Original article

Overexpression of *CXCL8* gene in Saudi colon cancer patients

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A B S T R A C T

Colorectal cancer (CRC) is one of the leading causes of death in Saudi Arabia. CRC mostly affects older age groups, but now a days it also appears frequently at a young age. However, the complete genetic etiology of CRC remains unknown. To identify the genetic factors responsible for this cancer type and to search for biomarkers for early diagnosis and prevention, we collected sixteen CRC tumor tissue samples and six normal colon tissues and extracted mRNA and synthesized cDNA. We then performed microarray transcriptomic profiling of Saudi patients with colon cancer. Gene expression was analyzed using Partek Genomics Suite, and principal component analysis (PCA) was performed to separate the different clusters of colon cancer and healthy tissues. Distinct differences in gene expression profiles were observed between colon cancer and normal tissue samples. Subsequently, we validated gene expression using real-time PCR. We found that the C-X-C motif chemokine ligand 8 (*CXCL8*) gene was expressed most in CRC samples. *CXCL8* expressed 25.6 folds more in CRC tissues than in healthy tissues. In conclusion, we found that *CXCL8* is the chief biomarker gene that is expressed most in CRC and plays an important role in tumor progression and metastasis.

1. Introduction

Colorectal cancer is the most common type of cancer in Saudi Arabia. According to the Saudi Cancer Registry report 2015, CRC is the most common cancer affecting men (14.9%) and the second most prevalent cancer (12.2%; Cancer incidence report, 2015). The International Agency for Research on Cancer in their online database of Globocan 2018 confirmed that there was a persistent trend in the Saudi population in 2018, with an overall CRC incidence of 14.4% (Bray et al., 2018).

Early detection of cancer at stages I and II has a better prognosis than that at stages III and IV (Li et al., 2020). The five year survival rate is 90% in patients with early detected CRC at stage I, while this percentage decreases significantly in patients where CRC is detected at stage IV (approximately 12% only) (Miller et al., 2019). Hence, it is critically important to diagnose CRC at the earliest stage possible, and it can only be performed through the advent of new biomarkers that may be both reliable and easy to detect. Currently, reliable diagnosis is made only through histological examinations (Łukaszewicz-Zając et al., 2020) and because of the implementation of bowel cancer screening programs, the prognosis for CRC has improved significantly in the last decade, but this method also has major limitations (Brenner et al., 2014). Colonoscopy procedures, however, have less patient compliance and are invasive, especially when polyp removal is necessary.

Carcinoembryonic antigen (CEA) is used as the best biochemical marker in clinical practice. The enhanced CEA values indicate the presence of many types of cancers, including CRC, but they have a poor prognosis. Enhanced CEA levels of >2.5–3 ng/ml are considered abnormal, have poor prognosis, and are usually associated with many different cancers. Surgical corrections or effective chemotherapy significantly decreased CEA levels. Therefore, CEA levels are important in follow-up studies and also indicate the presence of metastasis in other organs, especially the liver (Duffy, 2001). However, CEA levels are not only elevated in cancers but also in certain medical conditions such as pulmonary emphysema, chronic bronchitis, inflammatory bowel disease, and ulcerative colitis (Park et al., 2008). Hence, it cannot be used as a conclusive marker due to its lack of sensitivity and specificity. Therefore, there is a need to identify new biochemical markers that are non-invasive and have better diagnostic and prognostic capabilities.

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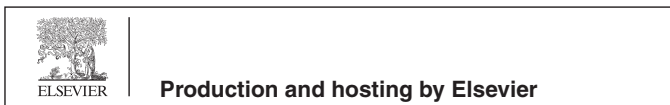
The crucial role of certain chemokines has also been reported in CRC development and progression (Bie et al., 2019; Awaji et al., 2019). Certain chemokines and their receptors have been demonstrated to regulate leukocyte migration during inflammation, tissue injury, immune responses, and cancer progression in CRC (Ransohoff, 2009; Rot and von Andrian, 2004; Raman et al., 2007).

CXCL8, also known as interleukin-8 (IL-8), was the first chemokine identified (Baggiolini, 2015). It arises mainly from neutrophils, macrophages, monocytes, endothelial cells, epithelial cells, and T-cells (Russo et al., 2014). Therefore, its involvement through its receptors seems to be crucial in CRC progression, and in recent studies, CXCL8 signaling in the tumor microenvironment has been shown to play a significant regulatory role in the metastasis and progression of CRC (Xiao et al., 2015).

In this study, we attempted to identify biomarkers that are non-invasive and more specific for CRC diagnosis and prognosis. We performed transcriptomic profiling of CRC tissues to analyze gene expression patterns.

2. Materials and methods

2.1. Sample collection

We collected 16 CRC tissue samples and six normal colon tissues from King Abdulaziz University Hospital, Jeddah. We ascertained the clinical information of the patients through a centralized medical records system and interviewing clinicians and patients. After collection, the tissue samples were immediately transferred to the Center of Excellence in Genomic Medicine Research (CEGMR) for immediate laboratory processing of RNA extraction to maintain the quality of RNA. The extracted RNA was kept at −80 °C freezer until analysis. All the prescribed ethical guidelines were followed according to the Declaration of Helsinki, and the study was approved by the ethical committee of CEGMR.

2.2. mRNA extraction and cDNA preparation

Total RNA was extracted using RNeasy® kits (Qiagen, Hilden, Germany). Special attention was given to ensure extraction of sufficient quantity and high quality of RNA, as these can affect the transcriptomic study and any variation or contamination in the samples can alter the results significantly. The quantity and quality of the eluted RNA were checked using a NanoDrop NDS-1000 Spectrophotometer (Thermo Scientific). Subsequently, cDNA was prepared using the iScript™ cDNA Synthesis Kit (BIO RAD). Each sample loaded in the Affymetrix machine contained the same amount of cDNA with strict quality control.

2.3. Microarray transcriptomics profiling

Affymetrix-based microarray transcriptomics profiling was performed using HuGene-1_0-st-v1 (GeneChip™ Human Gene 1.0 ST Array). The resultant gene expression data were analyzed using the Partek® Genomics Suite® var 7.0. Principal component analysis (PCA) was performed to visualize the high-dimensional array data and to identify the outliers in the study using a scatter plot where each point represented a sample/chip.

2.4. Molecular pathway analysis

The Ingenuity Pathway Analysis tool was used to study the differentially expressed genes. Based on the knowledge database, this tool determines the canonical pathways, molecular networks, affected phenotypes, and up/down regulation molecules.

2.5. Real time PCR

We validated the expression of CXCL8 using an Applied Biosystems StepOnePlus Real-Time PCR instrument (ThermoFisher Scientific, Waltham, MA USA). Primers were designed for target (CXCL8) and reference (GAPDH1) genes, and quantification was performed using PowerUp™ SYBR™ Green Master Mix (Thermo Fisher Scientific, Waltham, MA USA) according to the manufacturer's protocol. DataAssist™ Software, following the comparative Ct (ΔΔCt) method, was used for data analysis and accurate quantification of relative gene expression of CXCL8 in control and tumor samples.

2.6. Protein-protein interaction study

To get insight into the CXCL8 interaction proteins We generated the STRING model (https://string-db.org/). STRING interactions are derived from genomic context predictions, laboratory experimental values, co-expression molecules, automated text-mining, and knowledge derived from previous databases.

## 3. Results

3.1. Patient characteristics

In the present study, we recruited 16 CRC patients from the western region of Saudi Arabia. The male-to-female ratio was 1:1, and the tumor was located in the left (n = 8), right (n = 6), and rectal regions (n = 2). Most of the patients were older (≥50 years = 11; <50 years = 5), including all stages (stage I = 4; stage II = 6; stage III = 3; stage IV = 3) (Table 1).

3.2. Microarray transcriptomics profiling

GeneChip™ Human Gene 1.0 ST Array revealed 635 differentially expressed genes (DEGs). PCA showed tight clustering of the CRC tissue samples, with clear differences between normal and CRC tissue expression profiles (Fig. 1). CXCL8 was found to be the most expressed gene with a 25.6 times in expression. The receptor of CXCL8, CXCR1, was also found to be expressed 7.9 folds higher than that in normal tissues. The second receptor of CXCL8, CXCR2, was not significantly expressed. The expression of the other important chemokine gene chemokine (C-X-C motif) ligand 1 (CXCL1) was found to be 6.2 folds more expressed in tumor tissues than in normal tissues (Table 2).

Table 1
Characteristics of CRC patients.

S. No.	Sample ID	Age	Gender	Tumor Location	Tumor Stage
1	CR-310	34	Female	Left	III
2	CR-320	63	Male	Right	I
3	CR-326	76	Female	Right	I
4	CR-330	32	Female	Left	II
5	CR-444	54	Female	Right	IV
6	CR-754	50	Female	Left	II
7	CR-1190	49	Male	Left	II
8	CR-1296	76	Female	Left	II
9	CR-1438	51	Male	Rectal	II
10	CR-1440	49	Female	Right	IV
11	CR-1682	65	Male	Right	II
12	CT-1730	61	Male	Left	III
13	CR-1732	68	Male	Rectal	II
14	CR-1874	65	Female	Right	I
15	CR-2088	68	Male	Left	IV
16	CR-4642	55	Male	Left	III
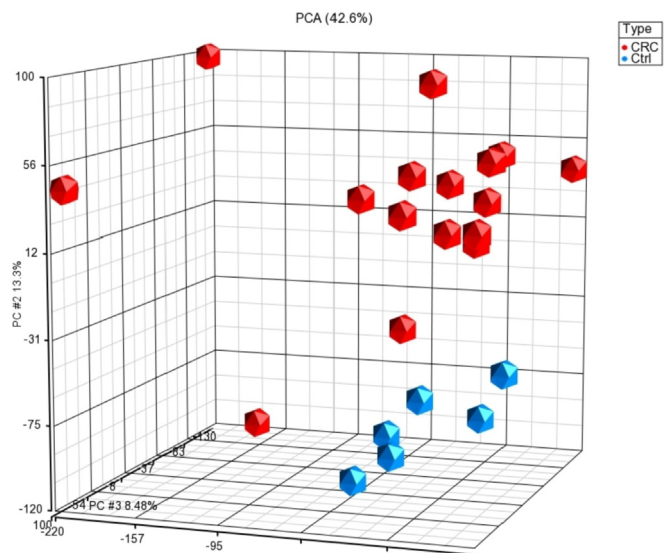
Fig. 1. Scatter plot where each point represents a chip/sample. The CRC samples show distinct cluster (red color) than the normal control tissue samples (blue color), representing different expression pattern for cancerous and normal tissues.
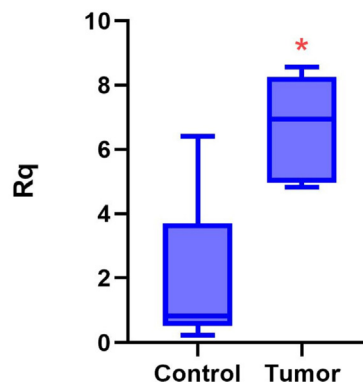
3.3. Molecular pathways analysis and integration of data

Ingenuity pathway analysis of differentially expressed genes revealed many canonical pathways associated with CRC, such as CXCL-8 signaling, granulocyte adhesion and diapedesis pathways, and the role of tissue factors in cancer (Fig. 2).


Fig. 3. Real time PCR "Box diagram" showing expression of CXCL8 in control and tumor tissues. Tumor Rq value was 6.5 ± 2.05.

3.4. Real time PCR

Validation of the CXCL8 gene was performed by real time PCR (qPCR) by calculating the mean Rq values of genes (Rq = 6.5 ± 2.05). qPCR confirmed the microarray-based overexpression of CXCL8 in CRC tissues (Fig. 3).

3.5. CXCL8 interaction study

The STRING-based protein–protein interaction model of CXCL8 predicted the associated molecules, including CXCR1, CXCR2,

Table 2
Expression of important chemokines, CXCL1, CXCL8 and its receptor CXCR1 in colorectal cancer tissue samples.

Gene Symbol	Gene Name	Fold-Change	p-value	Cytoband
CXCL8	chemokine (C-X-C motif) ligand 8	25.6	0.0000128	4q13.3
CXCR1	chemokine (C-X-C motif) receptor 1	7.9	0.0002481	2q35
CXCL1	chemokine (C-X-C motif) ligand 1	6.2	0.0001003	4q13.3
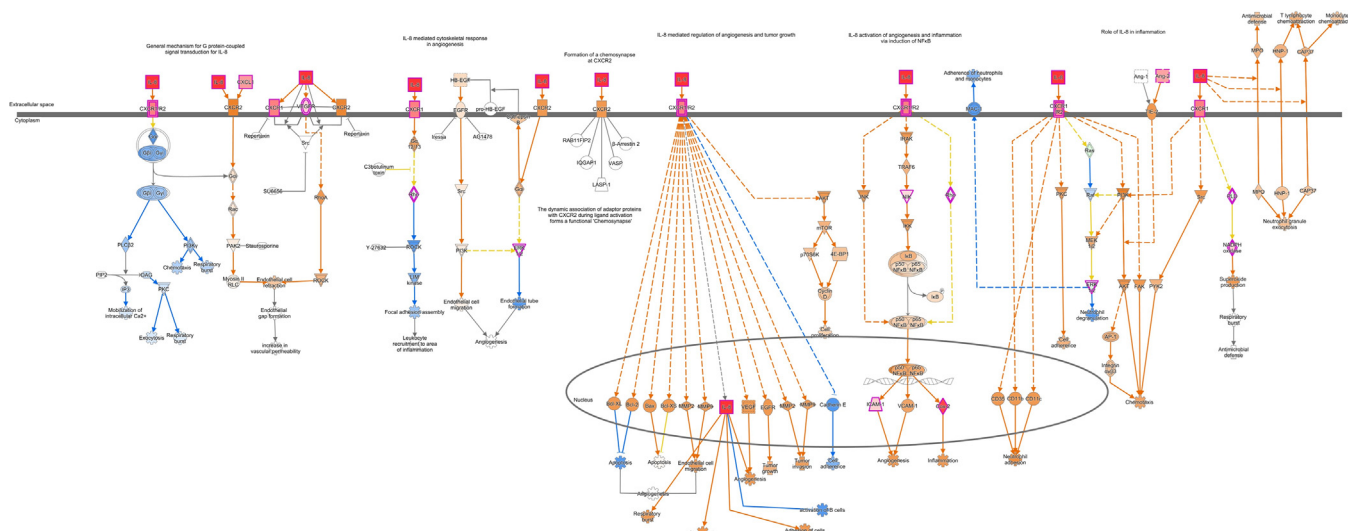
Fig. 2. CXCL8 Signaling Pathway. The activated canonical pathway was predicted for overexpressed genes (fold change) in dataset: mitogen-activated protein kinase 4 (NIK, 2.16), intercellular adhesion molecule 1 (ICAM1, 2.91), angiopoietin 2 (ANG2, 5.7), C-X-C motif chemokine ligand 1 (CXCL1, 6.25), C-X-C motif chemokine receptor 1 (CXCR1, 7.92), prostaglandin-endoperoxide synthase 2 (CoX-2, 11.7) and C-X-C motif chemokine ligand 8 (IL-8, 25.61).
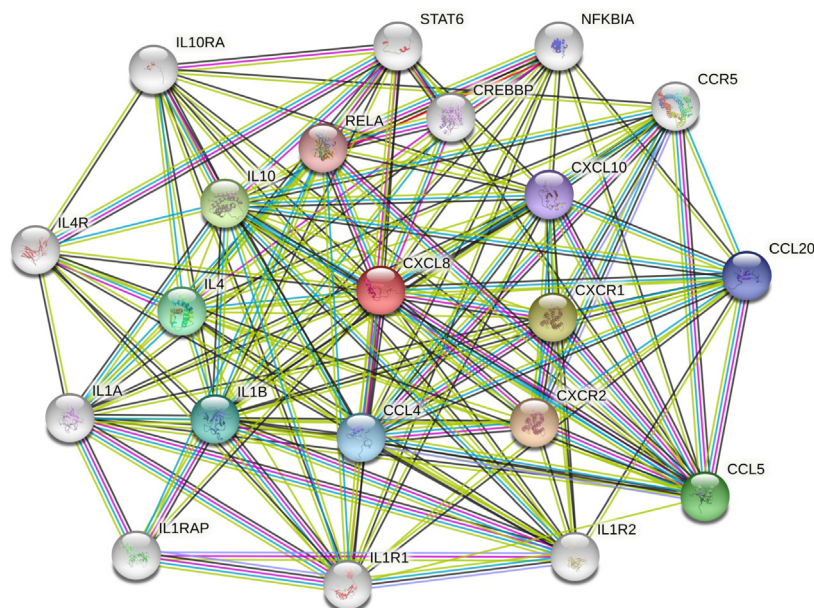
Fig. 4. STRING image for the interaction of CXCL8 with other proteins (https://string-db.org/network/9606.ENSP00000306512).

IL-10, IL-4, IL1-B, CCL5, and RELA. CXCL8 has been shown to play a central role in these interactions and may play a vital role in controlling the expression and function of other genes involved in CRC (Fig. 4).

4. Discussion

Chemokines are soluble chemotactic cytokines that are chiefly manufactured by leukocytes and tissue cells and are classified according to their roles in the inflammatory response and immune system (Zlotnik and Yoshie, 2000; Mortier et al., 2012; Anders et al., 2014). Chemokines through their receptors play vital functions in a multi-step process that is involved in tissue repair, wound healing, and protection against pathogens. Conversely, unchecked inflammatory processes are associated with the development of various malignancies (Shachar and Karin, 2013; Zaja-Milatovic and Richmond, 2008). Moreover, various pathways are linked with chemokine inflammation-related pathways, such as protein kinase B (PKB) and tumor necrosis factor alpha (TNF-α) (Cabrero-de et al., 2018; Ruiz de Porras et al., 2016). Therefore, recent studies have indicated that some chemokines and their receptors participate in each CRC development step.

CXCL8 is a key chemokine that performs different biological functions by binding to two of its major receptors, chemokine (C-X-C motif) receptor 1 and 2 (CXCR1 and CXCR2). In our transcriptomic study, we found that CXCL8 was highly expressed (25.6 folds) in CRC tissue samples as compared to normal samples. CXCL8 expression was much higher than that of the other enhanced expressed genes. This suggests that this gene has a special and important role in CRC tissue samples, making it unique and critical in CRC development and progression compared to other genes. Similarly, CXCR1 receptor expression was also found to be higher (7.9 fold) in CRC tumor samples than in controls, while there was no significant difference in CXCR2 receptor expression. This suggests that CXCL8, through its receptor CXCR1, plays a central role in these tumor tissues. Xiao et al. (2015) also found that CXCL8 is overexpressed in CRC and that it causes resistance of CRC cells to anoikis (Xiao et al., 2015). Shen et al. (2017) also provided further in vivo evidence that CXCL8 is overexpressed in CRC

and eventually promotes proliferation, invasion, and tumor growth (Shen et al., 2017).

CXCL8 signaling with its receptor CXCR1/2 has been shown to have regulatory functions in the tumor microenvironment, and a subsequent cascade of steps leads to metastasis and tumor progression (Inoue et al., 2000). To study the role of CXCL8 protein–protein association networks, we generated a STRING model (Szklarczyk et al., 2019) that clearly showed the central role played by CXCL8 with interacting proteins in the development of colon cancer.

In recent times, research on the identification of novel biomarkers has become increasingly vital for the early detection of cancers and evaluation of chemotherapeutic strategies in personalized medicine.

5. Conclusion

CXCL8 is highly overexpressed in CRC tissues and may prove to be a good candidate as an early detection biomarker. The CXCL8 pathway analysis also shows the importance of this gene in regulating a cascade of genes and receptor targets, thus proving its importance as a crucial factor in CRC development and progression. However, more comprehensive studies are needed with a larger cohort size and combining results from other latest techniques such as array comparative genomic hybridization, RNA sequencing, and in vivo cell models to further validate the role of CXCL8 in CRC.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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