Original Paper

Interleukin-8 mRNA Expression in Locally Advanced Colorectal Cancer Patients

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ABSTRACT: Purpose: Interleukin-8 (IL-8) has been proven to promote progression of malignant tumours and control angiogenesis processes. We aim to determine and compare interleukin-8 (IL-8) gene expression level in colorectal tumors (CCR) and peritumoral samples obtained through endoscopic biopsy. Material and methods: Total mRNA was obtained from both tumoral and peritumoral tissue samples collected from patients diagnosed with colorectal cancer. Through Quantitative Real Time PCR, IL-8 gene expression was assessed in both pathologic tissue and adjacent normal mucosa. Results: In our cohort, IL8 expression was higher in adjacent normal mucosa than in tumoral tissue, in all the samples. Further studies on larger groups are required to validate our results.

KEYWORDS: Interleukin-8 mRNA, advanced colorectal cancer, endoscopy, preoperative analysis

Introduction

Colorectal cancer (CRC) is the third worldwide mortality cause among cancers in men and women [1,2]. The carcinoma progression sequence is widely accepted and starts from benign polyps or dysplastic lesions which evolve to advanced adenoma and invasive carcinoma [3]. Multiple genes and chronic inflammation are involved in CRC pathogenesis and progression which usually evolves for a couple of years or decades [4,5]. Depending on the extent of tumour growth, the treatment for CRC is complex and consists of surgical excision and radio-chemotherapy for locally advanced cases [6].

In order to tailor treatment at an early stage, research directions have looked into the identification of genetic risk factors and development of predictive models. An early diagnosis of CRC, before spreading to the regional lymph nodes occurs, could increase the five-year survival at 90%, but the node-positive disease will be associated with a 64% survival rate [1,7]. As they are easily collectable with an excellent evaluation reproducibility in clinical practice, the biomarkers are challenging for prediction. Studies confirm that genetic biomarkers are related to tumor load, hypoxia and inflammation and can help to predict response to chemo-radio therapy (CRT) in rectal cancer [8].

Chemokines are a group of small and basic molecules that regulate cell mobility and interaction of various types of leukocytes [9]. Interleukins are a type of cytokine that control growth and differentiation, cell migration and inflammatory responses from the immune system [10]. Interleukin-8 (IL-8) is a member of the chemokine family, responsible for attracting and activating neutrophils during immune reactions [11] and is therefore able to induce an inflammatory reaction [12]. The expression level of IL-8 in different human samples has been shown to promote malignant progression of tumours [13] and to be regulated by inflammatory signals, such as tumour necrosis, or chemical and environmental stresses, as the exposure to chemotherapy agents and hypoxia [14]. In the physiopathology mechanism of cancer, IL-8 has been related with the regulation of angiogenesis and modulation of tumour immune response [15].

The aim of our study was to assess IL-8 gene expression level before radiotherapy and surgery in both normal and tumoral tissue samples collected from subjects with colorectal cancer through.

Material and methods

Patient selection

We included in our study patients diagnosed with colorectal tumours that undergone endoscopic evaluation at the Research Center of Gastroenterology and Hepatology at the University of Medicine and Pharmacy of Craiova between January 2014 and May 2015. The study was performed in agreement with the Romanian bioethical legislation and was approved by the Ethics Committee of University of Medicine and Pharmacy of Craiova. Eligible patients for this study were men and women diagnosed with primary colorectal tumours, aged between 18 and 90 years old.

Each patient had signed an informed consent for tissue sampling and genetic analysis. Patient data was kept anonymous and included familial, personal and clinical information for each enrolled subject. All patients had undergone endoscopic ultrasound examination (EUS) for staging purpose and endoscopic biopsies for histopathological diagnosis and genetic tests. We excluded patients with previous radio-chemotherapy or with a history of inflammatory bowel disease before the diagnosis of CRC.

Tissue sample collection

Multiple biopsies from the normal mucosa (N) and the tumour (T) were collected during the EUS for each patient. The samples for histological diagnostic were immersed in formalin and prepared for a senior pathologist evaluation at the Pathology Department of the County Clinical Emergency Hospital of Craiova. The paired biopsies used for the transcriptomic study were collected in RNA later (Ambion), stored at 4°C for 12-24 hours, and then kept at -80°C, until mRNA was extracted and purified, in the Human Genomic Laboratory of the University of Medicine and Pharmacy of Craiova.

Gene expression assessment

PureLink® RNA Mini Kit from Ambion (Lyfe Technologies, USA) was used to isolate and purify total mRNA. mRNA concentration was measured by spectrophotometry (Eppendorf Biophotometer, Eppendorf, AG, Hamburg, Germany), while purity was assessed with Agilent 2010 Bioanalyzer (Agilent Technologies Inc, USA).

Total mRNA obtained from 40 paired samples was assessed. The total mRNA obtained only from 19 samples appeared intact met the quality criteria and was used for reverse transcription. Complementary DNA (cDNA) was synthetized using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) The cDNA obtained during the first phase of Two-step real time quantitative polymerase chain reaction (qRT-PCR) was further amplified and quantified using the TaqMan® Gene Expression Master Mix (Applied Biosystems, Foster City, CA) and specific TaqMan Gene Expression Assays for target genes and for endogenous control gene (IL8-Hs00174103_m1 and GAPDH-Hs99999905_m1).

Statistical analysis was performed using Graph Pad Prism 5 (Graph Pad Software Inc, San Diego, CA). We used Wilcoxon matched pairs signed rank test that found significant results (p=0.00129).

Results

Initially, the study group included 40 patients. After mRNA integrity evaluation, a total number of 19 patients were selected, who respected the criteria of inclusion and had high purity of isolated mRNA. The expression of the target gene was normalized to the GAPDH (Glyceraldehyde 3-phosphate dehydrogenase), an endogenous control gene.

After demographic analysis, a total of 17 men and 2 women, aged from 25 to 80 years old (mean age 60 years) were included. EUS evaluation (Table 1) showed a majority of T3 tumors (12 patients, 63%) without any T1 tumours. EUS also had shown 32% of the subjects (6 patients) had no pathological lymphadenopathies and 68% (13 patients) presented loco-regional satellite spread. The most frequent were moderately differentiated adenocarcinomas (47%), followed by well-differentiated (32%), while the low-differentiated adenocarcinoams represented 21% of our study population. There were 5 patients (26,5%) included in our study who had portions of necrosis on the tumor tissue sample examined by the pathologist.

The expression of IL-8 was assessed in 19-paired samples of CRC tumours and adjacent normal mucosa. qRT-PCR analysis revealed that IL8 is expressed in both tumour and normal mucosa. IL8 expression was higher in non-invaded normal tissue samples compared with paired tumour in all the samples (Figure 1)

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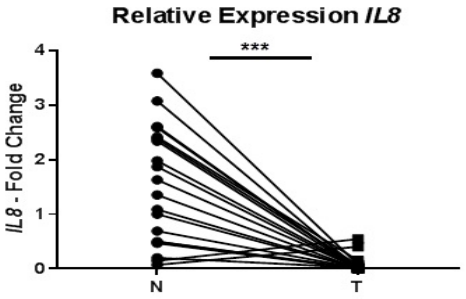
Table 1. Clinico-pathological features of the 19 patients studied group

Average age	**60 years** (25-80)
Men	17 (89%)
Women	2 (11%)
EUS tumour T status	
1	0
2	4 (21%)
3	12 (63%)
4	3 (16%)
EUS tumour N status	
0	6 (32%)
1	9 (47%)
2	4 (21%)
HP differentiation degree	
G1	6 (32%)
G2	9 (47%)
G3	4 (21%)

Fig. 1. Comparative expression of IL8 mRNA in paired tumour and normal mucosa (n=19). Data are presented as relative mRNA expression of target gene to GAPDH. Wilcoxon matched-pairs signed rank test, p<0.0001



Discussions

Certain studies showed that IL-8 supports both angiogenesis, as well as metastatic spread of cancer cells [16,17,18]. IL-8 is produced by some malignant cells, including colorectal cancer cells [19] and represents a tumour microenvironment regulator, which can contribute to tumour progression [20,21,22]. IL-8 receptor on tumour's surface produces signals that activate transcription factors which modify the survival rate of the malignant cells [24,25,26,27].

In the current study, we emphasized that IL-8 genic expression level is decreased in tumour tissue and increased in normal tissue. The originality of this study is represented mainly by the preoperative moment of sampling, not found in similar studies from literature and second, by the use of endoscopic method to obtain tissue samples. Tissue sample dependent errors should normally be minimal for our study because there are data that prove that fresh frozen samples provide higher quantity and quality of RNA [28].

A recent study compared IL-8 expression level in normal and tumour tissue samples collected during surgery. The authors found IL-8 mRNA levels increased in normal mucosa compared with tumour and metastatic tissue [29]. This kind of samples are much larger than those taken during endoscopy and could have included various layers of the colon or rectal wall. A bias of this study could be the fact that even if at the end of the surgery the tissue samples were immediately frozen, we should also count the delay between the moment of perioperative vascular ligature normally effectuated at the beginning of an oncological surgery and the moment of specimen excision achieved later depending of the technique and the team experience.

The same study indicated a differential IL-8 gene expression in correlation with some clinic-pathological features. Lower levels of IL-8 mRNA expression were found in multiple groups of patients as those younger than 60 years, the group of well differentiated tumors, the group of tumors with a diameter smaller than 4 centimeters and for patients with the preoperative carcinoembryonic antigen (CEA) value below 3,4ng/ml [29]. There could be an explanation for our lower differential expression of IL-8 mRNA in tumoral tissue, but we could not study a more homogenous group.

There are data that confirmed that patients with tumours with a high degree of microsatellite instability (MSI-H) appear to achieve a better survival than those with cancers that are microsatellite stable (MSS) [30,31,32]. IL-8 gene expression seemed to be down-regulated in MSI-H cancer cell lines and up-regulated in the MSS-H cancers. Augmented mRNA values of pro-inflammatory cytokines indicate an activated anti-tumour immune response [33]. The microsatellite statute as a personal prognostic detail helps multidisciplinary oncological teams to decide the type of chemotherapy and the algorithm for individual oncological therapy. It should have been challenging to know if the lower level of IL-8 expression in tumoral tissue samples from our patients could have been associated with a specific MSI statute.

Another interesting study explored the role of chemokines for KRAS and BRAF mutant gene and their wild type. The pro-angiogenic CXCL8 had a high expression in patients with mutated KRAS and BRAF genes compared to wild type

[34]. The KRAS and BRAF statute was not studied in our group, neither in the group of patients of Nastase's study, but this could be another correlation that could explain the differences between our results [29].

Cycling hypoxia has been shown to induce aggressive tumour cell phenotype and radio resistance, more significantly than chronic hypoxia [35,36]. The existence of acutely hypoxic cells in tumours was first observed several decades ago [37] and was attributed to transient changes in blood perfusion [38]. Intermittent blood flow in tumours also decreases the effectiveness of chemotherapy by limiting the delivery of drugs to tumour cells [39]. Cycling hypoxia also affects the effectiveness of anticancer therapies, most predominantly radiotherapy [40,41,42,43]. Vaupel et al showed that for those known hypoxic tumours such as melanoma, proinflammatory cytokines and chemokine expression was significantly suppressed under cycling hypoxia [36].

A limitation of our analysis was the studied population which included a heterogeneous group with only advanced tumours. Further studies are necessary to include a similar population of T1 and T2 tumours. The results concentrate on a qualitative analysis of IL-8 expression in CRC. The tissue samples were small and imposed exclusions from the genetic evaluation. Thus, comparative studies should clarify if the expression of IL-8 gene should be reported to the transtumour wall thickness collected during surgery, or another study needs to compare the differences between endoscopic biopsies values and per-surgical samples values.

Conclusions

Further extensive studies are needed on larger groups in order to clarify the expression of IL-8 mRNA in tumours. A study with a larger population would have been important for more accurate conclusions. Also, a correlation between the genetic expression level of IL-8 with KRAS and BRAF mutant genes and their wild gene type, or evaluating the microsatellite statue of tumours could have shown challenging results.

Nevertheless, despite its limitations, this study is the first to evaluate the expression of IL-8 mRNA using preoperatively fresh frozen endoscopic tissue samples, an excellent method that provides an earlier evaluation for this prognostic marker.

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