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The Emerging Mutational Landscape of G-proteins and G-protein Coupled Receptors in Cancer

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Abstract

Aberrant expression and activity of G proteins and G protein coupled receptors (GPCRs) are frequently associated with tumorigenesis. Deep sequencing studies show that 4.2% of tumors carry activating mutations in *GNAS* (encoding $G\alpha_s$), and that oncogenic activating mutants in genes encoding $G\alpha_q$ family members (*GNAQ* or *GNA11*) are present in ~66% and ~6% of melanomas arising in the eye and skin, respectively. Furthermore, nearly 20% of human tumors harbor mutations in GPCRs. Many human cancer-associated viruses also express constitutively active viral GPCRs. These studies indicate that G proteins, GPCRs and their linked signaling circuitry represent novel therapeutic targets for cancer prevention and treatment.

Introduction

The G protein coupled receptor (GPCR) family of proteins comprises approximately 4% of the encoded human genes: with over 800 members, it is the largest family of cell surface receptors involved in signal transduction. These proteins are characterized by a 7-transmembrane domain structure with an extracellular N-terminus and an intracellular C-terminus. GPCRs play critical roles in a variety of physiological processes including cardiac function, immune responses, neurotransmission, and sensory functions (such as vision, taste and olfaction), but their aberrant activity or expression also contributes to some of the most prevalent human diseases [1]. Indeed, GPCRs are the direct or indirect target of over 25% of therapeutic drugs on the market [2, 3].

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GPCRs function as key transducers of signals from the extracellular milieu to the inside of the cell. A variety of molecules ranging from photons to lipids to small proteins serve as ligands for different GPCRs, all capable of inducing conformational changes that promote receptor activation. Initial signal transduction is largely accomplished by the receptor coupling to and activating heterotrimeric G proteins, which then mediate the activation of a number of second messenger systems, small GTPases and an intricate network of kinase cascades. Ultimately, activation of these GPCR-regulated signaling circuits can lead to changes in gene transcription, cell survival and motility, and normal and malignant cell growth.

G protein and GPCR Signaling

The widely accepted model for GPCR activation involves binding of an agonist ligand at the extracellular side of the receptor, which induces a conformational change in the receptor and alters the position of its transmembrane helices and intracellular loops. In this active conformation, the agonist-occupied receptor couples to the heterotrimeric G proteins which promotes release of GDP from the Gα subunit, followed by loading of GTP and dissociation from Gβγ and from the receptor [4]. Then, GTP-bound Gα as well as Gβγ stimulate their cognate effectors as long as Gα remains loaded with GTP and the Gβγ effector interface remains available for direct interactions with its effectors. Regulators of G protein signaling (RGS) proteins turn off the switch represented by active Gα by promoting the GTPase activity of this subunit. Eventually, GDP-bound Gα re-associates with Gβγ, returning the complex to an inactive state. The newly reassembled inactive heterotrimer can couple again with available agonist-stimulated GPCRs. This process is amplified and regulated at its different signaling nodes, enforcing a tight temporal and spatial control of GPCR signaling that activates multiple targets depending on the specific G protein involved. Moreover, recent discoveries in GPCR biology support the idea that receptors can exhibit different conformational states, which activate variable intracellular signaling pathways and that are stabilized by different classes of ligands; ligand efficacy appears independent of affinity and varies between full agonists, partial agonists, inverse agonists, and allosteric modulators. As such, GPCRs can be viewed as molecular rheostats rather than simple on/off switches[4].

Different active conformations of GPCRs can stimulate different G protein-dependent and independent pathways, or elicit variable intensities of the downstream responses[4]. This dynamic range in receptor activity can be exploited therapeutically, enabling the use of biased or allosteric modulators to selectively inhibit certain activities while preserving others. Furthermore, the activation of GPCRs is also influenced by their oligomerization state and subcellular localization, and their downstream effects are expanded by the presence of recently recognized G protein-independent pathways transduced via GPCR-interacting proteins, such as arrestins[5]. The G proteins themselves can be activated independent of GPCRs by other mechanisms including receptor tyrosine kinases, non-receptor guanine nucleotide exchange factors (GEFs), and other intracellular modulators that can elicit growth and proliferative properties[6, 7]. Asymmetric cell division, for instance, which involves heterotrimeric G proteins but is independent of GPCRs, can contribute to cancer progression due to its role in stem cell polarized division and proliferation[8]. $G\alpha_i$, in particular, is a

component of the complex that determines the alignment of the mitotic spindle with respect to the cellular polarity axis of dividing stem or progenitor cells [9].

Detailed three dimensional structures of several GPCRs in various activation states have recently been solved, adding to our understanding of GPCR structure and function. Established GPCR structures now include inactive and activated forms of rhodopsin, adrenergic, and adenosine receptors; as well as inactive conformations of chemokine, dopamine, histamine, and sphingosine phosphate receptors and protease activated receptor-1 (recently reviewed by Palczewski and colleagues[10]). The crystal structures of active adenosine A2A receptors[11] and a quaternary complex of active agonist-occupied β2-adrenergic receptor bound to nucleotide free heterotrimeric $G\alpha_s$ protein have also been published[12]. In addition, of particular interest for oncologists, the structure of CXCR4, a critical regulator of cell migration implicated in cancer metastasis, has recently been revealed. This structure, visualized at a resolution of 2.5 to 3.2 angstroms, is consistent with a constitutive homodimeric organization in which interacting residues at the fifth transmembrane (TM) alpha-helix (TM5) and TM6 form the dimeric interface[13].

Based on structural data, it appears that in the absence of their cognate agonist, many members of the family A GPCRs maintain an inactive conformation through interactions between their TM3 and TM6. In some GPCRs these TM helices are bridged intracellularly by polar interactions established between the highly conserved E/DRY motif on TM3 and a glutamate residue on TM6, forming what is called an "ionic lock"[4, 14]. Upon ligand binding, transmembrane α-helices adjust their position. TM6, in particular, moves outward from the center of the bundle, loses contact with TM3 and moves closer to TM5. This conformational change leads to formation of a new pocket between TM3, TM5 and TM6 that binds to the C-terminus of a Gα-subunit[12]. Mutation of multiple residues at the interhelical interface of TMs 3, 5 and 6 shift the conformational equilibrium of the GPCR towards the G protein accessible state and hence lead to increased ligand-independent receptor activity. This phenomenon is observed for virally-encoded oncogenic GPCRs[15] (Box 1) as well as many human GPCRs[16]. For example, mutations of Val247 occupying the TM6.40 position leads to constitutive activity in chemokine receptor CXCR1[17] and, quite importantly, in the thyroid stimulating hormone (TSH) receptor, TSHR, as well. In the latter receptor, mutants at $Leu629^{6.40}$ or adjacent $Thr632^{6.43}$ are among the most common TSHR mutants in thyroid cancer (Figure 2, Supplemental Tables 1-4).

Historical Perspective on GPCRs and G proteins as Proto-oncogenes

Early evidence for a role of GPCRs in tumorigenesis stems from work describing the *mas* protooncogene over 30 years ago. Expression of *mas*, which encodes a putative GPCR, had the ability to transform and induce foci in NIH 3T3 cells, and also promote tumorigenicity in nude mice[18]. Similarly, ectopic expression of 5HT1c serotonin receptors in NIH3T3 cells led to their malignant transformation[19]. However, due to the initial absence of mutations found in *MAS1* and *5HT1C* in human cancers, the potential contributions and relevance of GPCRs in cancer was not fully appreciated. Overexpression of muscarinic acetylcholine receptors (mACHRs) alone was shown to be insufficient for oncogenic transformation of NIH 3T3 cells, but in combination with the agonist carbachol, foci were readily induced,

thus demonstrating directly that normal GPCRs can act as ligand-dependent oncogenes[20]. Furthermore, m1, m3 or m5 mACHRs receptor subtypes coupled to G_q possessed transforming capacity, whereas receptor subtypes that coupled to G_i (m2 and m4) did not[20]. These studies introduced GPCRs as a new class of membrane proteins with oncogenic properties, and highlighted the importance of excess ligand availability and G protein coupling specificity as determinants of oncogenic potential of GPCRs. These findings also raised the possibility that activating mutations in GPCRs may render them transforming. While mutation of α_{1B}-adrenergic receptor to generate a ligand-independent, constitutively active receptor could also recapitulate the transforming properties and oncogenic potential of ligand-activated receptor[21], the identification of constitutively activating TSHR mutations in ~30% of thyroid adenomas[22] provided the direct link between mutated GPCRs and human cancer.

Consistent with the role for GPCRs in normal and tumor growth, constitutively active mutants of *GNAI* (encoding $G\alpha_i$ subunits), *GNAQ* (encoding $G\alpha_q$ subunits), *GNAO1* (encoding $G\alpha_0$), *GNA12* (encoding $G\alpha_{12}$) and *GNA13* (encoding $G\alpha_{13}$) were shown to transform cells in a variety of experimental systems. Activated $G\alpha$ proteins have also been identified in several disease states (reviewed in [23,24]). For example, activated $G\alpha_s$ mutants lead to autonomous hyperproliferation of cells in multiple endocrine glands in McCune-Albright syndrome [25]. *GNAS* mutations that promote hyperplasia of endocrine cells have been reported in human thyroid and pituitary tumors [26, 25]. Activating mutations in *GNAI2* (encoding $G\alpha_{i2}$) in a subset of ovarian sex cord stromal tumors and adrenal cortical tumors are known[27]. GTPase defective mutants of $G\alpha_q$, $G\alpha_{12}$ and $G\alpha_{13}$ can efficiently transform cells [28-30,31]. These findings provided an early indication that activating mutations in G proteins and GPCRs have the potential for enhancing proliferation and promoting tumorigenesis.

Widespread Mutations in G proteins and GPCRs

Unbiased systematic approaches, including deep sequencing of tumor samples, are revealing genomic alterations that might stratify cancer patients into specific treatment groups. In addition, these studies have highlighted the oncogenic potential of GPCRs and their signal transducers.

Mutant G proteins

As discussed above, mutant $G\alpha_S$ proteins are known to be transforming, but recent deep sequencing approaches have firmly indicated that mutations in *GNAS* occur in growth hormone-secreting pituitary tumors (28%) and thyroid adenomas (5%). Moreover, these recent sequencing studies show that *GNAS* is also mutated in a wide variety of additional tumor types, including colon cancer (4%), pancreatic tumors (12%), hepatocellular carcinoma (2%), parathyroid cancer (3%) and a few others (3% in cancers of the ovary, 2% in endometrial cancers, 1% in lung cancer). Indeed, *GNAS* is mutated in 4.4% of the 9,486 tumor sequences deposited to date in the COSMIC database, making it one of the most frequently mutated G proteins in human cancer (Table 1). Furthermore, the vast majority of these mutations cluster around two hotspot residues, R201 and Q227, which result in constitutive signaling activity by reducing the rate of GTP hydrolysis of the active GTP-

bound $G\alpha_S$[26, 32, 33] (Table 2, Figure 1). In some cases, these activating mutations in *GNAS* are found in a specific tumor type or disease state. For example, in the case of pancreatic tumors, *GNAS* mutations are found in 66% of intraductal papillary mucinous neoplasms (IPMN), a precursor of pancreatic adenocarcinoma, in a mutually exclusive fashion with *KRAS* mutations[34, 35]. Similar *GNAS* mutations were found in invasive lesions arising from these mutant *GNAS* IPMNs, thereby defining a *GNAS*-driven pathway for pancreatic neoplasia[35]. *GNAS* is also mutated in 33% of biliary tract tumors sequenced to date (Cosmic v62), but these mutations occur exclusively in liver fluke-associated cholangiocarcinoma, a fatal bile duct cancer associated with parasitic infection in Southeast Asia[36]. As $G\alpha_S$ can mediate the effects of inflammatory mediators such as cyclooxygenase 2 (COX-2)-derived prostaglandins[37], it is tempting to speculate that gain of function mutations in *GNAS* may control pro-inflammatory gene expression programs in a cell autonomous fashion, thus mimicking the impact of chronic inflammation on tumor development. This possibility is nicely reflected in colon neoplasia in which COX-2 overexpression and function has an important pro-tumorigenic role[38, 39]. Furthermore, *GNAS* is mutated in approximately 6% of all colon adenomas and adenocarcinomas in which this gene was sequenced[40], and detailed patient history analysis suggest that *GNAS* represents a driver oncogene in a subset of these highly prevalent cancers[40].

Similarly, hotspot mutations in *GNAQ* and *GNA11* occur in 3.3% of 8,778 samples analyzed in COSMIC v62 and 2.3% of 6,237 samples analyzed in COSMIC v62. These mutations are mutually exclusive and activate the same signaling cascades, such that in over 5.6% of all cancers in COSMIC v62, this GPCR mediated signaling pathway is disrupted (Tables 2). The majority of these mutations affect Q209 and R183, residues required for GTPase activity; although both mutations impair GTP hydrolysis, the R183 mutations are still sensitive to RGS-dependent signaling termination, making it a less crippling mutant[41, 42]. Thus, the most frequent mutations observed in *GNAS*, *GNAQ* and *GNA11* render them GTPase defective and constitutively active leading to prolonged signaling. Of interest, ~66% of ocular melanomas harbor mutations in *GNAQ* or *GNA11* (Table 1), where it is now considered to represent the driver oncogene[42], thus providing a clear example of a human malignancy that is initiated by gain of function mutations in $G\alpha_q$ and $G\alpha_{11}$ proteins. Although less well studied, *GNAQ* and *GNA11* mutations are also frequently found in tumors arising from the meninges (59%), particularly in leptomeningeal melanocytic lesions [43], in most blue nevi of the skin (83%), and in a subset of cutaneous melanomas linked to chronic sun-induced damage (~6% [44] and Table 1).

Mutations in other Gα genes, *GNAI1* (encoding $G\alpha_{i1}$), *GNAI2*, *GNAI3* (encoding $G\alpha_{i3}$), *GNAO1*, *GNAT1* (encoding $G\alpha_{t1}$), *GNAT2* (encoding $G\alpha_{t2}$), *GNA12*, *GNA13*, *GNA14* (encoding Gα14), *GNA15* (encoding $G\alpha_{15}$), and *GNAL* (encoding $G\alpha_{olf}$) have been found in cancers, albeit at a much lower frequency (Supplemental Table 1). For example, several mutations in *GNAI2*, including R179H, which corresponds to the R201 and R183 mutations in *GNAS* and *GNAQ* or *GNA11*, have been found in a few tumors. In many cases, however, detailed analysis of the relevance of these mutations is not possible due to the limited availability of data for these genes. Furthermore, some of these mutations are not predicted to result in constitutive activity and their exact effect needs further characterization.

Nonetheless, we can learn important information from these naturally occurring mutants. For example, the R243H mutation in *GNAO1* reported in breast tumors has normal GTPase activity, but it can exchange GDP for GTP at a faster rate compared with wild type GNAO1 and thereby functions as an oncogene[45].

While the presence of activating hot spot mutations in *GNAS*, *GNAQ*, and *GNA11* in cancer are clear, further experimentation is required to establish the oncogenic relevance of the less frequently mutated G-proteins. Interestingly, however, the analysis of the somatic mutation rates for G-proteins compared against the background mutation rates in each tumor tissue type in which these mutations occur suggests that mutations in several of these G-proteins are likely of biologically significance (Supplemental Table 2). This may be of particular relevance to *GNA12* and *GNA13*, which have been identified as potentially oncogenic G-proteins in the past (reviewed in [23,24]), but only a small number of mutations have been identified in these genes thus far (Supplemental Tables 1 and 2). Mutations in *GNA13* are highly statistically significant in cancers derived from hematopoietic and lymphoid tissues, specifically in Burkitt lymphoma and diffuse large B cell lymphoma, and to a lesser extent in other cancer types. Certainly, further work will be required to examine whether cancer-associated *GNA12* and *GNA13* mutations display transforming potentials. Many cancers exhibit mutations in GPCRs coupled to $G\alpha_{12}$ and $G\alpha_{13}$, which may also explain why additional gain of function mutations in these G protein α subunits may not be frequently observed. Similarly, *GNA15*, a poorly studied Gαq gene family member is significantly mutated in skin melanomas, which do not often harbor *GNAQ* or *GNA11* mutations (Supplemental Table 1 and 2, data from COSMIC v62). Besides mutations in Gα proteins, to date few mutations have been identified in Gβ and Gγ G protein subunits (Supplemental Table 1), and their oncogenic relevance requires further characterization.

Mutant GPCRs

A surprising finding from a recent systematic analysis of somatic mutations in cancer genomes was the discovery that GPCRs are mutated in approximately 20% of all cancers[46]. Tumors harboring somatic mutations in GPCRs include those arising from large intestine, skin, ovary, upper aerodigestive tract, prostate, breast, thyroid, central nervous system, lung, stomach, haematopoietic and lymphoid tissue, pancreas, liver, kidney, urinary tract, autonomic ganglia, biliary tract (Supplemental Table 1, data from COSMIC v62). Mutations in GPCRs are also evident in metastases from tumors such as melanoma, lung, prostate, large intestine and pancreas (Supplemental Table 1). Examples of the most frequently mutated GPCRs in cancer and their tissue of origin are listed in Table 3 and Supplemental Table 1, respectively.

From this large and ever growing body of sequence information some interesting patterns emerge. TSHR, which is the most frequently mutated GPCR in thyroid cancer (Figure 2, Table 3 and Supplemental Tables 1 and 2) is also mutated in large intestine, lung and ovarian cancers, but the role of these TSHR receptor variants has yet to be explored. Luteinizing hormone receptor (LHCGR), a close homologue of TSHR, is the 23rd most mutated non-olfactory GPCR in cancer, and is particularly evident in breast, lung, and colon cancers (Supplemental Table 1), while a related GPCR, follicle stimulating hormone

receptor (FSHR), is mutated in cancers of the large intestine. Other TSHR-related receptors, leucine-rich repeat-containing GPCR 4 (LGR4), LGR5 and LGR6, some of which are expressed in particular subsets of adult stem cells [47], are also mutated in colon carcinoma and in melanoma, suggesting a potential role in cancer initiation from these stem cell populations. Smoothened (SMO) is a seven-transmembrane receptor that is negatively regulated by the twelve-transmembrane receptor Patched (PTCH)[48, 49]. This inhibition is relieved when Hedgehog (HH) family members bind to PTCH, initiating a signaling pathway that culminates with the activation of the transcription factor GLI[50]. Non-overlapping mutations in PTCH and SMO are often responsible for the initiation of sporadic basal cell carcinoma[51, 52]. Furthermore, an activating SMO W535L mutation initially identified in basal cell carcinoma was also recently reported in meningiomas[53, 54]. SMO is also mutated in cancers arising in the colon and central nervous system and many other cancers types (Supplemental Tables 1 and 3), and emerging information strongly support that continuous SMO signaling is involved in tumor progression[55]. Unlike activating substitutions, inactivating mutations in some GPCRs may result in loss of potential tumor suppressive activity and thus contribute to the development of cancers. This mechanism was recently described for inactivating mutations in the melanocortin 1 receptor (MC1R), which is important for pigment production and its defective function increases the risk of melanoma development[56].

Perhaps one of the most surprising findings from the mutational analysis of GPCRs in cancer is the high frequency of alterations in the coding sequence for members of the poorly studied adhesion family of GPCRs. This group, comprising 33 receptors (30 of which are orphan), is characterized by the presence of a long amino terminal region thought to have a role in cell-to-cell and cell-to-matrix interactions[57-59]. This GPCR receptor family includes GPR98 (also known as very large G protein-coupled receptor 1 (VLGRI), GPR112, and members of the brain-specific angiogenesis inhibitor (BAI), EGF LAG seven-pass (CELSR1-3), and the latrophilin (LPHN1-3) subfamilies of adhesion GPCRs, all of which are mutated often in multiple human cancers (Table 3). Among them, GPR98 is one of the most frequently mutated GPCRs in cancer (Table 3). It is the largest GPCR, and its ligand and physiological functions are currently unknown. However, GPR98 mutations are known to cause febrile seizures and one form of Usher syndrome, the most common genetic cause of combined blindness and deafness[60]. The function of GPR112 is still ill defined. BAIs were initially named because of the observation that the extracellular fragment of BAI1 inhibited angiogenesis in experimental models[61]. BAI1 binds to externalized phosphatidylserine on apoptotic cells to promote apoptotic cell engulfment[62]. The physiological roles of BAI1-BAI3 GPCRs are under active investigation[63]. CELSR1 is a member of the flamingo subfamily of nonclassic-type cadherins and is involved in cell-cell contact-mediated communication, planar cell polarity in early embryogenesis and epidermal wound healing[64, 65]. LPHN1 is a calcium-independent receptor for α-latrotoxin, a black widow spider toxin that triggers massive neurotransmitter release from neurons and neuroendocrine cells. Initially, all these adhesion GPCRs were described as candidate tumor suppressor genes. Most of these receptors are characterized by the presence of an N-terminal auto-inhibitory GPCR proteolytic sequence (GPS) as part of a recently identified large ~320 amino acid structural feature termed the GPCR Autoproteolysis INducing (GAIN)

domain[66]. Once cleaved, the large N-terminal region appears to remain associated with the 7-TM GPCR region, preventing its activation, but on binding to certain ligands it is possible that the cleaved N-terminus might disassociate, thereby initiating G protein mediated downstream signaling[59]. Cancer associated mutations in the GAIN domain of BAI3 and another adhesion receptor, CL1, have been analyzed; while these mutations did not seem to affect autoproteolysis or cell-surface localization of the receptor associated with the GAIN domain, these mutations may alter other properties or functions that are yet uncharacterized[66]. In this context, it is tempting to speculate that certain mutations in the extended N-termini of adhesion GPCRs may reduce the affinity for their cleaved 7-TM region, which may result in their constitutive activation. This concept and other possible mechanisms that can explain the potential selective tumorigenic advantage of cells harboring mutations in the adhesion family of GPCRs will likely receive increased attention in the future.

The second most frequently mutated GPCRs are members of the glutamate family of G protein-linked receptors, GRM1-8, which have an interesting cancer-specific distribution. In an initial study, GRM8 was found to be mutated in 8% of non-small cell lung cancer (NSCLC) of the squamous subtype, but GRM1 was mutated in 7% of NSCLC adenocarcinomas[46]. This finding has stimulated additional, more focused efforts. Another study examining whether mutant endogenous GPCRs are linked to melanoma progression used a systematic exon capture and massively parallel sequencing approach on 734 GPCRs[67]. Of the 11 genes determined to have at least 2 somatic mutations, the most frequently mutated genes were *GRM3* and *GPR98*, affecting 16.3% and 27.5% of the melanomas examined, respectively. The high ratio of non-synonymous to synonymous mutations in *GRM3* and the identification of the same mutation in multiple individuals, suggested that these mutations could be driver mutations as opposed to nonselected passenger mutations. Of interest, activating mutations in GRM3 increased the sensitivity of melanomas to MEK inhibitors[67]. This receptor family is of particular interest given its transforming potential and the excess availability of its ligand, glutamate, in the context of the tumor microenvironment[68], suggesting that GRMs may be readily activated at the surface of tumor cells expressing both wild type and mutant GRM proteins.

Aligned with this perspective of a growth advantage in cells displaying mutations in GPCRs for which the ligand accumulates within the tumor, a large fraction of cancers exhibit mutations in GPCRs for lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P), as well as receptors for the neurotransmitter acetylcholine (Table 3). In this regard, an interesting emerging observation is the presence of hotspot mutations in their coding sequences. Indeed, certain conserved residues display a higher mutational rate (Supplemental Table 3), suggesting a possible role in receptor signaling initiation, termination, coupling specificity, or even the possibility that these mutations may result in gain of function such as constitutive activity, all of which warrants considerable investigation. This observation may also apply to the recently identified mutations in *MAS1* and its related GPCRs (*MRGPRD*, *MRGPRE*, *MRGPRX1*, *MRGPRX2*, *MRGPRX3* and *MRGPRX4*). This group of genes is in the top ten mutant GPCRs found in colon cancer, and are present to a lesser extent in other cancer types. Other close relatives to this group include

the large family of olfactory receptors, which have been found to be mutated in multiple cancer types. However, these GPCRs appear not to be highly expressed in tumor cells and little is known about their functions or the potential consequences of their mutations, leaving wide-open areas for investigation. A complete list of non-olfactory GPCR mutants detected in cancer is provided in Supplemental Table 4. Though the oncogenic significance of GPCR mutations warrants further studies, analysis of their somatic mutation rates compared against the background mutation rates in tumor samples identified several significantly mutated GPCRs, suggesting a role for these in cancer (Supplemental Table 2).

The Functional Impact of Aberrant Expression

An interesting issue raised by the early studies on the *mas* oncogene and the serotonin and muscarinic receptors is that GPCRs do not need to be mutated to contribute to tumor progression: their aberrant expression or over-expression can exert oncogenic properties providing that locally released or circulating ligands are available. For example, the chemokine receptor, CXCR4 is not normally expressed on breast epithelial cells, but is often expressed on breast cancer cells and its ligand CXCL12 (also known as SDF-1) is constitutively expressed at sites of breast cancer metastases[69] and metastases from other tumour types. The role of chemokines, including CXCL12, CCL5 (also known as RANTES) and CXCL8 (also known as IL-8), and their cognate GPCRs, CXCR4, CCR5 and CXCR2, respectively, in the establishment of a permissive tumor microenvironment, immune evasion and cancer metastasis is also now well documented[70]. Furthermore, the role of COX-2 derived prostaglandins such as prostaglandin E2 (PGE2) and their GPCRs, primarily EP2 and EP4, linking chronic inflammation to increased risk of cancer development, is well known and can explain the cancer preventive activity of non-steroid anti-inflammatory drugs (NSAIDs) in colorectal cancer in genetically predisposed patients, as well as in the general population[38]. Similarly, lipid mediators such as LPA and S1P achieve a high local concentration in multiple cancer types, thereby contributing to angiogenesis, lymphangiogenesis, cancer growth, and metastasis, when acting on their GPCRs, LPARs and S1PRs, respectively, which are expressed in cancer, stromal, immune and endothelial cells[71, 72].

Indeed, many cancers exhibit aberrant overexpression of GPCRs and G proteins, whose complexity and clinical relevance have just begun to be appreciated. Increased expression of G proteins can result in enhanced and/or prolonged signaling downstream of GPCRs thereby influencing tumor growth and progression. Increases in the expression of select G proteins could also lead to changes in the coupling specificity of GPCRs, which could have dramatic impact on their entire signaling profile. For example, in triple negative breast cancers that overexpress $G\alpha_{12}$ and $G\alpha_{13}$, CXCR4 binds $G\alpha_i$ and also to heterotrimeric $G\alpha_{12}$ or $G\alpha_{13}$. This additional interaction with $G\alpha_{12}$ or $G\alpha_{13}$ leads to RHOA activation and cytoskeletal changes important for cell migration and metastatic spread[73]. Meta-analysis of publicly available gene array datasets (https://www.oncomine.org) revealed a large overexpression of $G\alpha_{12}$ and $G\alpha_{13}$ in breast, oral, esophageal, and colon cancer, and $G\alpha_s$ in bladder and colorectal cancer, among others. However, this information needs to be treated with caution, given the need to assess the appropriateness of the tissue controls used for each individual study. Of direct relevance, the analysis of extensive collections of matched patient normal

and cancer DNA (The Cancer Genome Atlas, TCGA, http://cancergenome.nih.gov) indicates that a remarkable fraction of colorectal and gastric cancers harbor DNA copy number gains in *GNAS*, and that cancers of the brain, central nervous system and kidney frequently harbor copy number gains in *GNAI1*; both of these genes rank in the top 1% of genes for copy number gains in the respective cancers, which suggests that overexpression of these G proteins may confer a growth advantage during cancer initiation and progression. As datasets from these DNA collections continue to expand, future gene copy number analysis of GPCRs and G proteins in each cancer type may provide further insight into this still poorly explored process.

Perspectives

Although a large body of evidence supported the role of GPCRs in tumor promotion and cancer progression and metastasis, the presence of genetic alterations in G proteins and GPCRs were initially restricted to only few neoplastic lesions, primarily in endocrine tumors. Hence, GPCRs and their downstream signaling pathways have traditionally received limited attention as direct targets for anti-cancer treatments. However, recent deep sequencing efforts have revealed an unanticipated widespread presence and high frequency of mutations in GPCRs and G proteins in many prevalent human malignancies. Many of these mutations have been already linked to cancer progression. These include hotspot mutations in genes for G protein α subunits, particularly *GNAS*, *GNAQ* and *GNA11*, which result in GTPase defective, constitutively active G proteins that promote the persistent activation of their direct downstream signaling targets. Activating mutations in TSH G protein linked receptors and SMO are also now well documented, and their direct cancer relevance is well established. The most frequent somatic mutations in GPCRs involve the glutamate metabotropic receptors (GRM) and the poorly studied adhesion family of GPCRs, together with mutations in receptors for bioactive lipid mediators that often accumulate in the tumor microenvironment, such as LPARs and S1PRs. While it is still unknown whether mutations in these GPCRs contribute to cancer initiation or progression, their rate of somatic mutations is significantly higher than the background mutation rate of the cancer types in which these genetic alterations were identified. This provides a strong rationale for the potential role of these GPCRs in cancer, and hence the foundation for further investigation in this exciting area of research.

The high prevalence of somatic hotspot mutations in genes for *GNAS*, *GNAQ* and *GNA11* is quite remarkable, and aligned with the proliferative capacity of these G proteins and their linked receptors in the tissues in which these activating mutations arise. For example, oncogenic *GNAS* mutants drive the hyperplastic growth of pituitary somatotrophs and thyroid cells (thyrocytes), two cell types in which cAMP stimulates growth and hormone secretion (reviewed in [23,24]). Hence, adenylyl cyclase activation and cAMP accumulation resulting from persistent $G\alpha_s$ activity likely represents the driver oncogenic pathway in these tissues. This also raises the possibility that *GNAS* activating mutants might act as oncogenes only in a limited number of tissues in which cAMP stimulates proliferation. Alternatively, $G\alpha_s$ may activate additional pro-inflammatory pathways in many cancer types in which *GNAS* mutations have been recently identified, including malignancies arising in the colon, pancreas, liver, parathyroid, ovary, endometrium, and lung, or *GNAS* may promote the

aberrant growth of a particular subset of self-renewing cells that are sensitive to cAMP-dependent proliferation within these organs.

The situation is more complex for *GNAQ* and *GNA11*, which are now considered the driver uveal melanoma oncogenes[42,44]. How $G\alpha_q$ and its coupled receptors, such as those activated by endothelin, a potent mitogen in melanocytes[74], transduce proliferative signals is still not fully understood, due to the complexity of the G_q-regulated signaling circuitry. For example, the G_q protein family and G_q-coupled GPCRs can stimulate multiple second messenger generating systems, and can also transactivate tyrosine kinase growth factor receptors, such as the EGF receptor[75]. Given the broad implication of growth factor receptor signaling in cell growth and transformation, this particular receptor cross talk and the resulting signaling output downstream of GPCRs is expected to be directly relevant to the transforming ability of G proteins and GPCRs in multiple tumor types. In particular for ocular melanomas, recently available evidence suggests that in addition to G_q-dependent activation of phospholipase C and the consequent rise in intracellular [Ca^{2+}] and protein kinase C activation, $G\alpha_q$ controls nuclear events resulting in cell proliferation by activating a network of Rho GTPases and MAPK cascades impinging on transcription factors and co-activators, such as c-Jun, c-Fos, and Yap[76,77]. Which of these pathways contribute to the malignant growth and metastatic spread of uveal melanomas is under current investigation. On the other hand, it is unclear why ocular melanocytes are more susceptible to transformation by the *GNAQ* oncogene than cutaneous melanocytes. An interesting possibility arises from the observation that that *GNAQ* or *GNA11* are mutated in nearly 83% of the blue nevi[42,44], which are highly pigmented melanocytic skin lesions that rarely progress into cancer. Thus, it is possible that aberrant $G\alpha_q$ function in dermal melanocytes may trigger cell differentiation or senescence, thereby protecting these cells from the transforming potential of *GNAQ* and *GNA11* mutants. Alternatively, ocular melanocytes may be enriched for a subset of cells that are particularly susceptible to the oncogenic activity $G\alpha_q$ and its coupled receptors, a possibility that may also have important clinical implications for other cancer types exhibiting activating *GNAQ* and *GNA11* mutations.

Emerging structural information of different GPCR families may soon provide the framework for the precise mapping of GPCR mutant sites from which the current picture of mutant GPCRs and their functional links to specific signaling pathways will be objectively defined. Furthermore, the contribution of this large number of mutant GPCRs to cancer initiation and progression can now be challenged in biologically relevant experiments. Nevertheless, we may still be underestimating the incidence and impact of G proteins and GPCR mutations in some cancer types, as their gene families were often not fully sequenced in some of the initial cancer genome analysis efforts. In addition, recent unbiased approaches based on new available deep DNA and RNA sequencing methods and systems biology analysis, are beginning to reveal alterations in entire G protein-regulated pathways, not just specific molecular components, in individual cancer patients[46]. This further supports the emerging notion that GPCR-dependent signaling circuits are indeed directly linked to malignant transformation and/or contribute to a variety of aberrant processes relevant to cancer progression and metastasis. Furthermore, it is evident that not only mutations in GPCR, but their aberrant expression, overexpression, or signal reprogramming in cancer

cells can be important contributors to cancer development and progression. Thus, novel therapeutic strategies aimed at targeting GPCRs and their regulated signaling networks could benefit cancer patients who are treated according to the molecular signatures in their tumors. This may include new strategies to develop signaling selective "biased" antagonists as well as allosteric modulators that can function as inverse agonists to halt persistent signaling from constitutively active receptor mutants, ultimately targeting the GPCR-regulated molecular networks associated with cancer.

Overall, as GPCRs are directly and indirectly the target of >25% drugs in the market, this information can be exploited for the development of novel strategies targeting GPCRs, G proteins, or their aberrant signaling circuitry for cancer prevention and treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Box 1

Virally encoded GPCRs as Human Oncogenes

Early studies of virally-encoded oncogenes provided the foundation of our current understanding of cancer biology. Although the relevance of viral infection to human cancer development was often debated, we now know that at least six human viruses, Epstein-Barr virus (EBV; also known as human herpes virus 4 (HHV-4)), hepatitis B virus (HBV), hepatitis C virus (HCV), human papilloma virus (HPV), human T-cell lymphotropic virus (HTLV-1), and Kaposi's associated sarcoma herpes virus (KSHV; also known as HHV-8) contribute to 10%-15% of the cancers worldwide[79]. In this regard, many human viruses harbor open reading frames encoding G protein coupled receptors (GPCRs) in their viral genomes, indicating that these signaling circuits are required for replicative success[15]. EBV encodes one GPCR, termed BILF1, and human cytomegalovirus (HCMV; also known as HHV-5) expresses multiple GPCRs, including US28, US27, UL33 and UL78. KSHV encodes a receptor commonly known as KSHV vGPCR (or ORF74), whose closest human homologs are CXCR1 and CXCR2, the receptors for IL-8 (also known as CXCL8) and CXCL1 (also known as Gro-α) chemokines[80]. KSHV vGPCR is constitutively active due to the presence of a several structural changes, including a mutation (Asp142Val) within its DRY motif at the intracellular end of TM3, and contributes to KS development through its potent transforming and pro-angiogenic functions (reviewed in[15]). Emerging findings implicate virally-encoded GPCRs as a crucial element in cancer pathogenesis, and suggest that strategies to block their function and specific signaling circuitries may help identify novel options for cancer treatment (reviewed in [15]).

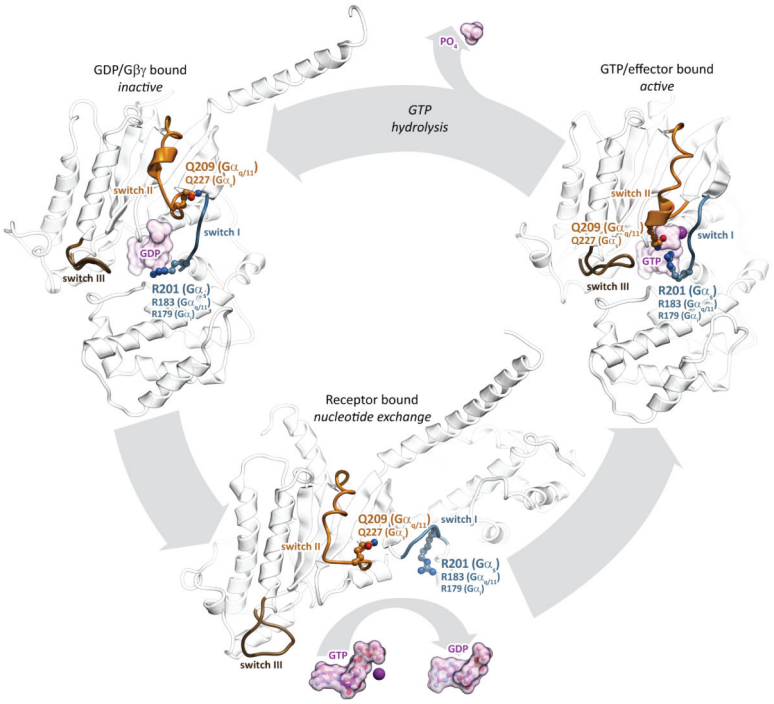

Figure 1. The residue positions most frequently mutated in cancers in the context of different functional states of the G protein α-subunits

Agonist-occupied G protein coupled receptors (GPCRs) couple to heterotrimeric G proteins, thereby promoting the release of GDP from the Gα-subunit, followed by loading of GTP and dissociation from Gβγ (Receptor bound, *nucleotide exchange*). Then, GTP-bound active Gα stimulates its cognate effectors (GTP/effector bound, *active*) as long as the Gα-subunit remains loaded with GTP. Gα proteins then hydrolyze GTP to GDP, a process often accelerated by RGS proteins, thus turning off the switch represented by the active Gα-subunit. Eventually, GDP-bound Gα re-associates with Gβγ, returning the complex to an inactive state (GDP/ Gβγ bound, *inactive*). The newly reassembled inactive heterotrimer can couple again with available agonist-stimulated GPCRs. The mutation hot-spots are the conserved arginine (blue) and glutamine (orange) residues in conformational switch regions I and II, respectively. These residues are involved in the interaction with Gβγ subunits in the inactive, GDP-bound state of the Gα[81] and in the nucleotide exchange in the receptor bound state (as observed in the ternary complex structure with a GPCR[82]). In the GTP-bound state, the direct interaction of these residues with GTP positions the conformational switches optimally for engagement of the effector proteins[83]. Finally, and most importantly, these residues are directly involved in GTP hydrolysis and consequent G protein inactivation. By interfering with GTP hydrolysis, the prevalent cancer driving mutations result in constitutive activation of the Gα-subunits and persistent stimulation of their downstream signaling pathways.

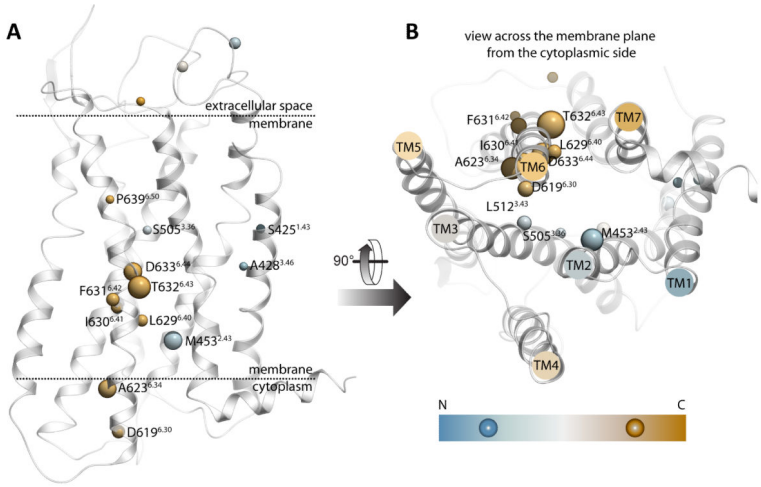

Figure 2. Cancer-related mutations in human thyroid stimulating hormone receptor, TSHR, projected onto a 3D model

The image shows a view along the membrane plane (**A**) and across the membrane plane from the intracellular side (**B**). The receptor is shown in ribbon form; the most frequently mutated positions are shown as spheres and colored from N- to C-terminus. The size of each sphere is proportional to the frequency of tumors with mutations in the corresponding position. The most frequent mutation cluster is located on the intracellular side of the sixth alpha helix of the transmembrane region (TM6) likely resulting in constitutive ligand-independent activity of the receptor.

Table 1

Frequency and tissue distribution of mutations in genes encoding the G proteins, $G\alpha_s$, $G\alpha_q$ and $G\alpha_{11}$, in tumors

Number of samples harboring mutations and the total number of samples where the gene was assessed for presence of mutations is reported. The high prevalence of non-synonymous mutations over synonymous changes indicates a drive role for the mutations in these genes. "N.D." indicates not determined. Data are obtained from COSMIC v62[78].

G-protein family	**$G\alpha_s$**		**$G\alpha_q$ ($G\alpha_q$, $G\alpha_{11}$)**			
Genes:	***GNAS***		***GNAQ***		***GNA11***	
% tumors with somatic mutations (number/ total)	4.40%	(422/9486)	3.40%	(295/8778)	2.50%	(155/6237)
% synonymous mutations	0.10%	(6/9486)	0.00%	(4/8778)	0.10%	(4/6237)
Mutations by tissue:						
Not Specified	0.0%	(0/121)	1.3%	(1/77)	0.0%	(0/76)
Adrenal gland	4.7%	(9/193)	N.D.	N.D.	N.D.	N.D.
Autonomic ganglia	0.9%	(1/107)	0.0%	(0/265)	0.0%	(0/73)
Biliary tract	26.3%	(5/19)	0.0%	(0/11)	0.0%	(0/11)
Bone	0.0%	(0/142)	0.0%	(0/75)	N.D.	N.D.
Breast	0.0%	(0/571)	0.0%	(0/712)	0.0%	(0/444)
Central nervous system	0.4%	(2/496)	0.0%	(0/499)	0.0%	(0/495)
Cervix	0.0%	(0/25)	0.0%	(0/29)	0.0%	(0/12)
Endometrium	1.9%	(4/214)	0.0%	(0/204)	0.5%	(1/204)
Eye	0.0%	(0/111)	32.3%	(228/706)	33.2%	(132/397)
Gastrointestinal tract	0.0%	(0/1)	N.D.	N.D.	N.D.	N.D.
Haematopoietic and lymphoid tissue	0.4%	(4/1035)	0.0%	(0/588)	0.0%	(0/541)
Kidney	1.0%	(5/488)	0.1%	(1/842)	0.2%	(1/429)
Large intestine	4.3%	(34/793)	0.7%	(3/460)	0.3%	(1/361)
Liver	1.6%	(9/565)	0.0%	(0/221)	0.0%	(0/89)
Lung	0.7%	(6/918)	0.5%	(4/832)	0.2%	(1/566)
Meninges	N.D.	N.D.	39.3%	(11/28)	20.0%	(5/25)
Oesophagus	0.0%	(0/110)	0.0%	(0/155)	0.0%	(0/87)
Ovary	3.3%	(16/485)	0.2%	(1/537)	0.3%	(1/399)
Pancreas	11.8%	(56/473)	0.0%	(0/315)	0.0%	(0/307)
Parathyroid	3.2%	(2/63)	N.D.	N.D.	N.D.	N.D.
Pituitary	27.9%	(228/816)	N.D.	N.D.	N.D.	N.D.
Placenta	0.0%	(0/2)	N.D.	N.D.	N.D.	N.D.
Pleura	0.0%	(0/6)	0.0%	(0/7)	0.0%	(0/1)
Prostate	0.3%	(1/348)	0.3%	(1/378)	0.4%	(1/273)
Salivary gland	0.0%	(0/2)	N.D.	N.D.	N.D.	N.D.
Skin	0.0%	(0/112)	4.8%	(44/908)	1.3%	(12/910)
Small intestine	25.0%	(1/4)	N.D.	N.D.	N.D.	N.D.
Soft tissue	0.0%	(0/89)	0.0%	(0/169)	0.0%	(0/37)

G-protein family	**$G\alpha_s$**		**$G\alpha_q$ ($G\alpha_q$, $G\alpha_{11}$)**			
Genes:	***GNAS***		***GNAQ***		***GNA11***	
Stomach	0.4%	(1/282)	0.0%	(0/294)	0.0%	(0/247)
Testis	28.6%	(2/7)	N.D.	N.D.	N.D.	N.D.
Thyroid	4.8%	(33/692)	0.0%	(0/248)	0.0%	(0/63)
Upper aerodigestive tract Urinary tract	1.5% 1.6%	(2/130) (1/63)	0.9% 0.0%	(1/112) (0/106)	0.0% 0.0%	(0/112) (0/78)
Vulva	0.0%	(0/3)	N.D.	N.D.	N.D.	N.D.

Table 2

Hotspot mutations in *GNAS, GNAQ,* and *GNA11*

Amino acid residues affected by recurrent mutations in *GNAS, GNAQ,* and *GNA11*are listed along with the relative distributions of specific amino acid changes. Data are obtained from COSMIC v62[78].

Gene (G-protein)	Mutations (amino acid changes)	% Tumor samples with hotspot mutations	% of mutated	Number of mutations
GNAS **($G\alpha_s$)**	**Overall**	**4.2%***		**404/9486**
	Q227		**10.60%**	**43/404**
	Q227L		4.95%	20
	Q227R		2.72%	11
	Q227H		2.23%	9
	Q227K		0.50%	2
	Q227E		0.25%	1
	R201		**88.12%**	**356/404**
	R201C		63.86%	258
	R201H		22.77%	92
	R201S		1.73%	7
	R201L		0.50%	2
GNAQ **($G\alpha_q$)**	**Overall**	**3.3%***		**285/8778**
	G64		**0.70%**	**2/285**
	G64V		0.70%	2
	Q209		**94.38%**	**269/285**
	Q209P		52.79%	142
	Q209L		44.98%	121
	Q209R		1.12%	3
	Q209H		0.37%	1
	Q209K		0.37%	1
	Q209Y		0.37%	1
	R183		**5.20%**	**14/285**
	R183Q		4.83%	13
	R183*		0.37%	1
GNA11 **($G\alpha_{11}$)**	**Overall**	**2.3%***		**161/6237**
	Q209		**95.95%**	**142/148**
	Q209L		92.56%	137
	Q209P		2.70%	4
	Q209K		0.67%	1
	R183		**4.05%**	**6/148**
	R183C		3.38%	5
	R183H		0.67%	1

Table 3

Select frequently mutated families of G protein coupled receptors (GPCRs) in cancer

Number of protein altering mutations observed in select GPCR genes, the number of samples surveyed for the presence of mutations and the percentage of protein altering changes are indicated. Data are obtained from COSMIC v62[78].

Gene name	Protein ID	Length (protein)	Total Number of Unique Samples	Number of Protein Altering Mutations	Total Number of Samples	Number of Silent Mutations	%Protein Altering
GPCRs of interest							
TSHR	P16473	764	320	322	5381	13	96.1%
CASR	P41180	1078	53	59	3615	22	72.8%
SMO	Q99835	787	52	53	6617	8	86.9%
FSHR	P23945	695	51	53	4047	19	73.6%
LHCGR	P22888	699	44	46	4111	9	83.6%
CCKBR	P32239	447	44	44	4097	15	74.6%
PROKR2	Q8NFJ6	384	36	37	3615	15	71.2%
NMUR2	Q9GZQ4	415	32	32	4046	12	72.7%
GPR149	Q86SP6	731	29	30	3615	16	65.2%
PTGFR	P43088	359	25	25	4049	6	80.6%
MAS1L	P35410	378	18	19	4047	8	70.4%
P2RY2	P41231	377	19	19	4024	6	76.0%
MAS1	P04201	325	18	18	4046	3	85.7%
P2RY8	Q86VZ1	359	17	17	4308	6	73.9%
BDKRB2	P30411	391	14	15	4254	7	68.2%
VIPR1	P32241	457	7	8	3614	5	61.5%
Adhesion-related GPCRs							
GPR98	Q8WXG9	6306	152	196	3656	46	81.0%
GPR112	Q8IZF6	3080	140	158	3691	40	79.8%
BAI1	O14514	1584	38	40	4634	13	75.5%
BAI2	O60241	1585	38	39	4047	12	76.5%
BAI3	O60242	1522	134	151	4734	38	79.9%
CELSR1	Q9NYQ6	3014	60	64	4048	27	70.3%
CELSR2	Q9HCU4	2923	54	56	4048	20	73.7%
CELSR3	Q9NYQ7	3312	54	59	4038	20	74.7%
LPHN1	O94910	1474	20	20	4046	11	64.5%
LPHN2	O95490	1459	81	91	4090	20	82.0%
LPHN3	Q9HAR2	1447	80	88	4029	28	75.9%
Glutamate receptors							
GRM1	Q13255	1194	91	96	4602	30	76.2%
GRM2	Q14416	872	20	20	4047	12	62.5%
GRM3	Q14832	879	73	80	4088	23	77.7%
GRM4	Q14833	912	32	33	4047	11	75.0%

Gene name	Protein ID	Length (protein)	Total Number of Unique Samples	Number of Protein Altering Mutations	Total Number of Samples	Number of Silent Mutations	%Protein Altering
GRM5	P41594	1212	66	68	4471	21	76.4%
GRM6	O15303	877	35	36	4109	18	66.7%
GRM7	Q14831	915	59	60	4047	12	83.3%
GRM8	O00222	908	87	93	4141	26	78.2%
LPA receptors							
LPAR1	Q92633	364	16	17	3546	4	81.0%
LPAR2	Q9HBW0	351	7	7	4025	3	70.0%
LPAR3	Q9UBY5	353	20	20	4024	2	90.9%
LPAR4	Q99677	370	32	34	3642	4	89.5%
LPAR5	Q9H1C0	372	5	5	3592	2	71.4%
LPAR6	P43657	344	9	10	4658	4	71.4%
S1P receptors							
S1PR1	P21453	382	26	29	4047	13	69.0%
S1PR2	O95136	353	10	10	4046	5	66.7%
S1PR3	Q99500	378	25	25	4470	8	75.8%
S1PR4	O95977	384	10	10	4097	1	90.9%
S1PR5	Q9H228	398	8	8	4046	4	66.7%
Muscarinic receptors							
CHRM1	P11229	460	4	4	3614	5	44.4%
CHRM2	P08172	466	46	52	3615	13	80.0%
CHRM3	P20309	590	42	42	3656	7	85.7%
CHRM4	P08173	479	14	14	3524	6	70.0%
CHRM5	P08912	532	15	15	3614	5	75.0%