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ORIGINAL ARTICLE

CD74 is a survival receptor on colon epithelial cells

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Abstract

AIM: To investigate the expression and function of CD74 in normal murine colon epithelial cells (CEC) and colon carcinoma cells.

METHODS: Expression of CD74 mRNA and protein were measured by reverse transcriptase-polymerase chain reaction (RT-PCR), Western blotting and fluorescence-activated cell sorter (FACS). The effect of migration inhibitory factor (MIF) on the survival of normal CEC from C57BL/6, NOD/SCID, and CD74 deficient mice both *in vitro* and *in vivo*, and on the CT26 carcinoma cell line was analyzed by (quantitative) qRT-PCR, RT-PCR, Western blotting and FACS.

RESULTS: CD74 was found to be expressed on normal CEC. Stimulation of CD74 by MIF induced a signaling cascade leading to up-regulation of Bcl-2 expression, resulting in a significant increased survival of CEC. CD74 was also expressed on the CT26 colon carcinoma cell line and its stimulation by MIF resulted in enhanced cell survival, up-regulation of Akt phosphorylation and Bcl-2 expression.

CONCLUSION: CD74 is expressed on CEC and colon carcinoma cells and serves as a survival receptor in these cells. These results may have implications on colorectal cancer research.

Key words: CD74; Migration inhibitory factor; Colon epithelial cells

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INTRODUCTION

CD74 (Invariant chain; Ii) is a type Ⅱ integral membrane protein, which acts as a chaperone for major histocompatibility complex (MHC) class Ⅱ protein expression[1]. It is a non-polymorphic type Ⅱ integral membrane protein; the mouse protein is comprised of a 30 amino acid (aa) N-terminal cytoplasmic tail, followed by a single 24 aa transmembrane region and an approximately 150 aa long lumenal domain. The CD74 chain was initially thought to function mainly as an MHC class Ⅱ chaperone, which promotes endoplasmic reticulum (ER) exit of MHC class Ⅱ molecules, directs them to endocytic compartments,

prevents peptide binding in the ER, and contributes to peptide editing in the MHC class Ⅱ compartment[1]. A small proportion of CD74 is modified by the addition of chondroitin sulfate (CD74-CS), and this form of CD74 is expressed on the surface of antigen presenting cells (APCs), including monocytes and B cells. In addition to APCs, other cells of the gastrointestinal tract, such as epithelial cells, express class Ⅱ MHC proteins and CD74 and act as APCs[2]. The role of CD74 in the epithelium is not fully defined. It was formerly accepted that the functions of CD74 in these cells have been correlated with its role in antigen processing and presentation in conventional APCs. However, CD74 may play additional roles in epithelial cells. CD74 expression on normal human colon epithelial cells (CEC) is still controversial, as the presence of this molecule was demonstrated using immunohistochemistry by some groups[3], but not by others[4,5]. On human colonic epithelial cell lines it was found to be expressed only after interferon-γ treatment[6]. In mice, CD74 expression on CEC is also not clear, although CD74 was not demonstrated on normal murine CEC[7]. In one study, CLIP (class Ⅱ invariant chain peptide) was shown to be expressed in a complex with MHC class Ⅱ, suggesting that CD74 is expressed on these cells as well[8]. Recently, it was demonstrated that CD74 is expressed on CEC of *APC*$^{Min/+}$ mice. However, these mice bear a point mutation in the murine homolog of the *APC* gene and develop multiple intestinal adenomas[9]. In humans, on the contrary, there was no CD74 expression on normal CEC, but it was expressed on CEC of sporadic colorectal adenomas[10].

It was shown previously that macrophage migration inhibitory factor (MIF) binds to the CD74 extracellular domain, a process that results in the initiation of a signaling pathway in a CD44 dependent manner[11-13].

In our previous studies, we showed that CD74 expressed on B cells is directly involved in shaping the B cell repertoire[14-16] through a pathway leading to the activation of transcription mediated by the nuclear factor-κB (NF-κB) p65/RelA homodimer and its co-activator, TAFII105[17]. We demonstrated that CD74 stimulation with anti-CD74 antibody or MIF leads to NF-κB activation, enabling entry of the stimulated B cells into the S phase, an increase in DNA synthesis, cell division, and augmented expression of anti-apoptotic proteins. These findings indicated that surface CD74 functions as a survival receptor[13,18,19].

In addition, CD74 is expressed at high levels from an early stage of the B cell leukemia, B-CLL. The activation of CD74 on human B-CLL cells by MIF, initiates a signaling cascade that contributes to tumor progression. This pathway induces NF-κB activation, resulting in the secretion of interleukin 8, which in turn promotes cell survival. Blocking of this pathway leads to decreased cell survival. Thus, CD74 expressed on the surface of B-CLL cells plays a critical role in regulating the survival of these malignant cells[20].

MHC class Ⅱ expression was initially thought to be limited to a restricted set of cells collectively known as APCs. However, in addition to conventional APCs, other cell types, including mucosal epithelial cells, were subsequently reported to express class Ⅱ MHC molecules and to present antigens[21].

Surface expression of newly synthesized CD74 is followed by rapid internalization to the endosomal pathway. Experiments that investigate cell surface CD74 are complicated by the fact that CD74 remains on the cell surface for a very short time. The surface half-life of CD74 was calculated to be less than 10 min[22,23].

In this article, we followed CD74 expression in colonic intestinal epithelial cells in the mouse. We show that CD74 is expressed on CEC derived from C57BL/6 and on the CT26 colon carcinoma cell line and serves as a survival receptor on these cells. This finding may suggest a role for CD74 in colon cancer development.

MATERIALS AND METHODS

Mice

C57BL/6, C57BL/6 CD74 deficient[24], C57BL/6 MIF deficient[25], CD44 deficient NOD/SCID mice (Jackson Lab), were used. All animals were used at 6-10 wk of age.

All animal procedures were approved by the Animal Research Committee at the Weizmann Institute of Science.

Intraperitoneal MIF administration

Recombinant murine MIF was purified from an expression system as previously described and contaminating endotoxin removed by C8 chromatography[26]. Mice were injected daily ip with MIF (400 ng) or with PBS, as indicated. Mice were sacrificed after 3.5 or 24 h and CEC were isolated.

Isolation of CECs

CEC were isolated using a modification of the method described previously[27]. Briefly, mice were sacrificed, colons were immediately removed and washed with phosphate buffered saline (PBS) until all content was removed. Colons were inverted and washed gently with Roswell Park Memorial Institute solution. Mucus was removed by incubation for 10 min in 1 mmol/L DTT. Specimens were treated with Dispase Ⅱ (Roche Diagnostics; 3 mg/mL) in DMEM for 30 min (vortexing every 5 min) at 37℃. CEC were isolated from the remaining tissue by passage through a metal filter. In order to purify CEC, cells were centrifuged on a discontinuous Percoll gradient for 30 min, 2000 r/min. Cells found on the top 0%-30% gradient are CEC[28]. Isolated cells were washed in PBS. CEC isolated in this fashion contained over 90% viable cells as determined by Trypan blue exclusion. A total of 92%-95% of the cells stained with the anti-epithelial cell marker anti-pan cytokeratin-26 (FITC conjugated) (Sigma-Aldrich). The remaining contaminating cells represented $CD3^+$ and B_220^+ cells.

CT26 cell line

CT26 murine colon carcinoma cells were grown as monolayer cultures in DMEM-10%, fetal bovine serum (FBS) (Invitrogen) supplemented with 100 IU/mL penicillin and

100 μg/mL streptomycin. Cells were maintained in a 37℃ incubator with 5% CO_2-humidified air.

Stimulation of cells by MIF

CECs (3-6 × 10^6 cells/well) isolated from CD57BL/6 mice, were cultured in 12-well plates at 37℃ in DMEM medium supplemented with 10% FCS, 2 mmol/L glutamate, 300 U/mL penicillin, 300 μg/mL streptomycin, with or without 400 ng/mL of MIF for 17 h.

1.5 × 10^6 cells (CT26 cell line) were plated in complete medium into dishes of 6-well cell culture plates, and were allowed to adhere for 24 h. After washing the cells twice with PBS, they were incubated with MIF (400 ng/mL) for 3 or 6 h (as indicated in the text) in serum-free medium.

RNA isolation and reverse transcription

Total RNA was isolated from cells using the Tri Reagent Kit (MRC), according to the manufacturer's instructions. Reverse transcription was carried out using Superscript Ⅱ RT (Gibco-BRL).

Primers that were used in polymerase chain reaction (PCR) reactions: Bcl-2: 5'-CACCGAACACTTGATTCTG, 3'-AGATCTCTGGTTGGGATTC; Cyclin E: 5'-GAAAATCAGACCACCCAGAGCC, 3'-GAAATGATACAAAGCAGAAGCAGCG; CD74: 5'-GGAGTACCCGCAGCTGAAGGGG, 3'-GAAGATAGGTCTTCCATGTCCAGTG; HPRT: 5'-GAGGGTAGGCTGGCCTATGCCT, 3'-GTTGGATACAGGCCAGACTTTGTTG.

Real time PCR

Levels of mRNA of Actin, Bcl-2 and Cyclin E, were analyzed by quantitative real-time reverse transcriptase (RT)-PCR using a Light-Cycler instrument (Roche Diagnostics, Mannheim, Germany). Total RNA was isolated from cells using the Tri Reagent Kit (MRC). Reverse transcription was carried out using Superscript Ⅱ RT (Gibco-BRL). The reaction volume (10 mL) contained 3 mmol/L $MgCl_2$, LightCycler HotStart DNA SYBR Green I mix (Roche Diagnostics), specific primer pairs, and 2.5 mL of cDNA. Conditions for PCR were as follows: 10 min at 95℃ followed by 60 cycles of 15 s at 95℃, 15 s at 60℃, and 15 s at 72℃. PCR was performed in triplicates. Primer sequences were as follows: Bcl-2: 5'-GCTACCGTCGTGACTT-3', 5'-GCCGGTTCAGGTACTC-3'; Cyclin E: 5'-GTAACATAAGCAAACTG-3', 5'-TTCTTCTGGATTGGCTAA-3'; Actin: 5'-CAGTAACAGTCCGCCT-3', 5'-GTGACGTTGACATCCG-3'; β-actin levels were used to normalize samples for calculation of the relative expression levels of the genes.

Western blotting analysis

To detect whether CD74 protein is expressed by CEC and to examine levels of Bcl-2 protein, cells were lysed in RIPA lysis buffer (10 mmol/L Tris, pH 7.2, 150 mmol/L NaCl, 1% deoxycholate, 1% Triton X-100, 0.1% SDS, 5 mmol/L EDTA) containing complete protease inhibitor cocktail [10 μg/mL leupeptin, 10 μg/mL aprotinin, 10 μg/mL pepstatin, 10 μg/mL chymostatin (Roche), 1 mmol/L PMSF (Sigma), and 20 mmol/L N-etheyl-melamide (Sigma)], for 30 min on ice and then centrifuged at 14000 *g* at 4℃ to remove cell debris. Lysates were separated by 12% (w/v) SDS-PAGE. The proteins were then transferred onto a nitrocellulose membrane and probed with anti-CD74 (FL-293; Santa Cruz) or anti-Bcl-2 (C-2; Santa Cruz), followed by horseradish peroxidase-conjugated anti-mouse (Jackson Laboratories).

To detect changes in Akt phosphorylation, stimulated cells were lysed in buffer containing: 25 mmol/L Tris, pH 7.4; 2 mmol/L vanadate; 75 mmol/L glycophosphate, pH 7.2; 2 mmol/L EDTA; 2 mmol/L EGTA; 10 mmol/L NaPPi; and 0.5% NP-40 in the presence of protease inhibitors. Lysates were separated by 10% (w/v) SDS-PAGE. The proteins were transferred onto a nitrocellulose membrane and probed with anti-p-Akt antibody (Cell Signaling Technology) followed by peroxidase-conjugated anti-mouse (Jackson Labs). The membrane was then stripped and reprobed with anti-tubulin antibody (Sigma), followed by peroxidase-conjugated anti-mouse (Jackson Laboratories).

Immunofluorescence and flow cytometry

Staining of IECs was performed using anti-CD74 (Santa Cruz), anti pan-cytokeratin (Sigma-Aldrich).

Cell survival assays

Annexin and PI staining: Cells were centrifuged, washed, and stained with annexin (BD Biosciences) and propidium iodide (PI) for 15 min at room temperature. The extent of annexin and PI staining was analyzed by FACS. Unstained cells were classified as living cells; annexin stained cells are apoptotic, and PI stained cells were considered necrotic.

FLICA staining: Analysis of apoptosis. To detect earlier stages of apoptosis, intracellular caspase 3 and 7 activity was analyzed using the FLICA (Fluorochrome Inhibitors of Caspases) Apoptosis Detection kit from Immunochemistry Technologies (Bloomington, MN). The kit contains carboxyfluorescein-labeled valylalanylaspartic acid fluoromethyl ketone, which tightly binds activated caspases. Cells were harvested and incubated with a FLICA solution in the complete medium at 37℃ for 1 h. After washing twice cells were analyzed by FACS.

Statistical analysis

Results are represented as averages of several experiments (as indicated) ± SE. Comparison between groups was done by Student's *t*-test.

RESULTS

CD74 is expressed on mouse colonic epithelial cells

To determine whether CD74 is expressed in normal CEC, we first analyzed its mRNA and protein levels in control and CD74 deficient (CD74-/-) CEC. As shown in Figure 1, CD74 mRNA (Figure 1A) and protein (Figure 1B) were detected in CEC, while they were not observed in the CD74 deficient cells. Isolation of CEC from C57BL/6

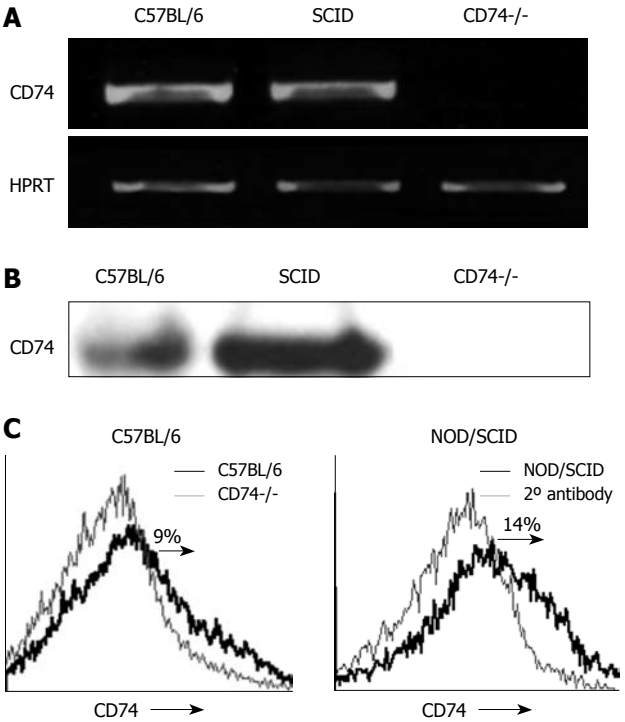

Figure 1 CD74 is expressed on naive CEC isolated from C57BL/6 mice. CEC were isolated from C57BL/6, NOD/SCID and CD74-/- mice. A: RNA was purified and CD74 or HPRT mRNA were analyzed by RT-PCR. The results presented are representative of four separate experiments; B: Cells were lysed and proteins were analyzed by Western blotting; C: Cells were stained with anti cytokeratin and anti CD74 antibodies, and analyzed by FACS. Histograms show CD74 expression on cytokeratin positive cells. CEC: Colon epithelial cells; RT-PCR: Reverse transcriptase-polymerase chain reaction; FACS: Fluorescence-activated cell sorter.

resulted in a population that was more than 90% pure, containing less than 5% B cells (data not shown), which expressed relatively high levels CD74. To eliminate contamination by B cell CD74, we analyzed CD74 mRNA and protein levels in CEC isolated from NOD/SCID mice, which lack B and T cells. As demonstrated in Figure 1, CD74 mRNA (Figure 1A) and protein (Figure 1B) were detected in CEC derived from NOD/SCID mice as well, showing that normal CEC express CD74. We next analyzed, by FACS analysis, CD74 cell surface expression on normal CEC. As shown in Figure 1C, CD74 is specifically expressed on normal CEC derived from C57BL/6 or NOD/SCID mice. Thus, CD74 is expressed on normal CEC.

CD74 serves as a survival receptor on CEC

We have previously shown that CD74 stimulation results in augmented expression of anti-apoptotic proteins, resulting in induction of cell survival[13,18]. In order to examine whether CD74 serves as a survival receptor on CEC, we followed the downstream cascade initiated by MIF in CEC. CEC from C57BL/6 mice were incubated *in vitro* for 17 h with MIF or with PBS, and the percentage of live and apoptotic cells in each group was analyzed using annexin-PI staining. As shown in Figure 2, MIF stimulation resulted in elevation of the proportion of live CEC (42%, $n = 4$, $P = 0.003$) and reduction in the apoptotic population.

To further show the *in vivo* role of MIF and CD74 in CEC survival, MIF was injected ip to control C57BL/6 and NOD/SCID mice and Bcl-2 mRNA and protein levels were determined. As can be seen in Figure 3A and B, a significant elevation in Bcl-2 mRNA and protein levels were detected following MIF stimulation compared to PBS-treated cells. The MIF-induced cascade was CD74 dependent, since in its absence, MIF was not able to increase Bcl-2 levels.

Since CEC survive poorly *in vitro*, and following 17 h incubation a large proportion of the cells are dead, we followed CEC survival immediately after their isolation. Thus, 24 h after MIF injection to C57BL/6, NOD/SCID and CD74-/- mice, cells were isolated and their cell death was analyzed using FLICA, which detects early stages of apoptosis by analyzing intracellular caspase 3 and 7 activity. The percentage of apoptotic cells was significantly higher in the PBS treated cells compared with the MIF treated cells derived from C57BL/6 and NOD/SCID mice (Figure 3C), while the CD74-/- cells were insensitive to MIF treatment. Results of four experiments are summarized in Figure 3D.

It has been recently shown that CD44 forms a complex with CD74 which regulates MIF-induced B cell survival[11,13]. CD44 expressed on CEC was previously characterized as a negative regulator of apoptosis as well[29]. Accordingly, CD44 deficient cells (CD44-/-) demonstrated an elevated basal apoptotic rate compared to control mice. In addition, there was no increase in CEC survival upon MIF stimulation (Figure 3C and D), suggesting that CD44 is a crucial component in the CD74-induced survival cascade.

Cell cycle progression is regulated by cyclin dependent kinases (Cdks). Cdks are constitutively expressed during the cell cycle and are activated upon specific cyclin binding. Different cyclins are differentially expressed during various stages of the cell cycle. This transient expression activates Cdks and regulates cell cycle progression. To further determine whether CD74 regulates cell entry to the S-phase, we followed cyclin E, which is expressed upon S-phase initiation. MIF (400 ng) was injected to C57BL/6 and NOD/SCID mice. 3.5 h later, CEC were isolated and their cyclin E mRNA levels were analyzed. As demonstrated in Figure 4A and B, cyclin E mRNA levels were upregulated in both C57BL/6 and NOD/SCID derived cells. These results demonstrate that following CD74 stimulation, CEC cells synthesize DNA, enter S phase, and probably divide.

Taken together, our results demonstrate that CD74 serves as a survival receptor on CEC. MIF binding to CD74 induces a signaling cascade resulting in Bcl-2 and cyclin E expression and survival in normal CEC.

In order to evaluate whether our finding may have implications on malignant cells, we further assessed the expression and function of CD74 in the colon carcinoma cell line CT26. We found that CD74 is expressed on the CT26 cell line (Figure 5A) and MIF stimulation reduces their apoptosis (Figure 5B and C), by upregulating Bcl-2 mRNA (Figure 5D and E), protein (Figure 5F) and cyclin E (Figure 5G) mRNA levels.

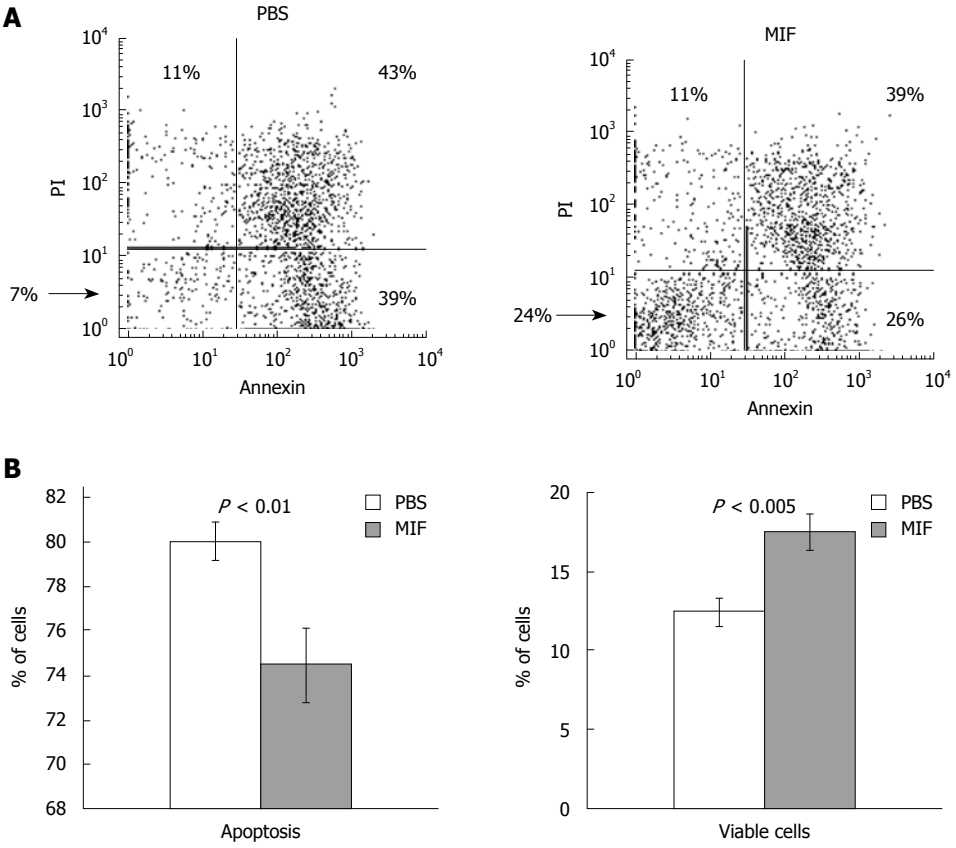

Figure 2 ***In vitro*** **stimulation with MIF induces CEC survival.** CEC from C57BL/6 mice were incubated in the presence or absence of MIF (400 ng/mL). A: After 17 h, cells were collected and analyzed for survival by annexin and propidium iodide staining; B: Graphs summarizing the results of four independent experiments. MIF: Migration inhibitory factor; PBS: Phosphate buffered saline.

We have previously shown that in B cells the CD74 induced survival cascade involves Akt phosphorylation[18]. In intestinal epithelial cells, it has been shown that phosphorylation of Akt enhances cell survival as well[30] and is closely associated with CRC[31,32]. We therefore analyzed Akt phosphorylation in CT26 cells. As shown in Figure 5H, MIF elevated Akt phosphorylation 1 min following stimulation.

Together, our results suggest that MIF and CD74 regulate colon tumor cell proliferation and survival.

DISCUSSION

The importance of CD74 as a survival receptor on B cells, and its role in the pathogenesis of certain malignancies is a subject of intense study. In the colon, CD74 is expressed on malignant cells, and its expression correlates with tumor grade[5]. Recent evidence suggests that up-regulation of CD74 and MIF on human colon adenomas is in correlation with dysplasia of the epithelial cells[10]. However, contradictory results regarding the presence of CD74 on normal human CEC were published through the years. In one report, the authors were able to detect the presence of CD74 by using immunohistochemistry, whereas, in a more recent study, using similar methodology, CD74 was not detected. In this article, we show CD74 expression in mouse normal CEC at the levels of mRNA, protein, and cell surface expression. Furthermore, we found that stimulation of CD74 by its natural ligand, MIF, results in increased survival of CEC. This can be at least partially attributed to enhanced expression of the survival gene, Bcl-2, which was found to be up-regulated in MIF-stimulated CEC. These findings suggest a role for CD74 on normal CEC.

Demonstration of CD74 on CEC is complicated by two main phenomena. First, CD74 has a very short half-life on these cells, and secondly, CEC are difficult to handle. These cells typically undergo apoptosis and/or necrosis, shortly after isolation[33]. Therefore, experiments involving incubation of these cells for more than a few hours usually result in death of most of the cells. In addition, isolates of CEC are never free of contaminating cells, specifically B cells from the lamina propria that also express CD74. In order to overcome these limitations, we performed our experiments shortly after CEC isolation. In experiments where we tested stimulation by MIF, we used mainly *in vivo* stimulation by ip injections of MIF for various amounts of time. To avoid contamination with B cells, we also repeated most of the experiments in NOD/SCID mice, which lack B cells. Our results show that CD74 is expressed on normal CEC cells.

Due to the difficulties discussed above, we used multiple models in order to demonstrate the positive effect CD74 has on CEC survival. In these experiments, we analyzed by FACS the percentage of living and apoptotic CEC. We demonstrated that stimulation by MIF either

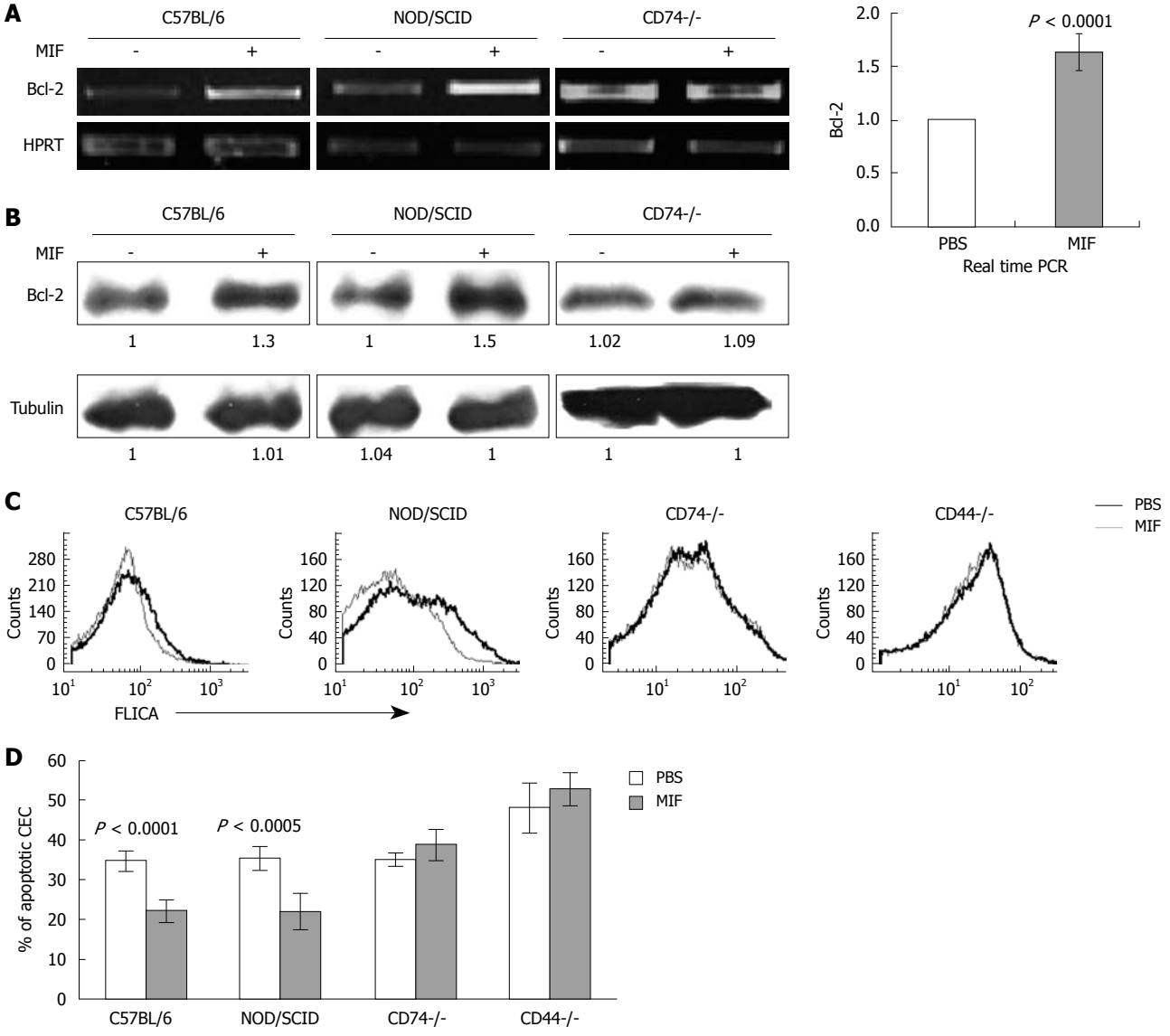

Figure 3 MIF stimulation elevates Bcl-2 and survival in CEC and their *in vivo*. CEC were isolated from CD74-/-, C57BL/6 and from NOD/SCID mice that were i.p injected with MIF (400 ng) or PBS 3.5 h before sacrifice. A: CEC were isolated, and RNA was purified; Bcl-2 or HPRT mRNA were analyzed by RT-PCR. The results presented are representative of four different experiments. The graph is a summary of four separate experiments of quantitative real time PCR using primers for Bcl-2 in CEC isolated from C57BL/6; B: Cells were lysed and Bcl-2 protein expression was analyzed by Western blotting analysis; C, D: C57BL/6, NOD/SCID, CD44-/- and CD74-/- mice were injected with MIF (400 ng) or PBS. After 24 h, mice were sacrificed and their CEC were isolated and stained with FLICA. C: Histograms show FLICA staining in MIF injected (grey line) compared to PBS injected (black line) CEC; D: Graph representing the average of five different experiments, demonstrating decreased apoptosis CEC in MIF treated C57BL/6 and NOD/SCID mice, compared to PBS treated animals.

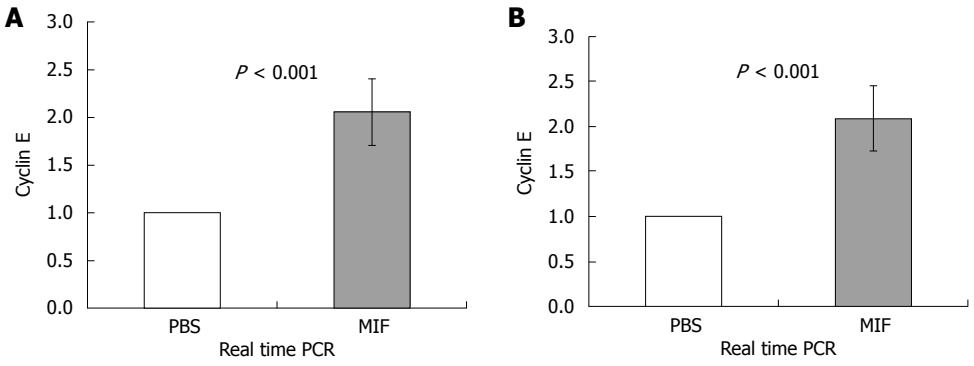

Figure 4 MIF induces cyclin E expression. CEC were isolated from C57BL/6 (A) and NOD/SCID (B) mice that were i.p injected with MIF (400 ng) or PBS 3.5 h prior to isolation of CEC. A, B: Quantitative real time PCR was performed using primers for cyclin E and β-actin. β-actin levels were used to normalize samples for calculation of the relative expression levels of cyclin E. Results are expressed as a fold of change in cyclin E expression at stimulated cells compared to non stimulated cells, which was defined as 1. Results shown are summary of four separate experiments.

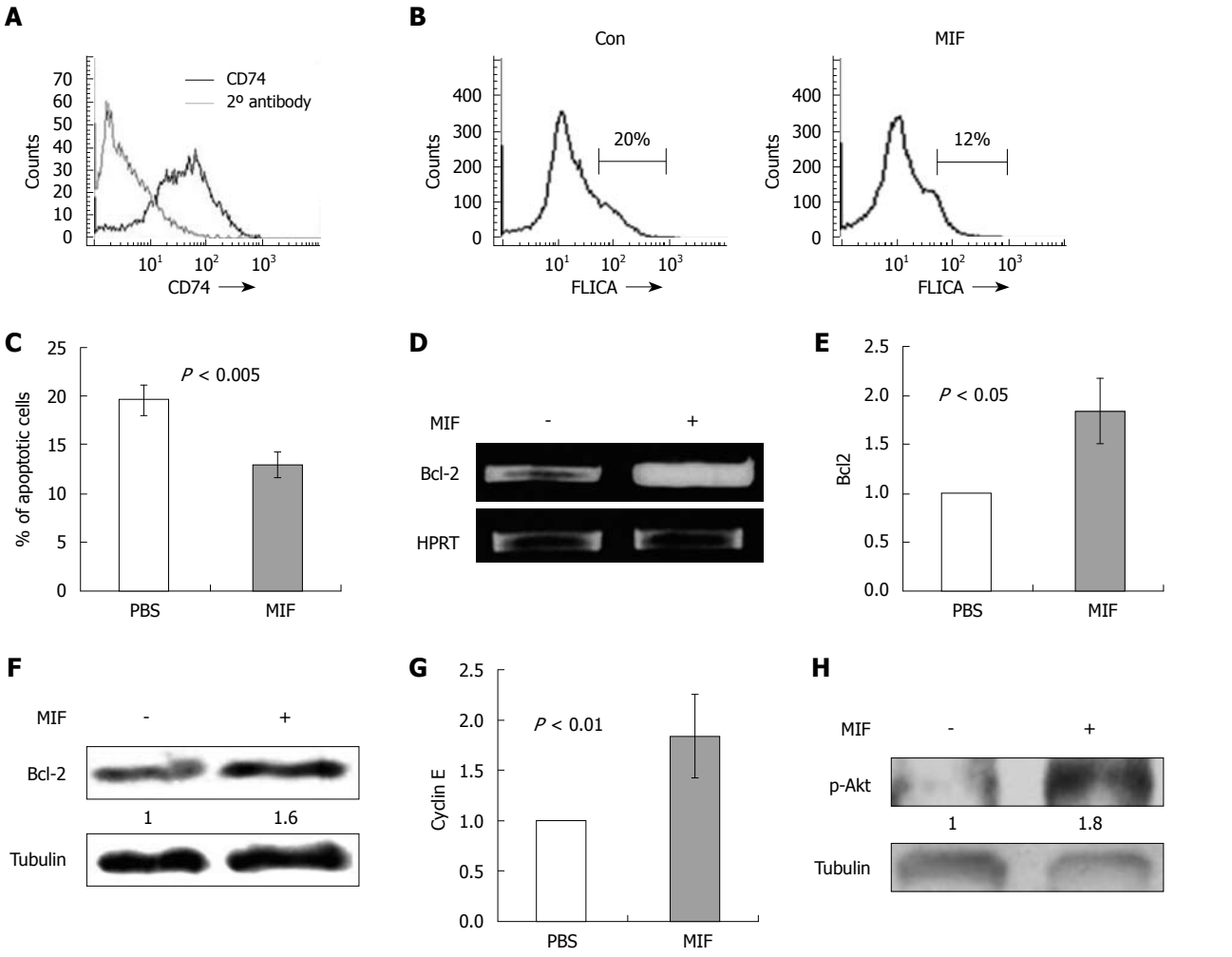

Figure 5 CD74 is expressed on CT26 cells and regulates their survival. A: CT26 cells were stained with anti CD74 antibodies, and analyzed by FACS. Histogram shows CD74 expression on these cells; B: Serum-starved CT26 cells were incubated in the presence or absence of MIF (400 ng/mL) for 6 h. Histograms show FLICA staining for apoptotic cells in both groups; C: Graph representing the average of five different experiments, demonstrating decreased apoptosis in MIF treated compared to untreated CT26 cell line; D: Serum-starved CT26 cells were either kept untreated or treated with MIF (400 ng/mL) for 3 h and levels of Bcl-2 mRNA were analyzed by RT-PCR. The results presented are representative of five different experiments; E: Quantitative real time PCR was performed using primers for Bcl-2. β-actin levels were used to normalize samples for calculation of the relative expression of Bcl2 level. Results are expressed as a fold of change in Bcl-2 expression at stimulated cells compared to non stimulated cells, which was defined as 1. Results shown are a summary of four separate experiments; F: Following 6 h of starvation the CT26 cells were lysed and Bcl-2 protein expression was analyzed by Western blotting analysis; G: Quantitative real time PCR was performed using primers for cyclin E and β-actin. β-actin levels were used to normalize samples for calculation of the relative expression levels of cyclin E. Results are expressed as a fold of change in cyclin E expression at stimulated cells compared to non stimulated cells, which was defined as 1. Results shown are a summary of four separate experiments; H: CT26 cells were incubated in the presence or absence of MIF (400 ng/mL) for 1 min. Cells were fast-frozen and lysed as described in Methods. Lysates were separated on 10% (wt/vol) SDS/PAGE and blotted with anti-p-Akt antibody (results presented are representative of three separate experiments) followed by HRP-conjugated anti-mouse antibodies. The membranes were then stripped and blotted with anti-tubulin.

by ip injection *in vivo* or by incubation of isolated CEC with MIF *in vitro*, resulted in elevated CEC survival and decreased apoptosis. These results strengthen our hypothesis that CD74 is a survival receptor on CEC and suggests that MIF is the natural ligand of CD74 on CEC, as described for B lymphocytes[13,18,19].

MIF is expressed throughout the human gastrointestinal tract, and has previously been implicated in control of apoptosis of non-epithelial and epithelial cells[34,35]. MIF induces Bcl-2 expression and cell survival. It was previously shown that CD74 forms a complex with CD44 in normal[11,12] and tumor cells[36]. We show here that CD44 is crucial for the MIF/CD74 induced survival cascade, since stimulation of CD44 deficient cells by MIF did not affect cell death.

Evidence for a role of CD74 in colorectal cancer was suggested by its expression on carcinoma cell lines of the colon[37], and by the observation that in human colorectal carcinomas, the grade of the tumor correlated with the level of CD74 expression on the transformed CEC[5]. Interestingly, both MIF and CD74 have been associated with tumor progression and metastasis. It was reported that MIF mRNA is over-expressed in various tumors[38,39] and MIF has also been associated with the growth of malignant cells[40]. Numerous studies have demonstrated the overexpression of CD74 in various cancers[20,41-46]. CD74 expression may also serve as a prognostic factor in many of these cancers, with higher relative expression of CD74 behaving as a marker of tumor progression[47]. Our findings further support a possible role for CD74 in

CRC. Additionally, CD44, which we showed essential in the MIF-CD74 survival cascade, is overexpressed in CRC, and has an anti-apoptotic effect on these cells, and probably involved in disease progression and metastases[29,48]. Moreover, we show that CD74 is expressed on the CT26 cell line and its stimulation by MIF enhances Akt phosphorylation, cyclin E and Bcl-2 expression resulting in their survival.

Our work clearly shows that CD74 is expressed on mouse intestinal epithelial cells, and serves as a survival receptor on these cells. Our results further show that CD74 may regulate survival of colorectal carcinoma tumor cells. Since we found that CD74 is a survival receptor on CEC, any change in gene expression that causes enhanced expression of CD74 or MIF may result in increased risk of colorectal cancer. Thus, CD74 may be found in the future to serve not only as a marker of colorectal cancer, but also as a therapeutic target.

COMMENTS

Background

CD74 is a protein that is expressed in and on some of the cells of the immune system, such as B lymphocytes and antigen presenting cells. This protein is known for its function in helping the immune cells to process and present foreign bodies. During recent years it was found that CD74 serves additionally as a survival receptor on cells of the immune system, and that its stimulation by its natural ligand - migration inhibitory factor (MIF) - prevents apoptosis (self destruction) of the cells.

Research frontiers

CD74 was found to be markedly expressed on numerous tumors - hematologic as well as epithelial; in some of these tumors it can serve as a tumor marker and its level of expression may be related to the prognosis. Moreover, activation of CD74 on B lymphocytes in B cell leukaemia, results in tumor progression, and blocking this pathway leads to decreased cell survival, and thus may be applicable as a therapeutic intervention. Whether CD74 is expressed on colon intestinal epithelial cells is controversial, although it was shown to be expressed on epithelial cells of colorectal adenomas.

Innovations and breakthroughs

The authors' work clearly shows that CD74 is expressed on mouse intestinal epithelial cells and serves as a novel survival receptor on these cells. They show that CD74 may regulate survival of colorectal carcinoma as well. Since they found that CD74 is a survival receptor on colonic epithelial cells, any change in gene expression that causes enhanced expression of CD74 or MIF may result in increased risk of colorectal cancer.

Applications

As CD74 is a survival receptor on colon epithelial cells and on malignant cell lines from mice, CD74 may be found in the future to serve not only as a marker of colorectal cancer, but also as a therapeutic target. Future research should focus on the role of CD74 in colorectal cancer models in animals and on human tissues.

Peer review

This paper is well written and documents expression on CD74 receptor mRNA and protein in colon epithelial cells. The study is very well done and straightforward. The experiments are of good quality.

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