

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

Characterization of G protein coupling mediated by the conserved D134^{3,49} of DRY motif, M241^{6,34}, and F251^{6,44} residues on human CXCR1

Xinbing Han^{a,*}, Yan Feng^{b,1}, Xinhua Chen^c, Craig Gerard^a, William A. Boisvert^{d,e,*}^a Boston Children's Hospital, Harvard Medical School, 300 Longwood Avenue, Boston, MA 02115, United States^b First Affiliated Hospital, Xinjiang Medical University, Urumqi, Xinjiang 830054, China^c Beth Israel Deaconess Medical Center, Harvard Medical School, 330 Brookline Avenue, Boston, MA 02215, United States^d Center for Cardiovascular Research, John A. Burns School of Medicine, University of Hawaii, 651 Ilalo Street, Honolulu, HI 96813, United States^e Kazan Federal University, Kazan, Russia

ARTICLE INFO

Article history:

Received 5 November 2014

Revised 27 February 2015

Accepted 3 March 2015

Keywords:

Chemokine receptor

CXCR1

G protein coupled receptor

Gα15

Gβi

Constitutive activity

ABSTRACT

CXCR1, a receptor for interleukin-8 (IL-8), plays an important role in defending against pathogen invasion during neutrophil-mediated innate immune response. Human CXCR1 is a G protein-coupled receptor (GPCR) with its characteristic seven transmembrane domains (TMs). Functional and structural analyses of several GPCRs have revealed that conserved residues on TM3 (including the highly conserved Asp-Arg-Tyr (DRY) motif) and TM6 near intracellular loops contain domains critical for G protein coupling as well as GPCR activation. The objective of this study was to elucidate the role of critical amino acid residues on TM3 near intracellular loop 2 (i2) and TM6 near intracellular loop 3 (i3), including S132^{3,47} (Baldwin location), D134^{3,49}, M241^{6,34}, and F251^{6,44}, in G protein coupling and CXCR1 activation. The results demonstrate that mutations of D134^{3,49} at DRY motif of CXCR1 (D134N and D134V) completely abolished the ligand binding and functional response of the receptor. Additionally, point mutations at positions 241 and 251 between TM6 and i3 loop generated mutant receptors with modest constitutive activity via Gα15 signaling activation. Our results show that D134^{3,49} on the highly conserved DRY motif has a distinct role for CXCR1 compared to its homologues (CXCR2 and KSHV-GPCR) in G protein coupling and receptor activation. In addition, M241^{6,34} and F251^{6,44} along with our previously identified V247^{6,40} on TM6 are spatially located in a “hot spot” likely essential for CXCR1 activation. Identification of these amino acid residues may be useful for elucidating mechanism of CXCR1 activation and designing specific antagonists for the treatment of CXCR1-mediated diseases.

© 2015 The Authors. Published by Elsevier B.V. on behalf of the Federation of European Biochemical Societies. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

CXCR1 is an important member of the chemokine receptor family that mediates migration of neutrophils primarily, but is also important for acute and chronic inflammation, proliferation and development of lymphocytes [1,2]. Its most important ligand,

Abbreviations: CXCR1, CXC receptor 1; GPCR, G protein-coupled receptor; DRY motif, Asp-Arg-Tyr motif; IL-8, interleukin 8; TMs, transmembrane domain; i2, intracellular loop 2; i3, intracellular loop 3; PLC, phospholipase C; WT, wild type; K_d, affinity constants; IP, inositol phosphate; PTX, pertussis toxin

* Corresponding authors at: Boston Children's Hospital, Harvard Medical School, Enders Building, Rm 408, 300 Longwood Avenue, Boston, MA 02115, United States. Tel.: +1 (617) 355 6958 (X. Han). University of Hawaii, John A. Burns School of Medicine, 651 Ilalo Street, BSB311C, Honolulu, HI 96813, United States. Tel.: +1 (808) 692 1567 (W.A. Boisvert).

E-mail addresses: XinbingHan12@gmail.com (X. Han), wab@hawaii.edu (W.A. Boisvert).

¹ These authors contributed equally to this work.

<http://dx.doi.org/10.1016/j.fob.2015.03.001>

2211-5463/© 2015 The Authors. Published by Elsevier B.V. on behalf of the Federation of European Biochemical Societies. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

IL-8, can not only bind to CXCR1, but also to its homologues CXCR2 and KSHV-GPCR [3]. CXCR1 and CXCR2 share 76% amino acid identity [1,2], are highly expressed on the surface of neutrophils and can trigger chemotactic signals in neutrophils in response to IL-8. Despite their high homology and similar co-expression on neutrophil that mediates chemotaxis, sequence differences between CXCR1 and CXCR2 lead to distinct activation patterns and subsequent regulation of physiological and pathophysiological processes involved in inflammation and cell proliferation. CXCR1 is specific for IL-8, whereas CXCR2 also binds to other CXC chemokines, and differential activation and regulation of CXCR1 and CXCR2 by IL-8 monomer and dimer have been demonstrated [4]. IL-8 promotes bacterial killing by neutrophils via CXCR1 but not CXCR2 at the site of inflammation [5], and impairment of CXCR1 increases susceptibility to bacterial infection [6]. Although both CXCR1 and CXCR2 promote tumor growth and angiogenesis, it is noteworthy that only CXCR2 plays an important