Institut Pasteur
Microbes and Infection 16 (2014) 864–870
www.elsevier.com/locate/micinf

Transcriptional regulation of CCL20 expression

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Received 2 June 2014; accepted 7 August 2014
Available online 14 August 2014

Abstract

Chemokines are key mediators of leukocyte recruitment during immunoregulatory and proinflammatory responses. CCL20 is a cysteine–cysteine chemokine that was originally shown to be chemotactic for immature dendritic cells, effector or memory $CD4^+$ T lymphocytes, and B lymphocytes. Additionally, CCL20 and its only receptor (CCR6) are exploited by cancer cells for migration and metastatic spread and play important roles in the development and progression of cancer. However, it still remains unclear how the activity of the CCL20/CCR6 axis is controlled and regulated at the transcriptional level. The CCL20 promoter region contains a transcription start site, a nuclear factor (NF)-κB binding site, a CCAAT/enhancer-binding proteins binding site, an activator protein-1 binding sites, and a specificity protein 1 (Sp1)-binding site. In this review, we outline recent advances in our understanding of the structure of the CCL20 promoter region and discuss the transcriptional regulation of the CCL20 promoter.

Keywords: CCL20; CCR6; Transcriptional regulation

1. Introduction

Chemokines are a large family of cytokines that play important roles in a variety of immunoregulatory and proinflammatory responses. Depending on the position of two conserved cysteines near the *N*-terminus, chemokines are classified as cysteine–cysteine (CC; adjacent cysteines) or CXC [cysteines separated by one non-conserved amino acid (X)]. The biological effects of chemokines are mediated by a group of seven transmembrane G protein-coupled receptors. Chemokine receptors often recognize several chemokines, and chemokines often bind to multiple receptors. Currently, chemokines can be categorized into two major functional groups: inflammatory and immune/homeostatic chemokines. The inflammatory chemokines mainly recruit neutrophils, monocytes, and eosinophils and play major roles in acute and chronic inflammation. The immune/homeostatic chemokines mainly attract lymphocytes and dendritic cells (DCs). These chemokines seem to be involved in the genesis, homeostasis, and function of the immune system [1,2]. In addition, the immune/homeostatic chemokines are recognized as critical components for the normal development of lymphoid tissues, immune cell maturation/development, and immunological homeostasis [3].

Chemokine genes tend to be clustered, with human CXC chemokines located around chromosome 4q12-13 and CC chemokines around chromosome 17q11.2 [1]. Unlike other traditional CC chemokines, the gene that encodes cysteine–cysteine motif chemokine ligand 20 (CCL20) is localized to chromosome 2q33-q372, and CCR6 is the only known receptor for CCL20 [4]. The CCL20/CCR6 axis has been shown to be an important mediator of various inflammatory diseases [5–7]. Moreover, CCR6 is the key chemokine

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http://dx.doi.org/10.1016/j.micinf.2014.08.005

receptor essentially implicated in driving interleukin (IL)-17-producing T helper cell (Th17) migration to sites of inflammation [8]. Recently, it has been suggested that CCL20 also plays a key role in cancer development and metastasis and may mediate the tumor-promoting effects of the inflammatory immune response [9–11]. Furthermore, Rs4458204_A located ~41.5 kb upstream of CCL20 (2q36.3) was found to be associated with breast cancer-specific death at a genome-wide significance level. Notably, the strength of the association of rs4458204_A with the survival of ER-negative breast cancer patients treated with chemotherapy became stronger after adjustment for tumor characteristics and type of treatment [12].

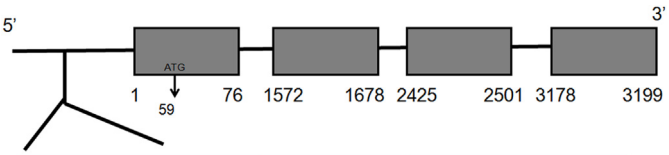
As a unique feature among the CC chemokine family, CCL20 has attracted increasing attention in recent years. Many investigations about biological function of CCL20 showed that it can act as both an inflammatory and homeostatic chemokine. However, the precise regulation of CCL20 expression remains a challenge. In this review, we focus on the genetic structures of the CCL20 promoter region and discuss transcriptional regulation of CCL20 expression.

2. The genetic structure and expression of CCL20

Human CCL20 was first reported by three independent groups. Hieshima et al. [13] identified the CCL20 gene from HepG2 hepatocarcinoma cells and a human liver cDNA library and thus named the gene "liver and activation-related chemokine (LARC)." Analogously, Rossi et al. identified the CCL20 gene in differentiated monocytes and therefore called the gene "macrophage inflammatory protein (MIP)-3α" [14]. Hromas et al. cloned the CCL20 gene from pancreatic islet cells and designated the gene "Exodus" [15].

CCL20 is encoded by the small inducible cytokine family A (CC), member 20 (*SCYA20*) gene. In contrast to other CC chemokine genes, the human CCL20 gene was found in a continuous segment between bases 74179 and 81794 located on chromosome 2q33-37 and containing four exons and three introns. The putative transcription start site was located approximately 81 bases upstream of the initiating amino acid ATG or AUG [16]. However, Hieshima et al. reported that the transcription start site was located 58 bp upstream of the start codon AUG [13]. Moreover, the CCL20 promoter region contains a nuclear factor (NF)-κB binding site (between −105 and −91), CCAAT/enhancer-binding proteins binding site (two proximal binding sites −716 to −724 and −734 to −748),AP-1 binding sites (between −124 and +33), specificity protein 1 (Sp1)-binding site (−52 to −58),an SV40 T antigen-binding site (at base 2168), epithelium-specific transformation-specific transcription factor-1 (ESE-1; nucleotides −143 to −154), a TATA box, and the transcription factor IID (TFIID)-binding site (designated base 299 at 18 bases upstream of the putative transcription start site) [17,18] The genetic structure diagram of CCL20 promoter is shown in Fig. 1.

Despite its original designation as LARC, CCL20 was later found in several tissues and a range of immune cells. CCL20 is constitutively expressed by a variety of epithelial cell types



Name	Site	Base sequence
NF-κB binding site	-105 to -82	GGGAAAACC
CCAAT/Enhancer Binding Proteins binding sites	-716 to -724 -734 to -748	TTGATCAAT CCCATGTGGCAACAC
AP-1	-124 to +33	TGATTAA
C-Ets binding site	-162 to +33	AAGCAGGAAGTT
Sp-1	-52 to -58	CGCCTTC

Fig. 1. Genetic structure diagram of the CCL20 promoter. The complete sequence of the CCL20 gene contains four exons and three introns. The exon sequences are indicated as grey boxes, and the intron sequences are indicated as horizontal lines. The lengths of sequences are numbered in bp. The putative transcription start is marked as +1 and indicated by an arrow. The start codon ATG is located at position +59. The potential binding sites for transcription factors are summarized.

including keratinocytes, pulmonary epithelial cells, and intestinal epithelial cells and by cells in some human organs such as lungs, lymph nodes, and appendix-associated lymphoid tissue, but surprisingly, CCL20 is virtually nonexistent in spleen or bone marrow [13]. Simultaneously, CCL20 can be strongly induced by many inflammatory factors, and the inducibility of CCL20 has been detected in most types of immune cells, including peripheral blood mononuclear cells (PBMCs), monocytes, macrophages, T lymphocytes, DCs, neutrophils, eosinophils, mast cells, colonic epithelial cells, lower airway epithelial cells, keratinocytes, and melanocytes [19]. Recently, cardiac fibroblasts were found to produce CCL20 after stimulation with tumor necrosis factor (TNF)-α, IL-1β, and IL-17 *in vitro* [14,20]. The unique CCL20/CCR6 axis is involved in regulating several aspects of the immune response, including the ability to mediate the recruitment of immature and mature DCs as well as antigen-presenting cells (APCs) to the sites of epithelial inflammation. Furthermore, CCR6 mediates the homing of both $CD4^+$ T (Th) cells and DCs to the gut mucosal lymphoid tissue [21].

3. Transcriptional regulation of CCL20 expression

As described above, the CCL20 promoter region contains a nuclear factor (NF)-κB binding site (between −105 and −91), CCAAT/enhancer-binding proteins binding site (two proximal binding sites −716 to −724 and −734 to −748), AP-1 binding sites (between −124 and +33), specificity protein 1 (Sp1)-binding site (−52 to −58) and so on. In this section, we discuss the effect of transcription factor on regulation CCL20 expression.

3.1. NF-κB

NF-κB is a transcription factor complex containing the proteins p50 (NF-κB1), p52 (NF-κB2), p65 (RelA), c-Rel, and RelB [22]. Upon stimulation by pro-inflammatory or microbial