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Basic Study

Oxidized low-density lipoprotein stimulates CD206 positive macrophages upregulating CD44 and CD133 expression in colorectal cancer with high-fat diet

Zheng SM *et al*. Ox-LDL stimulates CD206 positive macrophages

Abstract

BACKGROUND

Oxidized low-density lipoprotein (ox-LDL), which abnormally increased in the serum of colorectal cancer (CRC) patients who follow a high-fat diet (HFD), may be one of the risk factors of development of CRC. Ox-LDL exerts a regulatory effect on macrophages and may influence CRC through the tumor microenvironment. The role of ox-LDL in CRC is remained unclear.

AIM

To investigate the role of ox-LDL through macrophage in CRC associated with HFD.

METHODS

The expression of ox-LDL and CD206 was detected in colorectal tissues of CRC patients with hyperlipidemia and HFD-fed mice by immunofluorescence. We stimulated the macrophages with 20 μg/mL ox-LDL and assessed the expression levels of CD206 and the cytokines by cell fluorescence and quantitative polymerase chain reaction. We further knocked down LOX-1, the surface receptor of ox-LDL, to confirm the function of ox-LDL in macrophage. Then LoVo cells were co-cultured with the stimulated macrophages to analyze the CD44 and CD133 expression by western blot.

RESULTS

The expression of ox-LDL and the CD206 were significantly increased in the interstitium of colorectal tissues of CRC patients with hyperlipidemia, and also upregulated in the HFD-fed mice. Moreover, the increased level of CD206 and decreased level of inducible nitric oxide synthase were observed in macrophages after ox-LDL continuous stimulation. The regulation was inhibited when the surface receptor LOX-1 was knocked down in macrophage. Ox-LDL could induce CD206+ macrophages, which resulted in high expression of CD44 and CD133 in co-cultured LoVo cells.

CONCLUSION

ox-LDL stimulates CD206+ macrophages to upregulate CD44 and CD133 expression in CRC associated with a HFD.

Key Words: Oxidized low-density lipoprotein; CD206 positive macrophages; CD44; CD133

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Core Tip: Obesity increases the risk of colorectal cancer (CRC), but the mechanism remains unknown. CD206+ macrophages promote CRC. It has been established that the prevalence of CRC was higher in high-fat diet (HFD) people and HFD mice with up-regulated CD206+ macrophages levels in colorectal tissue. Oxidized low-density lipoprotein (ox-LDL) is a lipid peroxide produced, which have been found increasing in the serum ox-LDL level in CRC patients. Importantly, ox-LDL exerts a regulatory effect on macrophages and may regulate CRC through the tumor microenvironment. Our study showed that ox-LDL stimulates CD206+ macrophages to up-regulate CD44 and C133 expression in HFD associated CRC.

INTRODUCTION

Obesity is a widespread social phenomenon. There is sample evidence to suggest that obesity increases the risk of colorectal cancer (CRC)[1]. A prospective cohort study of 85256 women found that obese individuals had a 1.93 times higher risk of CRC by age 50 than normal-weight individuals[2]. Excessive intake of dietary fat, namely a high-fat diet (HFD), is the main cause of obesity and one of the important reasons for the increased incidence of CRC[3]. However, the specific mechanism of CRC remains

unknown. It is currently believed that a HFD promotes intestinal cancer by increasing the number and malignant potential of intestinal stem cells[4,5].

Interestingly, it has been shown that a HFD could activate systemic inflammation and increase the malignant potential of intestinal tumors by upregulating the expression of macrophages[6,7]. It has been established that macrophages exhibit multiple phenotypes, like CD 206, CD163 and inducible nitric oxide synthase (iNOS), *etc.*[8]. Importantly, some kind of macrophages promote cell repair and cell proliferation, like CD206+ macrophage[9]. An increasing body of evidence suggests that CD206+ macrophages in the tumor microenvironment promote CRC development, and a positive correlation has been documented between the CD206+ macrophages level and the degree of tumor malignancy[10]. Moreover, metastasis can be promoted through CD206+ macrophage interactions with CRC cells[11]. Liu *et al*[7] found that the prevalence of CRC was higher in people on a HFD with upregulated CD206+ macrophage levels in colon tissue. Moreover, when mice with intestinal microflora disorder were fed a HFD, it was found that CD206+ macrophages in the colon tissues correlated with the number of colon tumors and the degree of malignancy, suggesting that the increased CD206+ macrophage level induced by HFD could exert a significant promoting effect on CRC.

The HFD can cause low-grade inflammation and oxidative stress in the whole body, leading to increased lipid levels such as cholesterol and low-density lipoprotein[6]. Oxidized low-density lipoprotein (ox-LDL) is a lipid peroxide produced under oxidative stress that can be used to assess oxidative stress and lipid metabolism in the body. Several studies on obese people have found that the serum ox-LDL level in CRC patients was higher than in the control group with a healthy intestinal tract[12-14]. Importantly, ox-LDL exerts a regulatory effect on macrophages and may regulate CRC through the tumor microenvironment[15]. However, the mechanisms underlying macrophage regulation in CRC by ox-LDL remain unclear. In addition, the regulatory role of macrophages on tumors may be related to tumor stem cells. In this regard, Yang *et al*[16] found that CD44 levels were gradually increased in lung cancer tissue with an increase in CD206+ macrophage. Lv *et al*[17] also found that CD133 levels were

upregulated in thyroid cancer with the increasing CD206+ macrophages. CD206+ macrophages share a similar relationship with CD44 and CD133 in CRC. In this study, the expression and corresponding effects of ox-LDL in colorectal tissue from hyperlipidemia patients were studied to explore the regulatory effects of ox-LDL on macrophages and tumor stem cell markers CD44 and CD133 in the colorectal interstitium. Our findings provide a new mechanism of increased CRC susceptibility with HFD.

MATERIALS AND METHODS

Patient samples

Colonoscopy was performed on hyperlipidemia patients with CRC, with no prior radiotherapy, chemotherapy or surgery. The healthy colorectal tissue was collected from the patient underwent colonoscopy for physical examination. Samples of CRC tissue (CA, n = 16, male: female = 10:6) and normal colorectal tissue (normal, n = 20, male: female = 11:9) were collected. The average age of all patients was 57 ± 6 years old. All tissue samples were examined by experienced pathologists. All the CRC tissue we collected were adenocarcinoma. After sampling, tissue samples were fixed in 4% paraformaldehyde. All patients were treated at the Guangdong Provincial People's Hospital, and the tissue samples were collected by the same endoscopist. The study was approved by the Ethics Committee of Guangdong Provincial People's Hospital for human samples. Informed consent was obtained from all patients before the beginning of the study.

Animal model

Six C57/BL6 mice aged 4 wk assigned to the HFD group were fed a HFD (#H10141, China) for a total of 12 wk. Another six C57/BL6 mice aged 4 wk were assigned to the control group and fed a normal diet (normal grade, #02, China) for a total of 12 wk. After 12 wk, they were sacrificed for colorectal tissue harvesting. All tissues were

immediately transferred to 4% paraformaldehyde. Animal experiments were also approved by the Ethics Committee of Guangdong Provincial People's Hospital.

Specimen processing

After the above clinical and animal specimens were fixed at room temperature for 24 h, tissue sections were prepared as follows. The tissues were first dehydrated then embedded with paraffin. The paraffin-embedded tissues were cut into 3 μm-thick sections and cross-sections of the intestinal tissue were observed.

Animal histological analysis

3 micron-thick mice tissue slices were cut and dried in a 65 °C oven for 120 min. The slices were stained with hematoxylin-eosin, then dehydrated and transparent. Finally, the slices were dried and sealed with neutral gum. All samples were examined by experienced pathologists.

Immunofluorescence staining

The specimens were dried in a 65 °C constant temperature oven for 2 h for dewaxing and dehydration, then soaked in deionized water for 5 min. The tissue specimens were soaked in sodium citrate solution overnight in a 60 °C water bath to expose the antigen. The sections were immersed in phosphate buffer solution (PBS) and then incubated with 10% goat serum at 37 °C for 1 h for antigen blocking. The slices were incubated at 4 °C for 12 h with the corresponding primary antibody, then rewarmed at room temperature and washed with PBS. The cells were incubated with 100 uL/well working solution containing Alexa Fluor 594-conjugated goat anti-rabbit secondary antibodies at room temperature for 1 h in the dark. 4,6-diamino-2-phenylindole (DAPI; Thermo Fisher Science, United States) was used for nuclear counterstaining. The stained slides were imaged using an inverted fluorescence microscope (magnification, × 400; Olympus Corporation).

Calculation of ox-LDL value of immunofluorescence (IF): Three × 400 magnification fields were randomly selected for each section. Three fields were randomly selected from each section to observe the interstitium of colorectal tissue under a 400 × microscope. For iNOS-F4/80 or CD206-F4/80 double staining, the number of iNOS, CD206 and F4/80 positive cells and total cells were counted, and the ratio of iNOS and F/480, or CD206 and F4/80 was calculated. For ox-LDL-CD206 or LOX-1-CD206 double staining, the number of positive cells and total cells was counted, and their ratio was calculated. The mean value of the ratio from three fields was the positive cell rate of each section. All the analyses were double-blind and graded by two or more observers.

Cell experiment

The human colorectal adenocarcinoma cell line (LoVo) and mouse monocyte-macrophage leukemia cell line (RAW 264.7) were purchased from the American Center for Typical Culture Preservation (ATCC, United States). The human monocytic leukemia cell line was purchased from Wuhan Penoside Company (THP-1, #CL-0233, China). The maintenance medium for cell culture was Dulbecco modified Eagle medium (DMEM, Gibco) containing glucose (4.5 g/L), 10% fetal bovine serum, 100 U/mL penicillin and 100 mg/mL streptomycin. All cells were cultured under standard cell culture conditions of 37 °C, 5% CO_2 and 95% humidity.

Lipoprotein induction and RNA extraction: THP-1 cells were inoculated on 6-well plates, and 50 μg/mL ox-LDL was added 24 h and 72 h after sample collection. The confluency of cells in both groups was maintained at 60%-70% at the beginning of treatment, while the control group did not receive any treatment. The samples were centrifuged (800 rpm, 3 min) and washed with sterile PBS, and recentrifuged (800 rpm, 3 min). Then total RNA of tissues or cells was extracted using TRIzol reagent, according to the manufacturer's instructions. The samples were stored at -80 °C.

Cell fluorescence: RAW264.7 cells were inoculated on 12-well plates and treated with 50 μg/mL ox-LDL for 72 h when the cell confluency reached 60%-70%, while the control group did not receive any treatment. After 72 h, the culture medium was discarded, and

the cells were treated with 4% paraformaldehyde for 15 min. The cells were washed with PBS, sealed with 10% sheep serum at 37 °C for 1 h and incubated with PBS solution dissolved in 1% BSA and 0.1% TritonX-100 followed by primary antibody [Rabbit CD206 antibody (1:100, #18704-1-AP, United States)] at 4 °C for 12 h. 12 h later, the cells were removed washed with PBS, and incubated with the secondary antibody AlexaFluor594 (1:500) at room temperature in the dark for 1 h. The tissues were washed with PBS, and one drop of DAPI was added to each well, and images were obtained under the corresponding fluorescence channel using an inverted fluorescence microscope. The method used to calculate the IF-positive cell rate was the same as above.

Co-culture and protein extraction: LoVo cells and THP-1 cells were inoculated in the lower and upper chambers of 12-well Transwell plates, respectively. The cell concentration was 60%-70% and ox-LDL (#S24879, China) for 72 h. There were no THP-1 cells in the upper compartment of the culture plate in the control group, and the other conditions were the same as for the treatment group. 72 h later, the culture medium was discarded, and cells were gently moistened with PBS. Total protein of LoVo cells in each group was extracted with RIPA lysis buffer on ice. Protein concentration was detected by the BCA method, and protein samples were stored at -80 °C.

Quantitative polymerase chain reaction

After the RNA samples were stored at -80 °C, the concentration of the samples was detected first, and the samples were diluted, ensuring that the concentrations were consistent. The RNA was reverse-transcribed to cDNA in a 20 μL system, and the total RNA content was kept below 1000 ng. Then the cDNA was used as the template for quantitative polymerase chain reaction (qPCR) amplification. ABI 7300 Real-time PCR software was used to analyze the PCR results and detect the Ct value of the sample. GAPDH was used as an internal reference gene, and the relative quantitative analysis was carried out by the $2^{-\Delta\Delta Ct}$ method. The primer sequences used are in Table 1.

LOX-1 small interference RNA transfection

Small interference RNA (siRNA) was used to inhibit the expression of LOX-1 in macrophage. LOX-1 siRNA or siRNA negative control (GenePharma, GenePharma, Suzhou, China) was introduced into THP-1 cells *via* liposome 3000 (3 μL/mL) for 24 h. The sequence of LOX-1 siRNA is shown in Table 2.

Western blot

10 μg protein samples were taken from each well, separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to PVDF membrane and sealed with 5% skim milk. 1 h later, anti-CD44 antibody (1:5000, # AB189524, United Kingdom) and anti-GAPDH antibody (1:10000, # AB9458, United Kingdom) were added. After incubating with the primary antibody, the membrane was washed with TBST. The samples were then incubated with horseradish peroxidase-labeled secondary antibody (1:3000) for 1 h at room temperature. After washing with TBST, immune reactive bands were analyzed by protein band exposure with Enhanced Chemiluminescence reagents.

Statistical analysis

Microsoft Excel 2019 worksheet was used to summarize the experimental results and generate tables. Adobe Photoshop 2020 software was used to process the images, and GraphPad Prism 8.0.2 software was used for statistical analysis. A Chi-square test or unpaired *t*-test was performed to compare two groups. The one-way ANOVA test was performed to compare more than two groups, and the Dunnett *post-hoc* test was used for multiple comparisons. The data were assessed for normality before conducting an unpaired *t*-test and One-way ANOVA test. A P-value < 0.05 was statistically significant. All tests were carried out more than three times.

RESULTS

Ox-LDL expression was increased in the interstitium of CRC tissue

To explore the distribution and expression of ox-LDL in CRC, we collected tissue samples from CRC patients with hyperlipidemia (n = 16) and normal subjects (n = 20) and performed IF staining for ox-LDL. The results showed that compared with normal colorectal tissues, the expression of ox-LDL was significantly increased in the interstitium of CRC tissues (Figures 1A and 1G). It has been reported that LOX-1 is the surface receptor of ox-LDL in macrophage[12]. LOX-1-positive cells were also abundantly detected in CRC tissues (Figures 1C and 1I).

CD206+ macrophages were increased in the interstitium of CRC

Double-IF for CD206-F4/80 or iNOS-F4/80 showed that the number of CD206+ macrophages increased significantly in the interstitium of CRC (Figures 1E, 1F, 1K and 1L). Besides, the double-IF also showed the number of CD206+/ox-LDL+ cells and CD206+/LOX-1+ cells increased abundantly in CRC (Figures 1B, 1D, 1H and 1J).

Establishment of HFD mice model

4-wk-old C57 mice were fed a 60% HFD, and mice of the same age were fed a normal diet as controls. After 20 wk, the mice were sacrificed, and colorectal tissue specimens were harvested (n = 6). The colorectal length of controls mice and HFD-fed mice was measured, respectively. We found that the colorectal length of HFD mice was relatively shorter (Figures 2A and 2B). Subsequently, the tissue samples were sectioned and stained with hematoxylin-eosin. The staining results showed that glandular nuclei and interstitial cells in the colorectal tissues of HFD mice were slightly enlarged but to a milder degree than usually observed with intraepithelial neoplasia (Figure 2C).

HFD increased ox-LDL expression and CD206 positive macrophages in the colorectal interstitium of mice

After establishing the HFD-fed mouse model, we performed double-IF staining for CD206-F4/80 or iNOS-F4/80 in the colorectal tissue sections of the control and HFD-fed mice. We found that CD206 positive macrophages were significantly increased in the

colorectal interstitium of HDF-fed mice (Figures 3A-D). The number of CD206+/ox-LDL+ cells and CD206+/LOX-1+ cells also increased significantly in the colorectal interstitium of HDF-fed mice (Figures 3E-H).

Continuous ox-LDL stimulation promoted CD206+ macrophages

To explore whether ox-LDL is related to macrophage polarization, we stimulated human monocytic leukemia cells (THP-1) with ox-LDL for 24 h and 72 h *in vitro*. qPCR results showed that the expression level of CD206 gradually increased with increased stimulation time. However, the expression level of iNOS increased in a short period and then decreased significantly below the initial level (Figure 4A). In addition, ox-LDL was used to stimulate mouse leukemic monocyte/macrophage cell line (RAW 264.7) for 72 h, and IF detection for CD206 was conducted. The results showed that CD206+ macrophages significantly increased in RAW 264.7 cells three days after ox-LDL stimulation (Figures 4B and 4C). Overall, we found that CD206+ macrophages gradually increased with continuous stimulation with ox-LDL, while Inos+ macrophages initially increased then decreased in the later stages.

In order to confirm the relationship between ox-LDL and CD206+ cells, we transfected LOX-1 siRNA into THP-1 cells to inhibit the expression of LOX-1, the specific receptor of ox-LDL (Figure 4D). Our results suggested that after 72 h of ox-LDL stimulation, the regulation like inhibition of iNOS expression and the promotion of CD206 expression, was significantly weakened in THP-1 cells transfected with LOX-1 siRNA (Figure 4E). Further examination of the function of CD206+ macrophages showed that after 72 h of ox-LDL stimulation, the levels of CD206+ macrophage-related cytokines interleukin (IL)-4, IL-10 and tumor necrosis factor (TNF)-β increased significantly in THP-1 cells except IL-13 (Figure 4F).

Macrophages promote the expression of tumor stem cell markers CD44 and CD133 in an ox-LDL-stimulated high-fat microenvironment

To investigate whether the occurrence of CRC is related to macrophages in the colorectal interstitium and a high-fat microenvironment, we simultaneously inoculated human colorectal adenocarcinoma cell (LoVo) and THP-1 cells in transwell culture plates supplemented with 20 μg/mL ox-LDL for 72 h (Figure 5A). Thus, a high-lipid microenvironment was established to stimulate macrophages *in vitro* (n = 5). Western blot results showed that CD44 and CD133 expression were significantly increased in LoVo cells co-cultured with THP-1+ ox-LDL compared with ox-LDL alone (Figures 5C and 5D). We further transfected LOX-1 siRNA or siRNA negative control into THP-1 cells, and provided ox-LDL stimulation and co-cultured with LoVo cells again (Figure 5B). Our experiment demonstrated that after ox-LDL stimulation, the levels of CD44 and CD133 in LoVo cells were inhibited when we knocked down LOX-1 in THP-1 cells (Figures 5E and 5F).

DISCUSSION

An increasing body of evidence suggests that HFD increases the risk of CRC. Importantly, studies have demonstrated the relationship between HFD and cancer by establishing animal models. HFD is often associated with abnormal oxidative stress and elevated lipid levels. Ox-LDL is a metabolite that reflects oxidative stress and lipid metabolism and is associated with various tumors. Ma *et al*[18] hypothesized that ox-LDL could promote gastric cancer metastasis and demonstrated that ox-LDL could promote vascular proliferation and lymphatic metastasis in gastric cancer by activating the nuclear factor-kappa B signaling pathway in animal and cell experiments. Ox-LDL may play a potential role in promoting CRC in HFD. It has been reported that ox-LDL is correlated with CRC in patients with dyslipidemia, and the serum ox-LDL level of CRC patients is higher than in subjects without tumors[19]. A study from Egypt found that the serum ox-LDL level of obese colon cancer patients was higher than in obese patients with healthy intestines; a positive correlation was found between the serum ox-LDL level and the degree of tumor malignancy[13]. Furthermore, it was found that the serum ox-LDL of patients after surgery was significantly lower than before surgery[14]. These

findings suggest that ox-LDL is a potential predictor and prognostic biomarker of CRC. However, the above experiments were conducted using blood serum tests of CRC patients. To the best of our knowledge, no study has documented ox-LDL expression in colorectal tissue. Herein, we provided compelling evidence that ox-LDL was abnormally expressed in CRC patients with hyperlipidemia at the tissue level. In this regard, the ox-LDL level was upregulated in the cancer tissues of CRC patients, especially in the interstitium. Besides, we demonstrated that LOX-1, the surface receptor of ox-LDL, was also upregulated in CRC tissues. Consistently, increased ox-LDL and LOX-1 levels were documented in the colorectal tissues of HFD-fed mice, suggesting the regulatory role of ox-LDL in the tumor microenvironment in CRC.

Macrophages, the most important immune cells in the tumor microenvironment, exhibit multiple phenotypes and exert various function[8]. It has been shown that iNOS+ macrophages inhibit tumor progression mainly by playing a pro-inflammatory role[20], while CD206+ macrophages promote cell repair and cell proliferation and growth, thus promoting tumor progression[9]. Different macrophages exhibit dynamic changes in the tumor microenvironment, and CD206+ macrophages are closely related to CRC development[21]. Existing clinical studies have reported that the CD206+ macrophage level is positively correlated with the TNM stage, the number of metastasized lymph nodes and degree of vascular invasion[22], and high CD206+ macrophage expression suggests poor prognosis. Han *et al*[11] found that CD206+ macrophages in the tumor microenvironment could promote CRC metastasis through interaction with CRC cells. CD206+ macrophages have been reported to be elevated in colorectal tissues in patients with hyperlipidemia. A higher prevalence of CRC was detected in subjects with HFD in a retrospective cohort study conducted by Liu *et al*[7]. In the present study, CD206+ macrophages were upregulated in colorectal tissues in patients with hyperlipidemia. In addition, animal experiments also confirmed that an increase in the number of intestinal tumors in HFD-fed mice was accompanied by a higher level of CD206+ macrophage, which correlated with the degree of malignancy of the tumor[7]. Our study demonstrated that CD206+ macrophages were highly expressed in the interstitium of

CRC tissues compared with normal tissues. These results suggest that the elevated level of CD206+ macrophages induced by HFD exerted a stimulatory effect on CRC. Our experiment found that the number of CD206+/ox-LDL+ cells and CD206+/LOX-1+ cells were highly expressed in the colorectal interstitium of HDF-fed mice and CRC patients. In addition, studies confirmed that ox-LDL exert a regulatory effect on macrophages, and iNOS+ macrophages increased 24 h after ox-LDL induction in vitro[15]. Our experiment further found that CD206+ macrophages occurred in THP-1 cells under continuous ox-LDL stimulation. A high level of CD206+ macrophages was observed in RAW264.7 cells after ox-LDL stimulation for 72 h. Further examination the function of CD206+ macrophages showed that after ox-LDL stimulation for 72 h, the levels of CD206+ macrophage-related cytokines IL-4, IL-10 and TNF-β increased significantly in THP-1 cells. Our examination demonstrated that following the knock down of LOX-1, the number of CD206+ macrophages mediated by ox-LDL were significantly depressed. Based on our results, we hypothesized that ox-LDL could promote the progression of CRC by continuous stimulation of macrophages to induce CD206+ macrophages.

In addition, ox-LDL is also associated with tumor stem cells. Yang *et al*[23] found that ox-LDL could increase the malignancy of tumor stem cells in bladder cancer, thus promoting the development of bladder cancer. Active cell proliferation has been documented in gastrointestinal tissue from HFD-fed mice, with increased malignancy of tumor stem cells, which has been attributed to the inflammatory environment in colorectal tissue[4,5,24]. The high level of CD206+ macrophages in HFD-fed mice may play a certain role in this process. After THP-1 cells were stimulated by ox-LDL for 72 h and co-cultured with LoVo, the level of the tumor stem cell marker CD44 and CD133 significantly increased in LoVo cells. Further, when we knocked down LOX-1 in macrophages, the increasing level of CD44 and CD133 were not that obvious in CRC cells, confirming that ox-LDL mediated the CD206+ macrophages to upregulate CD44 and CD133 expression in colon cancer cells.

CONCLUSION

In this study, we hypothesized that HFD could induce ox-LDL and its surface receptor LOX-1 accumulation in CRC tissue, suggesting the regulatory role of ox-LDL on the microenvironment of CRC. Furthermore, continuous stimulation of ox-LDL on macrophages induced CD206+ macrophages, which could further promote the increase of CD44 and CD133 levels in CRC cells. However, there were many limitations in our study. First of all, this study was a single-center study, and the sample size of included clinical specimens was relatively small. Indeed, a larger sample size and a multi-center prospective study are needed to increase the robustness of our findings. Moreover, the mechanism underlying the effects of ox-LDL and the relevant signaling pathways were not explored, warranting further studies. In conclusion, we demonstrated that HFD causes ox-LDL accumulation in the colorectal tissue and upregulates CD44 and CD133 expression in colorectal cells by inducing CD206+ macrophage. These findings provide evidence of a new mechanism of increased CRC susceptibility with a HFD.

ARTICLE HIGHLIGHTS

Research background

Oxidized low-density lipoprotein (ox-LDL), abnormally increased in the serum of patients with colorectal cancer (CRC) associated with a high-fat diet (HFD), may be one of the risk factors. Ox-LDL exerts a regulatory effect on macrophages, associated with cancer stem cells and may regulate CRC through the tumor microenvironment. The role of ox-LDL in CRC is remained unclear. It's necessary to explore the function of ox-LDL to find out the pathogenesis of in CRC with HFD.

Research motivation

Our experiment detected the expression of ox-LDL in human colorectal cancerous tissues and colorectal tissues of hyperlipidemic mice and explored the function of ox-LDL in the macrophages in tumor microenvironment. Our key point is that ox-LDL up-regulates CD44 and CD133 in CRC with HFD mediates by macrophages. Our study will

reveal the function of ox-LDL in up-regulating in CRC with HFD mediates by promoting CD206+ macrophages in tumor microenvironment, which provides a new idea for the mechanism of CRC with HFD.

Research objectives

This study is aimed to investigate the role of ox-LDL through macrophage in CRC associated with HFD. Our studies found that after continuous stimulation of ox-LDL promotes CD206 positive macrophages then upregulate CD44 and CD133 expression in CRC with HFD.

Research methods

The expression of ox-LDL and CD206 was detected in colorectal tissues of CRC patients with hyperlipidemia and HFD-fed mice by immunofluorescence. We stimulated macrophages with 20 ug/mL ox-LDL and assessed CD206 and the cytokines expression levels by cell fluorescence and quantitative polymerase chain reaction. We further knocked down LOX-1, the surface receptor of ox-LDL, to confirm the function of ox-LDL in macrophage. Then LoVo cells were co-cultured with the stimulated macrophages to analyze the CD44 and CD133 expression by western blot.

Research results

The expression of ox-LDL and the CD206 were significantly increased in the interstitium of colorectal tissues of CRC patients with hyperlipidemia, and also upregulated in the HFD-fed mice. Moreover, the increased level of CD206 and decreased level of inducible nitric oxide synthase were observed in macrophages after ox-LDL continuous stimulation. The regulation was inhibited when the surface receptor LOX-1 was knocked down in macrophage. Ox-LDL could induce CD206+ macrophages, which resulted in high expression of CD44 and CD133 in co-cultured LoVo cells.

Research conclusions

Our studies found that HFD could induce ox-LDL accumulation in CRC tissue, suggesting the regulatory role of ox-LDL on the microenvironment of CRC. Continuous stimulation of ox-LDL on macrophages induced CD206+ macrophages, which could further promote the increase of CD44 and CD133 levels in CRC cells.

Research perspectives

Our next project is to collect more and bigger samples and look forward to make a convincing analysis the correlation between ox-LDL and progression and survival of the enrolled patients in the near future. We will explore the potential signal pathways related to ox-LDL promoting M2-type macrophages by using the technology of single cell sequencing and/or RNA-Seq assay. We are confident that there will be exciting data in the near future.

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ORIGINALITY REPORT

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SIMILARITY INDEX

PRIMARY SOURCES

1	Mingzhu Zhu, Junbao Du, Angie Dong Liu, Lukas Holmberg, Selena Y Chen, Dingfang Bu, Chaoshu Tang, Hongfang Jin. "L-cystathionine inhibits oxidized low density lipoprotein-induced THP-1-derived macrophage inflammatory cytokine monocyte chemoattractant protein-1 generation via the NF-κB pathway", Scientific Reports, 2015 Crossref	14 words — < 1%

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