

The role of G protein-coupled receptor signaling pathways and mutations in cancer development and immune response

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Abstract

G protein-coupled receptors (GPCRs) play a crucial role in cellular signaling, regulating various physiological processes. Abnormal expression and mutations of GPCRs have been implicated in several types of cancer, influencing tumor initiation, progression, and immune response. In this review, we present an overview of recent research on GPCR involvement in cancer, and discuss the evidence supporting whether mutations in GPCRs act as cancer driver or passenger. Accumulation of GPCR mutations in some highly conserved structural motifs and the mutually exclusiveness observed between G_i-coupled GPCRs and GNAS-activating mutations indicate their potential driving role in cancer. However, the functional redundancy of GPCR signaling networks, together with the widespread but low frequency distribution of GPCR mutations indicate that they may rather act as passengers. The future of GPCR drug discovery hinges on overcoming challenges related to data availability and the integration of GPCR research with broader cancer studies using multi-omics approaches.

The role of G protein-coupled receptor signaling pathways and mutations in cancer development and immune response

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Abstract: G protein-coupled receptors (GPCRs) play a crucial role in cellular signaling, regulating various physiological processes. Abnormal expression and mutations of GPCRs have been implicated in several types of cancer, influencing tumor initiation, progression, and immune response. In this review, we present an overview of recent research on GPCR involvement in cancer, and discuss the evidence supporting whether mutations in GPCRs act as cancer driver or passenger. Accumulation of GPCR mutations in some highly conserved structural motifs and the mutually exclusiveness observed between G_i-coupled GPCRs and GNAS-activating mutations indicate their potential driving role in cancer. However, the functional redundancy of GPCR signaling networks, together with the widespread but low frequency distribution of GPCR mutations

indicate that they may rather act as passengers. The future of GPCR drug discovery hinges on overcoming challenges related to data availability and the integration of GPCR research with broader cancer studies using multi-omics approaches.

Keywords G protein-coupled receptor (GPCR), cancer, driver mutation, passenger mutation, cellular signaling

1. Introduction

G protein-coupled receptors (GPCRs) are the largest and most diverse group of membrane receptors in eukaryotes (Grisshammer, 2017). The common structure of GPCRs consists of an extracellular N terminus, seven alpha-helical transmembrane domains (TM1-7) connected by three intracellular loops and three extracellular loops, and an intracellular C terminus (Baldwin, Schertler, & Unger, 1997). With this structure, GPCRs can translate extracellular stimuli into an intracellular response, mainly through heterotrimeric G proteins consisting of α , β , and γ sub-units. G proteins interact with other proteins, which activate a diverse array of downstream signaling pathways (Fredriksson, Lagerström, Lundin, & Schiöth, 2003). As such, GPCRs play important roles in the physiology of all major peripheral organ systems, and dysregulation of GPCRs are associated with various human diseases including type 2 diabetes (Hua Li *et al.*, 2013), Alzheimer’s disease (Nickols & Conn, 2014), hypertension (G. C. Sun *et al.*, 2015), heart failure (Cannavo, Liccardo, & Koch, 2013), and cancer (Young, Waitches, Birchmeier, Fasano, & Wigler, 1986). Therefore, GPCRs have received significant attention in drug discovery, and are targeted by nearly 34% of all FDA approved drugs {Hauser, 2017 #177} (Hauser, Attwood, Rask-Andersen, Schiöth, & Gloriam, 2017) {Hauser, 2017 #177}. Over the past decades, GPCR-related signaling cascades have been linked to critical cellular processes such as proliferation, angiogenesis, and immune responses, all of which are pivotal in tumorigenesis and metastasis (Wu *et al.*, 2019). Moreover, abnormal expression and function of GPCRs have been identified in various cancer types, both in cancer cells and cancer-associated immune cells, presenting these receptors as potential biomarkers for cancer diagnosis and prognosis (Chaudhary & Kim, 2021; P. A. Insel *et al.*, 2018). However, current use of drugs targeting GPCRs in cancer therapy remains limited, with only a few in clinic (Table 1) and more in clinical trials, which have been summarized by Usman and others (Usman, Khawer, Rafique, Naz, & Saleem, 2020). Investigation of GPCRs as anti-cancer drug targets features various receptors and an array of small molecules and antibodies, exhibiting potential in different cancer types including prostate cancer, ovarian cancer, pancreatic cancer, and melanoma (Jacquelot, Duong, Belz, & Zitvogel, 2018; Kaye *et al.*, 2012; Linehan *et al.*, 2018; Shepard & Dreicer, 2010). However, their potential remains largely untapped. Sequencing methods have revealed a list of genes driving tumor initiation and demonstrated GPCR overexpression in various cancer types (Lappano & Maggiolini, 2011). Recent studies have also raised the question whether mutations in GPCRs are driving cancer progression or if they represent passenger mutations with little impact (Kan *et al.*, 2010; Kandath *et al.*, 2013; Lawrence *et al.*, 2013). In this review, we discuss the involvement of GPCR signaling in cancer development and immune response, and the mutational landscape of G proteins and GPCRs. Subsequently, we provide evidence of GPCR mutations as cancer driver or passenger genes. Lastly, we summarize the challenges and opportunities of targeting GPCRs in cancer therapy. **Table 1** Currently FDA approved anti-cancer drugs targeting GPCRs.

Drugs	Target	Ligand	Cancer
Cabergoline	Dopamine receptor D1 (DRD1)	Small molecule	Neuroendocrine tumor
Lanreotide	Somatostatin receptor (SSTR)	Hormone	Pancreatic cancer
Degarelix	Gonadotropin releasing factor hormone receptor (GnRH)	Hormone	Prostate cancer
Plerixafor	C-X-C chemokine receptor 4 (CXCR4)	Small molecule	Multiple myeloma
Vismodegib (Erivedge)	Smoothed receptor (SMO)	Small molecule	Locally advanced, an
Raloxifene	Estrogen receptor (ER)	Small molecule	Breast cancer
Sonidegib (Odomzo)	Smoothed receptor (SMO)	Small molecule	Locally advanced, an

Drugs	Target	Ligand	Cancer
Mogamulizumab	C–C Chemokine receptor 4 (CCR4)	Antibody	T cell lymphoma

* Adapted from “*The current status of anti-GPCR drugs against different cancers (Usman et al., 2020)*”.

2. GPCR signaling in cancer

2.1 Classical GPCR signaling pathways

Activation of GPCRs represents a pivotal molecular event in cellular signaling cascades. Upon ligand binding, these receptors undergo conformational changes, which catalyze the dissociation of GDP from the G protein G_{α} subunit, followed by the binding of GTP to G_{α} and the subsequent separation of G_{α} from the $G_{\beta/\gamma}$ subunits (Pierce, Premont, & Lefkowitz, 2002). This separation allows G_{α} to modulate downstream effector molecules, such as adenylyl cyclase (AC) or phospholipase C (PLC), initiating a diverse array of intracellular responses that lead to changes in cell proliferation, migration, or cell survival (Figure 1) (Kamps & Coffman, 2005). $G_{\alpha_c} / G_{\alpha_i}$ can either upregulate or downregulate AC activity, modulating cyclic AMP (cAMP) production and subsequent activation of protein kinase A (PKA). PKA is a key intracellular mediator that induces the phosphorylation of target proteins. G_{α_x} induces PLC activation, leading to intracellular calcium mobilization and diverse responses. $G_{\alpha_{12/13}}$, while less understood, has emerged as a key player in cell migration, cytoskeletal dynamics, and oncogenic transformation (Rasheed *et al.*, 2022). Following GPCR activation, G protein-coupled receptor kinases (GRKs) play a crucial role in the phosphorylation of GPCRs and subsequent recruitment of β -arrestins. These protein have been shown to cause distinct cellular responses, including receptor desensitization, internalization that prevent further G protein coupling, but also activation of signaling cascades such as the mitogen-activated protein kinase (MAPK) pathway (S. K. Shenoy & Lefkowitz, 2005) (Gurevich & Gurevich, 2019). Hence, together G proteins and β -arrestins orchestrate a finely tuned cellular response upon GPCR activation by extracellular stimuli.

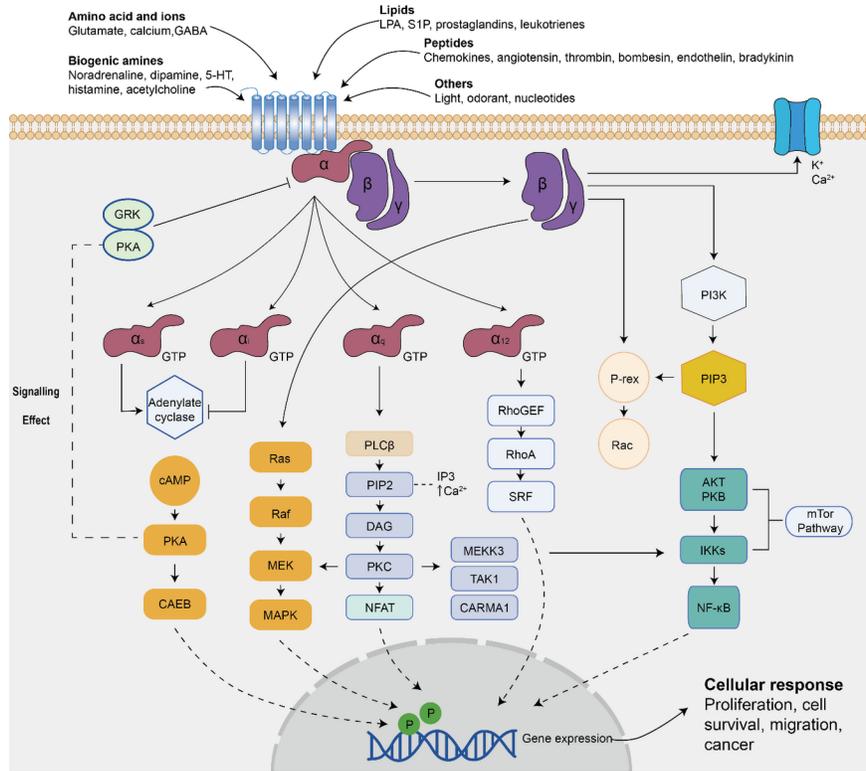


Figure 1 G protein-mediated signaling pathway of GPCRs. Ligand-induced conformational change leads to activation of the heterotrimeric G protein, resulting in dissociation of the G_{α} subunit from the $G_{\beta\gamma}$ subunits. Downstream effects of secondary messengers lead to an ultimate cellular response. Figure was made with adobe illustrator and adapted from Dorsam and Gutkind (Robert T Dorsam & J Silvio Gutkind, 2007).

2.2 Aberrant GPCR expression in cancer

Comparing to healthy tissues, aberrant expression of GPCRs are frequently observed in several types of tumors (Kübler & Albrecht, 2018; Perez Almeria, Setiawan, Siderius, & Smit, 2021). Especially in tumors of the neuroendocrine system, overexpression of GPCRs is highly prevalent, such as MC2R, 5-HT4R, LH-CGR, GnRHR, TRHR, GLP1R, GIPR, and GRP101 (Lacroix *et al.*, 2024). It has also been reported that in pancreatic ductal adenocarcinoma, overexpression of multiple GPCRs, such as HRH1, LPAR5 and CCR6, tends to be more prevalent than common oncogenic mutations such as KRAS and TP53 (Sriram, Moyung, Corriden, Carter, & Insel, 2019). In addition, Arora *et al.* found that almost every cancer subtype is characterized by a highly specific GPCR-ligand co-expression signature, and they identified clusters of GPCR-ligand pairs showing a prevalence of concordant upregulation or downregulation across cancer subtypes. Furthermore, in some subtypes featured with the co-downregulated GPCR axes, concomitant mutations of several tumor suppressor genes are present. While in the concordantly upregulated axes, cancer subtypes with $G_{\alpha_{12/13}}$ prevalence are characterized by mutations of the KRAS, PIK3CA, and MLLT3 oncogenes. Importantly, they also found that the expression of GPCR genes is associated with lower or higher survival of cases depending on cancer subtype, while some receptors show consistent associations among subtypes. For example, ADORA2A is associated with higher survival across four cancer tissues (pancreas, breast, skin, and head and neck), while GPCRs such as OXTR, ADORA2B, GPR3, FZD6 are invariably associated with poorer survival. To be noted, there is not always consistency between the association of receptor expression on patient survival with the direct effects of receptor activation/inhibition on cancer cells. For example,

when HEPG2 cells were treated with either an ADORA2A or ADORA2B inhibitor, they observed that the cell viability was significantly decreased in a dose-dependent manner for both (C. Arora *et al.*, 2024). Many factors including cancer subtype, receptor crosstalk, and cell-cell interactions within the tumor microenvironment can play a role, making drug effects on patients hard to predict. Despite the complexity, these findings indicate that aberrant GPCR expression could play an important role in cancer progression and prognosis.

2.3 GPCR signaling in cancer development

In cancer, GPCR signaling can impact crucial characteristics of cancer development, such as uncontrolled cell growth, achieving replicative immortality, resisting apoptosis, initiating invasion and metastasis (New & Wong, 2007; O’Hayre, Degese, & Gutkind, 2014). The role that different downstream signaling pathways play is summarized below.

AC - cAMP pathway

As mentioned above, GPCRs regulate adenylate cyclase (AC) activity through $G_{\alpha c} / G_{\alpha i}$ subunit and thereby change the level of intracellular cyclic AMP (cAMP). The rise in cAMP activates PKA, leading to the phosphorylation of target proteins involved in various cellular processes, including cell proliferation, gene expression, cell survival and differentiation. Dysregulation of the AC-cAMP pathway has been found to be a contributing factor in cancer development (Ahmed, Alghamdi, Islam, Lee, & Lee, 2022).

PLC - IP3/DAG pathway

The phospholipase C (PLC) can be activated by GPCRs- $G_{\alpha \chi}$ protein pairs. PLC catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) into inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG) (Rebecchi & Pentylala, 2000). IP3 diffuses to the endoplasmic reticulum, binds to its receptor and triggers the release of calcium ions (Ca^{2+}), which along with DAG act as a secondary messenger to modulate downstream effectors like protein kinases and phosphatases. Thereby, this pathway influences cell growth, apoptosis, and cell migration, and thus plays an important role in the development of cancer (Tyutyunnykova, Telegeev, & Dubrovskaya, 2017).

Ras - MAPK pathway

Research has demonstrated that GPCR activation can promote cell proliferation and inhibit apoptosis through the Ras-MAPK-pathway (Santarpia, Lippman, & El-Naggar, 2012). Ras proteins can be activated by the $G_{\alpha \chi}$ or $G_{\beta \gamma}$ subunit, triggering the Raf-MEK-MAPK kinase cascade. The mitogen-activated protein kinases (MAPK) pathway are highly involved in tumorigenesis, thus becoming potential therapeutic targets for cancer treatment.

PI3K - PKB/AKT pathway

Through $G_{\beta \gamma}$ subunit, GPCRs also engage in crosstalk with the phosphoinositide 3-kinase - protein kinase B/AKT (PI3K-PKB/AKT) pathway, another critical signaling cascade implicated in cancer development (Fumarola, Bonelli, Petronini, & Alfieri, 2014; New, Wu, Kwok, & Wong, 2007). GPCR-induced PI3K stimulation may lead to AKT phosphorylation, which enhances cell survival and resistance to apoptosis. This pathway is commonly dysregulated in a variety of cancers (R. Liu *et al.*, 2020).

$\Omega \nu \tau$ - β -catenin pathway

Wnt- β -catenin pathway is a signaling cascade crucial for cell fate determination and stem cell maintenance (Zhang & Wang, 2020). Dysregulated GPCR signaling can induce abnormal activation of Wnt- β -catenin signaling, which supports the properties of cancer stem cells and accelerates tumor growth (Nag *et al.*, 2018).

2.4 GPCR signaling in cancer immune response

Apart from cancer cell behavior, the absence or presence of immune response is an important factor that determines the progression of cancer. The following section introduces key GPCRs that have been identified to have impact on cancer immune response.

Chemokine receptors

Chemokines play a crucial role in the regulation of immune responses, also in the context of cancer, where the tumor microenvironment relies on a delicate balance of immune cell recruitment and activation (Ozga, Chow, & Luster, 2021). The interaction between chemokines and their corresponding GPCRs is a fundamental mechanism governing immune cell trafficking and positioning. For instance, CCL2 and CXCL12 bind to CCR2 and CXCR4/CXCR7 on immune cells respectively, directing the migration of monocytes, macrophages, and T cells toward the tumor site (Kohli, Pillarisetty, & Kim, 2022). Some chemokines have been reported to exert pro-cancer effects such as CCL2/CCL7/CCL8/CCL13, while others have shown anti-cancer effects such as CCL14 and CCL16 (Korbecki *et al.*, 2020). The pro- and anti-cancer effects of chemokines-chemokine receptors network were regulated in a context dependent manner, and therefore abnormal expression or function of chemokine receptors may disrupt the finely tuned signaling of immune responses (Strazza & Mor, 2020).

Prostanoid Receptors

Prostaglandins can be generated by tumor cells as well as cells in the surrounding tissue, and they exert diverse physiological functions including inflammation and immune responses. In the context of cancer, prostanoid receptors play a multifaceted role in shaping the tumor microenvironment (Harizi, 2013). For example, PGE2 binds to EP2 and EP4 receptors, which suppress anti-tumor immune responses by inhibiting the production of pro-inflammatory cytokines and promoting the expansion of immunosuppressive regulatory T cells (Ricciotti & FitzGerald, 2011). Conversely, stimulation of EP3 receptors may have opposite effects, enhancing certain aspects of the immune response (Semmlinger *et al.*, 2018). The intricate balance among these receptors influences the immune landscape within the tumor, and targeting prostanoid receptors has emerged as a potential strategy to enhance the efficacy of cancer immunotherapy (Nie, Wang, & Nie, 2023).

Sphingosine-1-Phosphate Receptors (S1PR)

Sphingosine-1-phosphate (S1P) receptors regulate fundamental biological processes such as cell proliferation and migration. In the context of cancer, S1P receptors have been implicated in modulating immune cell trafficking, activation, and overall composition of the tumor microenvironment (Maceyka, Harikumar, Milstien, & Spiegel, 2012). S1P receptors, particularly S1PR1 and S1PR3, are involved in releasing lymphocytes from lymphoid organs and regulating their migration to tumors. Activation of S1P receptors on immune cells can also impact their proliferation and functionality (Nagahashi, Abe, Sakimura, Takabe, & Wakai, 2018). Small molecules that modulate S1P receptors activity, including sphingosine analogs and selective receptor agonists or antagonists, are being explored for their potential in cancer immunotherapy (Maceyka *et al.*, 2012).

Lysophosphatidic Acid Receptors (LPA)

Lysophosphatidic acid (LPA), a bioactive lipid, is involved in various physiological processes, including cancer and immune response (Geraldo *et al.*, 2021). LPA is frequently elevated in the tumor microenvironment, affecting immune cell migration, survival, and cytokine production, shaping the immune landscape within tumors. LPA-LPAR interactions also influence the recruitment of immune cells, impacting both innate and adaptive immune responses (S. C. Lee *et al.*, 2020).

Adenosine Receptors

Adenosine is often elevated in the tumor microenvironment, and its binding to adenosine A_{2A} and A_{2B} receptors (A_{2A}AR and A_{2B}AR) on immune cells leads to suppression of anti-tumor immune responses (Haskó, Linden, Cronstein, & Pacher, 2008; Leone & Emens, 2018). Activation of the A_{2A}AR inhibits the

activity of cytotoxic T cells and natural killer cells while promoting the expansion of immunosuppressive regulatory T cells (C. Sun, Wang, & Hao, 2022). And activation of the $A_{2B}AR$ has been found to lower the abundance of B cells, inhibit natural killer cells activity and cytokine production (Faraoni *et al.*, 2023; Han, Dong, Hu, Wang, & Wang, 2024). The adenosine receptors have become a focus area in cancer immunotherapy research, with efforts to develop selective antagonists targeting $A_{2A}AR$ and $A_{2B}AR$ (Leone & Emens, 2018). Currently, several $A_{2A}AR/A_{2B}AR$ antagonists are under clinical trials for different types of cancer (Chen, Chang, Yu, & Sung, 2024; Franco, Rivas-Santisteban, Navarro, & Reyes-Resina, 2021).

Opioid Receptors

Opioid receptors are integral components of the endogenous opioid system, which regulates pain perception and various physiological functions. Receptors mu (MOR), delta (DOR), and kappa (KOR) mediate the effects of endorphins, enkephalins, and dynorphins, which are endogenous opioid peptides (S. S. Shenoy & Lui, 2023). While known for their role in pain modulation, opioid receptors have also been implicated in the regulation of immune responses and cancer progression (Boland & Pockley, 2018). The interaction between opioid receptors and the immune system is complex, with evidence suggesting both immunosuppressive and stimulatory effects. The immunosuppressive potential of opioids, particularly through MOR activation, has raised interests in blocking the receptor to revive anti-tumor immune responses (Gondoh *et al.*, 2022; Lennon, Moss, Singleton, & Riou, 2012).

3. Mutational landscape of G proteins and GPCRs in cancer

Besides aberrant expression, mutations are another key factor that cause dysregulation of GPCR signaling. GPCRs are mutated in approximately 20% of all cancers, and recurrent mutations in particular GPCRs are linked to the advancement of cancer (Kan *et al.*, 2010). Genetic mutations in the coding regions of GPCRs may lead to changes in ligand binding affinity, receptor expression, or the efficiency of G protein coupling, which further affect downstream signaling (Stoy & Gurevich, 2015). In the following section, we will present the mutational landscape of G proteins and GPCRs, with a focus on widespread mutations identified in cancer.

3.1 Widespread mutations in G proteins

G proteins play an instrumental role in regulating cellular signal transduction. G_{α_c} , G_{α_i} , $G_{\alpha_{\chi/11}}$ and $G_{\alpha_{12/13}}$ are four main types of G_{α} subunits. Sequencing has identified many encoding mutations of G proteins, where non-synonymous mutations are highly prevalent over synonymous mutations and mostly affect constitutive activity (CA) of GPCR signaling (O’hayre *et al.*, 2013). Overall, *GNAS* (G protein Subunit Alpha S) is mutated in 4.45% of all tumor sequences deposited in the Catalogue of Somatic Mutations in Cancer (COSMIC), making it the most frequently mutated G proteins in human cancer (Table 2) (Forbes *et al.*, 2017). *GNAS* mutations are commonly linked to endocrine-related tumors, including certain types of pancreatic and thyroid cancers, pituitary adenomas and others. Most of the well-known *GNAS* mutations are clustered around two hotspot residues, R201 and Q227, leading to sustained activation of the G_{α} subunit and downstream signaling pathways (Turan & Bastepe, 2015). This sustained signaling can promote cell proliferation and inhibit apoptosis, contributing to tumor initiation and progression. *GNAQ* (G Protein Subunit Alpha Q) mutations are notably associated with uveal melanoma, a rare but aggressive form of eye cancer. In uveal melanoma, activating mutations in the hotspot residues Q209, and R183 lead to persistent activation of the MAPK pathway, driving uncontrolled cell growth (Onken *et al.*, 2008). Unlike many other cancers, these *GNAQ* mutations are prevalent and are often early events in uveal melanoma, making them attractive targets for precision medicine. *GNA11* (G Protein Subunit Alpha 11) is closely related to *GNAQ*, and mutations in *GNA11* are also implicated in uveal melanoma, highlighting the redundancy and shared pathways of these G proteins in certain cancers (Piaggio *et al.*, 2022). **Table 2** Mutational landscape of G proteins in cancer. Table was adapted from O’Hayre et al. (O’hayre et al., 2013).

Mutation	Gene name	% affected tumors	Hotspot residues	Mechanism of action
<i>GNAS</i>	G protein Subunit Alpha S	4.45%	R201 Q227	Reduce rate of GTP hydrolysis of
<i>GNAQ</i>	G Protein Subunit Alpha Q	3.36%	Q209 R183	Activate GEF, Trio and Rho GT
<i>GNA11</i>	G Protein Subunit Alpha 11	2.49%		

3.2 Widespread mutations in GPCRs

Studies have identified widespread mutations of GPCRs in cancer (Sriram *et al.* , 2019). By analyzing 5,103 samples of 20 tumor types from The Cancer Genome Atlas (TCGA), Sriram *et al.* found that approximately 65% of tumors have at least 1 nonsilent GPCR mutation. This frequency is higher than the previously reported 20% by Kanet *et al.* (Kan *et al.*, 2010), which may lie in different sampling methods. Kan’s study included 441 tumor samples (183 breast cancers, 134 of lung cancers, 58 ovarian, 58 prostate and 8 pancreatic cancers), for which only 156 GPCRs were analyzed. While more samples were included in Sriram’s study, and almost all GPCRs annotated by GtoPdb were analyzed, including taste and vision receptors but not olfactory GPCRs. On one hand, this comprehensive analysis suggests a previously underappreciated role for GPCRs in cancer. On the other hand, given the large number of GPCR family members, even if certain receptors have very low mutation frequency in cancer, the overall mutation rate of GPCRs may be overestimated. Apart from the overall mutational burden, they also found GPR98/ADGRV1 the most frequently mutated GPCR, occurring in more than 8% of TCGA samples, and that approximately 40% SKCM tumors have a GPR98 mutation. Similarly, reoccurring high-impact GPCR mutations, predominantly found in class A GPCRs, are observed in UCEC, SKCM, LUAD, COAD, and STAD (Huh *et al.* , 2021).

These GPCR mutations in cancer can lead to various biological consequences. For example, mutated receptors like the thyroid-stimulating hormone receptor (TSHR) and lutropin receptor (LHR) share a common ability to increase cAMP (Bonomi *et al.* , 2001; G. Liu *et al.* , 1999; Miyai, 2007). The activation of MAPK/ERK and mTor pathway was affected by mutants of the melanocortin 1 receptor (MC1R) (JA & Bishop, 2005; Turan *et al.* , 2012). Mutants of the melanocortin 2 receptor (MC2R) exhibit a unique defect in trafficking to the cell surface (Fluck, Martens, Conte, & Miller, 2002). And the mutated smoothed receptor (SMO) was found to change the constitutive activity of the Hedgehog pathway (Reifenberger *et al.* , 1998; Wang *et al.* , 2014). The frequently observed mutated GPCRs in cancer are shown in Table 3, which highlights the variety of GPCR signaling pathways involved in cancer.

Table 3 Frequently observed mutated GPCRs in cancer. This list is adapted from the review: “An Insight into GPCR and G-Proteins as Cancer Drivers” by Kim *et al.* (Chaudhary & Kim, 2021).

Receptor	
Thyroid-stimulating hormone receptor (TSHR)	C
Melanocortin 1 receptor (MC1R)	C
Melanocortin 2 receptor (MC2R)	C
Lutropin receptor (LHR)	C
Smoothed receptor (SMO)	C
Follicle-stimulating hormone receptor (FSHR)	C
Glutamate family of G protein-linked receptors (GRM1–8)	C
Muscarinic acetylcholine receptor (mAChR)	C
Lysophosphatidic acid receptor (LPAR)	C
Sphingosine 1-phosphate receptor (S1PR)	C
Abbreviations: ICL- Intracellular loop, ECL- Extracellular loop, TM- Transmembrane α -helix, CA- constitutive activity	

3.3 Structural distribution of cancer-associated GPCR mutations versus natural variants

It is notable that the "hotspots"—well-defined mutation clusters—are not as common in GPCRs as in those oncogenes such as KRAS and tumor suppressor genes such as TP53, indicating a diverse landscape of genetic alterations (Baeissa, Benstead-Hume, Richardson, & Pearl, 2017). Part of the diversity originates from the non-synonymous natural variants, which represent genetic alterations in GPCRs that result in amino acid changes in healthy people. These variants play a significant role in the functional diversity observed among GPCRs across different individuals and populations. Across all GPCR families, there is a higher prevalence of non-synonymous natural variants in the N-terminus, C-terminus, and transmembrane (TM) domains compared to the extracellular or intracellular loops (A. Lee *et al.*, 2003). In addition, the highly conserved DRY and NPxxY motifs have been identified in the non-synonymous polymorphism analysis, which indicates that mutations in these structural motifs are inherent features in the diversity of GPCR function across different individuals and populations (Kim, Duc, & Chung, 2018). However, even with correction for natural variants, recent pan-cancer analysis has demonstrated that GPCRs still feature significant accumulation of mutations in some highly conserved structural motifs such as E/DRY, CWxP, NPxxY of class A GPCR, and HETx, GWGxP, PxxG of class B GPCR (Bongers *et al.*, 2022; Do, Haldane, Levy, & Miao, 2022). Bongers *et al.* found that conserved residues undergo a higher mutational pressure in cancer patients, which was not observed in natural variants, indicating their importance in cancer progression.

Most of the conserved motifs in GPCRs mediate their inactive conformation, and mutations at these motifs would therefore alter receptor function and stability. For example, the 'E/DRY' motif plays a pivotal role in receptor activation and signaling of class A GPCRs (Rovati, Capra, & Neubig, 2007). The ionic lock formed by the aspartic acid and the glutamic acid residue stabilizes the inactive state of the receptor. Upon ligand binding, conformational changes disrupt this ionic lock, allowing the transition to the active state and initiate downstream signaling. Conformational changes caused by mutations in the E/DRY motif could lead to alternations of receptor function, including gain of constitutive activity or loss of function (Huang & Tao, 2014; Römppler, Yu, Arnold, Orth, & Schöneberg, 2006). For example, cancer-associated CCR2 mutations in the DRY motif lead to a reduction or complete absence in G protein activation (den Hollander *et al.*, 2023). Similar phenotype has been observed for the muscarinic acid (M1 and M5) receptors (Lu, Curtis, Jones, Pavia, & Hulme, 1997), gonadotropin-releasing hormone (GnRH) receptor (K. K. Arora, Cheng, & Catt, 1997), cannabinoid 2 receptor (CB2R) (Feng & Song, 2003), and the adrenergic receptors (Chung *et al.*, 2002; Samama, Cotecchia, Costa, & Lefkowitz, 1993). In addition, mutations outside the conserved motifs may also affect receptor function. One example is the N-terminal TSHR mutation found in toxic thyroid adenomas, which resulted in basal activation of the protein kinase A pathway (Nanba *et al.*, 2012).

4. Cancer-associated mutations of GPCRs – driver or passenger

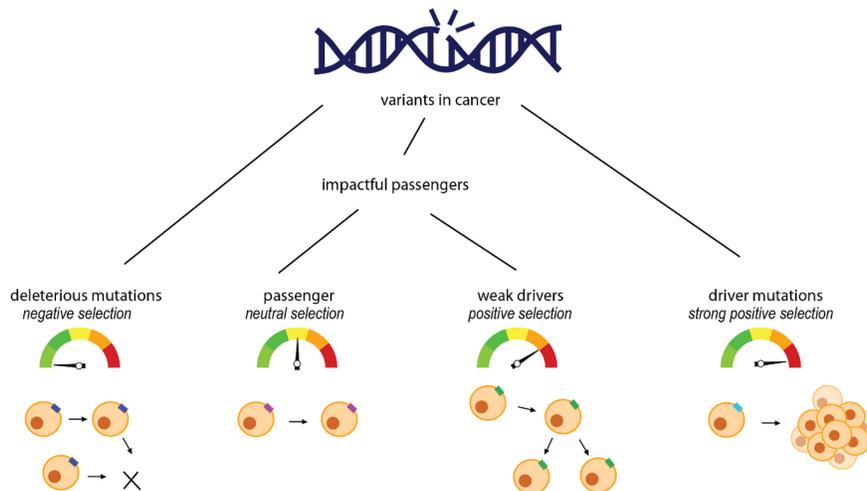


Figure 2 Mutational variants of GPCRs in cancer cells, listed as deleterious mutations, passenger, weak drivers, and driver mutations. Variants of GPCRs are shown with different colors on cell membrane. Impact of mutations are shown as dial with effect on cell proliferation.

4.1 Definitions of cancer driver and passenger mutations

Driver mutations are the primary architects of oncogenesis, which confer a selective advantage to the affected cells and thereby steer cells towards uncontrolled growth and proliferation (Vogelstein *et al.*, 2013). This advantage results from the activation of critical signaling pathways, such as those regulating cell cycle progression, apoptosis evasion, and DNA repair mechanisms (Bailey *et al.*, 2018). In a tumor, there are typically two to eight mutations in "driver genes", while the remaining mutations are considered passengers that do not provide any selective growth advantage (Bozic *et al.*, 2010). Passenger mutations are genetic alterations that occur incidentally during the chaotic genomic landscape of cancer development. Unlike driver mutations, they are carried along as collateral consequences of the genomic instability inherent in cancer cells (Kumar *et al.*, 2020). While passenger mutations may not directly contribute to the oncogenic process, their presence can serve as a molecular fingerprint, aiding in the characterization and classification of tumors (Salvadores, Mas-Ponte, & Supek, 2019).

4.2 Positive and negative selection in tumor growth

As illustrated in Figure 2, positive selection refers to the process by which genetic alterations (including driver mutations) conferring a growth or survival advantage to cancer cells become predominant. On the other hand, cells carrying deleterious mutations are rendered a survival disadvantage and thus are eliminated from the tumor population over time, the so-called negative selection (Bányai, Trexler, Kerekes, Csuka, & Patthy, 2021). Negative selection contributes to the maintenance of genomic stability within cancer cells. Together with positive selection, this purifying process is crucial for the overall evolution of tumor, allowing it to acquire and retain genetic alterations that promote its growth while discarding those that impede it. For example, research has demonstrated that several chemokine receptors (e.g. CCR2, CCR5, CX3CR1) exhibit robust indications of purifying selection in cancer (Bányai *et al.*, 2021). Cells with passenger mutations are mostly under neutral selection, while in some cases passenger mutations can act as weak drivers. For example, they are involved in relapses of acute promyelocytic leukemia by impeding drug response (Lehmann-Che *et al.*, 2018). On the other hand, there is evidence indicating that the accumulation of passenger mutations

could slow cancer progression related to enhanced immunity (McFarland *et al.* , 2017). As such, whether a mutation is a passenger or driver, and to which direction it promotes the selection process is highly context-dependent. Of note, mutations that are currently seen as passenger may still hold the potential to play an important role in cancer development and treatment, and thus should not be neglected.

4.3 Evidence of GPCR mutations as cancer driver gene

Because of the complex signaling network of GPCRs, illustrating the functional impact of genetic alterations may require investigation for each specific receptor, and a one-size-fits-all approach may be difficult. As discussed in section 3, Q209 and R183 mutation of *GNAQ* lead to persistent activation of the MAPK pathway and drive uncontrolled cell growth in uveal melanoma (Onken *et al.* , 2008). In comparison, less strong evidence has been found for GPCRs. Currently, studies have demonstrated that many GPCRs are involved in cancer progression and immune response, and have identified mutations positively or negatively affecting the downstream signaling pathways (examples in Table 3), but a clear role of specific GPCR mutations in cancer is missing. However, indications can be observed on a more general scale. Research indicates that a majority of G_i-coupled GPCRs exhibit mutations in the DRY motif, leading to loss of function. Interestingly, these mutations are always found to be mutually exclusive with GNAS-activating mutations (Raimondi *et al.* , 2019). This raises the intriguing possibility that mutations in G_i-coupled GPCRs may mimic GNAS-activating mutations in increasing intracellular cAMP levels and thereby promoting cancer progression.

4.4 Evidence of GPCR mutations as passenger gene

GPCRs often participate in intricate signaling networks where multiple receptors can activate similar downstream pathways. In this case, the so-called “redundancy” arises from the existence of alternative receptors and ligands that can compensate for the loss or alteration of a particular GPCR, which allows the cell to maintain essential functions without compromising its signaling integrity (Thompson, Canals, & Poole, 2014). Therefore, if a GPCR mutation occurs in a region that is functionally redundant with other receptors, the mutant GPCR may not exert a unique or critical influence on the cellular signaling cascade. Consequently, these mutations are less likely to confer a selective growth advantage to cells and act as passenger mutations in cancer (Pon & Marra, 2015). Apart from this, GPCRs are widely expressed in different tissue and cell types (P. Insel *et al.* , 2012). However, because of the widespread distribution of GPCR mutations across various cancer types, each tumor showcases a distinct repertoire of mutated GPCRs occurring at very low frequencies. Mutations that lack a distinct impact on critical signaling pathways within a specific tissue are more likely to be passenger mutations (Hao & Tatonetti, 2016). Therefore, we can conclude that while certain GPCRs may act as drivers, most mutations contribute to the broader genomic complexity without directly driving oncogenic processes.

5. Challenges and opportunities of targeting GPCRs in cancer

Targeting GPCRs including those harboring mutations in cancer therapy presents a dual landscape of challenges and opportunities. One significant challenge lies in the diversity of GPCRs and their intricate signaling networks including crosstalk with various cellular processes. This will bring potential off-target effects and unintended consequences on normal physiological functions, which make it complex to develop broad-spectrum therapeutic interventions. Furthermore, identification of cancer specific GPCR mutations while distinguishing them from natural variants is another hurdle, requiring advanced genomic and bioinformatics analyses. One of the primary challenges in this field is the limited availability and accessibility of comprehensive datasets. GPCR research suffers from relatively small and scattered datasets, which can impede the identification of robust associations between GPCR mutations and cancer. For example, when comparing the mutational landscape of GPCRs with the mutations in kinases, GPCRs mostly have widespread mutations with few identified clustering, while kinases feature distinct mutational hotspots (Dixit *et al.* , 2009). The absence

of clearly defined structural hotspot mutations in GPCRs imply that targeting GPCRs in cancer is more challenging compared to the well-studied approach targeting kinases. However, promising opportunities are present in cancer drug development targeting GPCRs, especially those overexpressed in cancer cells (R. T. Dorsam & J. S. Gutkind, 2007) and those involved in anti-cancer immunomodulation (Qiu, Yu, & Ma, 2024). In recent years, antibodies have shown the potential to revolutionize GPCR-targeted therapies with their high specificity and affinity. Modified antibodies directed against specific GPCRs can serve as precision tools, enabling treatment with reduced off-target effects. For example, the first-in-class CCR4 antibody drug named Mogamulizumab has been approved for treatment of T-cell leukemia-lymphoma with enhanced antibody-dependent cell-mediated cytotoxicity (ADCC) activity (Beck & Reichert, 2012). Additionally, novel allosteric modulators present a unique opportunity in GPCR-targeted cancer therapy. By modulating GPCR activity via non-competitive binding sites, allosteric modulators allow for a more nuanced regulation of signaling pathways. This fine-tuned control can offer advantages in specificity and selectivity, potentially avoiding side effects associated with orthosteric ligands (Bartfai & Wang, 2013). Another opportunity lies in the usage of unbiased “GPCRome” datasets. The concept of the GPCRome refers to the comprehensive exploration of GPCR gene expression, copy number variation, mutational signatures and functions, offering a system level understanding of their roles in cancer biology (Wu *et al.*, 2019). Leveraging the GPCRome could facilitate the discovery of novel biomarkers for early diagnose of cancer, and also accelerate drug discovery by identifying previously overlooked GPCRs as potential therapeutic targets, highlighting their signaling network, and uncovering their interactions within the tumor microenvironment. For example, Arora *et al.* performed a comprehensive analysis of extracellular GPCR networks in cancer transcriptomic datasets, and found that many ligand-receptor axes, including muscarinic, adenosine, 5-hydroxytryptamine and chemokine receptors, are associated with patient survival and can be exploited to inhibit cancer cell growth (C. Arora *et al.*, 2024). Furthermore, the advent of single-cell GPCRomics has allowed researchers to unravel the heterogeneity within cancer cell populations (P. A. Insel *et al.*, 2019), and AI-driven structural biological studies have enhanced our ability to understand the complexity of GPCR signaling networks (Matic, Miglionico, Tatsumi, Inoue, & Raimondi, 2023). Besides, experimental tools such as the PRESTO-Tango assay facilitates systematic interrogation of GPCR signaling by coupling receptor activation to a reporter system, uncovering novel druggable targets (Kroeze *et al.*, 2015). Alternative methods such as DNA-encoded library (DEL) screening and CRISPR-based profiling offer high-throughput platforms to identify GPCR ligands and evaluate the functional consequences of genetic alterations (Cai, El Daibani, Bai, Che, & Krusemark, 2023; Kapolka *et al.*, 2020). Lastly, quantitative mass spectrometry in combination with proximity labeling techniques such as BioID or APEX enables precise mapping of context-dependent GPCR signaling networks and post-translational modifications (Paek *et al.*, 2017). These advancements of *in silico* and experimental techniques will together make GPCR targeting in cancer a promising field.

6. Concluding remarks

GPCR signaling pathways are involved in almost every aspect of tumorigenesis and progression, including the cancer immune response. Mutations of GPCRs and G proteins are found in various cancer types. However, little evidence currently supports a direct link between specific GPCR or G protein mutations with cancer development, while most research provide circumstantial evidence that GPCR mutations can act as weak driver or passenger genes. On one hand, accumulation of GPCR mutations in some highly conserved structural motifs and the mutually exclusiveness observed between G_i-coupled GPCR and GNAS-activating mutations indicate their potential driving role in cancer. On the other hand, the functional redundancy of GPCR signaling networks, together with the widespread but low frequency distribution of GPCR mutations indicate that they are more likely to act as passenger in cancer development and do not have distinct biological consequences. The future of GPCR drug discovery for cancer hinges on overcoming challenges related to data availability and the integration of GPCR research with broader cancer studies. With regard to this, GPCRomics research aim to explore and characterize functionally important endogenous GPCRs associated with health and disease. Through large-scale genomic analyses, researchers have uncovered novel GPCR

mutations and polymorphisms associated with various cancers, shedding light on potential biomarkers for early diagnosis and prognosis. As research progresses, unraveling the complexities of GPCR involvement in cancer progression will pave the way for more effective and personalized care.

Abbreviations

Table of Abbreviations

AC	Adenylate cyclase
AKT	Protein kinase B
Asp	Aspartic acid
CA	Constitutive activity
Ca ²⁺	Calcium
cAMP	Cyclic adenosine monophosphate
CCL	C-C chemokines
CCR	C-C Chemokine receptor
CCR2	C-C chemokine receptor type 2
CCR5	C-C chemokine receptor type 5
COSMIC	Catalogue of Somatic Mutations in Cancer
CXCR	C-X-C chemokine receptor
CXCR1	C-X-C Motif Chemokine receptor 1
DAG	Diacylglycerol
DRD1	Dopamine receptor D1
ECL	Extracellular loop
ER	Estrogen receptor
ERK	Extracellular signal-regulated kinases
FDA	Food and Drug administration
GEF	Guanine nucleotide exchange factors
GNA11	G Protein Subunit Alpha 11
GNAQ	G Protein Subunit Alpha Q
GNAS	G protein Subunit Alpha S
GnRH	Gonadotropin releasing factor hormone receptor
GOF	Gain of function
GPCR	G protein-coupled receptor
GRK	G protein-coupled receptor kinase
GRMM1-8	Glutamate family of G protein-linked
ICL	Intracellular loop
IP3	Inositol 1,4,5-trisphosphate
LHCG	Lutropin receptor
LOF	Loss of function
LPA	Lysophosphatidic acid receptors
mAChR	Muscarinic acetylcholine
MAPK	Mitogen-activated protein kinase
MC1R	Melanocortin 1 receptor
MC2R	Melanocortin 2 receptor
PI3K	Phosphoinositide 3-kinase
PIP2	Phosphatidylinositol 4,5-bisphosphate
PKA	Protein Kinase A
PLC	Phospholipase C
S1P	Sphingosine-1-phosphate

Table of Abbreviations

SMO	Smoothened receptors
SMO	Smoothened receptor
SSTR	Somatostatin receptors
TM	Trans membrane α -helix
TSHR	Thyroid-stimulating hormone receptor
VEGF	Vascular endothelial growth factor
UCEC	Uterine Corpus Endometrial Carcinoma
SKCM	Skin Cutaneous Melanoma
LUAD	Lung adenocarcinoma
COAD	Colon adenocarcinoma
STAD	Stomach adenocarcinoma

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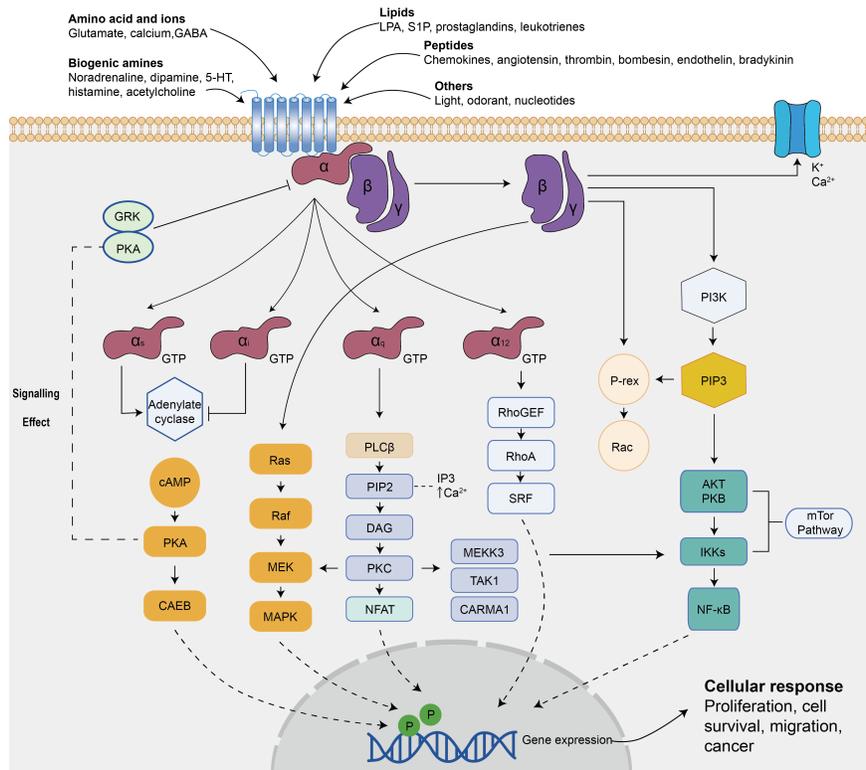
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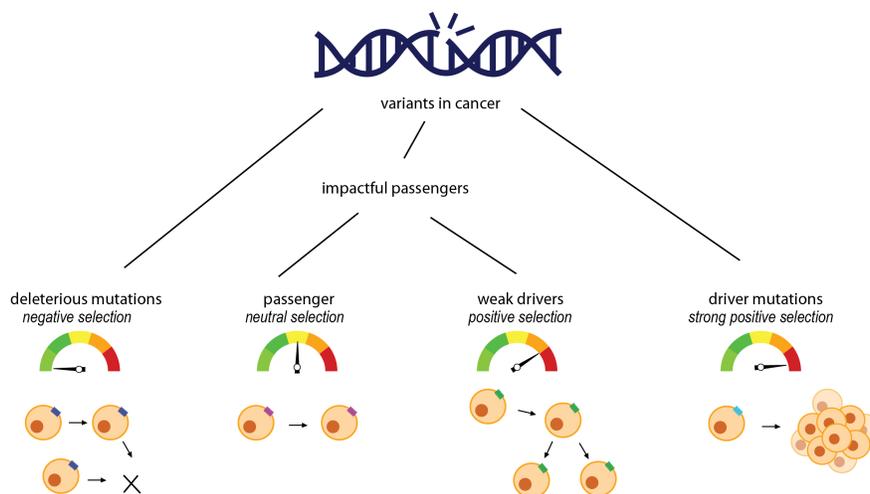
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