experimental period. The food intake was decreased in cold and capsaicin exposure group as compared to the DMH-treated group. Figure 2C and E showed that the thymus weight in cold and capsaicin exposure group was lower than that of the normal group, although no significant difference in thymus weight was observed among DMH-treated group, cold exposure, and capsaicin treatment group.

3.2 Morphological study of colon tumor and histopathological analysis

Seven months after the experiment started, no visible colon tumor was found in normal control. Most of the colonic tumors were developed in the distal colon than in the proximal colon of Wistar rats. As shown in Fig. 2D and F, the average volume tumor of DMH-treated group was 15.24 ± 12.45 mm^3, and that of the cold exposure and capsaicin treatment groups was34.58 ± 27.76 mm^3 and 22.70 ± 18.02 mm^3, respectively. These data suggest that there was a bigger volume to rats of the cold exposure and capsaicin treatment than the DMH treatment. There was no difference in tumor incidence among groups (Table 1). The tumor multiplicity was higher among cold exposure and capsaicin groups as compared to DMH group due to the increase number of tumors. A trend towards the increase of invasive tumors was observed in the cold and capsaicin group. Furthermore, the pathological type of cold group was the most

serious. Histopathological analysis showed that most tumor were either tubular adenocarcinomas or poor-differentiated mucinous adenocarcinomas (Fig. 3).

Table 1
Incidence and multiplicity of various tumors induced in different treatment groups

	Total Number of tumors	Tumor multiplicity	Incidence (%)		
			Adenomas	Tubular Adenocarcinoma	Mucinous Adenocarcinoma
Control	Nil	-	-	-	-
DMH	8	1.17 ± 0.41	1/8 (12.5%)	6/8 (75%)	1/8 (12.5)%
Cold exposure	21	2.83 ± 0.75**	2/21 (9.5%)	12/21 (57.1%)	7/21 (33.3)%
Capsaicin	13	2.00 ± 0.91*	2/13(7.1%)	9/13 (69.2%)	2/13(23.41%

Tumor Multiplicity: average number of all tumors in each tumor-bearing rat. Multiplicity Values are expressed as mean ± SD. * DMH compared with Cold exposure and Capsaicin-treated group. * $p < 0.05$, ** $p < 0.01$.

3.3 Effect of Cold exposure and Capsaicin on HDAC Activity

The expression of HDAC1, 2, 3, and 8 in colonic mucosa was showed in Fig. 4A and B. The protein expression levels of HDAC 1, 2, 3, and 8 were significantly elevated in colonic tissue from DMH-treated rats. Compared to those in the DMH group, the HDAC 1, 2, 3 and 8 expressed in cold and capsaicin exposure groups were further increased. These findings implicated that the activation of the HDACs may be involved in the CAC induced by DMH and cold exposure and capsaicin treatment could exacerbate the expression levels of HDACs.

3.4 Effect of Cold exposure and Capsaicin on Colon mucosa H3K9ac, H3K18ac, H3K27ac, and H4K16ac Proteins Level

The effect of Cold exposure and Capsaicin H3K9ac, H3K18ac, H3K27ac, and H4K16ac in colon mucosa was shown in Fig. 5. Compared to those in the control group, H3K9ac, H3K18ac, and H3K27ac in the DMH group were increased. In contrast, exposure of rats with cold water and capsaicin resulted in a loss of H4K16 acetylation in colon mucosa during colorectal carcinogenesis. Compared with the DMH group, cold and capsaicin exposure group further increased levels of H3K9ac, H3K27ac but had no effect on H3K18ac levels. However, the protein level of H4K16ac was lower than those in the DMH-treated group, but the statistical difference among groups was not significant.

3.5 Effect of Cold exposure and Capsaicin on Colon mucosa TLR2 and TLR4 Proteins Level

The expression of TLR2 and TLR4 protein levels and mRNAs in colonic mucosa were shown in Fig. 6. Cold and capsaicin exposure group had significantly higher levels of TLR2 and TLR4 protein and mRNAs in colon mucosa as compared with control. Compared to those in the DMH group, expression of TLR2 and TLR4 protein levels and mRNAs in cold and capsaicin exposure groups were further increased.

4. Discussion

Recent several epidemiological studies have associated cold temperatures and capsaicin consumption with an increased risk of several cancers (Du, Lv, Zha, Hong, & Luo, 2020; Michal Freedman et al., 2015; Voskarides, 2019). Our findings demonstrated that cold exposure and low-does capsaicin administration aggravates the ectopic expression of histone acetylation level and histone-modifying enzymes through the structure of chromatin, making rats-induced by DMH more susceptible to CRC. Moreover, cold and capsaicin exposure further increased TLR2 and TLR4 expression as well as increased histone H3 acetylation of TLR2 and TLR4 in the colonic mucosa of DMH-induced rats but not normal rats. These results suggest that higher HDAC expression in the colonic mucosa and ectopic expression of histone acetylation may be involved in chromatin remodeling, which might play a fundamental role in the pathogenesis of CRC.

More recently, evidence supports an emerging view that environmental factors such as dietary, and cold environment are among the top risk factors that predispose to CRC. Further, it has been reported that cold environmental temperature can be potential cancer-causing factors and people live in the cold environment have a high risk of cancer incidence and mortality (Sharma, Verma, Joshi, Panwar, & Mandal, 2015). Kokolus and colleagues found that when tumor-bearing mice are housed at thermoneutral temperature (30°C-31°C), they observed that reduce tumor formation, growth, and metastasis (Kokolusa et al., 2013). Capsaicin is the major pungent alkaloid of chili peppers (Naves et al., 2019). Conflicting reports exists on antitumoral or carcinogenic effects of capsaicin (Friedman et al., 2019). Some studies have shown that capsaicin at 50mg/kg reduced CRC risk in DMH-induced rats models by inhibiting the cytotoxicity, genotoxicity, proliferation, and apoptosis of cancer cell/tissue (Caetano et al., 2018). They also showed that high dose capsaincin decrease the proportion of tubular adenocarcinoma In DMH-induced CRC(Caetano et al., 2021). Additionally, Capsaicin has also shown have a protective role against the development of many types of human cancers (H. Li, Krstin, Wang, & Wink, 2018; Zheng et al., 2016). On the other hand, capsaicin has been reported to have tumor-promoting activities in skin, lung, and colon cancer in different chemically-induced carcinogenesis models (Geng et al., 2016; Z. Liu et al., 2015; Nalinia, Sabithaa, Viswanathanb, & V.P. Menona, 1998). An intro reported by Liu et al showed that low concentrations of capsaicin enhanced migratory and invasive capability of HCT116 cells by upregulating the expression of tumor-associated NADH oxidase (tNOX)(N. C. Liu et al., 2012). Similarly, we found that cold exposure and long-term administration of capsaicin at a low does

promote tumor growth and CRC tumorigenesis in animal models. It may be possible that the anti-cancer activity of capsaicin depends on its concentration and further study is need to precisely delineate the effects of capsaincin in CRC.

Recent research has shown that regulation of specific HDAC isoforms and aberrant epigenetic alterations imposed by diet might not only mechanisms responsible for neoplastic cell transformation but also be implicated in the development of cancer (Esteller, 2007; Nebbioso, Tambaro, Dell'Aversana, & Altucci, 2018). Understanding the complex biology of lysine acetylation and its regulators is thus essential for CRC pathogenesis and treatment. The overexpression of Class I HDACs have been reported in many cancer types including colon carcinoma (Weichert et al., 2008). Furthermore, the expression was enhanced in strongly proliferating and poorly differentiated tumors, and upregulation of HDAC correlated with poor prognosis of CRC (Ashktorab et al., 2009; Y. Li & Seto, 2016). In addition, H3K9ac, H3K18ac, and H3K27ac were reported to be significantly up-regulated in colorectal adenomas and cancers as compared to their normal counterparts (Karczmarski et al., 2014). Moreover, several studies have suggested that alteration of histone acetylation patterns is also predictive of histological subtype, prognosis, and cancer recurrence (David B. Seligson et al., 2009; Tamagawa et al., 2013). For instance, the hypoacetylation of H3K9, H3K18, and H4K16 strongly relevant to the clinical outcome of prostate cancer (D. B. Seligson et al., 2005). While elevated global histone acetylated histone H3 (H3ac) in colon cancer tissues was reported to predict poor overall survival of patients (Hashimoto, Yamakawa, Kimura, Usuba, & Toyono, 2013). Our results showed that the expression of H3K9ac, H3K18ac, and H3K27ac in the colons of DMH-induced CRC rats was significantly increased, and cold exposure and capsaicin treatment further increased this phenomenon. As compared with the normal group, the acetylation of H4K16 was clearly down-regulated in DMH, cold exposure, and capsaicin treatment group. We postulated that up-regulated HDACs lead to abnormal patterns of histone acetylation, which is associated with the deregulation of gene transcription thereby contributing to the CRC progression.

There are many molecular pathways involved in CRC development and different levels of evidence support that chronic inflammation plays an essential role in cancer development and progression (Diakos, Charles, McMillan, & Clarke, 2014; Grivennikov, Greten, & Karin, 2010; Monteleone, Pallone, & Stolfi, 2012). Aberrant activation of TLRs has been shown to increase the risk of colorectal cancer is caused by disruption of chronic inflammation, immune response, and epithelial barrier homeostasis, which predisposes individuals to develop CRC (Cario, 2010; T. T. Li, Ogino, & Qian, 2014; Xiang et al., 2012). Thus, its aberrant activation can be implicated in the pathogenesis of intestinal diseases, such as Inflammatory Bowel Disease (IBD), colitis-associated cancer (CAC), and colorectal cancer (CRC). The abnormal activation of toll-like receptors (TLRs) leads to an impairment of immune homeostasis, which contributes to colorectal cancer (CRC) development. It is reported that TLR2 and TLR4 are upregulated in CRC and correlated with a poor prognosis in patients with CRC (Nihon-Yanagi, Terai, Murano, Matsumoto, & Okazumi, 2012; Xu et al., 2011). Additionally, It has been shown that genetic variation in TLR2 and TLR4 gene interaction with dietary factors increased susceptibility to CRC and multiple single-nucleotide polymorphisms (SNPs) in these genetic profiles was associated with CRC prognosis (Kopp, Vogel, Tjonnel, & Andersen4, 2018; Slattery, Herrick, Bondurant, & Wolff, 2012). Furthermore, recent research

investigating the role of TLRs in diseases indicating that DNA-methylation, histone modification epigenetic has emerged as a new mechanism involved in the regulation of TLRs (Hennessy & McKernan, 2016). Takahashi et al. (Takahashi, Sugi, Hosono, & Kaminogawa, 2009) suggested that epigenetic modification, including histone acetylation and DNA methylation act as a negative regulator of TLR4 gene transcription when associated with ZNF160, a repressive-associated transcription factor in human intestinal epithelial cells. TLR2 was reported to decrease methylation of the proximal human TLR2 promoter was resulting in an up-regulation of TLR2 (Haehnel, Schwarzfischer, Fenton, & Rehli, 2002). Here we observed that cold and capsaicin exposure further increased the expression of TLR2 and TLR4 in the colon mucosa and this could be related to the epigenetic mechnism. Histone modification might be one of the regulators of TLR2 and TLR4. Further studies are necessary to identify the potential genetic locus regulated by histone modification upon cold exposure and administration of capsaicin in DMH-induced CRC through chromatin immunoprecipitation (ChIP) and next-generation sequencing (ChIP-seq) assay.

5. Conclusion

In summary, as showed in Fig. 7 the present study indicated that cold exposure and long-term administration of capsaicin at a low does further aggravate the abnormal expression of HDACs and global histone modification as well as TLRs and that such changes are strongly correlated with the development and progression of CRC. A better understanding of the epigenetics and TLRs on cold exposure and capsaicin treatment rats may help to clarify the mechanism of tumorigenesis which could afford a novel therapeutic approach for CRC patients. Lastly, Further investigations are required to understand the epigenetic mechanisms of TLR regulation and the role of TLRs in the pathogenesis, prevention, and treatment of CRC.

Declarations

Author’s Contributions:

Jingchun Qin and Huixuan Li wrote the first draft of the manuscript. Bin Wen designed the study. Jingchun Qin, Huixuan Li, and Weitao Yu conducted the experiment and analyzed the data. Li Wei contributed to review and edit the paper. Bin Wen revised the manuscript. All the authors have read and agreed to the published version of the manuscript.

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Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of interest:

The author declares that there are no conflicts of interest.

Ethics approval

This study was approved by the institution's Ethics
Review Board (S2017037).

Consent for publication

Not applicable

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Figures

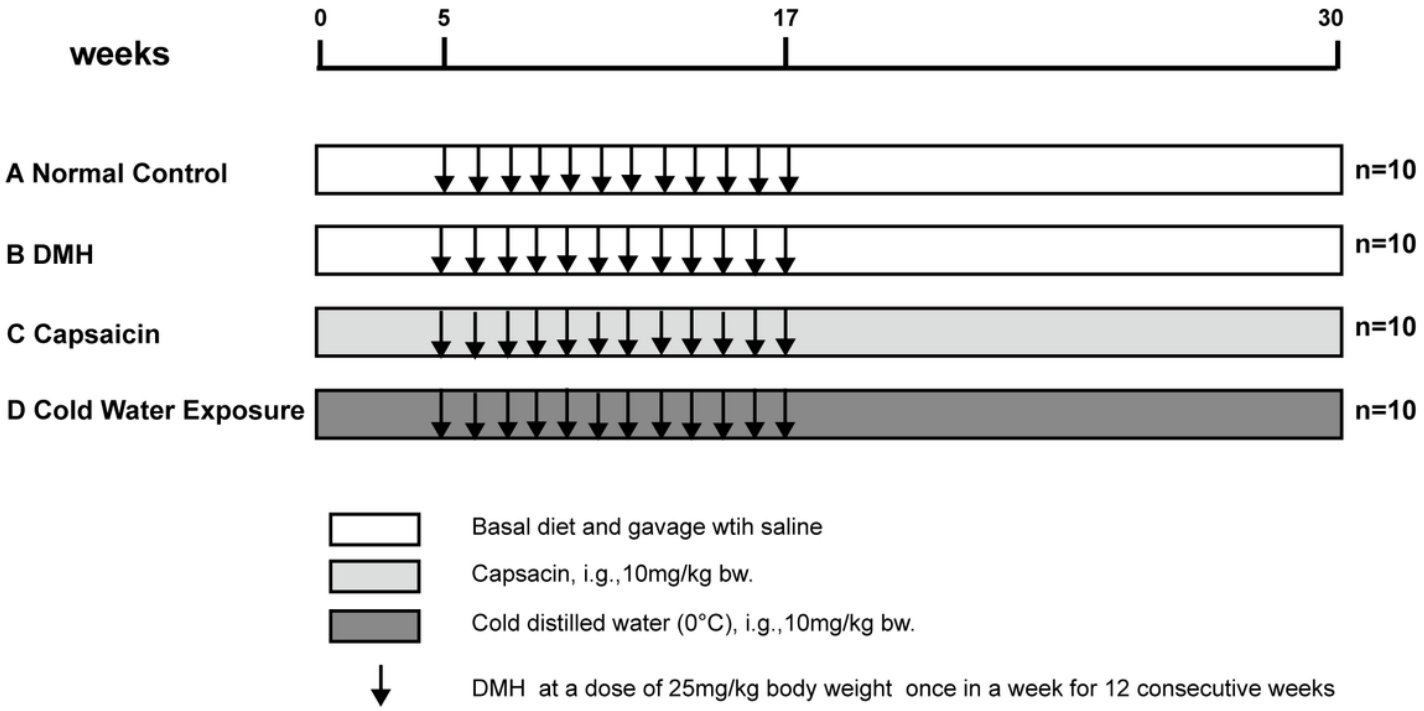

Figure 1

Schematic representation of the experimental protocol.

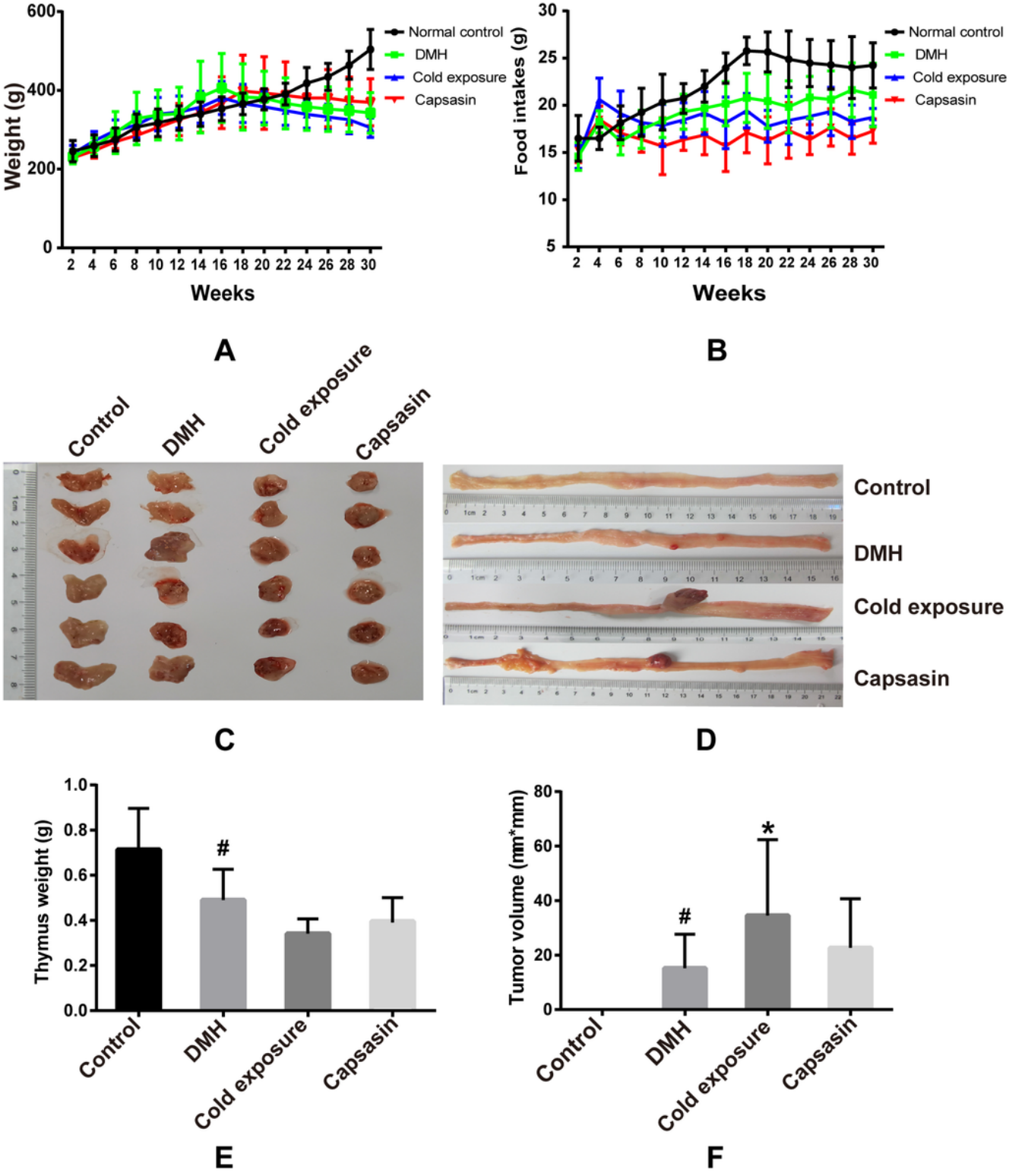

Figure 2

Bodyweight, food intake, thymus weight and tumor volume in normal rats□cold exposure and capsaicin-treated rats. (A) Effects of cold exposure and capsaicin on the bodyweight of rats. (B) Effects of cold exposure and capsaicin on the food intake of rats. (C, E) Change in thymus weight and thymic morphology. (D, F) Macroscopic image of the colonic tumors and tumor volumes in different treatment

groups. Data were expressed as the mean ± S.D. Comparisons: # Control compared with DMH, * DMH compared with Cold exposure and Capsaicin-treated group. * $p < 0.05$, ** $p < 0.01$, # $p < 0.05$, ## $p < 0.01$.

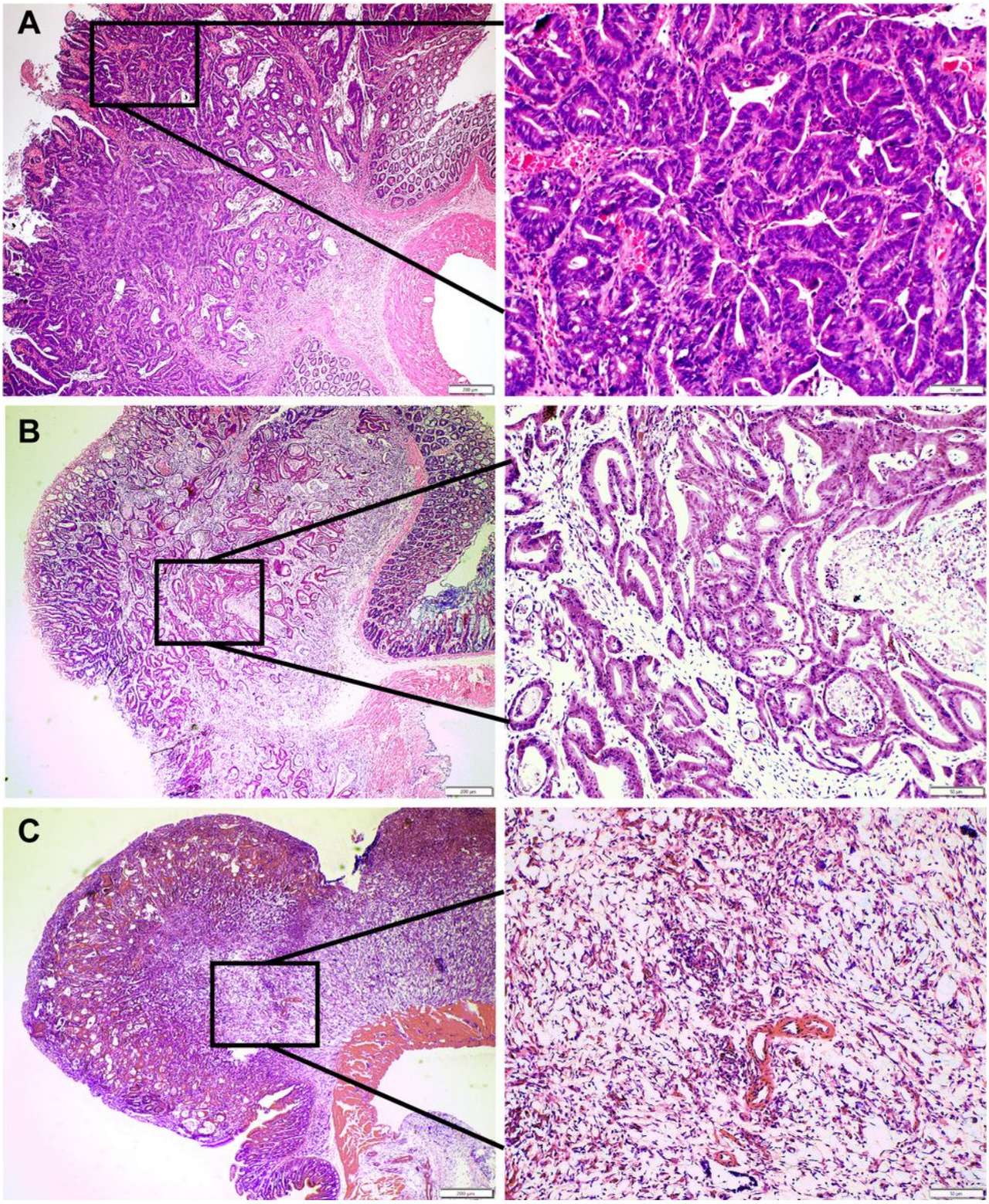

Figure 3

Representative sections stained with H&E showing the histopathology of the colonic neoplastic lesions.

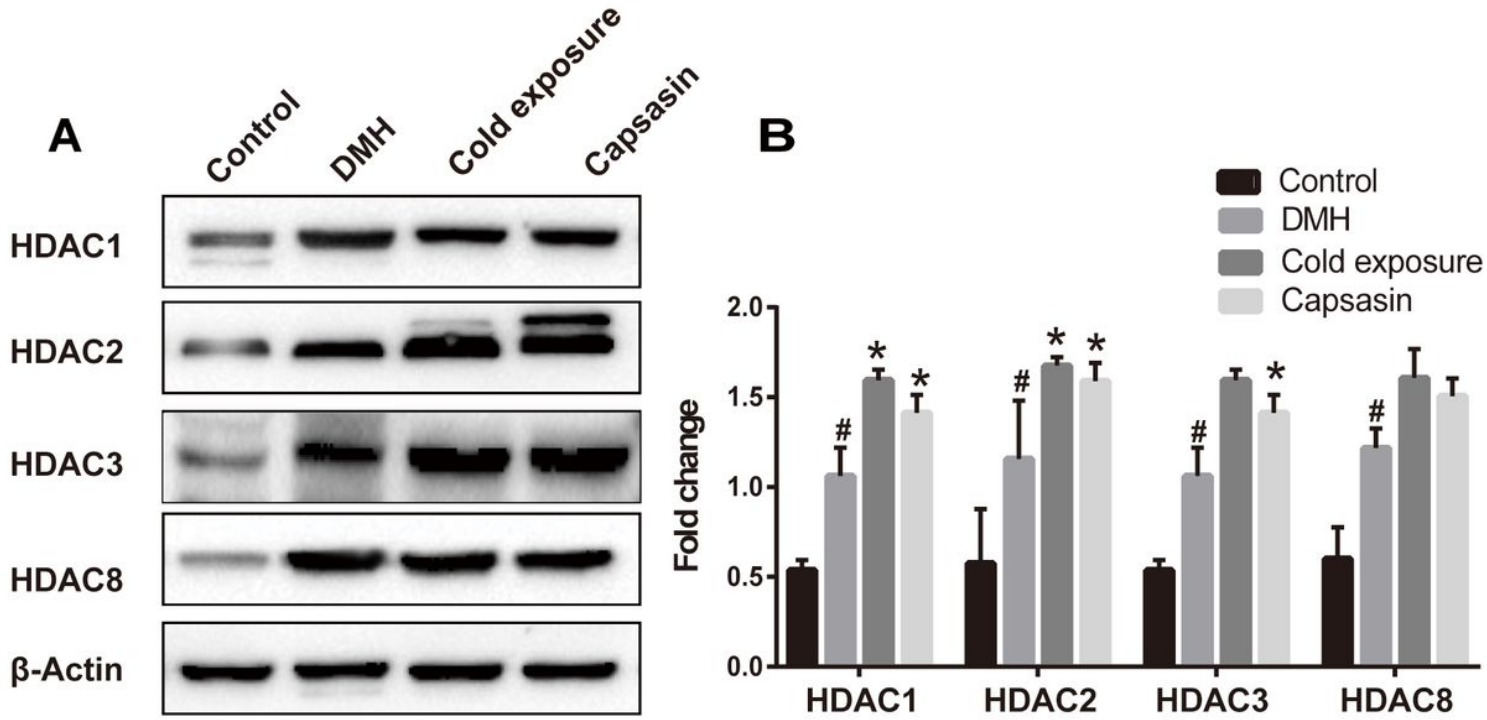

Figure 4

The effect of cold and capsaicin exposure on the protein of HDAC1-3、8. (A) The figure shows representative Western immunoblot images. (B) Semi-quantitative analysis of HDAC1-3, 8 immunoblots was normalized by corresponding β-actin bands. Cumulative values are reported as means ± S.E. from three separate experiments. Comparisons: # Control compared with DMH, * DMH compared with Cold exposure and Capsaicin-treated group. * $p < 0.05$, ** $p < 0.01$, # $p < 0.05$, ## $p < 0.01$.

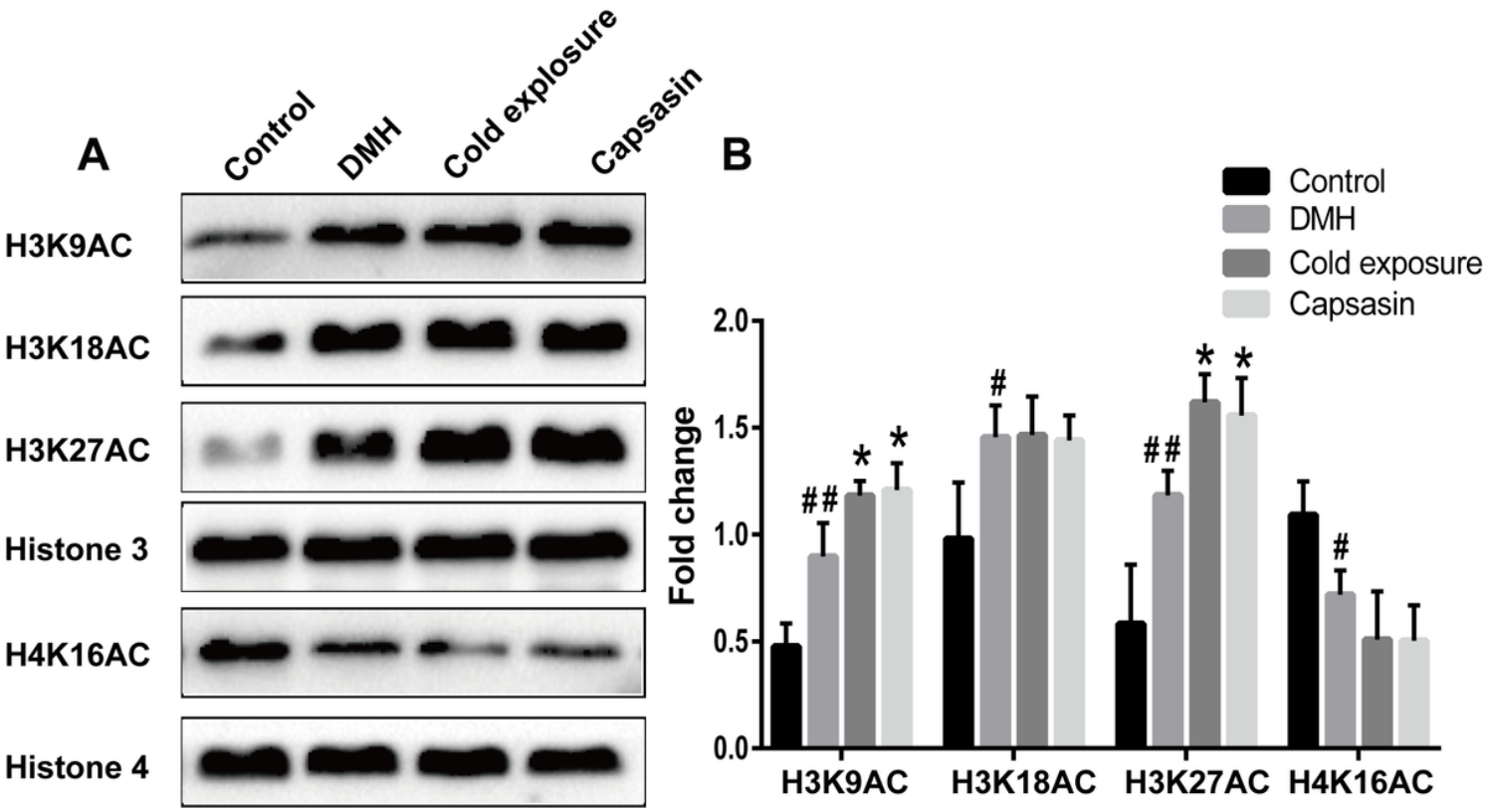

Figure 5

Western blot analysis of histone H3 and H4 modifications in the colonic mucosa of different treatment groups. (A) Representative Western immunoblot images are shown. (B) Semi-quantitative analysis of immunoblots was normalized by corresponding Histone 3 or Histone 4 bands. Data were presented as the mean ± S.D form three separate experiments. Comparisons: # Control compared with DMH, * DMH compared with Cold exposure and Capsaicin-treated group. * $p < 0.05$, ** $p < 0.01$, # $p < 0.05$, ## $p < 0.01$.

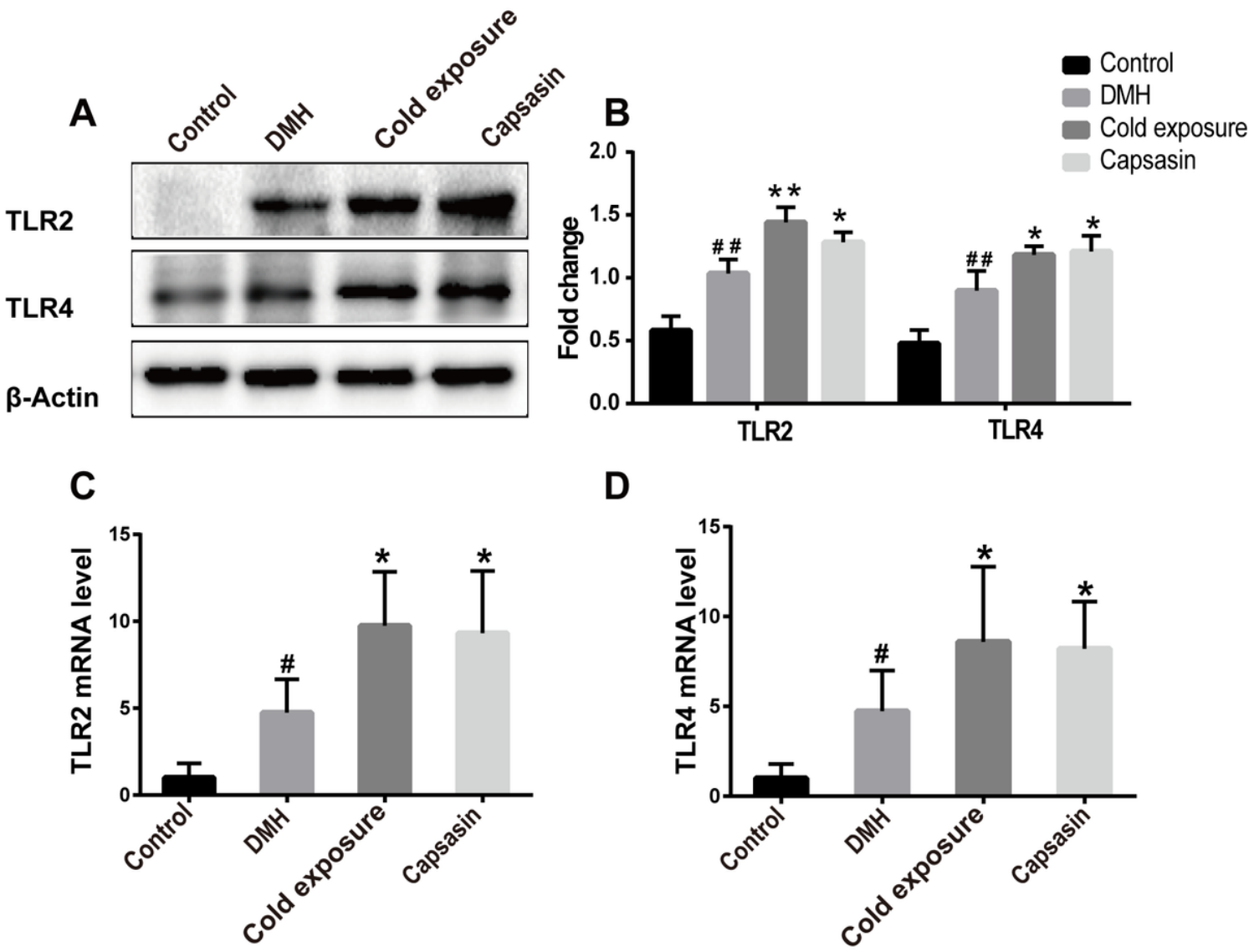

Figure 6

Effect of cold and capsaicin exposure on the expression of TLR2 and TLR4 proteins in different treatment groups. (A) Representative Western immunoblot images are shown. (B) Semi-quantitative analysis of immunoblots was normalized by corresponding β-actin bands. (C, D) The mRNA expression levels of TLR2 and TLR4 in the colon tissues of rats in different groups. Data were presented as the mean ± S.D form three independent experiments. Comparisons: # Control compared with DMH, * DMH compared with Cold exposure and Capsaicin-treated group. * $p < 0.05$, ** $p < 0.01$, # $p < 0.05$, ## $p < 0.01$.

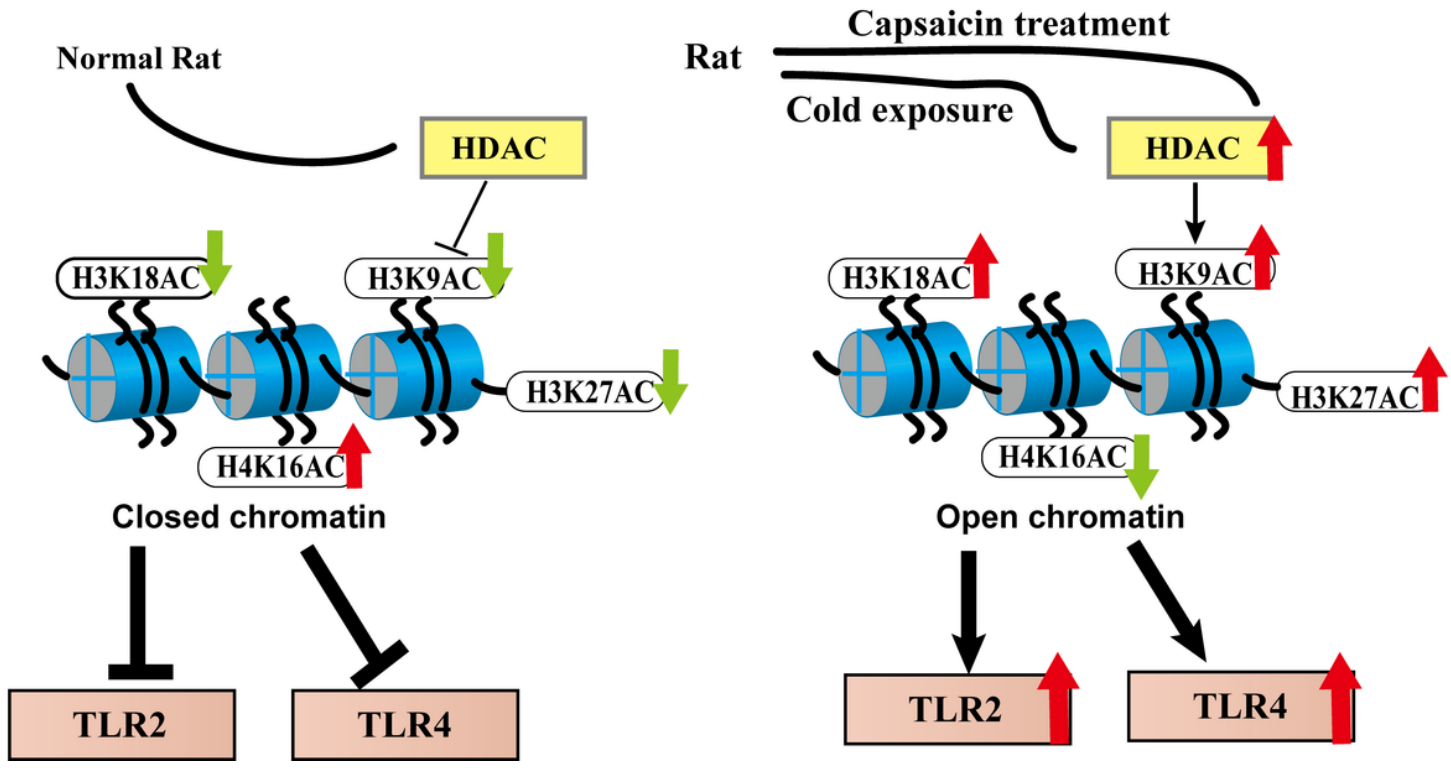

Figure 7

Schematic diagram depicting the role of histone acetylation and Toll-like receptors in promoting colorectal cancer pathogenesis. In comparison with the normal rats, rat exposure of cold and capsaicin has higher levels of HDACs activity in the colon mucosa, which results in abnormal expression of histone H3-K9, H3-K18, H3-K27, and H4-K16 acetylation. The innately higher levels are associated with the relaxation of chromatin of TLR2 and TLR4 genes. These results implicate a crucial role of histone acetylation-induced chromatin remodeling and can also positively regulate the expression of TLRs thereby exacerbating the CRC progression.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- HDAC.wb.pdf
- Histoneacetylation.wb.pdf
- TLR.wb.pdf