RESEARCH HIGHLIGHT

# Chemokine receptors and their interactors in HIV-1 replication: potential therapeutic targets

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**Chemokine receptors CXCR4 and CCR5 are co-receptors indispensable for human immunodeficiency virus type 1 (HIV-1) entry and subsequent infection in host cells. Antiretroviral therapies based on the viral proteins have been developed, and significant achievements have been made in the treatment of HIV/AIDS patients based on the HAART regimens. However, a lot of concerns are still present, the purge of latent viral reservoirs and cure of AIDS are currently impossible, and prophylactic vaccines are not yet available. Most recently, HIV-1 entry has been understood much more and targeting viral entry based on chemokine receptors represents an interesting prospective. In this research highlight, we review the role of HIV-1 co-receptors-interacting proteins during chemokine receptor signal activation and assembly, as well as present new results about how they can regulate the replication of the virus.**

***Keywords:*** HIV-1; chemokine receptors; NHERF1; DRiP78; signaling

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## Introduction

Long-term evolution has driven the sequential events of HIV infection by the interaction between virus and host. The viral envelop-target cell receptors binding acts as the portal for the viral entry and the establishment of productive infection. The viral envelope is assembled in spike-like structures formed by trimeric complexes of gp120 and gp41, which permits virion attachment to host cells and fusion processes. To activate HIV entry, gp120 must sequentially wage two host cellular receptors, CD4 [1, 2] and an alternative co-receptor of the chemokine receptor family member, CXCR4 or CCR5. Upon binding to CD4, gp120 undergoes conformational changes, leading to the exposure of gp120-hidden epitopes that can now interact with chemokine co-receptor such as CXCR4 or CCR5 [3-8], of the seven transmembrane receptor (7-TM-R) family.

Over the past three decades, the highly active antiretroviral therapy (HAART),based on drugs targeting HIV-1 replication stages of viral life cycle, have significantly improved HIV/AIDS treatment. Nevertheless, some therapeutic issues based on current available ART drugs are still prominent. Up to now, a major concern with HIV-1 is that it can adapt and become resistant to drugs blocking attachment, as well as the drugs targeting HIV entry except the fusion inhibitors at cell surface [9].HIV-1 chemokine co-receptors are G protein-coupled receptors (GPCRs) which signal via multiple subunits assembled into a complex. Recently, the attempt to understand the signaling complexes interacting with HIV-1 chemokine receptors has been made [10, 11]. Given that chemokine receptors can associate with

several other receptors through dimerization, it is important to understand the differences between the proteins that can interact with the different receptor dimer complexes. One important aspect of chemokine receptors, in relation to HIV infection, is that chemokine receptor internalization appears to be an extremely important step driving HIV viral entry. In fact, removal of HIV chemokine co-receptors either by genetic variations or via targeted receptor removal from cell surface (via internal retention or internalization from plasma membrane) indicate that one could be HIV resistant in absence of chemokine receptor plasma membrane expression [12]. In this review, we intend to present the influence of signaling components (proteins that interact with chemokine receptors during transport and cell-surface expression of HIV chemokine receptors) on both HIV-related signaling or HIV entry into cells. Previous studies from our group show variations in the partners found in different chemokine receptor complexes (CXCR4 dimers, CXCR4-CCR5 dimers, CCR5 dimers), suggesting that some interactors demonstrate specificity towards certain receptor signaling complexes. Therefore, identifying receptor interactors may provide promising new targets that would allow some "function sparing"; e.g. showing texture in how a therapy can actually focus on some aspects of receptor signaling while leaving others intact [13].

## Chemokine receptors and their role in HIV

The binding of HIV-1 envelop glycoproteins to chemokine receptor is important for productive viral infection, composed of HIV-1-cell membrane fusion and subsequent signal cascades. When HIV-1 gp120 recognizes and binds to the host cell surface CD4, the envelop complex undergoes a structural change. The conformation change results in the exposure of chemokine receptor binding domain in gp120 that allows interactions with the targeted chemokine receptor [14, 15].This secondary interaction leads to a more stable two-pronged attachment, which allows the distal hydrophobic fusion peptide of gp41 to insert into the target cell membrane [14, 15]. The heptad repeats (HR) in gp41, HR1 and HR2, then interact, causing the deformation of the extracellular portion of gp41 into a hairpin coiled-coil complex. This loop complex brings the viral and cellular membranes close together, allowing fusion of the two closely apposed membranes and subsequent entry of the viral capsid. After HIV-target cell fusion, the virus undergoes uncoating, by which the RNA genome and various enzymes, including reverse transcriptase, integrase, ribonuclease, and protease, are released into the target cell [14].During the microtubule-based transport of uncoated viral products to the nucleus, the viral single-strand RNA genome is reversely transcribed into double-strand DNA, and then interacts with viral enzymes to form a pre-integration complex (PIC) that is then integrated into a host chromosome and goes through serial later events to complete the full process of viral replication.

Virus entry and invasion into host cell triggers cell signal transduction to establish the productive infection [16, 17].Chemokine receptor-activated signal cascades play a critical role in HIV infection and AIDS pathogenesis [10]. More recently, it has been shown that the HIV-1 gp120-mediated signal transduction from the chemokine co-receptor CXCR4 is an absolute requirement for HIV-1 infection of blood resting $CD4^+$ T cells, by which promotes cortical actin remodeling, thus facilitates HIV-1 entry and intracellular migration [18].

## Current HIV Therapies and Concerns

Despite the undeniable positive effects of anti-retroviral therapies, important limitations have arisen including emergence and transmission of multi-resistant strains, along with side effects. The first HIV entry inhibitor (enfuvirtide) approved for clinical use has unfortunately encountered HIV resistance in patients harboring mutations in the HR1 or HR2 helices of gp41 [19].The targeting of host cell receptors such as CXCR4 by antagonists such as AMD3100 were tested but did not provide the anticipated results, since it could also mobilize hematopoietic stem and progenitor cells[20].Several agents of the "viroc" family, which target host cell receptors such as CCR5, have been or are currently under clinical trials. Although these small molecule entry-inhibitors can block virus binding to receptors, multiple developmental challenges remain before they become available for HIV infection treatments. Trials of aplaviroc were halted because of liver toxicities and virologic failure[21], while vicriviroc studies in treatment-naive patients showed treatment failure [22] or unexpected occurrence of malignancies such as lymphomas[23]. Maraviroc, the first co-receptor inhibitor, was approved for use in the USA and EU after only 6 years after its identification. Maraviroc has not only proved to be a valuable addition to the ever-growing anti-retroviral drug armamentarium, but also the data from associated studies have deepened our understanding of HIV tropism and the relationship between viral tropism and disease progression, the drug resistance to Maraviroc was also observed[24]. All these different concerns about ligand therapy suggest that other targets, outside the receptor binding interface should be identified to restrict the adaptation process of HIV during infection.

It is now common knowledge that a given cell can express homodimers, heterodimers and higher order oligomers for different receptors[25]. This type of receptor complexes are increasingly being found to have serious consequences for

signaling and receptor function[25]. It is possible that individual receptors could "crosstalk" through allosteric effects on the receptor(s) in the receptor complex and thus signaling could depend on whether one or more the receptors are activated. It has even been suggested that GPCR heteromerization could alter the properties of the receptors involved such that they respond differently to pharmacologic agents, revealing a possible source of unexpected pharmacological diversity[8].Precise characterization of receptor-mediated signaling pathways will be crucial for the development of therapeutic targets outside the ligand interface. A therapeutic strategy may require regulation of one specific effector pathway, but drugs aimed at the ligand binding site of GPCRs that are coupled to multiple effectors often lack specificity in this regard, and thus, can produce undesirable cross-reactivity and side effects. However, the presence of unique signaling partners within a particular pathway (for example, G proteins with a specific subunit composition, regulatory molecules, chaperones, scaffolds or effectors) suggests that there are unique components that govern interactions between these proteins. Previous studies from our group with chemokine receptors suggest that some of these interactors will interwork specifically with some GPCR complexes composed of various chemokine receptors, while not with others [11, 26, 27]. Understanding the role of these interactors in the different receptor complexes could help in the design of new drugs/strategies for HIV/AIDS therapy that would alter signaling pathways dependent on scaffolds or chaperones interacting with chemokine receptors.

## Chemokine receptor-interactors and their roles in HIV-1 replication

Chemokine receptors are known to be highly important for HIV infection. First, with the primary receptor CD4, they recognize the virion and allow its docking to cells. Secondly, it has been shown that up to 80% of CCR5-tropic HIV-1 infection could be dependent on CCR5 internalization [28]. Several of current strategies use CCR5 ligands, which can either block the interaction between the virus and the receptor, or promote receptor internalization, but also induce unintended signal transduction. Therefore, strategies that would trigger CCR5 internalization without any signaling pathway activation would be highly useful and recommendable. Furthermore, a CCR5 variant that isnot expressed at cell plasma membrane is present in the population and does not cause any harm in non-pathophysiological conditions [29]. Some chemokine receptor-interactors are believed to be a pivotal regulator ofthe expression and internalization of CCR5 or CXCR4, as well as on the HIV entry into cells driven by the expression of those receptors.

In our studies of specific interactors of receptor dimers, we used bimolecular fluorescence complementation assay, where each receptor is tagged with a portion of the yellow fluorescent protein (YFP). Upon receptor dimerization, the two individually non-fluorescent segments associate and generate fluorescence. In absence of dimerization, each construct is unable to generate fluorescence. When measuring a signaling pathway that is directly activated by the receptor, we can proceed with a co-immunoprecipitation, by using antibodies directed against the functional GFP (which immunoprecipitates only the dimers with the functional GFP) and then, immunoblotting for the signaling pathway of choice. It is this particularity of the assay that drives most of our choices of signaling pathways to study although we can still study the overall effect of the overexpression of the heterodimers on any pathway, but with an understanding that a signal from other dimers could be recorded, such as with receptors bearing non-functional GFP parts (e.g. two CXCR4 with the first portion of the GFP that would dimerize, but not produce a fluorescent GFP, for example). Despitethis limitation, there are several signaling pathways interacting directly with chemokine receptors that can be studied and provide useful information on the overall chemokine receptors signal transduction.

Recently, we have been identified and characterized interaction partners of HIV chemokine co-receptors (CXCR4 or CCR5) dimers that could regulate the expression of chemokine receptors at cell membrane, via internal trafficking or plasma membrane internalization of the receptor, and potentially of HIV entry into cells [11, 26, 30].Adaptors and scaffolding proteins are highly important for the function of membrane receptors and effectors. Their roles are highly diverse and among them is the regulation of the spatial and thereby functional association with various co-receptors, G proteins, effectors and other downstream partners. Regulation of GPCRs is frequently governed by adaptors or scaffolding protein interactions with its cytoplasmic C-tail, in which several motifs can be identified for interactions. One of such motifs is (D/E)-(S/T)-X-Φ (X, unspecified amino acid; Φ, hydrophobic amino acid, generally leucine, valine or isoleucine) [31], present in the sequence of several GPCRs. Chemokine receptor CCR5 bears a motif similar to the PDZ protein domain (EISVGL) [32], while CXCR4does not (SFHSS).An PDZ-containing adaptor protein, NHERF1 ($Na^+/H^+$ exchanger regulatory factor 1), was identified to interact with CCR5 homodimer, but not with the CXCR4 homodimer or CXCR4-CCR5 heterodimer [11]. NHERF1 increases CCR5-induced arrestin2 recruitment following ligand stimulation, and it is involved in the internalization of CCR5. The NHERF1-CCR5 interaction is mediated by the PDZ2 domain of NHERF1, and the CCR5 internalization could be blocked by the PDZ2

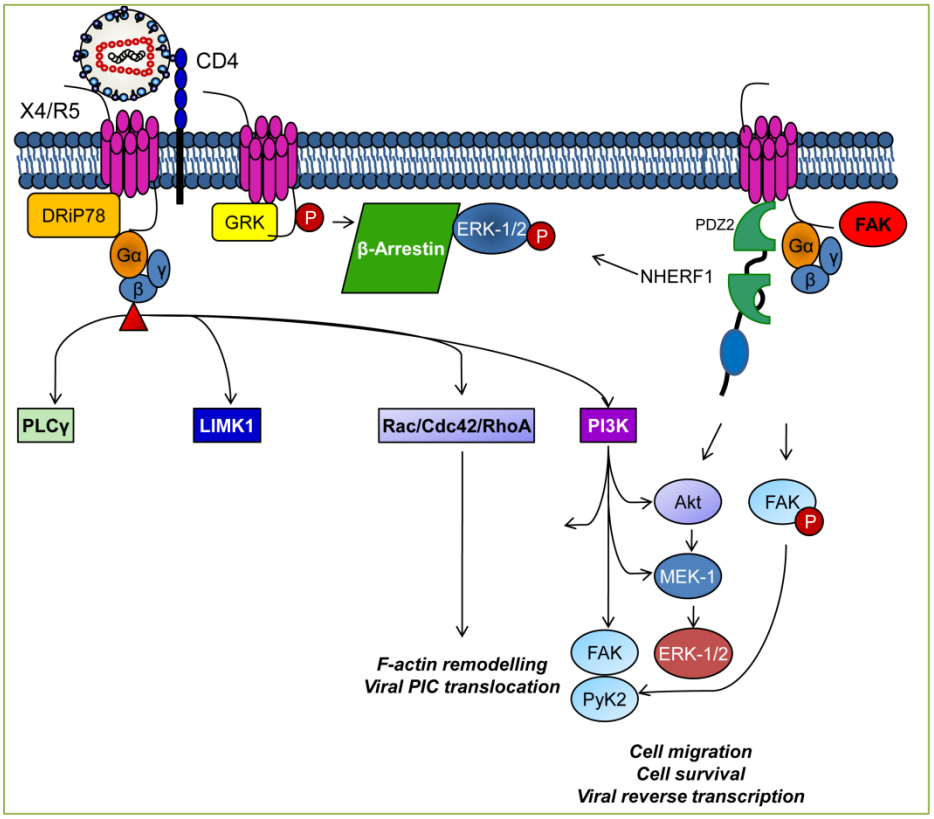


**Figure 1.Chemokine receptors and signaling via interacting molecules during HIV-1 infection.** Chemokine receptors CXCR4 or CCR5 interacts with scaffold protein NHERF1 and chaperon DRiP78, thus activates or potentiates some cell signal cascades involved in HIV-1 infection and replication in host cell. NHERF1 interacts with CCR5 homodimer via the PDZ2 domain (Right side). NHERF1 promotes activation of MAPK/Erk and RhoA signaling by HIV-1 gp120-stimulation or viral infection. NHERF1 could also increase gp120-induced FAK recruitment to CCR5 moderately, thus activates the subsequent FAK-mediated cell migration. The potentiated RhoA signal was also proved by the observation of enhanced filament actin remodeling by NHERF1. Thus, the effect of NHERF1 on HIV-1 replication may be uniquely involved in the chemokine co-receptors signaling. Moreover, NHERF1 increases CCR5 recruitment of arrestin2 following stimulation and is involved in the internalization of CCR5 (Middle). DRiP78 could interact with CXCR4 or CCR5 homodimer. It also participates in the signal assembly of G protein subunits (Gα) and CCR5 (Left, upper panel).

domain . Moreover, NHERF1 could potentiate RANTES (a natural CCR5 ligand)-induced Erk-1/2 phosphorylation via CCR5 and that this signal activation requires NHERF1 but not arrestin2 [11]. Extra residues are part of this motif, which could slightly interfere with the binding of NHERFl, or affect the stability and selectivity of other proteins to interact with the receptor through this motif.

We also demonstrated that NHERFl potentiated HIV-1 envelope glycoprotein gpl20 induced CCR5 internalization [30]. Since gp120 is one of the HIV envelope viral protein interacting with CCR5, its binding may trigger some signaling events mediated by CCR5. A couple of cell signaling events have been demonstrated to occur immediately after the binding of HIV envelope to $CD4^+$ target cells (Figure 1). Phosphorylation of many of these proteins involved in the signal cascades and the formation of focal adhesion complexes occur following T-cell activation and in response to chemotactic stimuli, and are associated with alterations in cytoskeletal architecture [10]. In light of observations that point to the importance of activation of cytoskeletal elements in facilitating HIV entry and subsequent reverse transcription, the ability of HIV envelope to induce cytoskeletal rearrangement may represent a critical step toward productive infection establishment. Interestingly, NHERF1 is an important regulator of actin organization, along with RhoA. NHERF1 interacts with ezrin and modulates ezrin activation, in turn, this interaction and activation may initiate the activation of RhoA signaling. Therefore, NHERF1-dependent recruitment of ezrin to the cell membrane would mediate its anchorage to the actin filaments and activate RhoA-dependent signaling, thus

modulating HIV entry. We observed the promoted activation of MAPK/Erk and RhoA signaling by NHERF1 in HIV-1 gp120-treated cells. Intriguingly, gp120-induced FAK recruitment to CCR5 was moderately increased in the presence of NHERF1, thus activates the subsequent FAK-mediated cell migration [30]. The potentiated RhoA signal was also proved by the observation of enhanced filament actin remodeling by NHERF1. Although the PDZ domain-containing protein 8 (PDZD8) is reported to interact with HIV-1 capsid [33, 34], no interaction was observed between NHERF1 and capsid (unpublished data). Thus, the effect of NHERF1 on HIV-1 replication may be uniquely involved in the chemokine co-receptors signaling.

Plasma membrane expression of receptors is dynamic. Biosynthesis at the endoplasmic reticulum level and the trafficking of newly synthesized receptors will affect the number of cell surface receptors expressed, along with regulation by endocytosis. Therefore, another way to modulate the number of receptors at plasma membrane is to block their expression at the cell surface, either by inhibiting the anterograde trafficking of the receptor, or by affecting the maturation of the receptor and its folding. We have identified several chaperones that can interact with chemokine receptors CXCR4 and CCR5, and tested their role on cell surface expression of those receptors by using shRNAs or dominant negative isoforms of the chaperones. As predicted, some chaperones affect the maturation and trafficking of the receptors to plasma membrane,and DRiP78 (Dopamine Receptor-interacting Protein 78) was one of the chaperones that interact with CXCR4 or CCR5 homodimer but not the CXCR4-CCR5 heterodimer [26]. DRiP78 has been shown to interact with several GPCRs and its role is generally associated with signaling events such as ER export and G protein subunits assembly [35-37]. CCR5 possesses the $F(x)_6LL$ motif in its C-terminal tail, previously recognized to mediate DRiP78 interaction with receptors. CXCR4 has a variation of that motif, with a FxxxxF motif. Our data have shown that DRiP78 can promote the formation of homologous dimers complex, but cannot influence the assembly of heterologous dimers. DRiP78 also participates in the signal assembly of G protein subunits and CCR5 [26]. Our study showed that DRiP78 regulates the signal complex of co-receptors CXCR4/CCR5 specifically, and influences the CCR5-mediated cell migration. The molecular chaperone DRiP78 may represent a new class of target, that regulates the expression level of receptor in the cytoplasm, and finally affect the binding on the cell surface of chemokine and the virus infecting mediated by chemokine receptor, like HIV-1. However, the influence of DRiP78 on HIV-1 virus entry and replication has not yet been studied. Therefore, DRiP78 could potentially be a good target to reduce the expression of those receptors to the cell surface.

More recently, we designed two short hairpin RNAs (shRNAs) targeting the DRiP78 and NHERF1, respectively, and constructed the pLenti6/BLOCK-iT-DEST lentiviral plasmids expressing DRiP78 or NHERF1 shRNA[38].The packaged lentiviruses were used to transduce the widely used HIV-1 model cell line GHOST(3).Then, cells with stable knockdown were established through selecting transduced cells with Blasticidin. This study, for the first time, reported the establishment of the GHOST(3) with DRiP78 and NHERF1 knockdown, which is the first stable cell line with HIV-1 co-receptor-interacting molecular defects. Using the GHOST(3) cells with the DRiP78-stable knockdown, we challenged cells with the CXCR4-tropic strains $HIV\text{-}1^{IIIB}$ and $HIV\text{-}1_{NL4\text{-}3}$ and the quantitative p24 ELISA showed that the replication of HIV-1 was abrogated in GHOST(3) cells expressing DRiP78 shRNA (unpublished data). The DRiP78 shRNA displayed a comparative inhibition activity to the anti-HIV-1 drug AZT. In addition, the DRiP78 knockdown could reduce the phosphorylation of MAPK/Erk and cofilin in GHOST(3) cells 48 h post-infection. Quantitative PCR demonstrated thatDRiP78 appears to block HIV-1 replication post-transcription, but before pre-integration complex (PIC) nuclear translocation and integration (unpublished data). The data with the DRiP78 shRNA in GHOST(3) cells suggest that the host DRiP78 is probably a necessity for CXCR4-mediated HIV-1 infection and replication. Further work is called to elucidate the mechanism used by DRiP78 to regulate HIV-1 infection and replication.

Taken together, the results of our studies combined with observations by others suggest important roles of chemokine receptors and their interacting molecules in HIV-1 replication. Understanding the mechanisms involved in the regulation of signaling induced by the receptors in presence/absence of these chaperones and other signaling partners represents an important step in the development of new therapeutic strategies directed at the receptors, but outside the ligand binding pocket and thus potentially limiting the resistance to treatments for HIV-1 infected patients.

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## Conflict of interests

The authors declared no conflict of interests.

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