CHAPTER TWENTY-FIVE

Virally Encoded G Protein-Coupled Receptors: Overlooked Therapeutic Opportunities?

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Contents

ABBREVIATIONS

AIDS acquired immune deficiency syndrome
CCL17/TARC chemokine (C–C motif) ligand 17/thymus- and activation-regulated chemokine
CCL19/ELC chemokine (C–C motif) ligand 19/EBI 1 ligand chemokine
CCL2/MCP-1 chemokine (C–C motif) ligand 2/monocyte chemotactic protein 1
CCL21/SLC chemokine (C–C motif) ligand 21/secondary lymphoid tissue chemokine

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CCL22/MDC chemokine (C–C motif) ligand 22/macrophage-derived chemokine
CCL3/MIP-1α chemokine (C–C motif) ligand 3/macrophage inflammatory protein-1α
CCL4/MIP-1β chemokine (C–C motif) ligand 4/macrophage inflammatory protein-1β
CCL5/RANTES chemokine (C–C motif) ligand 5/regulated upon activation, normal T-cell expressed, and secreted
CCR1 chemokine (C–C motif) receptor 1
CD4 cluster of differentiation 4
CREB cAMP response element binding
CX_3CL1/Fractalkine chemokine (C–X_3–C motif) ligand 1/Fractalkine
CXCL1/GROα chemokine (C–X–C motif) ligand 1/growth-regulated alpha protein
CXCL10 chemokine (C–X–C motif) ligand 10
CXCL12 chemokine (C–X–C motif) ligand 12
CXCL3 chemokine (C–X–C motif) ligand 3
CXCL4 chemokine (C–X–C motif) ligand 4
CXCL5 chemokine (C–X–C motif) ligand 5
CXCL7 chemokine (C–X–C motif) ligand 7
CXCL8/IL-8 chemokine (C–X–C motif) ligand 8/interleukin-8
CXCR2 chemokine (C–X–C motif) receptor 2
CXCR4 chemokine (C–X–C motif) receptor 4
EBI2 Epstein–Barr virus-induced receptor 2
EBV Epstein–Barr virus
GPCR G protein-coupled receptor
GPR17 human G protein-coupled receptor 17
GPR39 human G protein-coupled receptor 39
HCMV human cytomegalovirus
HHV-7 human herpesvirus 7
HIV-1 human immunodeficiency virus-1
KSHV Kaposi's sarcoma herpesvirus
MC1R melanocortin 1 receptor
NFAT nuclear factor of activated T cells
ORF74 open reading frame 74
PLC phospholipase C
R33 rat cytomegalovirus G protein-coupled receptor 33
U12 G protein-coupled receptor encoded by HHV-7 *U12* gene
U51 G protein-coupled receptor encoded by HHV-7 *U51* gene
UL33 G protein-coupled receptor homolog UL33
UL78 G protein-coupled receptor homolog UL78
US27 G protein-coupled receptor homolog US27
US28 G protein-coupled receptor homolog US28
vGPCR virally encoded GPCR

1. INTRODUCTION

Viruses use a great variety of tools to invade a host organism. Large DNA viruses, such as Herpesviruses, encode proteins that mimic the G protein-coupled receptors (GPCRs) of the host. These virally encoded GPCRs (vGPCRs) are commonly homologues of mammalian chemokine receptors.[1–5] The vGPCRs' efficient interaction with the host's signaling machinery provides advantages to the virus. The expression of chemokine-like vGPCRs by viruses illustrates the adaptation of viruses to a complex system of cellular intercommunication in their host and even includes functions beyond immune evasion.[3]

The modulation of cellular functions by vGPCRs has been extensively reviewed.[1–5] This review focuses on (1) the main characteristics of cellular reprogramming caused by vGPCRs, (2) the current development of synthetic ligands that target vGPCRs, and (3) a discussion of the importance of these ligands as chemical probes used to dissect the signaling properties of vGPCRs and their involvement in oncogene transformation or development of atherosclerosis. GPCRs are the target of more than one-third of all drugs on the market,[6] and therefore, an understanding of the precise mechanism of signal transduction and negative regulation of vGPCRs by synthetic small weight inverse agonists should provide a strategy to block the actions of these vGPCRs and to develop practical therapies for diseases caused by Herpesviruses.

2. STRUCTURE, FUNCTION, AND PHYSIOLOGICAL CONSEQUENCES OF vGPCRs

vGPCRs are seven-transmembrane receptors with often striking sequence homology to host chemokine receptors, particularly in the helical regions. Host chemokine receptors are responsible for coordinating the immune system surveillance and the response to infection and inflammation.[7] Chemokine receptors bind chemokines (chemoattractant cytokines) that regulate the trafficking and effector functions of leukocytes. Each immune cell type carries a specific expression pattern of chemokine receptors. The induction of expression of particular chemokines determines which immune cells will migrate during infection and inflammation. vGPCRs may represent immune-evasion strategies to inactivate inflammatory cytokines, to redirect the immune response, and to improve the survival and spreading of the virus.[3,4]

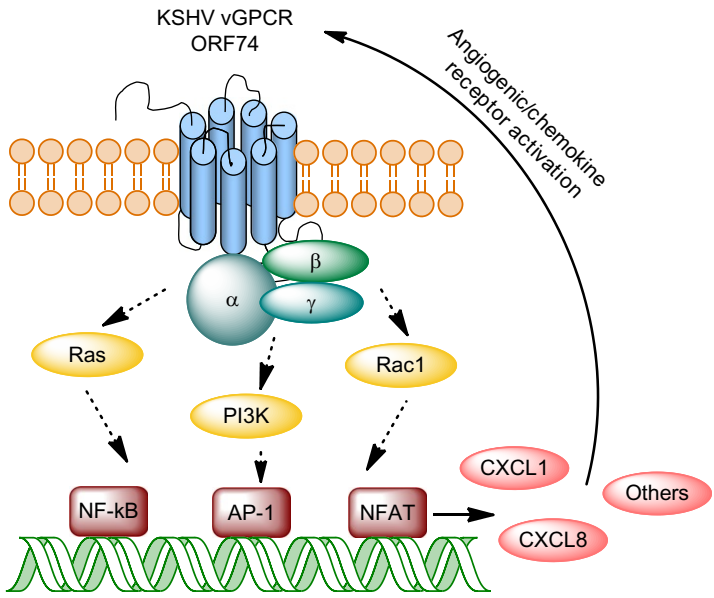


Figure 25.1 Interaction of vGPCRs with the host's signaling machinery, leading to reprogramming of cell signaling, activation of several transcription factors, and subsequent production of growth factors and chemokines.

vGPCRs interact efficiently with the signaling machinery of the host cell, which consequently often leads to pronounced biological response in the absence of a bound ligand (constitutive activity) (Fig. 25.1). The chemokine ligands of several vGPCRs are known.[1,3] The reprogramming of cell signaling by vGPCRs often results in prosurvival and angiogenic effects, inflammation, transformation, proliferation, and increased viral replication.[1,3,4,8]

2.1. Kaposi's sarcoma-associated herpesvirus

A γ-herpesvirus, Kaposi's sarcoma-associated herpesvirus (KSHV) is one of seven currently known human oncoviruses. It was first identified in AIDS patients suffering with the otherwise rare Kaposi's sarcoma.[9] KHSV is found in nearly 100% of tumors isolated from Kaposi's sarcoma patients. Moreover, it is associated with primary effusion lymphoma and multicentric Castleman's disease.[10,11] KSHV encodes one vGPCR, referred to as ORF74 (open reading frame 74).[9] ORF74, a homologue of the human chemokine CXC receptor 2, which binds a broad range of human chemokines from the CXC and CC family, is required for increased viral

replication in response to chemokines and efficient reactivation from latency.[12,13] The sole expression of ORF74 is sufficient to induce the development of Kaposi's sarcoma-like lesions in transgenic mice.[14] Selective elimination of vGPCR-expressing cells in established allografts in nude mice resulted in tumor regression.[15]

Work from several laboratories has demonstrated that ORF74 promotes cell proliferation, enhances cell survival, modulates cell migration, stimulates angiogenesis, and recruits inflammatory cells in expressing cells as well as in neighboring (bystander) cells (reviewed in Ref. 16). The molecular mechanisms by which this powerful viral oncogene rewires the cell-signaling network are very complex. Not only is ORF74 highly constitutively active, but also its activity can be further potentiated by chemokines, such as chemokine (C–X–C motif) ligand 8/interleukin-8 (CXCL8/IL-8) and CXCL1/GROα.[17] Interestingly, chemokines CXCL10 and CXCL12 act as inverse agonists, and chemokines CXCL4, CXCL5, and CXCL7 act as neutral antagonists.[18–20] ORF74 activates numerous transcription factors, such as cAMP response element-binding protein, nuclear factor-kappaB, activator protein-1, and nuclear factor of activated T cells, resulting in the expression of a variety of angiogenic growth factors and proinflammatory chemokines and cytokines (e.g., vascular endothelial growth factor and CXCL8/IL-8).[21–23] Cell transformation caused by ORF74 is thus mediated partially by a paracrine mechanism.

Despite strong evidence for the essential role of ORF74 as a viral oncogene and potential novel target for the treatment of patients with Kaposi's sarcoma, no small-molecule allosteric modulators of this oncogene have been reported.

2.2. Epstein–Barr virus

Epstein–Barr virus (EBV), another representative of the γ-herpesvirus group, causes a lifelong latent infection in healthy individuals. Burkitt's lymphoma, Hodgkin's lymphoma, and nasopharyngeal carcinoma are the main diseases associated with EBV.[24] These diseases are particularly common in immunocompromised patients who cannot control the proliferation of EBV-infected B cells because of immune suppression.[25,26]

EBV encodes two vGPCRs—BILF1 and Epstein–Barr virus-induced receptor 2 (EBI2). BILF1 has low homology with the human chemokine (C–X–C motif) receptor 4. BILF1 is an orphan receptor, but due to its high constitutive activity, BILF1 signaling networks are well

characterized.[4,8,27–30] It has been suggested that EBV may use BILF1 to control Gαi-activated pathways during viral lytic replication, thereby promoting disease progression.[28] In nude mice, BILF1 promoted tumor formation in 90% of the animals; the positive correlation between receptor activity and the ability to mediate cell transformation *in vitro* and tumor formation *in vivo* suggests that allosteric modulators which act as inverse agonists for BILF1 could inhibit cell transformation and be relevant therapeutic candidates.[27]

The second vGPCR of EBV, EBI2, is a highly constitutively active receptor that controls follicular B-cell migration and T-cell-dependent antibody production.[31–33] EBI2 has a low homology with lipid binding GPCRs.[34] In 2011, Liu *et al.*[35] identified oxysterols as endogenous ligands of this vGPCR. 7α,25-Dihydroxycholesterol (7α,25-OHC) is the most potent ligand and activator of EBI2 with a K_i of 0.45 nM. *In vitro* and *in vivo* studies showed (7α,25-OHC) can serve as a chemokine that directs EBI2-mediated migration of B cells.[35] Oxysterols are otherwise known to activate nuclear hormone receptors.[36,37] This discovery offers the opportunity to develop steroid-based synthetic derivatives to inhibit the constitutive activation of EBI2.

2.3. Human cytomegalovirus

The omnipresent human cytomegalovirus (HCMV), a member of β-herpesvirus group, causes a lifelong latent infection in healthy hosts. In patients with immature or suppressed immune systems (e.g., neonates, as well as AIDS, cancer, and transplant patients), HCMV can lead to severe and life-threatening disease. Studies of the effects of cytomegalovirus infection on cellular processes provide evidence that the virus contributes to the development of restenosis and atherosclerosis by increasing the migration of smooth muscle cells, inhibition of apoptosis, and augmentation of cellular proliferation.[38,39] The presence of the HCMV genome and antigens was confirmed in tumor cells (but not in adjacent normal tissue) of more than 90% of patients with malignancies like colon cancer, malignant glioma, prostate carcinoma, and breast cancer.[40–42] Also, HCMV infection in transplant patients contributes to transplant rejection.[43,44]

HCMV encodes four vGPCRs (US28, UL33, US27, and UL78) which demonstrate up to 33% sequence homology to human chemokine receptors.[1] US28 and UL33 are characterized by high constitutive activity; US27 and UL78 show no constitutive activity. US27, UL33, and UL78

are orphan receptors—no binding partners have been identified. US28 is the best characterized HCMV chemokine-like receptor which is known to bind to a broad spectrum of chemokines such as CCL2/MCP-1, CCL3/MIP-1α, CCL4/MIP-1β, CCL5/RANTES, and CX_3CL1/Fractalkine with sub-nanomolar affinity. These chemokines activate cell type- and ligand-specific US28-mediated signaling pathways.[45,46] The reprogramming of host cells by US28 leads to vascular smooth muscle cell migration and thus potentially accelerates atherosclerosis and promotes intestinal neoplasia in transgenic mice.[47–49] The view that US28 behaves as an oncogene and promotes tumorigenesis is not yet widely accepted.[45,47] UL33 is another homologue of human chemokine receptors and is characterized by high constitutive activity that may be used by HCMV to orchestrate multiple signaling networks within infected cells.[50] US28, UL33, and US27 are presumably also present in the viral membrane of HCMV.[51,52] The HCMV chemokine receptor homologue US27 is required for efficient spread by the extracellular route but not for direct cell-to-cell spread of HCMV.[53] The role of UL78 in the viral life cycle remains to be identified.

2.4. Other viruses expressing vGPCRs

Human herpesvirus 7 (HHV-7) belongs to the β-herpesvirus subfamily and infects children during infancy and then becomes latent. HHV-7 contains two genes (*U12* and *U51*) that encode putative homologues of cellular GPCRs.[54] *U12* and *U51* encode functional calcium-mobilizing receptors that bind CCL17/TARC, CCL19/ELC, CCL21/SLC, and CCL22/MDC.[55] Overall, these studies suggest that HHV-7 U51 is a positive regulator of virus replication *in vitro*, because it may promote membrane fusion and facilitates cell–cell spread of this highly cell-associated virus.[56]

3. ALLOSTERIC MODULATORS OF vGPCRs

Although a considerable amount of data demonstrates the important role of vGPCRs in viral dissemination and development of cancer, few efforts have been made to pharmacologically target vGPCRs. This section presents an overview of current molecular scaffolds that target US28 and EBI2. Allosteric modulators of vGPCRs described to date generally have only moderate affinity and limited selectivity. The development of highly potent and selective allosteric modulators of vGPCRs thus remains a challenge. Allosteric modulators offer considerable advantages over classical

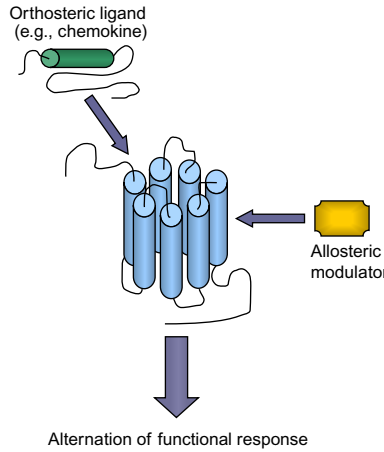


Figure 25.2 Allosteric modulation of vGPCRs. Allosteric modulators bind to a site topographically distinct from the orthosteric site on the receptor to alter either the affinity or efficacy of an endogenous ligand (chemokine) and thus shift the functional response. Allosteric modulators can also reduce the basal activity of the receptor in the absence of an endogenous ligand.

orthosteric ligands because they bind to a topographically distinct site from the orthosteric site (Fig. 25.2). Consequently, they alter GPCR conformation and interactive properties, both with respect to orthosteric ligands and downstream signaling partners in a positive or negative direction.[57,58] Additionally, allosteric binding sites are less conserved compared to orthosteric sites and thus offer the opportunity for better selectivity.

3.1. Allosteric modulators of US28

3.1.1 VUF2274 and its structural analogues

VUF2274 {5-(4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl)-2,2-diphenylpentanenitrile} (**1**) was the first reported inhibitor of US28 receptor constitutive activity (Fig. 25.3).[59,60] This compound fully inhibited the constitutive activity of US28 in the phospholipase C (PLC) pathway with a potency of 3.5 μM. Structure–activity relationship studies showed that a 4-phenylpiperidine moiety is essential for affinity and activity of **1**.[60,61] This compound was initially reported as a CCR1 receptor antagonist,[62] which shares 33% homology with the US28 receptor.[59,60,62] Compound **1** does not inhibit constitutive ORF74- and R33-mediated accumulation of inositol triphosphate, but it interacts with adrenergic, dopamine, muscarinic, and serotonin receptors.[59,62,63] Despite the suboptimal selectivity profile,

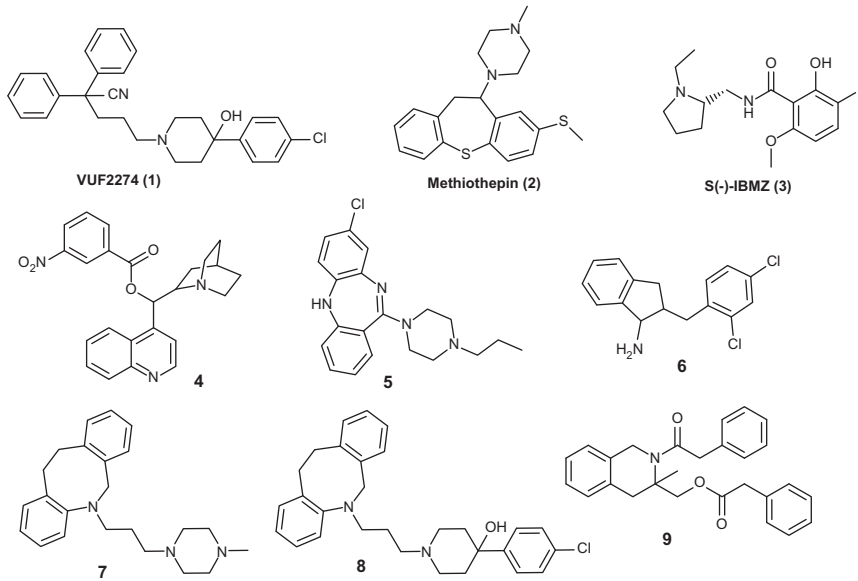


Figure 25.3 Allosteric modulators of the vGPCR of HCMV US28.

1 proved to be an interesting lead compound for the development of US28 inhibitors, leading to the discovery of novel compounds such as **8**.[59,64]

3.1.2 Dibenzothiepines, arylamines, and bicyclic compounds

Dihydrodibenzothiepines (e.g., methiothepin (**2**)), arylamines, such as *S*-iodobenzamide (**3**), and bicyclic compounds (e.g., cinchonidine derivatives, such as compound **4**) have been reported as modulators of US28 in the patent literature (Fig. 25.3).[65–68] Compounds **2** and **3** behave as agonists on US28.[65,67] For the remaining compounds, only the inhibition of fractalkine binding to US28 was reported, without any investigation of intrinsic activity. Continuous efforts to discover novel nonpeptidergic modulators that would inhibit constitutive activity of US28 resulted in a series of piperazinyldibenzodiazepine (e.g., **5**), cinchonidine (e.g., **4**), and indanylamine (e.g., **6**) derivatives (Fig. 25.3).[64] Although the successful modification of these scaffolds yielded a broad variety of structural analogues acting on US28, none of these modifications improved potency and efficacy. The only exception was structural hybridization of the tricyclic imipramine analogue **7** and the 4-phenylpiperidine moiety of **1**, which yielded a modulator **8** with negative efficacy comparable to **1**

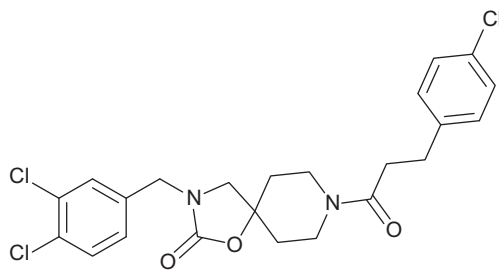
and increased antagonist potency against CCL5 binding (4.7 and 0.7 μM, respectively) (Fig. 25.3).[64]

3.1.3 Isoquinoline and isoquinolinone derivatives

The most recent molecular scaffolds reported to be efficacious inhibitors of US28 constitutive activity belong to the group of dihydrotetraisoquinolinone and tetrahydroisoquinoline **9** derivatives.[63] Compound **9** is a stronger partial agonist (a dose–response with maximal efficacy of −37% at 10 μM) in the reporter gene assay compared to **2** (−22%), although with an EC_{50} still in the micromolar range (3.4 μM).[63] These dihydroisoquinolinones demonstrate remarkable potency and efficacy on the dopamine D_{2L} receptor that underlies an obvious issue regarding the cross-reactivity with biogenic amine receptors similar to that reported for **1**.[62]

3.2. Allosteric modulator of EBI2

Rosenkilde *et al.* identified the first potent and efficacious inverse agonist, GSK682753A (**10**), of EBI2 (Fig. 25.4).[69] Inverse agonist **10** inhibits the constitutive activity of EBI2 in various functional assays with high potency (IC_{50} = 2.6–53.6 nM) and efficacy (−75%), determined from a dose–response curve at a concentration of 10 μM. The selectivity of **10** was tested only on a limited set of constitutively active receptors, including GPR39, GPR17, MC1R, ORF74, and the ghrelin receptor, on which **10** had no inverse agonist effect. In the future, **10** will serve as a potent lead compound for the development of novel inverse agonists of vGPCRs and serve as a useful tool for further characterization of EBI2.



GSK682753A (10)

Figure 25.4 Allosteric modulator of the EBV vGPCR EBI2.

4. ALLOSTERIC MODULATORS OF vGPCRs AT WORK

Suboptimal potency and selectivity of the small number of currently available allosteric modulators of vGPCRs limits their use as chemical probes, but isolated examples reviewed herein demonstrate the utility of these allosteric modulators for the development of therapies directed toward the treatment of vGPCR-mediated pathologies.

4.1. Inhibition of US28-mediated HIV entry

In vitro experiments in cell lines coexpressing vGPCR US28 and CD4 showed that US28 serves as a cofactor for HIV-1 entry.[70] The US28 inverse agonist **1** at a concentration of 1 μM inhibited 60% of the US28-mediated HIV-1 entry in cells coexpressing US28 and CD4.[59]

4.2. Inhibition of US28-mediated signaling in HCMV-infected fibroblasts

The infection of human foreskin fibroblasts with HCMV induces a consistent increase in PLC activity[71] which is thought to be mediated by US28.[45,59,71] The US28 inverse agonist **1** inhibited US28-mediated PLC activation with an $IC_{50} \sim 0.8$ μM.[59]

4.3. Suppression of EBI2-mediated proliferation of murine B cells

Mice that overexpressed EBI2, specifically in B cells, were generated to mimic the expression pattern observed upon EBV infection.[69] EBI2 constitutive activity by **10** suppressed basal migration and antibody-induced proliferation of EBI2 expressing B cells *ex vivo* with an IC_{50} value 0.28 μM.[69]

5. CONCLUSIONS

vGPCRs are seven-transmembrane receptors with frequently striking sequence homology to host GPCRs. The reprogramming of cell signaling by vGPCRs often results in prosurvival and angiogenic effects, inflammation, transformation, proliferation, and increased viral replication that ultimately lead to lifelong infections and chronic diseases like atherosclerosis and cancer. Despite the fact that GPCRs are excellent drug targets, vGPCRs have received little attention. The development of potent and selective vGPCR allosteric modulators would have a significant impact on the

deciphering of molecular mechanisms of negative vGPCR regulation and consequently on the development of therapies for various diseases caused by Herpesviruses.

The few studies reporting small weight inhibitors of vGPCRs indicate that selective targeting of these receptors represents a challenge due to sub-optimal affinity and with it related selectivity issues. Alternatively, antibodies neutralizing or blocking vGPCRs could be used in proof-of-concept studies. After these issues are resolved and the drug candidates show favorable preclinical profile, these drugs could be used in clinical trials on the subset of patients carrying the desired biomarkers, but not primarily as a preventive therapy. This type of personalized medicine (the combination of vGPCR inhibitors and, e.g., currently available cancer treatments) is expected to improve treatment outcome.

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