Chemokines in tumor proximal fluids

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Chemokines are chemotactic cytokines produced by leukocytes and other types of cells including tumor cells. Their action is determined by the expression of cognate receptors and subsequent signaling in target cells, followed by the modulation of cytoskeletal proteins and the induction of other responses. In tumors, chemokines produced by neoplastic/stroma cells control the leukocyte infiltrate influencing tumor growth and progression. Tumor cells also express functional chemokine receptors responding to chemokine signals, promoting cell survival, proliferation and metastasis formation. Chemokines may be detected in serum of cancer patients, but due to the paracrine nature of these molecules, more significant concentrations are found in the tumor adjacent, non-vascular fluids, collectively called tumor proximal fluids. This review summarizes the expression of CC and CXC chemokines in these fluids, namely in interstitial fluid, pleural, ascitic, and cyst fluids, but also in urine, saliva, cerebrospinal fluid, cervical secretions and bronchoalveolar lavage fluid. Most comparative clinical studies reveal increased chemokine levels in high-grade tumor proximal fluids rather than in low-grade tumors and benign conditions, indicating shorter survival periods. The data confirm peritumoral fluid chemokines as sensitive diagnostic and prognostic markers, as well as offer support for chemokines and their receptors as potential targets for antitumor therapy.

Key words: tumor proximal fluids, chemokine, chemokine receptors, metastasis

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INTRODUCTION

Chemokines are a superfamily of 8- to 20-kDa chemotactic cytokines classified into four subfamilies on the basis of the number and relative positions of conserved N-terminal cysteine residues within the polypeptide. They are designated as C, CC, CXC, and CX_3C chemokines. The two major subfamilies are the CC chemokines, in which the cysteine residues are adjacent, and the CXC family, in which these residues are separated by one amino acid (Fig. 1). The chemokines of these two subfamilies are produced by leukocytes and by several types of cells, such as endothelial cells, epithelial cells, fibroblasts, and tumor cells.

Historically, the discovery of chemokines resulted from the studies of cytokine-like chemotactic activities initially attributed to IL-1. It appeared that chemokines were chemoattractants with cell specificity, as was shown on the first purified chemokine, a neutrophil chemoattractant named IL-8 (ref.[1]). The cell migration of target cells is determined by the level of cognate receptor expression at the plasma membrane of these cells and the receptor's responsiveness to chemokines. Today there are about 50 identified chemokines and 20 chemokine receptors.

Directed cell migration is primarily facilitated by the cell's ability to sense an external gradient of chemokines and chemotactic growth factors. It is widely accepted that these molecules are secreted into the extracellular space and retained there by binding to extracellular matrix glycosaminoglycans, thereby establishing immobilized gradients[2]. A chemotactic gradient can also be built by modifying the activity of chemokines through MMP9-mediated cleavage, or it can be shaped by removing the chemoattractant by scavenger receptors[3]. Due to the optical clarity of the zebrafish embryos, the migration of neutrophils and primordial germ cells can be imaged at a high resolution based on the dynamic expression of the homologs of the human CXCL8 and CXCL12, respectively, and their receptors[4].

The receptors for chemokines are G protein-coupled receptors. The ligand-receptor interactions may be unique (single ligand and single receptor) or promiscuous (single ligand/multiple receptors, or multiple ligands/single receptors). The activation of chemokine receptors leads to the production of second messengers, cytoplasmic calcium mobilization, and the activation of multiple downstream signaling cascades. This signaling can in turn modulate cytoskeletal protein configuration and integrin affinity. Cell migration is a spatially organized process that begins with the polarization of the cell, developing a distinguish-

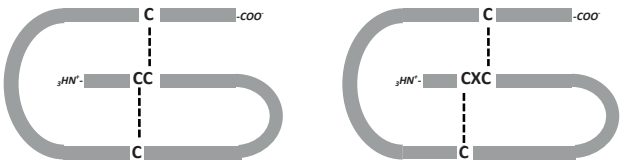

Fig. 1. Schematic representation of disulfide bridges between conserved cysteine residues (C) in the CC- and CXC- classes of chemokines.

able leading edge and trailing end, followed by changes in cell adhesion[3].

Since Wirchow's seminal observation that leukocytes are present in tumor tissues, empirical evidence has underscored inflammation as both a cause and a consequence of cancer. Chronic inflammation is linked to various phases implicated in tumorigenesis, such as cellular proliferation, transformation, apoptosis evasion, cancer cell survival, invasion, angiogenesis and metastasis[5]. It has been estimated that about 25% of all cancers are etiologically associated with chronic inflammation and infection. Chronic inflammation is also associated with the suppression of the host's innate and adaptive immune systems, which are essential for effective antitumor responses[6].

Chemokines produced by neoplastic and/or stromal cells control the nature of the inflammatory infiltrate by actively recruiting cells of the innate and adaptive immune systems. Some chemokines promote conditions favorable for tumor growth and progression, while others have antitumor activity. For example, CXCL8/IL-8 promotes tumor growth by inducing leukocyte cell migration, in contrast, CXCL10 can have angiostatic properties. Chemokines recruit tumor-associated macrophages (TAMs) that promote tumor progression, but when TAMs are recruited massively and appropriately activated, they can exert antitumor activity[6].

Many tumor cells also express functional chemokine receptors, undetectable on their normal counterparts. These receptors respond to chemokine signals by promoting cell survival, proliferation, adhesion, or migration, but also direct metastasis, the most common cause of death in cancer patients, as reported recently[7] (Fig. 2). The sites of distant metastasis are not random since certain tumors tend to develop metastases in specific organs where the corresponding ligands are secreted[8]. An important factor in cancer progression is the dysfunction of apoptosis. Tumor cells modify their microenvironment composition in several ways to avoid immune attack. These procedures include the alteration of surface molecule expression and the recruitment of immunosupressive cells. The pivotal role in cancer survival is played by T-reg cells, in lymphoma recruited by several factors such as chemokine CCL22 produced by cancer cells, and chemokine CXCL13 produced by the follicular helper cells[9].

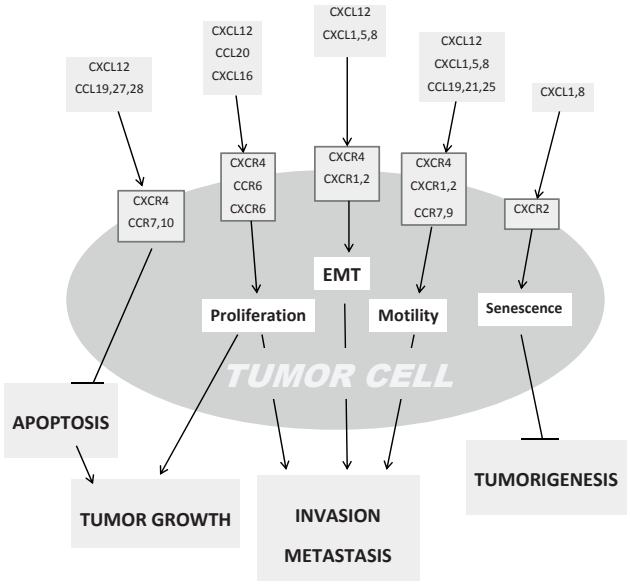

Fig. 2. Effects of chemokines on tumor cells.
Whereas most chemokines activating cancer cell chemokine receptors promote proliferative and invasive properties of cancer cells, specific CXCR2-binding chemokines can promote the arrest of cellular growth by inducing cellular senescence and delay the early phase of tumorigenesis. (EMT = epithelial-mesenchymal transition). Adapted from Mukaida et al., 2014 (ref.[7]).

The above data present chemokines as pathogenetic factors with tumorigenic activity. Elevated levels of circulating cytokines in the serum of cancer patients often correlate with advanced disease and diminished survival rate[10-13], but in many cases fail to discriminate between cancerous and non-cancerous conditions[14-16]. It appears that the concentrations of chemokines in other tumor-related body fluids are higher and more closely related to the tumor microenvironment than plasma. With regard to the paracrine nature of inflammatory cytokines, appearance in plasma may be considered as a byproduct of local production. In addition, the chemokine plasma concentrations are continuously reduced by the binding to cell receptors and to specific binding proteins, and by the kidney clearance[17].

This review extends the scope of our previous review article focused on CXCL8/ IL-8 (ref.[18]) to other clinically relevant chemokines, using the PubMed database and previous results from our laboratory[19]. The relevant articles (n=103) completed by the clinical/laboratory medicine handbooks provided the basis for this review.

CHEMOKINES IN TUMOR-ASSOCIATED BIOLOGICAL FLUIDS

It has become increasingly obvious that not only tumor cellular proteins (i.e. the *proteome*), but also proteins secreted or shed into the tumor microenvironment (i.e. the *secretome*) play a key role in the processes that shape the malignant nature of a tumor. A major advantage of studying the secretome, compared to the cellular proteome, is that proteins secreted by tumor cells are more likely to end up in body fluids in a measurable amount. The term cancer secretome comprises a multitude of sample types. The most straightforward and, as a result, the most studied secretome type, is the conditioned medium from cancer cell lines. However, cell lines are an *in vitro* system which ignores the contributions of the host-tumor microenvironment and thus provides no insight into the evolution of the disease[20]. A more complex image of tumor microenvironment is achieved through an analysis of tumor interstitial fluid (TIF), obtained by incubating small pieces of tumor tissue in a salt solution, or by microdialysis. Due to the presence of signaling molecules, the composition of TIF is more closely related to tumor progression and metastasis. Alternatively, components of the cancer secretome may be detected in the manifold

tumor adjoining extravascular fluids, collectively called *tumor proximal fluids* or tumor extravascular fluids[21]. They represent a reservoir of tumor secreted proteins *in vivo* without the large dynamic range and complexity of plasma or serum[22]. Some tumor proximal fluids are released into preformed body cavities or spaces (pleural, peritoneal space), or newly formed cysts. Cancer-related chemokines may also be found in different organ secretory products such as urine, saliva, cerebrospinal fluid, cervical fluid, and bronchoalveolar lavage/breath condensate fluids.

A valuable supplementation of clinical studies appear to be experiments using animal models. For example, the action of chemokines may be tested following the injection of cells engineered to express variable levels of a specific chemokine into the pleural cavity of mice

In the following paragraphs, numerous reports on the identification as well as the diagnostic/prognostic value of chemokines in individual tumor proximal fluids are summarized.

Interstitial fluid (ISF)

A fundamental method used for the identification of secretome products of interstitial fluids appears to be microdialysis. The diffusion of low molecular substances is almost complete at low flow rates.

In buffered saline perfusing invasive breast carcinoma, nine chemokines were detected[23], for details see Tables I, II. In adjoining studies, a significant positive correlation was found between CXCL8 and estradiol in hormone-dependent breast cancer in vivo[24]. ER^+ cancers produced high levels of extracellular CCL2 and CCL5, associated with infiltration by tumor-associated macrophages. These effects were inhibited by anti-CCL2 and CCL5 therapy[25]. Brain tumor patients undergoing craniotomy showed markedly elevated concentrations of CCL2, CCL3, CCL4, CXCL8, and CXCL10 in peritumoral tissue, decreasing over 96h following surgery[26].

Pleural fluid (PE)

A pleural effusion (PE) is an excessive accumulation of fluid in the pleural space and can be caused by a variety of diseases. The alteration of systemic factors that influence the formation and absorption of pleural fluid, e.g. by cardiac failure, cause a transudative PE, the alteration of local factors that influence the formation and absorption of pleural fluid, e.g. by infection or cancer, cause an exudative PE. The reported incidence of PEs in lung cancer patients varies between 25% and 53% (ref.[27]).

One report showed higher levels of CCL2 in malignant PEs than benign conditions[28]. In a more recent study, cancer cells engineered to express high or low levels of CCL2, injected into the pleural cavity of mice, positively affected the volume of PE, monocyte and macrophage recruitment, vascular permeability, and neoangiogenesis[29]. Concentrations of CCL22 in malignant PE were significantly higher than corresponding CCL22 serum values. Pleural fluid from lung cancer patients was chemotactic for regulatory T cells, and this activity was partly blocked by anti-CCL22 (ref.[30]).

In our previous study, PEs from lung cancer patients were analyzed for 13 inflammatory markers, among them chemokines CCL2 and CXCL8, and compared with PEs of non-malignant origin[19]. It appeared that the chemokines were highly expressed in PEs of paraneoplastic origin compared to transudates and serum levels. The predictive value for CXCL8 with respect to patient's overall survival was the best of all markers, yielding a correlation coefficient r=-0.36. Interestingly, in the sub-

Table 1. CC Chemokines in tumor proximal fluids.

Chemokine	Synonym	Receptor	Fluid / Ref.
CCL2	MCP-1	CCR2	ISF (21,23,24), PE (17,26,27), AF (32-35), CF (45), CSF (45,79)
CCL3	MIP-1a	CCR1	ISF (24), AF (32,33)
CCL4	MIP-1beta	CCR1,5	ISF (24), AF (32,35), CSF (81)
CCL5	RANTES	CCR5	ISF (21,23), AF (32,34)
CCL7	MCP-3	CCR2	ISF (21)
CCL8	MCP-2	CCR1,2,5	ISF (21), AF (32)
CCL10	MRP-2	CCR1	AF (35)
CCL11	Eotaxin-1	CCR2,3,5	ISF (21), AF (35)
CCL13	MCP-4	CCR2,3,5	ISF (21)
CCL14	HCC-1	CCR1	S (74,75)
CCL15	MIP-5	CCR1,3	AF (35), CS (85)
CCL17	TARC	CCR4	CSF (81)
CCL18	MIP-4	CCR8	AF (33,35), U (58)
CCL19	ELC	CCR7	S (76)
CCL21	SLC	CCR7	S (76)
CCL22	MDC	CCR4	PE (28), AF (32), CF (49)
CCL23	MIP-3	CCR1	ISF (21)
CCL28	MEC	CCR3,10	S (77)

Interstitial f luid (ISF), Pleural fluid (PE), Ascitic fluid (AF), Cyst fluid (CF), Urine (U), Saliva (S), Cerebrospinal fluid (CSF), Cervical secretions (CS), Bronchoalveolar lavage fluid (BAL)

Table 2. CXC Chemokines in tumor proximal fluids.

Chemokine	Synonym	Receptor	Fluid / Ref.
CXCL1	GRO1	CXCR2	U (57), S (76)
CXCL5	ENA-78	CXCR2	S (76)
CXCL8	IL-8	CXCR1,2	ISF (21,22,24), PE (17,29,30), AF (13,33-40), CF (13,46-53), U (58-63), S (66,68-76), CSF (46,81,82), CS (86,87), BAL (89,90), EBC (93)
CXCL10	IP-10	CXCR3	ISF (24), S (74,75), CSF (81)
CXCL12	SDF-1	CXCR4,7	PE (31), AF (41-44), U (64), S (76)

Interstitial fluid (ISF), Pleural fluid (PE), Ascitic fluid (AF), Cyst fluid (CF), Urine (U), Saliva (S), Cerebrospinal fluid (CSF), Cervical secretions (CS), Bronchoalveolar lavage fluid (BAL), Exhaled breath condensate (EBC)

group of metastatic lung cancer patients the correlation coefficient increased to r=-0.64. The high expression of CXCL8 in malignant PEs in comparison with transudate and tuberculous PEs was confirmed by Zaki and Ashour[31]. Similarly, higher levels of CXCL8 were also determined in mesothelioma PEs when compared with PEs in congestive heart failure[32]. The CXCL8 hyperproduction appears to be a common phenomenon in tumor proximal fluids. Typically, higher CXCL8 levels are found in more aggressive, expanding tumors of different origin when compared to low-grade tumors and premalignant conditions[18].

Expression analyses of both transcripts (TR1, TR2) of CXCL12 and its cognate receptor CXCR4 were carried out in pleural fluid pellet by RT-PCR and Western blot analysis. It was shown that CXCL12-TR1, but not CXCL12- TR2 or CXCR4 were higher in malignant PEs compared to transudates[33].

Ascitic fluid (AF)

The accumulation of fluid within the peritoneal cavity, called ascites, results from an imbalance between the influx and efflux of fluid from the peritoneal compartment. An early study of the expression of CC chemokines and their cognate receptors using both the mRNA and protein estimation revealed a complex chemokine/chemokine receptor network in ovarian cancer ascites[34]. Epithelial ovarian cancer is the leading cause of death among gynecological cancers. Most women presenting with advanced ovarian cancer have ascites, which in itself constitutes a unique form of tumor environment. AFs of these patients contain significantly higher levels of CXCL8 and CCL18 when compared with ascitic fluids from nonovarian carcinoma patients[35]. In a recent study, CCL2 and CCL5, too, were found elevated in most malignant ascites fluids[36].

The presence of chemokines in AF from a patient with metastatic melanoma was associated with a large influx of activated T cells, including $CD8^+$ T cells. Despite of recruitment of large numbers of activated $CD8^+$ T cells into the tumor environment, T cell hyporesponsiveness and additional negative regulatory mechanisms apparently limited the effector phase of the anti-tumor immune response[37].

CXCL8 concentrations were significantly higher in malignant ascitic fluids compared with AFs accompanying benign disorders[15,38,39]. CXCL8 ascitic levels were also significantly higher when compared with serum levels of cancer patients[40,41], and correlated with tumor grade[41,42].

CXCL12 levels were elevated in ovarian cancer patients compared to patients with serous cyst, as well as in FIGO III and IV patients compared with FIGO stage I patients. No significant differences were found in the plasma CXCL12 levels between different tumor grades[43]. Signals mediated by CXCL12 and its receptor CXCR4 were apparently involved in cancer progression by activating cancer cells, by inducing angiogenesis, and by recruiting T regulatory and plasmacytoid immune cells. Interestingly, both CXCL12 and CXCR4 were controlled by the tumor-associated inflammatory mediator prostaglandin E_2 (ref.[44]).

AFs from gastric cancer patients with peritoneal carcinomatosis contained high concentration of CXCL12 and were positive for the expression of CXCR4. It was found that CXCL12 stimulates cell proliferation, and induces phosphorylation of Akt and ERK in a CXCR4-expressing gastric carcinoma cell line. Hence, the CXCR4/CXCL12 axis may serve as a potential therapeutic target[45]. Increased concentrations of CXCL12 were also found in ascitic fluid from hepatocellular carcinoma patients. Cancerous ascitic fluid induced migration of hepatocellular cancer cell lines suggesting CXCR4/CXCL12 may play a role in metastasis by promoting the migration of tumor cells[46].

Cyst fluid (CF)

Cyst fluids are liquids contained in sac-like structures within tissues. Most cysts are harmless, formed by inflammatory and obstructive processes, or represent congenital abnormalities. Malignant cysts accompany cyst-forming neoplasms, such as ovarian or pancreatic tumors and malignant gliomas. CFs from patients with malignant glioma were rich in CCL2 (ref.[47]), CXCL8 protein was determined in CF of the brain glioblastomas and in CF of a metastatic tumor of the brain[48].

Cystic fluid CXCL8 levels from malignant ovarian tumors were significantly higher than benign tumors and

endometriomas. These differences were not reflected by serum CXCL8 levels[15,49,50]. Analysis of ovarian CFs for a series of cytokines revealed higher expression of CXCL8 and CCL22 in malignant CFs compared with benign samples. In the high-grade malignant serous group, an inverse relationship between CXCL8 levels and survival, yielding correlation coefficient of -0.68, was found[51].

The CXCL8 concentrations in pancreatic CF aspirates were higher in the CF from patients in the high-risk group compared to the low-risk group[52]. Similarly, CXCL8 levels in high-grade pancreatic tumors were 2.8 times higher than the levels in the low-grade lesions[53]. CXCL8 was also identified in a chondroblastoma cyst[54]. The ratios of expression of 15 pairs of the cytokines in odontogenic cysts and tumors of the jaw could assist in establishing diagnosis of lesions that were difficult to discern clinically and radiografically[55].

Urine (U)

The analysis of urine is a traditional method used to diagnose and predict the clinical outcome of a wealth of urinary tract diseases. Yet, a critical evaluation of commonly applied standards, namely urinary creatinine, osmolality, and protein, is needed[56].

CCL18 performed best as a biomarker for bladder cancer detection compared to urinary PAI-1 and CD44 (ref.[57]). The diagnostic value of CCL18 for the bladder cancer diagnosis was confirmed in a following study, but no association between CCL18 and a bladder cancer grade or stage was observed[58].

Urinary CXCL1 levels were found higher in patients with invasive bladder cancer than in those with noninvasive tumors and normal control, confirmed by analyses of secretory products from highly invasive and poorly invasive bladder carcinoma cell lines[59].

Urine-based evaluation of potential bladder cancer markers identified CXCL8 as the most prominent marker with 95% confidence interval[60]. Based on the previous detection of IL-8 in bladder carcinoma cell lines, urinary IL-8 levels were measured in patients differing in disease origin and stage. Urinary IL-8 levels were typically higher in subjects with invasive than in those with low-stage cancer[61-63]. Urine CXCL8 levels in patients with non-Hodgkin's lymphoma corrected to creatinine concentration were significantly higher in comparison with controls. In contrast, no significant differences were found in serum CXCL8 between the patients and controls[16]. The urinary CXCL8 performed best in bladder cancer detection in comparison to MMP-9 and VEGF, showing 90% sensitivity and 86% specificity[64]. The combination of three markers, namely CXCL8, VEGF, and apolipoprotein E, reached 90% sensitivity and 97% specificity in non-invasive bladder cancer detection[65].

Of six known CXCL12 isoforms generated by alternative mRNA splicing, beta-isoform appeared to be an independent predictor of metastasis and disease specific mortality in bladder cancer. The beta-isoform was detected in both bladder tissues and urine specimens. In exfoliated urotelial cells, beta-isoform was shown to have 91% sensitivity and 74% specificity for the detection of bladder cancer[66].

Saliva (S)

Saliva is easy to access and its collection is the least invasive for the patient of all body fluids. In the past two decades, the combination of emerging biotechnologies and salivary diagnostics has extended the range of saliva-based diagnostics from oral diseases to manifold diseases including cancer[67]. The most common oral cancer is oropharyngeal squamous cell carcinoma (OSCC), which makes up 90% of all oral cancers.

The early studies investigating cytokine biomarkers for OSCC recognized a high diagnostic and prognostic value of CXCL8, manifested by the increase of both salivary CXCL8 protein[68] and CXCL8 mRNA (ref.[69]). This observation was later confirmed by exploring a larger patient group[70]. The degree of elevation was consistent with OSCC histologic grading[71], and significant when compared to the precancer, chronic periodontitis, oral lichen planus, and healthy control groups[72-74]. In a similar fashion, CXCL8 levels in squamous cell carcinoma of the tongue correlated with increased metastasis and poor prognosis[75].

In addition to CXCL8, CXCL10 and CCL14, too, were significantly elevated in saliva samples of oral cancer patients[76,77].

Sahingur and Yeudall(ref.[78]) summarized data on the relationship between periodontal disease and oral cancer, based on studies communicating an association between chronic periodontitis and cancer. Number of chemokines and their receptors seemed to be implicated in oral squamous cell carcinogenesis, in particular CXCL1, -5, -8, -12 from the CXC subgroup, and CCL19, -21, and CCR7, from the CC subgroup.

The chemokine CCL28, a CCR10/CCR3 ligand, is commonly expressed by epithelial cells in different mucosal tissues. Surprisingly, CCL28 protein expression was found significantly lower in pleomorphic adenoma and adenolymphoma of salivary glands compared to normal adjacent tissues. The saliva CCL28 protein levels were also reduced when compared to healthy volunteers. A similar pattern of expression of CCL28 was formerly detected in colon tumors. It was hypothesized that the reduced expression of CCL28 might decrease the recruitment of antitumor immunocompetent cells to the affected salivary glands[79].

Cerebrospinal fluid (CSF)

The cerebrospinal fluid (CSF) is secreted from the chorioid plexuses in the cerebral ventricles and is absorbed through the arachnoidal villi in the subarachnoid space. CSF analyses contribute largely to the diagnosis of the brain and meningeal disorders, including the diagnosis of primary and secondary tumors of the CNS (ref.[80]). It should be noted that brain metastasis is estimated to occur in 10-30% of all cancer patients.

The CCL2 concentrations in CSF samples from patients with malignant glioma were significantly higher

than CSF CCL2 levels from patients with benign glioma and from patients with no tumor[47]. In leukemia with CNS metastasis, the high-CCL2 patients had shorter event-free survival. In general, CSF CCL2 levels were found more pronounced than serum levels, suggesting CSF analysis may be more potent than serum analysis in predicting CNS metastasis and the disease outcome[81]. However, the CSF CCL2 was also found increased after induction of leukemia chemotherapy, suggesting that standard leukaemia treatment may cause a subclinical inflammation and neurotoxicity[82].

Melanoma brain metastasis reconfigure the chemokine and cytokine CSF profile, increasing CCL4, CCL17, CXCL8, and CXCL10 on one hand, and reducing CCL22 on the other hand[83].

Cerebrospinal fluid CXCL8 was found both in neoplastic and infectious diseases of the human CNS[48], and its increased levels distinguished leptomeningeal metastases from systemic malignancies without CNS metastases[84]. Typically, higher CSF CXCL8 levels in metastatic brain tumors were associated with short-term survival[85].

Cervical secretions (CS)

Secretions of the uterine cervix are rich in immunoregulatory proteins including cytokines and chemokines. Various tools for collecting CS were tested, among them ophthalmic sponges, yielding a very beneficial recovery[86].

It appears that cervical intraepithelial neoplasia (CIN) is strongly associated with human papillomavirus (HPV) infection. CCL11was found increased in HPV positive patients, whereas CCL15 was found increased in both HPV and CIN patients[87]. CXCL8 levels were higher in endocervical and/or vaginal secretions of CIN patients, compared to patients with bacterial vaginosis and to controls[88]. In patients with cervical cancer, CS CXCL8 concentrations exceeded serum levels[89].

Bronchoalveolar lavage fluid (BAL) and exhaled breath condensate (EBC)

Bronchoalveolar lavage fluid is obtained by repeated washings with aliquots of sterile saline using a flexible fibre-optic bronchoscope positioned in a subsegmental bronchus. BAL contains a broad spectrum of proteins, which are either released locally by epithelial or inflammatory cells, or enter through plasma exudation[90]. In a recent study, significantly higher levels of CXCL8 were found in BAL of lung cancer patients when compared to patients with nonspecific chronic inflammation and normal controls. These levels positively correlated with the number of neutrophils and lymphocytes[91]. In lung cancer patients, higher serum and BAL CXCL8 levels were associated with shorter survival[92].

Exhaled breath condensate (EBC) is a non-invasive method of sampling airway lining fluid. In contrast to small molecule biomarkers identified in EBC, proteomic analysis identified just a limited number of protein biomarkers due to methodological hurdles . Among identified chemokines, CXCL8 seems to be significantly elevated in lung cancer compared to controls[93].

CONCLUSION

This review summarizes currently available data on the presence and diagnostic and prognostic value of chemokines in tumor proximal fluids. Certain chemokines may serve as humoral markers to differentiate between benign and malignant affections. Moreover, the expression of selected chemokines in tumor proximal fluids is directly proportional to tumor grade and, according to numerous observations, indirectly proportional to overall survival[19,51,85,92]. In regard to the diagnostic and prognostic role of chemokines it should be noted, however, that many chemokines are post-translationally modified by proteolytic cleavage, which may render an agonist more active or inactive or even convert the active chemokine into a receptor antagonist. Consequently, more refined methods are needed to indicate not only intact chemokines, but also those modified posttranslationally[76,77].

The investigation of tumor proximal fluid provides a unique opportunity to directly study the composition of tumor microenvironment, and to gain more insight into tumor-host interactions without distortion following the passage of regulatory molecules into the systemic circulation. An important topic is the relationship between chemokines and superior regulatory molecules. For example, investigation of ISF samples of breast cancer tissue using microdialysis revealed a significant positive correlation between CXCL8 and estradiol, suggesting that estradiol plays a critical role in the regulation of CXCL8 (ref.[24]). In a recent study exploring experimental breast cancer, estradiol enhanced macrophage influx and angiogenesis through increased release of CCL2, CCL5, and VEGF, indicating the potential of novel therapies targeting responsible chemokine pathways[25]. In this respect, the targeted silencing of CCL2 gene in breast cancer non-responding to conventional therapy inhibited primary tumor growth and metastasis, associated with a reduction in recruitment of M2 macrophages[94]. Alternatively, in ascites fluid from ovarian cancer patients, both CXCL12 and CXCR4 were controlled by the tumor-associated inflammatory mediator prostaglandin E_2. COX2 inhibition blocked CXCL12 production, providing a rationale to target PGE_2 signaling in ovarian cancer therapy[44].

The action of chemokines is closely associated with the composition of inflammatory infiltrate in solid tumors. Regulatory T cells, found in tumor microenvironments, may suppress T cell responses to tumors and thereby promote the growth of human lung cancer. Chemokine CCL22, detected at high concentrations in malignant PEs, appears to induce regulatory T cell migration into the pleural space[9,30].

Chemokine receptors expressed on the surface of cancer cells represent suitable targets for the generation of new anti-tumor drugs controlling cancer invasion and metastasis. The most widely expressed chemokine receptor among cancers is likely CXCR4 (ref.[95]). Preclinical development of novel CXCR4 antagonists is currently in progression[96-102]. However, it must be kept in mind that CXCR4 plays a critical role in embryogenesis, homeo-

stasis, and inflammation in the fetus. Therefore, caution should be taken when inhibition of the SDF-1-CXCR4 signaling pathway is applied in human subjects[103].

Search strategy and selection criteria

Data for this Review were identified by search of the PubMed database and references from relevant articles using the search terms "chemokine and cancer" and "chemokine and a respective fluid type". Only articles published in English between 1990 and 2016 were included. Abstracts and reports from meetings were not included.

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