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ORIGINAL ARTICLE

CRP and LOX-1: a Mechanism for Increasing the Tumorigenic Potential of Colorectal Cancer Carcinoma Cell Line

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Abstract

Chronic inflammation and dyslipidemia are associated with an increase in the incidence of colorectal cancer (CRC). Serum C-reactive protein (CRP) and oxidized low-density lipoprotein (oxLDL), as Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) ligands, increase during inflammation and dyslipidemia, respectively. To evaluate the effects of CRP on the expression of important genes involved in the development of CRC, the CRC cell line, LS174T, was treated with the commercial CRP. Based on the Real-time PCR data, in the presence of CRP, *LOX-1*, *CEA*, *MMP1*, and *MMP2* mRNA expression significantly increased, compared to the control group. Moreover, in the presence of CRP, secretion, and expression of CEA in the cell lysate and conditioned media increased in a concentration-dependent manner. The results of flow cytometry showed that expression of LOX-1 receptors at the cell surface increased significantly in the presence of 10 mg/L of CRP. However, inhibition of LOX-1 receptors with a specific monoclonal antibody reduced the effects of CRP on protein/mRNA expression. In conclusion, Increased CRP level, can potentially elevate the expression of important genes in CRC by stimulating LOX-1 receptors.

Keywords Carcinoembryonic antigen · Matrix metalloproteinase · C-reactive protein · Oxidized low density lipoprotein · LOX-1 receptor · Tumorigenic potential

Abbreviations

LOX-1 or OLR1	Lectin-like oxidized low-density lipoprotein receptor-1
CEA	Carcinoembryonic antigen
MMP	Matrix Metalloproteinase

Introduction

Colorectal cancer (CRC) was identified as the third most common cancer among women and men in 2017. CRC is also the second and third cause of cancer-related mortality among American men and women, respectively [1]. Obesity and chronic inflammation also play an important role in various aspects of cancer, including its onset, progression, metastasis, and clinical features [2]. Epidemiological studies show that 25% of human cancers worldwide are associated with chronic inflammation or infection [3]. Moreover, evidence shows that chronic inflammation is a cause of different malignancies. For instance, inflammatory bowel disease can lead to CRC [4].

Although chronic inflammation is associated with many cancers, most people with chronic inflammatory diseases do not develop cancer, suggesting that chronic inflammation alone cannot promote cancer. However, in epithelial cells, chronic inflammation can increase genetic changes by increasing the production of reactive oxygen and nitrogen species. Especially in cells that have the reduced capacity to repair damaged DNA (mismatch repair), apoptosis and cell cycle control, it can cause cancer [5, 6].

Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) is a type II membrane protein, which has an extracellular carboxyl end with a structure similar to C-type lectin. Initially, it was recognized as the main oxidized low-density lipoprotein (oxLDL) receptor in endothelial cells [7]. C-

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reactive protein (CRP) is a marker of systemic inflammation and a main acute-phase protein. In addition to oxLDL, CRP protein acts as a LOX-1 ligand [8].

In 2013, a meta-analysis of prospective studies on the incidence of all cancers and CRP showed a higher risk of cancer in people with a higher CRP level [9]. Today, CRP is used as a predictive marker for many cancers, including CRC. A study by Lin et al. in 2013 showed that CRC patients with a CRP level above 5 mg/L had a poorer prognosis than patients with a CRP level below 5 mg/L [10].

Carcinoembryonic antigen (CEA) is a widely used tumor marker in cancer patients. This tumor marker also plays an important role in the CRC biology. Clinical studies on CRC patients have shown that risk of synchronous [11] and metachronous liver metastasis is higher in patients with a higher CEA concentration [12]. Moreover, different experimental studies have shown that CEA can induce liver metastasis by stimulating Kupffer cells with a mechanism independent of cell adhesion [13, 14]. A recent clinical study showed a significant relationship between the level of inflammatory markers and plasma concentrations of CEA [15].

A study by Liang et al. showed that in endothelial cells, TNF-α causes LOX-1 upregulation and increases the adhesion and migration of breast cancer cells [16]. On the other hand, an increase in the level of oxLDL, as the main risk factor for atherosclerosis, increases the risk of many cancers. Moreover, in a study on the effects of hyperlipidemia on susceptibility to breast cancer, treatment of the human breast cancer cell line with oxLDL increased the proliferation of cells and expression of *LOX-1* gene in a concentration-dependent manner [17].

In a study by Gonzalez-Chavarria et al., proliferation and angiogenesis of cancer cells were inhibited by the LOX-1 knockdown in the C4–2 prostate cancer cell line. With an increase in LOX-1 expression, the proangiogenic markers, MMP2, MMP9, and VEGF, also increased in this cell line [18, 19]. Also, LOX-1 increase the migratory and invasive abilities of cancer cells [20, 21].

CRP and oxLDL increase due to inflammation and obesity, respectively, and both interact with LOX-1 receptors [22, 8]. Although studies have been conducted on the effects of oxLDL as a LOX-1 ligand in cancer, no study has been conducted to investigate the effects of CRP as a LOX-1 ligand. Considering the significance of CEA in CRC, in the present study, we used a cell line with high CEA expression to investigate the effects of CRP on CRC biology. In addition CRP as a LOX-1 ligand was used to investigate its effects on CEA expression and several other genes (eg, *cyclin D1*, *c-myc*, *MMP1*, and *MMP2*) involved in the molecular mechanisms of CRC development and progression.

Materials and Methods

The colorectal cancer cell line, LS174T (NCBI no.C568), was obtained from the cell bank of Pasteur Institute of Iran. The culture media, including RPMI, trypsin, and fetal bovine serum (FBS), were obtained from Gibco Co. Agarose was provided by YTA Co., DEPC and DMSO were supplied by CinnaGen Co. (Iran). In addition, sodium Azide free CRP was provided by My Biosource Co. (USA).

The primary mouse anti-human LOX-1 antibody and secondary goat anti-mouse IgG-FITC were provided by R&D Co. Total RNA was extracted using RNA extraction solution provided by GeneAll Co. (Korea). The cDNA was synthesized using the kit provided by Takara Co. (Japan). The primers were manufactured by Bioneer Co. (Korea), and the SYBR Green master mix, plates, and cap were provided by ABI Co. Finally, the CEA measurement kit was supplied by CanAg Co. (Sweden).

Cell Culture and Cell Treatment with CRP Antigen The single-layer culture of LS174T colorectal cancer cell line in RPMI, containing 10% FBS, 100 U/mL of penicillin, and 100 μg/mL of streptomycin, was incubated at 37 °C (95% humidity and 5% CO_2). To evaluate the effect of CRP on the colorectal cancer cell line, 1.5×10^5 cells were cultured in 3-cm dishes. At the subconfluent stage, the treated cells were incubated for 24 h after adding 5, 10, and 20 mg/L of CRP. Furthermore, to block the LOX-1 receptor, 10 μg/mL of anti-LOX-1 antibody was added to one of the cell groups 1 h before adding CRP to the cells. After the treatment, the cells were used for the analysis of gene expression via Real-time PCR and flow cytometry. All experiments were performed three times individually, and each time it was done in the form of a triplicate.

CEA Measurement in the Culture Medium and Cell Lysate After incubation of cells in the treatment and control groups, the collected conditioned media were centrifuged for 10 min at 15000 rpm (4 °C) in order to harvest the cell residues. The supernatant was stored in a − 70 °C freezer. To lyse the cells, lysis buffer, containing 100 mM tris-HCl, 100 mM NaCl, and Triton X-100 (1%), was used. The cell lysate was transferred to a microtube and placed on ice for 15 min and centrifuged at 13000 rpm for 10 min at 4 °C. The supernatant was kept at −70 °C until analysis of CEA concentration. The CEA levels in the cell culture medium and cell lysate were measured by ELISA kit. CEA concentration was reported as nanogram per milligram of total protein.

RNA Extraction and Real-Time PCR The manufacturers' instructions for the use of RNA extraction solution and cDNA synthesis kit were applied to extract total RNA and synthesize cDNA. In brief, after determining the purity of RNA at the absorbance ratio of 260/280 nm, its integrity was determined

using RNA electrophoresis in 1.5% Agarose gel, containing 2% formaldehyde. Then, 2 μg of total RNA was used for reverse transcription with the cDNA synthesis kit in the presence of random hexamers and oligo (dT).

The primers were designed using Allel ID 7.5 software. To prevent nonspecific binding primers were designed exon-exon junction manner (Table 1). Real-time PCR was performed for analyzing the expression of *LOX-1*, *CEA*, *cyclin D1, c-myc*, *MMP1*, and *MMP2* genes, using a thermal cycler (ABI 7900 HT) in triplicate. The final reaction volume for each of the samples was 10 μL, containing 3.7 μL of RNAse free water, 5 μL of 2X SYBR Green master mix, 0.3 μL of each primer at a concentration of 10 μM, and 1 μL of cDNA.

The Real-time PCR program was as follows: 10 min of initial denaturation at 95 °C, 40 cycles of denaturation at 95 °C for 15 s, 40 s of annealing, and 30 s of polymerization at 60 °C. The quantitative analysis of genes was performed using SYBR green I fluorescence dye and comparative cycle threshold (Ct) method. The GAPDH gene was used as the internal control. Calculation of quantitative expression of genes in the samples was performed using the $2^{-\Delta\Delta ct}$ method.

Flow Cytometry Analysis For analyzing the expression of LOX-1 receptor after cell treatment with different concentrations of CRP, the cells were isolated using Hanks' balanced salt solution (without calcium or magnesium). 5×10^5 cells was placed in 100 μL of buffer (PBS with 2% FBS), which contained LOX-1 antibody. The cells remained on ice for 30 min. They were then washed twice with buffer and incubated for 30 min in the dark with goat anti-mouse IgG1 antibody. After washing the cells and resuspension in buffer and analyzed flow cytometry device (CyFlow, Partec, Germany), the percent of positive cell was evaluated. The results were analyzed by flow Max software.

Statistical Analysis After the experiments and data collection, SPSS version 18 and GraphPad Prism5 were used to perform one-way ANOVA (comparison of means by Dunnett's multiple comparison test), two-way ANOVA, and independent sample *t* test, if necessary. $P < 0.05$ was considered significant. Graphs were plotted using GraphPad Prism5.

Results

CRP as a Ligand for LOX-1 The flow cytometric method was used to investigate the effects of CRP antigen, as a ligand for LOX-1 receptor candidates (Fig. 1). Studies have shown that this receptor increases at the cell surface in the presence of oxLDL ligands [23]. The analysis of LOX-1 receptor expression in cell treatment with 5 and 10 mg/L of CRP showed (Fig. 2) that cell treatment with 5 mg/L of antigen increased the expression of this receptor on the cell surface, although this increase was not significant compared to the control group ($P = 0.192$, Fig. 2).

On the other hand, by increasing the level of CRP antigen to 10 mg/L, expression of LOX-1 receptor increased significantly in the cells ($P = 0.015$). The percentage of LOX-1-positive cells increased from 66.5% in the control group to 78.9% in the treatment group. Moreover, in cells treated with 10μg/mL of anti-LOX-1 antibody at 1 h before adding 10 mg/L of CRP, the effects on the expression of LOX-1 receptor significantly decreased, compared to the group which was only treated with 10 mg/L of CRP ($P = 0.013$). The results of flow cytometry showed that LOX-1 receptor expression increased concentration-dependently in LS174T cells in the presence of CRP. However, receptor inhibition with a specific monoclonal antibody, LOX-1, prevented the effects of CRP on protein expression in the cell surface.

Table 1 Real time-PCR primers

Gene	GenBank accession number	Sequence of the selected primer pair1	Length of amplicon (nt[2])
LOX-1	NM_004795	TAGAAACCCTTGCTCGGAAGC	**136**
CEA	NM_001291484	TGCCAGATCCAGTCTTGCG AAATCACGCCAAATAATAACG	**161**
MMP1	NM_002421.3	AACCAGCACTCCAATCAT CACAAACCCCAAAAGCGTGT	**193**
MMP2	NM_001127891.2	TCGGCAAATTCGTAAGCAGC GCCAAGTGGTCCGTGTGAAG	**210**
c-myc	NM_002467	CTTCAGCGTTGCCGCCCA CTGCTTAGACGCTGGATT	**96**
cyclin D1	NM_053056	GGTCATAGTTCCTGTTGGT TCGGTGTCCTACTTCAAAT	**98**
GAPDH	NM_001098209	CACTTCTGTTCCTCGCA GGAAGGACTCATGACCACAG CACGTTGGCAGTGGGGAC	**208**

[1] Forward primer is shown as upper sequence in the respective primer pair. Reverse primer is the lower sequence

[2] is nucleotide

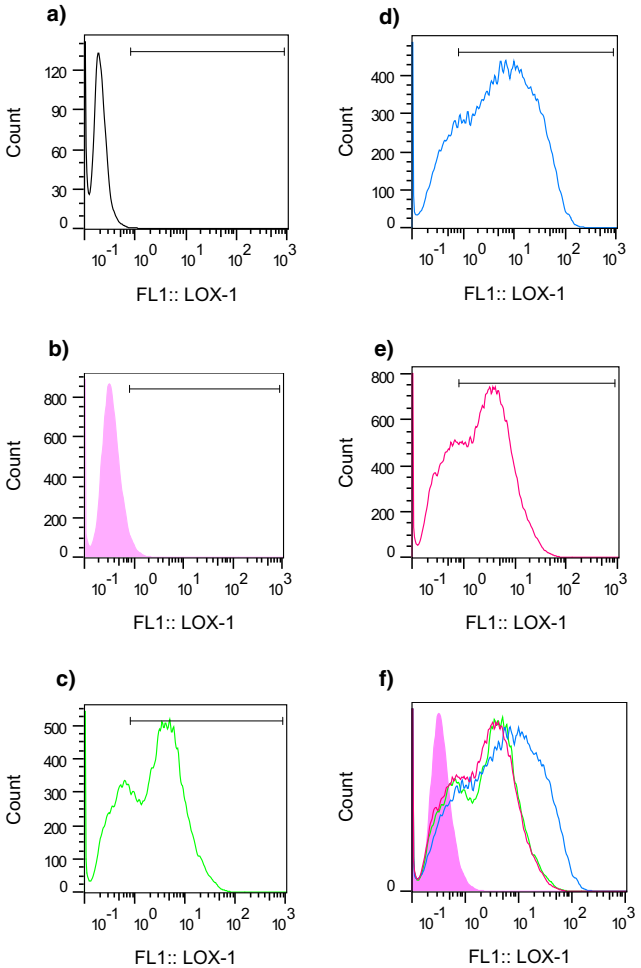

Fig. 1 Histogram of LOX-1 cell receptor expression in LS174T cells via flow cytometry. **a**, **b**, **c**, and **d** represent unstained cells, isotype controls, untreated cells, and treated cells, respectively, and **e** represents the cells which were incubated with anti-LOX-1 antibody at 1 hour before treatment with 10 mg/L of CRP antigen. The duration of cell treatment was 24 hours

Increase in LOX-1 mRNA Expression in the LS174T Cell Line To evaluate the effects of CRP on the mRNA expression of LOX-1 receptors, the LS174T cells were treated with different CRP levels (5, 10, and 20 mg/L), as well as 10 mg/L of CRP, at treatment intervals of 12, 24, and 48 h. After extraction of total RNA, synthesis of cDNA, and real-time PCR, the expression of this gene in different groups was quantitatively compared to the control group. The analysis of mRNA expression at CRP levels of 5, 10, and 20 mg/L showed that the expression of LOX-1 gene was 1.79, 2.18, and 2.37 times higher than that of the control group (Fig. 3a). Furthermore, comparison between the treatment group receiving 10 mg/L of CRP antigen and the group receiving blocking antibodies plus 10 mg/L of CRP showed that the receptor gene expression significantly decreased in the presence of antibody despite the presence of CRP ($P < 0.001$). The comparison of relative expression changes between the groups was carried out at 12, 24, and 48 h. The difference between the treated and untreated groups was significant at 24 and 48 h (Fig. 3b).

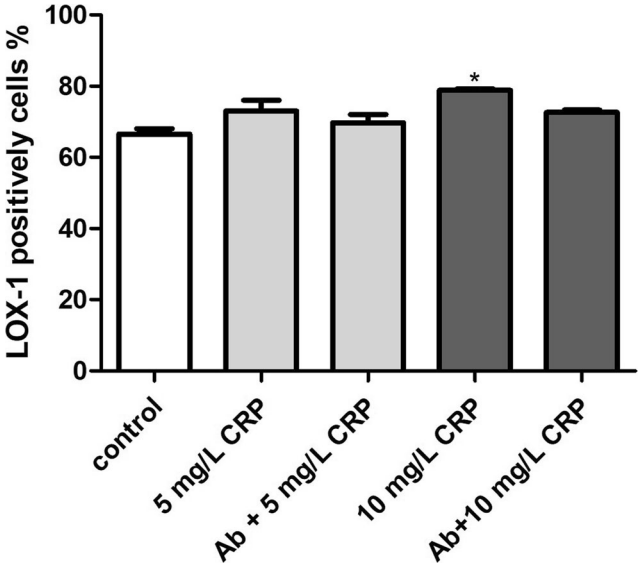

Fig. 2 Comparison of LOX-1 positive cells in different groups of treatment with CRP. The results represent 3 replicates which are expressed as mean ±SD (* represent $P < 0.05$; Ab denotes anti-LOX-1 antibody)

Nevertheless, two-way ANOVA showed that these differences were not time-dependent ($P = 0.12$).

CRP Antigen Act as a New Simulator of CEA Expression and Secretion To study the effect of CRP on the expression of effective genes in the progression and metastasis of CRC, in addition to LOX-1, several genes were examined including CEA. The relatively high expression and secretion of CEA in the LS174T cells allowed accurate estimation of tumor marker secretion from the cells to the culture medium, as well as protein expression in the cell lysate via ELISA assay. In the present study, different groups were compared after treating the cells with different CRP levels at different intervals with a constant level of CRP (10 mg/L). The results of CEA measurements in the conditioned media showed that 5, 10, and 20 mg/L of CRP increased CEA secretion in the cell culture media (Fig. 4a). The increase of secretion in the treatment group with 20 mg/L of antigen was more significant than that of the control group ($P = 0.036$). The analysis of CEA measurements in the groups also showed a similar rising pattern (Fig. 4b). In the treatment groups receiving 10 and 20 mg/L of CRP, CEA expression was significantly different from the control group ($P = 0.836$, $P < 0.01$, and $P < 0.001$ for CRP levels of 5, 10, and 20 mg/L, respectively). Although there was a significant difference in CEA expression between the groups receiving 5 with 10 mg/L of CRP, there was no significant difference between the groups receiving 10 and 20 mg/L of CRP; this finding shows that the highest CRP level for CEA expression was 10 mg/L.

Furthermore, the CEA level in the cell lysate and conditioned media (Fig. 4c and d) in the groups, which were treated with 10 mg/L of Bovine Serum Albumin (BSA), Anti-LOX1 antibody, 10 mg/L of CRP, and CPR plus Anti-LOX1 antibody for 24 h, were compared with the untreated group. The analysis of

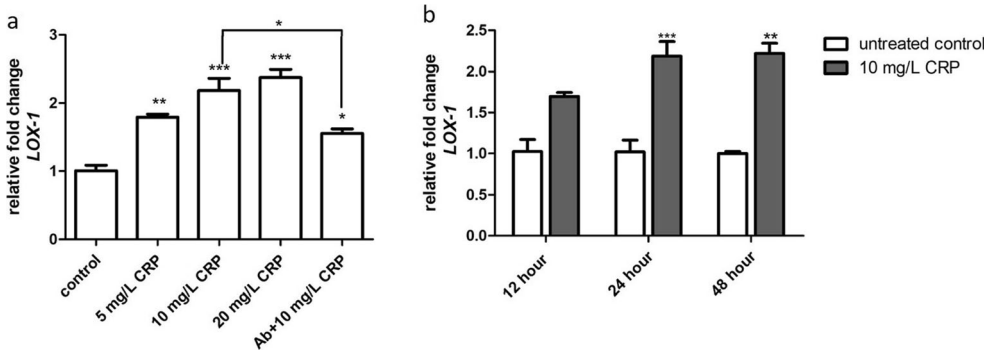

Fig. 3 The relative expression of LOX-1 gene in presence of different concentrations of CRP (**a**, incubation time was 24 h) and at different time intervals (**b**) (CRP 10 mg/L). The results indicate 3 replicates and are expressed as mean ±SD (*, **, and *** represent $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively; Ab denotes anti-LOX-1 antibody)

CEA protein level in the cell lysate and conditioned media in the presence of BSA and Anti-LOX1 antibody showed no significant difference with the untreated group in terms of CEA level.

The results of CEA measurement in the conditioned media showed that CEA secretion and cellular content of CEA increased at 12, 24, and 48 h after treatment with 10 mg/L of CRP antigen. There was a significant difference in cellular CEA between the group treated with 10 mg/L of CRP antigen and the control group at 24 and 48 h ($P = 0.358$ at 12 h; $P < 0.001$ at 24 and 48 h). However, CEA secretion and cellular concentration were not affected by the incubation time.

The CEA expression at CRP levels of 5, 10, and 20 mg/L was respectively 1.21, 2.2, and 2.77 times higher than that of the control group after treating the LS174T cell line with CRP antigen, extracting total RNA, synthesizing cDNA, performing real-time PCR, and analyzing the results (Fig. 5a).This increase in expression was inhibited in the group treated with 10 μg/L of anti-LOX-1 and 10 mg/L of CRP.

To evaluate the effect of time on CEA expression, the LS174T cells were treated with 10 mg/L of CRP for 12, 24, and 48 h. After RNA extraction, cDNA formation, real-time PCR, and data analysis, it was determined that CEA expression increased in the treatment group, compared to the control group at all intervals (Fig. 5b). However, in general, CEA expression was not affected by time ($P = 0.34$).

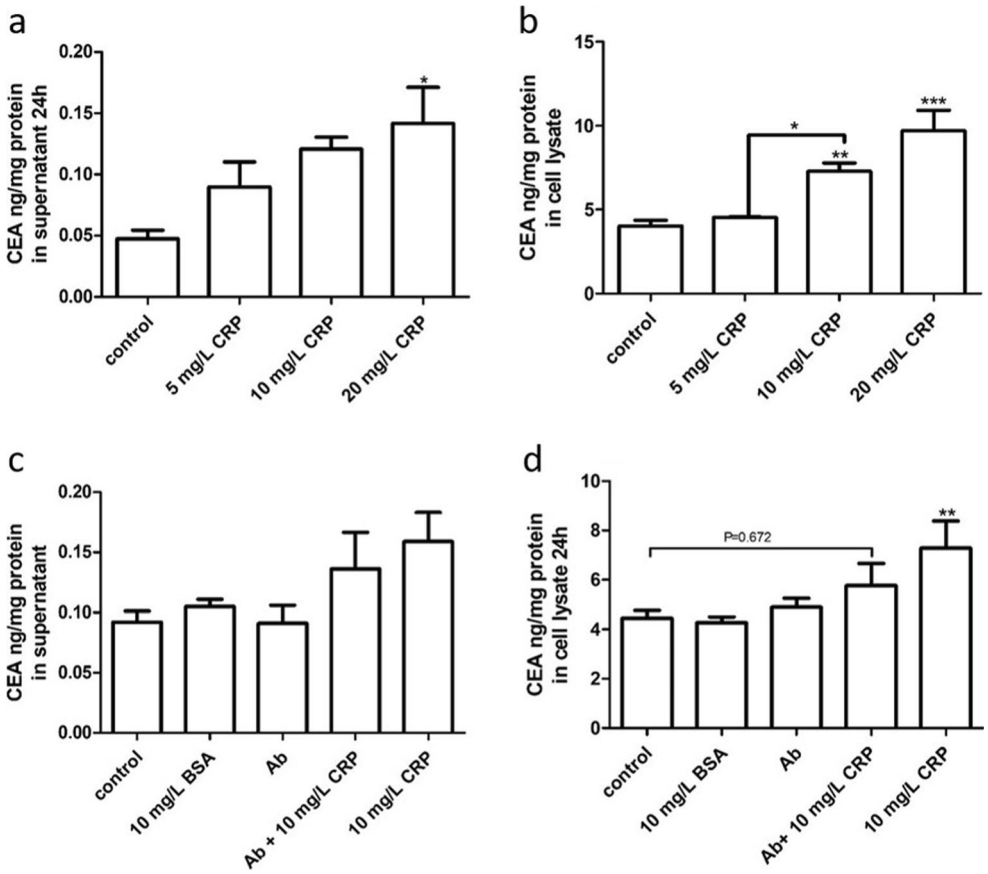

Fig. 4 Effects of different CRP antigen levels on the expression and secretion of CEA protein in the cell lysate (**a** and **c**) and conditioned media (**b** and **d**). CEA concentration were analyzed in the cell lysate and conditioned media using ELISA kits. The results indicate 3 replicates and are illustrated as mean ±SD (*, **, and *** represent $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively; Ab denotes anti-LOX-1 antibody)

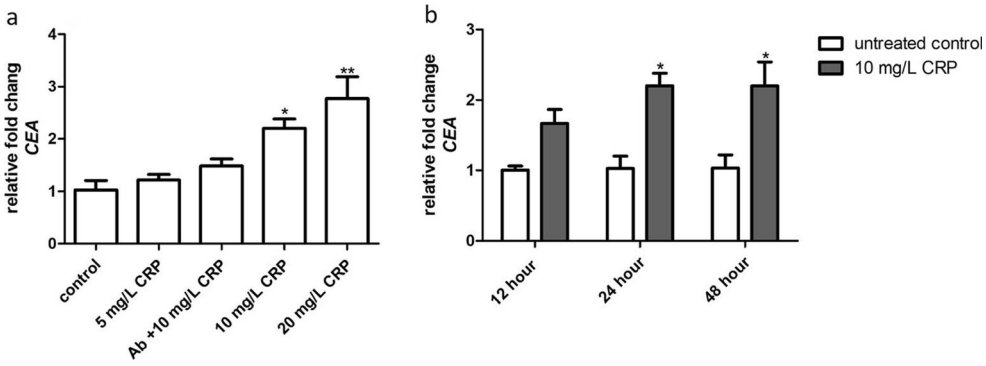

Fig. 5 Effects of CRP on the CEA expression in different CRP concentration (**a**, incubation time was 24 hours) and time intervals (**b**, 10 mg/L of CRP). The results indicate 3 replicates and are expressed as mean ±SD (*$P <$ 0.05; **$P < 0.01$; Ab denotes anti-LOX-1 antibody)

The Effect of CRP Is Not through the Wnt / β-Catenin Signaling We evaluate the effect of CRP on the Wnt/ β-catenin signaling downstream genes, cyclin D1, and c-myc, by Real-time PCR in LS174T cell line. The level of mRNA expression in *c-myc* and *cyclin D1* proto-oncogenes was quantitatively analyzed after 24 h of treatment with 5, 10, and 20 mg/L of CRP with and without anti-LOX-1 antibody. The results of real-time PCR showed that *c-myc* and *cyclin D1* expression was not significantly different between the groups ($P = 0.16$ for *c-myc* and $P = 0.69$ for *cyclin D1*) (Fig. 6a).

CRP Induces MMP1 as Time and Dose Dependent Manner The mRNA expression of *MMP1* gene in the group treated with 10 mg/L of CRP showed a significant increase compared to the control group (Fig. 6b). At 12 and 24 h, gene expression had 3.14 and 6.7-fold increase, respectively. In the presence of 10 μg/mL of anti-LOX-1 antibody, this increase was significantly inhibited. Furthermore, our results showed that the effect of CRP on increasing *MMP1* expression was time-dependent (two-way ANOVA; $P = 0.001$). The *MMP2* mRNA expression showed 2.8- and 5.5-fold increase in the presence of 10 mg/L of CRP at 12 and 24 h, respectively (Fig. 6c); interestingly, this effect diminished in the presence of anti-LOX-1 antibody. Regarding the effect of time on the expression of *MMP2* in the presence of CRP, the results did not show a significant difference (two-way ANOVA; $P = 0.062$).

Discussion

One of the main causes of mortality in CRC patients is liver metastasis [24]. Therefore, investigation of molecular mechanisms involved in the progression of cancer and metastasis is important in improving the therapeutic approaches and increasing the survival of patients. Inflammatory processes and elevated plasma levels of CRP play an important role in various cancers including CRC [25–28]. Studies show that there is a correlation between the increase in inflammatory biomarkers and reduction in the survival of metastatic patients with CRC.

CRP has been suggested as one of the prognostic biomarkers of CRC [28–30]. Recently, it has been shown that systemic inflammatory biomarkers and CEA level in the blood of patients with metastatic CRC is significant in their response to chemotherapy [15] . The present study showed that treatment of colorectal cancer cell lines with CRP can increase the expression of important genes involved in CRC progression, including *LOX-1*, *CEA*, *MMP1*, and *MMP2*. The

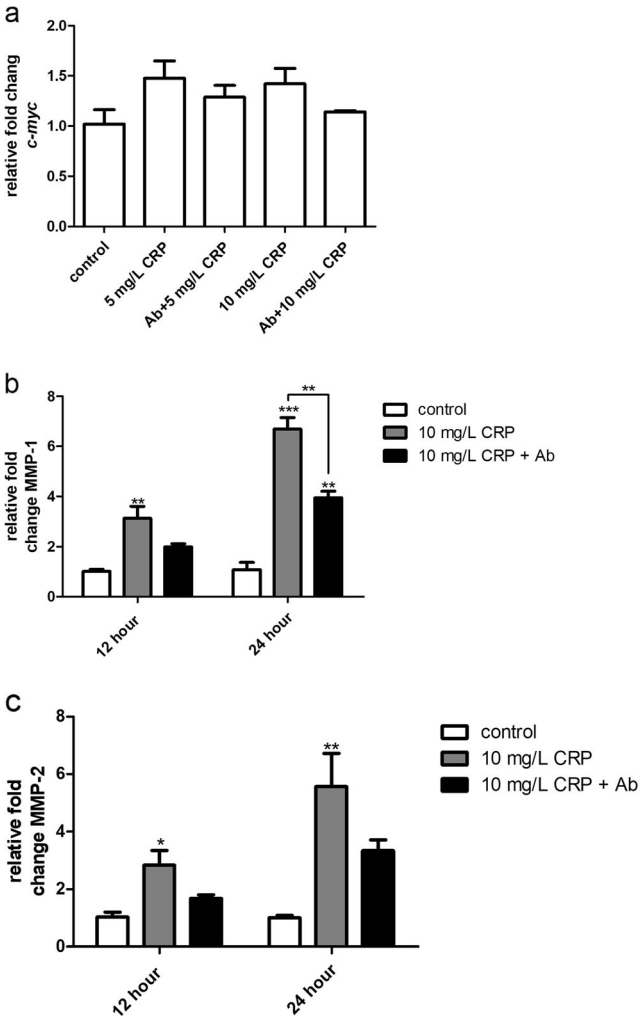

Fig. 6 Effects of CRP on c-myc (**a**), MMP1 (**b**) and MMP2 (**c**) expression. The results indicate 3 replicates and are expressed as mean ±SD (*, **, and *** represent $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively; Ab denotes anti-LOX-1 antibody)

findings showed that in the presence of anti-LOX-1antibody, CRP activity decreased in gene expression. Therefore, considering the direct function of CRP as a LOX-1 ligand, it may be one of the potential mechanisms associated with inflammation in CRC.

Although CRP has been already introduced as a LOX-1 ligand in endothelial cells [7], this study showed that it can also act as the LOX-1 ligand in the colorectal cancer cell line. It has been revealed that LOX-1 expression increases in several types of cancer. Studies have often focused on the relationship between hyperlipidemia and cancer progression due to oxLDL as the main receptor ligand [16, 18, 31]. The present study emphasized on the importance of CRP and the association between inflammation and cancer through this receptor. It was also observed that in the presence of 10 mg/L of CRP, the level of LOX-1 positive cells increased by nearly 13% on the cell surface (Figs. 1 & 2).

In the present study, the increase in the LOX-1 mRNA expression was concentration-dependent (Fig. 3). In a study by Zhang et al., this receptor was involved in the development and invasion of pancreatic cancer. Moreover, it was defined as one of the candidates for monitoring metastasis to lymph nodes [32]. In a study by Murdocca et al. on DLD-1 colorectal cancer cells using the wound-healing/invasion assay, the knockdown of LOX-1 receptor with siRNA or anti-LOX-1 antibody led to a decline in the rate of cell proliferation and migration [21].

Our study showed that the expression of important genes involved in the development of CRC increased in the presence of CRP. Furthermore, in terms of CEA, the protein measurements in the cells and conditioned media significantly increased (Fig. 4). CEA concentration in the blood of patients with CRC is affected by various factors, including CEA expression in tumor tissues, CEA clearance of the liver, tumor size, and stage of disease [33]. Naghibalhossaini et al. studied the role of glycosylphosphatidylinositol phospholipase D (GPI-PLD) in CEA secretion [34]. They used synthetic compounds as stimulators and inhibitors of GPI-PLD to study the role of this enzyme. According to the findings, the clinical significance of GPI-PLD in CRC is not completely clear. However, CRP, as a biomarker of systemic inflammation, has been shown to be related to many cancers in epidemiological studies. On the other hand, this study, for the first time, showed that CRP increased the expression and secretion of CEA. Considering the significant role of CEA in CRC liver metastasis and the results of the present study on cell treatment with CRP (associated with an increase in the expression and secretion of CEA), stimulation of this receptor at high concentrations of CRP may play a significant role in liver metastasis.

MMPs belong to a family of zinc-dependent endopeptidases, which mediate the extracellular matrix turnover and are involved in invasion and metastasis. Kim et al. showed that high expression of MMP1 in primary tumor was a strong prognostic factor for liver metastasis in CRC [35]. In the present study, after the treatment of LS174T cells with CRP, the expression of *MMP1* gene was also examined. It was observed that gene expression was multiplied in a concentration- and time-dependent manner.

Another member of the family of zinc-dependent endopeptidases, *MMP2*, was also significant in the development of CRC [36]. In this regard, the results of our study are consistent with the study by Gonzalez et al., which concluded that an increase in LOX-1 expression increased the expression of MMP-2 in prostate cancer [18].

In the present study, to determine the effects of LOX-1 receptor, the cells were treated with 10 μg/mL of Anti-LOX1 antibody at 1 h before adding CRP, according to the manufacturer's guidelines. Although this method does not completely inhibit the blocking of receptors, the effects of CRP on increasing the expression of LOX-1 and other genes, as well as CEA protein, were significantly inhibited. In 2016, Murdocca et al. showed that addition of anti-LOX-1 Antibody to the cell culture medium significantly reduced cell growth [21]. In other studies, inhibition was achieved by gene knockdown and RNA interference; cell growth decreased in both conditions [37].

It has been observed that excessive expression of *cyclin D1* is associated with tumorigenicity [38]. Several studies have been performed on the status of *c-myc* in various cancers. The increase in the expression of *c-myc* in gastric and esophageal cancers is associated with reduced survival [39, 40]. According to the literature, the level of *c-myc* proto-oncogene increases in CRC tumors [41]. In the present study, mRNA expression of *c-myc* increased, although it was not statistically significant (Fig. 6).

Unlike many other cancers, there may be one different subtype of CRC with a better prognosis in patients with very high levels of WNT and *c-myc* expression [42]. Therefore, since this type of cell line exhibits high CEA expression and patients with higher CEA have poorer prognosis [13, 14], increase in *cyclin D1* and *c-myc* through LOX-1 is not possibly important pathway in this cell line.

A study by Chu et al. showed that in endothelial cells, oxLDL and CRP synergistically reduced NO production and affected vascular function [42]. We believe that the effects of CRP on LOX-1 in cancerous epithelial cells are more complex, and there may be an interaction between oxLDL and CRP in binding to this receptor. However, the relatively low stability of oxLDL and limitations in the manufacturing and purchasing of this product are among the shortcomings of the present study. Considering the small number of patients with cancer in this study, it was not possible to collect sufficient samples to perform further studies. Regarding the importance of CEA in colorectal cancer, we used a cell line with high expression and secretion of this tumor marker. In this case, the CEA measurement with commercially available ELISA kits in conditioned media is more accurate. Among the available colorectal cancer cell lines, only the LS174T, a variant of LS 180, has this feature.

The results of the present study showed that treatment of CRC cell line with CRP could stimulate the LOX-1-dependent pathways and activate genes, which are involved in liver metastasis by increasing the expression of *CEA*, *MMP1*, and *MMP2*. Therefore, further studies are suggested on blocking LOX-1 receptors with Anti-LOX1 antibody or appropriate antagonists as a new therapeutic strategy for patients predisposed to cancer.

The advantage of using LOX-1 inhibitor in CRC inhibition can be simultaneous inhibition of *CEA* and *MMP* expression against cancer progression and metastasis of liver and other tissues. It should be noted that the inhibitory effects of LOX-1 antibodies on cellular concentration and CEA expression only appear in the presence of CRP (Fig. 4). Therefore, use of this therapeutic approach is unlikely to impair the normal functioning of the body.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that there are no conflicts of interests exist.

Ethical Approval The study was approved by the ethics Committee of Semnan University of Medical Sciences.

Informed Consent This research not involving human participants or animals.

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