Cancer Letters 364 (2015) 173–180

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Original Articles

Activation of mucosal mast cells promotes inflammation-related colon cancer development through recruiting and modulating inflammatory CD11b$^+$Gr1$^+$ cells

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ARTICLE INFO

Article history:
Received 2 March 2015
Received in revised form 9 May 2015
Accepted 12 May 2015

Keywords:
Mucosal mast cells
Colitis-related colon cancer
CD11b$^+$Gr1$^+$ cells
Mouse mast cell protease-1

ABSTRACT

Mast cells (MCs) have been reported to be one of the important immunoregulatory cells in promoting the development of colitis-related colon cancer (CRC). It is not clear which MC subtypes play critical roles in CRC progression from colitis to cancer because mucosal mast cells (MMCs) are distinct from connective tissue mast cells (CTMCs) in maintaining intestinal barrier function under homeostatic and inflammatory conditions. In the current study, we found that MMC numbers and the gene expressions of MMC-specific proteases increased significantly in an induced CRC murine model. The production of mast cell protease-1 (mMCP-1) after MMC activation not only resulted in the accumulation of CD11b$^+$Gr1$^+$ inflammatory cells in the colon tissues but also modulated the activities of CD11b$^+$Gr1$^+$ cells to support tumor cell growth and to inhibit T cell activation. Blocking the MMC activity in mice that had developed colitis-related epithelium dysplasia, CD11b$^+$Gr1$^+$ infiltration was reduced and CRC development was inhibited. Our results suggest that MMC activation recruited and modulated the CD11b$^+$Gr1$^+$ cells to promote CRC and that MMCs can be potential therapeutic targets for the prevention of CRC development.

Introduction

Intrinsic genetic lesions are critical in tumor formation; nevertheless, the importance of chronic inflammation in cancer development has been highlighted recently [1–3]. MCs are abundant at sites exposed to the external environment, such as the intestine, and considered to be important sentinel cells for the inflammatory stimuli. Early studies demonstrated that these cells were vital in medicating bacterial clearance at the sites of infection by producing TNF-α and recruiting neutrophils [4,5]. Their immunomodulatory functions have been observed in a variety of inflammatory diseases, including allergy and autoimmune diseases [6].

Innate and adaptive immune cells shape tumor growth [2,7]. The roles of MCs in tumor development have attracted attention in recent years. MC infiltration has been documented in several types of human tumors. However, the role of MCs in the tumor microenvironment is still debated [8–11]. The mechanisms that underline the opposite effect of MCs on cancer are unclear.

Clinical and experimental animal studies suggest that an altered intestinal epithelium creates a tumorigenic microenvironment that boosts tumor progression [12,13]. In fact, different types of MCs are distinct in maintaining the intestinal barrier function under homeostatic and inflammatory conditions [14,15]. Two major MC subtypes, MMCs and CTMCs, have been described so far [16,17]. The MMCs mainly reside within the mucosa of the intestinal and respiratory tracts and contain mouse mast cell protease (mMCP)-1 and mMCP-2 (chymases); they rarely express tryptase. The CTMCs reside in the submucosa of the gastrointestinal tract and dermis and express the chymases mMCP-4 and mMCP-5, and the tryptases mMCP-6, mMCP-7, and carboxypeptidase A (CPA) [18,19]. Under homeostatic conditions, the overall MC numbers are low, with an approximate 1:1 ratio of MMCs to CTMCs. However, the ratio changed to approximately 5:1 MMCs to CTMCs under inflammatory conditions, with the numbers increased approximately 20–25-fold [20]. Consistent with the increased number of MMCs, remarkably increased mMCP-1 levels were observed [14]. In a colitis-related colon (CRC) animal model, it was found that the mice lacking MCs were less susceptible to inflammation-associated colorectal carcinogenesis [21]. However, it is unclear which MC subtype is mainly involved in the progression from colitis to cancer.

Abbreviations: CRC, colitis-related colon cancer; MC, mast cells; MMC, mucosal mast cells; CTMC, connective tissue mast cells; mMCP, mouse mast cell protease; DSS, dextran sodium sulfate; AOM, azoxymethane; DSCG, disodium cromoglycate.

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http://dx.doi.org/10.1016/j.canlet.2015.05.014

In this study, we reported that the activation of MMCs not only resulted in the accumulation of $CD11b^+Gr1^+$ inflammatory cells in the colon tissues but also modulated the activities of $CD11b^+Gr1^+$ cells to create a microenvironment for tumor growth. Blocking the MMC activity *in vivo* at the stage of colitis-related epithelium dysplasia reduced $CD11b^+Gr1^+$ infiltration and inhibited CRC development in the murine model.

Materials and methods

Mice

Male C57BL/6 or Balb/c mice, 8–10 weeks, were purchased from the Institute of Laboratory Animal Sciences, Chinese Academy of Medical Sciences (CAMS, Beijing, China). All procedures involving mice were approved by the Institutional Animal Care and Use Committee at the Cancer Institute, CAMS.

CRC induction for analysis of mouse MC proteases

CRC was induced as reported [22]. Briefly, azoxymethane (AOM; Sigma-Aldrich, MO) was injected intraperitoneally at 12.5 mg/kg body weight. One week later, the mice were exposed orally to 2.5% dextran sodium sulfate (DSS, M.W. = 36,000–50,000 LLC, MP Biomedicals, OH) for 5 days, followed by a 16-day fresh water interval. The continuous 21-day was designated as one DSS cycle. Animals sacrificed at the end of the first DSS cycle were designated as AD1, and those sacrificed at the end of cycles 2 and 3 were designated as AD2 and AD3, respectively. At AD1, AD2, and AD3, four mice were sacrificed, the distal 2 cm colon or polyps area were harvested for RNA isolation using Trizol reagent (Takara, Dalian, China). As controls, four untreated mice were processed in the same way. Gene expression array was carried out with a 4×44 K Agilent Whole Mouse Genome Oligo Microarray Kit (G4122F). All data were normalized and analyzed with GeneSpring Software.

Colon tissues obtained from another independent experiment were processed and analyzed individually by quantified real-time PCR to confirm the findings in transcriptome analysis. Primers used for quantitative PCR (Supplementary Table S1) were GeneCopoeia All-in-One qPCR Primers purchased from FulenGen (Guangzhou, China). Relative expressions were calculated based on GAPDH.

Blocking MMC activation using MC stabilizers

Oral exposure to DSS induced acute colitis in mice [23,24]. To avoid the acute effects of DSS on MC activation, we adopted a novel CRC animal model reported by Tanaka et al. [25] to investigate the MMC in CRC development. Male C57BL/6 mice received intraperitoneal AOM. One week later, they were exposed orally to 2.5% DSS for 7 days. Then fresh water was replaced without further treatment. Eight weeks after DSS withdrawal, we treated the mice with two MMC stabilizers, DSCG or doxantrazole [26–28]. One group of mice (n = 10) received DSCG daily at 100 mg/kg body weight dissolved in 100 μl saline for one week by gavage; one group (n = 10) received doxantrazole daily by intraperitoneal injection at 5 mg/kg body weight dissolved in 100 μl 5% $NaHCO_3$ for three days. The reagents were purchased from Sigma-Aldrich. As controls, one group (n = 10) received 100 μl saline by gavage for one week; one group (n = 10) was injected intraperitoneally with 100 μl 5% $NaHCO_3$ for 3 days. Mice were sacrificed 4 weeks after completing the treatment. Treatment experiments were repeated twice.

Five mice at each of the following time points were sacrificed: 3 days, 3 weeks, 8 weeks, and 12 weeks after the DSS withdrawal. Each colon was opened longitudinally and any fecal contents were cleared out with ice-cold saline. The colon length and weight were measured, the numbers and sizes of polyps were macroscopically assessed individually.

Depleting CD11b+Gr1+ cells in vivo

We depleted the $CD11b^+Gr\text{-}1^+$ cells *in vivo* based on the report [29] using anti-mouse Gr1 antibodies (RB6-8C5; eBioscience). Eight and nine weeks after DSS withdrawal, each mouse received 200 μg RB6-8C5 antibody or rat IgG isotype intraperitoneally. Mice were sacrificed 4 weeks after completing treatment.

Flow cytometry analysis (FACS) of the infiltrated cells in the colon

When no tumor was macroscopically observed in longitudinally opened colons, intraepithelial lymphocytes (IEL) and lamina propria lymphocytes (LPL) were isolated as described [30]. When tumors were ready to be identified, the tumorous tissues and tumor-adjacent tissues were collected separately and processed as performed for LPL isolation. Digested tissues were filtrated through a 100 μm pore size mesh (BD Company, CA) to remove debris. Isolated cells were stained with FITC-conjugated anti-mouse CD45, PE.Cy7-conjugated anti-mouse CD11b, and APC-conjugated anti-mouse Gr1. Data were acquired in LSR-II (BD Company, CA) and analyzed by using FlowJo software (Tree Start, OR). The analyses were based on the gating of CD45-positive cells.

Staining MCs in colon tissues

MCs were stained as reported using toluidine blue [25]. The expressions of mMCP-1 and mMCP-6 were stained using immunohistochemistry. Briefly, sectioned tissues were treated in PBS solution containing 1% Triton X-100 for 30 min after antigens were retrieved in 0.01 M citrate buffer (pH = 6) to improve antigen exposure. The section was then incubated with 50 μg/ml rat anti-mouse mMCP-1 monoclonal antibody (eBioscience, San Diego, CA), or with 1:500 diluted goat anti-mouse mMCP-6 polyclonal antibodies (Santa Cruz, CA) in PBS solution containing 0.05% Tween-20 and normal rat and goat serum overnight at 4 °C. The same amount of rat IgG or goat IgG was used as an isotype control. For mMCP-1 staining, the section was incubated with 1:500 diluted biotinylated goat anti-rat at room temperature for 1 h, followed by HRP–avidin (ZSGB-Bio, Beijing, China) for 30 min. For mMCP-6 staining, the section was incubated with 1:50 diluted HRP-conjugated rabbit anti-goat for 2 h. The sections were colored with 3-Amino-9-ethylcarbazole (AEC, Sigma, MO) solution for 5–15 min and analyzed in Aperio Scanscope and Console software version 10 (Aperio Technologies, Vista, CA).

Quantification of mMCP-1 in serum and in colon tissues

To quantify the mMCP-1 in colon tissues, interstitial liquid was prepared as reported [31]. Every 100-mg tissue sample was cut into small pieces in 400 μl ice-cold normal saline and incubated on ice for 5 min. The mMCP-1 concentrations in the interstitial liquid and in the serum were measured using mMCP-1 ELISA kits (eBioscience, CA) according to the manufacturer's instructions.

Assay of mMCP-1 on inflammatory cell recruitment in vivo

The mice received 10 ng recombinant mMCP-1 diluted into 0.5 ml DMEM medium, or 0.5 ml DMEM medium alone, intraperitoneally. Cells in peritoneal cavity were collected and counted 22 h after the injection, stained with FITC-conjugated anti-mouse CD45, PE-conjugated anti-mouse CD11b, APC-conjugated anti-mouse Gr1, and PercpCy5.5-conjugated anti-mouse Ly6c. These assays were repeated four times.

Assays of $CD11b^+Gr1^+$ cell activity treated with recombinant mMCP-1

Inflammatory cells from Balb/c mice were collected from the peritoneal cavity 22 h after injecting 1 ml 4% thioglycollate (Sigma, St. Louis, MO) solution as in our previous report [32]. $CD11b^+Gr1^+$ cells were sorted in FACSAria (BD Company, CA) and cultured (5×10^5/ml) in DMEM containing 10% FBS in the presence or absence of 10 ng/ml recombinant mMCP-1 for 48 h. Conditioned medium was collected to determine its activity on colon cancer cell proliferation (CT26 cell line, Balb/c background). The treated $CD11b^+Gr1^+$ cells were used as stimulators on allogeneic (C57BL/6 background) T cell proliferation. The cell proliferation was determined using a CCK8 kit (Dojindo Lab, Japan) according to the manufacturer's instructions. Experiments were repeated three times.

Statistical analysis

All statistical analyses were performed using the GraphPad InStat 3 program (La Jolla, CA). Student's *t*-test or the Mann–Whitney U test was used for comparison between groups. Results are expressed as the mean ± SD or medians with interquartile range. Differences were considered to be statistically significant for $p < 0.05$.

Results

MMCs and CTMCs in the tumor tissues

Animal colon adenoma was induced by the combination of AOM and DSS exposures (Supplementary Fig. S1A). Histological analysis showed the evolutionary process from inflammation to dysplasia to adenoma in the mouse colon (Supplementary Fig. S1B). Consistent with the other reports, the adenoma was mainly observed in the middle to distal colon at AD3. Transcriptome analysis of the colon tissues demonstrated that the expression of genes encoding MC-specific proteases increased dramatically in the mice with adenoma compared with naive mice and with the mice with chronic inflammation (Fig. 1A). The up-regulation of these genes in the colon tissues was verified by real-time PCR (Supplementary Fig. S1C).

Compared with the mRNA expression of MC-specific proteases in the adjacent non-tumor colon tissues, MMC-specific mMCP-1 and mMCP-2 were the most highly upregulated in the tumor tissue (Fig. 1B). We then investigated the MC numbers and their distributions. In the colon tissues, MCs were rare in the naive mice. However, MC numbers increased significantly in the adenoma

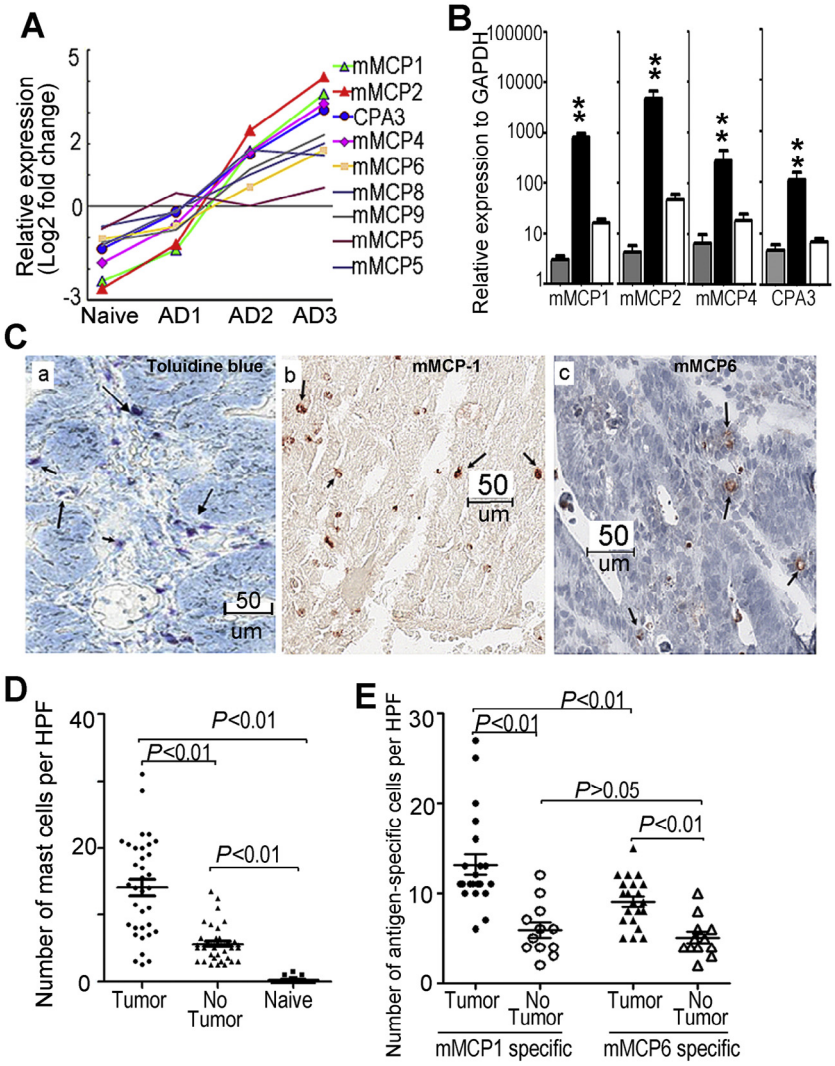

Fig. 1. MMCs and CTMCs in murine CRC induced by AOM + DSS. (A) Relative expression of mast cell-specific proteases by microarray determined at different time points. Colon tissues collected at the end of the first DSS cycle were assigned as group AD1, and those collected at the end of cycles 2 and 3 were AD2 and AD3, respectively. Untreated mice (Naive) were used as the baseline expression. (B) Expression of MMC-specific mMCP-1, mMCP-2 and CTMC-specific mMCP-4, CPA in the tumors (filled bars) and tumor-adjacent tissues (empty bars). The colon tissues collected from naive mice were used as control (gray bars). (C) Representative images of mast cells in tumor tissue: (a) mast cells stained by toluidine blue (purplish blue-staining); (b) mMCP-1 specific MMCs and (c) mMCP-6 specific CTMCs stained by immunohistochemistry (red-staining). (D) Numbers of total mast cells, (E) mMCP-1 specific MMCs, mMCP-6 specific CTMCs were quantified under microscope in tumor or tumor-adjacent tissues (No-tumor). Each dot represents the average of 3 sections from one mouse. Five untreated mice with the same weeks were used as control (Naive). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

compared with the adjacent non-tumor tissues, mainly observed within the epithelium (Fig. 1C-a, D).

To distinguish the subtypes of increased MCs, we stained the tumor and tumor-adjacent tissues with antibody against mMCP-1 (Fig. 1C-b), which is specifically expressed by MMCs, or with antibody against mMCP-6 (Fig. 1C-c), which is specifically expressed by CTMCs. Both mMCP-1-positive cells and mMCP-6-positive cells were higher in the tumor tissues than in tumor-adjacent tissues. However, more mMCP-1-positive MMCs than mMCP-6-positive CTMCs were observed in the tumor tissues, but not in the non-tumor tissues (Fig. 1E).

MMCs were activated during CRC development

The mice with adenoma (AD3) had significantly increased serum mMCP-1, the MMC-specific product, averaging 14.3 ± 1.3 ng/ml, than the naive mice (0.5 ± 0.1 ng/ml). We then processed the tissues and collected interstitial liquid. The mMCP-1 in tumors was 47.8 ± 7.5 ng per 100 mg tissues, which was significantly higher than that in the colon of naive mice (2.1 ± 0.4 ng/100 mg tissues).

Oral exposure to DSS might activate MMC directly. We therefore adopted the novel CRC animal model [25] (Fig. 2A) to investigate the role of MMC in CRC development. Histology analysis demonstrated that inflammatory cell infiltration was obvious 3 days after DSS withdrawal and was still remarkable 3 weeks after that (Fig. 2B-a). Epithelium dysplasia was observed after 8 weeks (Fig. 2B-b) and all of the mice developed colon tumors 12 weeks after DSS withdrawal in the middle to distal colon (Fig. 2B-c).

Compared with the naive mice, serum mMCP-1 increased gradually 3 weeks, 8 weeks and 12 weeks after DSS withdrawal, but no changes were seen after AOM and 3 days after DSS withdrawal (Fig. 2C). In colon tissue, the mMCP-1 showed the same pattern as in the serum. However, 3 days after DSS withdrawal, the mMCP-1 levels in colon tissue increased significantly relative to that of the naive mice (Fig. 2D). These results suggest that MMCs might be an important player in CRC development.

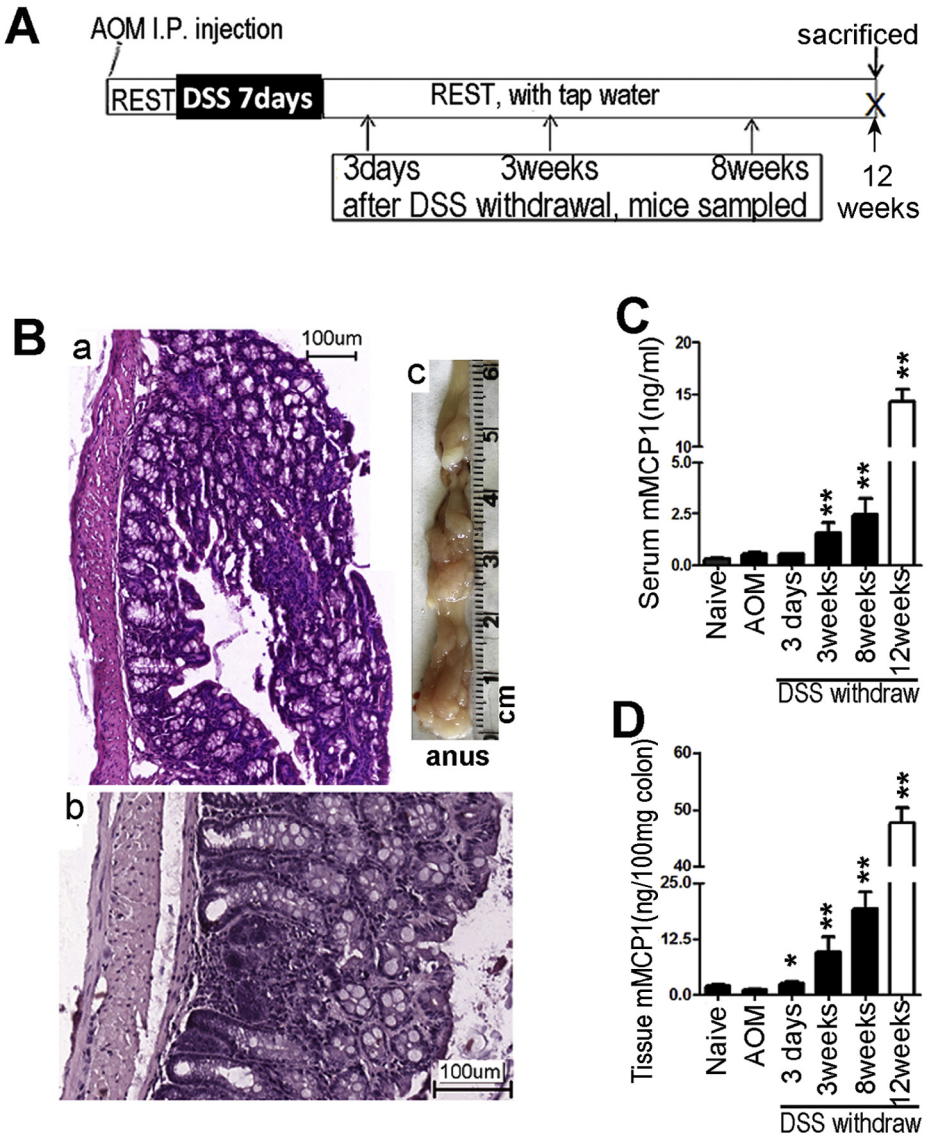

Fig. 2. Production of MMC-specific mMCP-1 during the course of CRC development. (A) Diagram of the adopted novel CRC animal model induced based on Ref. 25. (B) Representatives of the colon tissues examined 3 weeks (a), 8 weeks (b), and 12 weeks after DSS withdrawal. (C) Serum levels; (D) tissue levels of mMCP-1 determined at different time points after AOM injection or after DSS withdrawal. Each column represents the average of 3 individual mice at each time point. *: $P < 0.05$; **: $P < 0.01$.

Blockade of MMC activation inhibited CRC development

We treated the mice with DSCG or with doxantrazole to validate MMCs' roles in CRC development (Fig. 3A). The drugs were given at 8 weeks after DSS withdrawal, when the mice developed low-grade epithelium dysplasia (LGD) with chronic inflammation in their colon tissues (as shown in Fig. 2B-b). In controls, the mice were sham treated with saline or with $NaHCO_3$ (Fig. 3A).

The mice treated with either DSCG or with doxantrazole had a smaller colon weight than the sham-treated mice ($P < 0.05$, Supplementary Fig. S2). Total tumor numbers and those greater than 4 mm in diameter decreased significantly in the drug-treated relative to the sham-treated mice (Fig. 3B). In parallel, mMCP-1 levels in serum (Fig. 3C) and in the interstitial liquid of colon tissues (Fig. 3D) were reduced significantly in the drug-treated relative to the sham-treated mice. These results suggest that blockade of MMCs' activity could decrease CRC development in the murine model.

MMC activation resulted in CD11b$^+$Gr-1$^+$ cell accumulation during CRC development

Massive infiltration of inflammatory cells was observed in the adenoma tissues. There was a positive correlation between CD45$^+$ inflammatory cell numbers and tissue mMCP-1 levels in the colon ($P = 0.03$, Fig. 4A). Phenotype analysis demonstrated that more than 70% of CD45$^+$ cells were CD11b$^+$Gr1$^+$ in the tumor tissues, while it was less than 50% in the tumor adjacent tissues (Fig. 4B). We then analyzed this population at different stages during CRC development. Compared with acute colitis (3 days after DSS withdrawal), more CD11b$^+$Gr1$^+$ cells were observed in the epithelium, but not in the lamina propria at the stage of chronic colitis (3 weeks after the DSS withdrawal). Notably, more CD11b$^+$Gr1$^+$ cells presented in stages of dysplasia (8 weeks after the DSS withdrawal) and in the tumors, most prominently in the epithelium (Fig. 4C and Supplementary Fig. S3). After the mice were treated with MC stabilizers, paralleled

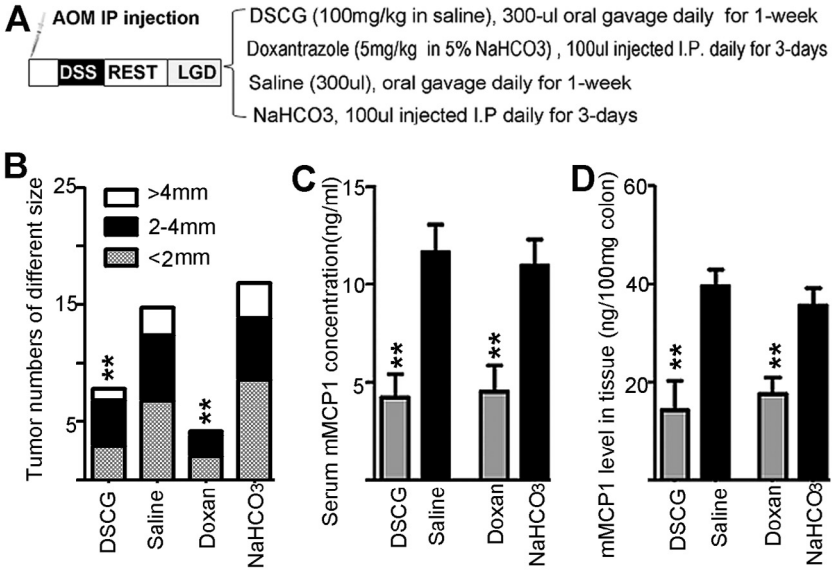

Fig. 3. Blocking MMC activity on CRC development in murine model. (A) Diagram of the treatment in the mice when they developed low grade dysplasia (LGD) induced by AOM + DSS. Each group contained 10 mice. (B) Tumor numbers in different sizes, (C) mMCP-1 levels in serum, and (D) in colon tissues of the drug or sham-treated mice. **: $P < 0.01$.

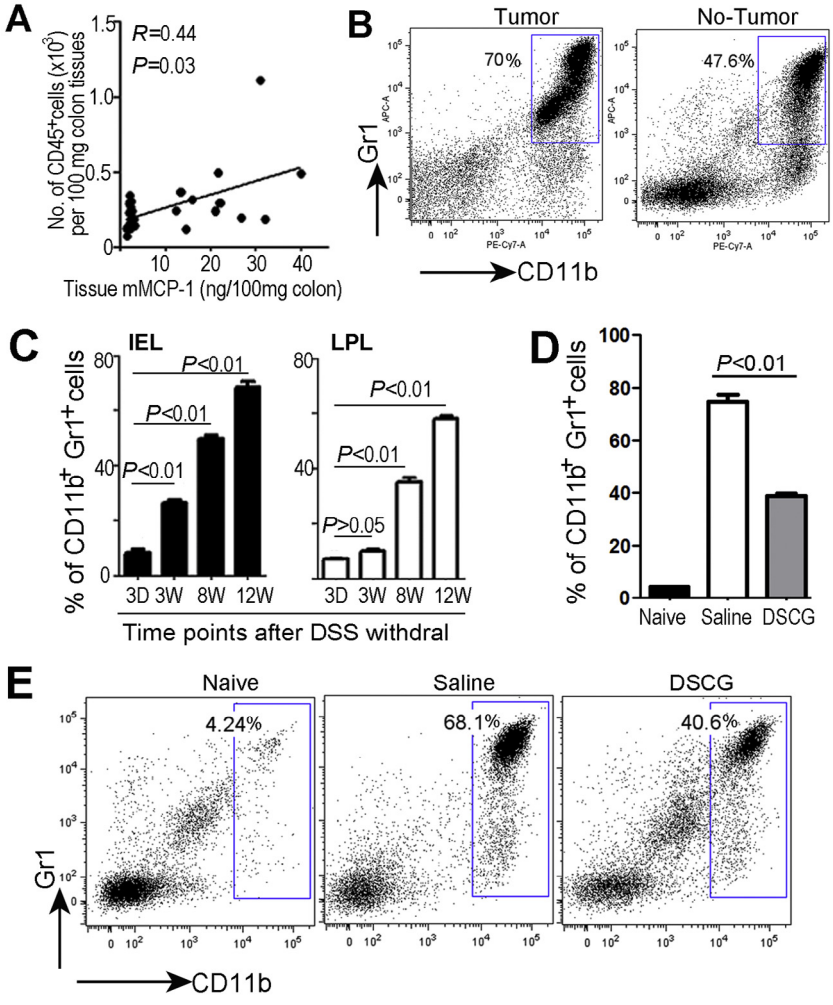

Fig. 4. Infiltration of $CD11b^+Gr1^+$ cells in the colon tissues. (A) Correlation of numbers of infiltrated inflammatory cells and mMCP-1 levels in the colon tissues. (B) Representative of the $CD11b^+Gr1^+$ cells in tumor and tumor-adjacent (No-Tumor) tissues. C) Percentage of $CD11b^+Gr1^+$ cells in epithelium (intraepithelial lymphocytes, IEL) or in lamina propria (lamina propria lymphocytes, LPL) at different time points after DSS withdrawal. Calculation of the $CD11b^+Gr1^+$ cell percentage was based on $CD45^+$ cells. D) Percentage of $CD11b^+Gr1^+$ cells in the tumor tissues of sham (Saline) or DSCG treated mice. (E) Representative of these mice. Five untreated mice with the same weeks were used as control (Naive).

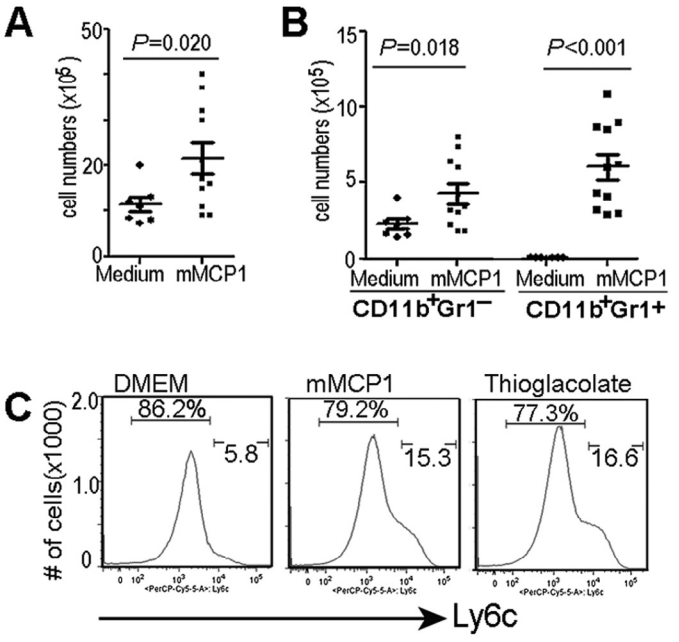

Fig. 5. Effect of recombinant mMCP-1 on CD11b⁺Gr1⁺ cell recruitment *in vivo*. Balb/C mice either received 0.5 ml DMEM medium alone (n = 8) or the same volume containing 10 ng mMCP-1 (n = 12). Twenty hours after the injection, cells infiltrated into the peritoneal cavity were collected using 5 ml of DMEM medium. (A) The total cell numbers, (B) the numbers of CD11b⁺Gr1⁺ cells or CD11b⁺Gr1⁻ cells in each group of the mice with FACS staining in different treated group. (C) Expression of Ly6c in the CD11b⁺Gr1⁺ cells. The mice received 1 ml of 4% thiogylcollate was used as control.

with reduced mMCP-1 levels (Fig. 3D), the percentage of CD11b⁺Gr1⁺ cells decreased significantly in the colon tissues (Fig. 4D, E).

To confirm the effect of activated MMC on the accumulation of CD11b⁺Gr1⁺ cells, we injected recombinant mMCP-1 into mouse peritoneal cavity, where no MMCs reside. Inflammatory cell numbers increased in the mice that received 10 ng mMCP-1 (Fig. 5A). Notably, the CD11b⁺Gr1⁺ cells but not CD11b⁺Gr1⁻ cells increased significantly in the mice that received mMCP-1 (Fig. 5B). In comparison with the mice that received medium alone, the percentage of CD11b⁺Gr1⁺Ly6c^{high} subpopulation increased in the mice that received mMCP-1, similar to that in the mice that received thioglycollate (Fig. 5C). However, when we sorted the CD11b⁺Gr1⁺ cells for chemotaxis assays, no direct effect of mMCP-1 on these cells was observed *in vitro* (Supplementary Fig. S4).

CD11b⁺Gr1⁺ cells treated with mMCP-1 enhance tumor cell proliferation and inhibit T cell activation

We then cultured the CD11b⁺Gr1⁺ cells with or without mMCP-1 to determine the effect of mMCP-1 alone, CD11b⁺Gr1⁺ cells alone, and the mMCP-1-treated CD11b⁺Gr1⁺ cells on colon tumor cell proliferation and on allogeneic T cell activation. The CT26 cell proliferation showed no change when they were treated with the medium collected from CD11b⁺Gr1⁺ cells alone or with mMCP-1 alone. However, CT26 cell proliferation increased significantly with conditioned medium from mMCP-1 treated CD11b⁺Gr1⁺ cells (Fig. 6A). On the contrary, allogeneic T cell proliferation stimulated by mMCP-1-treated CD11b⁺Gr1⁺ cells was inhibited compared with that by CD11b⁺Gr1⁺ cells alone (Fig. 6B).

Removing CD11b⁺Gr1⁺ cells inhibited CRC development

The above results suggest that activation of MMCs modulated CD11b⁺Gr1⁺ cell activity, which enhanced the tumor cell proliferation

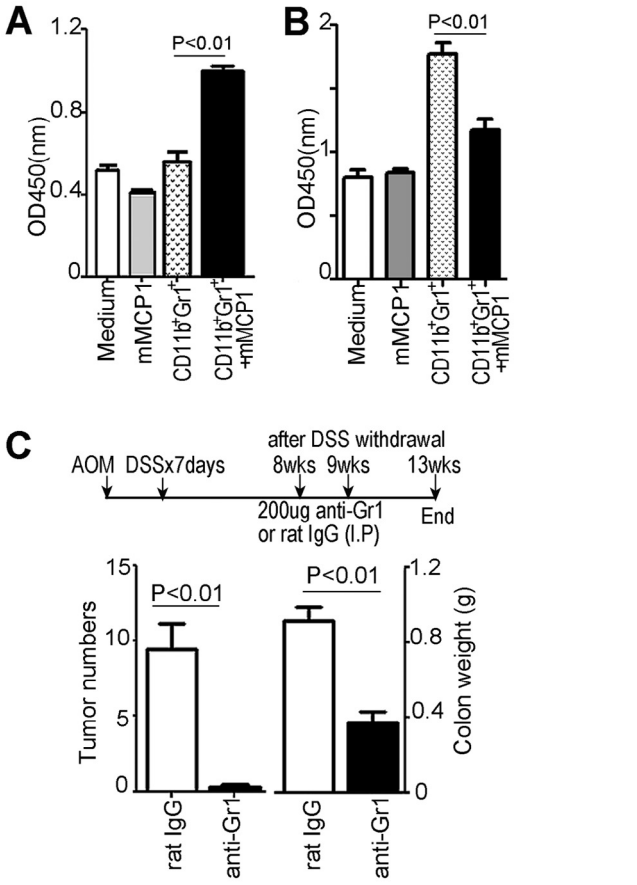

Fig. 6. Effect of recombinant mMCP-1 on CD11b⁺Gr1⁺ cell activity. The sorted CD11b⁺Gr1⁺ cells (at concentration of 5×10^5/ml) were cultured in the presence or absence of mMCP-1. Parallel, mMCP-1 was included in the medium without cells included (diagram provided in Supplementary Fig. S5). (A) The conditioned medium was collected and diluted with normal culture medium at 1:1 ration. Colon cancer cell line, CT 26 cells (at the concentration of 5×10^4/ml), were cultured in the presence of the above collected medium for another 48 hours and the cell proliferation was determined by using CCK8 kit. (B) Allogeneic (C57BL/6 background) T cells were prepared and mixed with the above cells for 68 hours at the ratio of one CD11b⁺Gr1⁺ cell to two T cells. The T cell proliferation was determined by using CCK8 kit. The cell proliferation was quantified by determining the absorbance at OD450nm. Experiments were repeated 3 times. (C) The mice were treated with rat anti-murine Gr1 antibodies or rat IgG (each group contained 5 mice). The mice were then sacrificed 4 weeks after the antibody treatment was completed.

and inhibited the T cell activity that promoted CRC development. We then depleted CD11b⁺Gr1⁺ cells in the mice that had developed dysplasia by injecting anti-Gr1 antibody. Four weeks after the antibody treatment, the tumor numbers in the anti-Gr1 antibody-treated mice decreased significantly compared with the mice treated with isotype control (Fig. 6C).

Discussion

In this study we found that MC numbers and MC-specific protease expressions increased significantly in tumors compared with the adjacent non-tumor tissues. Notably, more mMCP1-positive MMCs than mMCP6-positive CTMCs were observed in tumor but not in tumor-adjacent tissues. Significantly increased mMCP-1 was determined in tumors. Blocking the activities of MMCs, mMCP-1 levels both in serum and in colon tissues decreased, infiltration of CD11b⁺Gr1⁺ cells was alleviated, and the tumor numbers were reduced. After administration of recombinant mMCP-1 *in vivo* the numbers of CD11b⁺Gr1⁺ but not CD11b⁺Gr1⁻ cells increased significantly. The mMCP-1 conditioned medium from CD11b⁺Gr1⁺ cells but

not the medium from $CD11b^+Gr1^+$ cells alone or mMCP-1 alone was able to support colon cancer cell proliferation. In addition, allogeneic T-cell proliferation stimulated by mMCP-1-treated $CD11b^+Gr1^+$ cells was inhibited compared with that by $CD11b^+Gr1^+$ cells alone. Removing the $CD11b^+Gr1^+$ cells without affecting the MMCs in mice the tumor growth was inhibited. These data demonstrated that MMCs are important players in CRC development, which might modulate the $CD11b^+Gr1^+$ cell activity in the inflamed colon tissues.

It was found that there was a large pool of MC progenitors in murine small intestine and the establishment of MC progenitor reservoir depended on the $\alpha4\beta7$ integrin expressions. MMC hyperplasia associated with *Trichinella spiralis* infection in small intestine was proposed due to the established MC progenitor reservoir [33]. Whether the increase of MMCs during CRC development in the murine model was related to the small intestine reservoir and the involvement of integrins needs to be addressed in the future.

The way of inflammatory cells and their products in affecting the tumor development has been explored [1]. It remains largely unknown how these cells are accumulated in promoting the CRC development. MCs are abundant at sites exposed to the external environment, such as the intestine [6]. MMCs and CTMCs were found to have different functions in the intestine [14,15]. The distinction between MMCs and CTMCs is acquired during the local tissue development of the subsets rather than being fixed either by the genetic makeup of their progenitors or their classical location [33]. Previously, we found that degranulation of skin MCs (CTMC subtype) boosted the homing of monocyte-derived dendritic cells [34]. MMCs were found different in their proteases and their production of eicosanoids and the other mediators after activation [33]. In our current study, we found a positive correlation between $CD45^+$ inflammatory cell numbers and tissue mMCP-1 levels in the colon. More than 70% of $CD45^+$ cells were $CD11b^+Gr1^+$ in the tumor tissues, while this value was less than 50% in the tumor-adjacent tissues. MMC products seemed important in mobilizing inflammatory $CD11b^+Gr1^+$ cells during CRC development. However, the effect of mMCP-1 might be indirect as no chemotaxis of mMCP-1 on these cells was observed. The cleavage activity of mMCP-1 on specified amino acids of some proteins has been documented and basement membrane proteins and cell adhesion proteins were considered to be highly interesting potential substrates for mMCP-1 [35]. Our current study suggests that the activation of MMCs induced $CD11b^+Gr1^+$ cell accumulation in the inflamed colon tissues probably by impairing the barrier function of intestinal mucosa. However, we cannot exclude the effect of luminal bacteria translocation after the MMC activation, which in turn regulates the infiltrated cell function to boost tumor development.

An altered intestinal epithelium creates a favorable tumorigenic microenvironment [12] and the microbe and the microbial products within the mucosa cause the activation of the immune cells, which in turn drives the tumor growth [13]. The roles of MCs in promoting tumor development were recently observed [19,21,36]. During our manuscript preparation, Pucillo and colleagues reported that MCs boost the activity of myeloid-derived suppressor cells, the heterogeneous cells with the phenotypes of $CD11b^+Gr1^+$ expression, and contribute to the development of the tumor-favoring microenvironment [37]. However, it is unclear which subtype of the MCs plays critical roles in tumor initiation or becomes involved in the progression from colitis to cancer [21,36]. Our current study demonstrated that the activation of MMCs not only recruited the $CD11b^+Gr1^+$ cells but also modulated the cell activity to promote CRC development. Some soluble factors from mMCP-1-treated $CD11b^+Gr1^+$ cells supported colon cancer cell proliferation. In addition, T cell activation was also inhibited when the $CD11b^+Gr1^+$ cells were treated by mMCP-1. Therefore, a favorable cancer-promoting inflammatory microenvironment was created in the presence/activation of MMCs. Indeed, some other studies suggested that MCs regulate both innate and adaptive immunity through many types of release mediators after activation, such as histamine [38], turning the immune responses toward tumor progression [16].

Inhibition of MC activity with a therapeutic purpose has been widely exploited using histamine antagonists against allergic reactions. In recent decades, many new targets of MCs have been identified, offering new therapeutic opportunities for numerous inflammatory diseases and perhaps for cancers [16]. Our current study provided data on the prevention of CRC development by blocking MMC activity using current clinically used drugs. MCs can serve as potential therapeutic targets for preventing CRC development.

Acknowledgments

This work was supported by the “973” project: 2011CB504205 and the National Natural Science Foundation of China (30973387).

Conflict of interest

None.

Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.canlet.2015.05.014.

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