RESEARCH HIGHLIGHT

vGPCR, The Great Escape

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Kaposi Sarcoma (KS), a connective tissue cancer that may affect the skin and internal organs, is associated with human herpes virus 8 (HHV-8) infection. Among the oncogenes encoded by HHV8, the viral G protein coupled receptor (vGPCR ORF74) was found instrumental for sarcomagenesis initiation and progression. Indeed, vGPCR displays permanent activation, and is sufficient to induce tumor development in mice. However, the molecular mechanisms controlling vGPCR expression and activation remain poorly understood. Here, we present recent data from our group highlighting the presence of an endocytosis motif (Y_{326}GLF) in the vGPCR C-terminal domain that orchestrates the receptor cellular localization, as well as its signaling and paracrine actions. We further show that this YGLF motif controls TLR4 surface expression, and may thus assure immune surveillance. In conclusion, this work shed light on the importance of vGPCR cellular localization and trafficking for its pathogenicity.

***Keywords*:** G protein coupled receptor; endocytosis; signaling; NF-κB; herpes virus

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The human herpes virus 8 (HHV8), also known as the Kaposi sarcoma herpes virus (KSHV), is the causative agent for both Kaposi Sarcoma (KS) and two kinds of non-Hodgkin lymphomas, namely primary effusive lymphoma and multicentric Castleman disease. The viral G protein coupled receptor (vGPCR, ORF74) has now been established to initiate KS and chiefly orchestrate its progression [1]. At the molecular level, vGPCR sequence and modus operandi resemble to the C-X-C chemokine receptor CXCR2 [2], although vGPCR harbors multiple mutations that contribute to its constitutive and permanent activation. Moreover, vGPCR expression is sufficient to initiate cell transformation *in vitro* and tumor development in mice [3-5]. vGPCR activates multiple signaling cascades, that subsequently trigger cell survival, proliferation, and migration in a wide range of cell models, including endothelial and lymphatic vascular cells [6-11]. Although elevated and aberrant PI3K/mTOR and NF-κB signaling nexi were associated with vGPCR oncogenic abilities, little is known about the ruses employed by this receptor to preserve its actions in sarcomagenesis. Recent studies from our group provide molecular insights on the regulation of vGPCR trafficking and signaling [12].

To understand how vGPCR dynamics is regulated, we performed an *in silico* analysis of vGPCR and CXCR2 sequences. We found that vGPCR exclusively exhibits a docking site for the adaptor protein 2 (AP2) in its C-terminal domain (Y_{326}GLF). Indeed, vGPCR coalesces

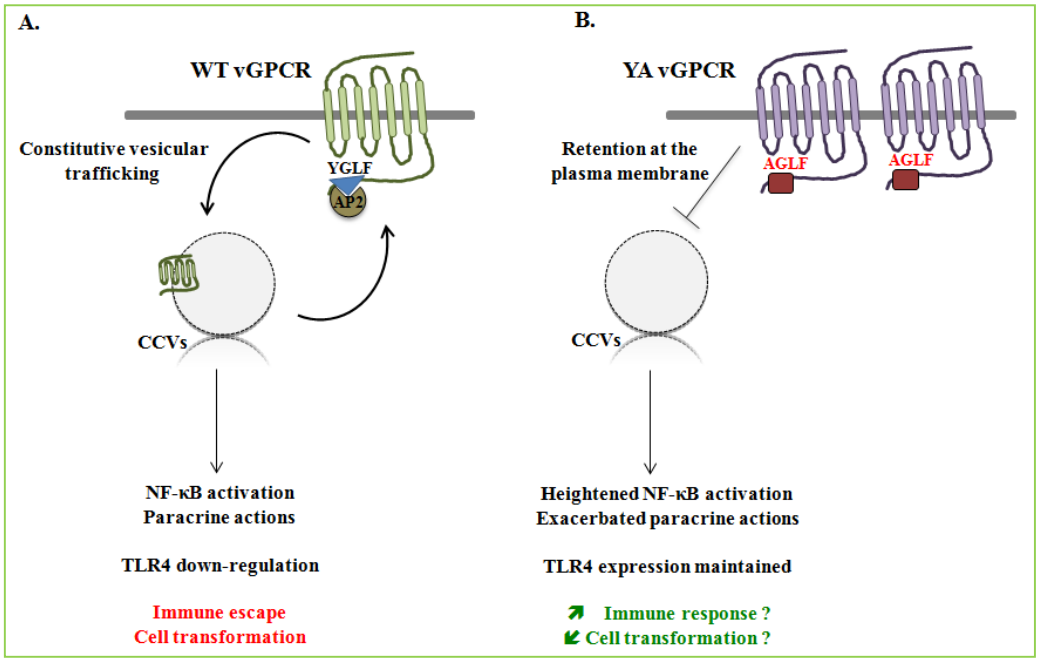

Figure 1. Role of the YGLF motif in vGPCR trafficking and signaling. A. Viral G-coupled protein receptor (vGPCR) harbors a classical AP2 binding motif $-_{326}$YGLF- in its C-terminal tail that directs its trafficking, signaling, paracrine activity, and might impact immune evasion and cell transformation. **B.** The introduction of a point mutation (Y-A) in this domain hinders the AP2 complex interaction with vGPCR and fastens it at the plasma membrane. This unleashes its signaling activity and exacerbates paracrine communication. This may have deleterious effects for the virus life cycle. For instance, YA mutant maintains TLR4 expression, most likely favoring immune responses. Additionally, its action on cell transformation remains under question.

with clathrin-coated vesicle (CCV) components, including AP2α, AP2β, AP2γ, EPS15, and clathrin heavy chain (Figure 1). Likewise, vGPCR was observed in internal vesicles in various cell types, while ectopically or endogenously expressed[12]. Keeping with this, interfering genetically with CCV function promotes vGPCR retention at the plasma membrane. To further assess the involvement of this motif in vGPCR trafficking, an AP2 binding mutant ($Y_{326}A$) was generated, and its dynamics was examined. Although both WT and YA forms of vGPCR were expressed at similar level, YA vGPCR surface availability surpasses WT vGPCR one, and the mutant barely accumulated in CCVs (Figure 1B). In this context, a HHV8 encoded small mitochondrial membrane protein, named K7, was found to specifically interact with vGPCR and to promote its sequestration in the endoplasmic reticulum (ER) and its subsequent degradation [13]. Hence, vGPCR might constantly shuttle between the plasma membrane and internal compartments, such as ER and CCVs. Overall this mechanism might help vGPCR to escape from negative feedback loops that control physiological GPCR localization and activity.

As vGPCR mediates its oncogenic and transforming abilities notably through the NF-κB axis [14, 15], we asked whether the introduction of the YA mutation might impact its signaling activity. Strikingly, $Y_{326}A$ substitution multiplied the NF-κB level of activation and exacerbated vGPCR-governed paracrine communication (Figures 1A-B). Interestingly, the AP1 and NFAT promoter activities were also elevated (our unpublished observations). Indeed, conditioned media (CM) prepared from HeLa cells expressing the YA mutant increased IκBα phosphorylation in THP1 monocytes, as compared to the CM from WT vGPCR expressing cells [12]. The YA-related secretome further altered cell architecture, increased cell migration and boosted proliferation in monocytes. It also promoted T cell migration and elevated endothelial permeability. Collectively our data support a model in which the YA mutation stabilizes vGPCR at the plasma membrane and inflates its outside-in and inside-out signaling (Figure 1).

However, one can ask the benefit for the virus to have selected a somehow less active form? As KSHV persistently infects its host, it has to fraud the immune system. In this scenario, it was shown that the Epstein-Barr virus GPCR BILF1 physically interacts with major histocompatibility complex class I (MHCI) molecules and reduces their surface expression level, favoring in turn immune evasion[16]. Although vGPCR did not modify MHCI levels (our un- published observations and[16]), the pathogenic receptor might contribute to the virus' ability to evade immune surveillance through Toll Like Receptor 4 (TLR4) downregulation[17, 18]. As previously reported,

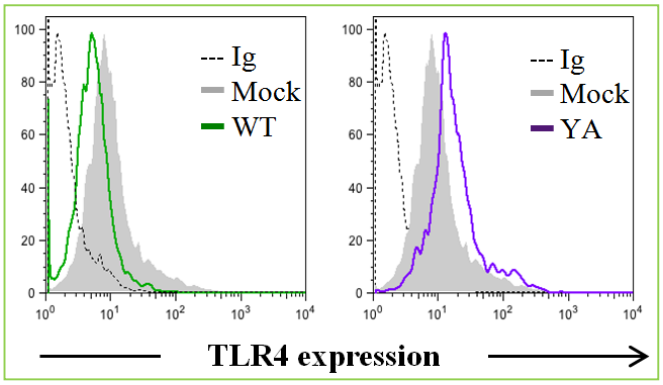

Figure 2. Impact of the $Y_{326}A$ mutation on TLR4 surface availability. 293 cells stably expressing HA-tagged TLR4 (Invivogen) were co-transfected with AU5-tagged WT or YA vGPCR. TLR4 expression was analyzed by flow cytometry (10000 events) using anti-HA antibodies (Sigma). Mock-transfected cells were used as control. Thus, in contrast to the WT form, the YA vGPCR mutant fails to suppress TLR4 expression, as part of the immune escape response.

TLR4 surface expression was reduced in WT vGPCR expressing HeLa cells, when compared to the mock controls. Surprisingly, the YA mutant fails to do so (Figure 2). This piece of data highlights a putative advantage for the presence of a functional YGLF site within vGPCR. This effect of YA vGPCR on TLR4 might be ascribed to a direct signaling and/or to a paracrine action via deregulated cytokine secretion. Additionally, v GPCR mislocalization might weigh on transforming abilities and oncogenic progression, as strengtheningparacrine communication might be deleterious for virus life cycle. Our work unmasks how vGPCR cellular localization impacts on its signaling and paracrine actions, which might alter communication and immune responses within the tumor microenvironment.

Conflicting interests

The authors have declared that no competing interests exist.

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