Distinct roles of extracellular domains in the Epstein-Barr virus-encoded BILF1 receptor for signaling and major histocompatibility complex class I downregulation

The Epstein-Barr virus (EBV) BILF1 gene encodes a constitutively active G protein-coupled receptor (GPCR) that downregulates major histocompatibility complex (MHC) class I and induces signaling-dependent tumorigenesis. Different BILF1 homologs display highly conserved extracellular loops (ECLs) including the conserved cysteine residues involved in disulfide bridges present in class A GPCRs (GPCR bridge between transmembrane helix 3 [TM-3] and ECL-2) and in chemokine receptors (CKR bridge between the N terminus and ECL-3). In order to investigate the roles of the conserved residues in the receptor functions, 25 mutations were created in the extracellular domains. Luciferase reporter assays and flow cytometry were used to investigate the G protein signaling and MHC class I downregulation in HEK293 cells. We find that the cysteine residues involved in the GPCR bridge are important for both signaling and MHC class I downregulation, whereas the cysteine residues in the N terminus and ECL-3 are dispensable for signaling but important for MHC class I downregulation. Multiple conserved residues in the extracellular regions are important for the receptor-induced MHC class I downregulation, but not for signaling, indicating distinct structural requirements for these two functions. In an engineered receptor containing a binding site for Zn^{2+} ions in a complex with an aromatic chelator (phenanthroline or bipyridine), a ligand-driven inhibition of both the receptor signaling and MHC class I downregulation was observed. Taken together, this suggests that distinct regions in EBV-BILF1 can be pharmacologically targeted to inhibit the signaling-mediated tumorigenesis and interfere with the MHC class I downregulation.

**Importance**

G protein-coupled receptors constitute the largest family of membrane proteins. As targets of >30% of the FDA-approved drugs, they are valuable for drug discovery. The receptor is composed of seven membrane-spanning helices and intracellular and extracellular domains. BILF1 is a receptor encoded by Epstein-Barr virus (EBV), which evades the host immune system by various strategies. BILF1 facilitates the virus immune evasion by downregulating MHC class I and is capable of inducing signaling-mediated tumorigenesis. BILF1 homologs from primate viruses show highly conserved extracellular domains. Here, we show that conserved residues in the extracellular domains of EBV-BILF1 are important for downregulating MHC class I and that the receptor signaling and immune evasion can be inhibited by drug-like small molecules. This suggests that BILF1 could be a target to inhibit the signaling-mediated tumorigenesis and interfere with the MHC class I downregulation, thereby facilitating virus recognition by the immune system.

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