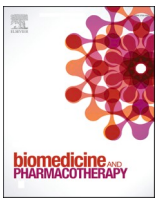
Biomedicine & Pharmacotherapy 152 (2022) 113252

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

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



The presentation and regulation of the IL-8 network in the epithelial cancer stem-like cell niche in patients with colorectal cancer

Guanglin Cui [a,b,*], Gui Li [a], Zhigang Pang [a], Jon Florholmen [c], Rasmus Goll [c]

[a] Research Group of Gastrointestinal Diseases, The Second Affiliated Hospital of Zhengzhou University, Zhengzhou, China
[b] Faculty of Health Science, Nord University, Campus Levanger, Levanger, Norway
[c] Department of Gastroenterology, University Hospital of North Norway, University of Tromsø, Tromsø, Norway

ARTICLE INFO

Keywords:
Cancer stem cell
Niche
IL-8
Colorectal adenoma
Cancer

ABSTRACT

Background: Accumulative evidence suggests that the biological behavior of cancer stem-like cells (CSCs) is regulated by their surrounding niche, in which cytokines function as one of the main mediators for the interaction between CSCs and their microenvironment in the colorectal cancer (CRC).
Methods: We characterized the presentation of CSCs and the interleukin (IL)− 8 network in the adenoma/CRC epithelium using quantitative real-time PCR (q-PCR), immunohistochemistry (IHC) and double immunofluorescence. In addition, the capacity of IL-1β to stimulate epithelial IL-8 production in colon cancer Caco-2 cells was examined *in vitro* and the IL-8 product was measured by enzyme-linked immunosorbent assay (ELISA).
Results: IHC observation showed increased expression of both CSCs and IL-8 in the adenoma and CRC epithelium, and q-PCR results revealed that increased expression of IL-1β transcript was strongly correlated with increased IL-8 transcript levels in both adenoma and CRC tissues. Double immunofluorescence images demonstrated the coexpression of the IL-8 receptors IL-8RA and IL-8RB with LGR5 labeled CSCs in CRC tissue sections. Consistently, *in vitro* experiments showed that coculture of Caco-2 cells with IL-1β at concentrations of 1, 5, 10 and 20 ng/ml resulted in a dose-dependent release of IL-8, which could be specifically inhibited by cotreatment with the IL-1β receptor antagonist.
Conclusions: These results demonstrate activation of the IL-8 network in the niche of CSCs from the precancerous adenoma stage to the CRC stage, which is potentially stimulated by IL-1β in CRC cells.

1. Introduction

Colorectal cancer (CRC) is a common cancer that threatens human life with an estimated 1.8 million newly diagnosed cases and an estimated 0.8 million deaths in 185 countries in 2020 [1]. Statistical data also show that recurrence and metastasis are the two major reasons why the current treatments temporary and incomplete and why poor prognosis [2]. It is currently thought that CRC is derived from a small group of cancer cells with stem cell-like properties, termed cancer stem cells (CSCs) [3,4]. Increasing in vitro and in vivo evidence suggests that CSCs are closely associated with the invasion, recurrence, and metastasis of CRC [5–14]. Therefore, specifically targeting and modulating the biological activity of CSCs might be an effective therapeutic for the management of CRC with recurrence and metastasis [15,16].

Compelling evidence has revealed that intestinal stem-like cells reside within a specialized inflammatory microenvironment that contains high amounts of immune cells and cytokines [17–19], these components act in a coordinated manner to play an essential regulatory role in stem cell renewal, differentiation and the early onset of cancer [17,18,20,21]. Similar to physiological intestinal stem-like cells, a direct crosstalk between CSCs and their inflammatory microenvironment has also been demonstrated [13,22–29], in which cytokines function as the

Abbreviations: ALDH1, aldehyde dehydrogenase 1; CRC, colorectal cancer; CSCs, cancer stem-like cells; ELISA, enzyme-linked immunosorbent assay; IHC, immunohistochemistry; IL, interleukin; IL-1R1, interleukin-1 receptor type 1; IL-1RA, interleukin-1 receptor antagonist; IL-8RA, interleukin-8 receptor A; IL-8RB, interleukin-8 receptor B; IRs, immunoreactivities; LGR5, leucine-rich repeat-containing G-protein-coupled receptor 5; PBS, phosphate buffered saline; q-PCR, quantitative real-time PCR.

* Correspondence to: Faculty of Health Science, Nord University, Campus Levanger, Norway or Research Group of Gastrointestinal Diseases, the Second Affiliated Hospital of Zhengzhou University, Zhengzhou, China.
E-mail address: guanglin.cui@nord.no (G. Cui).

https://doi.org/10.1016/j.biopha.2022.113252
Received 12 April 2022; Received in revised form 2 June 2022; Accepted 2 June 2022
Available online 7 June 2022

Table 1
Basic clinicopathological information of patients with CRC.

	Case	TNM			Invasion depth				Lymph node	
		I	II	III	T1	T2	T3	T4	Positive	Negative
CRC	37	9	16	12	2	9	22	4	14	23

Table 2
q-PCR primer/probe sequences of house-keeping gene β-actin, IL-1β and IL-8.

Assay		Primer	Sequence
β-actin	TaqMan	Forward	5′ TGCCGACAGGATGCAGAAG 3′
		Reverse	5′ GCCGATCCACACGGAGTACT 3′
		Probe	FAM 5′ AGATCAAGATCATTGCTCCTCCTGAGCGC 3′ TAMRA
IL-1β	TaqMan	Forward	5′ CCTGAGCTCGCCAGTGAAA 3′
		Reverse	5′ TTTAGGGCCATCAGCTTCAAA 3′
		Probe	FAM 5′ ATGGCTTATTACAGTGGCAATGAGGATGACTTG 3′ TAMRA
IL-8	TaqMan	Forward	5′ TCTTGGCAGCCTTCCTGATT 3′
		Reverse	5′ TTTCTGTGTTGGCGCAGTGT 3′
		Probe	FAM 5′ CTGCAGCTCTGTGTGAAGGTGCAGT 3′ TAMRA

main mediators and play a critical role in the communication between CSCs and surrounding immune cells [27–30]. Such interactions can markedly affect the properties and features of CSCs and determine the recurrence and metastasis of CRC [3,13,26,30,31]. All these findings in turn result in growing research interest in the investigation of cytokines that can specifically impact the biological behavior of CSCs within the tumor microenvironment.

Previous studies have demonstrated that interleukin (IL)− 8 is a potent promoting factor in many human cancers including CRC [12, 32–38]. A high level of IL-8 expression in human CRC tissues was particularly relevant to the properties and features of CSCs involved in tumor invasion and metastasis [39,40], which was mediated by the IL-8 receptors IL-8RA and IL-8RB [9,10,12,25,40]. Recent studies have provided considerable evidence to suggest that the promoting effect of IL-8 on CRC occurs via a direct impact on the generation and maintenance of CSCs [9–11,13,40,41], in addition to its effects on the mobilization of immature myeloid cells, angiogenesis and epithelial–mesenchymal transition [12,37,42–45]. Furthermore, accumulative evidence has revealed that the activation of the IL-8 network, modulated by a set of cytokines, such as IL-1β [46–48], in the tumor microenvironment contributes to the architecture of a supportive inflammatory niche for CSCs [9,10,26,40]. Therefore, the evaluation of the IL-8/IL-8 receptor network surrounding CSCs may help to provide novel insights into the regulatory mechanisms of CSCs via cytokines and design novel therapeutics to target CSCs in the future.

Given the above background, the objective of this study was to characterize the presentation and regulation of the IL-8 network surrounding the CSC niche from the precancerous adenomas to the CRC stage.

2. Materials and methods

2.1. Patients

A total of 40 colorectal polyps (male/female ratio 27/13, average age 62 years, range 44–92 years) excised by colonoscopy and 37 CRCs (male/female ratio 33/4, average age 69 years, range 42–89 years) excised by surgery; 21 (male/female ratio 13/8, average age 54 years, range 26–76 years) morphologically normal colorectal mucosa samples without pathological evidence by colonoscopic and microscopic examinations collected from the Departments of Gastroenterology & Surgery respectively, between August 2003 and January 2008 at University Hospital of North Norway were included in this study. No adenoma/CRC

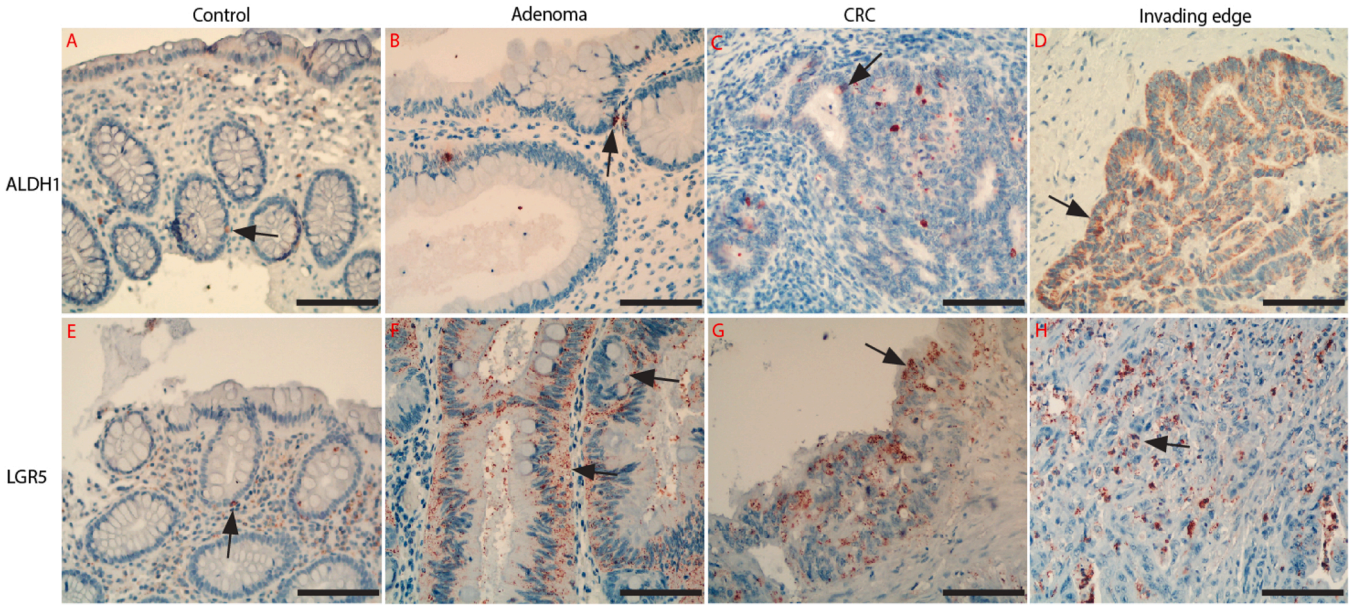


Fig. 1. Photographic representations of CSCs labeled by ALDH1 and LGR5 in the adenoma and CRC epithelium examined with immunohistochemistry (IHC). Low densities of CSCs labeled by ALDH1 (Fig. 1A, arrow pointed) and LGR5 (Fig. 1E, arrow pointed) were observed in the epithelium of controls. High intensities of CSCs labeled by ALDH1 and LGR5 were shown on epithelial surface of both the adenoma (arrows pointed in Fig. 1B for ALDH1 & Fig. 1F for LGR5 respectively) and CRC (arrows pointed in Fig. 1C for ALDH1 & Fig. 1G for LGR5 respectively). Notably, abundant CRC cells positive for ALDH1 (Fig. 1D, arrow pointed) and LGR5 (Fig. 1H, arrow pointed) were shown in the invading edge of CRCs. (Fig. 1A-H: IHC, counterstained with hematoxylin, original magnification 200 ×).

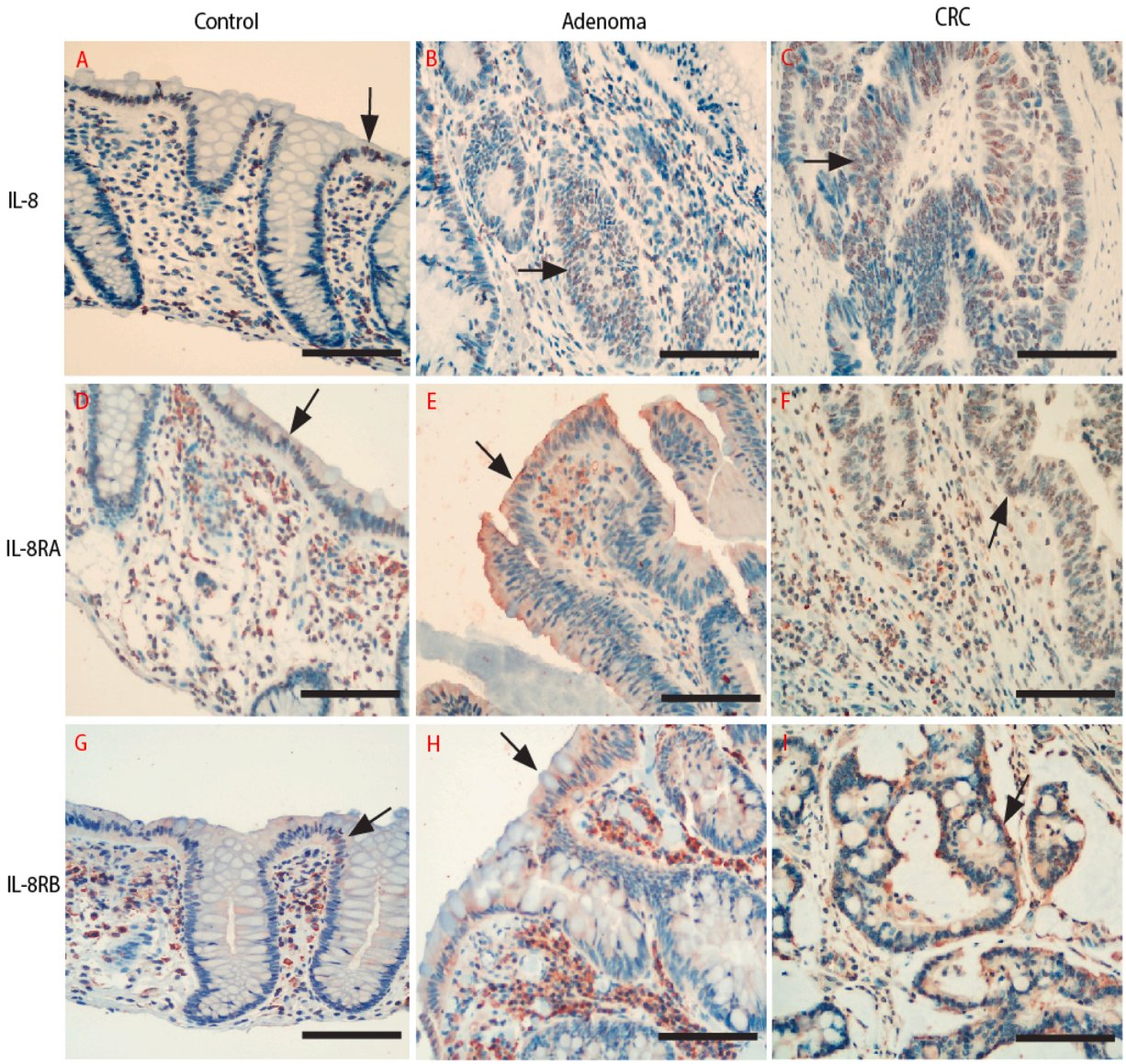


Fig. 2. Photographic representations of IL-8 and IL-8 receptors IL-8RA/IL-8RB expressions in the adenoma and CRC epithelium examined with IHC. In the controls, low intensity of IL-8 positive cells was located in the control epithelium (Fig. 2 A, arrow pointed). In both the adenoma and the CRC sections, increased IL-8 immunoreactivity was distributed in the adenoma (Fig. 2B, arrow pointed) and CRC epithelium (Fig. 2 C, arrow pointed); Similarly, the expression of IL-8 receptor IL-8RA (Fig. 2D, arrowhead pointed) and IL-8RB (Fig. 2 G, arrowhead pointed) were observed in the control epithelium at a low intensity. However, both IL-8RA and IL-8RB immunoreactivities were highly expressed in the adenoma (refer to Fig. 2E for IL-8RA & Fig. 2H for IL-8RB, arrowhead pointed) and CRC epithelium (refer to Fig. 2 F for IL-8RA & Fig. 2I for IL-8RB, arrowhead pointed). (Fig. 2A-I: IHC, counterstained with hematoxylin, original magnification 200 ×).

patients or control subjects had a history of regular use of immunomodulatory treatments or chemotherapy. Histologically, all 40 polyps were confirmed to be adenomas and 37 CRC patients were adenocarcinomas, and the basic clinical pathological features of CRC patients are summarized in Table 1. The biopsies were prepared and embedded in paraffin routinely. Sections were cut at 4 μm, and then stained with hematoxylin and eosin (H&E). The conventional histological diagnosis for all the biopsies was examined at the Department of Pathology, University Hospital of North Norway. The study was approved by the Regional Ethical Committee of Northern Norway, permission for the storage of human tissues and data was given by the Norwegian Department of Health and the Norwegian Bureau of Data Surveillance, and written informed consent was obtained from the patients.

2.2. Quantification of IL-1β and IL-8 mRNAs in normal and CRC tissues by real-time PCR

Biopsies were collected in *RNAlater* solution (Ambion Europe, Cambridgeshire, UK) and total RNA was extracted by using the *TRIzol* method (Invitrogen Life Tech., Carlsbad, MA, USA) and reverse transcription was performed using *SuperScript II* (Invitrogen Life Tech., Carlsbad, MA, USA) [49,50]. Real-time PCR was performed on an *ABI-prism 7900* sequence detector using a *TaqMan Gold*™ PCR core reagents kit (Applied Biosystems/Roche, Branchburg, NJ, USA) in 25 μL volume according to our previously published method [49]. The primer sequences for IL-1β, IL-8 and the housekeeping gene (beta-actin) are listed in Table 2 as published previously [49,51–53]. IL-1β and IL-8 mRNA expression in CRC tissues was measured by the cycle threshold cross point (CT) value relative to that of normal mucosa as fold difference (N)= $2^{-\Delta\Delta CT}$, $\Delta CT = CT_{IL\text{-}1\beta \text{ or IL-8 gene}}\text{-}CT_{beta\text{-}actin}$, $\Delta\Delta CT = \Delta CT$ adenoma/$_{CRC}$-average ΔCT $_{normal}$ as described in our recent publication

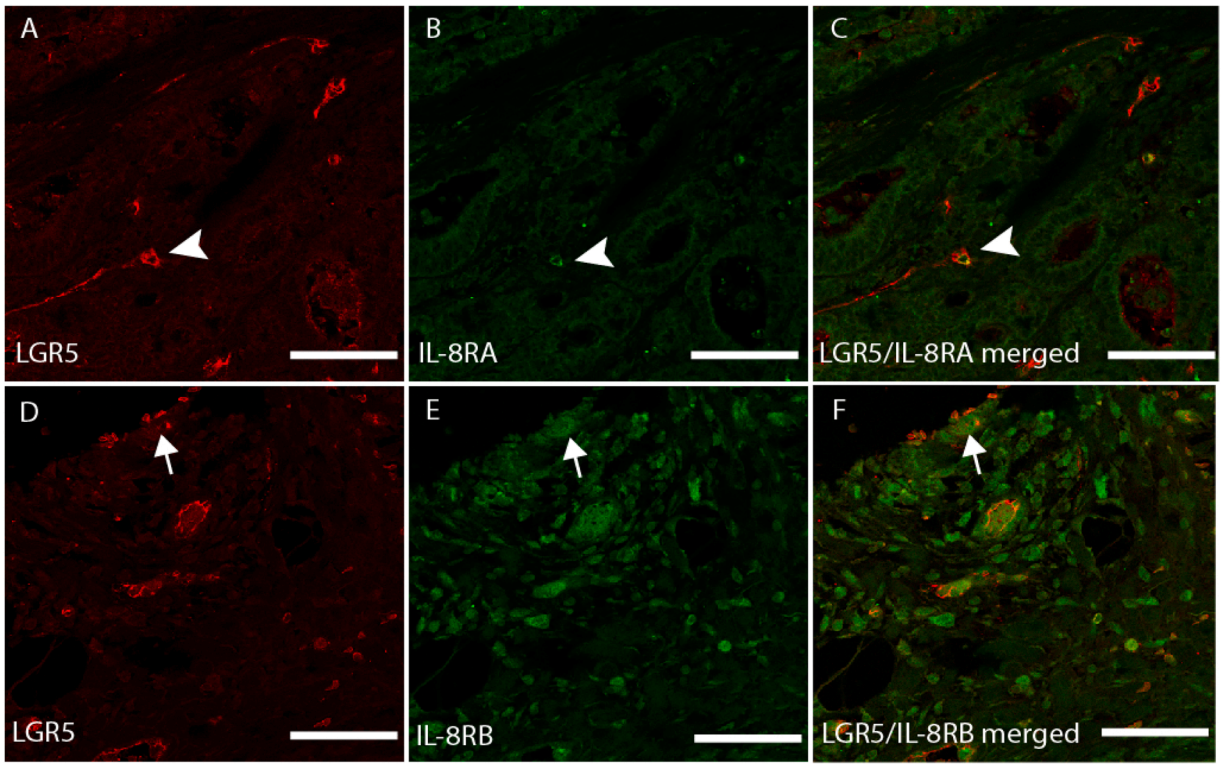


Fig. 3. Double immunofluorescences visualize the expression of IL-8RA and IL-8RB on LGR5 labeled CSCs in the CRC sections. Double immunofluorescence images showed that IL-8RA- (visualized by Texas red, *red color* in Fig. 3A) and IL-8RB-immunoreactivity (visualized by Texas red, *red color* in Fig. 3D) were frequently expressed on LGR5 labeled CSCs (visualized by FITC, *green color* in Fig. 3B & E respectively) in the CRC epithelium (merged images in Fig. 3C & F). (Fig. 3A-F, confocal images, original magnification 200 ×; counterstaining was not applied).

Table 3
Increased folds of IL-1β and IL-8 transcript levels in the adenoma and CRC tissues relative with controls ($2^{-\Delta\Delta CT}$ method).

mRNA	Control	Adenoma	*P1* (Adenoma vs. Control	CRC	*P2* (CRC vs. Control)
IL-1β	1.98 ± 0.78	20.41 ± 7.14	< 0.01	47.33 ± 18.95	< 0.01
IL-8	2.17 ± 0.77	2.37 ± 0.50	> 0.05	1977 ± 981.8	< 0.01

P values were obtained from the Mann–Whitney test.

[52].

2.3. Immunohistochemical examinations of CSCs and the expression of IL-8 and its receptor IL-8RA and IL-8RB in both the adenoma and CRC epithelium

Immunohistochemistry (IHC) was performed in 4 μm paraffin sections from controls, adenomas, and CRC tissues using a Vectastatin *Elite* ABC Kit (Vector Lab., Burlingame, CA, USA) as described previously [5,54,55]. The following primary antibodies were used: rabbit anti-human leucine-rich repeat-containing G-protein-coupled receptor 5 (LGR5) (to label CSC, MBL International, Woburn, MA 01801, USA), mouse anti-human aldehyde dehydrogenase 1 (ALDH1) (to label CSC, BD Bioscience, San Jose, CA, USA), rabbit anti-human IL-8 (to label IL-8 expressing epithelial cells, working dilution 1:100; R&D system, Minneapolis, MN, USA), mouse anti-human IL-8RA (working dilution 1:100; BD Pharmingen, San Diego, CA, USA) and mouse anti-human IL-8RB (working dilution 1:50, R&D system, Minneapolis, MN, USA). The antibodies were incubated at 4 ºC overnight. 3-Amino-9-ethylcarbazole (AEC; Vector Laboratories, Burlingame, CA, USA) was used as a chromogen and slides were slightly counterstained with Mayer's hematoxylin. Negative control slides for IHCs were processed routinely: (1) primary antibodies were substituted with the isotype-matched control antibodies; (2) secondary antibody was substituted with phosphate buffered saline (PBS).

2.4. Double immunofluorescence (DIF) with confocal microscopy to examine the expression of IL-8 receptors in CSCs

To examine the expression of IL-8RA and IL-8RB on CSCs in CRC, DIF was performed with antibodies against IL-8RA/LGR5 and IL-8RB/LGR5 in 10 selected CRC tissue sections according to the protocol described in our previous publications [19,56]. The immunoreactivity (IR) of LGR5 was developed using a Texas red-conjugated secondary antibody, and IL-8RA or IL-8RB-IRs were developed using a fluorescein isothiocyanate (FITC)-conjugated secondary antibody (both antibodies obtained from Jackson ImmunoResearch Lab., West Grove, PA, USA). Nuclear counterstaining was not applied. Negative controls were processed by (1) substituting primary antibodies with the isotype-matched control antibodies; (2) examining cross-reactivity by crossing different secondary antibodies. Stained sections were observed and photographed with a confocal microscope (LSM-700, Carl Zeiss, Jena, Germany) under × 200 medium-power magnification.

2.5. In vitro studies to examine the regulatory effect of IL-1β on epithelial IL-8 production in colon cancer cells

2.5.1. Caco-2 colon cancer cell line culture

To determine the stimulatory effect of IL-1β on IL-8 production in colon cancer epithelial cells, the Caco-2 cell line, derived from a human colon adenocarcinoma (ATCC, Rockville, MD), was used between passages 40 and 50. Cells were maintained in DMEM containing 1% glucose, 25 mM HEPES, 10% (v/v) heat-inactivated FBS (HyClone Perbio-Sciences, Erembodegem, BE), 2% (v/v) L-glutamine 200 mM and

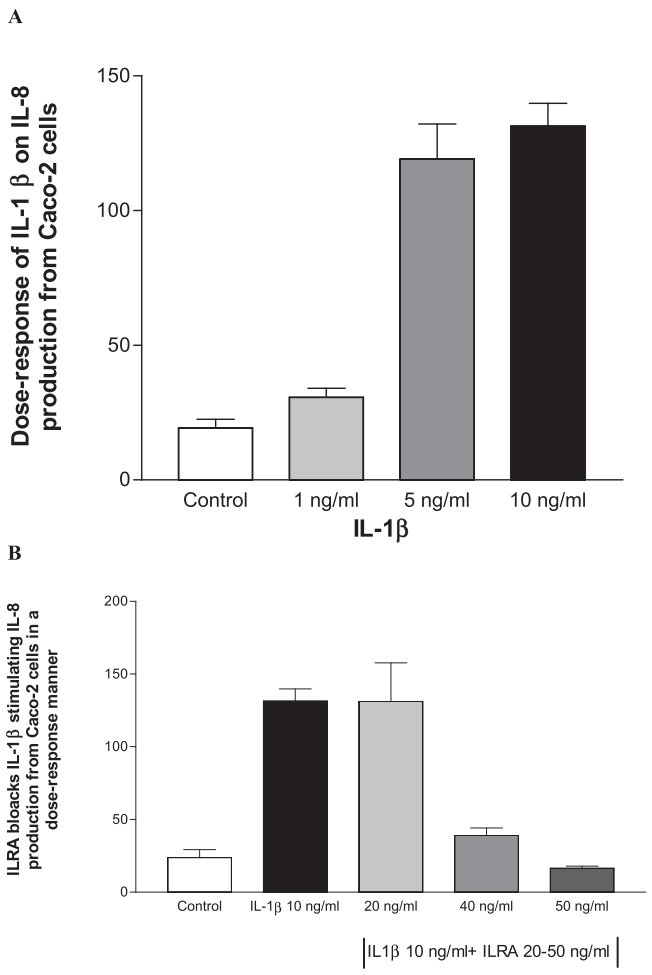


Fig. 4. The stimulatory effect of IL-1β on IL-8 production from Caco-2 cells *in vitro*. Data from *vitro* experiments showed that coculture of Caco-2 cells with IL-1β at concentrations of 1, 5, 10 and 20 ng/ml resulted in a dose-dependent release of IL-8 measured by ELISA (Fig. 4A). Which could be specifically inhibited by the cotreatment with the IL-1β receptor antagonist (IL-1Ra) in a dose-dependent manner (Fig. 4B).

1% (v/v) nonessential amino acids (NEAAs) (Invitrogen, Carlsbad, CA). Cells were grown in 175 cm2 flasks (Greiner Bio-One, Strickenhausen, DE) in an atmosphere of 5% CO2/95% air (v:v) at 37 °C. For experiments, cells were seeded at a density of 180 × 103 cells/well on type I collagen (Sigma–Aldrich) precoated 12-well plates (Nunc, Roskilde, DK) and cultured in standard medium supplemented with 1% pen (10 × 103 U/ml)-Strep (10 mg/ml) for 6 days. The cells were then washed with phosphate buffered saline (pH 7.2) and each treatment was applied for 24 h in culture medium containing 1% (v/v) FBS.

2.5.2. *The stimulatory effect of IL-1β on IL-8 production in cultured Caco-2 cells*

To determine the stimulatory effect of IL-1β on IL-8 secretion in Caco-2 cells, we performed a dose-response study of IL-1β on Caco-2 cells. Cells were either treated or not with IL-1β at a series of concentrations of 1 ng/ml, 10 ng/ml, and 20 ng/ml. To determine the blocking effect of the IL-1 receptor RA (IL-1RA) antagonist, cells were incubated with the IL-1RA at a series of concentrations of 150 ng/ml, 250 ng/ml, and 350 ng/ml individually for one hour before incubation with IL-1β at a concentration of 15 ng/ml.

2.6. *Enzyme-linked immunosorbent assay (ELISA) for the detection of IL-8 levels in the supernatant of treated Caco-2 cells*

After the experiment, the cells were washed with phosphate-buffered saline, collected, and centrifuged (1 min at 12,000 g). The pellet was then resuspended in Reporter Lysis Buffer (Promega Corp., Madison, WI) and frozen at − 20 °C for 30 min, followed by thawing, centrifugation (2 min at 16,000 g) and collection of the supernatant. The IL-8 protein level was evaluated using a sandwich ELISA method (BD Biosciences Pharmingen, San Diego, CA) and quantified in pg/ml using the standard provided with the kit. The results were expressed in relation to the negative control (untreated cells) to facilitate comparison between different concentrations.

2.7. *Morphological evaluation and statistical analysis*

The populations of stem-like cells labeled by ALGH1 or LGR5 in the adenoma/CRC epithelium were semiquantified on a scale of 0–3: grade 0 indicated an absence of positive cells; grade 1 indicated a 1–5% presence of positive cells; grade 2 indicated a 6–25% presence of positive cells; and grade 3 indicated a 26–100% presence of positive cells according to the published method [57].

The results are expressed as the mean ± SEM unless otherwise stated. The Kruskal–Wallis test was used to evaluate the statistical significance of stem-like cell populations among the control, adenoma and CRC groups, and the Mann–Whitney test was used to evaluate the statistical significance of IL-1β and IL-8 mRNA levels among the control, adenoma, and CRC tissues. Spearman's correlation coefficient was used to analyze the relationships between IL-1β and IL-8 mRNA levels in both adenoma and CRC tissues. A value of $P < 0.05$ was considered significant.

3. Results

3.1. *The presentation pattern of cancer stem-like cells, IL-8, and IL-8 receptors in the CRC epithelium*

CSCs labeled by LGR5 and ALDH1 were observed in the adenoma/CRC epithelium. Compared with that of controls (Fig. 1 A&D), IHC observations showed an expanding expression pattern of CSCs in both the adenoma and CRC sections, in which many CSCs labeled by ALDH1 and LGR5 were diffusely observed on the surface of adenoma (Fig. 1B for ALDH1; Fig. 1 F for LGR5) and CRC epithelium (Fig. 1C for ALDH1; Fig. 1G for LGR5) respectively. Notably, CSCs labelled by ALDH1 (Fig. 1D) and LGR5 (Fig. 1H) were more abundant in the invading edges of CRC tissues. The populations of ALDH1- and LGR5-labeled stem-like cells in the adenoma/CRC epithelium showed a gradually increasing trend (control vs. adenoma vs. CRC: 0.92 ± 0.01 vs. 1.68 ± 0.56 vs. 1.87 ± 0.68, $P < 0.05$; Kruskal–Wallis test).

Interestingly, immunoreactivities (IRs) of IL-8 and its functional receptors IL-8RA/IL-8RB were high in both adenoma/CRC cells and stromal cells. As the emphasis of this study was the IL-8 microenvironment surrounding the niche of CSCs, we focused on IL-8/IL-8 receptor expression in the adenoma/CRC epithelial cells. Compared to controls (Fig. 2A), intensive IL-8-IR was observed in both the adenoma (arrow pointed in Fig. 2B) and CRC epithelium (arrow pointed in Fig. 2C). Similarly, the IRs of IL-8RA and IL-8RB were observed in the control (arrows in Fig. 2D for IL-8RA and 2 G for IL-8RB), adenoma (arrows in Fig. 2E for IL-8RA and 2 H for IL-8RB) and CRC epithelium (arrows in 2 F for IL-8RA &2I for IL-8RB).

3.2. *The expression of IL-8 receptors IL-RA and IL-8RB on the surface of CSCs in CRC*

Double immunofluorescence staining with IL-8RA/LGR5 and IL-8RB/LGR5 antibodies demonstrated that the IL-8 receptor IL-8RA was occasionally (Fig. 3A) and that IL-8RB (Fig. 3D) was frequently

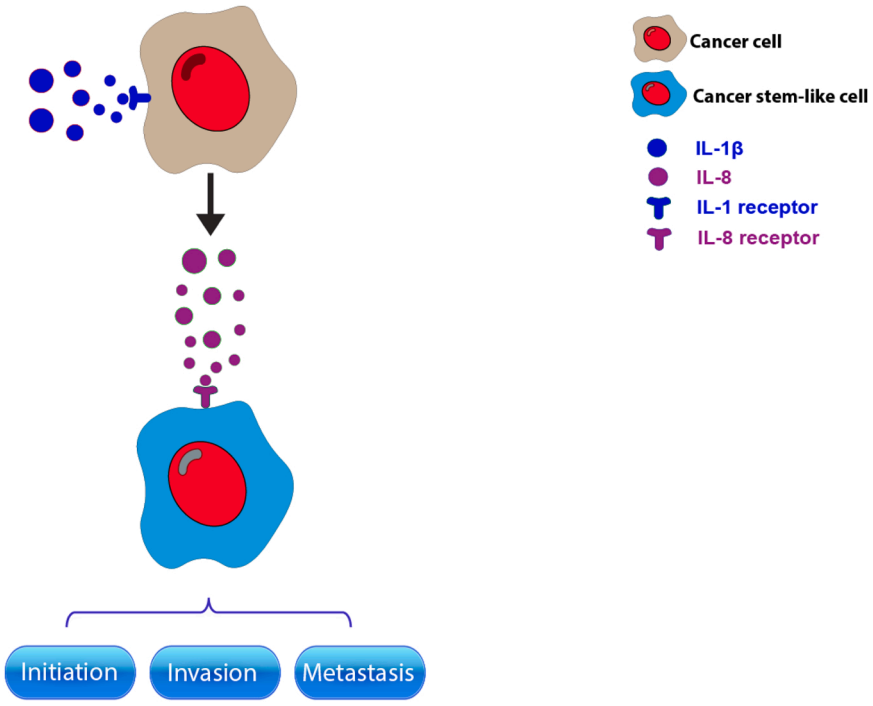


Fig. 5. Schematic summarized the presentation and regulation of tumor-derived IL-8 microenvironment surrounding the niche of CSCs in the CRC epithelium. Accumulative evidence suggested that the production of IL-8 from cancer cells was stimulated by IL-1β, which might further affect the biological behavior of CSCs through IL-8 receptors expressed on the surface of CSCs in a paracrine manner.

coexpressed with LGR5 (Fig. 3B & E) labeled CSCs (merged images in Fig. 3C & F), implying a possible regulatory effect of IL-8 on CSCs.

3.3. The regulation of IL-8 signaling by IL-1β in cancer cells in vivo and in vitro

3.3.1. IL-1β and IL-8 transcripts increased from the adenoma stage to the CRC stage

The IL-1β/IL-8 milieu surrounding the niche of CSCs was examined in both adenoma and CRC tissues. As shown in Table 3, q-PCR results revealed significantly increased levels of IL-1β and IL-8 mRNAs from the adenoma stage to the CRC stage relative to the controls.

Spearman's correlation coefficient analysis showed that the levels of IL-1β mRNA were strongly correlated with the level of IL-8 mRNA in both the adenoma ($R=0.5578$, $P = 0.0002$) and CRC tissues ($R=0.5917$, $P < 0.0001$).

The dose-dependent stimulatory effect of IL-1β on IL-8 release from cultured Caco-2 colon cancer cells *in vitro*.

In cultured Caco-2 cells, administration of IL-1β at concentrations of 1, 5, 10 and 20 ng/ml resulted in a dose-response release of IL-8 as measured by ELISA (Fig. 4A). This effect could be specifically blocked by cotreatment with an IL-1β receptor antagonist (IL-1Ra) in a dose-dependent manner (Fig. 4B).

4. Discussion

Our current results suggested that the IL-8 network surrounding the CSC niche is strongly activated from the adenoma stage to the CRC stage, which suggest that the IL-8 network is involved in the regulation of CSC biological behavior in the adenoma/CRC microenvironment.

CSCs are a subpopulation of tumor cells responsible for tumor initiation, progression, and metastasis [5,8,58,59]. Previously, many studies have confirmed the presence and significance of CSCs in both the adenoma and CRC stages [4,8,14,60–66]. We characterized CSCs labeled by different stem cell surface markers, e.g., Musashi, CD133, LGR5 and ALDH1, and reported significant temporal and spatial changes in CSCs throughout the human colorectal adenoma-carcinoma sequence [5]. Extensive studies have now reported that the biological behavior of CSCs is regulated by their inflammatory cytokine milieu in human cancers [22,67,68]. Several studies revealed that IL-8 could directly affect the biological behavior of CSCs e.g., stemness, recurrence and metastasis in several types of human cancer [9,10,13,40], mediated by the IL-8 receptors IL-8RA and IL-8RB [9–11,26]. In this study, we show that IL-8 and its receptors IL-8RA/RB are highly expressed in both the adenoma and CRC epithelium, where CSCs are located. Furthermore, we identify that IL-8 receptors, particularly IL-8RB, are frequently expressed in CSCs. These findings suggest that adenoma/CRC cell-derived IL-8 might affect CSC properties through IL-8 receptors in a possible paracrine action pathway.

Furthermore, q-PCR results showed that IL-1β levels were elevated in both adenoma and CRC tissues, and IL-8 levels were only elevated in CRC tissues. Since the promoting effect of IL-1β on colorectal carcinogenesis has been reported (refer to recent reviews [48,69,70]), this finding might suggest that a strong activated IL-1β level in the adenoma stage is involved in the transformation of precancerous adenomas to CRCs. Notably, our further analysis revealed that IL-1β levels were strongly correlated with IL-8 levels in both the adenoma and CRC stages. This finding implies a potential regulatory effect of IL-1β on IL-8 production in the adenoma/CRC microenvironment. Indeed, our *in vitro* experiments support such a claim. The epithelial IL-8 protein level in Caco-2 cells stimulated with IL-1β was significantly increased in a dose-dependent manner, which could be specifically blocked by the IL-1β receptor antagonist IL-1RA. These results suggest that an active IL-8 milieu surrounding the CSC niche is, at least partially, regulated by IL-1β. Both Vazquez-Iglesias et al. [71] and Huang et al. [72] recently identified that stem cell markers, e.g., CD133 and CD44, were expressed on the surface of Caco-2 cells, and suggest that Caco-2 cells might have the properties and features of CSCs. Therefore, the possibility of IL-1β stimulating the secretion of IL-8 from CSCs in an autocrine manner cannot be excluded. Fig. 5 shows a schematic summarizes the presentation and regulation of the IL-8 network surrounding the niche of CSCs in the CRC epithelium based on our current findings.

In addition to its impact on CSCs [26,40], recent studies have also revealed that IL-8 could serve as an important regulatory factor for mesenchymal stem cells in an autocrine or paracrine manner and be involved in the process of tumor angiogenesis [73,74]. Therefore, it has been hypothesized that targeting IL-8/IL-8 receptor signaling might inhibit the activity of both epithelium-resident CSCs and stroma-resident mesenchymal stem cells in patients with cancer [13,26]. In the future, precise targeting approaches on IL-8 receptors expressed on CSCs are needed.

Finally, it is worth mentioning that the IL-8 network is generated by a number of cells including CRC cells, immune cells and stromal cells surrounding the niche of CSCs [11,39,75]. Thus, IL-8 produced from other types of cells, e.g., stromal cells, particularly these IL-8-positive stromal cells aggregated in the stroma close to the CSC-resident epithelium (the so called subepithelial stroma region), might also participate in the regulation of CSCs in a paracrine manner [11].

5. Conclusion

Our data outlined an activated IL-8 network surrounding the niche of CSCs from the adenoma stage to the CRC stage, which is potentially regulated by IL-1β. Taken together with previous findings, we propose that one of the pathways by which tumor-derived IL-8 contributes to tumor invasion and metastasis is possibly through a regulatory effect on CSCs in adenoma/CRC tissues. These findings may help us gain novel insights into the regulatory mechanisms of cytokines of affecting the biological behavior of CSCs and design novel therapeutics to target CSCs in the future.

Funding

This study was supported by the National Natural Science Foundation of China (Program No. 81071969) and the Medical Research Program, Northern Norway Regional Health Authority (Helse Nord RHF), Norway (Program No. SFP-44-04). The funders did not play any role in paper design, data collection, data analysis, interpretation, writing of the paper.

Authors' contributions

Guanglin Cui had the idea for this project and performed the most experiments, Gui Li and Zhigang Pang performed *in vitro* experiments and data analysis. Jon Florholmen and Rasmus Goll joined the data analysis and discussion. All the listed authors contributed to this manuscript in writing and final approvement.

Conflict of interest statement

The authors declare no conflicts of interests.

Acknowledgments

This project was supported by the grants from National Natural Science Foundation of China (81071969) and Medical Research Program, Northern Norway Regional Health Authority (SFP-44–04).

References

[1] H. Sung, J. Ferlay, R.L. Siegel, M. Laversanne, I. Soerjomataram, A. Jemal, F. Bray, Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA Cancer J. Clin. 71 (3) (2021) 209–249.
[2] F. Bray, J. Ferlay, I. Soerjomataram, R.L. Siegel, L.A. Torre, A. Jemal, Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA Cancer J. Clin. 68 (6) (2018) 394–424.
[3] B.M. Boman, E. Huang, Human colon cancer stem cells: a new paradigm in gastrointestinal oncology, J. Clin. Oncol. 26 (17) (2008) 2828–2838.
[4] M.J. Munro, S.K. Wickremesekera, L. Peng, S.T. Tan, T. Itinteang, Cancer stem cells in colorectal cancer: a review, J. Clin. Pathol. 71 (2) (2018) 110–116.
[5] G. Cui, G. Xu, L. Zhu, Z. Pang, W. Zheng, Z. Li, A. Yuan, Temporal and spatial changes of cells positive for stem-like markers in different compartments and stages of human colorectal adenoma-carcinoma sequence, Oncotarget 8 (28) (2017) 45311–45322.
[6] S.J. Leedham, N.A. Wright, Expansion of a mutated clone: from stem cell to tumour, J. Clin. Pathol. 61 (2) (2008) 164–171.
[7] M. Quante, T.C. Wang, Stem cells in gastroenterology and hepatology, Nat. Rev. Gastroenterol. Hepatol. 6 (12) (2009) 724–737.
[8] A.M. Baker, T.A. Graham, G. Elia, N.A. Wright, M. Rodriguez-Justo, Characterization of LGR5 stem cells in colorectal adenomas and carcinomas, Sci. Rep. 5 (2015) 8654.
[9] L. Chen, J. Fan, H. Chen, Z. Meng, Z. Chen, P. Wang, L. Liu, The IL-8/CXCR1 axis is associated with cancer stem cell-like properties and correlates with clinical prognosis in human pancreatic cancer cases, Sci. Rep. 4 (2014) 5911.
[10] C. Corro, M.E. Healy, S. Engler, B. Bodenmiller, Z. Li, P. Schraml, A. Weber, I. J. Frew, M. Rechsteiner, H. Moch, IL-8 and CXCR1 expression is associated with cancer stem cell-like properties of clear cell renal cancer, J. Pathol. 248 (3) (2019) 377–389.
[11] J.M. David, C. Dominguez, D.H. Hamilton, C. Palena, The IL-8/IL-8R axis: a double agent in tumor immune resistance, Vaccines (2016).
[12] S. Asfaha, A.N. Dubeykovskiy, H. Tomita, X. Yang, S. Stokes, W. Shibata, R. A. Friedman, H. Ariyama, Z.A. Dubeykovskaya, S. Muthupalani, R. Ericksen, H. Frucht, J.G. Fox, T.C. Wang, Mice that express human interleukin-8 have increased mobilization of immature myeloid cells, which exacerbates inflammation and accelerates colon carcinogenesis, Gastroenterology 144 (1) (2013) 155–166.
[13] J.K. Singh, B.M. Simoes, R.B. Clarke, N.J. Bundred, Targeting IL-8 signalling to inhibit breast cancer stem cell activity, Expert Opin. Ther. Targets 17 (11) (2013) 1235–1241.
[14] C.A. Gonzalez-Villarreal, A.G. Quiroz-Reyes, J.F. Islas, E.N. Garza-Trevino, Colorectal cancer stem cells in the progression to liver metastasis, Front Oncol. 10 (2020) 1511.
[15] Y.S. Ma, W. Li, Y. Liu, Y. Shi, Q.L. Lin, D. Fu, Targeting colorectal cancer stem cells as an effective treatment for colorectal cancer, Technol. Cancer Res Treat. 19 (2020), 1533033819892261.
[16] R. Gupta, L.K. Bhatt, T.P. Johnston, K.S. Prabhavalkar, Colon cancer stem cells: potential target for the treatment of colorectal cancer, Cancer Biol. Ther. 20 (8) (2019) 1068–1082.
[17] M. Biton, A.L. Haber, N. Rogel, G. Burgin, S. Beyaz, A. Schnell, O. Ashenberg, C. W. Su, C. Smillie, K. Shekhar, Z. Chen, C. Wu, J. Ordovas-Montanes, D. Alvarez, R. H. Herbst, M. Zhang, I. Tirosh, D. Dionne, L.T. Nguyen, M.E. Xifaras, A.K. Shalek, U.H. von Andrian, D.B. Graham, O. Rozenblatt-Rosen, H.N. Shi, V. Kuchroo, O. H. Yilmaz, A. Regev, R.J. Xavier, T. Helper, Cell Cytokines modulate intestinal stem cell renewal and differentiation, Cell 175 (5) (2018) 1307–1320, e22.
[18] A. De Lerma Barbaro, G. Perletti, I.M. Bonapace, E. Monti, Inflammatory cues acting on the adult intestinal stem cells and the early onset of cancer (review), Int J. Oncol. 45 (3) (2014) 959–968.
[19] G. Cui, Z. Li, J. Florholmen, R. Goll, Dynamic stromal cellular reaction throughout human colorectal adenoma-carcinoma sequence: A role of TH17/IL-17A, Biomed. Pharm. 140 (2021), 111761.
[20] S. Michael, C. Achilleos, T. Panayiotou, K. Strati, Inflammation shapes stem cells and stemness during infection and beyond, Front Cell Dev. Biol. 4 (2016) 118.
[21] C. Buhrmann, P. Kraehe, C. Lueders, P. Shayan, A. Goel, M. Shakibaei, Curcumin suppresses crosstalk between colon cancer stem cells and stromal fibroblasts in the tumor microenvironment: potential role of EMT, PLoS One 9 (9) (2014), e107514.
[22] C. Andrews, M.H. McLean, S.K. Durum, Cytokine tuning of intestinal epithelial function, Front Immunol. 9 (2018) 1270.
[23] Y. Wang, R. Kim, S.J. Hwang, J. Dutton, C.E. Sims, N.L. Allbritton, Analysis of interleukin 8 secretion by a stem-cell-derived human-intestinal-epithelial-monolayer platform, Anal. Chem. 90 (19) (2018) 11523–11530.
[24] L.F. Mager, M.H. Wasmer, T.T. Rau, P. Krebs, Cytokine-induced modulation of colorectal cancer, Front Oncol. 6 (2016) 96.
[25] Y. Ning, H.J. Lenz, Targeting IL-8 in colorectal cancer, Expert Opin. Ther. Targets 16 (5) (2012) 491–497.
[26] H. Korkaya, S. Liu, M.S. Wicha, Regulation of cancer stem cells by cytokine networks: attacking cancer's inflammatory roots, Clin. Cancer Res 17 (19) (2011) 6125–6129.
[27] D. Bayik, J.D. Lathia, Cancer stem cell-immune cell crosstalk in tumour progression, Nat. Rev. Cancer 21 (8) (2021) 526–536.
[28] C. Ciardiello, A. Leone, A. Budillon, The crosstalk between cancer stem cells and microenvironment is critical for solid tumor progression: the significant contribution of extracellular vesicles, Stem Cells Int 2018 (2018), 6392198.
[29] A. Dutta, D. Sengupta, S. Paul, S. Chakraborty, S. Mukherjee, T. Das, A new insight into tumour immune-evasion: crosstalk between cancer stem cells and T regulatory cells, Ann. Oncol. (2019).
[30] L. Muller, A. Tunger, I. Plesca, R. Wehner, A. Temme, D. Westphal, F. Meier, M. Bachmann, M. Schmitz, Bidirectional Crosstalk Between Cancer Stem Cells and Immune Cell Subsets, Front Immunol. 11 (2020) 140.
[31] Y. Li, L. Wang, L. Pappan, A. Galliher-Beckley, J. Shi, IL-1beta promotes stemness and invasiveness of colon cancer cells through Zeb1 activation, Mol. Cancer 11 (2012) 87.
[32] A. Li, S. Dubey, M.L. Varney, B.J. Dave, R.K. Singh, IL-8 directly enhanced endothelial cell survival, proliferation, and matrix metalloproteinases production and regulated angiogenesis, J. Immunol. 170 (6) (2003) 3369–3376.

[33] A. Li, M.L. Varney, J. Valasek, M. Godfrey, B.J. Dave, R.K. Singh, Autocrine role of interleukin-8 in induction of endothelial cell proliferation, survival, migration and MMP-2 production and angiogenesis, Angiogenesis 8 (1) (2005) 63–71.
[34] A. Li, M.L. Varney, R.K. Singh, Expression of interleukin 8 and its receptors in human colon carcinoma cells with different metastatic potentials, Clin. Cancer Res 7 (10) (2001) 3298–3304.
[35] Y.C. Chung, Y.F. Chang, Significance of inflammatory cytokines in the progression of colorectal cancer, Hepatogastroenterology 50 (54) (2003) 1910–1913.
[36] A. Yuan, J.J. Chen, P.L. Yao, P.C. Yang, The role of interleukin-8 in cancer cells and microenvironment interaction, Front Biosci. 10 (2005) 853–865.
[37] Y. Ning, P.C. Manegold, Y.K. Hong, W. Zhang, A. Pohl, G. Lurje, T. Winder, D. Yang, M.J. LaBonte, P.M. Wilson, R.D. Ladner, H.J. Lenz, Interleukin-8 is associated with proliferation, migration, angiogenesis and chemosensitivity in vitro and in vivo in colon cancer cell line models, Int J. Cancer 128 (9) (2011) 2038–2049.
[38] Q. Liu, A. Li, Y. Tian, J.D. Wu, Y. Liu, T. Li, Y. Chen, X. Han, K. Wu, The CXCL8-CXCR1/2 pathways in cancer, Cytokine Growth Factor Rev. 31 (2016) 61–71.
[39] G. Cui, A. Yuan, R. Goll, B. Vonen, J. Florholmen, Dynamic changes of interleukin-8 network along the colorectal adenoma-carcinoma sequence, Cancer Immunol. Immunother. 58 (11) (2009) 1897–1905.
[40] F. Conciatori, C. Bazzichetto, I. Falcone, G. Ferretti, F. Cognetti, M. Milella, L. Ciuffreda, Colorectal cancer stem cells properties and features: evidence of interleukin-8 involvement, Cancer Drug Resist. 2 (4) (2019) 968–979.
[41] C. Bazzichetto, F. Conciatori, I. Falcone, F. Cognetti, M. Milella, L. Ciuffreda, Advances in tumor-stroma interactions: emerging role of cytokine network in colorectal and pancreatic cancer, J. Oncol. 2019 (2019), 5373580.
[42] R.I. Fernando, M.D. Castillo, M. Litzinger, D.H. Hamilton, C. Palena, IL-8 signaling plays a critical role in the epithelial-mesenchymal transition of human carcinoma cells, Cancer Res 71 (15) (2011) 5296–5306.
[43] C.W. Luo, I.L. Hsiao, J.Y. Wang, C.C. Wu, W.C. Hung, Y.H. Lin, T.Y. Chen, Y.C. Hsu, T.L. Cheng, M.R. Pan, Cell motility facilitated by mono(2-ethylhexyl) phthalate via activation of the AKT-beta-catenin-IL-8 axis in colorectal cancer, J. Agric. Food Chem. 66 (37) (2018) 9635–9644.
[44] Z. Zhao, S. Wang, Y. Lin, Y. Miao, Y. Zeng, Y. Nie, P. Guo, G. Jiang, J. Wu, Epithelial-mesenchymal transition in cancer: Role of the IL-8/IL-8R axis, Oncol. Lett. 13 (6) (2017) 4577–4584.
[45] X. Long, Y. Ye, L. Zhang, P. Liu, W. Yu, F. Wei, X. Ren, J. Yu, IL-8, a novel messenger to cross-link inflammation and tumor EMT via autocrine and paracrine pathways (Review), Int J. Oncol. 48 (1) (2016) 5–12.
[46] Y.S. Hwang, M. Jeong, J.S. Park, M.H. Kim, D.B. Lee, B.A. Shin, N. Mukaida, L. M. Ellis, H.R. Kim, B.W. Ahn, Y.D. Jung, Interleukin-1beta stimulates IL-8 expression through MAP kinase and ROS signaling in human gastric carcinoma cells, Oncogene 23 (39) (2004) 6603–6611.
[47] G.Y. Kim, J.W. Lee, H.C. Ryu, J.D. Wei, C.M. Seong, J.H. Kim, Proinflammatory cytokine IL-1beta stimulates IL-8 synthesis in mast cells via a leukotriene B4 receptor 2-linked pathway, contributing to angiogenesis, J. Immunol. 184 (7) (2010) 3946–3954.
[48] J. Li, L. Huang, H. Zhao, Y. Yan, J. Lu, The role of interleukins in colorectal cancer, Int J. Biol. Sci. 16 (13) (2020) 2323–2339.
[49] G. Cui, T. Olsen, I. Christiansen, B. Vonen, J. Florholmen, G. Rasmus, Improvement of Real-time PCR for quantifying TNF-a mRNA expression in inflamed colorectal mucosa-an approach to optimize procedures for clinical use, Scand. J. Clin. Lab. Investig. 66 (3) (2006) 249–259.
[50] W. Hua, A. Yuan, W. Zheng, C. Li, J. Cui, Z. Pang, L. Zhang, Z. Li, R. Goll, G. Cui, Accumulation of FoxP3+ T regulatory cells in the tumor microenvironment of human colorectal adenomas, Pathol. Res Pr. 212 (2) (2016) 106–112.
[51] G. Cui, R. Goll, T. Olsen, S.E. Steigen, A. Husebekk, B. Vonen, J. Florholmen, Reduced expression of microenvironmental Th1 cytokines accompanies adenomas-carcinomas sequence of colorectum, Cancer Immunol. Immunother. 56 (7) (2007) 985–995.
[52] R. Goll, F. Gruber, T. Olsen, G. Cui, G. Raschpichler, M. Buset, A.M. Asfeldt, A. Husebekk, J. Florholmen, Helicobacter pylori stimulates a mixed adaptive immune response with a strong T-regulatory component in human gastric mucosa, Helicobacter 12 (3) (2007) 185–192.
[53] G. Cui, A. Yuan, Z. Sun, W. Zheng, Z. Pang, IL-1beta/IL-6 network in the tumor microenvironment of human colorectal cancer, Pathol. Res Pr. 214 (7) (2018) 986–992.
[54] G. Cui, A. Yuan, L. Zhu, J. Florholmen, R. Goll, Increased expression of interleukin-21 along colorectal adenoma-carcinoma sequence and its predicating significance in patients with sporadic colorectal cancer, Clin. Immunol. 183 (2017) 266–272.
[55] G. Cui, A. Yuan, R. Goll, J. Florholmen, IL-17A in the tumor microenvironment of the human colorectal adenoma-carcinoma sequence, Scand. J. Gastroenterol. 47 (11) (2012) 1304–1312.
[56] G. Cui, A. Yuan, Z. Li, R. Goll, J. Florholmen, ST2 and regulatory T cells in the colorectal adenoma/carcinoma microenvironment: implications for diseases progression and prognosis, Sci. Rep. 10 (1) (2020) 5892.
[57] B.G. Jang, B.L. Lee, W.H. Kim, Distribution of LGR5+ cells and associated implications during the early stage of gastric tumorigenesis, PLoS One 8 (12) (2013), e82390.
[58] A. Patsalias, Z. Kozovska, Personalized medicine: stem cells in colorectal cancer treatment, Biomed. Pharm. 141 (2021), 111821.
[59] C. Hervieu, N. Christou, S. Battu, M. Mathonnet, The role of cancer stem cells in colorectal cancer: from the basics to novel clinical trials, Cancers (2021).
[60] N. Barker, R.A. Ridgway, J.H. van Es, M. van de Wetering, H. Begthel, M. van den Born, E. Danenberg, A.R. Clarke, O.J. Sansom, H. Clevers, Crypt stem cells as the cells-of-origin of intestinal cancer, Nature 457 (7229) (2009) 608–611.
[61] F. Zhou, Y.D. Mu, J. Liang, Z.X. Liu, D. Zhou, W.L. Ning, Y.Z. Li, D. Ding, J. F. Zhang, Aldehyde dehydrogenase 1: a specific cancer stem cell marker for human colorectal carcinoma, Mol. Med Rep. 11 (5) (2015) 3894–3899.
[62] M. Todaro, M.G. Francipane, J.P. Medema, G. Stassi, Colon cancer stem cells: promise of targeted therapy, Gastroenterology 138 (6) (2010) 2151–2162.
[63] Z. Kozovska, V. Gabrisova, L. Kucerova, Colon cancer: cancer stem cells markers, drug resistance and treatment, Biomed. Pharm. 68 (8) (2014) 911–916.
[64] F.B. Rassouli, M.M. Matin, M. Saeinasab, Cancer stem cells in human digestive tract malignancies, Tumour Biol. 37 (1) (2016) 7–21.
[65] I. Hong, S.W. Hong, Y.G. Chang, W.Y. Lee, B. Lee, Y.K. Kang, Y.S. Kim, I.W. Paik, H. Lee, Expression of the cancer stem cell markers CD44 and CD133 in colorectal cancer: an immunohistochemical staining analysis, Ann. Coloproctol. 31 (3) (2015) 84–91.
[66] L.F. Fan, W.G. Dong, C.Q. Jiang, D. Xia, F. Liao, Q.F. Yu, Expression of putative stem cell genes Musashi-1 and beta1-integrin in human colorectal adenomas and adenocarcinomas, Int J. Colorectal Dis. 25 (1) (2010) 17–23.
[67] K. Kurokawa, Y. Hayakawa, K. Koike, Plasticity of intestinal epithelium: stem cell niches and regulatory signals, Int J. Mol. Sci. 22 (1) (2020).
[68] Z. Culig, Cytokine disbalance in common human cancers, Biochim Biophys. Acta 1813 (2) (2011) 308–314.
[69] D. Briukhovetska, J. Dorr, S. Endres, P. Libby, C.A. Dinarello, S. Kobold, Interleukins in cancer: from biology to therapy, Nat. Rev. Cancer 21 (8) (2021) 481–499.
[70] C. Rebe, F. Ghiringhelli, Interleukin-1beta and cancer, Cancers (2020).
[71] L. Vazquez-Iglesias, L. Barcia-Castro, M. Rodriguez-Quiroga, M. Paez de la Cadena, J. Rodriguez-Berrocal, O.J. Cordero, Surface expression marker profile in colon cancer cell lines and sphere-derived cells suggests complexity in CD26(+) cancer stem cells subsets, Biol. Open 8 (7) (2019).
[72] G.-C. Huang, C.-Y. Su, Y.-C. Chang, Y.-J. Chen, H.-W. Fang, Establishment of surface marker expression profiles for colorectal cancer stem cells under different conditions, Trans. Cancer Res. 9 (4) (2020) 2503–2510.
[73] Y. Hou, C.H. Ryu, J.A. Jun, S.M. Kim, C.H. Jeong, S.S. Jeun, IL-8 enhances the angiogenic potential of human bone marrow mesenchymal stem cells by increasing vascular endothelial growth factor, Cell Biol. Int. 38 (9) (2014) 1050–1059.
[74] L. Wang, Y. Li, X. Zhang, N. Liu, S. Shen, S. Sun, Y. Jiang, P. Li, H. Jin, L. Shen, Paracrine interleukin-8 affects mesenchymal stem cells through the Akt pathway and enhances human umbilical vein endothelial cell proliferation and migration, Biosci. Rep. 41 (5) (2021).
[75] D.G. Leuning, N.R.M. Beijer, N.A. du Fosse, S. Vermeulen, E. Lievers, C. van Kooten, T.J. Rabelink, J. Boer, The cytokine secretion profile of mesenchymal stromal cells is determined by surface structure of the microenvironment, Sci. Rep. 8 (1) (2018) 7716.