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 tumours carry activating mutations in *GNAS* (encoding $G\alpha_s$), and that oncogenic activating mutations 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 tumours harbour mutations in GPCRs. Many human cancer-associated viruses also express constitutively active viral GPCRs. These studies indicate that G proteins, GPCRs and their linked signalling circuitry represent novel therapeutic targets for cancer prevention and treatment.

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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 seven-transmembrane domain structure with an extracellular amino terminus and an intracellular carboxyl terminus. GPCRs have crucial roles in various 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 more than 25% of therapeutic drugs on the market[2,3].

GPCRs function as key transducers of signals from the extracellular milieu to the inside of the cell. Various entities, ranging from photons to lipids to small proteins, serve as ligands for different GPCRs, and all are 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, the activation of these GPCR-regulated signalling circuits can lead to changes in gene transcription, cell survival and motility, and normal and malignant cell growth.

G protein and GPCR signalling

The widely accepted model for GPCR activation involves the 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 the 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 signalling (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 signalling nodes, thus enforcing a tight temporal and spatial control of GPCR signalling 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 signalling pathways and which are stabilized by different classes of ligands; ligand efficacy seems to be independent

G-protein-coupled receptors (GPCRs). A family of receptor proteins that have seven-trans-membrane domains, an extracellular amino terminus and an intracellular carboxyl terminus. They respond to stimuli outside the cell and transduce signals into the cells through interactions with intracellular signalling proteins, including G proteins.

G proteins
A family of guanine-nucleotide-binding proteins that are important for signal transduction. Their activity is regulated by binding and hydrolysing GTP such that the active state is GTP-bound, whereas the inactive form is in a GDP-bound state. Heterotrimeric G proteins consist of α-, β- and γ-subunits.

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 as 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 independently of GPCRs by other mechanisms, including by receptor tyrosine kinases, non-receptor guanine-nucleotide exchange factors (GEFs) and other intracellular modulators that can elicit growth and proliferative properties[6,7]. For example, asymmetric cell division, which involves heterotrimeric G proteins but is independent of GPCRs, can contribute to cancer progression owing 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 the chemokine receptor CXCR4, which is a crucial regulator of cell migration that is 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 in the fifth transmembrane (TM) α-helix (TM5) and TM6 form the dimeric interface[13].

Based on structural data, it seems that in the absence of their cognate agonists, many members of the family A GPCRs maintain an inactive conformation through interactions between their TM3 and TM6 helices. In some GPCRs these TM helices are bridged intracellularly by polar interactions that are 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]. On ligand binding, transmembrane α-helices adjust their position. TM6, in particular, moves outwards from the centre of the bundle, loses contact with TM3 and moves closer to TM5. This conformational change leads to the 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 TM3, TM5 and TM6 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) and for many human GPCRs[16]. For example, mutations of V247, which occupies the TM6.40 position ($V247^{6.40}$; the superscript number indicates the Ballesteros–Weinstein numbering for conserved GPCR residues), lead to constitutive activity in chemokine receptor CXCR1 (REF. 17). Similarly and importantly, mutations in the thyroid-stimulating hormone (TSH) receptor, TSHR, at $L629^{6.40}$ or the adjacent $T632^{6.43}$ are among the most common TSHR mutations in thyroid cancer (FIG. 1; Supplementary information S1–S4 (tables)).

At a glance

- Recent cancer genome deep sequencing efforts have revealed an unanticipated high frequency of mutations in G proteins and G-protein-coupled receptors (GPCRs) in most tumour types.
- A striking 4.2% of all tumour sequences deposited to date show activating mutations in *GNAS* (a complex locus that encodes $G\alpha_s$). Transforming mutations in *GNAS* have been well documented in human thyroid and pituitary tumours, and recent sequencing efforts have shown these mutations to be present in a wide variety of additional tumour types, including colon cancer, hepatocellular carcinoma, and parathyroid, ovarian, endometrial, biliary tract and pancreatic tumours.
- Mutually exclusive activating mutations in *GNAQ* or *GNA11* (encoding $G\alpha_q$ family members) occur in 5.6% of tumours, and they are present in ~66% and ~6% of melanomas arising in the eye and skin, respectively, where they can act as driver oncogenes.
- Hotspot mutations in $G\alpha_s$ (R201 and Q227) as well as $G\alpha_q$ and $G\alpha_{11}$ (R183 and Q209) disrupt the GTPase activity, thereby leading to constitutive activity and persistent signalling.
- Nearly 20% of human cancers harbour mutations in GPCRs.
- The most frequently mutated GPCRs include thyroid-stimulating hormone receptor (TSHR), Smoothened (SMO), glutamate metabotropic receptors (GRMs), members of the adhesion family of GPCRs and receptors for bioactive lipid mediators such as lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P) that accumulate in the tumour microenvironment.
- Many GPCR mutations are still uncharacterized with respect to their potential contribution to tumorigenesis and cancer progression.
- Aberrant expression, overexpression or signal reprogramming of GPCRs and G proteins in tumour cells can contribute to cancer development and progression. These alterations may arise from cancer-specific changes in gene copy number, as well as from other genetic, epigenetic and post-translational changes resulting in higher protein expression, thereby enhancing tumour progression and metastasis.
- Detailed three dimensional structures of GPCRs in various activation states can now help to explain the functional impact of cancer-associated GPCR mutations, and guide the rational design of signalling-selective GPCR agonists, antagonists and allosteric modulators.
- G proteins, GPCRs and their linked signalling circuitry represent novel therapeutic targets for cancer prevention and treatment.

GPCRs and G proteins as proto-oncogenes

Early evidence of a role for GPCRs in tumorigenesis stems from work describing the *MAS1* proto-oncogene over 30 years ago. Expression of *MAS1*, which encodes a putative GPCR, had the ability to transform and induce foci of NIH3T3 cells, and also to promote tumorigenicity

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 contribute to 10–15% of cancers worldwide[78]. These viruses are Epstein–Barr virus (EBV; also known as human herpesvirus 4 (HHV-4)), hepatitis B virus (HBV), hepatitis C virus (HCV), human papilloma virus (HPV), human T-cell lymphotropic virus 1 (HTLV-1) and Kaposi's-associated sarcoma herpesvirus (KSHV; also known as HHV-8). In this regard, many human viruses harbour open reading frames encoding G-protein-coupled receptors (GPCRs) in their viral genomes, indicating that these signalling 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 that is commonly known as KSHV vGPCR (or ORF74), for which the closest human homologues are CXCR1 and CXCR2, which are receptors for the chemokines interleukin 8 (IL-8) and CXCL1 (also known as GROα)[79]. KSHV vGPCR is constitutively active owing to the presence of several structural changes, including a mutation (D142V) within its DRY motif at the intracellular end of the third transmembrane α-helix (TM3), and contributes to Kaposi's sarcoma development through its potent transforming and pro-angiogenic functions (reviewed in REF. 15). Emerging findings implicate virally encoded GPCRs as a crucial element in cancer pathogenesis, and suggest that strategies to block their function and specific signalling circuitries may help to identify novel options for cancer treatment (reviewed in REF. 15).

in nude mice[18]. Similarly, ectopic expression of 5HT1C serotonin receptors in NIH3T3 cells led to their malignant transformation[19]. However, owing 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 cholinergic receptors (CHRMs) alone was shown to be insufficient for the oncogenic transformation of NIH3T3 cells, but in combination with the agonist carbachol, foci were readily induced, thus directly demonstrating that normal GPCRs can act as ligand-dependent oncogenes[20]. Furthermore, CHRM1, CHRM3 or CHRM5 receptor subtypes coupled to $G\alpha_q$ possessed transforming capacity, whereas receptor subtypes that coupled to $G\alpha_i$ (CHRM2 and CHRM4) 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 the oncogenic potential of GPCRs. These findings also raised the possibility that activating mutations in GPCRs may render them transforming. Indeed, 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], and 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 tumour growth, constitutively active mutants of *GNAI* (encoding $G\alpha_i$ subunits), *GNAQ* (encoding $G\alpha_q$ subunits), *GNAO1* (encoding $G\alpha_o$), *GNA12* (encoding $G\alpha_{12}$) and *GNA13* (encoding $G\alpha_{13}$) were shown to transform cells in various experimental systems. Activated Gα proteins have also been identified in several disease states (reviewed in REFS 23,24). For example, activated $G\alpha_s$ mutants lead to autonomous hyperproliferation of cells in multiple endocrine glands in McCune–Albright syndrome[25]. Mutations in *GNAS* (a complex locus that encodes $G\alpha_s$) that promote hyperplasia of endocrine cells have been reported in human thyroid and pituitary tumours[26,25]. Activating mutations in *GNAI2* (encoding $G\alpha_{i2}$) in a subset of ovarian sex cord stromal tumours and adrenal cortical tumours 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 to enhance proliferation and promote tumorigenesis.

Widespread mutations in G proteins and GPCRs

Unbiased systematic approaches, including deep sequencing of tumour samples, are revealing genomic alterations that might underlie tumour progression and 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 tumours (28%) and thyroid adenomas (5%). Moreover, these recent sequencing studies show that *GNAS* is also mutated in a wide variety of additional tumour types, including colon cancer (4%), pancreatic tumours (12%), hepatocellular carcinoma (2%), parathyroid cancer (3%) and a few others (3% in cancers of the ovary, 2% in endometrial cancers and 1% in lung cancer). Indeed, *GNAS* is mutated in 4.4% of the 9,486 tumour sequences deposited to date in the Catalogue of somatic mutations in cancer (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 signalling activity by reducing the rate of GTP hydrolysis of the active GTP-bound $G\alpha_s$[26,32,33] (FIG. 2; TABLE 2). In some cases, these activating mutations in *GNAS* are found in a specific tumour type or disease state. For example, in the case of pancreatic tumours, *GNAS* mutations are found in 66% of intraductal papillary mucinous neoplasms (IPMNs), which are precursors of pancreatic adenocarcinoma, in a mutually exclusive manner 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 tumours sequenced to date (COSMIC v62), but these mutations occur exclusively in liver-fluke-associated cholangiocarcinoma, which is 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 (COX2)-derived prostaglandins[37], it is tempting to speculate that gain-of-function mutations in *GNAS* may control pro-inflammatory gene expression programmes in a cell-autonomous manner,

Regulators of G protein signalling
(RGS). GTPase-accelerating proteins that lead to heterotrimeric G protein inactivation by promoting hydrolysis of the GTP of Gα to GDP.

Arrestins
A family of proteins that interact with the carboxyl termini of G-protein-coupled receptors and help to mediate receptor desensitization, internalization, recycling and signalling.

Guanine-nucleotide exchange factors
(GEFs). Proteins that stimulate the release of GDP to allow exchange for GTP, thereby promoting G protein activation.

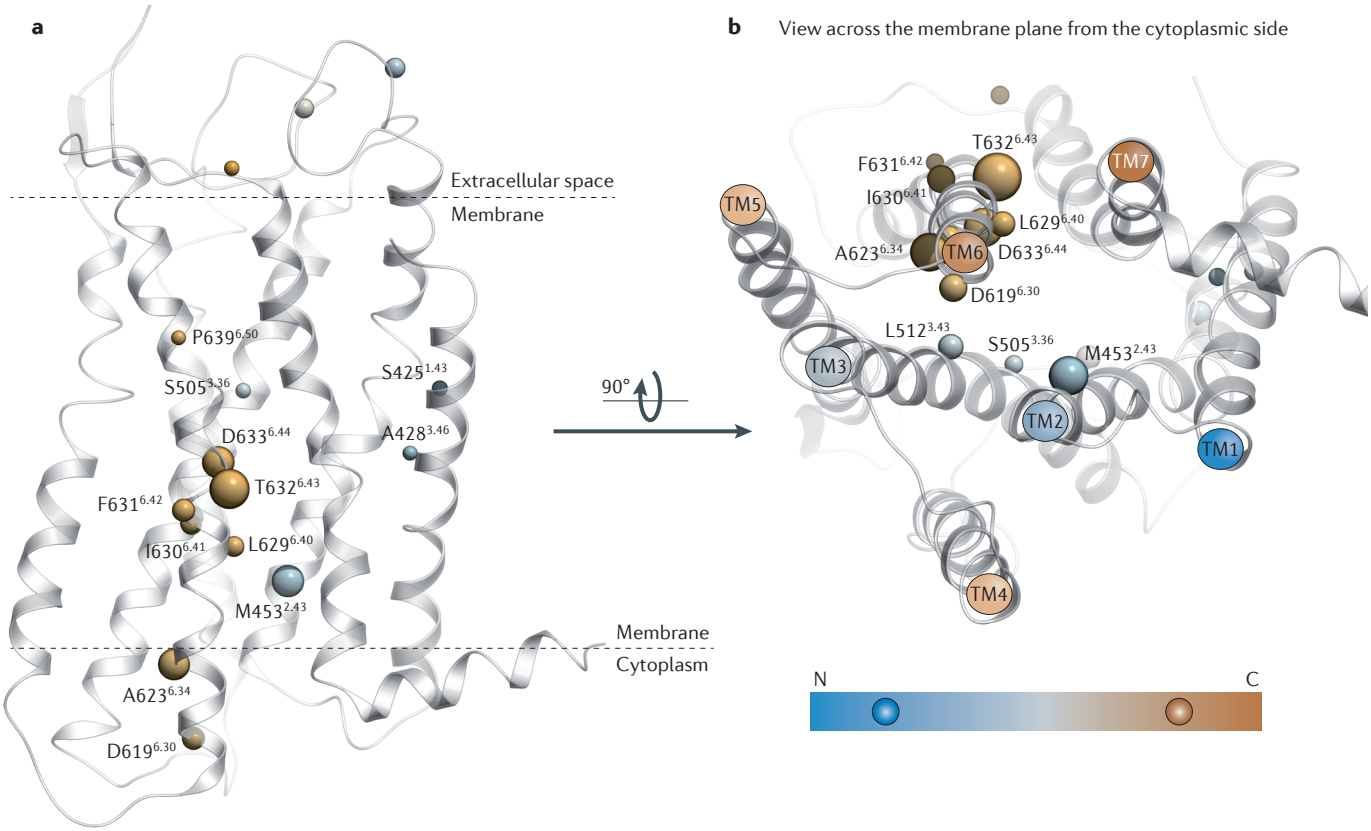

Figure 1 | **Cancer-related mutations in human TSHR projected onto a three-dimensional model.** The image shows a view along the membrane plane (**a**) and across the membrane plane from the intracellular side (**b**). Human thyroid stimulating hormone receptor (TSHR) is shown in ribbon form; the most frequently mutated positions are shown as spheres and coloured from amino-terminal to carboxy-terminal. The size of each sphere is proportional to the frequency of tumours with mutations in the corresponding position. The most frequent mutation cluster is located on the intracellular side of the sixth transmembrane α-helix (TM6), probably resulting in constitutive ligand-independent activity of the receptor. Superscript numbers indicate Ballesteros–Weinstein numbering for conserved G-protein-coupled receptor (GPCR) residues.

thus mimicking the impact of chronic inflammation on tumour development. This possibility is nicely reflected in colon neoplasia, in which COX2 overexpression and function has an important protumorigenic 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 analyses suggest that *GNAS* represents a driver oncogene in a subset of these highly prevalent cancers[40].

Similarly, hotspot mutations in *GNAQ* occur in 3.3% of 8,778 cancer samples and in *GNA11* in 2.3% of 6,237 cancer samples, according to COSMIC v62. These mutations are mutually exclusive and activate the same signalling cascades, such that in >5.6% of all cancers in COSMIC v62, this GPCR-mediated signalling pathway is aberrantly activated (TABLE 2). Most of these mutations affect Q209 or R183, which are residues that are required for GTPase activity; although both mutations impair GTP hydrolysis, the R183 mutations are still sensitive to RGS-dependent signalling termination, making it a less-crippling mutant[41,42]. Thus, the most frequent mutations observed in *GNAS, GNAQ* and *GNA11* render the proteins GTPase-defective and constitutively active, leading to prolonged signalling. Of interest, ~66% of ocular melanomas harbour mutations in *GNAQ* or *GNA11* (TABLE 1), and these are now considered to represent the driver oncogenes of this cancer type[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 tumours arising from the meninges (59%), particularly in leptomeningeal melanocytic lesions[43], in most blue naevi of the skin (83%), and in a subset of cutaneous melanomas linked to chronic sun-induced damage (~6%)[44] (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 $G\alpha_{14}$), *GNA15* (encoding $G\alpha_{15}$) and *GNAL* (encoding $G\alpha_{olf}$) have been found in cancers, albeit at a much lower frequency (Supplementary information S1 (table)). For example, several mutations in *GNAI2,* including R179H — which corresponds to the R201 mutations in *GNAS* and the R183 mutations in *GNAQ* or *GNA11* — have been found in a few tumours. However, in many cases detailed analysis of the relevance of these mutations is not possible owing to the limited availability of sequencing data for these genes. Furthermore, some of

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 tumours**

Tissue	Mutations in *GNAS*		Mutations in *GNAQ*		Mutations in *GNA11*	
	%	Numbers	%	Numbers	%	Numbers
Mutations across all tumour types						
Tumours with somatic mutations	4.45%	422 of 9486	3.36%	295 of 8778	2.49%	155 of 6237
Tumours with synonymous mutations	0.06%	6 of 9486	0.05%	4 of 8778	0.06%	4 of 6237
Mutations in individual tumour types						
Not specified	0.0%	0 of 121	1.3%	1 of 77	0.0%	0 of 76
Adrenal gland	4.7%	9 of 193	ND	ND	ND	ND
Autonomic ganglia	0.9%	1 of 107	0.0%	0 of 265	0.0%	0 of 73
Biliary tract	26.3%	5 of 19	0.0%	0 of 11	0.0%	0 of 11
Bone	0.0%	0 of 142	0.0%	0 of 75	ND	ND
Breast	0.0%	0 of 571	0.0%	0 of 712	0.0%	0 of 444
Central nervous system	0.4%	2 of 496	0.0%	0 of 499	0.0%	0 of 495
Cervix	0.0%	0 of 25	0.0%	0 of 29	0.0%	0 of 12
Endometrium	1.9%	4 of 214	0.0%	0 of 204	0.5%	1 of 204
Eye	0.0%	0 of 111	32.3%	228 of 706	33.2%	132 of 397
Gastrointestinal tract	0.0%	0 of 1	ND	ND	ND	ND
Haematopoietic and lymphoid tissue	0.4%	4 of 1035	0.0%	0 of 588	0.0%	0 of 541
Kidney	1.0%	5 of 488	0.1%	1 of 842	0.2%	1 of 429
Large intestine	4.3%	34 of 793	0.7%	3 of 460	0.3%	1 of 361
Liver	1.6%	9 of 565	0.0%	0 of 221	0.0%	0 of 89
Lung	0.7%	6 of 918	0.5%	4 of 832	0.2%	1 of 566
Meninges	ND	ND	39.3%	11 of 28	20.0%	5 of 25
Oesophagus	0.0%	0 of 110	0.0%	0 of 155	0.0%	0 of 87
Ovary	3.3%	16 of 485	0.2%	1 of 537	0.3%	1 of 399
Pancreas	11.8%	56 of 473	0.0%	0 of 315	0.0%	0 of 307
Parathyroid	3.2%	2 of 63	ND	ND	ND	ND
Pituitary	27.9%	228 of 816	ND	ND	ND	ND
Placenta	0.0%	0 of 2	ND	ND	ND	ND
Pleura	0.0%	0 of 6	0.0%	0 of 7	0.0%	0 of 1
Prostate	0.3%	1 of 348	0.3%	1 of 378	0.4%	1 of 273
Salivary gland	0.0%	0 of 2	ND	ND	ND	ND
Skin	0.0%	0 of 112	4.8%	44 of 908	1.3%	12 of 910
Small intestine	25.0%	1 of 4	ND	ND	ND	ND
Soft tissue	0.0%	0 of 89	0.0%	0 of 169	0.0%	0 of 37
Stomach	0.4%	1 of 282	0.0%	0 of 294	0.0%	0 of 247
Testis	28.6%	2 of 7	ND	ND	ND	ND
Thyroid	4.8%	33 of 692	0.0%	0 of 248	0.0%	0 of 63
Upper aerodigestive tract	1.5%	2 of 130	0.9%	1 of 112	0.0%	0 of 112
Urinary tract	1.6%	1 of 63	0.0%	0 of 106	0.0%	0 of 78
Vulva	0.0%	0 of 3	ND	ND	ND	ND

The table reports the number of samples harbouring mutations and the total number of samples in which the gene was assessed for the presence of mutations. The high prevalence of non-synonymous mutations relative to synonymous changes indicates a driver role for the mutations in these genes. Data are obtained from the Catalogue of somatic mutations in cancer (COSMIC) v62 (REF 83). CNS, central nervous system; ND, not determined.

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 tumours 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].

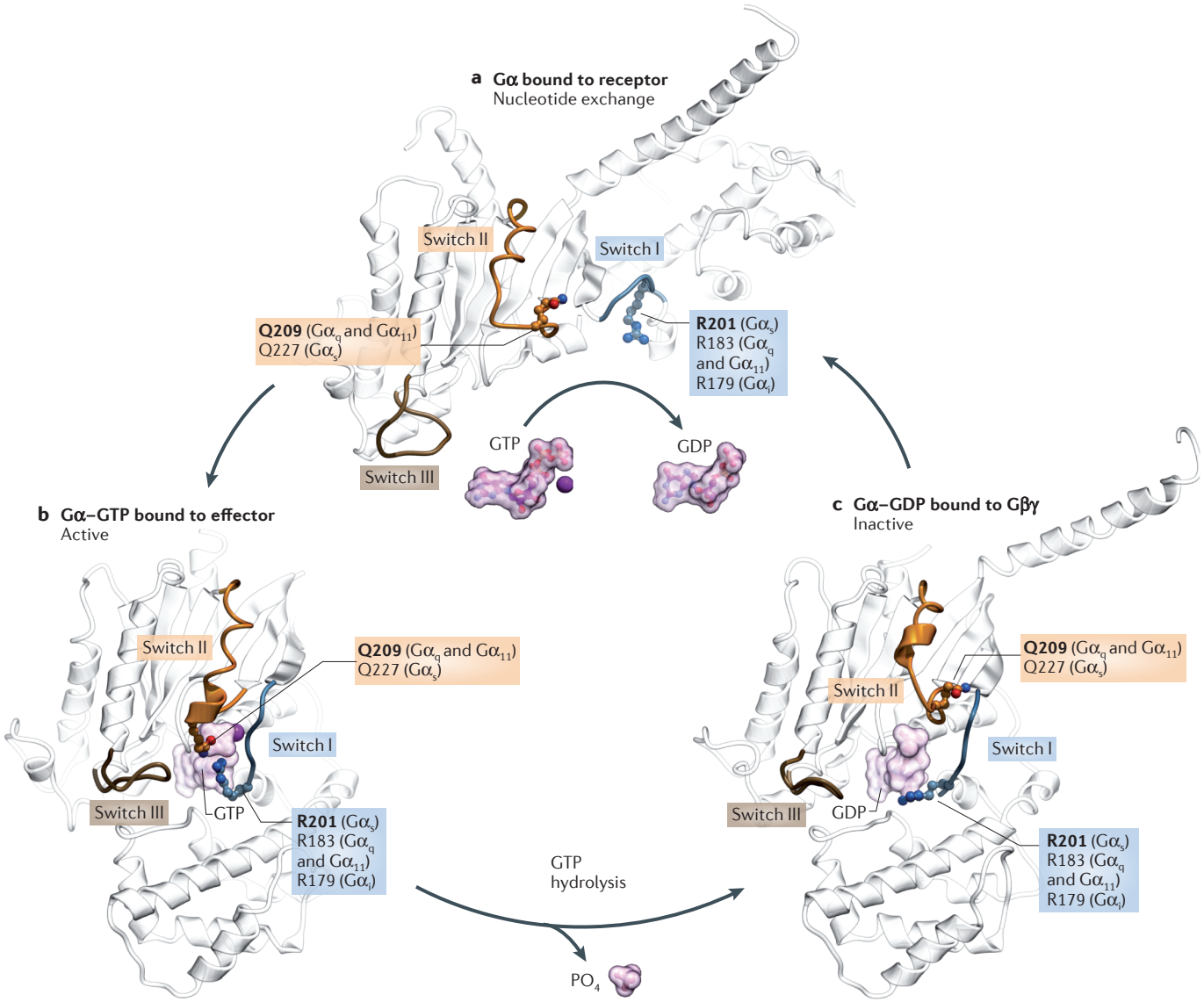

Figure 2 | **The residue positions most frequently mutated in cancers in the context of different functional states of the G protein α-subunits.** Agonist-occupied G-protein-coupled receptors (GPCRs) couple to heterotrimeric G proteins, thereby promoting the release of GDP from the Gα subunit, followed by loading of GTP and dissociation from Gβγ (**a**). Then, GTP-bound active Gα stimulates its cognate effectors for as long as the Gα subunit remains loaded with GTP (**b**). Gα proteins then hydrolyse GTP to GDP, a process that is often accelerated by regulators of G protein signalling (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 (**c**). The newly re-assembled inactive heterotrimer can couple again with available agonist-stimulated GPCRs. The mutation hotspots 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 subunit[80] and in nucleotide exchange in the receptor-bound state (as observed in the ternary complex structure with a GPCR[81]). In the GTP-bound state, the direct interaction of these residues with GTP positions the conformational switches optimally for engagement with the effector proteins[82]. 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 signalling pathways.

Although the presence of activating hotspot mutations in *GNAS*, *GNAQ* and *GNA11* in cancer is 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 tumour tissue type in which these mutations occur suggests that mutations in several of these G proteins are probably biologically important (Supplementary information S2 (table)). This may be of particular relevance to *GNA12* and *GNA13*, which have been previously identified as potentially oncogenic G proteins (reviewed in REFS 23,24), but only a few mutations have been identified in these genes thus far

Table 2 | **Hotspot mutations in *GNAS*, *GNAQ* and *GNA11***

Gene (protein)	Somatic mutation amino-acid change	Mutated samples (%)	Mutated samples (number)
GNAS ($G\alpha_s$)	Total *GNAS* hotspot mutations across all samples	4.26%	404 of 9486
	Total Q227 mutations	10.60%	43 of 404
	Q227L	4.95%	20 of 404
	Q227R	2.72%	11 of 404
	Q227H	2.23%	9 of 404
	Q227K	0.50%	2 of 404
	Q227E	0.25%	1 of 404
	Total R201 mutations	88.12%	367 of 404
	R201C	63.86%	266 of 404
	R201H	22.77%	92 of 404
	R201S	1.73%	7 of 404
	R201L	0.50%	2 of 404
GNAQ ($G\alpha_q$)	Total *GNAQ* hotspot mutations across all samples	3.25%	285 of 8778
	Total Q209 mutations	94.38%	269 of 285
	Q209P	52.79%	142 of 285
	Q209L	44.98%	121 of 285
	Q209R	1.12%	3 of 285
	Q209H	0.37%	1 of 285
	Q209K	0.37%	1 of 285
	Q209Y	0.37%	1 of 285
	Total R183 mutations	5.20%	14 of 285
	R183Q	4.83%	13 of 285
	R183*	0.37%	1 of 285
	G64V	0.70%	2 of 285
GNA11 ($G\alpha_{11}$)	Total *GNA11* hotspot mutations across all samples	2.37%	148 of 6237
	Total Q209 mutations	95.95%	142 of 148
	Q209L	92.56%	137 of 148
	Q209P	2.70%	4 of 148
	Q209K	0.67%	1 of 148
	Total R183 mutations	4.05%	6 of 148
	R183C	3.38%	5 of 148
	R183H	0.67%	1 of 148

Amino acid residues affected by recurrent somatic mutations in *GNAS*, *GNAQ* and *GNA11* are listed along with the relative distributions of specific amino-acid changes. Data are obtained from the Catalogue of somatic mutations in cancer (COSMIC) v62 (REF 83). R183* indicates a nonsense mutation at position R183.

(Supplementary information S1,S2 (tables)). Mutations in *GNA13* are highly statistically significant in cancers that are derived from haematopoietic and lymphoid tissues, specifically in Burkitt's 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 can transform cells. 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*, which encodes a poorly studied $G\alpha_q$ family member, is significantly mutated in skin melanomas that do not often harbour *GNAQ* or *GNA11* mutations (Supplementary information S1,S2 (tables); data from COSMIC v62). Besides mutations in Gα proteins, to date few mutations have been identified in Gβ and Gγ G protein subunits (Supplementary information S1 (table)), 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]. Tumours harbouring somatic mutations in GPCRs include those arising from the 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 and biliary tract (Supplementary information S1 (table); data from COSMIC v62). Mutations in GPCRs are also evident in metastases from tumours such as melanomas or lung, prostate, large intestine and pancreatic tumours (Supplementary information S1 (table)). Examples of the most-frequently mutated GPCRs in cancer and their tissue of origin are listed in TABLE 3 and Supplementary information S1 (table), 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 (FIG. 1, TABLE 3 and Supplementary information S1,S2 (tables)) is also mutated in large intestine, lung and ovarian cancers, but the role of these TSHR variants has yet to be explored. Luteinizing hormone receptor (LHCGR), which is a close homologue of TSHR, is the 23rd most frequently mutated non-olfactory GPCR in cancer, and is particularly evident in breast, lung and colon cancers (Supplementary information S1 (table)), whereas 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. This suggests a potential role for these stem-cell populations in cancer initiation. 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 signalling 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 that was initially identified in basal-cell carcinoma was also recently reported in meningiomas[53,54]. SMO is also mutated in cancers that arise in the colon and central nervous system and many other cancer types (Supplementary information S1,S3 (tables)), and emerging information strongly support that continuous SMO

Table 3 | **Selected frequently mutated families of GPCRs in cancer**

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

signalling is involved in tumour progression[55]. Unlike activating substitutions, inactivating mutations in some GPCRs may result in loss of potential tumour-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 N-terminal region that is thought to have a role in cell–cell and cell–matrix interactions[57–59]. This GPCR receptor family includes GPR98 (also known as VLGR1), GPR112, and members of the brain-specific angiogenesis inhibitor (BAI), cadherin EGF LAG seven-pass G-type receptors (CELSR1–3) and the latrophilin (LPHN1–3) subfamilies of adhesion GPCRs, all of which are often mutated 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, which is the most common genetic cause of combined blindness and deafness[60]. The function of GPR112 is still poorly 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 BAI1–3 GPCRs are under active investigation[63]. CELSR1 is a member of the flamingo subfamily of non-classic-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, which is a black-widow spider toxin that triggers extensive neurotransmitter release from neurons and neuroendocrine cells. Initially, all these adhesion GPCRs were described as candidate tumour 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 seems to remain associated with the seven-transmembrane GPCR region, thus 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 signalling[59]. Cancer-associated mutations in the GAIN domain of BAI3 and another adhesion receptor, CL1, have been analysed; although 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 to be characterized[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 seven-transmembrane regions, which may result in their constitutive activation. This concept and other possible mechanisms that can explain

Table 3 (Cont.) | **Selected frequently mutated families of GPCRs in cancer**

Gene	Protein identifier (amino acid length)	Total number of samples	Total number of unique samples	Number of protein-altering mutations	Number of synonymous mutations	Protein-altering mutations (%)
Glutamate receptors						
GRM1	Q13255 (1194)	4602	91	96	30	76.2%
GRM2	Q14416 (872)	4047	20	20	12	62.5%
GRM3	Q14832 (879)	4088	73	80	23	77.7%
GRM4	Q14833 (912)	4047	32	33	11	75.0%
GRM5	P41594 (1212)	4471	66	68	21	76.4%
GRM6	O15303 (877)	4109	35	36	18	66.7%
GRM7	Q14831 (915)	4047	59	60	12	83.3%
GRM8	O00222 (908)	4141	87	93	26	78.2%
LPA receptors						
LPAR1	Q92633 (364)	3546	16	17	4	81.0%
LPAR2	Q9HBW0 (351)	4025	7	7	3	70.0%
LPAR3	Q9UBY5 (353)	4024	20	20	2	90.9%
LPAR4	Q99677 (370)	3642	32	34	4	89.5%
LPAR5	Q9H1C0 (372)	3592	5	5	2	71.4%
LPAR6	P43657 (344)	4658	9	10	4	71.4%
S1P receptors						
S1PR1	P21453 (382)	4047	26	29	13	69.0%
S1PR2	O95136 (353)	4046	10	10	5	66.7%
S1PR3	Q99500 (378)	4470	25	25	8	75.8%
S1PR4	O95977 (384)	4097	10	10	1	90.9%
S1PR5	Q9H228 (398)	4046	8	8	4	66.7%
Muscarinic receptors						
CHRM1	P11229 (460)	3614	4	4	5	44.4%
CHRM2	P08172 (466)	3615	46	52	13	80.0%
CHRM3	P20309 (590)	3656	42	42	7	85.7%
CHRM4	P08173 (479)	3524	14	14	6	70.0%
CHRM5	P08912 (532)	3614	15	15	5	75.0%

The number of protein-altering mutations observed in selected G-protein-coupled receptor (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 the Catalogue of somatic mutations in cancer (COSMIC) v62 (REF 83). *BAI*, brain-specific angiogenesis inhibitor; *BDKRB2*, bradykinin receptor B2; *CASR*, calcium-sensing receptor; *CCKBR*, cholecystokinin B receptor; *CELSR*, cadherin EGF LAG seven-pass G-type receptor; *CHRM*, cholinergic receptor, muscarinic; *FSHR*, follicle-stimulating hormone receptor; *GRM*, glutamate receptor, metabotropic; *LHCGR*, luteinizing hormone/choriogonadotropin receptor; LPA, lysophosphatidic acid; *LPAR*, LPA receptor; *LPHN*, latrophilin; *MAS1L*, *MAS1*-oncogene-like; *NMUR2*, neuromedin U receptor 2; *P2RY*, purinergic receptor P2Y, G-protein-coupled; *PROKR2*, prokineticin receptor 2; *PTGFR*, prostaglandin F receptor; S1P, sphingosine-1-phosphate; S1PR, S1P receptor; *SMO*, smoothened; *TSHR*, thyroid-stimulating hormone receptor; *VIPR1*, vasoactive intestinal peptide receptor 1.

the potential selective tumorigenic advantage of cells harbouring mutations in the adhesion family of GPCRs will probably receive increased attention in the future.

The second most frequently mutated GPCRs are members of the glutamate metabotropic receptor (GRM) family of GPCRs, 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 cancers (NSCLCs) 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 that were determined to have at least two 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 non-selected 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 tumour microenvironment[68], suggesting that GRMs may be readily activated at the surface of tumour cells expressing both wild-type and mutant GRM proteins.

Aligned with this perspective of a growth advantage for cells displaying mutations in GPCRs for which the ligand accumulates within the tumour, a large proportion 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 mutation rate (Supplementary information S3 (table)), suggesting a possible role in receptor signalling initiation, termination or coupling specificity, or even the possibility that these mutations result in a gain of function such as constitutive activity, all of which warrant 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 that are found in colon cancer, and are present to a lesser extent in other cancer types. Other close relatives of this group include the large family of olfactory receptors, which have been found to be mutated in multiple cancer types. However, these GPCRs seem not to be highly expressed in tumour 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 mutations detected in cancer is provided in Supplementary information S4 (table). Although the oncogenic importance of GPCR mutations warrants further studies, analysis of their somatic mutation rates compared against the background mutation rates in tumour samples identified several significantly mutated GPCRs, suggesting a role for these in cancer (Supplementary information S2 (table)).

The functional impact of aberrant expression

An interesting issue that was raised by the early studies of the *MAS1* oncogene and the serotonin and muscarinic receptors is that GPCRs do not need to be mutated to contribute to tumour progression: their aberrant expression or overexpression can exert oncogenic properties provided that locally released or circulating ligands are available. For example, CXCR4 is not normally expressed on breast epithelial cells, but is often expressed on breast cancer cells, and its ligand CXCL12 (also known as SDF1) 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 interleukin 8 (IL-8; also known as CXCL8)) and their cognate GPCRs (CXCR4, CCR5 and CXCR2, respectively) in the establishment of a permissive tumour microenvironment, immune evasion and cancer metastasis is also now well documented[70]. Furthermore, the role of COX2-derived prostaglandins such as prostaglandin E2 (PGE2) and their GPCRs (primarily EP2 and EP4) in linking chronic inflammation to an increased risk of cancer development, is well known and can explain the cancer-preventive activity of non-steroidal anti-inflammatory drugs (NSAIDs) in colorectal cancer in genetically predisposed patients and in the general population[38]. Similarly, lipid mediators such as LPA and S1P achieve a high local concentration in multiple cancer types, and their GPCRs (LPARs and S1PRs, respectively) are expressed in cancer, stromal, immune and endothelial cells; these ligand–GPCR interactions contribute to angiogenesis, lymphangiogenesis, cancer growth and metastasis[71,72].

Indeed, many cancers exhibit aberrant overexpression of GPCRs and G proteins, the complexity and clinical relevance of which have just begun to be appreciated. Increased expression of G proteins can result in enhanced and/or prolonged signalling downstream of GPCRs, thereby influencing tumour growth and progression. Increases in the expression of particular G proteins could also lead to changes in the coupling specificity of GPCRs, which could have a dramatic impact on their entire signalling profile. For example, in triple-negative breast cancers that overexpress $G\alpha_{12}$ and $G\alpha_{13}$, CXCR4 binds to $G\alpha_{i}$ 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 that are important for cell migration and metastatic spread[73]. Meta-analysis of publicly available gene-expression microarray data sets from Oncomine revealed that $G\alpha_{12}$ and $G\alpha_{13}$ are overexpressed in breast, oral, oesophageal and colon cancers, and that $G\alpha_{s}$ is overexpressed in bladder and colorectal cancers, among others. However, this information needs to be treated with caution, given the need to assess the appropriateness of the tissue controls that were used for each study. Of direct relevance, the analysis of extensive collections of patient-matched DNA samples from cancer and normal tissues (as part of The Cancer Genome Atlas (TCGA) project)

indicates that a remarkable proportion of colorectal and gastric cancers harbour DNA copy number gains in *GNAS*, and that cancers of the brain, central nervous system and kidney frequently harbour copy number gains in *GNA11*; 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 data sets 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 tumour promotion and in cancer progression and metastasis, the presence of genetic alterations in G proteins and GPCRs was initially restricted to only a few neoplastic lesions, primarily endocrine tumours. Hence, GPCRs and their downstream signalling pathways have traditionally received limited attention as direct targets for anticancer 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 signalling targets. Activating mutations in TSH GPCRs and SMO are also now well documented, and their direct cancer relevance is well established. The most frequent somatic mutations in GPCRs involve the GRM family and the poorly studied adhesion family of GPCRs, together with mutations in receptors for bioactive lipid mediators that often accumulate in the tumour microenvironment, such as LPARs and S1PRs. Although it is still unknown whether mutations in these GPCRs contribute to cancer initiation or progression, their rate of somatic mutation 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 *GNAS*, *GNAQ* and *GNA11* is quite remarkable, and is aligned with the proliferative capacity of the encoded G proteins and their coupled 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 REFS 23,24). Hence, adenylyl cyclase activation and cAMP accumulation resulting from persistent $G\alpha_s$ activity probably represents a driver oncogenic pathway in these tissues. This also raises the possibility that activated *GNAS* mutants might act as oncogenes only in a limited number of tissues in which cAMP stimulates proliferation. Alternatively, $G\alpha_s$ might 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 to be uveal melanoma oncogenes[42,44]. How $G\alpha_q$ and its coupled receptors, such as those activated by endothelin (which is a potent mitogen in melanocytes[74]) transduce proliferative signals is still not fully understood owing to the complexity of the $G\alpha_q$-regulated signalling circuitry. For example, the $G\alpha_q$ protein family and $G\alpha_q$-coupled GPCRs can stimulate multiple second-messenger generating systems, and can also transactivate tyrosine kinase growth factor receptors, such as the epidermal growth factor receptor (EGFR)[75]. Given the broad implication of growth factor receptor signalling in cell growth and transformation, this particular receptor crosstalk and the resulting signalling output downstream of GPCRs is expected to be directly relevant to the transforming ability of G proteins and GPCRs in multiple tumour types. In particular, for ocular melanomas, recently available evidence suggests that in addition to the $G\alpha_q$-dependent activation of phospholipase C and the consequent rise in the intracellular Ca^{2+} concentration 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 that impinge on transcription factors and co-activators, such as JUN, FOS and YAP[76,77]. Which of these pathways contribute to the malignant growth and metastatic spread of uveal melanomas is under current investigation. It remains unclear why ocular melanocytes are more susceptible than cutaneous melanocytes to transformation by the *GNAQ* oncogene. An interesting possibility arises from the observation that *GNAQ* or *GNA11* are mutated in nearly 83% of blue naevi[42,44], which are highly pigmented melanocytic skin lesions that rarely progress to 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, which is 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 signalling 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 newly available DNA and RNA deep sequencing methods and systems biology analysis, are beginning to reveal alterations in entire G-protein-regulated pathways, not just in specific molecular components, in individual cancer patients[46]. This further supports the emerging notion that GPCR-dependent signalling circuits are indeed directly linked to malignant transformation and/or contribute to a variety of aberrant processes that are relevant to cancer progression and metastasis. Furthermore, it is evident that not only mutations in GPCRs, 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 signalling networks could benefit cancer patients who are treated according to the molecular signatures in their tumours. This may include new strategies to develop signalling-selective 'biased' antagonists as well as allosteric modulators that can function as inverse agonists to halt persistent signalling from constitutively active receptor mutants, ultimately targeting the GPCR-regulated molecular networks that are associated with cancer.

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

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Competing interests statement
The authors declare competing financial interests. See Web version for details.

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