Minireview *Highlight article*

Chemokine-like factor 1: A promising therapeutic target in human diseases

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Impact statement

CKLF1, a recently identified chemokine, has been reported by a number of studies to play important roles in quite many diseases. However, the potential pathways that CKLF1 may be involved are not manifested well yet. In our review, we showed the basic molecular structure and major functions of this novel chemokine, and implication in human diseases, such as tumors. To attract more attention, we summarized its signaling pathways and clearly present them in a set of figures. With the overview of the experimental trial of CKLF1-targeting medicines in animal models, we hope to provide a few important insights about CKLF1 to both medical researchers and pharmacy.

Abstract

Chemokines are a family of small molecular-weight proteins, being well-known with their important roles in the process of allergic response, immune regulation, cell proliferation, and differentiation. Almost two decades ago, a novel member of this family, chemokine-like factor 1 (CKLF1) was identified. In recent years, it draws much attention of researchers because of its key roles in many types of tissues and its potential implications in a large number of diseases. Up to now, CKLF1 has been shown to have two main functions: broad-spectrum chemotactic activity, and proliferation- and differentiation-promoting abilities. In this review, we would introduce the basic structural features of CKLF1 and its biological functions, and then elaborate the relationships between CKLF1 and human diseases. The cell signaling pathways CKLF1 may be involved in would be discussed and summarized in details. Furthermore, we present the trials of CKLF1-targeted treatments in animal disease models, hoping to provide a few important insights about CKLF1 to both medical researchers and pharmacy, and finally conclude that CKLF1 is a potent, and very promising, therapeutic target.

Keywords: Chemokines, chemokine-like factor 1, human diseases, tumors, signaling pathways, targeted therapy

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Introduction

Cytokines are a family of soluble proteins with small molecular weight, which are secreted by both immune cells and non-immune cells while being induced by immunogens, mitogens, or other stimulants. In the process of immune response, immune cells stimulate and restrain each other through many kinds of cytokines with different biological effects. An ordered cytokine network regulates the immune response and maintains the homeostasis of the immune system.[1] According to their characteristics of functions, cytokines can be divided into chemokines, colony-stimulating factors, tumor necrosis factors, growth factors, interleukins, interferons, and so on.

As a type of cytokines, chemokines are polypeptide signaling molecules which have the ability to promote the directed chemotaxis of nearby reaction cells. Such molecules are all approximately 8–13 KD in mass. According to the expression of its amino terminal cysteine motif, chemokines have been divided into four categories: CC chemokine, owning two adjacent cysteine (C) residues in the motif; CXC chemokine and CX3C chemokine, with one or three amino acid (X) to separate the two cysteine residues, respectively; and then C chemokine, which has only one cysteine residue near the NH_2 end.[2]

Being the largest category of chemokines, CC chemokines mainly attract monocytes and subset of lymphocytes

to sites of chronic inflammation. The most common characterized CC chemokine is monocyte chemoattractant protein 1 (MCP-1)/CCL2, which is a potent agonist for monocytes, basophils, and dendritic cells. The others include macrophage inflammatory protein (MIP)-1α/CCL3, MIP-1β/CCL4, and so on.[2] In the second category of chemokines, interleukin-8(IL-8)/CXCL8 is the most well-known one. IL-8 can attract leukocytes to the acute inflammatory sites and activate monocytes.[3] Unlike the first two groups of chemokines, CX3C group has only one member, fractalkine/CX3CL1, which is shown to be up-regulated in activated vascular endothelial cells. It mediates the rapid arrest of cells flowing.[4] Lymphotactin/XCL1 is the alone member from the fourth category and has a vital effect in dendritic-cell-mediated cytotoxic immune response.[5]

In the past few decades, several novel chemokines, with different structural features comparing to typical chemokines, have been identified. These atypical chemokines are grouped as chemokine-like factor (CKLF) family.[6] In 2001, Han *et al.* identified a new cytokine, namely chemokine-like factor 1 (CKLF1). Since then, they discovered more chemokine-like molecules, which were designated as chemokine-like factor super family (CKLFSF) together with CKLF1. Because at least one spliceosome of genes in this family shared a common domain, called MARVEL, CKLFSF1–8 were thereby renamed as the chemokine-like MARVEL transmembrane domain-containing 1-8(CMTM1-8), by the international human genome naming committee.[7] Up to now, CKLFSF consists of nine genes which are CKLF and CMTM1-8.

CKLF, as the first identified member of this family, is located at chromosome 16q22.1. It has four RNA splicing isoforms, namely CKLF1-4, encoding 99, 152, 67, and 120 amino acids, respectively. Among them, CKLF2 has the full-length transcript of CKLF. It shares two exons with other three isoforms. CKLF1, as the most researched isoform, has three exons and two helical regions (Figure 1(a)). CKLF1-4 has different expression patterns in the cells, among which CKLF1 and CKLF2 are highly expressed, while CKLF3 has the lowest expression level. Besides, CKLF1 and CKLF3 are secreted molecules, whereas CKLF2 and CKLF4 are belonging to trans-membrane molecules.[8]

Till now, rat and murine homologues of human CKLFs have been found.[9,10] There are two rat and four murine homologues, which are rat CKLF1, rat CKLF2, and murine CKLF2, 4, 5, 6. Among them, rat CKLF1 and murine CKLF4 are most similar with human CKLF1 (Figure 1(b)). Although such CKLF isoforms from rat, mice, and human have different tissue distributions and contain different motifs, their bioactivity has been conserved to some extent.[9]

In recent years, more and more evidence indicate that CKLF1 is involved in the occurrence and development of many human diseases, including bronchial asthma, cerebral ischemia, and tumors. In this review, we will not only introduce the structural features of CKLF1 and its biological functions, elaborate the relationships between CKLF1 and human diseases, but also summarize the cell signaling pathways of CKLF1. The CKLF1-targeted therapy trials in animal disease models would be presented and discussed in the last. We hope to provide a few important insights about CKLF1 to medical researchers and pharmacy as well.

Special biological structure of CKLF1

CKLF1 cDNA encodes 99 amino acids, with the length of 530 bp.[8] Its protein is highly alkaline hydrophobic. It has been indicated that CKLF1 is a secretory protein, having similarity with CC family chemokines TARC and STCP-1, and they share a functional chemokine receptor, CCR4.[11] However, CKLF1 is different from other chemokines of CC family as followed: (1) mature CKLF1 protein only has a continuous CC structure, and its carboxyl terminal has no other cysteine; (2) the similarity with other chemokines is not very significant; (3) it has at least three other different RNA transcript isoforms; (4) it can stimulate the growth of skeletal muscle cells.[8]

CKLF1, with CCR4 as its functional ligand in human, contains at least two peptides, C27 and C19.[12] Compared with C27 peptide, the N-terminal of C19 peptide lacks eight amino acids, which makes the effect of C27 peptide on chemotaxis and calcium flux be stronger than that of C19 peptide. To be noticed that, C19 peptide was indicated to have inhibitory effects on chemotaxis conversely, mediated by CCR3 and CCR4 in mouse and human separately. In addition, it was found that Lys residues in C19 peptide may

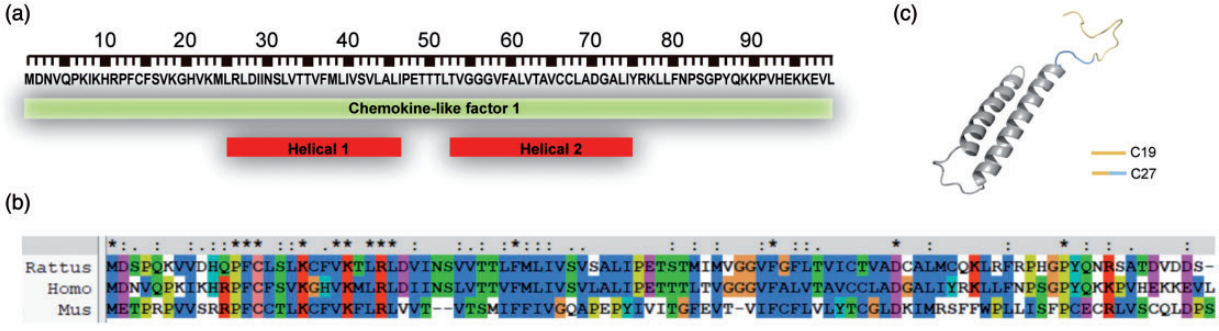

Figure 1. (a) Schematic structure of chemokine-like factor 1 (CKLF1). (b) Amino acid sequence alignment of CKLF1 with ClustalX software, across human, rat, and mouse. (c) 3D protein structure of CKLF1 predicted by PyMOL software. Its C19 and C27 peptides were marked. (A color version of this figure is available in the online journal.)

have a crucial role in the recognition of C19 peptide and heparin, and the binding activity of C27 peptide to heparin was higher than C19 peptide.[13] To have a clear picture of CKLF1, we predicted its 3D protein structure here, and showed the positions of C19 and C27 (Figure 1(c)).

CKLF1 expression profile

CKLF1 and its isoforms are highly expressed in adults' spleen, lung, testis, ovary, peripheral blood leucocyte, placenta, and pancreas. Relatively, they are expressed lower in human skeletal muscle, liver, thymus, colon, and prostate. And in adult brain tissue, kidneys, heart and small intestine, CKLF1 is almost undetectable.[8] Comparing with the adults, the expression levels from lung, renal, liver and spleen in fetal are lower, however, the expression levels in brain, heart, thymus, skeletal muscle are higher. This suggests that CKLF1 may be involved in human organ development and the physiological process of human body.

Biological functions of CKLF1

Chemotactic activity

White blood cells move directionally along the concentration gradient of chemical stimuli, which is called chemotactic reaction. Under inflammatory conditions, the expression of specific chemokines with potent chemotactic activity is markedly upregulated.[14] As shown in previous studies, CKLF1 has broad chemotactic activity on various cells (summarized in Table 1).

Han *et al.*[8] found that CKLF1 could significantly induce chemotaxis to neutrophils, lymphocytes, and monocytes *in vivo*, which was consistent with the chemotactic results *in vitro*. In addition, another study also indicated that CKLF1 could induce the infiltration of inflammatory cells and the proliferation of airway epithelial cells in lung, which can cause pulmonary inflammatory lesions.[15] In line with the above, it was found that human CKLF1 could promote the proliferation and migration of human aortic endothelial cells, and this chemotaxis could be blocked by the pertussis toxin.[29] With CELLocate cell localization method, Wang *et al.* showed that CKLF1 could exert chemotaxis on SH-SY5Y cells and then inducing the migration movement. Besides, their research also found that CKLF1 promoted the migration of primary cortical neurons in rats by means of inducing actin polymerization in a dose-dependent manner.[17]

Promoting proliferation and differentiation

CKLF1 has proliferation- and differentiation-promoting abilities in many cell types, such as skeletal muscle cells, bone marrow cells, vascular smooth muscle cells, and hematopoietic stem/progenitor cells[8](Table 1). Han *et al.*[8] found that CKLF1 could promote the proliferation of mouse skeletal muscle cells *in vitro* and also enhance proliferation and differentiation *in vivo.* Intriguingly, CKLF2 can promote the proliferation and differentiation of mouse C2C12 cells.[22] We can conclude that CKLF family plays a key role in the differentiation and development of skeletal muscle and this may be instructive for treating human muscle wasting.

A recent study found that in the state of vascular inflammation, CKLF1 was involved in the migration and proliferation of vascular smooth muscle cells, which leaded to the formation of neointimal.[23] Besides, CKLF1 exhibited enhancement effect of growth in human umbilical vein endothelial cells; meanwhile, C27 peptide showed more effect of promoting proliferation than C19 peptide.[24]

Surprisingly, although most CC family chemokines have inhibitory effect on the proliferation of hematopoietic stem cells, CKLF1 has obvious pro-proliferation and pro-differentiation effects in human low-density bone marrow cells and mouse bone marrow cells. For example, it could promote the proliferation of bone marrow CD34+ cells and increase the peripheral blood CD34+ cells in a rat myocardial infarction model.[21] Besides, a synergistic effect with GM-CSF had been observed in the bone marrow cells.[25] In accordance with this, the ability of GFU-GM colony formation was also shown to be increased in Ke *et al.*'s[26] study, suggesting that CKLF1 can promote proliferation and colony formation of human hematopoietic progenitor cells.

Other functions

In addition to the functions mentioned above, CKLF1 has also other abilities (Table 1). A study found that

Table 1. Functions of CKLF1.

Functions	Targeting cells	Related diseases	References
Chemotactic activity	Neutrophils, lymphocytes and monocytes	Asthma, acute respiratory distress syndrome, and severe acute respiratory syndrome	8,15,16
	SH-SY5Y cells and cortical neurons	Promote neurological development	17
	Lymphocyte and macrophage	Cerebral ischemia/reperfusion	18–20
Promote proliferation and differentiation	Macrophage and fibroblast	Pulmonary fibrosis	15
	Bone marrow CD34+ cells	Treating myocardial infarction	21
	Murine skeletal muscle cells	Treating muscle wasting	8,22
	Rat vascular smooth muscle cells	Atherosclerosis and restenosis	23
	Dermal microvascular endothelial cells	Psoriasis	24
	Mouse bone marrow hematopoietic stem/progenitor cells	Treating hematopoietic disorders	25,26
Others	Inhibit the synthesis of DNA, collagen and proteopolysaccharides	Rheumatoid arthritis	27
	Seminiferous tubule injury	Infertility	28

conditioned medium from 293 T cells with overexpression of CKLF1 could inhibit the synthesis of DNA, collagen, and proteopolysaccharides in rabbit articular chondrocytes, and increase the transcription of induced nitric oxide synthase (iNOS), which may be very important in the initiation and development of inflammation.[27] Last but not least, CKLF1 may have functions in the reproductive system of mice. As Zhong *et al.* showed in their work, the testicular tissues of male mice with exotic expression of CKLF1 were significantly changed. In their mice, spermatozoa number was reduced, and several pathological changes happened in the seminiferous tubules, which eventually resulted in infertility.[28] Taken together, CKLF1 can affect the synthesis of DNA, collagen and proteopolysaccharides and spermatogenesis.

Relationships between CKLF1 and the tumors

CKLF1 has been reported by a number of studies previously to show its implication in many human diseases. For example, CKLF1 was shown to contribute to airway damage and pulmonary fibrosis in lung.[15] And through mediating vascular smooth muscle cells (VSMCs) migration, CKLF1 showed a boosting effect in intimal hyperplasia.[30] Considering that the relationships between CKLF1 and many human diseases have been reviewed before, we will take some different types of tumors as main examples here, to show the important but also complicated roles of CKLF1 in tumors and tumor development.

As a hot topic in tumor field, tumor microenvironment (TME), firstly proposed by Lord, is mainly composed of tumor cells, stromal cells, extracellular matrix, and cytokines.[31] The main features of TME are hypoxia, chronic inflammation, and immunosuppression. Chemokines can directly or indirectly affect tumor stem cells, thereby affecting the tumor cell proliferation, stemness, and angiogenesis. Chemokines-chemokine receptor signaling pathways act on various stages of tumor occurrence and development, and exert bidirectional regulation on tumor biological behaviors. As found before, CCL2, CCL3, and CCL5 possess direct pro-tumor effects, whereas CXCL8, CXCL9, and CXCL10 function as tumor suppressors.[32] So what and how many roles does CKLF1 play in tumors? Promoting and/or inhibiting tumors?

Malignant tumors

Chemokines can orchestrate the interaction among parenchymal liver cells and nonparenchymal cells, and the disordered cellular interactions can change the hepatic microenvironment to a proinflammatory, profibrotic, proangiogenic, and thus preneoplastic milieu.[33] Recently, a research found that the expression level of CKLF1 was much higher in cancerous tissues than that in non-cancer tissues from patients with liver hepatocellular carcinoma (LIHC), and CKLF1 was more highly expressed in advanced tumors than in low-stage tumors.[34] Here we confirmed that CKLF1 was up-regulated in LIHC patients, based on large-scale RNA_Seq data from TCGA (Figure 2 (a)).[35] Excepting tumor staging, CKLF1 was also shown to be associated with vascular invasion, and patient survival.[34] Besides, based on xenotransplantation experiments in nude mice, CKLF1 was shown to not only promote the carcinogenesis and metastasis potential of liver cells, but also accelerated the progress of hepatocellular carcinoma (HCC) by activating the IL6/STAT3 pathway and then prevent adriamycin-induced apoptosis.[34]

In addition, CKLF1 expression was much more in ovarian cancer samples, comparing with normal control (Figure 2(a)). Moreover, CKLF1 can be also detected in normal ovarian tissue, though in very low level, which may be related to its normal physiological chemotaxis.[36]

Not to be upregulated, CKLF expression was downregulated in lung cancer tissues, among which in squamous cell carcinoma tissues the expression was significantly lower than that in lung adenocarcinoma tissues,[37] which was confirmed by analyzing a gene expression omnibus (GEO) dataset (Figure 2(b)).[38] Similarly, CMTM3 and CMTM5 from the same CKLFSF family with CKLF1 were also found to be downregulated in a spectrum of different carcinoma cell lines, with hypermethylation on their promoter regions.[39,40]

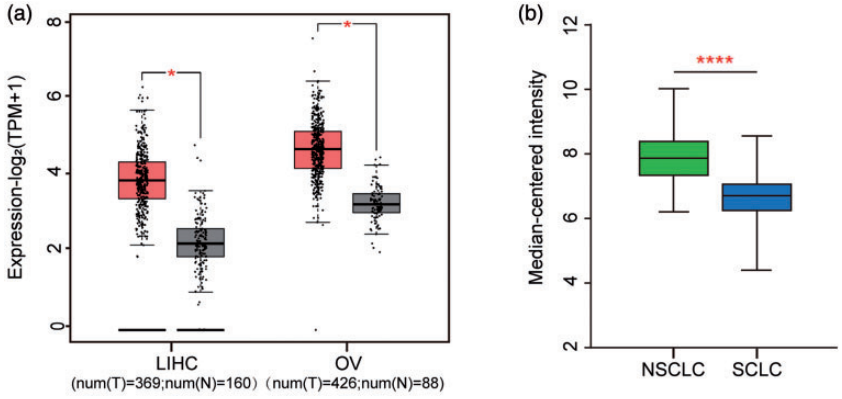

Figure 2. (a) Examplified expression pattern of chemokine-like factor 1 (CKLF1) in two different tumors. TCGA dataset was employed and figure was made via a web-tool, GEPIA2. (b) Differential expression of CKLF and its variants between non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). Microarray data were downloaded from gene expression omnibus (GEO), with accession numbers of GSE8894. N=normal with grey color, T=tumor with red color. NSCLC, $n = 61$; SCLC, $n = 72$. *$P < 0.01$, ****$P < 0.0001$. LIHC: liver hepatocellular carcinoma; OV: ovarian serous cystadenocarcinoma. (A color version of this figure is available in the online journal.)

Taken together, we can speculate that CKLF1 may be an important chemical chemokine that reflects the occurrence, progression, biological behavior, and prognosis of tumors, and affects the biological behavior and prognosis of malignant tumors.

Benign tumors

Keloid is one type of benign skin tumor. During the process of skin damage and self-healing, collagen anabolic function loses normal restraint control which induces excessive proliferation of collagen fibers and then eventually the formation of keloid.[41] At present, the pathogenesis of keloid is still not clear. By comparing skin samples of keloid patients, scar patients, and normal people, Zhang *et al.* found that the protein expression of CKLF1 in keloid patients was significantly increased, accompanied by up-regulated mRNA expression. They concluded that CKLF1 expression and inflammation may be one of the causes of scar tissue formation, and CKLF1 may contribute to the identification of keloid susceptible individuals.[42]

Aortic aneurysm refers to the expansion or bulging of the local aortic wall, could reach 1.5 times of the normal diameter. When it occurs in the abdominal aorta, it is called abdominal aortic aneurysm (AAA). It was suggested that local inflammation in the aorta of AAA patients may have important influence in the development of AAA. Especially, the infiltration of monocyte-macrophage, lymphocyte activation, and chemotaxis induced by cytokine is very crucial.[43,44] It was found that osteopontin and CKLF1 expression levels were higher in rats with AAA than that from sham and control, which induced the upregulation of matrix metalloproteinase-2 (MMP2). The elevated MMP2 would accelerate the degradation of the extracellular matrix of the arteries, eventually leading to AAA.[45]

Overall, CKLF1 expression can be dysregulated in both malignant tumors and benign ones. And this dysregulation is not only related with tumor occurrence, but also tumor stages. With more datamining work of pan-cancer in TCGA, people may see more complex dysregulation patterns of CKLF1. It gives us a hint that the role of CKLF1 would be quite divergent and dependent on the tumor or disease model of interest.

Relationships between CKLF1 and other diseases

In the respiratory system, CKLF1 may be involved in the development of allergic rhinitis,[46] asthma,[16] airway damage, and pulmonary fibrosis.[15] In animal models of such diseases, researchers found that CKLF1 got highly expressed. In particular, when blocking or antagonizing the interaction between CKLF1 and CCR4, the severity of these diseases would be reduced.[47] CKLF1 is also involved in the occurrence of cardiovascular and cerebrovascular diseases. It has been found that increased expression of CKLF1 on vascular smooth muscle cells would promote cell migration and proliferation, and further induce the appearance of atherosclerosis in mice.[23,30] The similar outcomes were also found in human samples.[48] Chen's laboratory conducted serial in-depth researches on the role of CKLF1 in cerebral ischemia/reperfusion injury. They found that CKLF1 was involved in cerebral ischemia by affecting the blood–brain barrier,[19] the inflammatory response,[20] and energy metabolism[49] at the damaged site. Recently, they showed that CKLF1 could promote the polarization of microglia/macrophages towards M1 at early stage of cerebral ischemic injury, which would further deteriorate the inflammatory response.[50]

Potential signaling pathways CKLF1 involved in

CKLF1 can bind to CCR4 on the cell surface and play a series of biological roles. CCR4 is a seven-transmembrane G protein-coupled receptor, which is expressed in various tissues and located on the surface of T cells, NK cells, monocytes, and eosinophils. In addition to binding to CKLF1, CCR4 can also interact with thymus and activation-regulated chemokine (TARC) and macrophage-derived chemokine (MDC). Several studies have found that the interaction of CCR4 and its ligands played an important role in a variety of inflammatory diseases, and was closely related to the metastatic mechanisms in several malignant tumors.[51] A large number of Treg cells with CCR4 expressed exit in tumor microenvironment. CCR4 can specifically activate the aggregation and infiltration of Treg cells by binding with its ligands TARC and MDC, causing immune escape and adverse clinical consequences, which has been confirmed in ovarian, gastric, and breast cancer.[52] In this section, we will summarize and present how and what signaling pathways CKLF1 would be involved in, with the scenario of three different diseases.

NF-κB is an important dimeric nuclear transcription factor, which broadly exists in nearly all tissues and cell types of animals. It participates in the body's inflammatory response and immune response and is essential for coordinating inflammatory responses. With a lot evidence supported, NF-κB signaling pathway was found to be involved in CKLF1-induced asthma pathological changes[16] (Figure 3). Bronchial asthma is an inflammatory allergic disease, which mainly happens in type II helper T cells (Th2) lymphocytes, and is characterized by airway inflammation, airflow obstruction, and airway hyperresponsiveness. Th2 cells can secrete much additional IL-4, IL-5, IL-9, and IL-13 to disturb the innate and acquired immune system, then lead to asthma.[53] In a mouse asthma model, CKLF1 was shown to up-regulate the expression of IKK in the cytoplasm of lung tissue, while reversely reduce the expression of IκB. Similarly, in rat arterial injury models, when the balance of vascular smooth muscle cells (VSMCs) proliferation and apoptosis was disturbed following artery injury of CKLF1, intimal hyperplasia was then initiated through PI3K/AKT/NF-κB signaling pathway.[16]

More complicatedly, CKLF1 can induce ischemic encephalopathy by playing around in two signaling pathways, MAPK and AKT/NF-κB. In focal cerebral ischemia and reperfusion of rats, CKLF1 was shown to be up-regulated. And the use of anti-CKLF1 antibodies could reduce infarct size and water content, via interfering the activation of the MAPK signaling pathway (including p38, ERK and JNK), which would eventually inhibit the expression of ICAM-1 and VCAM-1 and reduce the

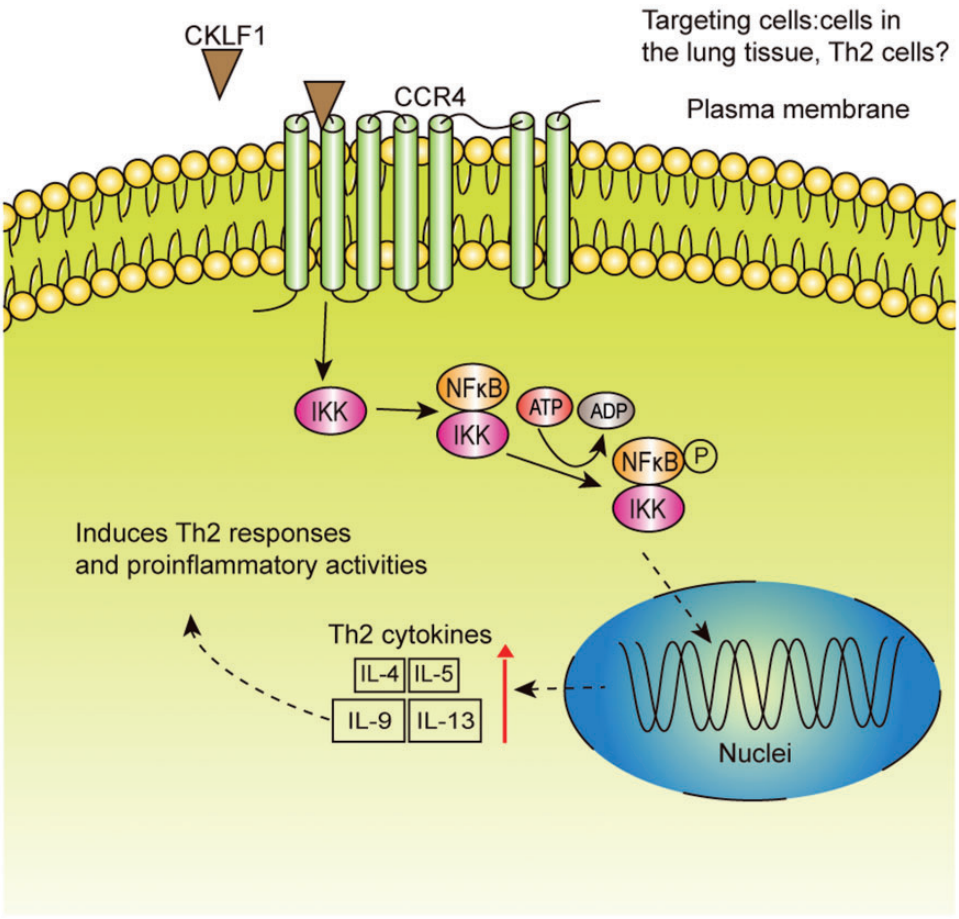

Figure 3. Schematic overview of chemokine-like factor 1 (CKLF1) involved in asthma via NF-κB signaling pathway. In a mouse model of asthma, CKLF1 expression was increased in lung tissue, meanwhile, NF-κB was less localized in cytoplasm but more in nucleus which may induce Th2 cells to secrete pro-inflammatory cytokines. (A color version of this figure is available in the online journal.)

production of TNF-α, IL-1β, MIP-2, and IL-8.[20] Moreover, from different studies, it was suggested that anti-CKLF1 antibodies could reduce the apoptosis of ischemia area and improve glucose metabolism via the AKT signaling pathway.[49] As a CKLF1 antagonist, IMM-H004 was shown to have protective activities in ischemic brain and ischemic stroke with cardiopulmonary complications. IMM-H004 could reduce the release of inflammatory factors in the heart, brain, and lung tissue via the NF-κB signaling pathway, such as IL- 1β, TNF-α, Caspase-3, Bax, Bcl-2, and so on[54] (Figure 4).

When it comes to tumorigenesis and tumor development, PI3K/Akt/NF-κB and JAK/STAT signaling pathway shall be involved. NF-κB is proposed as a tumor driver. Activated NF-κB pathway can promote positive feedback release of inflammatory signaling molecules (such as IL-6, IL-8 and TNF-α), increase expression of anti-apoptotic genes (such as Bcl-2, Bcl-xL and BIRC5), and induce the expression of mitotic proteins (such as c-Myc and Cyclin D1). Besides, it was suggested that PI3K/Akt/NF-κB could mediate epithelial-mesenchymal transition, decrease the apoptosis of tumor cells, and promote tumor cell proliferation, such as lung tumor and prostate tumor.[55,56] Excepting PI3K/AKT/NF-κB signaling pathway, JAK/STAT signaling pathway also played a key role in tumor development. JAK/STAT signaling pathway is very essential for many cytokine receptor systems, and it regulates cell growth, survival, differentiation, and resistance to pathogens. Abnormal IL-6 signaling and activation of JAK mutations are implicated in the pathogenesis of autoimmune diseases, cancers (such as prostate cancer, multiple myeloma), and inflammation.[57,58] In a mouse HCC model, CKLF1 was indicated to promote the occurrence and metastasis of cancer.[34] This study found that high expression of CKLF1 increased the levels of STAT3-related cytokines (such as IL17A, TNF-α and VEGF), stimulated the expression of anti-apoptosis and mitogenic proteins (Bcl-xL and MYC), and rendered tumor cells to go through aerobic glucose fermentation transformation (Figure 5). Such changes would disappear, once the STAT3 expression blocker static was employed as a treatment. To be noticed that, different with CKLF1 antibodies or antagonists, overexpression of CKLF1 could prevent the doxorubicin-induced apoptosis of HCC cells.

Overview of CKLF1-targeted therapeutic applications

As stated above, the potential signaling pathways CKLF1 is involved would be differential under different disease models. Hence, deciphering the mechanism of signal transduction in the context of diseases would be needed to

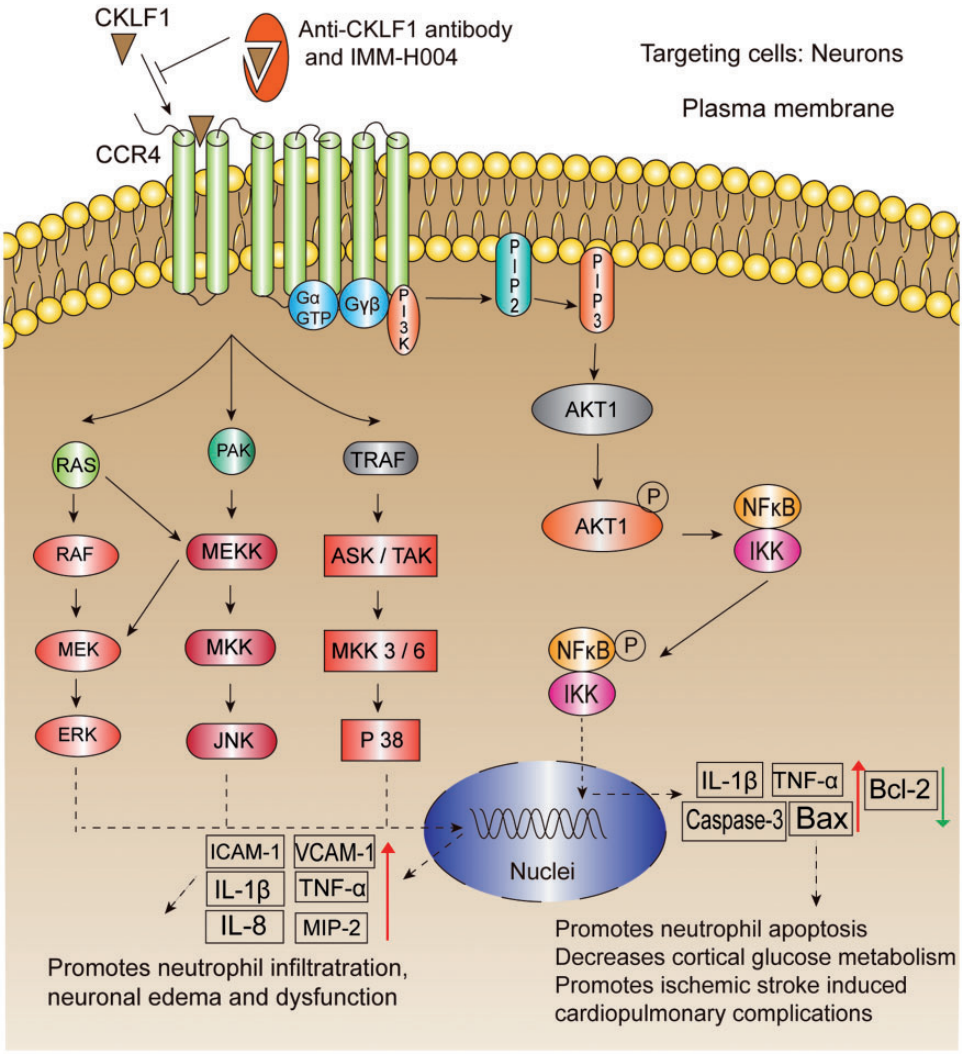

Figure 4. Schematic overview of chemokine-like factor 1 (CKLF1) involved in ischemic encephalopathy via MAPK and AKT/NF-κB signaling pathway. In the rat ischemia-reperfusion model, CKLF1 expression was increased at the ischemic site. When anti-CKLF1 antibody and IMM-H004 were added, the inflammatory response at the ischemic site was reduced, neuron degeneration and apoptosis and glucose metabolism got rescued. (A color version of this figure is available in the online journal.)

deepen our knowledge of the pathogenesis and to provide new diagnosis and treatment methods. The agonists and inhibitors of signal transduction molecules are the starting point for the development of signal transduction-based drugs.

To learn from other chemokines, we would firstly summarize the clinical trials targeting chemokines and/or their receptors. In the whole scenario of chemokines-targeted therapies, there are two most successful applications, intervening CCL2-CCR2 (Table 2) and CXCL12-CXCR4 axis (Table 3). CCL2-CCR2 signaling was suggested to be involved in tumors, diabetes, atherosclerosis, and so on. While using CCL2/CCR2 inhibitors and CCR2 monoclonal antibodies, diabetes and atherosclerosis can get relieved to a certain extent. However, there is no observed effect to treat tumors, which, we assume, may be due to the complexity of tumors.[59] As for CXCL12-CXCR4 axis, it contributes to all processes in tumor biology. Plerixafor (AMD3100), a CXCR4 inhibitor, was developed to treat HIV but found to increase the white blood cells in patients, and then the trial had to be terminated. However, nowadays plerixafor, combined with other chemotherapy drugs, are used to treat acute myeloid leukemia (AML) and definite curative effects have been achieved. Similarly, as a new-generation peptide CXCR4 inhibitor, BL-8040 has been proved to be effective in clinical trials of AML and pancreatic cancer. Besides, anti-CXCR4 antibody and CXCL12 inhibitor also shed light on treating multiple myeloma and chronic lymphocytic leukemia.

While back to CKLF1, the potential treatment methods would be dependent on the diseases or the stages of the disease. In the cases, such as asthma, rhinitis, airway injury, stroke, arterial stenosis, etc., upregulation of CKLF1 expression played a vital role in their pathological mechanisms. Both antagonists and inhibitors of CKLF1 and CCR4 can be explored to treat these diseases. For example, the use of anti-CKLF1 antibodies (such as compound 41 and IMM-H004) in animal models can improve the pathological changes of asthma and reduce the abnormalities of cerebral and cardiopulmonary function from stroke.[20,36,49,69]

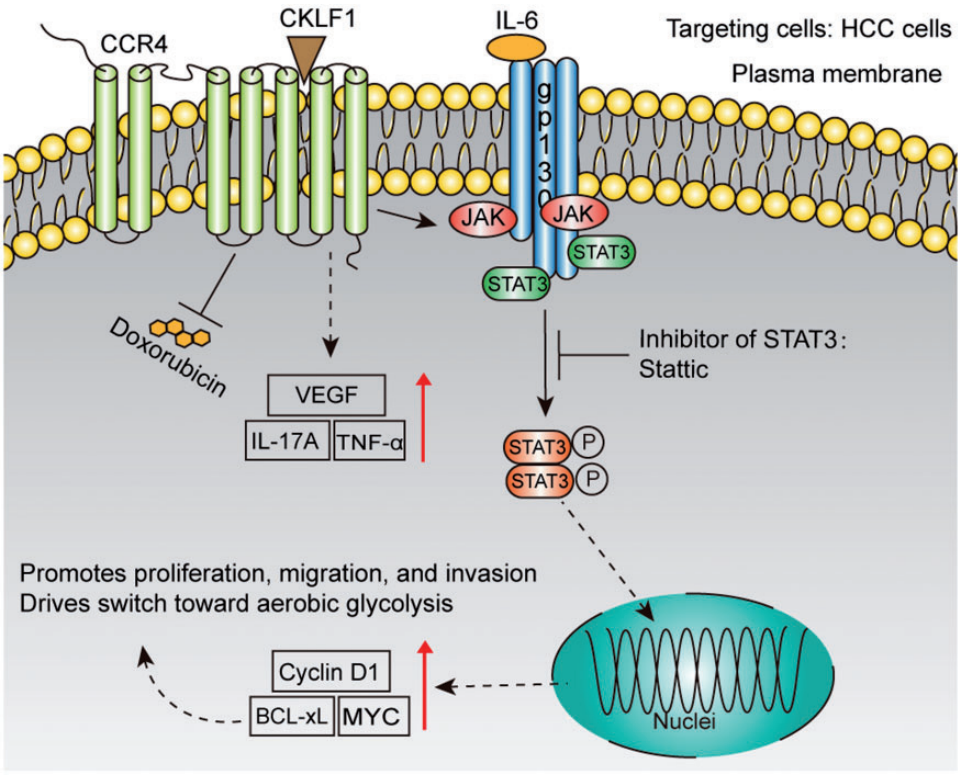

Figure 5. Schematic overview of chemokine-like factor 1 (CKLF1) involved in hepatocellular carcinoma (HCC) via IL-6/JAK/STAT3 signaling pathway. CKLF1 activated the IL-6/STAT3 signaling pathway, which enhanced HCC progression and aerobic glycolysis. With the use of static, the process was inhibited. Besides, CKLF1 decreased the efficacy of doxorubicin-induced HCC cells apoptosis and increased the levels of STAT3-related cytokines. (A color version of this figure is available in the online journal.)

Table 2. Effective clinical trials targeted the CCL2-CCR2 axis.

Target	Drugs	Treating diseases	ClinicalTrials.gov identifier	References
CCR2 inhibitor	CCX 140-B	Type2 diabetes and nephropathy	NCT01447147	[60]
CCL2 inhibitor	Emapticap Pegol (NOX-E36)	Type2 diabetes and proteinuria	NCT01085292 and NCT01547897	[61]
CCR2 monoclonal antibody	MLN1202	Atherosclerosis	NCT00715169	[62]

Table 3. Effective clinical trials targeted the CXCL12-CXCR4 axis.

Target	Drugs	Combined drugs	Treating diseases	ClinicalTrials.gov identifier	References
CXCR4 inhibitor	Plerixafor	Chemotherapy	AML	NCT01435343 and NCT01352650	[63,64]
	BL-8040 (BTK140)	Chemotherapy	AML	NCT01838395	[65]
CXCR4 antibody	Ulocuplumab (BMD-936564/MDX-1338)	Lenalidomide, dexamethasone and bortezomib	Multiple myeloma	NCT01359657	[66]
CXCL12 inhibitor	Olaptesed Pegol (NOX-A12)	Bendamustine and rituximab	Chronic lymphocytic leukemia	NCT01486797	[67]
	Olaptesed Pegol (NOX-A12)	Bortezomib and dexamethasone	Multiple myeloma	NCT01521533	[68]

After the administration of CCR4 antagonists (such as compound 6 b and compound 8a), the symptoms got relieved to some extents in allergic rhinitis and asthmatic mice.[70,71] Whereas unlike the C27 peptide, the C19 peptide exhibits an antagonistic effect. Several studies have found that in animal models of asthma, allergic rhinitis, arterial stenosis, and stroke, the C19 peptide showed very promising therapeutic potentency.[46,47,72,73] Although these findings were mainly based on animal models, it shall deserve particular attentions and more credits in future.

Conversely, while dealing with the disease cases where CKLF1 has an opposite role, different treatment methods should be considered. For example, CKLF1 could facilitate the mobilization of bone marrow CD34 + cells, which can be beneficial to relieve the acute myocardial infarction.[21] Besides, CKLF1 gene transfer could limit the mass of

myocardial infarction and improve post-infarction cardiac function.[74] These indicate that overexpression of CKLF1 could be used to treat acute myocardial infarction. Similarly, because CKLF1 can promote the proliferation and differentiation of mouse skeletal muscle cells[8,22] and bone marrow hematopoietic stem/progenitor cells,[25,26] in the future, researchers can activate local CKLF1, or overexpress CKLF1 via gene transfer in muscle fibers to treat muscular atrophy and hematopoietic disorders.

Conclusions

CKLF1, as a novel chemokine, is still in its infancy and needs further researches. By reviewing the previous studies, we can conclude that CKLF1 is related with many kinds of diseases, including allergic rhinitis, asthma, tumors, and so on. With tumors as the main example, we showed that the expression level of CKLF1 was quite divergent through different tumor types, and its atypical expression shall be related to the severity and prognosis of the disease. Therefore, CKLF1 can be considered as a potential biomolecule for diagnosis and evaluation of tumors, at least.

While exploring the therapeutic potencies of CKLF1-targeted medicines, there should be some key points to be taken into considerations. Firstly, what if three other prominent spliced variants of CKLF1 are also affected, while it was inhibited? The second concern is whether any chance exists that intervening binding of CKLF1 and CCR4 may also "off-target" other chemokine factors. Last, but not the least, there could be disease type-dependent and/or disease stage-dependent effects from CKLF1-targeted therapies, in the view of the fact that CKLF1 is only dysregulated in some tumors. Therefore, whether to use antagonist or agonist of CKLF1 needs to be deeply researched in the context of a certain disease or a certain stage from that disease. For this aim, it needs further work in future to address the role of CKLF1 in both *ex vivo* and *in vivo* disease models and to decipher the cell signaling pathways behind, so as to widen the scope of our knowledge of this potent, and very promising, therapeutic target.

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DECLARATION OF CONFLICTING INTERESTS

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