Original Paper

Identification of Potential Therapeutic Targets Among CXC Chemokines in Breast Tumor Microenvironment Using Integrative Bioinformatics Analysis

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Key Words

Chemokine • Breast cancer • Tumor microenvironment • Prognosis

Abstract

Background/Aims: Breast cancer is a common cause of cancer mortality throughout the world. The cross-talk between cancer cells and interstitial cells exerts significant effects on neoplasia and tumor development and is modulated in part by chemokines. CXC is one of four chemokine families involved in mediating survival, angiogenesis, and immunosensitization by chemoattracting leukocytes, and it incentivizes tumor cell growth, invasion and metastasis in the tumor microenvironment. However, the differential expression profiles and prognostic values of these chemokines remains to be elucidated. **Methods:** In this study, we compared transcriptional CXC chemokines and survival data of patients with breast carcinoma (BC) using the ONCOMINE dataset, Kaplan-Meier Plotter, TCGA and cBioPortal. **Results:** We discovered increased mRNA levels for CXCL8/10/11/16/17, whereas mRNA expression of CXCL1/2/3/4/5/6/7/12/14 was lower in BC patients compared to non-tumor tissues. Kaplan-Meier plots revealed that high mRNA levels of CXCL1/2/3/4/5/6/7/12/14 correlate with relapse-free survival (RFS) in all types of BC patients. Conversely, high CXCL8/10/11 predicted worse RFS in BC patients. Significantly, high transcription levels of CXCL9/12/13/14 conferred an overall survival (OS) advantage in BC patients, while high levels of CXCL8 demonstrated shorter OS in all BC sufferers. **Conclusions:** Integrative bioinformatics analysis suggests that CXCL8/12/14 are potential suitable targets for precision therapy in BC patients compared to other CXC chemokines.

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Introduction

Breast cancer (BC), the most prevalent cancer in the world among women, presents nearly 1.7 million new cases worldwide each year [1]. Due to remarkable advances made in diagnosis and therapy, the survival rates for BC patients have increased in regions with adequate medical research. In contrast, the efficacy of precision target therapies for BC remains stagnant due to multifaceted gene dysregulation and complex molecular mechanisms that involve multiple pathological and molecular subtypes. One of the classic molecular subtypes relies on expression of estrogen receptor (ER), progesterone receptor (PR) and epidermal growth factor receptor 2 (HER2) to provide important clinical and predictive therapeutic information [2, 3]. Researchers and clinicians are increasingly regarding cancer as an imbalance between normal cells and the immune system. The cross-talk between cancer cells and immune cells is a pivotal part of cancer growth, metastasis, and treatment effects [4]. Hence, identifying novel biomarkers in the immune system for BC is urgent and required to achieve the goal of individualized treatment.

Chemokines are a family of low molecular weight, secreted signaling proteins well known for modulating immune cell trafficking and stromal cell development. Chemokines are classified into diverse groups according to their structural and behavioral characteristics. All chemokines are divided into four main classes based on their cysteine (C) residue sequence: the CXC chemokines, the CC chemokines, the C chemokines and CX3C chemokines, in which X represents any amino acid [5]. The nearly 50 chemokines identified in humans are divided into two differential functional groups: inflammatory chemokines and homeostatic chemokines. Inflammatory chemokines are usually induced by harmful stimulants, such as infections or cell damage, while homeostatic chemokines are constitutively expressed in normal immune network regulation. Aside from being known for mediating chemotaxis, most chemokines exhibit various additional functions related to cell proliferation and survival and to the stimulation or inhibition of angiogenesis.

Seventeen CXC chemokines have been described in humans. Interestingly, CXC chemokines can be further separated based on the presence or absence of an ELR (Glu, Leu, Arg) motif, which is situated before the first conserved cysteine residue. Almost all ELR⁺ CXC chemokines are considered contributors to neutrophil aggregation and/or angiogenesis [6, 7]. In contrast, ELR⁻ CXC chemokines attract lymphocytes, such as T cells, B cells, and natural killer (NK) cells [8, 9]. Although several studies have uncovered a general profile for CXC chemokines in BC, identifying suitable targets for precision therapy remains a challenge that urgently needs attention in this field. In the present study, we implemented a deep bioinformatics analysis of the clinicopathological and survival data as it relates to CXC chemokines in patients with BC based on several large public databases in order to illustrate their prognostic and potential therapeutic value in cancer treatment.

Materials and Methods

ONCOMINE data-mining analysis

ONCOMINE (www.oncomine.org), an online web-based cancer database for RNA and DNA sequences, was used to facilitate data-mining the transcriptional expression of CXC chemokines in 20 types of cancer [10]. Transcriptional expression of CXCL chemokines in cancer samples were compared with those in normal individuals using Student's t-test. Statistically significant values and fold change were demarcated as $p < 0.05$ and 2, respectively.

bcGenExMiner v4.0

Breast Cancer Gene-Expression Miner v4.0 (bcGenExMiner v4.0) is a statistical mining tool of 36 noted genomic datasets and three classical mining functions: expression, prognosis, and correlation [11, 12]. Expression data was updated in March 2016 and compares production of a particular gene with clinical

parameters such as age, ER, PR, HER2, and so on. The prognosis module calculates the prognostic value of target genes in BC and offers potential novel prognostic markers.

Kaplan-Meier Plotter

The prognostic value of CXC chemokine mRNA transcription level was measured using an online open database, the Kaplan-Meier Plotter (www.kmplot.com) [13], which consists of gene expression profiles and survival information for 1,809 BC patients. To evaluate the relapse-free survival (RFS) of patients with BC, individuals were separated into two groups based on median gene expression (high vs. low) and validated by K-M survival curves. The mean value of each CXC chemokine's multiple probes was determined to generate K-M survival curves, and the number-at-risk is displayed below the curves.

TCGA data and cBioPortal

The Cancer Genome Atlas (TCGA) contains gene expression data obtained by sequencing and has accurate clinical and pathological data on many cancers [14]. The cBioPortal (www.cbioportal.org) is an open-access data set for exploring multiple cancer genes. The BC dataset contains data from 1105 cases, with pathologic diagnosis chosen by cBioPortal for further analyses of CXC chemokines [15, 16].

Results

Dysregulated expression of CXC chemokines in patients with BC

Sixteen CXC chemokines were identified using the ONCOMINE database, excluding CXCL15. As shown in Fig. 1, we first measured expression of CXC chemokines in 20 types of cancer and compared those to normal individuals. The mRNA expression of CXCL8/10/11/16/17 was significantly up-regulated in BC samples in multiple datasets. According to the Curtis dataset, CXCL8 overexpression was observed in a few types of BC compared with normal tissues, with a fold change in ductal BC of 2.593 [17], while Richardson reported ductal BC as having a CXCL8 fold change=4.856 (Table 1) [18]. Overexpression of CXCL10 has also been reported in all types of BC compared with normal breast tissue, including mucinous BC with

Fig. 1. Transcriptional levels of CXC chemokines in different types of cancers with the threshold of P-value $\leq 1E-4$, Fold Change ≥ 2 , and Gene Rank $\geq$ Top 10%. This information was attained from ONCOMINE and indicates the numbers of datasets with statistically significant ($p < 0.05$) mRNA high-expression (Red) or low-expression (Blue) of CXC chemokines (different types of cancer vs. corresponding normal tissue). Cell color was decided by the best gene rank percentile for the analyses within the cell, and the gene rank was analyzed by percentile of target genes in the top of all genes measured by each study.

fold change 2.166, mixed lobular and ductal BC with fold change 2.086, invasive lobular BC with fold change 2.383, and invasive BC with fold change 3.009, according to the TCGA dataset. Data from Curtis report CXCL10 levels in medullary BC with fold change=15.564, ductal BC with fold change=4.231, and invasive BC with fold change=4.398. In different datasets for CXCL10, we observed invasive ductal BC having a fold change=5.002 compared with normal breast reported by Curtis, and a similar trend was found by Karnoub [5.072] [19], Turashvili [4.877] [20], and Ma 4 [4.915] datasets [21].

CXCL11 is another chemokine of interest in the CXC family with respect to BC. mRNA expression of CXCL11 was found to be up-regulated in most types of BC compared to normal breast tissue. Transcriptional levels of CXCL11 in invasive ductal BC exhibited fold change=10.712 [19], while TCGA and Radvanyi reported slightly lower fold changes (5.867 and 7.089, respectively) [22]. Both sources report markedly increased expression of CXCL16 in invasive lobular BC and invasive ductal BC, with fold changes=2.369 and 2.225, respectively. However, only Finak reported that high expression of CXCL17 was associated with invasive BC, in contrast to normal tissue (fold change=2.257) [23].

Table 1. The significant changes of CXC-chemokines expression in transcription levels between different types of BC and breast tissues (ONCOMINE database)

	Type	Fold change	P value	t-test	REF
CXCL8	Ductal BC	2.593	0.028	2.184	Curtis [17]
	Ductal bc in situ epithelia	3.649	0.012	2.619	Ma [21]
	Ductal BC	4.856	3.71E-4	4.284	Richardson[18]
CXCL10	Intraductal cribriform B adenocarcinoma	3.313	0.047	2.736	TCGA
	Ductal BC	19.021	8.00E-12	9.558	Richardson[18]
	Mucinous BC	2.166	1.71E-7	7.611	TCGA
	Mixed lobular and ductal BC	2.086	1.14E-5	5.838	TCGA
	Invasive lobular BC	2.383	3.52E-12	7.870	TCGA
	Invasive BC	3.009	5.77E-17	9.973	TCGA
	Medullary BC	15.564	9.43E-14	11.593	Curtis [17]
	Ductal BC	4.231	7.79E-5	5.891	Curtis [17]
	Invasive ductal BC	5.022	4.40E-71	26.711	Curtis [17]
	Invasive BC	4.398	8.97E-6	5.467	Curtis [17]
	Invasive lobular BC	3.189	1.55E-27	12.159	Curtis [17]
	Invasive ductal BC	5.072	1.71E-4	5.760	Karnoub [19]
	Invasive ductal BC	4.877	0.029	2.489	Turashvili [20]
	Invasive lobular BC	5.006	0.047	2.115	Turashvili [20]
	Invasive ductal BC	4.915	0.017	2.349	Ma [21]
CXCL11	Ductal BC in situ	8.12	0.010	2.597	Ma [21]
	Ductal BC in situ epithelia	6.157	0.012	6.157	Ma [21]
	Invasive ductal BC	10.712	2.81E-5	5.279	Karnoub [19]
	Ductal BC	12.741	1.52E-9	7.494	Richardson[18]
	Invasive BC	8.811	1.69E-25	13.055	TCGA
	Invasive ductal BC	5.867	2.08E-31	15.594	TCGA
	Invasive lobular BC	5.268	8.07E-11	7.777	TCGA
	Medullary BC	2.189	7.13E-6	5.134	Curtis [17]
	Invasive ductal BC	7.089	0.009	3.222	Radvanyi [22]
	Invasive lobular BC	3.724	0.038	2.066	Radvanyi [22]
	Invasive mixed BC	4.123	0.029	2.422	Radvanyi [22]
	Ductal BC in situ stroma	6.014	0.004	3.200	Ma [21]
	Invasive ductal BC stroma	3.094	0.011	2.777	Ma [21]
	Invasive lobular BC	2.369	0.016	2.413	Radvanyi [22]
	Invasive ductal BC	2.225	0.014	2.571	Radvanyi [22]
CXCL17	Invasive BC	2.257	1.67E-12	10.984	Finak [23]

Table 2. Prognostic association of CXC-chemokines expression in breast cancer based on bc-GenExMiner v4.0. (Event: metastatic recurrence). Abbreviations: MR, metastatic recurrence

Chemokine	Event	p-value	HR	95%CI
CXCL1	MR	0.6277	0.98	0.93 - 1.05
CXCL2	MR	0.0020	0.90	0.84 - 0.96
CXCL3	MR	0.2543	0.96	0.91 - 1.03
CXCL4	MR	0.9770	1.00	0.94 - 1.06
CXCL5	MR	0.1935	0.95	0.88 - 1.03
CXCL6	MR	0.0565	0.94	0.89 - 1.00
CXCL7	MR	0.3720	0.97	0.91 - 1.04
CXCL8	MR	< 0.0001	1.20	1.13 - 1.28
CXCL9	MR	0.1288	0.95	0.90 - 1.01
CXCL10	MR	0.3621	1.03	0.97 - 1.09
CXCL11	MR	0.8691	1.01	0.94 - 1.07
CXCL12	MR	< 0.0001	0.81	0.76 - 0.86
CXCL13	MR	< 0.0001	0.83	0.78 - 0.89
CXCL14	MR	< 0.0001	0.87	0.82 - 0.92
CXCL16	MR	0.9349	1.00	0.90 - 1.10
CXCL17	MR	0.0512	1.09	1.00 - 1.20

Increased expression of CXCL2/12/13/14 and reduced expression of CXCL8 are correlated with preferable RFS or OS in BC patients

We separated all types of BC patients into one of two groups for chemokine expression (high vs low) based on median expression values for each chemokine across all samples. Fig. 1 presents transcription levels of CXC chemokines in different types of cancers with

Fig. 2. Kaplan-Meier curve reveals the relapse-free survival differences based on mRNA level of CXC chemokines in BC patients. (A) BC patients with over expression of CXCL1/2/3/4/5/6/7/12/14 presented with better relapse-free survival (RFS). (B) BC patients with decreased expression of CXCL8/10/11 presented with better RFS.

the threshold of P-value $\leq$ 1E-4, Fold Change $\geq$ 2, and Gene Rank $\geq$ Top 10%). This is true that IL-8 is not regulated in BC with this threshold. But IL-8 is overexpressed in BC with the threshold of P-value $\leq$ 0.05, Fold Change $\geq$ 2, and Gene Rank $\geq$ Top 10%. When the chemokine expression of cancer is higher than that of adjacent non-tumor, we defined it as high expression and vice versa. The Kaplan-Meier curves for survival data indicated that increased CXCL1/2/3/4/5/6/7/12/14 mRNA levels and reduced CXCL8/10/11 mRNA levels are

Fig. 3. Kaplan-Meier plotter reveals the overall survival differences based on mRNA level of CXC chemokines in BC patients. (A) BC patients with overexpression of CXCL9/12/13/14 presented with better overall survival (OS). (B) BC patients with decreased expression of CXCL8 presented with better OS.

Fig. 4. The relationship between mRNA expression of CXC chemokines and Scarff Bloom & Richardson grade status. (A) Diagrams shows the number of CXC chemokines identified as potential good/bad prognostic targets of BC between Kaplan-Meier Plotter and bcGenExMiner v4.0. (B) Global significant differences between groups was assessed by Welch's test to determine p value ($p < 0.05$).

strongly correlated with RFS ($p < 0.05$) in all BC types (Fig. 2). Furthermore, BC patients with higher transcriptional levels of CXCL9/12/13/14 or lower mRNA levels of CXCL8 demonstrated better overall survival (OS) (Fig. 3). To further investigate chemokine roles in

Fig. 5. The relationship between mRNA expression of CXCL2/8/12/13/14 and the protein expression of ER or PR and clinic pathological parameters of breast carcinoma (A) The relationship between mRNA expression of CXCL2/8/12/13/14 and clinic pathological parameters of breast carcinoma. (B) The relationship between mRNA expression of CXCL2/8/12/13/14 and protein expression of ER or PR.

the prognosis of BC, we also use bc-GenExMiner v4.0 to verify our findings. CXCL8 exerts the most significant negative effect on patient prognosis, and expression of CXCL2/12/13/14 was associated with better prognosis (Table 2).

The relevance between aberrantly expressed CXC chemokines and clinical pathological parameters of BC patients

Using the abovementioned databases, we next focused on whether CXCL2/12/13/14 and CXCL8 play pivotal roles in BC progression (Fig. 4A). Using bc-GenExMiner software, Welch's test was implemented to compare the aberrant expression of CXC chemokines between groups of patients based on different clinical pathological parameters. For age criterion (Fig. 5 A), there was no significant difference between ≤ 51 year and > 51 year groups, except for CXCL2/13, which demonstrated reduced levels in the older group. BC patients with negative nodal status showed lower expression of CXCL2 than did positive nodal patients.

As shown in Fig. 5B, ER-positive or PR-positive patients tended to express low levels of CXCL2/8/13 and high levels of CXCL12/14 compared with ER-negative or PR-negative patients. ER-positive and PR-positive cancer cells depend on estrogen for their growth, so they can be treated with drugs to block estrogen effects (e.g., tamoxifen) and generally have a better prognosis [24]. Therefore, these results indicate that low expression of CXCL2/8/13 and high expression of CXCL12/14 may predict a better prognosis. In addition, CXCL2/8/13 expression was positively associated with HER2 status. Triple negative breast cancer (TNBC) is the most lethal type of BC, with a molecular signature including ER (-), PR (-) and HER2 (-). We found that CXCL2/8/13 mRNA expression was significantly increased in TNBC patients, while CXCL12/14 mRNA expression were significantly reduced in TNBC patients; the same pattern of change was observed in basal-like BC (Fig. 5).

The Scarff, Bloom & Richardson grade (SBR) is an accepted prognostic factor that evaluates tubule formation, nuclear characteristics of pleiomorphism, and mitotic index. Patients with poorly differentiated tumors (grades II and III) have a decreased survival rate compared with those who have well-differentiated tumors (grade I). Based on SBR criterion (Fig. 4B, C), patients with high grade tumors (grades II and III) tended to express high levels of CXCL8 and low levels of CXCL2/12/14, consistent with our previous data. Apart from molecular subtypes, the Nottingham Prognostic Index (NPI) is also a useful prognostic model for BC patients. We found that lower expression of CXCL8 and higher expression of CXCL12/14 were correlated with the NPI. Combined with this information, multiple bioinformatics analyses

identify CXCL8/12/14 as potential suitable therapeutic targets for BC patients. To visualize potential genes co-expressed with CXCL2/8/12/13, we constructed the gene network shown in Fig. 6. The network is meant to identify potential interactions between CXCL2/8/12/13 and other key proteins. As shown in Fig. 5, we found that CXCL2 was associated with other chemokines, including CXCL1/3/4/6/11/12 and CCL28. CXCL8 was associated with IL15 and IL12. Of note, PIK3CA was related to both CXCL8 and CXCL12 expression. Erb-b2 receptor tyrosine kinase 2 (ERBB2), a key modulator of breast cancer

Fig. 6. Prospective gene networks of four differential CXC chemokines. To explore the regulation network of CXCL2/8/12/13, we show co-expressed genes of those key genes screened out by ONCOMINE.

progression, was involved with CXCL13. This network analysis provides a series of gene candidates to help elucidate the molecular mechanisms of CXC chemokine involvement in BC.

Discussion

CXC chemokines have primarily been identified as chemo-attractive cytokines that are mainly involved in the maturation and differentiation of leukocytes. It has become clear that chemokine expression in BC plays a role in multiple aspects of neoplasia, including proliferation, survival, apoptosis, chemoattraction, angiogenesis, invasion and metastasis. Furthermore, cell-to-cell communication between BC cells and interstitial cells impacts chemokine expression patterns in a variety of cell types, favoring a specific microenvironment that promotes tumor invasion and metastasis. There are conflicting reports on the roles of CXC chemokines within cancer. Recently, Nagarsheth et al. published a systematic review of chemokines in the cancer microenvironment and their relevance to cancer immunotherapy [4], indicating the crucial roles of CXC chemokine signaling pathways in multiple cancers and the efficacy of immunotherapies. However, the role of distinct CXC chemokines in the progression and metastasis of BC remain to be elucidated. To our knowledge, this is the first study to identify specific CXC chemokines with prognostic value and biological function in the tumor microenvironment using integrative bioinformatics (Table 4 and 5).

Literature Review of Dysregulated CXC chemokines in breast cancer

A mini review of these CXC chemokines, their location, characteristics and known downstream targets is discussed below to suggest possible functional roles in breast tumorigenesis.

Table 3. Summary of characteristics and role of CXC-chemokines in BC

Chemokine	Location	Source	Downstream	Possible role
CXCL1	Chr.4q13.3	cancer cell	MDSC	oncogene
CXCL2	Chr.4q21	cancer cell	macrophage	oncogene
CXCL3	Chr.4q13.3	MSC	cancer cell	oncogene
CXCL4	Chr.4q13.3	MDSC, platelets	cancer cell	oncogene
CXCL5	Chr.4q13.3	TAO	cancer cell,	oncogene
CXCL6	Chr.4q21	cancer cell	neutrophil	oncogene
CXCL7	Chr.4q13.3	cancer cell	MSC	oncogene
CXCL8	Chr.4q13.3	cancer cell	neutrophil	oncogene
CXCL9	Chr.4q21.1	cancer cell	NK cell, T cell	tumor suppressor
CXCL10	Chr.4q21.1	cancer cell, MEC	NK cell, T cell	tumor suppressor
CXCL11	Chr.4q21.1	macrophage	T cell	tumor suppressor
CXCL12	Chr.10q11.21	B cell	cancer cell, TAM	tumor suppressor
CXCL13	Chr.4q21.1	cancer cell	B cell	tumor suppressor
CXCL14	Chr.5q31	CAF	cancer cell	tumor suppressor
CXCL16	Chr.17q13	cancer cell	MSC	oncogene
CXCL17	Chr.19q13.2	cancer cell	MDSC	oncogene

Table 4. Prognostic association of CXC-chemokines expression in breast cancer based on Kaplan-Meier Plotter (Event: overall survival)

Variable	Cutoff value expression	Expression	P value	HR	No. Patients
CXCL1	113	1-26780	0.777	1.21 (0.98-1.5)	1402
CXCL2	173	3-8589	0.1692	0.86 (0.69-1.07)	1402
CXCL3	42	1-4710	0.3934	1.1 (0.89-1.36)	1402
CXCL4	67	2-1705	0.8958	0.99 (0.8-1.22)	1402
CXCL5	27.33	1-5166	0.6312	0.94 (0.76-1.18)	1402
CXCL6	55	1-2559	0.3397	0.9 (0.73-1.12)	1402
CXCL7	29	1-7447	0.6595	0.95 (0.77-1.18)	1402
CXCL8	141	6-28546	0.0003	1.48 (1.19-1.83)	1402
CXCL9	1266	20-27026	0.0091	0.75 (0.61-0.93)	1402
CXCL10	1673	16-31023	0.4117	0.91 (0.74-1.13)	1402
CXCL11	239.5	2-12392	0.8859	1.02 (0.82-1.26)	1402
CXCL12	1467.5	68-9818	0.0001	0.65 (0.52-0.8)	1402
CXCL13	499	2-36579	0.0003	0.67 (0.54-0.84)	1402
CXCL14	2253.5	5-46051	0.0495	0.73 (0.53-1)	626
CXCL16	552	63-5822	0.7503	1.05 (0.77-1.44)	626

Table 5. Prognostic association of CXC-chemokines expression in breast cancer based on Kaplan-Meier Plotter. (Event: relapse-free survival)

Variable	Cutoff value expression	Expression	P value	HR	No. Patients
CXCL1	109	1-26780	0.0104	0.87 (0.78-0.97)	3951
CXCL2	173	2-12317	0.0001	0.8 (0.72-0.89)	3951
CXCL3	42	1-4710	0.0154	0.87 (0.78-0.97)	3951
CXCL4	56	2-1980	1.30E-06	0.76 (0.69-0.85)	3951
CXCL5	24.67	1-5166	0.0015	0.84 (0.75-0.93)	3951
CXCL6	48	1-4136	2.60E-07	0.75 (0.67-0.84)	3951
CXCL7	22	1-7447	0.0145	0.87 (0.78-0.97)	3951
CXCL8	112.5	4-28546	5.70E-07	1.32 (1.18-1.47)	3951
CXCL9	1120	6-28997	0.96	1 (0.9-1.12)	3951
CXCL10	1429	16-31023	3.20E-03	1.18 (1.06-1.31)	3951
CXCL11	188	1-12392	0.0027	1.18 (1.06-1.32)	3951
CXCL12	1464	60-9818	2.00E-11	0.69 (0.62-0.77)	3951
CXCL13	380	1-37026	0.11	0.91 (0.82-1.02)	3951
CXCL14	2529.5	2-66318	2.10E-07	0.66 (0.57-0.77)	1764
CXCL16	597	63-5822	0.0052	0.8 (0.69-0.94)	1764

CXCL1/2/3/4/5/6/7/8/9/16/17 as oncogenes

CXCL1, the first member of the CXC chemokine family, is considered an oncogene in BC. Cancer cells that overexpress CXCL1 attract CD11b+Gr1+ myeloid cells into the tumor stroma, stimulating secretion of S100A8/9, which enhances cancer cell survival and chemoresistance [25]. A pro-inflammatory interleukin, (IL)-17, significantly increases secretion of CXCL1 from breast carcinoma cells, suppressing function of myeloid-derived suppressor cells (MDSCs). MiR-200 was found to have both direct and indirect mechanisms of targeting CXCL1 to inhibit cancer angiogenesis [26]. In an experimental animal model, after co-injection of CXCL1 with human adipose-derived mesenchymal stem cells (hADSCs), hADSCs promoted residual BC proliferation and metastasis via the PI3K/AKT/SOX2 axis [27]. CXCL2 shares 90% amino acid homology with CXCL1. As a pro-inflammatory and pro-oncogenic factor, BC patients with high CXCL2 mRNA levels have a prognostic disadvantage. Kronski et al. has shown that miR-181b modulates the expression of CXCL1 and CXCL2, inhibiting breast cancer growth and invasion [28]. Constitutive CXCL3 activation has been reported in some human cancers with a poor prognosis as a potential therapeutic target [29]. Bone-marrow-derived mesenchymal stem cells (MSCs) may facilitate breast cancer progression by releasing CXCL3 with transforming growth factor- α (TGF α) [30]. As few studies have focused on CXCL3, the underlining function of CXCL3 still needs additional research. CXCL4, known as platelet factor 4 (PF4), is released from activated platelets and binds with high affinity to heparin. In a mouse model, CXCL4 was shown to reduce breast cancer growth and proliferation of endothelial cells *in vivo* [31]. Of note, CXCL4 is produced by Ly6G+CD11b+ myeloid cells in the pre-metastatic stage, but production is lowered in the metastatic stage [32]. Controversially, Pucci et al. reported that CXCL4 induces platelet accumulation to promote lung adenocarcinoma in a demonstration of tumor-host communication [33]. There is little information on CXCL5 in BC. In the bone metastasis microenvironment, breast tumor associated osteoblasts (TAOs) secrete CXCL5 to mediate cancer development via the ERK/MSK1/Elk-1/Snail signaling pathway [34]. As mentioned above, (IL)-17 significantly increases secretion of CXCL1 and CXCL5 from breast carcinoma cells, suppressing the function of MDSCs [35]. As its former name granulocyte protein 2 (GCP-2) indicates, CXCL6 is a chemoattractant for neutrophils. Neutrophils within tumor stroma facilitate cancer invasion and metastasis. Due to lack of original research articles concerning the role of CXCL6 in BC, we speculate that CXCL6 may serve as a proto-oncogene in the tumor microenvironment [36]. CXCL7 is an isoform of pro-platelet basic protein (PPBP), which is released from activated platelets. In cell-to-cell communication between breast cancer stem cells (CSCs) and MSCs, CSCs produce IL6 to attract MSCs, which produce CXCL7 in response to IL6. In turn, CXCL7 induces the secretion of many cytokines from mesenchymal cells, including IL6, IL8, CXCL6 and CXCL5 [37].

CXCL8, previously called IL8, is a cytokine involved in cell-cell communication between cancer cells and neutrophils in the breast carcinoma microenvironment. Some transcription factors orchestrate key functions for increasing levels of CXCL8. The nuclear factor of activated T cells (NFAT) stimulates expression of CXCL8 by binding to its promoter and soliciting neutrophils *in vitro* and *in vivo* [38]. Tumor necrosis factor α (TNF α)-activated mesenchymal stromal cells accelerate breast tumor progression via neutrophil aggregation [39]. Considering hormone receptor criteria, CXCL8 deficiency inhibits hormone-resistant cell growth and invasion, in part attenuating the effect of Forkhead box protein A1 (FOXA1) function in ER+ breast cancer [40].

CXCL9/10/11/12/13/14 as tumor suppressors

CXCL9, known as monokine induced by gamma interferon (MIG), is a T-cell chemoattractant that is usually induced by IFN- γ . We found that high transcription levels of CXCL9 conferred an overall survival advantage in all BC patients, consistent with previous findings. Some researchers have already discovered that IFN- γ exhibits a powerful influence on the secretion and expression of CXCL9 and CXCL10 [41, 42]. The GeparSixto trial revealed that as an immune-activating factor, CXCL9 shows a positive correlation with PD-1 [43]. Brackett et al., found that the toll-like receptor-5 agonist entolimod quickly activates an

innate antitumor response predominantly by attracting CXCR3+ NK cells using CXCL9 and CXCL10 [44]. Our study confirms that high transcription levels of CXCL9 confer an overall survival advantage in all BC patients and is a promising prognostic factor.

Intratumoral hypoxia triggers metastasis by activation and release of hypoxia-inducible factors (HIFs). Under hypoxic conditions, breast cancer cells increase HIF activity via MSC and cancer cell communication. Additionally, co-existence of two kinds of cells induce expression of the chemokine CXCL10 in MSCs to support metastasis to lymph nodes and lungs [45, 46]. However, in recent studies, the idea of CXCL10 as a potent chemoattractant has received more attention. For example, type I IFNs trigger autocrine and paracrine circuits that accelerate the release of CXCL10 [47]. Mammary epithelial cells (MECs) create elevated expression of CXCL10 via type I IFNs as well [48]. Notably, combinatorial therapy with celecoxib and PD-1 mAb increases expression of CXCL9 and CXCL10 [49].

It has been demonstrated that CXCL11 has similar chemotactic functions as CXCL9 and is located near the CXCL9 gene on human chromosome 4. Unfortunately, not much research has focused on CXCL11 in BC. The stimulation of IFN genes (STING) triggers macrophage production of TNF α and high expression levels of the T cell-recruiting chemokines CXCL10 and CXCL11 [50].

Many different interstitial cell subsets are recruited into the tumor microenvironment via cell-to-cell communication between CXCL12 and its receptor CXCR4. However, CXCL12 and CXCR4 have disparate functions on breast tumorigenesis and outcomes depending on the specific immune cell type and conditions present [51]. Yu et al. demonstrated that transforming growth factor β (TGF β) impairs MSCs ability to secrete CXCL12 and therefore promotes breast cancer metastasis [52], consistent with our findings. Recently, Xia et al. found that B cells directly kill BC cells using CXCR4/CXCL12 pathways, indicating that CXCL12 functions as a tumor suppressor [53]. Bioinformatics analysis also suggested that CXCL12 is an independent prognostic indicator. Contradictorily, CXCL12 is robustly increased in perivascular sites after chemotherapy, where it also carries out chemotaxis for tumor-associated macrophages [54].

CXCL13, known as B lymphocyte chemoattractant (BCL), was frequently found in the interaction between cancer cells and B cells [55]. ROR γ t+ group 3 innate lymphoid also stimulated the expression of CXCL13 by stromal cells in the microenvironment and largely contributed to lymph node metastases [56]. Additionally, p53 deficiency was shown to activate CXCR5 expression in response to CXCL13 [57]. Nonetheless, Stoll et al. stated that expression of CXCL13 cardinaly reflects the levels of effector T cells within the tumor bed, suggesting that CXCL13 might have a suppressive role in tumor progression [58].

Previously, CXCL14's role as a negative modulator of cancer growth and metastasis seemed well established [59]; however, a recent investigation revealed that CXCL14 might actually assist in tumor development and progression. Epithelial CXCL14 levels are markedly correlated with ER+ tumors and lower proliferation status. Interestingly, total (epithelial and stromal) CXCL14 level is dramatically associated with decreased RFS [60]. Cross-talk between cancer cells and cancer-associated fibroblasts (CAFs) is also relevant to cancer research. CXCL14 is a potent executor of CAFs' pro-tumorigenic functions. Of note, nitric oxide synthase (NOS1) has increased expression in CAF and, with CXCL14, stimulates CAFs. NOS1 can speed up tumor growth with the assistance of CXCL14-expressing CAFs [61]. Furthermore, lower miR-29b expression in CAFs can accelerate metastasis by activating the p38-STAT1 pathway in breast cancer cells and up-regulating CXCL14 and CCL11 in CAFs. Additionally, STAT1 increases expression of CXCL14 and CCL11 at the transcription level [62]. Kaplan-Meier curves revealed that high CXCL14 mRNA levels were correlated with favorable relapse-free survival (RFS) in all types of BC patients, and high transcription levels of CXCL14 conferred an overall survival (OS) advantage in all BC patients. These results suggest that CXCL14 is a potential target for precision therapy in BC patients.

BC cells secrete CXCL16 that binds to CXCR6 on MSCs. In turn, MSCs secrete CXCL10 that binds to CXCR3 on cancer cells. This feedback loop drives accumulation of tumor-associated macrophages (TAMs) and MDSCs [46]. CXCR6 was found not only in stromal cells but was also

Fig. 7. Cross-talk between BC cells and immune cells mediated by CXC chemokines in the tumor microenvironment. The immune system is determined by the recruitment of various immune cells through secretion of CXC chemokines by tumor cells and other infiltrating immune cells in the tumor microenvironment. MDSC, myeloid-derived suppressor cell; MSC, mesenchymal stem cell; TAO, tumor associated osteoblast; NK cell, nature killer cell; MEC, mammary epithelial cell; CAF, cancer associated fibroblast; IL-6, interleukin-6.

constitutively expressed in cancer cells. BC cells mediate production of autocrine CXCL16, promoting cancer progression via pERK1/2 signaling [63]. On the other hand, stromal cells like CAFs can secrete CXCL16, a chemoattractant for monocytes that contributes to the aggressive phenotype of TNBC [64].

CXCL17 is also called VEGF co-regulated chemokine 1 (VCC-1) and dendritic cell- and monocyte-attracting chemokine-like protein (DMC). Cancer cell-derived CXCL17 recruits MDSCs to tumor sites and facilitates tumorigenesis and angiogenesis [65].

Comparison of mini review of CXC chemokines with our results

Overall, we illustrated that cross-talk between breast cancer cells and immune cells is mediated by CXC chemokines in the tumor microenvironment (Fig. 7 and Table 3). Although CXC chemokines are reported to affect OS and RFS in BC patients, the dominant regulatory factors and underlying mechanism are still unclear. The exact mechanism of CXC chemokine involvement in BC patient prognosis requires more investigation.

The above mini-review indicates that CXCL1/2/3/4/5/6/7 may function as proto-oncogenes in the tumor microenvironment, which contradicts our findings. The CXC chemokine expression profiles in the dataset are primarily from RNA sequences. These disparities may stem from differences between clinical samples and experimental conditions used in each study. Of note, in this study, we report that CXCL8 mRNA level is significantly overexpressed in breast cancer and increased in higher SBR grades, which predict rapid-growing, metastatic tumors. This result is consistent with previous reports. Furthermore, high levels of CXCL8 indicated shorter OS in all BC patients, representing a potential therapeutic target.

In the current study, we systemically analyzed transcriptional expression and prognostic values for all CXC chemokines in BC, supplying a more profound understanding of the complicated dysregulation in the molecular biology of BC. Integrative bioinformatics analysis suggests that CXCL8/12/14, compared to other CXC chemokines, are potential suitable targets for precision treatment in BC patients. However, other multiple clinical trials are required to validate the diagnostic potentials of these CXC chemokines. Additionally, more in-depth experiments, such as single cell sequencing, are necessary to explore the underlying mechanism of cross-talk between cancer cells and stromal cells in BC.

Abbreviations

BC (breast cancer); RFS (relapse-free survival); OS (overall survival); ER (estrogen receptor); PR (progesterone receptor); HER2 (epidermal growth factor receptor 2); NK (cells nature killer cells); TCGA (The Cancer Genome Atlas); TNBC (triple negative breast cancer); SBR (Scarff, Bloom & Richardson grade); NPI (Nottingham Prognostic Index); ERBB2 (Erb-b2 receptor tyrosine kinase 2); MDSCs (myeloid-derived suppressor cells); hADSCs (human adipose-derived mesenchymal stem cells); TGF α (transforming growth factor- α); TAOs (tumor associated osteoblasts); GCP-2 (granulocyte protein 2); PPBP (pro-platelet basic protein); NFAT (nuclear factor of activated T cells); TNF α (tumor necrosis factor α); FOXA1 (Forkhead box protein A1); HIFs (hypoxia-inducible factors); MECs (mammary epithelial cells); CAFs (cancer-associated fibroblasts); VCC-1 (VEGF co-regulated chemokine 1)

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Disclosure Statement

The authors disclose no competing interests in this research.

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