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ORIGINAL ARTICLE

Overexpression of interleukins IL-17 and IL-8 with poor prognosis in colorectal cancer induces metastasis

Mohsen Mohammadi[1] · Maria Kaghazian[2] · Omid Rahmani[3] · Koorosh Ahmadi[4] · Elham Hatami[5] · Katayoun Ziari[3] · Amir Talebreza[6]

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Abstract Current evidences indicated that a group of soluble mediators called chemokines is involved in tumor growth and metastasis. The association of IL-8 with tumor cell migration was previously found, and its expression was related to angiogenesis, tumor progression, and metastasis in many kinds of carcinomas in human and animal models. Furthermore, it has been showed that IL-17 plays its role as either a proteome of tumor progression or antitumor indifferent cancer models. To investigate the messenger RNA (mRNA) expressions of IL-8 and IL-17 in patients with colorectal cancer (CRC) and non-tumor tissue, quantitative real-time PCR was used in the study. Our results showed that expression of IL-8 mRNA was significantly increased in tumor tissues (mean ± SD 3.84 ± 0.08) compared with adjacent normal mucosa (mean ± SD 1.17 ± 0.75, $P = 0.001$). Furthermore, a higher expression of IL-17 mRNA was found in tumor tissues (mean ± SD 2.73 ± 0.69) when compared with normal tissues (mean ± SD 1.06 ± 0.07, $P = 0.001$). Our findings indicated that advanced tumor-node-metastasis (TNM) stage ($P = 0.024$) and histological grade (poorly differentiated, $P = 0.013$) and distant metastasis ($P = 0.001$) were correlated with expression of IL-8. Moreover, high expression of IL-17 showed significant association with early stage CRC (TNM) ($P = 0.038$). In conclusion, the expression of IL-8 and IL-17 mRNAs was significantly increased in tumor tissues compared with adjacent normal tissues. We found that advanced TNM stage and histological grade and distant metastasis were correlated with expression of IL-8, while high expression of IL-17 showed significant association with early stage CRC (TNM) stage and overexpression of IL-8 may be associated with progression of CRC.

Keywords IL-17 · IL-8 · Colorectal cancer · PCR · Expression

✉ Amir Talebreza
dr.amirtalebreza@gmail.com

[1] Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Lorestan University of Medical Sciences, Khorramabad, Iran

[2] Department of Biology, Jundishapur University of Medical Sciences, Ahvaz, Iran

[3] Department of Pathology, Be'sat Hospital, AJA University of Medical Sciences, Tehran, Iran

[4] Department of Emergency Medicine, Alborz University of Medical Sciences, Karaj, Iran

[5] Department of Pathology, Loghman Hakim Hospital, Sarakhs, Iran

[6] Department of Surgery, AJA University of Medical Sciences, Tehran, Iran

Introduction

Colorectal cancer (CRC) is known as one of the most frequent cancer worldwide and is one of the most common causes of cancer-related death [1–3]. Recently, growing evidence has indicated that the functional disturbance of the immune system is related to stages of tumor and prognosis of patients. It is required to find effective indicators for detecting cancer early and predicting prognosis. Increasing evidence shows that chemotactic cytokines may be linked to cancers and also may be potential markers for detecting cancers and predicting prognosis of disease [4]. Interleukin-8 (IL-8 or CXCL8) has been found to be involved in angiogenesis of many kinds of cells including neutrophils, endothelial cells, macrophages, and cancer cells [5–7].

IL-8 has been showed to be involved in the tumor cell migration, and its expression was related to angiogenesis, tumor progression, and metastasis in many kinds of carcinomas in human and animal models [8–11]. Current studies have

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indicated that IL-8 was overexpressed in non-small-cell lung carcinoma, melanoma, glioblastoma, and colorectal cancer through the expression of CXCR1 and CXCR2 on cancer cells and binding to them; it can be associated with angiogenesis, tumor progression, metastasis, and survival in many kinds of malignancies [12–14].

Interleukin-17 (IL-17) is an essential proinflammatory cytokine, and its high expression level was detected in many kinds of tumors [15, 16]. Several studies have shown that IL-17 plays its role as either a proteome of tumor progression or antitumor in various cancer models [17–19]. This study was aimed to evaluate the expression of IL-8 and IL-17 in CRC and its association with the clinicopathological features in patients with CRC.

Materials and methods

Sixty patients who underwent resection for colorectal cancer between 2009 and 2013 in the Tehran, Tabriz, and Mashhad hospitals were enrolled in this study. Surgical tumor tissue samples and adjacent normal mucosa were obtained from patients with colorectal cancer. Then, tissue samples were immediately frozen in liquid nitrogen until use. The tissues were stained with hematoxylin and eosin and evaluated histopathologically by independent pathologists (Fig. 1). The tissues were staged according to the tumor-node-metastasis (TNM) staging system of the International Union Against Cancer. The clinicopathological factors of the patients were showed in Table 1. The mean age of the patients was 62.8 years; of the 60 patients, 28 cases were male and 32 cases were female.

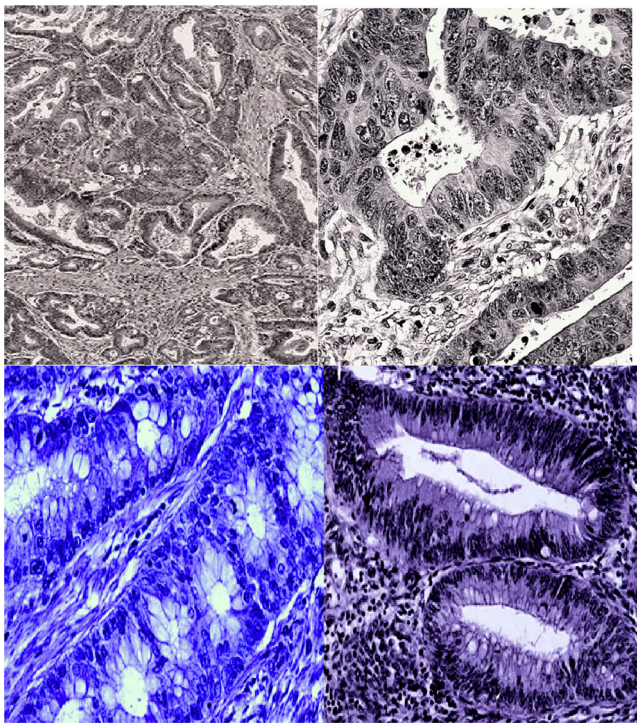

Fig. 1 Photomicrographs of colorectal cancers (H&E)

RNA extraction and qRT-PCR

Briefly, total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions. Then, cDNA synthesis and quantification of specific messenger RNA (mRNA) species were done. A SYBR Green PCR kit (Qiagen, Germantown, MD) and a Light Cycler 480 instrument (Roche Applied Science) were used to perform real-time reverse transcription polymerase chain reaction (RT-PCR). GAPDH was used as a reference gene, for normalization. Furthermore, relative RNA expression levels were evaluated using the comparative cycle threshold (CT) method.

Statistical analysis

Differences between expression levels were evaluated using the Student's *t* test, and correlation between RNA expressions and the clinicopathological features were also analyzed using the chi-squared test. Statistical analysis was considered to be statistically significant at $P < 0.05$

Results

qRT-PCR analysis showed that expression of IL-8 mRNA was significantly increased in tumor tissues (mean ± SD 3.84 ± 0.08) compared with adjacent normal mucosa (mean ± SD 1.17 ± 0.75, $P = 0.001$; Fig. 2). Furthermore, a higher expression of IL-17 mRNA was found in tumor tissues (mean ± SD 2.73 ± 0.69) when compared with normal tissues (mean ± SD 1.06 ± 0.07, $P = 0.001$; Fig. 2). The patients categorized into low and high expression groups in relation to the median value. The association between clinicopathological parameters and mRNA expression were summarized in Table 1.

Among the features that we evaluated, advanced TNM stage ($P = 0.024$) and histological grade (poorly differentiated, $P = 0.013$) and distant metastasis ($P = 0.001$) were correlated with expression of IL-8. There were no significant association of IL-8 with other factors including age ($P = 0.621$), sex ($P = 0.671$), and tumor size ($P = 0.523$). Moreover, high expression of IL-17 showed significant association with early stage CRC (TNM) ($P = 0.038$). But, no significant association was found with other clinical factors (Table 1).

Discussion

This study was aimed to evaluate the clinical significance of IL-8 and IL-17 in patients with CRC. Increased expression of

Table 1 The association between IL-8 and IL-17 mRNA expression and the clinicopathological parameters of CRC patients

Parameters	Number	IL-8 expression		IL-17 expression		*P* value (IL-8)	*P* value (IL-17)
		Low = 21	High = 39	Low = 27	High = 33		
Age group						0.621	0.731
<64 years	36	14	22	17	19		
>64 years	24	7	17	10	14		
Gender						0.671	0.62
Female	32	11	21	15	17		
Male	28	10	18	12	16		
Tumor size						0.523	0.586
<5	40	15	25	19	21		
≥5	20	6	14	8	12		
TNM staging						0.024	0.038
I	14	8	6	4	10		
II	20	10	10	8	12		
III	11	2	9	6	5		
IV	15	1	14	9	4		
Histological grade						0.013	0.632
Poor	26	5	21	7	9		
Moderate	18	9	9	11	13		
Well	16	7	9	9	11		
Distant metastasis						0.001	0.617
No	29	17	12	12	17		
Yes	31	4	27	15	16		

IL-8 has been found in neutrophils and endothelial cells, tumor-correlated macrophages, and cancer cells. In cancer cell lines, it has been confirmed that IL-8 signaling pathway has important role in proliferation, invasion, and migration [20–23].

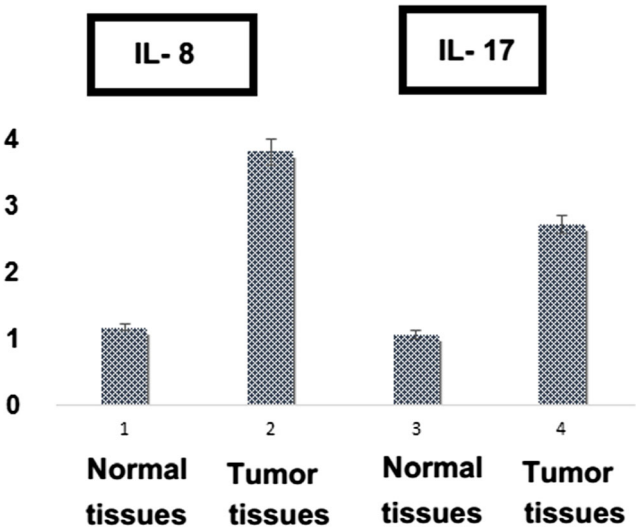

Fig. 2 The expression of IL-8 and IL-17 mRNAs was significantly increased in tumor tissues compared with adjacent normal mucosa ($P = 0.001$)

IL-8 has been found to be involved in the tumor cell migration, and its expression was related to angiogenesis, tumor progression, and metastasis in many kinds of carcinomas in human and animal models [8–11].

Consistent with a previously published data [24], qRT-PCR analysis indicated that expression of IL-8 mRNA was significantly increased in tumor tissues compared with adjacent normal mucosa in our study [24].

Recent studies have indicated that IL-8 was overexpressed in non-small-cell lung carcinoma, melanoma, glioblastoma, and colorectal cancer through the expression of CXCR1 and CXCR2 on cancer cells; it can be associated with angiogenesis, tumor progression, metastasis, and survival in many kinds of malignancies [12–14]. An et al. found that IL-8 protein expression was increased in clear cell renal cell carcinoma (ccRCC) and indicated that high IL-8 expression was positively linked to Fuhrman grade [25].

Among the features that we evaluated in the present study, advanced TNM stage and histological grade/poorly differentiated and distant metastasis were correlated with expression of IL-8. There were no significant association of IL-8 with other clinical parameters including, age, sex, and tumor size.

Increased expression of IL-8 has been demonstrated to be correlated with a poor outcome in patients suffering from colorectal cancer. Moreover, high expression variants of IL-8T-

251A polymorphism have been showed to be markedly related to risk of recurrence in patients with gastric and ovarian cancer as well as CRC patients that had stage III [26, 27]. In colorectal cancer cells in vitro and in vivo, Ning et al. reported that IL-8 mRNA was increased in IL-8 transfectants with 45~85-fold higher than parental cells [28]. IL-8 overexpression was reported to be associated with tumor size, Dukes stage, depth of infiltration, and shorter overall survival times in the malignant tissues [29, 30]. Moreover, Li et al. [24] reported that constitutive IL-8 expression is associated with aggressiveness of human colon carcinoma cells [31]. Current evidences showed that blocking IL-8 signaling with neutralizing antibodies can be associated with inhibition of the proliferation, angiogenesis, invasion, and metastasis in bladder cancer and melanoma [31, 32].

However, a study reported that metastatic prostate tumor cell lines do not express IL-8 at detectable levels [33]. Furthermore, in some studies, no association between IL-8 secretion and tumor growth kinetics or metastatic potential was demonstrated at all [34]. Despite these ongoing controversies, our results indicated that overexpression of IL-8 may be associated with progression of disease. Further studies are needed to evaluate the role of IL-8 in clinical samples.

Several studies have shown that IL-17 plays its role as either a proteome of tumor progression or antitumor in various cancer models [17–19]. But, current evidence has demonstrated that IL-17 can act as proteome of tumor initiation and progression in CRC [35].

Consistent with a previously published data, higher expression of IL-17 mRNA was found in tumor tissues when compared with normal tissues in our study [15, 36, 37]. They found overexpression of IL-17 or higher number of IL-17 producing cells in CRC compared with non-tumor tissues. Furthermore, in our study, high expression of IL-17 showed significant association with TNM stage, but no significant association was found with other clinical factors. Lin et al. indicated that the IL-17 mRNA level was higher in CRC than in adenoma and non-tumor tissue. They found that histological differentiation, Dukes staging (early stage), TIN infiltration, and better survival were linked to IL-17 expression and increased CD15+ neutrophils was positively associated with IL-17 expression [35]. However, the findings of Lin et al. is completely different from the findings of Liu et al. that showed that high expression of IL-17 was associated with high microvessel density and poorer OS but was not significantly related to location, age, sex, differentiation, or histological type [37]. High prevalence of IL-17 in MMR-proficient colorectal carcinomas was previously reported [38]. IL-17-producing cells have been demonstrated in non-small-cell lung carcinoma (NSCLC) [10], prostate cancer, breast cancer, ovarian cancer, hepatocellular carcinoma (HCC), and CRC [16, 39–41]. Comprehensive studies are required to determine clinical importance of IL-17 in patients with CRC.

In conclusion, our results indicated that expression of IL-8 and IL-17 mRNA was significantly increased in tumor tissues compared with adjacent normal tissues. We found that advanced TNM stage and histological grade and distant metastasis were correlated with expression of IL-8, while high expression of IL-17 showed significant association with early stage CRC (TNM) stage and overexpression of IL-8 may be associated with progression of disease.

Compliance with ethical standard

Conflicts of interest None

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