General and Comparative Endocrinology 215 (2015) 117–131

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Review

The CXC chemokine receptors of fish: Insights into CXCR evolution in the vertebrates

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ARTICLE INFO

Article history:
Received 19 May 2014
Revised 12 January 2015
Accepted 13 January 2015
Available online 23 January 2015

Keywords:
CXC chemokine receptors
Fish
Vertebrates
Evolution

ABSTRACT

This article will review current knowledge on CXCR in fish, that represent three distinct vertebrate groups: Agnatha (jawless fishes), Chondrichthyes (cartilaginous fishes) and Osteichthyes (bony fishes). With the sequencing of many fish genomes, information on CXCR in these species in particular has expanded considerably. In mammals, 6 CXCRs have been described, and their homologues will be initially reviewed before considering a number of atypical CXCRs and a discussion of CXCR evolution.

1. Introduction

Chemokine receptors interact with a group of peptide ligands of 8–10 kDa and are indispensable for coordination of cell migration in diverse physiological processes such as development, angiogenesis, immune defence and neuroendocrine regulation. They belong to the largest rhodopsin family of G protein-coupled receptors (GPCRs), representing approximately 60% of the total GPCR repertoire (Fredriksson et al., 2003). Structurally each consists of 7 transmembrane domains and multiple extracellular and intracellular loops that are involved in ligand binding and signalling. Based on the ligand they bind, chemokine receptors are classified into four subgroups, the CC chemokine receptors (CCRs), CXCRs, XCRs and CX_3CRs. In humans, 18 chemokine receptors, comprising 10 CCRs, 6 CXCRs, 1 XCR, and 1 CX_3CR, have been characterised and act as receptors for at least 44 chemokine ligands (Zlotnik and Yoshie, 2012). An additional 4 atypical chemokine receptors (ACKRs) are known to scavenge ligands and suppress chemotactic responses elicited by chemokine receptors. The number of chemokine receptors varies considerably among vertebrate taxa, with fewer chemokine receptors found in birds and a large expansion seen in teleost species. With an increasing wealth of sequence data available from sequenced genomes and EST databases, this review will take a comparative approach to provide an update of the advances in chemokine receptor gene discovery, with a focus on fish CXCRs.

The chemokine receptor family shares many common properties with other GPCR family members, including protein structure, the mode of action and shared downstream signalling pathways (Viola and Luster, 2008). They consist of an extracellular N-terminal region, three extracellular hydrophilic loops (ECLs), three intracellular loops (ICLs) and an intracellular C-terminal region. The N-terminal extracellular region, together with the first ECL, is the core domain that physically engages with chemokines and dictates specificity. Upon activation by ligands, the monomeric receptor or homodimeric/heterodimeric receptor complex undergoes conformational changes that initiates distinct cellular responses. A

Abbreviations: ACKR, atypical chemokine receptor; AGTR, angiotensin II receptor; AP-2, activated protein 2; CCR, CC chemokine receptors; CD, cluster of differentiation; CXC, CXC chemokine; CXCR, CXC chemokine receptor; CX_3CR, CX_3C receptor; ERK, extracellular regulated MAP kinase; FPR, formyl peptide receptor; FYCO1, FYVE and coiled-coil domain containing 1; GCPR, G protein-coupled receptors; GRK, G protein-coupled receptor kinase; IFN-γ, interferon gamma; IL, interleukin; IQCA1, IQ motif containing with AAA domain 1; LPS, lipopolysaccharide; MCSF, macrophage colony stimulating factor; NK cells, natural killer cells; PI3K, phosphatidylinositide 3-kinase; PolyI:C, polyinosinic–polycytidylic acid; RXFP, relaxin/insulin-like family peptide receptor; SDF, stromal cell-derived factor; SSTR, somatostatin receptor; Tc cells, cytotoxic T cells; Th, T helper; TNF, tumour necrosis factor; VHSV, viral hemorrhagic septicemia virus; XCR, XC chemokine receptor.

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http://dx.doi.org/10.1016/j.ygcen.2015.01.004
0016-6480/

heterodimeric receptor complex can be formed by two members of the chemokine receptor family or with the distantly related GPCRs (Muñoz et al., 2012), e.g. CXCR1/CXCR2 (Wilson et al., 2005) and CXCR2/opioid receptor (Parenty et al., 2008). The cellular signalling pathways are complex and G protein (α, β and γ subunits) dependent or involve members of the Janus kinase and signal transducers and activators of transcription family (Vila-Coro et al., 1999; Vroon et al., 2006). Upon activation, the C-terminal region interacts with G proteins, leading to the replacement of GDP with GTP in the Gα subunit, the disassociation of Gα from Gβγ, and phosphorylation of the C terminal intracellular region by G protein-coupled receptor kinases (GRKs) (Reiter and Lefkowitz, 2006). With the involvement of β-arrestin, the uncoupling of the Gα subunit triggers immediate internalization of the receptors which are transported into the endosomes where they are either recycled to the cell surface or are delivered to lysosomes for degradation (Marchese, 2014). These events eventually lead to activation of genes that are associated with cell mobilisation, growth or death.

2. CXCR1/2

The CXC receptor 1 and 2 (CXCR1 and CXCR2) have been well characterised in vertebrates. In mammals and birds, the two receptors are shared by the ELR+ CXC ligands, namely the CXCL8 family members that have proinflammatory roles in recruitment of neutrophils, monocytes and macrophages to sites of infection. Activation of CXCR1 and CXCR2 leads to a cascade of cellular events responsible for migration, homeostasis and adhesion of the target cells. CXCR1 primarily binds with CXCL6–8 whilst CXCR2 is less ligand specific, interacting with almost all of the ELR+ CXC chemokines including CXCL1–3 and CXCL5–8 (Fan et al., 2007; Stillie et al., 2009). Chemokine receptors and other GPCRs can be recycled to the cell surface after internalization with their ligands (Fan et al., 2003), a process that is mediated by cellular pathways involving multiple signalling molecules such as β-arrestins, Activating Protein (AP) 2, G protein-coupled Receptor Kinases (GRKs) and dynamin (Barlic et al., 1999). Recent studies have shown that CXCR1 and CXCR2 recruit GRK2 and GRK6 respectively, to regulate leucocyte functions (Raghuwanshi et al., 2012). This can lead to functional differences. For example, CXCR2 but not CXCR1 is involved in CXCL8 mediated angiogenesis and cancer growth (Heidemann et al., 2003).

The first fish CXCR1/2 homologue was identified in peritoneal leucocytes of carp (*Cyprinus carpio*), induced by treatment with sodium alginate, using suppression subtractive hybridization (Fujiki et al., 1999). Subsequently CXCR1 and CXCR2 have been found in a wide range of teleost fish species including rainbow trout (*Oncorhynchus mykiss*) (Xu et al., 2014a; Zhang et al., 2002), fugu (*Takifugu rubripes*) (Huising et al., 2003a,b); zebrafish (*Danio rerio*) (Deng et al., 2013; Oehlers et al., 2010); Chinese perch (*Siniperca chuatsi*) (Chen et al., 2009), miiuy croaker (*Miichthys miiuy*) (Xu et al., 2014b) and several elasmobranch species, including lesser spotted catshark (*Scyliorhinus caniculus*), basking shark (*Cetorhinus maximus*), great white shark (*Carcharodon carcharias*), cuckoo ray (*Raja naevus*) (Goostrey et al., 2005) and elephant shark (*Callorhinchus milii*) (Venkatesh et al., 2014), and more recently in the coelacanth (*Latimeria chalumnae*) (Xu et al., 2014a) (Fig. 1 and Supplementary file 1). Comparative analysis of CXCR protein sequences reveals that *C. milii* CXCR2 possesses the conserved motif (I[L]L[I]XL[I]L) and PDZ-like ligand domain (STTIL[I]) in the C-terminal region, which have been shown to be important for cellular signalling of human CXCR2 (Fig. 2) (Baugher and Richmond, 2008; Fan et al., 2001; Marchese, 2014). In general, the CXCR1 and CXCR2 genes are tandemly linked in the vertebrate genomes, although in teleost fish there are usually two loci for CXCR1 (i.e. encoding for CXCR1a and CXCR1b), and only one is linked to CXCR2. CXCR1/2 have not been reported in jawless fish but two predicted proteins with moderate sequence homology to CXCR1 and CXCR2 are identifiable in the sea lamprey (*Petromyzon marinus*) genome (Ensembl Acc. No.: ENSPMAP00000011202, annotated as CXCR3 in the Ensembl database) and could be the putative receptor for the CXCL8 identified in this species (Najakshin et al., 1999). In the phylogenetic tree (Supplementary file 2), this protein together with another putative lamprey receptor (Ensembl Acc. No.: ENSPMAP00000011160) is located in the branch containing CXCR1–5.

Although most jawed vertebrates typically possess both CXCR1 and CXCR2, teleost CXCR1 and CXCR2 are not 1:1 orthologues to their tetrapod counterparts (Saha et al., 2007). Several hypotheses have been proposed regarding how these receptors have evolved during vertebrate evolution. It is believed that CXCR1/R2 like genes were present before the divergence of the bony fish and tetrapods (Xu et al., 2014a), and this notion is supported by the high conservation of gene synteny of the CXCR1/2 genes in the genomes of different vertebrates; as seen in spotted gar (*Lepisosteus oculatus*), an extant species of Holostei. CXCR1 and CXCR2 are also tandemly linked and flanked by GPAR1 and TNS1, an arrangement seen in reptiles and mammals (Xu et al., 2014a) (unpublished data). The elephant shark genome contains two copies of putative tandemly clustered CXCR1/R2 genes (Venkatesh et al., 2014), one of which is a partial sequence. The phylogenetic relationship of one of the elephant shark CXCR1/2 like proteins with CXCR1 and CXCR2 is ambiguous since the NJ tree placed it in the CXCR2 clade whilst the Bayesian posterior probability and maximum likelihood bootstrap scores support the orthologous relationship of the elephant shark CXCR1/2 with teleost and coelacanth CXCR1 (Fig. 1 and Supplementary file 2). However, phylogenetic analysis alone cannot establish the evolutionary relationship of the two receptors between fish and tetrapods. It is possible that events such as gene conversion and lineage specific gene gain and loss combined with selection pressure from the ligands could contribute to the divergence of the two receptors in different vertebrate lineages (Shields, 2000; Xu et al., 2014a).

As the principal receptors for regulating the inflammatory process in mammals, CXCR1 and CXCR2 have an important role in regulating the trafficking of phagocytes such as neutrophils, monocytes and macrophages (Viola and Luster, 2008). The evidence gathered to date from fish studies are in agreement with these functions. In healthy fish, they are highly expressed in immune tissues including head kidney, spleen and blood, suggesting they are required for homeostasis of phagocytes (Chen et al., 2009; Deng et al., 2013; Oehlers et al., 2010). In rainbow trout, high constitutive expression is detected in neutrophils and B cells (Xu et al., 2014a) in agreement with the recent findings that fish B cells exhibit strong pro-inflammatory functions such as phagocytic and antimicrobial abilities (Li et al., 2006). CXCR2 has been shown to be involved in mediating neutrophil circulation in the blood under steady state conditions in zebrafish (Oehlers et al., 2010). In contrast, fish CXCR1 may be primarily responsible for the development and homing of neutrophils in the hematopoietic tissues (Oehlers et al., 2010). The discrepancies in CXCR1 and CXCR2 functions observed in fish may be attributed to the distinct signalling pathways they activate; each receptor is known to recruit distinct GRKs to mediate leucocyte inflammatory functions (Raghuwanshi et al., 2012). It will be of particular interest to determine whether the two receptors are expressed on the surface of the same cell or different cell types in future research.

Accumulating evidence suggests that fish CXCR1 and CXCR2 are involved in immune defence against bacterial, parasitic and viral infections. It is well known that their probable cognate ligands (CXCL8_L1 and CXCL8_L2) are induced after immune stimulation