Illuminating the Onco-GPCRome: Novel G protein–coupled receptor-driven oncocrine networks and targets for cancer immunotherapy

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G protein–coupled receptors (GPCRs) are the largest gene family of cell membrane–associated molecules mediating signal transmission, and their involvement in key physiological functions is well-established. The ability of GPCRs to regulate a vast array of fundamental biological processes, such as cardiovascular functions, immune responses, hormone and enzyme release from endocrine and exocrine glands, neurotransmission, and sensory perception (*e.g.* vision, odor, and taste), is largely due to the diversity of these receptors and the layers of their downstream signaling circuits. Dysregulated expression and aberrant functions of GPCRs have been linked to some of the most prevalent human diseases, which renders GPCRs one of the top targets for pharmaceutical drug development. However, the study of the role of GPCRs in tumor biology has only just begun to make headway. Recent studies have shown that GPCRs can contribute to the many facets of tumorigenesis, including proliferation, survival, angiogenesis, invasion, metastasis, therapy resistance, and immune evasion. Indeed, GPCRs are widely dysregulated in cancer and yet are underexploited in oncology. We present here a comprehensive analysis of GPCR gene expression, copy number variation, and mutational signatures in 33 cancer types. We also highlight the emerging role of GPCRs as part of oncocrine networks promoting tumor growth, dissemination, and immune evasion, and we stress the potential benefits of targeting GPCRs and their signaling circuits in the new era of precision medicine and cancer immunotherapies.

The G protein–coupled receptor (GPCR)[7] family of proteins includes over 800 members and comprises ~4% of the encoded human genome, making it the largest gene family involved in signal transduction (1, 2). Common to all GPCRs is the 7-transmembrane domain structure, which has an extracellular N terminus and an intracellular C terminus. The importance of the multiple biological roles GPCRs is reflected in the range of key physiological processes that they regulate, including vision, olfaction, neurotransmission, hormone and enzyme release, immune response, hemostasis, cardiac response and blood pressure regulation, epithelial cell renewal, stem cell fate decisions, tissue development, and homeostasis. In fact, dysfunction of GPCRs contributes to some of the most prevalent

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[7] The abbreviations used are: GPCR, G protein–coupled receptor; 7TM, 7 transmembrane; CNV, copy number variation; COAD, colon adenocarcinoma; COX, cyclooxygenase; DC, dendritic cell; DLBC, diffuse large B-cell lymphoma; GBM, glioblastoma multiforme; GEF, guanine nucleotide exchange factor; GISTIC, Genomic Identification of Significant Targets in Cancer; GOF, gain–of–function; KSHV, Kaposi's sarcoma herpesvirus; LOF, loss–of–function; MDSC, myeloid-derived suppressor cell; NGF, nerve growth factor; NK, natural killer cell; NSAID, nonsteroidal anti-inflammatory drug; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PD-1, programmed cell death protein 1; PGE2, prostaglandin E2; PKA, protein kinase A; SHH, Sonic hedgehog; SKCM, skin cutaneous melanoma; SMO, Smoothened; STAD, stomach adenocarcinoma; TAM, tumor-associated macrophage; TCGA, The Cancer Genome Atlas; Treg, regulatory T cell; UVM, uveal melanoma; VEGF, vascular endothelial growth factor; FDA, Food and Drug Administration; mAChR, muscarinic acetylcholine receptor; FZD, Frizzled; TSHR, thyroid-stimulating hormone receptor; PAR, protease-activated receptor; GRP, gastrin-releasing peptide; NMB, neuromedin B; SCLC, small cell lung cancer; CTCL, cutaneous T-cell lymphoma; FAK, focal adhesion kinase; MAPK, mitogen-activated protein kinase; DAG, diacylglycerol; mTOR, mammalian target of rapamycin; LPA, lysophosphatidic acid; S1P, sphingosine 1-phosphate; BCC, basal cell carcinoma; YAP, Yes-associated protein; ERK, for extracellular signal-regulated kinase; PI3K, phosphatidylinositol 3-kinase; GI, gastrointestinal; IL, interleukin; MHC, major histocompatibility complex; EGFR, epidermal growth factor receptor.

human diseases, which is reflected by the 475 currently approved drugs that target 108 unique GPCRs and represent 34% of all FDA-approved drugs (https://www.centerwatch.com/drug-information/fda-approved-drugs[8] (3, 4). Although drugs for GPCRs represent ~34% of the global therapeutic drug market (3, 4), only a handful of these are drugs for oncology; of the current FDA-approved anti-cancer drugs, only eight of these target GPCRs, as described in detail below. GPCRs have been a longstanding topic of interest in the *Journal of Biological Chemistry*, and here we will expand on the impact of GPCRs in cancer biology. This review will summarize the current knowledge of how GPCRs are altered in cancer and how these aberrations can contribute to cancer initiation and progression. We also bring forth an emerging role of GPCRs as part of autocrine and paracrine signaling processes, which we refer to collectively as oncocrine networks that drive tumor formation, growth, and immune evasion. We also highlight the potential benefits of targeting GPCRs in the new era of precision cancer immunotherapies.

Historical perspective

The first evidence demonstrating a role for GPCRs in tumorigenesis came over 30 years ago in 1986 when studies illustrated that the GPCR encoded by the *Mas1* gene (*MAS1*) produced tumors in nude mice (5). This finding was largely underappreciated, likely because in contrast to most oncogenes discovered at the time, these receptors did not harbor activating mutations, similarly to the behavior of WT 5HT1c receptors (HTR1C) that resulted in NIH3T3 cell transformation (6). Further work, however, revealed that WT GPCRs can become tumorigenic in a ligand-dependent fashion. This was best demonstrated in 1991 in studies depicting the oncogenic transforming ability of mAChRs in NIH3T3 cells only in combination with the agonist, carbachol, and exclusively for $G\alpha_q$-coupled mAChR subtypes (M1, M3, and M5, gene names *CHRM1*, *CHRM3*, and *CHRM5*, respectively) (7). With this, these studies brought to light the possibility of G protein– dependent oncogenic roles for GPCRs when activated by locally produced or circulating ligands and raised the possibility that activating mutations in key conserved GPCR residues could result in transforming potential even without agonist stimulation.

These early studies introduced GPCRs as a new class of receptors capable of oncogenic transformation. Aligned with this possibility, mutational alteration of α1B adrenergic receptor (*ADRA1B*) can lead to transformation, providing an enhanced ability for tumor generation in nude mice (8). The identification of activating mutations in the thyrotropin receptor gene (*TSHR*) in hyperfunctioning thyroid adenomas provided the first evidence that mutant GPCRs can initiate a neoplastic disease (9). Downstream of the receptor, somatic mutations that impair the GTPase activity of $G\alpha_s$ conferred constitutive activation of adenylyl cyclase, leading to development of hyperfunctioning thyroid adenomas and pituitary tumors (10–12). Although these lesions are benign in nature, and hence often neglected in cancer biology, recent studies demonstrated similar activating mutations in the $G\alpha_s$-encoding gene (*GNAS* oncogene) in multiple cancer types, including pancreatic and colorectal cancer (13–15). In addition, our systematic analysis of the transforming potential of G proteins revealed that the genes encoding the $G\alpha_{q/11}$ (*GNAQ* and *GNA11*) and $G\alpha_{12/13}$ (*GNA12* and *GNA13*) G protein α subunits harbor transforming potential (16–18), thus contributing to the more recent discovery of multiple G protein– driven cancers (see below).

Remarkably, many human cancer-associated viruses utilize GPCR signaling for their life cycle. These include Kaposi sarcoma-associated herpesvirus (KSHV/HHV8) (19, 20), human cytomegalovirus (21, 22), and Epstein-Barr virus (23), which encode receptors in their genomes that resemble human chemokine receptors, and deploy them to recruit immune cells and exploit the immune system for viral dissemination (reviewed in Ref. 24). Specifically, the discovery that the GPCR encoded by KSHV/HHV8, often referred to as vGPCR or ORF74, initiates Kaposi's sarcomagenesis provided the first link between GPCRs and virally-associated human malignancies (20, 25). Viral GPCRs can signal through $G\alpha$ proteins independent of ligand activation, and they take advantage of this "constitutive activation" to promote tumorigenesis and aid in tumor survival, growth, and metastasis (24, 26). The dawn of these studies opened a new door to establish the link between GPCRs and cancers.

Despite this large body of information, GPCRs were generally not thought to represent traditional "genetic drivers" in cancer, thus pursuing GPCRs in oncology was neglected for some time. In the past decade, however, studies bloomed linking GPCRs to many cancers and mechanisms of tumorigenesis, metastasis, and immune evasion. The goal of the comprehensive expression, mutation, and copy number alteration omics information presented in this review is to shed light on understudied GPCRs and G proteins in different cancers and, for leading experts in studying particular cancers, to direct more attention in considering GPCRs as potential therapeutic targets.

Canonical and noncanonical G protein and GPCR signaling

As a result of the use of alignment tools and gene ontology, 342 functional nonolfactory human GPCRs (1) have been documented and further classified into major receptor families based on sequence similarity and function (27, 28) (http://gpcrdb.org/). To date, over 600 inactivating and almost 100 activating mutations in GPCRs have been identified, which are responsible for more than 30 different human diseases (29). Consequently, GPCRs have remained a long-standing interest as pharmacological targets.

GPCRs bind a wide variety of agonists, including ions, amines, purines, lipids, peptides, and proteins. Upon agonist binding, a conformational change is induced in the extracellular loops of the transmembrane region for ligand binding and in the intracellular loops (primarily in the second, third, and fourth loops), which promotes receptor activation and G protein coupling (30–32). The basic signaling unit of a GPCR system includes five main components: the receptor; the trimeric

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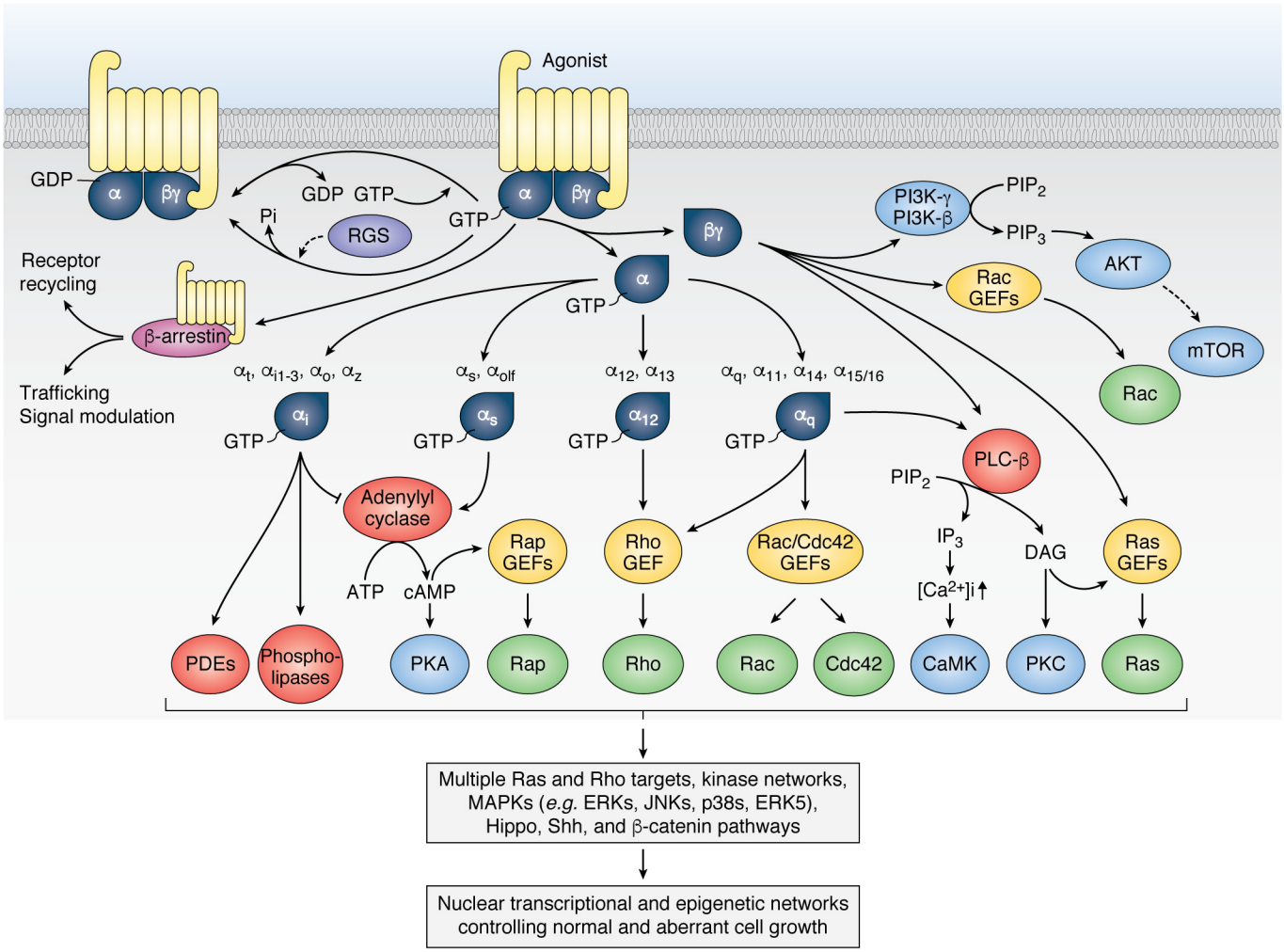


Figure 1. GPCR signaling. Agonist-activated GPCRs promote the dissociation of GDP bound to the α subunit of heterotrimeric G proteins and its replacement by GTP. Gα and G$\beta\gamma$ subunits can then activate numerous downstream effectors. The 16 human G protein α subunits can be divided into the four subfamilies, and a single GPCR can couple to one or more families of Gα subunits. Downstream effectors regulated by their targets include a variety of second messenger systems (*red*), GEFs (*yellow*), and Rho and Ras GTPases (*green*), which will result in the stimulation of multiple kinase cascades (*blue*) regulating key cellular functions. These include members of the MAPK, AKT, and mTOR, second messenger regulated kinases and phosphatases, and multiple kinases regulated by Rho and Ras GTPases. In addition, Gα_s-coupled receptors inhibit and G$\alpha_{12/13}$-, Gα_i-, and G$\alpha_{q/11}$-coupled receptors activate the transcription coactivator YAP and its related protein TAZ, the most downstream targets of the Hippo kinase cascade, as well as β-catenin and the Shh pathway, among others. Ultimately, these large numbers of effector molecules can have multiple effects in the cytosol and nucleus to regulate gene expression, cell metabolism, migration, proliferation, and survival by GPCRs, which can contribute to normal and malignant cell growth. See text for details.

$\alpha\beta\gamma$ G protein; an effector; RGSs (regulators of G protein signaling) that accelerate GTP hydrolysis and inactivate G proteins; and arrestins that control receptor fate and signal modulation (2). Once activated, the receptor binds the heterotrimeric G proteins, which promotes the release of GDP from the Gα subunit and the exchange for GTP and the functional dissociation of the GTP-bound α subunit from $\beta\gamma$ dimers (2, 31). Both parts remain attached to the plasma membrane but free from the GPCR to interact with downstream signaling proteins.

A defining feature of GPCRs is the ability to activate one or multiple Gα proteins, which can be subdivided into four major families based on sequence similarity: Gα_s, Gα_i, G$\alpha_{q/11}$, and G$\alpha_{12/13}$ (Fig. 1). As reviewed previously (33, 34), Gα_s activates adenylyl cyclases to catalyze the conversion of ATP to cAMP, which is produced as a second messenger and activates protein kinase A (PKA) and in some cells guanine nucleotide exchange factors (GEFs) for the small GTPase RAP1. Members of the Gα_i family primarily inhibit cAMP production, activate a variety of phospholipases and phosphodiesterases, and promote the opening of several ion channels. The G$\alpha_{q/11}$ family converts phosphatidylinositol 4,5-bisphosphate to DAG and inositol 1,4,5-trisphosphate to activate PKC and elevates intracellular Ca^{2+} levels. In a noncanonical fashion, G$\alpha_{q/11}$ also stimulates Rho GEFs thereby stimulating Rho GTPases (35, 36), whereas DAG activates Ras-GEFs (37). G$\alpha_{12/13}$ signaling involves a family of RhoGEFs harboring an RGS domain by which they associate with active G$\alpha_{12/13}$ and stimulate Rho GTPase (reviewed in Ref. 38). In turn, as depicted in Fig. 1, the coordinated activation of second messenger systems and Rho and Ras GTPases will result in the stimulation of multiple kinase cascades regulating key cellular functions. These include one or more members of the mitogen-activated protein kinases (MAPK) (*e.g.* ERK1 and ERK2, JNK1–3, p38α-δ, and ERK5, AKT, and mTOR), second messenger–regulated kinases (*e.g.* PKA, PKC, PKD, PKG, and CAMKs) and phosphatases (*e.g.* calcineurin), and multiple kinases regulated by Rho (*e.g.* ROCK, LIMK, PKN,

Citron kinase, PAKs, and MLKs) and Ras (*e.g.* BRAF, ARAF, and CRAF) GTPases, which in turn regulate nuclear events contributing to normal and malignant cell growth (reviewed in Refs. 33, 34). In addition, $G\alpha_s$-coupled receptors activate and $G\alpha_{12/13}$-, $G\alpha_i$-, and $G\alpha_{q/11}$-coupled receptors inhibit LATS1/2 kinases, which are key components of the recently described Hippo kinase cascade (39). LATS kinases phosphorylate and inhibit the transcription coactivator Yes-associated protein (YAP) and its related protein, TAZ, thereby causing their cytoplasmic retention and degradation (40). By inhibiting LATS1/2, $G\alpha_q$- and $G\alpha_{12/13}$-coupled GPCRs stimulate the ability of YAP/TAZ to promote the expression growth and anti-apoptotic genes (39). See below for exciting new information on how oncogenic $G\alpha_q$ proteins regulate the Hippo pathway and its therapeutic potential for $G\alpha_q$-driven malignancies.

Once functionally dissociated from the $G\alpha$ protein, $G\beta\gamma$ dimers also play a central signaling role, first described in the context of ion channel regulation. For example, $G\beta\gamma$ can inhibit some voltage-activated Ca^{2+} channels and activate G protein–activated inwardly rectifying K channels (GIRKs) (41). In the context of cancer signaling, $G\beta\gamma$ dimers were initially shown to mediate the activation of ERK downstream from GPCRs linked to $G\alpha_i$ (42). We now know that $G\beta\gamma$ stimulates PLCβ, adenylyl cyclases, PI3Ks (primarily PI3Kγ and PI3Kβ in cells lacking PI3Kγ expression) and GEFs stimulating the small GTPase Rac, such as PREX1 (33). By doing so, $G\beta\gamma$ signaling contributes to the prosurvival and migratory activity of many GPCRs, with emphasis on chemokine receptors involved in cancer metastasis, such as CXCR4 (see below and Ref. 43).

Ultimately, the signaling pathways stimulated by each GPCR depends on its G-protein–coupling specificity, which can be distinct for each ligand (often referred to as "biased agonism"), the intensity and duration of receptor activation, and the level of expression of each G protein subunit and the repertoire of signaling molecules expressed in each cell type. The most proximal signaling pathways stimulated by each G protein subunit are summarized in Fig. 1.

In addition to canonical signaling through heterotrimeric G proteins, some classes of GPCRs can initiate G protein–independent signal transduction. For example, some GPCRs also initiate intracellular signaling by engaging the scaffolding activity of β-arrestins, particularly for the activation of ERK and JNK3 (44). However, it is possible that G proteins may be required to initiate signal transduction, with β-arrestins playing a more important modulatory role in signal transmission, by shaping and fine-tuning dynamic GPCR responses (45).

G protein–independent signaling is well-exemplified by the Frizzled (FZD) family of receptors. In this case, the FZD ligand, WNT, stimulates a signal transduction cascade that results in β-catenin activation through the protein disheveled (DVl), which plays a key role in embryonic development and cancer. WNT proteins bind FZD and a single-pass transmembrane molecule, low-density lipoprotein receptor–related proteins 5 and 6 (LRP5/6), leading to the dimerization of the two receptors (46). The resulting conformational changes cause the phosphorylation of the cytoplasmic tail of LRP at multiple residues and the recruitment of GSK3β bound to scaffold protein Axin, whereas FZD associates with Dishevelled. This complex formation prevents the persistent phosphorylation and consequent degradation of β-catenin bound to its degradation complex, which includes Axin, the tumor suppressor APC, the kinases GSK-3α/β and CK1, and the E3-ubiquitin ligase β-TrCP, thereby stabilizing β-catenin and promoting its nuclear-signaling activity (46). The WNT/β-catenin pathway is frequently dysregulated in cancer, with particularly high incidence in colorectal cancer (47).

An interesting aspect of WNT signaling is that FZDs are persistently ubiquitinated and down-regulated by the transmembrane proteins ZNRF3 and RNF43, and that this negative effect can be circumvented by secreted proteins of the R-spondin family that bind ZNRF3/RNF43 together with the GPCRs LRG4 and LGR5, suppressing ZNRF3/RNF43 function and leading to enhanced WNT signaling indirectly (46). However, as recently reviewed, FZD can also activate G proteins of the $G\alpha_i$, $G\alpha_q$, and $G\alpha_{13}$ families, mediating many of the responses initiated by WNT exposure (48).

Another GPCR involved in development and cancer, particularly in basal cell carcinoma, is smoothened (SMO), which acts in the sonic hedgehog (SHH) pathway primarily by regulating the activity of the GLI transcription factor by a not fully understood mechanism in mammalian cells (49). Traditionally, this effect was considered to be G protein–independent, but GLI activation requires the inhibition of PKA, and growing evidence suggests that this aspect may require the activation of $G\alpha_i$ proteins or the inhibition of $G\alpha_s$ or its coupled receptors (reviewed in Ref. 47). How G protein signaling by FZD is coordinated in space and time with canonical β-catenin signaling, and how SMO regulates G protein–independent and G protein–regulated pathways to activate GLI and other signaling events in the context of cancer stemness and metastasis is an active area of current investigation (48–50). Its full elucidation may have important implications for the design of new pharmacological interventions in cancers that involve persistent G protein–independent and/or -dependent WNT and SHH signaling.

Mutational landscape of G proteins and GPCRs in cancer

The Cancer Genome Atlas (TCGA) is a comprehensive, publicly available database launched by the National Institutes of Health, which includes large-scale genome sequencing analyses through multiple omics platforms for a variety of cancer types (51). In addition to this, the TCGA database also includes array-based DNA methylation sequencing for methylation profiling and reverse-phase protein array for large-scale protein expression profiling. These platforms can add a multidimensional view to the landscape of GPCRs and G proteins in cancer. Here, we built on our prior cancer genome-wide study (13), performing an in-depth omics analysis of the mutational landscape of 33 cohorts of cancer patients in TCGA by new bioinformatics approaches (Table S1*B*).

The power of this analysis revealed that 20% of all human tumors sequenced contained mutations in genes encoding GPCRs. In particular, we used MutSig2CV, a now widely used computational biology tool that takes mutations discovered by DNA sequencing to illuminate genes that are statistically more frequently mutated relative to the background mutation rate of

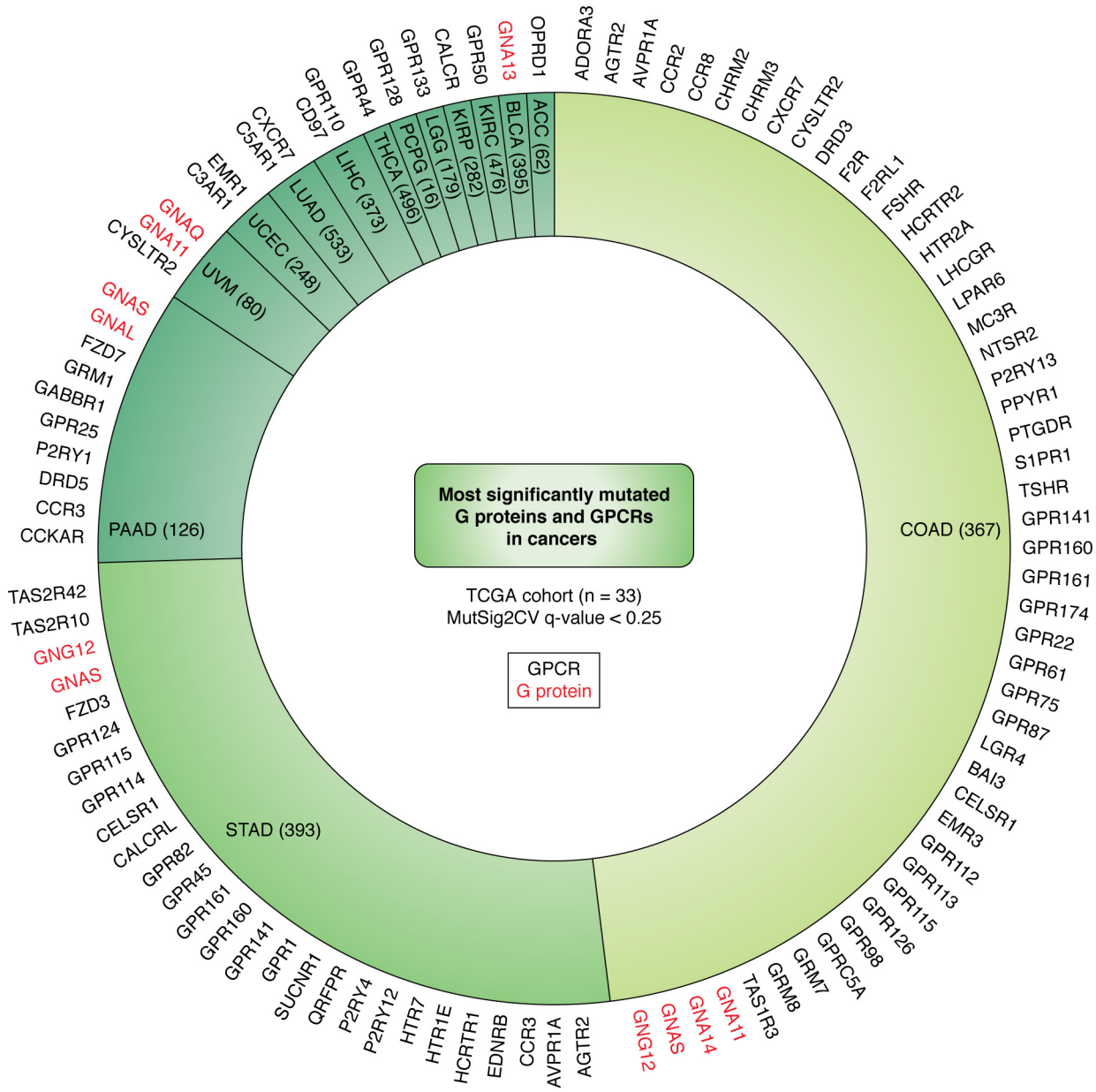


Figure 2. Top significant mutations of GPCRs and G proteins in cancer. From MutSig2CV analysis, the proportion of TCGA cohorts (sample number) with highly-significant (MutSig2CV *q*-value <0.25) mutations in genes encoding GPCRs (*black*) and G proteins (*red*) are shown. The statistically significant mutated genes for each cohort are plotted outside of the *pie*; cohorts are colored based on number of significant genes.

individual lesions (52). Many G proteins and GPCRs were found to be mutated. For visualizing the data, we used a very stringent criterion (MutSig2CV *q*-value <0.25) to identify the most statistically significant mutated genes in each cancer type. An unexpected observation was that among all cancer cohorts, cancers arising in the gastrointestinal (GI) tract, including colon adenocarcinoma (COAD), stomach adenocarcinoma (STAD), and pancreatic adenocarcinoma (PAAD) displayed the highest number of significantly mutated GPCRs and G proteins (Fig. 2 and Tables S2, *A* and *B*). This may be independent of the mutational burden of these tumors, which are lower than that of other typical highly-mutated cancers such as melanoma and lung cancer, for example (53). However, the phenotypic and biological outcome of these mutations remains largely unknown, and thus these findings provide a wealth of information for the development of hypothesis-driven approaches to investigate their cancer relevance.

In addition to our analysis of the most statistically significant mutated and genomically altered G proteins and GPCRs in cancer ($q < 0.25$), we have compiled the frequency of mutations of all G proteins and GPCR genes for each cancer type investigated in TCGA (Table S6). We expect that this color-coded table will provide easy access and visualization of the cancers in which G proteins and GPCRs of interest are most frequently mutated. We generated this table using the more recent and robust Multi-Center Mutation Calling in Multiple Cancers (MC3) Project TCGA PanCancer 2018 dataset (54). This database includes mutation-calling algorithms that account for variance and batch effects to enable more precise cross-tumor–type analyses (54). We have also provided a direct link for each

gene to their corresponding page in cBioPortal Cancer Genomics portal (http://www.cbioportal.org/)[8] (55, 240) for the visualization, analysis, and download of mutational information. The cBioPortal for Cancer Genomics is a web resource for dissecting and visualizing multidimensional cancer genomics data. These data include information about somatic mutations, copy number alterations, mRNA expression, DNA methylation, and transcript and protein abundance from multiple cancer omics studies (55). Please note that the percentage of mutated samples may vary with our analysis, as cBioPortal analysis uses different instances of the TCGA PanCancer dataset from 2013–2018 (56). We encourage our colleagues to follow the corresponding links to gain easy access to the following: (*a*) "Cancer Types Summary," in which all genomics alterations are displayed for all cancer types; (*b*) "Mutations," which provide a visual representation of the most frequently mutated and altered residues and a downloadable list of samples that includes their corresponding protein change mutations, mutation type, and CNV type; (*c*) "Survival," which shows the overall survival (length of time that the patients are alive) of cancer patients harboring genomic alterations *versus* those without (although, we recommend to perform this analysis for each particular cancer type of interest); and (*d*) "Expression," which provides a graphical representation of the mRNA expression level of each sample in every cancer type, together with their mutational status.

Significantly mutated G proteins in cancer

Whereas the contribution of each GPCR mutation in cancer is still under evaluation, the recent discovery of hot spot mutations in G proteins as oncogenic drivers in multiple highly prevalent cancer types has accelerated tremendously the research in this field. Indeed, many G protein genes (*GNAS*, *GNA11*, *GNAQ*, and *GNA13*) are part of the current ~400 gene panels of cancer-associated genes sequenced routinely by clinical oncology services in many cancer centers and by all large cancer genomic testing providers and institutional genomics cores. Among them, the summary of our MutSig2CV analysis revealed that *GNAS* is the most highly mutated G protein in human cancer (Table S2*B*). From this analysis, *GNAS* is significantly mutated in COAD (6.19%), PAAD (5.09%), and STAD (7.52%). As described above, *GNAS* is a known oncogene that was first described in growth hormone–secreting pituitary adenomas and has since been found to be mutated in a number of neoplasms, predominantly at the codon 201 hotspot (13, 57). Mutations occurring at arginine 201 of *GNAS* activate adenylate cyclase and lead to constitutive cAMP signaling by reducing the rate of GTP hydrolysis of the active GTP-bound $G\alpha_s$, as well as by adopting an active-like conformation even when bound to GDP (13, 58). In COAD, a synergistic effect with the MAPK pathway is likely, as *GNAS* is co-mutated with *KRAS* in a large portion of adenomas and carcinomas. Similarly, *GNAS* mutations are found in ~50% of low-grade appendiceal mucinous neoplasms (59) and are highly prevalent in a subset of pancreatic tumors, including intraductal papillary mucinous neoplasms and adenocarcinomas (14). In this regard, recent mouse models revealed that *GNAS* and *KRAS* mutations are necessary and sufficient to initiate this particular subtype of pancreatic adenocarcinomas (60, 61).

Emerging studies have begun to explain the functional impact of *GNAS* mutations. In 1991, *GNAS* mutations were discovered in McCune-Albright syndrome and pituitary tumors (62). In cancer, *GNAS* has been linked to pro-inflammatory functions, which could mimic the impact of chronic inflammation on tumor development. $G\alpha_s$ is well-documented to mediate the effects of inflammatory mediators like cyclooxygenase (COX) 2-derived prostaglandins. Its inflammatory role in cancer is best shown in colon neoplasia where *COX2*-derived prostaglandin E2 (PGE2) enhances colon cancer progression via activation of PI3K and AKT and relieving the inhibitory phosphorylation of β-catenin as part of $G\alpha_s$ oncogenic signaling (63). Activating mutations in *GNAS* have also been found in gastric adenocarcinomas, leading to activation of the Wnt/β-catenin signaling pathway (64).

Mutations in *GNAQ* and *GNA11* are most relevant in uveal melanoma (UVM) incidence, as 93% of patients harbor mutations in these genes encoding constitutively active $G\alpha_q$ family members (65, 66). All cancer mutations in $G\alpha_q$ or $G\alpha_{11}$ occur at either glutamine 209 or, in a smaller proportion, arginine 183 (Gln-209 and Arg-183, respectively; Arg-183 is the identical position to Arg-201 in $G\alpha_s$) (65, 66). Mutations affecting Gln-209 in *GNAQ* or *GNA11* are present in most primary UVM lesions and their metastases (66). Mutated residues impair GTPase activity (diminish GTP hydrolysis), which ultimately leads to prolonged signaling. Although initial studies supported a role of ERK signaling in UVM development, targeting this pathway did not improve the survival of UVM metastatic patients (67). Instead, our genome-wide RNAi screens revealed that the noncanonical activation of RhoGEFs, specifically TRIO, by $G\alpha_q$ mediates UVM progression (68). Furthermore, we discovered that the activation of YAP, the most downstream target of the Hippo pathway, by the novel TRIO–RHO signaling arm is essential for UVM, thus identifying a druggable target downstream from mutated $G\alpha_q$ (68).

GNAQ mutations are also associated with a smaller proportion of skin cutaneous melanoma (SKCM) and have been recently described in vascular tumors, such as hemangiomas and angiosarcomas (15, 69). *GNAQ* R183Q mutations are also specifically responsible for a frequent congenital neurocutaneous disorder characterized by port wine skin lesions that are vascularly-derived, which is known as Sturge-Weber syndrome (70). Thus, mutations in *GNAQ* appear to be responsible for numerous disease conditions for which there are no current targeted therapeutic options.

Mutations in *GNA13* have been characterized in both liquid and solid tumors and are present at high frequency in bladder carcinoma. In addition, recent genome-wide sequencing efforts have unveiled the presence of frequent mutations in *GNA13* in lymphomas, specifically Burkitt's lymphoma and diffuse large B-cell lymphoma (DLBCL) (71–73). These mutations in *GNA13* as well as in RhoA, a downstream target of $G\alpha_{13}$, have been shown to be inhibitory in nature, suggesting a tumor-suppressive role for $G\alpha_{13}$ and RhoA in Burkitt's lymphoma and DLBCL (71). In this case, loss–of–function (LOF) mutations rather than gain–of–function (GOF) mutations underlie the

oncogenic activity of *GNA13*, likely by disrupting the normal differentiation program of B cells (71). In contrast, WT *GNA13* overexpression has been implicated in many solid tumors, such as in gastric cancer (74), nasopharyngeal carcinoma (75), prostate cancer (76), and breast cancer (77). Furthermore, *GNA13* levels modulate drug resistance and tumor-initiation phenotypes in patient-derived head and neck squamous cell carcinoma cells *in vitro* and *in vivo* (78). In this case, *GNA13* or *GNA12* overexpression may enhance the proliferative and pro-migratory function of multiple GPCRs that converge to activate these G protein α subunits. A causal role of excessive $G\alpha^{12}$ signaling may be elucidated by a use of a recently developed $G\alpha_{12}$-coupled chemogenetic designer GPCR (Designer Receptors Exclusively Activated by Designer Drugs (DREADD)) (79).

Mutations in Gβ subunits are infrequent, and yet activating mutations in Gβ1 and Gβ2 (*GNB1* and *GNB2*, respectively) has been identified in myeloid and B-cell neoplasms, which act as an oncogenic driver and confer resistance to kinase inhibitors targeting typically mutated kinases in these malignancies, including BCR–ABL, BRAF, and JAK2 (80). Certainly, this information suggests that other Gβ subunit mutations may also harbor tumorigenic potential.

Mutated oncoGPCRome

The most frequently mutated GPCRs in each cancer type are depicted in Fig. 2 and are listed in Table S2*A* with the corresponding statistical significance (*q*-value) and frequency. As mentioned above, the high frequency of GPCR mutations specifically in tumors arising from the gastrointestinal tract is intriguing as it likely reflects their ability to stimulate organ-specific growth-promoting pathways in these cancers. Although a discussion of each specific GPCR is beyond the goals of our review, we will discuss new emerging concepts and specific cases that may exemplify the challenges and opportunities for future exploration in this area and its potential for drug discovery.

Whether mutations in GPCRs result in GOF or LOF, or represent passenger mutations with little impact on cancer progression, in most cases is still unknown. A complicating factor is that most GPCRs do not harbor hotspot mutations, meaning that mutations in each GPCR do not occur with high frequency in a single or limited numbers of codons, and in addition, each tumor exhibits a different repertoire of mutated GPCRs. To address this daunting question, we have recently developed new bioinformatics approaches analyzing GPCR mutations in the context of multiple sequence alignments (MSA) defining the conserved seven-transmembrane (7TM) domain, as well as considering 3D structures and interaction partners (241). We have used this approach to model the most significantly mutated GPCRs (Table S2*A*). Remarkably, visualization of the most mutated 7TM positions on a representative GPCR 3D structure revealed that most mutations occur in "hotspot structural motifs" rather than being randomly distributed (Fig. 3 and Table S3). This includes frequent mutations in the DRY arginine motif, which is as important for class A GPCR activation as it is responsible for the intramolecular polar contacts that keep the receptor inactive until ligand binding (81). Other structural mutation hotspots are found at or nearby highly-conserved

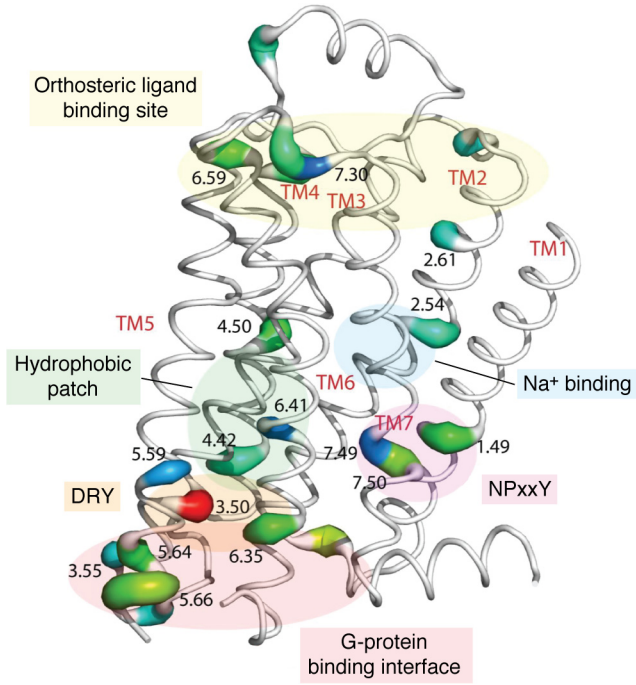


Figure 3. Significantly mutated genes in 7TM positions. 3D "putty" drawing of most mutated 7TM positions in significantly mutated genes from the TCGA database is shown. A prototypical GPCR structure (*i.e. ADRB2*, Protein Data Bank code 3NYA) is used for representation. Cartoon diameter and coloring (*blue* to *red*) are directly proportional to the number of unique samples carrying mutations at given 7TM positions. To identify these, mutated receptor sequences were aligned (using PFAM 7tm_1 Hidden Markov Model), and Ballesteros/Weinstein numberings were assigned (see Table S3). Conserved functional motives are highlighted and labeled.

GPCR regions, including the ligand and G protein–binding sites, as well as the NP*XX*Y and other conserved motifs that regulate in an allosteric way receptor's activation (82). Collectively, this supports that most cancer-associated mutations in GPCRs occur in "structural hotspots," similar to other oncogenes and tumor suppressor genes, a property that could have not been predicted from the analysis of individual GPCRs.

Although the functional impact of these alterations may need to be investigated for each GPCR, our recent computational analysis of cancer genomes indicates that most $G\alpha_i$-linked GPCRs exhibit DRY mutations that are inhibitory in nature (inhibit function), which typically occur mutually exclusively with *GNAS*9-activating mutations (241). This suggests the exciting possibility that mutations in $G\alpha_i$–GPCRs may mimic *GNAS* mutants leading to higher cAMP activity to drive tumorigenesis (241).

A particular challenge when analyzing the potential impact of cancer mutations is that longer genes exhibit a higher number of mutations, which would achieve statistical significance (MutSig2CV analysis) only when higher than the background mutation rate of individual lesions. This is well-exemplified by GPR98, which is the most frequently mutated GPCR across all cancer types and, concomitantly, is the GPCR with the highest number of amino acids. GPR98 is an adhesion receptor, and its ligand and physiological functions are currently poorly understood. *GPR98* mutations are known to cause febrile seizures and one form of Usher syndrome, the most common genetic cause of combined blindness and deafness (83, 84). *GPR98* has

been shown to have significant association with glioblastoma (GBM) (85) and lymphoblastic leukemia (86), and the evaluation of the impact of GPR98 mutations in cancer warrants further investigation. The family of metabotropic glutamate GPCRs, GRM1–8, are also frequently mutated in many cancer cohorts. Mutations of *GRM1*, *GRM5*, and *GRM3* have been shown in breast cancer and melanoma (87–89). In addition, their transforming potential and increased secretion of their ligand, glutamate, by the tumor microenvironment makes the GRM receptor family an intriguing area of study.

The analysis of the mutational landscape of GPCRs suggest that COAD harbors the highest incidence of significantly mutated receptors. Among them, thyroid-stimulating hormone receptor (*TSHR*) was the most frequently mutated GPCR, involving ~14% of COAD patients. Mutations in the P2Y purinoceptor 13 (*P2RY13*) gene were the most statistically significant in this cancer type and occurred in ~5% of COAD patients. *P2RY13* encodes for a purine receptor and has been shown to be overexpressed in acute myeloid leukemia samples but not involved in other nonhematologic malignancies (90). On a related note, mucosal biopsies from the colon of Crohn's disease and ulcerative colitis patients have shown abnormalities in *P2RY13*, which may suggest a role for the receptor in GI inflammatory diseases (91). The importance of TSHR-activating mutations in human neoplasia was first demonstrated in thyroid adenomas (9) and are also found in some thyroid carcinomas. However, the roles of both TSHR and $P2Y_{13}$ in COAD remain largely unexplored.

Recently, analysis of hotspot mutations in oncogenes uncovered a mutation in cysteinyl leukotriene receptor 2 (*CYSLTR2*) in a UVM cohort. This GOF mutation results in an L129Q substitution and leads to the $G\alpha_q$-coupled receptor to be constitutively active (92). This mutant protein is insensitive to leukotriene stimulation, constitutively activates $G\alpha_q$, and can promote tumorigenesis in melanocytes *in vivo* (92). According to MutSig2CV analysis, $CysLT_2$ is the most frequently mutated GPCR (3.75%) in UVM. While representing a small fraction of all UVM cases, these mutations in *CYSLTR2* are mutually exclusive with known drivers in UVM (*GNA11* and *GNAQ*) (92). Therefore, *CYSLTR2* mutations promote persistent $G\alpha_q$ activation substituting for *GNA11* and *GNAQ* mutations to drive aberrant $G\alpha_q$ signaling in UVM. This receptor is also mutated in COAD at a distinct amino acid, and hence its consequences (GOF or LOF) are still unknown. Recently, small molecules have been discovered and utilized against WT $CysLT_2$, but development of higher-affinity molecules or antibodies that can stabilize the mutated receptor in its inactive state will be required to explore the therapeutic benefit of targeting $CysLT_2$ in UVM.

Our current analysis also identified many adhesion receptors and class A GPCRs that are mutated with high frequency in cancer. The former includes GPR98, BAI3, ADGRL1, CELSR1, GPR125, GPR110, GPR112, and GPR126, which can now be prioritized for their individual analysis. A recent comprehensive mutagenesis screen in ADGRL1 revealed that many cancer-associated mutations result in GOF alterations and persistent activity (93).

Among the typical class A GPCRs, some of the more frequently mutated genes are muscarinic receptors M2 and M3 (*CHRM2* and *CHRM3*), multiple P2Y receptors, serotonin receptors (*HTR1E, HTR1F, HTR2A*, and *HTR7*), and adenosine receptors (*ADORA3*), among others, all of which could be activated by locally produced ligands as well. Notable mutated GPCRs also include the PAR2 receptor (*F2RL1*), which is often amplified and will be discussed below, as well as multiple orphan GPCRs whose coupling specificity and biological activity is still largely unknown. Given the emerging studies supporting the notion that aberrant GPCR activity leads to tumor initiation and progression, we expect that the emerging mutational information will guide new cancer-relevant studies addressing each of these frequently mutated GPCRs. Given that many ligands of GPCRs may be produced in significantly higher amounts in the hypoxic, metabolic, and acidic tumor microenvironment, the tumorigenic synergism between ligand availability and activating mutations in receptors should also be explored.

Gene copy number alterations and G protein and GPCR expression in cancer

In addition to mutations, alterations in gene expression and copy number of G protein and GPCR genes have been detected. Determining the contribution of such alterations to cancer initiation and progression remains a significant challenge, yet it may be critical both for the discovery of driver oncogenic processes and for the development of targeted therapeutics. Indeed, aberrant expression of many WT G proteins and GPCRs can contribute to cancer growth even if not mutated, often as part of oncocrine signaling networks (see below).

Somatic alterations are acquired at random during cell division, and some of these participate in tumorigenesis or tumor growth. Here, we used GISTIC (Genomic Identification of Significant Targets in Cancer), an algorithm that identifies genes targeted by somatic CNVs that may contribute to tumorigenesis by evaluating the frequency and amplitude of observed events (94). To illuminate the most relevant GPCR candidates in tumorigenesis, we also filtered the large list of CNVs for those that correlated with mRNA expression. Our analysis revealed that 28 out of 33 TCGA cancer cohorts included alterations of GPCR and G protein that are significantly correlated with mRNA expression of the corresponding genes ($R > 0.33$) (Tables S4, *A* and *B*, and S5, and Fig. 4).

Among the G proteins, copy number gain in *GNA12* is remarkably significant in ovarian cancer (OV). This cancer type is characterized by few driver mutations and by the accumulation of high concentrations of LPA in ascites fluids, which may work through $G\alpha_{12}$ to promote growth and metastasis (reviewed in Ref. 95). Similarly, *GNAI1* (encoding $G\alpha_{i1}$) is significantly amplified in breast-invasive carcinoma (BRCA), a cancer type in which many $G\alpha_i$-coupled GPCRs, including CXCR4, are well-established as metastatic drivers (see below). The significance of other genomic alterations in G proteins, including copy number gains in $G\beta$ subunits (*GNAB1*, *GNAB2*, *GNAB3*, and *GNAB5*) and $G\gamma$ (*GNG4*, *GNG5*, *GNG7*, *GNG12*,

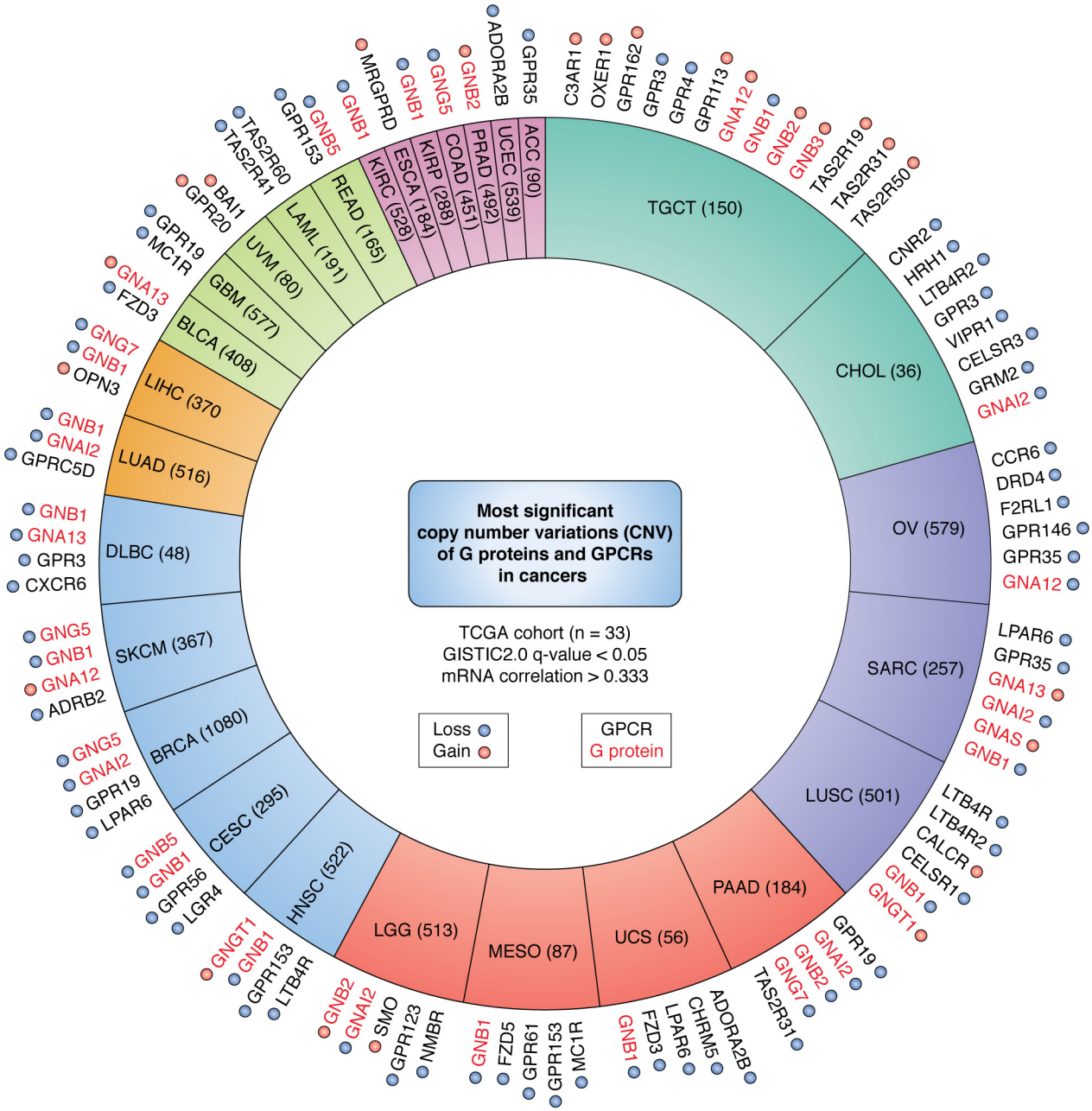


Figure 4. Top significant CNVs of GPCRs and G proteins in cancer. From GISTIC analysis, the proportion of TCGA cohorts (sample number) with highly significant (GISTIC *q*-value <0.05 and mRNA correlation >0.333) CNVs in genes encoding GPCRs (*black*) and G proteins (*red*) are shown. The significant genes for each cohort are plotted outside of the cohort pie; cohorts are colored based on the number of significant genes, and amplification is denoted by *red* highlighting, and deletion is denoted by a *blue* highlighting.

and *GNGT1*) in multiple cancers likely reflect the broad signaling capacity of G$\beta\gamma$ dimers (see Fig. 1).

Testicular germ cell tumor displayed the most genomic alterations in genes encoding GPCRs, which included mostly orphan, taste, and adhesion receptors. In contrast, *F2RL1*, the gene encoding -activated receptor (PAR) 2, was the most significantly altered gene in OV. PAR2 is a protease-activated receptor and is expressed in many organs. The ability of proteases to degrade extracellular matrices and to activate PARs render them important in the facilitation of tumor growth and metastasis (96, 97). Overexpression of *F2RL1* has been linked to some of the most diagnosed cancers, including lung, breast, colon, and pancreatic cancers (96, 98, 99). Functionally, PAR2 has also been linked to cancer cell migration and stimulates vascular endothelial growth factor (VEGF) production for angiogenesis (100, 101). Another unexpected observation was that most kidney cancers (KIPAN) exhibit highly-significant copy number gains in genes for multiple chemokine receptors (*CCR2*, *CCR5*, *CCR6*, *CCR9*, *CX3CR1*, and *CXCR6*) and histamine receptors (*HRH2*), among others. The frizzled family of GPCRs and LPA receptors (in particular *LPAR6*) were also genetically altered in multiple cancer types. Overall, although gene copy gains and losses may reflect cancer-associated genomic instability, most cancers exhibit a very specific pattern of copy number variations in G protein and GPCR genes, whose biological relevance can now be examined.

G proteins and GPCRs as tumor suppressor genes?

An interesting observation of the pattern of genomic alterations is that many cancers lose one or both copies of specific G protein and GPCR genes. This raises the possibility that certain G protein/GPCRs may act as tumor suppressors rather than oncogenes. Indeed, as described above, *GNA13* is significantly mutated in diffuse B-cell lymphoma and Burkitt's lymphoma, and detailed experimental analysis revealed that in all cases this involves LOF mutations, resulting in the inability of B cells to undergo terminal differentiation and hence increasing their uncontrolled growth (71). Analysis of candidate $G\alpha_{13}$-coupled GPCRs identified inactivating mutations in P2RY8 and LOF mutations in *RHOA* (71, 102). These mutations appear to be mutually exclusive, supporting the notion that in these B-cell malignancies *P2RY8–GNA13–RHOA* are part of a tumor-suppressive axis.

Surprisingly, while conducting conditional gene knockout studies of $G\alpha_s$ in the skin, we observed that mice develop massive basal cell carcinomas (BCC) in only 2 weeks after *Gnas* excision (103). This involved a widespread activation of the SHH pathway in $Gnas^{-/-}$ tumor lesions (103), thus phenocopying the effects of LOF mutations in *PATCHED* (*PTCH*) or GOF mutations in *SMO*, which are the best known BCC tumor suppressor and oncogenes, respectively. Activation of the SHH pathway is also typical of a subset of medulloblastomas, a childhood malignancy (104). Remarkably, homozygous *GNAS* gene loss was identified in a group of SHH subtype medulloblastomas that does not harbor mutations in *PTCH* or *SMO* (104). In these particular cancers that express SHH pathway components, $G\alpha_s$ and its downstream target PKA act as tumor suppressors by preventing the activation of GLI transcription factors (103), whereas in most GI tissues, *GNAS* and PKA signaling act as tumor promoters. The former implies that certain yet to be identified $G\alpha_s$-coupled receptors may exert a tumor-suppressive function in BCC. The latter raises the possibility that in GI cancers, $G\alpha_i$-coupled GPCRs may act as tumor suppressors and hence that their LOF mutations might be pro-tumorigenic. This is aligned with the large number of $G\alpha_i$-coupled GPCRs that are mutated in GI tumors; however, whether they exhibit GOF or LOF mutations has not yet been tested formally. These particular predictions are of high clinical relevance, as overactive cAMP/PKA activity in many GI tumors could be counteracted therapeutically by stimulating locally expressed $G\alpha_i$–GPCRs, whereas BCCs and SHH-subtype medulloblastomas may be treated by raising cAMP using phosphodiesterase inhibitors (as proposed in Ref. 104) or by stimulating locally (or systemically) $G\alpha_s$–GPCRs expressed in these tissues. These exciting possibilities will likely be explored in the near future.

pan-Cancer GPCRs expression

In addition to mutations, normal GPCRs can play a key role in cancer progression, and they can be targeted pharmacologically for therapeutic purposes. A typical problem when analyzing gene expression changes in cancer is that often both normal and cancerous tissues are heterogeneous, including multiple cell types. Hence, relative changes (fold changes and over- and underexpression) may reflect cellular heterogeneity more than the progression from a normal cell to its distinct cancer states. For example, comparison of GPCRs expressed in cutaneous melanoma with normal skin may grossly overestimate the relative changes in expression between normal and cancerous melanocytes, as the normal skin includes a very limited number of melanocytes. Moreover, although fold changes can provide useful information, this takes attention away from GPCRs that may exert important functions for cancer transformation through increased local ligand secretion or aberrant downstream signaling activity. A recent study has documented relative changes in GPCR expression in cancer (105). Instead, we focus here on illuminating absolute expression levels of each GPCR and provide visual representations to gauge absolute GPCR levels. Certainly, a limitation of this analysis is that the precise cells that express each GPCR within the tumors, such as cancer and tumor stromal cells (*e.g.* cancer associated fibroblasts, blood vessels, and immune infiltrating cells), will need to be established in future efforts, for example by the use of modern single cell sequencing approaches. Nonetheless, we expect that we can gain an unprecedented new perspective on GPCR expression patterns in human malignancies by utilizing information gained from this analysis.

Specifically, as shown in Fig. 5, an intriguing area of study is the expression of orphan GPCRs in cancer. The endogenous ligands of more than 140 of these receptors remain unidentified and/or poorly understood, thus, their natural function is currently largely unknown (106). Nevertheless, according to our pan-cancer analysis, orphan GPCRs are differentially expressed across cancer types, and they may exert multiple functions during cancer progression (Fig. S1*M*). For example, since a decrease in extracellular pH is a major tumor-promoting factor in the tumor microenvironment, an intriguing area of research is the group of proton-sensing GPCRs: GPR132, GPR65, GPR68, and GPR4, which are highly expressed in a large range of human cancers. Both GPR4 and TDAG8 (GPR65) have been shown to be overexpressed in many cancers and can cause malignant transformation of cells *in vitro* (107). GPR132 (also known as G2A) was previously shown to have tumor suppressor properties, as it prevents oncogenic transformations of pre-B cells by the BCR–ABL oncogene, similar to the role of *GNA13* in these cell types (108). However, GPR132 has been shown to be highly transforming in fibroblasts (109). Thus, proton-sensing GPCRs may display tumor-promoting or -suppressive functions depending on the cancer cell of origin and may also display pro-tumorigenic activity when activated in the tumor stroma (105). Interestingly, in our recent G protein–coupling predictor trained by a large experimental dataset, orphan GPCRs tend to show a higher proportion of coupling toward $G\alpha_{12/13}$ than other GPCR classes (79) further suggesting potential importance of orphan GPCRs in cancers that involve aberrant $G\alpha_{12/13}$ signaling.

The leucine-rich repeat-containing GPCRs (LGR) LGRs 4–8 are known for their role in development, bone formation, and remodeling, but LGR4 and LGR5 are also up-regulated in several cancer types (110). These receptors are expressed in multiple tissue-resident stem cells, and their overexpression may reflect the expansion of this cellular compartment as well as the establishment of cancer stem cell niches (110). Overexpression

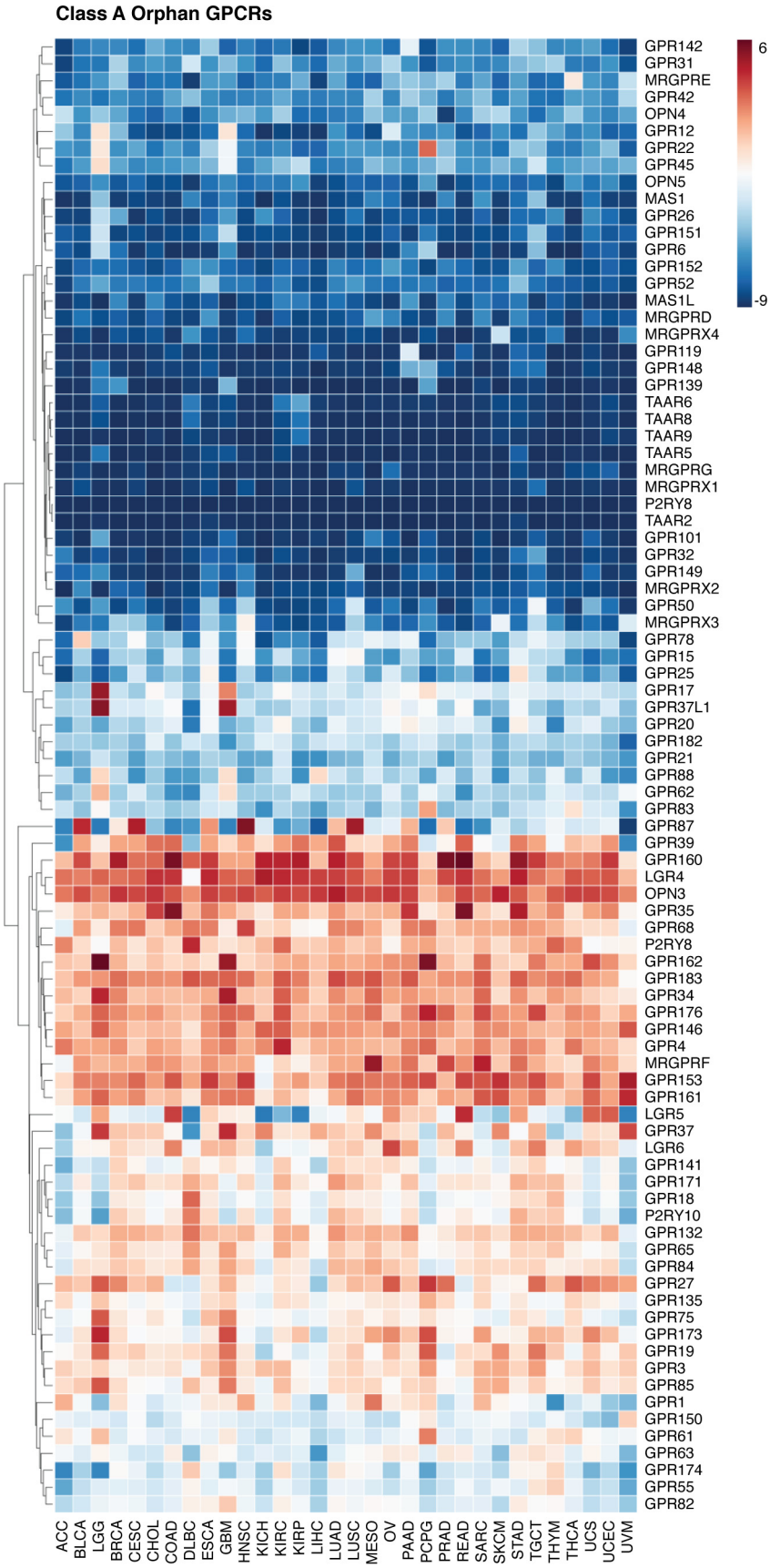


Figure 5. Expression of class A orphan receptors in cancer. Gene expression for class A orphan GPCRs from the UCSC TCGA PanCan Cohort RNA-seq dataset is shown. Expression values are summarized by defining transcripts per million (TPM), which normalizes for both gene length and sequencing depth. Expression values are $\log_2(\text{TPM} + 0.001)$ averaged within the primary tumor samples of each cancer. GPCRs are clustered based on similarity across cancer types.

of LGR4 and LGR5 in colon and ovarian tumors most likely enhances cell proliferation and metastasis (111, 112). Interestingly, many class A orphan GPCRs are rarely expressed across cancer types. These include the *MAS* oncogene, which can explain the limitations in analyzing its role in human cancer despite its initial identification during transfection experiments several decades ago. Others are expressed in a single cancer (*e.g.* GPR22 in pheochromocytoma and paraganglioma) or a few cancers (*e.g.* GPR17 and GPR37L1 that are expressed only in GBM and brain lower grade glioma), whereas others are expressed in most cancers, such as OPN3 and LGR4. These studies de-orphaning GPCRs and uncovering the function of additional overexpressed GPCRs may provide promising candidates for therapeutic intervention in cancer.

The pan-cancer expression of each GPCR class is depicted in Fig. S1, *A–N*. We hope that this information will be useful for hypothesis generation in our large community of scientists working in the field of GPCRs in academia and industry. Although this review will not provide a comprehensive analysis of each GPCR, a few concepts may be worth discussing. For example, expression of the purinergic $P2Y_{11}$ and adenosine A_{2A} receptors is widespread in all cancers, whereas GBM tumors express high levels of *ADORA1*, *ADORA2*, and *ADORA3*, all of which can be activated by adenosine in the tumor microenvironment. Multiple lipid receptors for S1P ($S1P_{1-3}$) and LPA (LPA_1, LPA_2, and LPA_6) are widely expressed as well. These receptors are intriguing because ligands for these receptors have been shown to accumulate in the tumor microenvironment (113, 114). Conserved residues in these receptors also display a high mutational rate, which suggests that they may play vital roles in receptor signaling initiation, termination, and coupling specificity (13).

This is also highly relevant for the 17 known GPCRs that specifically recognize intermediates or (by)products of cellular metabolism, which are often involved in nutrient sensing (115). These include receptors sensing amino acids and amino acid metabolites (GPR142, CasSR, GPR35, TAAR1, and FOPR1/2), bile acid (TGR5/GPBAR1), triglyceride metabolites (*e.g.* FFA1/GPR40, FFA4/GPR120, and GPR119), products of the intermediary metabolism and small carboxylic metabolites such as acetate and propionate (FFA2/GPR43 and FFA3/GPR41), butyrate (FFA2/GPR43, FFA3/GPR41, and HCA2/GPR109A), β-hydroxybutyrate (HCA2/GPR109A), β-hydroxyoctanoate (HCA3/GPR109B), lactate (HCA1/GPR81), succinate (GPR91), and capric acid (GPR84) receptors, as well as gut microbiota-derived products (*e.g.* short-chain fatty acids, such as acetate, propionate, and butyrate) (reviewed in Ref. 115). These receptors are highly expressed in multiple organs of the digestive tract and immune cells (116), and they may be persistently activated in the tumor microenvironment due to the high metabolic rate that characterizes most solid tumors.

The EP_4 (*PTGER4*) and EP_2 (*PTGER2*) receptors for the typical inflammatory mediator PGE2 (see below) are also widely expressed, whereas EP_3 (*PTGER3*) is mainly expressed in kidney cancer. PGE2 plays a critical role in epithelial regeneration following tissue injury and cancer growth, which occurs via PI3K/Akt and β-catenin pathways (63, 117). COX2 overexpression and enhanced PGE2 production is most notable in colorectal cancer, and COX2 blockade can help explain the cancer chemopreventive activity of aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) (118). However, direct roles for PGE2 in tumorigenesis have been demonstrated for many other human malignancies, including breast, lung, liver, and gastric cancers, among others. For example, in laboratory models of breast and gastric cancers, COX2 overexpression and alterations in Wnt signaling both led to increased tumorigenesis (119, 120). Moreover, EP_3 has been shown to be involved in angiogenesis in lung cancer cell lines by increasing VEGF and metalloproteinase-9 (MMP-9) expression (121).

Among the class of GPCRs for proteins (Fig. S1*E*), which includes chemokine receptors, CXCR4 is the most widely expressed. This may include many cancers that express CXCR4 under hypoxic conditions, as well as in blood vessels and immune cells (see below) (122–124). Other chemokine receptors that are highly expressed in immune cells (see below) were less well-represented, suggesting a more limited impact of immune infiltrating cells to the overall mRNA expression patterns in our pan-cancer analysis. The analysis of GPCRs activated by peptides (Fig. S1*F*) show a clear widespread expression in genes for thrombin PAR1 (*F2R*) and PAR2 (*F2RL1*) receptors and endothelin receptors (*EDNRB*), the latter with particularly higher expression in SKCM and uveal (UM) melanomas. HRH1, encoding H_1 histamine receptor, is the most widely expressed aminergic GPCR (Fig. S1*G*), whereas M1 muscarinic receptors (*CHRM1*) and β1-adrenergic receptors (*ADRB1*) are highly expressed in prostate cancer, the latter receptor being of unexpected importance for the most highly prevalent cancer among males (see below). Another interesting finding was the high level of expression of dopamine receptor 2 (*DRD2*) in a well-defined set of cancers, including GBM, considering that a new family of antagonists for this receptor has exhibited encouraging anti-tumor activity in multiple cancer types (125, 126).

Interestingly, from our analysis of Frizzled GPCRs, *SMO* is widely expressed in most cancers, beyond its initial main role in BCC. This might be due to *SMO* being expressed in cancer stromal cells that are present in most solid tumors (Fig. S1*G*) (127, 128). There is also widespread expression of FZD_6 and a more cancer-restricted expression of FZD_1 and FZD_4 (Fig. S1*H*).

Intriguingly, analysis of the sensory GPCRs revealed a high level of expression of the taste receptor, *TAS1R3*, across most cancer types, which has not been previously investigated (Fig. S1*J*).

The adhesion GPCR family has mainly been studied in immunological and developmental functions, but they have recently been linked to cancer (Fig. S1*M*). For example, EMR2 (*ADGRE2*) is overexpressed in human breast cancer, and increased nuclear expression of EMR2 is negatively correlated with tumor grade (129). Additionally, CD97 (*ADGRE5*) and GPR56 (*ADGRG1*) are the highest expressed adhesion GPCRs across all cancers, but they have only been studied in the context of melanoma, gastric, esophageal, and thyroid cancers (130–132). Additionally, GPR65 (*TDAG8*) and GPR133 (*ADGRD1*) have also been associated with human cancers and linked to tumor promotion (107, 133), but the role of this high-

ly-expressed family of GPCRs in tumor initiation and metastasis is still not fully understood.

Overall, we expect that the emerging pan-cancer information on GPCR expression will ignite new interest on their study in human malignancies.

GPCRs in metastasis and angiogenesis

Metastasis is one of the cancer hallmarks, in which tumor cells can acquire the ability to migrate and disseminate from the tumor to distant tissues. Cancer cells spread from the primary organ to secondary sites through lymphatic vessels and blood and are the result of a sequential, highly-organized, and organ-selective process. The precise mechanisms determining the directional migration and invasion of tumor cells into specific organs remain to be fully established, but chemokine receptors, all of which are GPCRs, have been the most popular place to look (134, 135). Chemokines are small, cytokine-like proteins that induce directional migration for immune cells through interaction with GPCRs. Chemokines are secreted by multiple organs and act in a coordinated fashion with cell-surface proteins to direct homing of immune cells to specific anatomical sites (136, 137). To serve a similar purpose, tumor cells can hijack chemokine receptor networks and migrate toward specific chemokines, facilitating metastasis to other organs, primarily the liver, lungs, brain, lymph nodes, and bone marrow (134). In addition, the tumor microenvironment includes chemokines that can enhance the motility and survival of cancer cells in an autocrine and paracrine fashion, a process that we refer as oncocrine signaling.

There are 23 distinct chemokine receptors in humans, and they are divided into four classes according to the type of chemokine with which they interact (CC, CXC, CX3C, or XC) (135, 138, 139). CXCR4 and CCR7 represent the best-studied chemokine receptors driving cancer metastasis, as they play active roles in tumor growth, invasion, angiogenesis, metastasis, and cancer relapse and therapeutic resistance (134, 140, 141). CXCL12, the chemokine for CXCR4, is highly expressed in multiple tissues, mainly lungs, liver, and bone marrow, and it is also secreted by tumor and stromal cells (134, 140). CXCL12 expression levels are highest in these common sites of metastasis, which could recruit cancer cells to these distant organs. CCR7 binds the ligands, CCL21 and CCL19, and guides the migration of lymphocytes and dendritic cells (DC) to lymph nodes (142). Expression of CCR7 in tumor cells has emerged as an important predictor of lymph node metastasis and poor prognosis in cervical cancer, gastric carcinoma, and breast cancer, among others (134).

CCR7 and CXCR4 are the main receptors typically present on metastasizing cells, but there are other chemokine receptors that may dictate a more organ-specific metastasis. For example, the small intestine is an organ that expresses high levels of CCL25 physiologically to guide CCR9+ lymphocytes to this tissue. Because melanoma, breast cancer, and ovarian cancer express high levels of CCR9, this receptor may play a pivotal role in the preferential metastasis of these tumors to the small intestine (143–145). Additionally, malignant melanomas express high levels of CCR10, a receptor that guides leukocytes to the skin, and consequently, CCR10 expression in melanoma may drive metastasis to the skin (146, 147). CXCR3 and CXCR5 have both been shown to play a role in lymph node metastasis (148, 149).

With increasing nutrients and oxygen demands by the tumor cell, solid tumors produce angiogenic factors that promote the migration and proliferation of endothelial cells to form new vessels. Many of these factors exert their functions through GPCRs expressed on endothelial cells, including thrombin, prostaglandins, S1P, and many chemokines. In addition, chemokines, like CCL2, CCL5, and CXCL8/IL-8, can recruit leukocytes and macrophages to the tumor site, which leads to production of VEGF and other angiogenic factors that contribute to the growth of tumor-associated blood vessels (134, 135). Production of inflammatory cytokines can also promote new vessel formation by elevating COX2 expression, and in turn prostaglandin E2 (PGE2) increases the expression of VEGF, CXCL8, and CXCL5 by tumor cells (150, 151).

In the case of thrombin, this serine proteinase plays a vital role in regulating hemostasis by converting fibrinogen into fibrin to stimulate platelet aggregation and coagulation (152). Thrombin carries out its effects through the PAR family of receptors, which exhibit the unique property of harboring a tethered ligand within the receptor that becomes exposed upon cleavage of the N-terminal extracellular region by thrombin (153). Thrombin promotes angiogenesis by increasing metalloproteinases and decreasing the ability of endothelial cells to adhere to the extracellular matrix (154, 155), while promoting the expression and activity of the VEGF receptor, VEGFR-2, through $G\alpha_{13}$ and its target RhoA (156). However, $S1P_1$ stimulates endothelial cell proliferation, survival, and migration and also regulates sprouting angiogenesis through cross-talk with VEGFR-2 and enhanced tissue hypoxia and VEGF production (157–159). The effects of S1P on angiogenesis largely depend on the GPCR it binds ($S1P_{1-5}$). S1P stimulates angiogenesis mainly through $S1P_1$ and $S1P_3$, and it mediates endothelial cell migration and formation of capillary structures through $G\alpha_i$ (or more likely its associated $G\beta\gamma$ subunits) activation of the small GTPase Rac1 (160). In contrast, $S1P_2$ has been shown to be involved in the suppression of angiogenesis most likely through the inhibition of Rac and cell migration (161). Altogether, GPCRs participate in angiogenesis either by promoting the proliferation, migration, and sprouting of endothelial cells or by the release of pro-angiogenic factors for new blood vessel formation, thereby increasing the blood supply to the growing tumors.

Key role for GPCRs in cancer immunology

In the last few years, cancer immunotherapy became one of the most exciting breakthroughs in cancer treatment. Recent revolutionary discoveries have highlighted the importance of the tumor microenvironment and its associated immune cells in cancer development and therapeutic resistance. Tumors can deploy multiple mechanisms to avoid immune recognition and an anti-tumor immune response, including the recruitment of myeloid-derived suppressor cells (MDSC) and conditioning of the surrounding microenvironment to become highly immune-suppressive by expressing cytokines, such as IL-6, IL-10, and transforming growth factor β (162). This can lead to the accu-

mulation of suppressive regulatory T cells (Tregs) and the polarization of macrophages toward an immune-suppressive phenotype, which is often referred to as M2 or tumor-associated macrophage (TAM) phenotype (163). A key emerging mechanism of tumor immunosuppression involves the induction of T-cell exhaustion through activation of T-cell checkpoints, including programmed death 1 (PD-1). Its ligand, programmed death-ligand 1 (PD-L1), is expressed by macrophages and some cancer cells, which can restrain T-cell activation and induce immunosuppresion (164–166). Together, these conditions contribute to the suppression of cytotoxic CD8+ T lymphocyte recruitment, survival, and function, and ultimately to the loss of an effective anti-tumor immune response. Although the aberrant function and dysregulated expression of GPCRs is now beginning to be linked directly to the tumor itself, the role of GPCRs on immune cells infiltrating tumors is still not fully understood and grossly underappreciated. Given the diversity of GPCRs and the variety of GPCR families, current studies have only scratched the surface of delineating GPCRs on immune cells in cancer. The importance of studying GPCRs in the context of cancer immunology is reflected by the multiple roles that this receptor family plays in inflammation, orchestrating immune cell trafficking and regulating the tumor microenvironment, as summarized in Fig. 6. A crucial first step in anti-tumor immunity is the migration of cytotoxic cells recognizing tumor antigens to the tumor, and this is mediated largely by chemokine receptors.

GPCRs orchestrate immune cell migration and recruitment

In the 1960s, it became clear that chemoattractants can bind and act directly on lymphocytes, and from there, GPCRs in the immune system became mostly known for their ability to steer cell migration toward chemokine gradients (167, 168). Cytotoxic immune cells, including natural killer (NK) cells and CD8 T cells that are specific for tumor antigens, are guided to the tumor where they secrete cytotoxic molecules to induce tumor cell death.

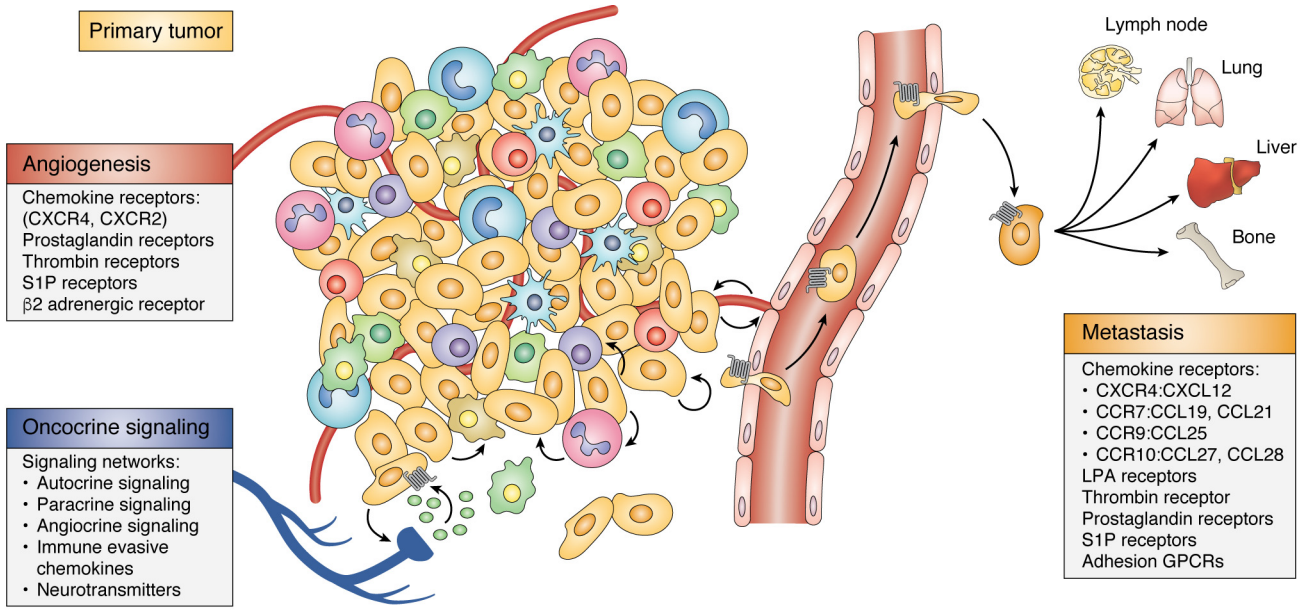
CXCR3 on CD8 T cells and NK cells binds the ligands CXCL9 and CXCLl0 to migrate into tumors (reviewed in Ref. 169). Indeed, increased CXCL9 and CXLC10 and tumor-infiltrating CD8 T cells correlates with improved survival and decreased cancer metastasis (170, 171). Furthermore, CXCL10, which is also known as interferon-induced protein 10 (IP10), as well as CXCL9 and CXCL11 are known to be induced by interferon α, β, and γ and are part of the "interferon gene signature" that is often used to predict a favorable response to anti-PD-1 treatment (172). This provides direct evidence highlighting the importance of chemokine and chemokine receptors in the new era of immunotherapies.

The activation of these antigen-experienced cytotoxic CD8+ T cells is driven by DCs that capture cancer cell antigens on their major histocompatibility (MHC) I or MHCII molecules and present them to naïve T cells to drive effector T-cell activation, bridging the innate and adaptive immune system (173). DCs are antigen-presenting cells that traffic to draining lymph nodes for stimulation and activation of T cells, and their mobilization is largely driven by CCR7 and its ligands CCL19 and CCL21 (174). The endocytosis of apoptotic cells has been shown to induce CCR7 expression and subsequent migration of DCs, and CCR7-mediated activation of Rac1 and Rac2 may have redundant functions in migration to the lymph nodes, as shown by the absence of DC mobilization in Rac1- and Rac2-deficient mice (175). Dendritic cells play a crucial role in immunosurveillance for elimination of cancer cells.

In addition to DC mobilization and recruitment of cytotoxic immune cells to the tumor, chemokine receptors also participate in promoting tumorigenesis by mediating the recruitment of immunosuppressive immune cells, specifically Treg cells and MDSCs (169). Treg cells express CCR4 and migrate to the tumor in response to CCL22 produced by macrophages and tumor cells (176). Blockade of this receptor with a therapeutic mAb (mogamulizumab) or small molecule inhibitors is under current evaluation for cancer treatment when combined with immune-checkpoint inhibitors (177). The hypoxic regions of the tumor microenvironment also generate CCL28, which can recruit CCR10-expressing Tregs (178). The immunosuppressive microenvironment of the bone marrow has been linked to high frequencies of Tregs. Tregs can be mobilized from the bone marrow into the periphery by granulocyte colony-stimulating factor (G-CSF), which promotes the degradation of CXCLl2, a ligand for CXCR4 (179). CXCL12 can lead to higher numbers of Tregs in the bone marrow, promoting an immunosuppressive environment that favors the establishment of metastatic niches, which may help explain why many cancers often metastasize to the bone marrow.

Chemokine receptors also direct MDSCs to the tumor thus favoring an immunosuppressive, tumor-promoting environment. Monocytic MDSCs, including TAMs, are recruited to the tumor by CCL2, CXCL5, and CXCL12 acting on CCR2, CXCR2, and CXCR4 (180). MDSCs have been also shown to secrete ligands for CCR5 to direct the migration of CCR5+ Tregs, although various studies have also shown that CCR5 guides MDSCs themselves as well (181–183). Moreover, CXCL8 and CXCL1 are often secreted by most solid tumors and by certain subsets of Tregs and act on granulocytic MDSCs that express CXCR1 and CXCR2, including neutrophils, to promote their recruitment to tumors. Because neutrophils secrete tumor- and angiogenesis-promoting molecules, CXCL8 is generally thought to contribute to the immunosuppressive environment leading to tumor angiogenesis and progression (169, 184).

In summary, a variety of chemokines dictate the recruitment of different immunosuppressive immune cells into the tumor microenvironment, and through these processes, tumors can evolve to avoid detection and destruction by the innate and adaptive immune system. This can, in turn, provide an opportunity to disable the immune evasive mechanisms by targeting chemokine receptors with an increasing repertoire of small molecule inhibitors and negative allosteric modulators (185) and/or with blocking antibodies. These therapeutic strategies are already in use or under clinical evaluation in multiple chronic inflammatory diseases, and their study in the context of cancer immunotherapy will likely represent one of the most exciting areas of future exploration in the GPCR targeting field.



Immune cells

Tumor-promoting immune cells	Receptors:	Recruited by:	Functions:	Anti-tumoral immune cells	Receptors:	Recruited by:	Functions:
Treg	CCR4 CCR10	CCL17, CCL22 CCL27, CCL28	Secretion of suppressive cytokines, impairment of CD8/NK cells, promotion of M2 macrophages, poor prognosis	Dendritic cell	CCR6, CCR7, CXCR4	CCL19, CCL21, CCL20, CXCL12	Professional antigen presenting cells, initiation of adaptive immune responses, stimulation and activation of CTLs
Granulocytic MDSC	CXCR1 CXCR2	CXCL8, CXCL1, CXCL5	Suppression of antigen-specific CD8 T cells by ROS and cytokines	NK cell	CXCR3	CXCL9, CXCL10, CXCL11	Rapid, cytotoxic responses without prior sensitization, ADCC, release of death ligands, and immunosurveillance
Monocytic MDSC	CCR2, CCR5 CXCR2, CXCR4	CCL2, CXCL5, CXCL12	Inhibition of T cell migration to tumor and anti-tumor responses through iNOS, ARG1, COX2 and others	CD8 T cell	CXCR3	CXCL9, CXCL10, CXCL11	Secretion of highly cytotoxic cytokines, release of death ligands and cytolytic granule exocytosis for induction of tumor cell death
M2 macrophage	CCR2 CCR5	CCL2, CCL5	Secretion of suppressive cytokines, promotion of EMT, inhibition of cytotoxic responses via arginase, iNOS, PGE2	M1 macrophage	CCR2 CCR5	CCL2, CCL5	Enhanced phagocytosis and antigen presentation, induction of T cell responses and pro-inflammatory cytokines

Figure 6. Function of GPCRs in cancer. *Top,* GPCRs contribute to both tumor promotion, angiogenesis, metastasis, and immune evasive functions in the tumor microenvironment. Multiple GPCR agonists released by the tumors or accumulating in the tumor microenvironment promote angiogenesis by stimulating GPCRs on endothelial cells. GPCRs play multiple roles in cell communication between tumors cells, tumor stroma, endothelial cells, and blood vessels and immune cells, as well as in response to neurotransmitters released as a consequence of tumor-induced axonogenesis and tumor innervation as part of autocrine and paracrine (oncocrine) signaling networks that drive tumorigenesis. GPCRs present on tumor cells assist in extravasation and migration of circulating tumor cells to promote metastasis to distant organ destinations. *Bottom*, chemokine receptors recruit a variety of immune cells to the primary tumor and release agents that both promote and suppress immune functions. Immune-suppressive cells promote tumor growth by inhibiting functions of cytotoxic immune cells or secreting hypoxic and anti-inflammatory molecules to sculpt the suppressive tumor microenvironment. Anti-tumor immune cells that are recruited to the tumor secrete highly cytotoxic molecules for tumor cell destruction. See text for details. (Abbreviations used are as follows: *ROS,* reactive oxygen species; *iNOS,* inducible nitric-oxide synthase; *ARG1,* arginase 1; *EMT,* epithelial to mesenchymal transition; *ADCC,* antibody-dependent cellular cytotoxicity.

Modulation of immunosuppressive GPCRs by the tumor microenvironment

The immunosuppressive and hypoxic nature of the tumor microenvironment can also largely influence the function of cytotoxic immune cells and the success of cancer immunotherapies. A driving force behind the malignancy and morbidity of cancer is its ability to proliferate unrestrained, by creating an immunosuppressive environment favoring tumor growth. The nucleoside adenosine is a potent physiologic and pharmacologic regulator that is released from injured and necrotic cells

by extracellular breakdown of ATP by the action of the ectonucleotidases CD39 and CD73 (reviewed in Ref. 186). Typical extracellular adenosine levels are low, but at injury sites with tissue breakdown and hypoxia, the adenosine levels can rise from nanomolar to micromolar concentrations. Extracellular adenosine can signal through four GPCRs: A_1, A_{2A}, A_{2B}, and A_3 adenosine receptors (ADORA1, ADORA2A, ADORA2B, and ADORA3, respectively) (187). A_1 and A_3 receptors signal through $G\alpha_i$ and lead to decreased cAMP. Activation of A_{2A} and A_{2B} receptors, which are expressed on immune and endothelial cells, leads to signaling through $G\alpha_s$ proteins, and A_{2B} can also signal through $G\alpha_q$ (188). Of the four adenosine receptors, A_{2A} receptor (encoded by the *ADORA2A* gene) is the predominantly expressed subtype in most immune cells. In general, stimulation of the A_{2A} receptor provides an immunosuppressive signal in T cells (187), NK cells (189), DCs (190), and neutrophils (191). A_{2A} receptor stimulation interferes with trafficking of T cells and NK cells by desensitizing chemokine receptors and reducing levels of pro-inflammatory cytokines (192). Blocking the adenosine-generating pathway has shown tumor regression in breast cancer, colorectal cancer, and melanoma (reviewed in Refs. 186, 193), and small molecule inhibitors of A_{2A} receptor as well as blocking antibodies anti-CD73 and anti-CD39 are under current evaluation for combination cancer immunotherapies (186, 194). Although these immunotherapies aim to boost immune cell activity in the immunosuppressive tumor microenvironment, it is also important to consider the effects of tumor-driven inflammation, largely driven by prostaglandins and prostaglandin receptors.

GPCRs link inflammation to cancer immune evasion

Inflammation occurs as the immune system responds to infection and injury to beneficially remove the offending factors and restore tissue structure and physiological function. However, with subsequent tissue injury, cells that have sustained DNA damage or mutagenic assault will continue to proliferate in microenvironments rich in inflammatory cells and growth/survival factors that support their growth. Prostaglandins are a group of physiologically-active lipid compounds found in almost every tissue in humans and animals, and they play a key role in the generation of an inflammatory response (195). They are enzymatically derived from arachidonic acid by the COX isoforms, COX1 and COX2, and are powerful vasodilators (196). PGE2 is the most abundant prostaglandin produced in cancers, and the prostanoid receptor family, which are GPCRs, includes the following: E prostanoid receptor 1 (EP_1, *PTGER1*), EP_2 (*PTGER2*), EP_3 (*PTGER3*), and EP_4 (*PTGER4*). Of these, EP_1 is coupled to $G\alpha_q$; EP_3 is coupled to $G\alpha_i$, and both EP_2 and EP_4 are coupled to $G\alpha_s$ (196). PGE2 binding to different EP receptors can regulate the function of many immune cell types, including macrophages, DCs, T cells, and B cells, as will be discussed here.

PGE2 produced by cancer cells has been linked to increased expression of *FOXP3* in Treg cells, promoting the immunesuppressive activity of Tregs (197). In addition to Tregs, PGE2 has also been linked to increased recruitment of MDSCs (198), decreased CD8 T-cell activation (199, 200), and increased expression of inhibitory markers, like PD-1 (198, 199, 201). PGE2 alters the differentiation, maturation, and cytokine secretion of DCs by up-regulating CD25 and indoleamine-pyrrole 2,3-dioxygenase and decreased expression of CD80, CD86, and MHCI maturation markers (202). Recently, NSAIDs that block COX2 and/or COX1 and COX2 were found to have beneficial effects on reducing the risk of developing esophageal, stomach, skin, and breast cancers, in addition to their best-established function in preventing colorectal cancer (203, 204). Hence, EP receptors may represent exciting targets for cancer immune prevention and treatment.

New "oncocrine" GPCRs networks

As outlined above, GPCRs play multiple roles in cancer progression, dissemination, angiogenesis, and immune evasion upon their activation by ligands produced by cancer cells or by the multiplicity of cells within the tumor stroma. This is often referred to as autocrine and paracrine signaling, respectively, and to angiocrine signaling when referring to the release of endothelial cell-derived factors, including GPCR ligands, during organ regeneration and homeostasis and cancer progression (205). However, these terms may not reflect fully the complexity of the cell communication networks deployed by cancer cells and their tumor microenvironment. Indeed, seminal findings have linked GPCRs to the cross-talk among multiple cell types to promote the survival and proliferation of cancer cells, instruct the growth of new blood vessels, evade immune surveillance, and metastasize to secondary organs. In turn, targeting GPCRs and their cancer-driving oncocrine networks may provide unique opportunities to disrupt intratumoral cell–cell communication, thereby initiating tumor collapse.

The concept that cell–cell communication circuits mediate GPCR-induced tumorigenesis was well-established by our prior studies on a virally-encoded receptor expressed by the Kaposi's Sarcoma (KS) virus (KSHV/HHV8). This receptor, known as KSHV vGPCR, is constitutively active and is alone sufficient to initiate angiosarcomas when expressed in endothelial cells and their progenitors (206). However, as for human KS, only few cells within the tumor express KSHV vGPCR, but instead vGPCR-expressing cells secrete multiple factors, including VEGF, IL-6, IL-8/CXCL8 and IL-1, thereby causing what was termed as "paracrine transformation" of the surrounding cells that express other KSHV genes (such as LANA) (25, 207, 208).

Perhaps one of the best-described oncocrine systems is that initiated by neuropeptides that act as oncogenic autocrine growth factors, such as bombesin-like peptides, including gastrin-releasing peptide (GRP) and neuromedin B (NMB), which bind to bombesin receptors, NMB receptor and GRP receptor, respectively (209, 210). Most small cell lung cancer (SCLC), prostate, and ovarian cancers express GRP and NMB mRNA, and bombesin-like peptide antagonists reduce the growth of their derived cell lines *in vitro* (211–214). Moreover, SCLC and prostate carcinoma cell lines also secrete arginine vasopressin and express vasopressin receptors (encoded by *AVPR1A*, *AVPR1B*, and *AVPR2*) and neurotensin receptors (encoded by *NTSR1* and *NTSR2*). Multiple studies have demonstrated the involvement of the cholecystokinin B receptor (CCK_2) autocrine system in human pancreatic carcinoma. The mRNA for

the receptor was detected in all pancreatic carcinomas, and growth of their derived cell lines was inhibited by antagonists blocking CCK_2 (215, 216). Most of the neuropeptide receptors act through $G\alpha_q$ proteins, highlighting again the transforming potential of $G\alpha_q$–GPCRs when activated locally by their ligands.

Remarkably, recent evidence has also demonstrated that oncocrine signaling by GPCRs mediates the well-established link between nerve innervation and growth of many malignancies (217, 218). For example, in pancreatic ductal adenocarcinoma, signaling through the β2 adrenergic receptor, which can be stimulated by circulating adrenaline and noradrenaline under stress conditions, leads to increased nerve growth factor (NGF) secretion by pre-cancer and cancer cells (219). In this case, neurotrophins, such as NGF, signal through Trk receptors to promote neuron survival and axonogenesis and tumor innervation, initiating a paracrine circuit through the local release of noradrenaline by the nerve ends. This can act on β2 adrenergic receptors in pancreatic ductal adenocarcinoma cells to promote increased growth and therapy resistance (219). A similar nerve-cancer paracrine mechanism, now referred to collectively as oncocrine, was recently described in stomach cancer (220). In this case, acetylcholine released from tuft cells and nerves induces NGF release from gastric epithelial cells, leading to enhanced innervation and subsequent release of acetylcholine by the nerves. Acetylcholine stimulates M3 muscarinic receptors (encoded by *CHRM3*) expressed on stomach pre-cancer cells to promote stomach cancer progression (220). Another interesting example of oncocrine signaling is the autocrine activation of muscarinic receptors in prostate cancer. In prostate cancer tissues, there is simultaneously an increased expression of choline acetyltransferase, the enzyme catalyzing the synthesis and secretion of acetylcholine, and M3 muscarinic receptors (*CHRM3*) (221). Experimentally, activation of acetylcholine receptors increases prostate cancer proliferation and migration (222). In addition to prostate cancer, muscarinic receptors have also been implicated in SCLC and CRC stimulating cancer cell growth with acetylcholine as an autocrine growth factor (223, 224).

Frizzled receptors may also play important oncocrine functions. For example, microglia actively recruit and support the growth of GBM and contribute to brain metastasis in a GPCR (WNT/FZD)-dependent manner (225). Together, these findings support a critical and underappreciated role of GPCRs as part of autocrine and paracrine ("oncocrine") mechanisms driving tumor progression, some of which can be readily targeted with existing approved drugs.

Opportunities to target the underexploited GPCR axis for precision cancer therapy

In this new era of precision medicine, we have unique opportunities to intervene pharmacologically to correct faulty signaling circuits while sparing the normal ones. In this context, GPCRs represent the most widely studied receptor family, but their oncogenic signaling pathways are still not fully understood. We hope that the present review highlighting the oncoGPCRome, including mutations, gene copy variations, and expression of GPCRs in each cancer type and cancer-associated immune cells will ignite new efforts targeting this receptor family in cancer. Indeed, GPCRs and G proteins are widely dysregulated in cancer and yet underexploited in oncology. This is reflected by the fact that only eight FDA-approved anti-cancer drugs target GPCRs directly (Drugs@FDA: FDA Approved Drug Products). These include the following. 1) Cabergoline, a small molecule dopamine 1 (D_1) receptor inhibitor, is approved for hyperprolactinemic disorders, either idiopathic or due to pituitary adenomas. 2) Sonidegib and 3) Vismodegib are small molecule antagonists of SMO and block Shh signaling and are approved for the treatment of locally advanced BCC that has recurred following surgery or radiation therapy, or BCC patients who are not candidates for surgery or radiation therapy. Vismodegib is also approved for metastatic BCC. 4) Lanreotide is a long-acting analogue of somatostatin, which stimulates somatostatin receptors (SST_{1-5}), and is indicated for the long-term treatment of acromegalic patients who do not respond to surgery and/or radiotherapy. 5) Degarelix is a gonadotropin-releasing hormone receptor antagonist that reduces testosterone production and is indicated for treatment of patients with advanced prostate cancer. 6) Plerixafor, a small molecule CXCR4 antagonist, is used in combination with G-CSF to mobilize hematopoietic stem cells to the peripheral blood for autologous transplantation in patients with non-Hodgkin's lymphoma and multiple myeloma. 7) Evista (raloxifene), a selective estrogen receptor modulator that also targets GPER1 (G protein–coupled estrogen receptor 30, GPR30, reviewed in Ref. 226) in post-menopausal women for breast cancer prevention, provides a valuable alternative target for estrogen receptor–negative breast tumors and is also a preventative method less toxic than the standard tamoxifen treatment regimen. 8) Mogamulizumab, a humanized mAb targeting CCR4, was approved in August, 2018, for mycosis fungoides and Sézary syndrome, two subtypes of cutaneous T-cell lymphoma (CTCL). In Japan, mogamulizumab is also approved for the treatment of relapsed or refractory CCR4+ T-cell leukemia/lymphoma (ATCLL) and CCR4+ CTCL. Although CTCL and ATCLL are rare nonmelanoma skin cancers, this represents a breakthrough in GPCR targeting using biologics (monoclonal antibodies) rather than GPCR modulation solely based on small molecule inhibitors.

Building on these successes, multiple monoclonal antibodies against GPCRs and/or small molecule inhibitors are now under clinical exploration for multiple cancer indications, and we can expect that new drug discovery efforts may soon enable targeting GPCRs to 1) prevent cancer in at-risk patients with premalignant lesions or genetic predisposition, 2) diminish therapy resistance, 3) increase the response to new revolutionary cancer immunotherapies, and 4) prevent tumor relapse in patients that have had a prior cancer, often referred to as cancer survivors.

Regarding the latter, it is estimated that there are currently at least 15 million cancer survivors in the United States alone (227). The emerging findings that disruption of GPCR-mediated oncocrine communication between cancer cells, cancer and stromal cells, cancer and immune cells, and cancer and local innervation can prevent cancer progression and metastasis may further support the clinical exploration of repurposing approved drugs as an adjuvant treatment in cancer patients and

cancer survivors. For example, a typical β blocker, propranolol, improves prognosis in skin melanoma (228) and decreases resistance to EGFR inhibitors in lung cancer (229). Based on these and additional findings, propranolol is currently under clinical evaluation as an adjuvant treatment in multiple cancer types.

This may just represent the tip of the iceberg, as there is ample experience in using GPCR-modulating agents for prolonged periods of time for a myriad of human diseases. Thus, we can anticipate that harnessing the power of patient health "big data" using computational analytic platforms and large epidemiological datasets can provide extraordinary opportunities for GPCR-based treatments in cancer through drug repurposing of approved GPCR-targeting therapies.

Antibodies targeting GPCRs could have broader applications, such as for cancer imaging and early detection, as well as to deliver cytotoxic agents and/or radiosensitizers. For example, the exclusivity of FZD_{10} expression in tumor cells over normal tissues was recently exploited to develop anti-FZD_{10} antibodies for radioimmunotherapy as a therapeutic β-particle radiation target for synovial sarcoma (230). As more monoclonal antibodies targeting GPCRs are developed, radioimmunotherapy could be utilized to target and destroy cancer cells exclusively expressing these receptors.

Another exciting opportunity is to target GPCRs as part of combination therapies to prevent or reverse cancer therapy resistance. Specifically, a major drawback of the use of targeted cancer therapies is that the prolonged inhibition of key signaling hubs, such as EGFR, PI3K, BRAF, and mTOR, often leads to the activation of an intricate network of negative and positive feedback loops, which may initiate compensatory bypass mechanisms overcoming the growth-suppressive activity of targeted therapies that inhibit oncogenic pathways (231, 232). As such, combinatorial inhibition of oncogene-effector and feedback pathways is a promising cancer treatment strategy (233). In this context, we can anticipate that GPCRs may provide unexplored opportunities for combination treatment options. For example, two large omics approaches using (*a*) genome-wide ORF expression approaches and (*b*) CRISPR/Cas9 activation methods, enhancing the expression of endogenous human genes revealed that GPCRs, G protein, and/or their downstream targets are the most highly represented class of molecules conferring BRAF inhibitor resistance in melanoma cells (234, 235). This information, combined with the expression analysis of GPCRs expressed in each cancer type, may facilitate the hypothesis-driven exploration of the role of GPCRs in resistance to targeted agents in melanoma and other malignancies.

Developing effective treatment options for cancers driven by constitutively-active mutants of G proteins α subunits is expected to be more challenging, as it may require the development of cell-permeable agents targeting these G proteins directly or interfering with their oncogenic signaling pathways. Regarding the former, a new generation of naturally-occurring cyclic depsipeptides, FR900359 and YM-254890, which block $G\alpha_q$ have shown encouraging activity in experimental UVM models (236–238). While providing an exciting proof of principle, likely toxicities associated with general $G\alpha_q$ blockade may limit its therapeutic use. Instead, the use of new computational pipelines have recently revealed that the kinase FAK (encoded by the *PTK2* gene) is synthetic-lethal with active *GNAQ*, which means that while FAK inhibition can be tolerated in most normal cells in adults, cancer cells driven by *GNAQ* depend on FAK for their survival and growth (239). The discovery of additional signaling vulnerabilities for *GNAQ* and *GNAS* oncogenes, for example by genome-wide synthetic lethality screens, may soon provide novel precision therapies for the multiple emerging G protein–driven human malignancies and disease conditions.

Overall, we are now witnessing a revolution in our understanding of cancer at the molecular and cellular level. In this context, targeting GPCRs and their oncogenic circuits as single agents or as part of new combination modalities may provide unprecedented opportunities for the development of novel cancer prevention strategies and targeted and immune therapies.

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