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G Protein Coupled Receptors and Heterotrimeric G Proteins as Cancer Drivers

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Abstract

G protein coupled receptors (GPCRs) and heterotrimeric G proteins play central roles in a diverse array of cellular processes. As such, dysregulation of GPCRs and their coupled heterotrimeric G proteins can dramatically alter the signalling landscape and functional state of a cell. Consistent with their fundamental physiological functions, GPCRs and their effector heterotrimeric G proteins are implicated in some of the most prevalent human diseases, including a complex disease such as cancer that causes significant morbidity and mortality worldwide. GPCR/G protein-mediated signalling impacts oncogenesis at multiple levels by regulating tumour angiogenesis, immune evasion, metastasis, and drug resistance. Here, we summarize the growing body of research on GPCRs and their effector heterotrimeric G proteins as drivers of cancer initiation and progression, and as emerging antitumoral therapeutic targets.

Keywords:

G protein coupled receptors, GTPases, cancer, signal transduction, metastasis, immune therapy, precision medicine, inflammation, angiogenesis, cancer metabolism

Abbreviations:

aGPCR: Adhesion G protein coupled receptor

ATP: Adenosine triphosphate

bFGF: Basic fibroblast growth factor

BMR: Background mutation rate

cAMP: Cyclic adenosine monophosphate

CNV: Copy number variation

COSMIC: Catalogue of Somatic Mutations in Cancer

COX: Cyclooxygenase

GAP: GTPase-accelerating protein

GDP: Guanosine diphosphate

GEF: Guanine nucleotide exchange factor

GOF: Gain of function

GPCR: G protein coupled receptor

GTP: Guanosine triphosphate

IAP: Islet activating protein

LOF: Loss of function

LPA: Lysophosphatidic acid

LRR: Leucine-rich repeats

MAPK: Mitogen-activated protein kinase

MMP: Matrix metalloproteinase

NAM: Negative allosteric modulator

NGF: Nerve growth factor

ORF: Open reading frame

PAM: Positive allosteric modulator

PG: Prostaglandin

PSA: Prostate specific antigen

RGS: Regulators of G protein signaling

ROS: Reactive oxygen species

RTK: Receptor tyrosine kinase

SRE: Serum response element

TSH: Thyroid stimulating hormone

TCGA: The Cancer Genome Atlas

VEGF: Vascular endothelial growth factor

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Introduction

G protein coupled receptors (GPCRs) represent the largest family of cell-surface receptors, with over 800 GPCRs and 35 heterotrimeric G protein subunits involved in transduction of diverse signalling cascades¹⁻³. GPCRs are characterized by a distinctive 7-transmembrane domain structure with an extracellular amino terminus and an intracellular carboxyl terminus, and by their ability to couple to heterotrimeric G proteins, comprised of α , β , and γ subunits, which activate a diverse array of downstream signalling pathways¹. GPCRs play key roles in many cellular and physiological functions, including in neurotransmission, cardiac response and blood pressure regulation, vision, olfaction, tissue development and immune regulation^{2,4}.

The human GPCR superfamily can be phylogenetically grouped into 5 subfamilies based on distinct structural features— Class A (rhodopsin), Class B1 (secretin), Class B2 (adhesion), Class C (glutamate), and Class F (frizzled/taste2)¹. Most GPCRs activate one or multiple $G\alpha$ proteins, which bind guanine nucleotides—GDP in their inactive form and GTP in their active form. $G\alpha$ proteins are subdivided into 4 major families: $G\alpha_i$, $G\alpha_{12}$, $G\alpha_s$, and $G\alpha_q$, with each family activating a distinct repertoire of signalling mechanisms⁴. GPCR activation is initiated by the binding of an agonist ligand to the extracellular domain of the receptor which induces a rapid conformational change in the extracellular and intracellular loops of the receptor⁵. This transition into the active conformation of the receptor results in coupling to the heterotrimeric G proteins and triggers the exchange of GDP for GTP on the $G\alpha$ subunit, promoting its dissociation from $G\beta\gamma$ dimers. Both $G\alpha$ -GTP bound and $G\beta\gamma$ subunit complexes then stimulate downstream signalling cascades, including the rapid generation of multiple second messengers by modulating the activity of ion channels, phospholipases, phosphodiesterases, and adenylyl cyclases². These second messenger generating systems and their downstream regulated kinases cascades are responsible for most of the rapid physiological responses elicited by GPCRs⁶⁻¹². In tandem to these processes, Regulators of G protein Signalling (RGS) proteins enhance the GTPase activity of the $G\alpha$ subunit, enabling the reassociation of the $G\alpha$ and $G\beta\gamma$ subunits into a bound heterotrimeric G protein, and returning the protein complex to a GDP-bound inactive state⁵. Moreover, arrestins are recruited to activated GPCRs to promote receptor endocytosis and can participate in downstream signalling as scaffolds for signalling complexes, and as molecular rheostats of G protein-driven signal transduction¹³. This model of GPCR function has been improved over the recent years to encompass various classes of ligands, including agonists, partial agonists, inverse agonists and allosteric modulators, and the detailed structure features of the corresponding GPCR conformations that can be stabilized upon binding¹⁴.

As GPCRs and their associated heterotrimeric G proteins are involved in a diverse array of signal transduction pathways and cellular processes, dysregulation in either can have significant impacts on cellular behaviour and the initiation of pathogenic processes. This is highlighted by large body of drugs in the market targeting GPCRs. Indeed, 34% of all FDA approved drugs currently on the market target GPCRs directly or indirectly¹⁵⁻¹⁷. This review will summarize the growing body of information establishing GPCRs and heterotrimeric G proteins as drivers of cancer and their roles in cancer initiation and progression.

Historical Perspective

The earliest evidence suggesting a role for GPCRs in tumorigenesis stems from work demonstrating that expression of a GPCR encoded by the *mas* oncogene (*MAS1* gene), had the ability to transform and induce foci formation in NIH3T3 fibroblasts, as well as develop tumors in nude mice¹⁸. This pivotal work was novel in contrast to the many known oncogenes at the time, most of which were discovered based on the transforming activity of oncogenic viruses. These findings were reinforced by the observation that ectopic expression of the 5HT1c serotonin receptor (*HTR1C*) led to transformation of NIH3T3 cells¹⁹. However, in both cases the receptors did not harbour any identifiable mutations, contrasting with most viral and human oncogenes, and these observations were not widely appreciated. Subsequent studies examining the transforming potential of GPCRs led to the discovery that coupling specificity and excess ligand availability were key determinants of the oncogenic activity of wild type GPCRs. Specifically, overexpression alone of muscarinic cholinergic receptors (CHRM_s), which span across Gα-coupling subtypes, was found to be insufficient to transform NIH3T3 cells. However, in the presence of the agonist carbachol foci were readily induced for Gαq-coupled CHRM_s, thereby establishing that wild-type GPCRs can act as agonist-dependent oncogenes based on their G protein coupling capacity²⁰. The α1B-adrenergic receptor (*ADRA1B*) was found to behave similarly by inducing neoplastic transformation when ectopically expressed in NIH3T3 cells, and triggering formation of foci in an agonist-dependent manner²¹. However, mutation of this receptor eliminated agonist dependency of receptor activation, rendering it constitutively active, thus raising the possibility that mutations may be a mechanism to enhance the oncogenic potential of GPCRs. As time has progressed, massive advances in the field of cancer genomics have transformed our understanding of oncogenesis and drivers of cancer. Aligned with this, GPCRs and heterotrimeric G proteins have emerged as candidate drivers supported by a diverse body of work underscoring the cancer relevance of mutations in GPCRs and heterotrimeric G proteins.

The Hallmarks of Cancer

The hallmarks of cancer were originally proposed as a conceptual framework to better describe and understand the fundamental underpinnings of neoplastic disease and have since revolutionized our paradigm of the intricate processes driving cancer^{22,23}. Composed of multiple interrelated processes, 1) sustaining proliferative signalling, 2) evading growth suppressors, 3) resisting cell death, 4) enabling replicative immortality, 5) inducing angiogenesis, 6) activating invasion and metastasis, 7) metabolic reprogramming, 8) evading immune destruction, and the enabling characteristics of 9) genome instability and 10) inflammation, this collection of capabilities cooperatively interact to facilitate the transformation of cells, and drive malignant growth and metastasis.

Aimed towards characterizing the unifying features of all cancers irrespective of tumor type, these guiding principles address the complex and dynamic interactions between and within the tumor and the tumor microenvironment, and have grown to encompass the numerous cell types that participate in these interactions, including normal cells whose functions can be co-opted to help drive tumorigenesis. Moreover, the dynamic and interrelated nature of these hallmarks necessitate the coordination of different signalling programs and their associated molecular mechanisms.

The remarkable functional repertoire of GPCRs and heterotrimeric G proteins, and their centrality to numerous cellular and physiological processes make them key participants in facilitating each of these hallmarks. In particular, given the complexity of signal transduction networks controlled by GPCRs, coupled with the cell-context specificity of signal integration and output, it is likely that GPCRs and heterotrimeric G proteins participate in multiple hallmarks within cancer cells and their tumor microenvironment. Specifically, there are likely to be numerous and overlapping mechanisms by which mutations and/or aberrant expression of GPCRs and heterotrimeric G proteins drive proliferative signalling, induce evasion of growth suppressors and enable replicative immortality, which will be described in detail below. The role of GPCRs in the remaining hallmarks will be described under each respective heading.

GPCRs in Cancer Initiation: Proliferative Signalling, Evasion of Growth Suppressors, and Replicative Immortality

GPCRs and Viral-Associated Cancers

Numerous viruses have evolved to take advantage of the diverse signalling outcomes downstream of GPCRs for their survival and propagation. Together, these mechanisms facilitate viral entry of the host cell, evasion of the host immune response, viral replication, and modulation

of host survival pathways in order to promote viral pathogenesis^{24,25}. Several viruses have been found to encode GPCRs in their genomes, including Kaposi's Sarcoma associated herpesvirus (KSHV), Human cytomegalovirus (HCMV), Human Herpesvirus 6 and 7, and Epstein-Barr Virus (EBV)²⁶⁻³². These viral GPCRs (*vGPCRs*) all encode GPCRs that are distantly related to human chemokine receptors²⁴.

In many cases, infections by these viruses can lead to development of viral-associated cancer. For example, KSHV, a γ-2-herpesvirus, is the causative agent of Kaposi's sarcoma (KS), most frequently found in immunosuppressed individuals, and a significant driver of mortality among AIDS patients^{25,27,28}. The KSHV vGPCR is most closely related to human CXCR1/2 receptors; however, it possesses several substitutions in residues which confer constitutive activity and enhanced coupling to G proteins^{24,33}. Indeed, expression of KSHV vGPCR is alone sufficient to induce angiosarcomas when expressed in endothelial cells^{25,27,34} (Figure 1). Studies examining the etiology of KS, have found that vGPCR can initiate Kaposi's sarcomagenesis and contribute to progression of KS lesions³⁴. KSHV has also been shown to play key roles in lymphoproliferative disorders, namely primary effusion lymphoma (PEL) and multicentric Castleman's disease (MCD), both of which are B cell lymphomas primarily seen in AIDS patients²⁴. Here, vGPCR has been shown to elicit a broad range of signalling and transcriptional events ultimately driving B cell recruitment and hyper-proliferation³⁵.

Viruses can also exploit host GPCRs to promote viral pathogenesis and survival by expressing virally encoded GPCR agonists or antagonists in order to modulate host cell signalling. For example, KHSV encodes multiple viral cytokines, which have significant sequence similarity to host chemokine agonists and antagonists and can bind to host GPCRs to mediate functions such as chemotaxis of immune cells, and resistance to apoptosis^{25,36,37}. Alternatively, these proteins can bind to and inactivate host-produced ligands in order to shut off or abrogate host immune response³⁸.

The Mutation Spectrum of GPCRs in Cancer

The generation of vast cancer sequencing resources including The Cancer Genome Atlas (TCGA), and the Catalogue of Somatic Mutations in Cancer (COSMIC) have revealed that GPCRs and heterotrimeric G proteins are collectively mutated in roughly 20% of all cancers, spanning across numerous tumor types³⁹⁻⁴². This is aligned with mutation frequencies of some of the most well-studied cancer-related pathways, including the receptor tyrosine kinase (RTK)/RAS/MAPK and p53 pathways, which are mutated at 46% and 29% of all cancers

respectively⁴³. Despite this staggering number, the biological function and consequence of many of these GPCR and heterotrimeric G protein mutations are as of yet largely unknown due to the numerous factors that influence the prediction of cancer-driving mutations. For example, the contribution of gene length must be weighed against the number of mutations in a given gene. Coupled with this, many of the mutations in GPCRs are not isolated to hotspot residues, further hindering efforts to predict their signalling and cellular impact and potential role in cancer. As a result, it has been difficult to infer the biological contexts in which mutations in GPCRs have cancer-driving effects as compared to passenger mutations with little functional impact. Towards this end, there have been numerous recent advances in cancer genomics and methodology aimed at identifying significantly mutated genes, and their associated biological consequences. For example, the MutSig suite of tools builds a model based on several characteristics, including the mutation burden of a gene relative to the background mutation rate (BMR) of each tissue, mutation clustering within a gene and potential hotspots, and the evolutionary conservation of mutations⁴⁴. These features are then integrated to obtain gene-level mutation significance^{44,45}. For our analysis (Table S3) we have included data generated using MutSig2CV, which refines the BMR predictions by incorporating parameters of mutational heterogeneity such as DNA replication timing and transcriptional activity⁴⁵. To complement measurements of mutation significance, other approaches have been used to predict the pathogenicity of mutations in cancer, including investigating phylogenetic conservation, allele frequency, proximity to specific genomic features, mapping mutations to protein domains, and assessing patterns of mutual exclusivity with other mutations⁴⁶. In tandem, the integration of rapidly growing mechanistic data are beginning to shed light on the molecular basis of how cancer-associated mutations may be playing a role, including the systematic mapping of cancer-related alterations on a pathway level⁴³.

Of particular relevance to GPCR-driven signalling, structure-function analyses of mutations in GPCRs have begun to provide insight on these observations and a new framework of the functional impact of GPCR mutations is emerging. Projection of cancer-related mutations for TSHR, for example, have revealed an accumulation of mutations clustered at the cytoplasmic tail of helix 6, suggesting that in the absence of a physical hotspot mutation, structural motif hotspots may be more prevalent manifestations of cancer-associated GPCR mutations³⁹. Indeed, our recent pan-cancer analysis of functional somatic mutations in gene families has demonstrated significant enrichment of mutations in a handful of highly conserved regions of GPCRs that regulate GPCR function and activation. These include mutations localized to the DRY motif, which mediates the inactive conformation of class A GPCRs, with particular emphasis on

recurrent mutations in DRY arginine, in addition to the nPxxy motif, both of which are critical regulators of GPCR activation. Mutations in these positions show statistically significant mutual exclusivity between motifs, as well as with activating hotspot mutations in the G α subunit further supporting the functional significance of these structure-based predictions⁴⁷. DRY arginine mutations are also mutually exclusive with many common mutant oncogenes that are downstream of GPCR-mediated signalling cascades, including AKT E17K, PIK3CA E545K and JAK2 V617F⁴⁷. In many cases where the full G-protein-coupling repertoire of GPCRs has not been fully elucidated, recent efforts to systematically predict the G protein-coupling selectivity of GPCRs via functional interaction studies have provided novel insights into these coupling determinants and the subsequent implications both from a structural, and signalling perspective⁴⁸. Integration of this information with other data types including aforementioned mutation and structure-function analyses will continue to widen and clarify our view of the impact of cancer-associated mutations in GPCRs.

Broken down phylogenetically, the adhesion and glutamate GPCR are highly mutated families of GPCRs in cancer; however, their role in cancer is not well defined³⁹. Adhesion GPCRs (aGPCRs) are named as such due to the extended extracellular N terminus containing structural domains such as thrombospondin repeats, and leucine-rich-repeats (LRRs), which participate in a variety of protein-protein interactions and can mediate adhesion to cellular matrix proteins⁴⁹. The aGPCR subfamily is widely mutated among cancer types in TCGA and a number of aGPCRs are involved in angiogenesis, metastasis, and other critical components of cancer initiation and progression^{50,51} (Figure 1). Among them, GPR98 (*ADGRV1*) is the most frequently mutated GPCR across all cancer types. In particular *ADGRV1* is mutated in roughly 45% of skin cutaneous melanoma, and is the longest GPCR by amino acid length; however, not much is known regarding its functional impact⁵² (Table S1). *ADGRE5*, also known as CD97, was the first aGPCR to be linked to cancer, as its expression was found to be a sensitive marker of dedifferentiation in thyroid carcinomas^{53,54}. While *ADGRE5* expression was nearly absent in normal thyrocytes, its levels increased in correlation with thyroid tumor stage^{53,54}. Its over-expression has since been identified in several other cancer types including pancreatic, gallbladder, and esophageal carcinomas, and is linked to metastatic aggressiveness in gastric, colorectal cancers, and particularly in glioblastoma⁵⁵. Glioblastoma in particular, is known to develop extensive intratumoral hypoxia, and GPR133 (*ADGRD1*) has been implicated to be critical for glioblastoma growth under hypoxic conditions⁵⁶.

Glutamate receptors, which bind glutamate as their ligand, have primarily been studied for their roles in the central nervous system but are becoming increasingly implicated in cancer^{57,58}. A pan-cancer analysis of somatic mutation patterns revealed a number of significantly mutated GPCRs, including *GRM8*⁴². In another case, a GPCR-targeted mutation analysis of melanomas revealed that *GPR98* (mentioned above) and *GRM3*, a metabotropic glutamate receptor, were the most frequently mutated genes⁵⁹. Expression of patient-derived *GRM3* mutants in melanoma cell lines significantly increased anchorage-independent cell proliferation, as well as cell migration *in vitro* and *in vivo* metastatic rate⁵⁹ (Figure 1). Similarly, ectopic expression of *GRM1* and *GRM5* have been found to induce spontaneous formation of melanoma in transgenic mouse models suggesting a role for GRMs in driving melanoma initiation⁶⁰⁻⁶². Glutamate and other amino acid-binding GPCRs, such as the taste receptors *TAS1R1*, and *TAS1R3*, can also act as nutrient sensors that survey the tumor microenvironment and extracellular milieu to assess nutrient availability. Under times of nutrient stress or deprivation, these GPCRs can regulate the activation of autophagic programs that enable cancer cells to persist^{63,64}.

One of the most prominent GPCRs implicated in neoplastic growth is the Thyroid-stimulating hormone (TSH) receptor (*TSHR*). *TSHR* is coupled primarily to G α s and to a lesser extent G α q, and is a key regulator of thyroid cell function, growth and hormone metabolism⁶⁵. Mutations in *TSHR* have been found to lead to numerous thyroid diseases, including hyper- and hypothyroidism and hyperfunctioning thyroid adenomas⁶⁶. Strikingly, activating mutations in *TSHR* are the predominant cause of solitary toxic thyroid adenomas, accounting for roughly 60-80% of all cases, and are found in roughly 30% of thyroid adenomas among other cancers^{67,68}. Aligned with this, activating mutations in *GNAS* and *TSHR* are commonly found in differentiated thyroid carcinomas^{67,69,70} (Figure 1).

TSHR and other hormone GPCRs, such as G-protein coupled estrogen receptor (*GPER1*, also known as GPR30), luteinizing hormone receptor (*LHR*), follicle-stimulating hormone receptor (*FSHR*), gonadotropin-releasing hormone receptor (*GNRHR*), and their cognate hormone ligands, are known to play multiple roles in modulating the tumor microenvironment and promoting tumor proliferation particularly for cancers with hormonal involvement such as thyroid, breast, ovarian, and prostate cancers. Indeed, prostate cancer induced by injection of testosterone was one of the earliest indications that hormone dynamics could control the initiation of cancer⁷¹.

Estrogen for example, is a hormone involved in many aspects of normal physiology and has been well-studied in the context of breast and ovarian cancers. Concomitant to the transcriptional

actions of classical estrogen receptors (ERs), GPER has been shown to mediate both transcriptional and rapid signal transduction events upon estrogen binding, activating the EGFR/MAPK pathways and resulting in the activation of c-fos regulated transcriptional networks in multiple tissue types^{72,73}. Interestingly, GPER has been shown to cooperate with ER α to promote mitogenic effects in a cell-context dependent manner, highlighting the multifaceted nature of estrogen signalling in cancer⁷⁴. Evista (raloxifene), which is FDA approved for the prevention of breast cancer in post-menopausal women, targets ERs, while also modulating GPER activity. Other hormone-binding GPCRs have been successfully targeted in oncology, including the gonadotropin-releasing hormone receptor (GnRH). Degarelix, a well-tolerated GnRH antagonist, has been used for the treatment of advanced prostate cancer by rapidly suppressing levels of gonadotropins, testosterone, and prostate-specific antigen (PSA) in patients⁷⁵. The profound efficacy of degarelix as a first-line monotherapy for the management of prostate cancer is a remarkable testament to the druggability of GPCRs and their use in oncotherapy.

Another seven-transmembrane receptor frequently mutated in cancer is Smoothened (SMO), which drives activation of the transcription factor GLI^{76,77}. SMO is negatively regulated by the twelve-transmembrane receptor patched (PTCH); however, this inhibition is relieved upon binding of PTCH to hedgehog family members, including sonic hedgehog (SHH)⁷⁶. Mutations that result in the activation of the PTCH-SMO-SHH signalling axis have been found to drive sporadic basal cell carcinomas (BCCs)^{78,79} (Figure 1). As of yet, the potential role of heterotrimeric G proteins in the signalling capacity of SMO have not been fully clarified⁷⁷. Studies have found that in some cell contexts, G α i and G α 12 are involved in activation of the pathway; however, the dependency of SMO on G protein coupling for its roles in cancer initiation and progression is not well understood and requires further investigation⁸⁰⁻⁸².

The Frizzled family of seven-transmembrane receptors (FZDs) are also heavily involved in cancer, as transducers of the Wnt signalling cascade⁸³. Wnt signalling can drive the activation of several transcriptional networks, primarily β -catenin and has been described to play a role in numerous cancer types, with emphasis in colorectal cancer^{83,84}. Indeed, the Frizzled family of GPCRs is collectively mutated in >15% of colon adenocarcinomas in TCGA (Figure 1, Table S2). FZD6 in particular is significantly mutated in colon adenocarcinoma (MutSig2CV q-value = 0.05) (Table S3). There is mounting evidence supporting the G protein coupling of Frizzleds as a critical component of the Wnt signalling pathway⁸⁵⁻⁸⁷. For example, inhibition of G α o and G α q has been found to disrupt Wnt-mediated stabilization of β -catenin and teratocarcinoma stem-cell

differentiation^{86,88}. Future work will likely expand the current view of G protein regulated Wnt signalling in cancer initiation and progression

While the complexity of the mutational landscape of GPCRs in cancer, which we refer to as the onco-GPCRome, is daunting, some interesting tissue-specific patterns are now emerging that may help explain their direct biological impact. Indeed, recent cross-cancer analysis of GPCR mutations in human malignancies has revealed that gastrointestinal (GI) cancers harbor the highest number of mutated GPCRs and heterotrimeric G proteins, irrespective of the mutational load of these cancers⁵². This is coupled with the recent finding that a significant portion of mutated GPCRs couple to G α s and G α i/o, more specifically, an enrichment of activating mutants of G α s-coupled receptors, and deleterious (inactivating) mutants of G α i/o-coupled receptors⁴⁷. Together, these mutation patterns converge on the oncogenic impact of elevated adenylyl cyclase activity and production of cyclic AMP (cAMP), which is stimulated by G α s and inhibited by G α i/o, suggesting a prominent role for enhanced cAMP signalling in cancer. The cAMP signalling network is involved in different aspects of tumorigenesis, and these findings establish a functional link between the mutation patterns in GPCRs and their candidate role as cell-context specific oncdrivers, with emphasis on GI cancers, including colorectal, stomach, and pancreatic adenocarcinomas. GPCRs are also known to possess copy number variation (CNV) or gene expression alterations, leading to their aberrant expression in numerous cancer types, representing a mutation-independent, and potentially targetable mechanism by which they can drive oncogenic signalling networks^{52,89}. Indeed, 28 out of 33 TCGA cancer cohorts have been found to have CNVs in GPCR and heterotrimeric G protein genes that are significantly correlated with the mRNA expression patterns of each respective gene⁵². The tissue, cell type, and even tumor stage-specific nature of these alterations further emphasize the complex and dynamic molecular forces underlying GPCR signalling in cancer. These mechanisms can contribute to diverse aspects of tumorigenesis, which will be discussed in greater detail below.

In contrast to the pro-tumorigenic role of many GPCRs, GPCR-driven signalling has in some cases, been shown to play a tumor suppressive role, highlighting the complexity and the cell-context dependency of these signalling events. This is highly aligned with tumor suppressive roles for heterotrimeric G proteins in certain contexts as well. For example, inactivation of G α 13 signalling, either by inactivating mutations in the G α 13-coupled Sphingosine-1-phosphate receptor 2 (S1PR2), or in G α 13 itself have both been identified to enhance tumor progression in diffuse large B cell lymphoma⁹⁰. Moreover, the Kisspeptin receptor (*KISS1R*) has been widely

implicated as a metastatic suppressor in numerous cancer types^{91,92}. Similarly, GPR68 was first found to be a metastatic suppressor in prostate cancer and was found to be significantly downregulated in metastatic tumors; however, further work has shown that GPR68 is highly upregulated in pancreatic cancer associated fibroblasts (CAFs) and represents an emerging drug target in this cancer type⁹³⁻⁹⁵ (Figure 1). In another case, targeting of dopamine receptors in cancer has been shown to have highly tumor-type specific effects, where both activation and antagonism have been found to have anti-tumor effects⁹⁶. However, in the particular case of glioblastoma, targeting of the dopamine receptor DRD2 has shown to be a highly promising drug target, with the compound ONC201 being investigated in clinical trials^{96,97}. Finally, ADGRB1-3, also known as the BAI family GPCRs, are silenced or mutated in numerous cancer types⁹⁸ (Figure 1). See below (**GPCRs and Genome Instability**) for more details on the effect of BAI1 loss on genomic instability.

Mutations in heterotrimeric G Proteins in Cancer

The unanticipatedly high frequency of mutations in GPCRs coupled with their complex expression and mutation patterns in different cancer types highlight the previously underappreciated role of GPCR signalling in cancer. Detailed work on the functional impact of GPCRs in cancer, exploiting novel computational biology pipelines, structure-function predictions, and functional analyses of the impact of cancer-associated mutations in GPCRs will contribute to establishing GPCRs as cancer-specific drivers. In contrast, the presence of hotspot mutations in heterotrimeric G proteins defines them as driver oncogenes in specific cancer types. Here we will summarize the role of heterotrimeric G proteins in cancer and as cancer drivers.

Signalling through Gαq G proteins—The GNAQ oncogenes

The Gαq family of heterotrimeric subunits, encoded by GNAQ, GNA11, GNA14 and GNA15, and their coupled receptors, are responsible for transducing many of the mitogenic signals initiated by growth factors acting on GPCRs^{4,99}. Members of the Gαq family activate phospholipase-Cβ (PLCB1-4), which cleaves PIP₂ into diacylglycerol and inositol 1,4,5 triphosphate. Generation of these second messengers triggers the mobilization of cytosolic calcium and all together, elicit the activation of numerous downstream signalling events via regulated kinases and transcriptional networks⁸⁻¹¹. Mutation of the Gαq subunit into a GTPase defective, constitutively active conformation was originally modelled based on previous studies examining mutations in the *ras* oncogene¹⁰⁰. These Gαq mutants induced malignant transformation in NIH3T3 cells and were found to be tumorigenic in nude mice¹⁰⁰.

Since this original discovery, aberrant G α q activity has been found to be involved in diverse pathological conditions including congenital hemangiomas, leptomeningeal melanocytic lesions, Sturge Weber syndrome, and recent cancer sequencing efforts revealed that *GNAQ* and *GNA11* are significantly mutated in uveal melanomas (UM) (MutSig2CV q-values = 6.08E-13) (Table S3). More specifically, activating mutations in G α q or G α 11, predominantly at Q209, are collectively found in >90% of UM where they act as driver oncogenes¹⁰¹⁻¹⁰⁵ (Table 1, Table S3, Table S4, Table S5). The G α q family is also mutated in roughly 10% of skin cutaneous melanomas (Table 1, Table S5). UM is the primary cancer of the eye in adults, affecting roughly 2500 patients in the US each year¹⁰⁶. Nearly 5% of UM patients who lack mutation in G α q or G α 11, possess mutually exclusive activating mutations in other levels of the G α q pathway, including in *CYSLTR2*, a G α q-coupled GPCR (MutSig2CV q-value = 0.005), or *PLCB4*, a downstream effector of G α q, firmly establishing hyper-activation of the G α q-pathway as the oncogenic driver of UM^{107,108} (Table S3). UM caused by mutation and hyperactivation of *CYSLTR2* remains one of the most striking examples of a GPCR-driven cancer type to date¹⁰⁹ (Figure 1).

Studies investigating the mechanisms by which aberrant G α q signalling drives tumorigenesis have revealed that compared to the transient stimulation of second messengers and mitogenic kinases after canonical G α q stimulation, mutant G α q requires a protein-protein interaction with the Rho-specific guanine nucleotide exchange factor (GEF) TRIO in order to sustain persistent signalling¹¹⁰. In *in vivo* UM models, knockdown of TRIO phenocopies reduction of G α q expression based on cell proliferation assays, tumorigenic potential as well as activation of JNK, p38 and AP-1-mediated transcription¹¹⁰. Remarkably, this signalling circuitry was independent of PLC β , the best-known target of G α q and led to the activation of YAP, a transcriptional co-activator regulated by the Hippo pathway. The Hippo pathway is a tumor suppressive pathway, broadly involved in cell proliferation and organ size control, and has been found to be frequently altered in cancer¹¹¹. In UM, YAP activity was demonstrated to be necessary for tumor growth and proliferation^{112,113}. Recently studies have shown that Focal Adhesion Kinase (FAK), is a central mediator of the G α q-driven oncogenic signalling circuitry by controlling the activation of YAP, thereby representing a potential therapeutic target for patients with UM¹¹⁴.

Mutations in G α q/11 at residue R183, the second most commonly mutated site in *GNAQ* have been found to cause Sturge-Weber Syndrome, a neurocutaneous disorder characterized by facial port-wine stains and ipsilateral occipital leptomeningeal angiomas, and are also sometimes found in UM patients¹⁰⁴ (Figure 2A,B). Similar to Q209, R183 is located in the GTP-binding region of

$\text{G}\alpha\text{q}$, whereby mutation interferes with the intrinsic GTPase activity and increases signalling activity; however, mutations at R183 have been found to be less potently activating than Q209 mutants¹⁰⁴. Activating $\text{G}\alpha\text{q}$ mutants have also been associated with congenital hemangiomas, and frequently found in a subset of other melanocytic neoplasms, including blue nevi, nevi of Ota, and primary melanocytic tumors of the central nervous system^{103,105}.

$\text{G}\alpha\text{q}$ has also been found to play diverse roles in the immune system, where $\text{G}\alpha\text{q}$ has been implicated to have a tumor suppressive role. Loss of *GNAQ* expression enhances both B and T cell proliferation *in vivo* and survival *in vitro*¹¹⁵⁻¹¹⁷. Strikingly, alteration of the $\text{G}\alpha\text{q}$ pathway by loss of $\text{G}\alpha\text{q}$ expression or recurrent loss of function (LOF) mutations at T96S or Y101, are found in roughly 25% of natural killer/T cell lymphoma (NKTCL), a malignant and highly aggressive subtype of non-Hodgkin's lymphoma¹¹⁸. Consistent with the impact of $\text{G}\alpha\text{q}$ loss in other immune cells, NK-specific knockout of $\text{G}\alpha\text{q}$ in mice imparted an intrinsic survival advantage of NK cells as compared to wild type NK cells. Moreover, expression of WT $\text{G}\alpha\text{q}$ in $\text{G}\alpha\text{q}$ -low NK cell lines promoted apoptosis, which could be ablated by concomitant expression of T96S $\text{G}\alpha\text{q}$ suggesting T96S $\text{G}\alpha\text{q}$ acts as a dominant negative mutant to promote NK cell tumorigenicity¹¹⁸. The proclivity of R183 and Q209 hotspot mutations in $\text{G}\alpha\text{q}$ to solid tumors compared to the incidence of T96 and Y101 in hematopoietic malignancies suggests a complex relationship between the oncogenic or tumor suppressive function of these mutations and the cell context in which they originate, highlighting the complex molecular events underlying $\text{G}\alpha\text{q}$ -driven oncogenic signalling.

*Signalling through $\text{G}\alpha\text{s}$ in cancer– The *GNAS* oncogene*

GNAS, encoding the $\text{G}\alpha\text{s}$ protein, is among the most frequently mutated G proteins in human cancer⁵². Mutation characterization across tumor types has revealed that *GNAS* is mutated in ~5% of sequenced tumors, enriched in appendix cancers (70%), pituitary tumors (27%)³⁹. Across TCGA, *GNAS* is most frequently mutated in endometrial carcinomas (7.3%), stomach adenocarcinomas (5.7%), adrenocortical carcinomas (5.5%), pancreatic adenocarcinomas (5.6%), esophageal carcinomas (4.9%) and colorectal cancers (4.7%) (Table S4). The majority of $\text{G}\alpha\text{s}$ mutations occur at a hotspot residue (R201), resulting in expression of a constitutively active, GTPase defective form of the $\text{G}\alpha\text{s}$ protein¹¹⁹. A secondary hotspot can be found at Q227 but is less prevalent than R201 mutants (Figure 2C). The functional significance of the R201 residue was first suggested from studies examining the mechanism of action of cholera toxin. It was revealed that cholera toxin ADP-ribosylates R201 of $\text{G}\alpha\text{s}$, irreversibly inhibiting its GTPase

activity, and resulting in its activation¹²⁰⁻¹²². Crystallographic studies of Gas have since confirmed that the R201 residue lies in its GTP-binding pocket and facilitates GTP hydrolysis¹²³.

Activation of Gas, either by stimulation of Gas-coupled GPCRs or by activating mutations, stimulates adenylyl cyclases, which leads to the production of cAMP and activation of protein kinase A (PKA) and subsequent signalling events^{39,124}. The tumorigenic effects of Gas were first demonstrated in a subset of growth hormone-secreting pituitary tumors harbouring R201C/H mutations^{39,125,126}. In these tumors, persistent signalling driven by mutant G α s bypassed the canonical requirement for stimulation by growth hormone, resulting in hormone-insensitive growth¹¹⁹. Indeed, the Gas subfamily (*GNAS* and *GNAL*) is mutated in nearly 7% of adrenocortical carcinomas in TCGA (Table 1, Table S5). Activation of the G α s pathway has been shown to cause numerous other syndromes with involvement of endocrine dysfunction including McCune-Albright syndrome, which is also characterized by fibrous dysplasia of the bone, in addition to Cushing's syndrome, Carney complex, and micronodular adrenocortical hyperplasia¹²⁷⁻¹²⁹.

Strikingly, our recent study found that there is widespread activation of the Gas pathway across a large proportion of cancer types in TCGA with a remarkable enrichment in cancers of the gastrointestinal tract⁴⁷. Consistent with this, the Gas subfamily is mutated in 5.24% of colorectal, 5.03% of pancreatic and 5.96% of stomach adenocarcinomas (Table 1, Table S5). Indeed, both *GNAS* and *GNAL* are significantly mutated in pancreatic adenocarcinoma (MutSig2CV q-values = 9.56E-04, and 1.77E-02 respectively) (Table S3). Remarkably, greater than 50% of colorectal cancer patients have aberrant activation of the G α s pathway, including activating mutation or gene amplification of *GNAS*, or in downstream effectors of Gas including PKA, and adenylyl cyclases, as well as by the activation of prostaglandin E2 receptors (*PTGER2*, 4), which are coupled to G α s, by inflammatory prostaglandins accumulating locally due to COX2 overexpression (described below)¹³⁰⁻¹³². These genetic alterations and autocrine activation play a central role in the deregulation and activation of the PKA, Wnt and MAPK pathways in the intestinal epithelium and are likely to contribute to tumorigenesis and colorectal cancer progression¹³³⁻¹³⁵ (Figure 1).

Interestingly, many G α s mutant cancers have been found to be highly mucinous. Aligned with this, *GNAS* expression has been found to induce expression of mucin genes¹³⁶. In particular, *GNAS* mutations are highly prevalent in gastrointestinal mucinous neoplasms, including intraductal papillary mucinous neoplasms, an invasive pancreatic cystic neoplasm that can

advance to pancreatic adenocarcinoma, as well as appendiceal mucinous tumors where signalling driven by G α s has been found to play a critical role¹³⁶⁻¹⁴¹.

G α s, and G α s-driven signalling also plays a critical role in stem cell populations, where it has been found to suppress self-renewal. In contrast to the pro-oncogenic effects of GOF mutations in G α s, in some stem-like cell states, G α s has a tumor suppressive role where LOF mutations, or G α s deletion has been found to drive tumor initiation and progression by de-repression of the Sonic Hedgehog and Hippo pathways^{142,143}. In particular, the loss of GNAS, or low G α s expression is tightly linked to poor overall survival in a subset of aggressive medulloblastomas (MBs) with alterations and activation of SHH signalling, and in neuroblastoma¹⁴⁴. Strikingly, deletion of GNAS in neural stem/progenitor cells consistently leads to MB-like tumors in mice by derepressing SHH signalling thus contributing to the development and malignancy of SHH-associated MB tumors¹⁴⁴. In epidermal and hair follicle progenitor cell populations, conditional deletion of G α s leads to rapid formation of BCC by repressing PKA-mediated inhibition of SHH and YAP signalling⁷⁸. Of note, activation of G α s in epithelial stem cells leads to exhaustion of hair follicle stem cells and subsequent hair loss, demonstrating the cell-context specific impact of G α s signalling⁷⁸.

Mutations in genes encoding Gai/o proteins in cancer

The G α i/o subfamily of G proteins elicit signalling through a broad range of effectors, including activation of MAPK and PI3K signalling. In opposition to G α s signalling, G α i inhibits adenylyl cyclase, leading to a decrease in cAMP¹⁴⁵. This was originally discovered through studies examining the mechanism of action of Islet Activating protein (IAP), also known as pertussis toxin, as it was isolated from *Bordetella pertussis*. IAP was found to release inhibition of adenylyl cyclase and enhance receptor mediated cAMP generation. Multiple groups studying the substrate of IAP found that IAP ADP-ribosylated G α i, thereby inactivating it, and enhancing adenylyl cyclase activity¹⁴⁶⁻¹⁴⁸. Similar to other G proteins, constitutively active mutants of G α i have been shown to possess the ability to transform cells and are known proto-oncogenes^{149,150}. Cancer derived activated mutants of GNAO1, encoding G α o, have also been found to promote oncogenic transformation and anchorage-dependent growth when expressed in cells¹⁵¹.

In particular, hotspot activating mutations in GNAI2, which has been termed as the gip2 oncogene, has been found in several tumor types, including ovarian and adrenal tumors, and has been shown to underlie a dysfunctional state in a subset of growth hormone-secreting pituitary

adenomas; however the small number of patients with this particular disorder have limited efforts to study the effect of germline *GNAI* variants and warrant further investigation¹⁵²⁻¹⁵⁴. Strikingly, *GNAI2* is in the top 1% of copy number gains in brain, central nervous system, and kidney cancers suggesting there could be a larger role for Gαi-driven signalling³⁹. In particular, many GPCRs that are well established as drivers of metastasis, such as CXCR4 (see below) couple to Gαi. Aligned with this, *GNAI2* (encoding Gai2) has been found to be highly amplified in breast invasive carcinoma⁵².

Remarkably, Gαi/o-coupled receptors are the most commonly deleteriously mutated class of GPCRs across cancers⁴⁷. Coupled with this, inactivating mutations in Gαi/o coupled receptors have been found to be mutually exclusive with activating mutations in Gαs, suggesting that they lead to the same functional outcomes, converging on increased cAMP activity⁴⁷. Indeed, a large subset of GPCRs mutated in GI-tract cancers are Gαi coupled, and the Gαi/o subfamily of G proteins are altered at similar frequencies in GI cancers to the Gαs subfamily; however, the functional significance of their mutations have not yet been comprehensively tested⁵² (Table 1, Table S5). Aligned with the recurrent incidence of Gαs-pathway activation in GI cancers, upregulated cAMP/PKA activity may be a common mechanism of tumorigenesis in these tissue types, and warrants further investigation, both from a signalling and clinical perspective.

Mutations in genes encoding Gα12 proteins in cancer

The Gα12 subfamily of G proteins is comprised of two α subunits, encoded by *GNA12* and *GNA13*. Expressed in almost every tissue type, Gα12 and Gα13 play critical roles in cell proliferation and cytoskeletal remodelling, including cell polarity, cell adhesion, migration, and invasion^{155,156}. The Gα12/13 subunits were originally discovered based on their amino acid sequence similarity to other Gα subunits and determined to be unique based on their insensitivity to pertussis toxin¹⁵⁷. Wild-type (WT) Gα12 was identified from a sarcoma-derived cDNA library screen to cause transformation of NIH3T3 cells, representing the only G protein subfamily whose overexpression is sufficient to be transformative without mutation¹⁵⁸. The transforming ability of WT Gα12 was found to be dependent on the presence of serum; however, mutation of *GNA12* leading to expression of an activated, GTPase deficient Gα12 was found to be potently transforming, and eliminated serum-dependence for transformation¹⁵⁹⁻¹⁶¹. Similar work demonstrating the potent transforming activity of Gα13 led to the designation of *GNA12* and *GNA13* active mutants collectively referred to as the *gep oncogene*^{160,162}.

$\text{G}\alpha_{12/13}$ have since been widely explored as drivers of cellular transformation, cancer progression and metastatic potential in a variety of cell types¹⁶³⁻¹⁶⁶. Signalling driven by $\text{G}\alpha_{12/13}$ is largely mediated via the RhoGEF family, including p115RhoGEF, PDZ-RhoGEF and Leukemia-associated RhoGEF (LARG) to mediate signalling to Rho¹⁶⁷⁻¹⁷². Initial studies on the mechanism by which active mutant of $\text{G}\alpha_{12}$ was fully transforming in fibroblasts demonstrated that $\text{G}\alpha_{12}$ strongly stimulated transcriptional activity through the c-fos promoter element and the serum response element (SRE), which was dependent on Rho¹⁷³. Further work has revealed that $\text{G}\alpha_{12/13}$ can signal through multiple effectors including β -catenin, Radixin, and MAPK among others^{155,174-176}. All together, these signalling pathways regulate a diversity of cancer-related transcriptional networks and cellular functions, including the activation of AP-1, STAT3, and YAP^{155,177-180}.

Induction of aberrant $\text{G}\alpha_{12/13}$ driven signalling by overexpression or mutation of $\text{G}\alpha_{12/13}$ or of $\text{G}\alpha_{12/13}$ coupled GPCRs such as protease activated receptor 1 PAR-1 (*F2R*), or thromboxane A2 receptor TA2R (*TBXA2R*), have been shown to be transforming, and significantly increase the invasive ability of different cancer types^{163,164,181-184}. Of interest, $\text{G}\alpha_{12/13}$, $\text{G}\alpha_{12/13}$ -coupled GPCRs and their effectors including Rho, are overexpressed in several cancer types including breast, prostate, and hepatocellular carcinomas¹⁸⁵⁻¹⁸⁷. In particular, *GNA13* is upregulated in several solid tumors and its expression levels have been found to modulate drug resistance in squamous cell cancers, such as head and neck squamous cell carcinoma (HNSCC)¹⁸⁸⁻¹⁹⁰. *GNA12* also exhibits significant copy number gain in ovarian cancer, which is a cancer type characterized by generally few driver mutations⁵².

It is well established that several receptors for bio-active lipids including LPAR and S1PR (*S1PR2-5*), and chemokine receptors including CXCR4 can couple to $\text{G}\alpha_{12/13}$ and are involved in the metastatic potential of cancers^{155,187,191}. Either overexpression of these receptors, or their cognate ligands can create an oncocrine feed forward loop promoting proliferation and migration of cancer cells. Consistent with this, blockade of $\text{G}\alpha_{12}$ signalling using breast cancer mouse models has been found to significantly reduce the metastatic ability of 4T1 mouse breast cancer cells, and significantly increase the metastasis-free survival of mice¹⁶³.

Interestingly, the $\text{G}\alpha_{13}/\text{RhoA}$ signalling axis has been found to have a tumor-suppressive role in a wide range of hematopoietic and lymphoid malignancies including Burkitt's lymphoma, and Diffuse Large B Cell Lymphoma (DLBCL)¹⁹²⁻¹⁹⁴. COSMIC analyses have revealed that *GNA13*,

and its downstream effector RhoA are significantly mutated in these cancer types with mutations distributed across the gene body as compared to easily recognizable hotspot mutations, which tend to be activating in nature^{195,196}. Across TCGA PanCancer studies, *GNA13* appears to have a potential hotspot mutation at R200, although it has not been formally tested and annotated as such by OncoKB¹⁹⁷ (Figure 2D). This cluster of mutations appears exclusively in bladder urothelial carcinomas, where *GNA13* is significantly mutated (MutSig2CV q-value = 0.003); however, the function is currently unknown⁴⁷ (Table S3). G α 12/13 has been well-studied in the context of DLBCL where it is mutated in 7.32% of patients in TCGA (Table 1, Table S5). In particular, *GNA13* is the most frequently mutated gene in germinal center derived B-Cell lymphomas, caused by aberrant expansion and dissemination of a subset of B cells that are highly regulated and tightly confined to germinal centers in lymphoid tissues^{198,199}. Mutations in upstream effectors, including the G α 12/13 coupled including Sphingosine-1-phosphate-receptor-2 (*S1PR2*), and *P2RY8*, a putative G α 12/13-coupling orphan GPCR, or in downstream effectors, including ARHGEF1 have also been found^{90,198,200} (Table S1).

Indeed, functional characterization of the most commonly detected G α 13, RhoA, and lymphoma associated *S1PR2* mutants showed significantly reduced signalling and downstream transcriptional activity, which could be rescued by expression of wild-type proteins *in vitro* and *in vivo*^{90,198}. The mechanism by which inactivation of the G α 13 signalling pathway promotes development of lymphoma has not been fully characterized; however, several studies have found that suppression of the G α 13/RhoA axis leads to an increase in phosphorylated-AKT in B cells¹⁹⁸. Consistent with this, elevated pAKT levels can be detected in immunohistochemistry of DLBCL tumors, and high pAKT is associated with poor survival in DLBCL patients, suggesting that targeting the PI3K/AKT pathway may be a viable treatment option for patients with G α 13 loss²⁰¹.

Mutations affecting G $\beta\gamma$ and RGS signalling in cancer

Functional participants of G protein signalling, including Regulator of G protein Signalling (RGS) family proteins and $\beta\gamma$ subunits of the heterotrimeric G proteins can also play pro-oncogenic roles by modulating or potentiating G protein-driven signalling²⁰². RGS family proteins are physiological inhibitors of G proteins through their intrinsic GTPase-accelerating protein (GAP) activity, enhancing GTP hydrolysis by the G α -subunit, and promoting the reassembly and deactivation of the heterotrimeric G protein complex⁵. Recent pan-cancer analyses have revealed transcriptomic dysregulation and hundreds of mutations in RGS proteins, enriched for those leading to loss of function, thereby promoting G protein activity via a previously unanticipated tumor suppressive

role, and mechanism of G protein signalling potentiation^{203,204}. For example, RGS7 is recurrently mutated in ~13% of melanomas, where it promotes anchorage-independent growth, migration and invasion²⁰⁵ (Table S4). Similarly, a close homolog RGS6, has been found to possess tumor suppressive functions in breast and bladder cancers^{206,207}.

Currently, 5 G β subunits and 12 G γ subunits have been described, which can dimerize in numerous unique $\beta\gamma$ combinations. Once activated by GTP binding, the G α subunit dissociates from G $\beta\gamma$ dimers, enabling G $\beta\gamma$ dimers to regulate distinct signalling axes including the PI3K/AKT and MAPK pathways^{2,208-211}. Recurrent, activating mutations in G β proteins, *GNB1* and *GNB2* have been discovered in multiple cancers, namely in haematological malignancies where they induce aberrant activation of PI3K/AKT and MAPK pathways²¹² (Table 1, Table S4, Table S5). Strikingly, expression of patient-derived *GNB1* mutants were found to be transforming *in vivo* by induction of myeloid and B cell malignancies in mouse models²¹³. Of note, distinct mutations tend to cluster within cancer types based on cell lineage, suggesting that there may be lineage-dependent cellular predispositions determining the impact of specific G β mutations.

G $\beta\gamma$ subunits have been also been found to drive cell migration and metastasis. Along with PIP₃, G $\beta\gamma$ can directly bind to and synergistically activate the guanine-nucleotide exchange factor (GEF) PREX1, which is a stimulator of the small GTPase RAC²¹⁴⁻²¹⁶. In addition, G $\beta\gamma$ have been found to activate different PI3K isoforms, primarily PI3K β and PI3K γ , through interaction with both p110 and p101 catalytic subunits of PI3K to transduce signalling^{217,218}. Of note, expression of G $\beta\gamma$ has been shown to be necessary for the transforming capacity of PI3K β , where binding-defective p110 mutants have been demonstrated to significantly attenuate the transforming potential and GPCR-driven chemotaxis of cells^{219,220}. In line with these findings, expression of this mutant in breast cancer cells significantly inhibited extravasation, matrix-degradation and macrophage-stimulated invasion of tumor cells, highlighting the potential role of G $\beta\gamma$ in paracrine signalling between tumor and immune cells^{221,222}. In this context, there is an emerging body of literature demonstrating the importance of G $\beta\gamma$ signalling in tumorigenesis and metastasis of cancer, in part by modulation and promotion of a pro-metastatic tumor microenvironment^{223,224}.

GPCRs and Cancer Immune Evasion

The immune system is intricately linked to cancer initiation and progression, and as such, cancer immunotherapy is now one of the fastest growing areas of translational and clinical research for cancer treatment. Indeed, the functional roles of GPCRs in inflammation, cell trafficking, and the

diversity of ligands they bind, establish GPCRs as major regulators of the tumor-immune microenvironment.

Chemokine receptors were initially discovered in the context of immune cell migration, where expression of chemokines in different tissues and/or in response to infection and tissue injury act to direct and recruit a diverse range of innate and adaptive immune cells²²⁵⁻²²⁷. This process can be hijacked by cancer cells that express chemokines such as CCL17, CCL22 and others, to attract immunosuppressive immune cells including T-regulatory cells (Tregs), which specifically express the respective receptor CCR4, to promote a tumor permissive environment and dampen immune recognition²²⁸⁻²³⁰ (Figure 1). This can create a potent feed-forward control system where immune suppressive cells, such as myeloid derived suppressor cells (MDSCs), which are recruited by a host of chemokines such as CXCL8, CXCL1, and CCL2, can further inhibit T cell activation and migration to the tumor^{231,232}. Together, these processes can polarize macrophages, to the M2, or tumor associated macrophage (TAM) phenotype, further dampening the tumor-immune microenvironment²³³.

In tandem, the infiltration of cytotoxic immune cells providing an anti-tumor response is also dependent on a repertoire of chemokines and chemokine receptors. The infiltration of CD8+ T cells and natural killer (NK) cells has been shown to have high prognostic value and it is mainly guided by CXCR3, which binds the CXCL9 and CXCL10 ligands in the tumor microenvironment²³⁴ (Figure 1). Additionally, conventional dendritic cells (cDCs), namely, Batf3+ cDC1s, have been shown to be the main DC subtype responsible for taking up dead tumor cells and cross-prime T cells, and their function largely depends on trafficking to the tumor by XCR1^{235,236}. The exclusivity of XCR1 expression on cDC1s may make it an ideal target for boosting the dendritic cell antigen presentation response.

Many by-products of tumor cell metabolism are ligands that bind GPCRs, suggesting that they may also dictate the success of cancer immunotherapies. More specifically, studies have shown that lipid compounds, nucleosides (adenosine), and prostaglandins, some of the main products of ATP breakdown and inflammation, bind GPCRs to suppress cytotoxic function of immune cells^{237,238}. As our understanding of normal and aberrant GPCR signalling deepens, assessing levels of bioactive GPCR ligands, in addition to chemokine and chemokine receptor expression could also help guide clinical strategies. For example, increased CXCL9, CXCL10 and CXCL11 are associated with improved survival, and are part of an interferon-induced gene expression

profile that has been used as a clinical-grade assay to predict favourable responses to PD-1 blockade²³⁹.

Tumor-immune interactions are increasingly becoming the target of therapeutic agents intended to break the cross-talk that enables cancer progression. Currently, there are several therapeutics targeting chemokine receptors such CXCR4, CCR2 and CCR4, and the adenosine-binding A2AR receptor (*ADORA2A*) being explored for the treatment of cancer^{240,241} (Figure 1). For example, mogamulizumab, an FDA-approved monoclonal antibody targeting CCR4 is being used for the treatment of lymphomas as monotherapy and is being tested in combination with other treatments²⁴². Targeting GPCRs for their immunomodulatory functions in cancer present an exciting and promising field that has the potential to revolutionize how cancer is treated in the future.

GPCRs and Tumor Promoting Inflammation

Chronic inflammation has been consistently linked to cancer. In particular, prostaglandins (PG) and specifically prostaglandin E2 (PGE2) are key mediators of inflammation, generated by cyclooxygenase (COX) enzymes from arachidonic acid^{243,244}. Binding of PGE2 to their cognate GPCRs, EP1-4 (*PTGER1-4*), induces inflammatory responses tightly linking GPCR-driven signalling to inflammation that is well established to contribute to tumor development and progression (Figure 1). COX2 mediated production of PGs, in particular PGE2 has been found to promote tumorigenesis in a broad range of cancer types, with a prominent role in gastrointestinal cancers²⁴⁵⁻²⁴⁷. In the context of colorectal cancer, increased expression of PGE2 has been found to drive polyp formation, proliferation and cell motility²⁴⁸. This has been suggested in part, to be due to PGE2-driven activation of β-catenin, which may synergize with APC inactivation commonly found in colon cancer^{133,249,250}. COX-2 and PGE2 driven expression of pro-angiogenic chemokines can further enhance the tumor growth and progression^{251,252}. Chronic inflammation, via COX2 overexpression, can also lead to immune suppression and promote tumor growth (see above)²⁵³⁻²⁵⁶.

Overexpression of COX-2 itself has been shown to be sufficient to induce tumorigenesis and invasiveness in various models^{257,258}. Conversely, there is strong epidemiological data linking decreased mortality for colorectal carcinoma patients taking NSAIDs, which are inhibitors of both COX-1 and COX-2^{132,259}. Aligned with this, COX inhibitors are being explored in multiple human clinical trials for cancer prevention and as an adjuvant therapy, and have shown promising results in colorectal cancer and other cancer types²⁶⁰⁻²⁶².

GPCRs and Cancer Metastasis

Migration and metastasis of cancer cells are highly coordinated processes that are tightly regulated by numerous signalling and transcriptional events²⁶³. Signalling through G protein-coupled chemokine receptors is central to many of these processes and consequently, has been widely implicated in driving several aspects of cancer metastasis. Upon binding of their chemokine ligands, chemokine receptors (CXCRs) can stimulate interaction, migration and trafficking of both immune and cancer cells within and between tissues in the body^{4,264}. Both chemokines and bioactive lipids can also induce the secretion of matrix metalloproteinases (MMPs) by tumor and immune cells to facilitate tumor cell extravasation^{231,244,265-267}.

Chemokine receptor-ligand dynamics locally within the tumor microenvironment and more broadly, in a systemic fashion can strongly influence tumor behaviour. These dynamics can drive dysregulated autocrine and paracrine signalling loops in cancer collectively referred to as oncocrine signalling^{52,264}. Interestingly, chemokine receptors and ligands are often aberrantly expressed at common sites of metastasis, driving tissue tropism during metastatic spread²⁶⁸. This has been well studied in the context of breast cancer, where it has been found that the chemokine receptors CXCR4 and CCR7 are overexpressed²⁶⁹ (Figure 1). Interestingly, their respective ligands, CXCL12/SDF-1 and CCL21 are highly expressed in the main sites of breast cancer metastasis, such as bone marrow and lung, driving the metastatic profile of this cancer²⁶⁹. Indeed, CXCR4/SDF-1 signalling, has been found to potently drive breast cancer migration and metastasis^{187,270-272} (Figure 1). This signalling axis is so potent that tumoral expression of CCR7 is one of the most prominent predictors of metastasis and poor prognosis in several different cancer types²⁶⁸. Numerous other chemokine and chemokine receptor dynamics have been found to impact metastasis in different settings, including CCR9, CCR10, and CXCR5, in addition to playing complementary and significant roles in driving inflammation in the tumor microenvironment^{52,273,274} (Figure 1).

Targeting CXCR4/SDF-1 signalling using pharmacological inhibitors such as plerixafor, which is an FDA approved CXCR4 antagonist for the treatment of patients with non-Hodgkin's lymphoma, and multiple myeloma, has shown to be effective in numerous cancer types, supporting the therapeutic potential of targeting this signalling axis^{275,276}. In addition to the well-established role for CXCRs in metastasis, other classes of GPCRs have also been extensively shown to act as metastatic drivers, including PARs, LPAR, and S1PRs, and this list will likely expand as we gain a better understanding of GPCR-driven signalling networks^{266,277-280}.

Concomitant with this, there is mounting evidence supporting the role of GPCRs as mediators of the oncocrine signalling networks driving tumor growth and neural innervation, particularly in prostate and stomach cancers^{281,282}. For example, activation of the $\beta 2$ adrenergic receptor (*ADRB2*) by adrenaline and noradrenaline has been found to promote secretion of nerve growth factor (NGF) by both cancerous and pre-cancerous cells²⁸³ (Figure 1). Release of NGF and other neurotrophins potently promote axonogenesis and subsequent tumor innervation, which establishes a paracrine signalling axis via the secretion of noradrenaline by nerves in the tumor microenvironment. This creates a dynamic feed-forward signalling mechanism by acting on cancer cells, in turn, stimulating their growth, promoting resistance to therapy, and reinforcing the expression of NGF²⁸⁴. The autocrine activation of muscarinic receptors is a similar oncocrine signalling axis in prostate and other cancers^{285,286}. Enhanced secretion of acetylcholine leads to aberrant activation of the M3 muscarinic receptor (*CHRM3*), which has been shown to drive cancer cell proliferation and migration^{287,288}. Aligned with this, amplification or gain of the M1 and M3 muscarinic receptors have recently been found to correlate with worse progression free survival in prostate cancer patients, and is more frequent in castration-resistant prostate cancers, which are significantly more challenging to treat compared to hormone-sensitive tumors²⁸⁹. Strikingly, activation of M1 and M3 signalling has recently been found to induce castration-resistant growth of prostate cancer cells, through the activation of a conserved FAK-YAP signalling axis²⁸⁹. Pharmacological inhibition of FAK was found to prevent YAP activation and castration-resistant growth, representing a promising therapeutic strategy against innervation-driven castration-resistance growth of prostate cancer²⁸⁹.

GPCRs and Tumor Angiogenesis

Stimulation of angiogenesis is a key component of cancer progression for solid tumors. Angiogenic factors released by the tumor, including prostaglandins and thrombin, often act on GPCRs in the stroma and surrounding endothelial cells, such as S1PR (*S1PR1-5*), PAR1 (*F2R*), and CXCR4-7, to trigger stimulation of angiogenic mediators such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF)²⁹⁰⁻²⁹⁵ (Figure 1). Thrombin-mediated stimulation of PAR2, for example, induces expression and release of VEGF, increased vascular permeability, and cleavage of fibrinogen to create a favourable environment for vascularization^{267,296-298}. EP3 stimulation on endothelial cells in particular, can stimulate production of matrix metalloproteinases (MMPs), which degrade extracellular matrix proteins, enabling neovascularization²⁶⁵. Release of chemokines such as CCL2, CCL3, and CCL5 can act similarly on the stroma in addition to recruiting immune cells to drive angiogenic growth^{226,244,268}.

In particular, interleukin 8 (IL8), encoded by *CXCL8* and known to bind CXCR1 and CXCR2, is a potent pro-angiogenic and pro-inflammatory chemokine that can be released from the tumor, the stroma, and from neutrophils, which together can create a robust feed-forward mechanism to elicit angiogenesis^{228,299} (Figure 1).

These dynamics are typically fine-tuned processes with robust responses induced by minute expression of signalling factors. As such, it is no surprise that tumor cells must co-opt these complex signalling networks to stimulate vascularization and drive cancer progression. Anti-VEGF treatment using the monoclonal antibody bevacizumab is commonly incorporated into cancer treatment approaches³⁰⁰. Moreover, co-targeting the apelin receptor APLNR in addition to VEGF blockade has shown to be a promising strategy to improve antiangiogenic therapies, particularly in glioblastoma, where APLNR has been shown to have a proangiogenic role^{301,302} (Figure 1). The dependency of tumors on vascularization, and the centrality of GPCRs to several of aspects of angiogenesis have made them an attractive target for the cancer therapy toolbox³⁰³. Inhibition of PAR1, CXCR2, CXCR4, and angiotensin receptors AGTR1 and AGTR2 have shown promising results and are all under current investigation as antiangiogenic cancer therapeutics³⁰⁴ (Figure 1). Targeting GPCRs in combination with anti-VEGF drugs may be an effective strategy to extend the clinical efficacy of each treatment alone by preventing the development of drug resistance and bridging the gap for patients with refractory cancer types.

GPCRs and Genome Instability

DNA damage and repair mechanisms underly the ability of cells to acquire genetic alterations that can drive cancer initiation and progression. One exciting emerging field suggests that GPCRs and their regulated signalling systems can functionally participate in DNA damage response and repair systems. Lysophosphatidic acid (LPA), for example, has been shown to mediate enhanced survival and mitigate chemotherapeutic and radiation-induced DNA damage in the intestinal epithelium via engagement of LPA₁ (*LPA1*), and LPA₂ (*LPA2*) receptors³⁰⁵. LPA₂ in particular has a potent pro-survival effect against gastrointestinal radiation-induced injury by regulating DNA damage response and repair pathways^{306,307} (Figure 1). Damage to the intestinal epithelium is a frequent and dose-limiting complication of common chemo- and radio-therapies. While exogenous LPA is rapidly processed and metabolized in the gastrointestinal tract limiting its activity, synthetic and metabolically stable LPA mimetics may be used as protective agents to promote gastrointestinal integrity during chemo- and radio-therapy³⁰⁶.

CXCR4, best-studied for its role in metastasis and modulation of the tumor microenvironment, has also been found to regulate cell survival in ovarian cancer. Blockade of CXCR4 with a small peptide antagonist was not only found to inhibit metastasis but induced ovarian cancer cell death by weakening DNA damage checkpoints and leading to mitotic catastrophe³⁰⁸. The CXCR4/CXCL12 signalling axis has also been shown to protect hematopoietic stem cells (HSCs) from oxidative stress injury by reducing mitochondrial reactive oxidative species (ROS) and subsequent genotoxic stress³⁰⁹.

Chronic stress is another mechanism that has been linked to development of numerous pathological conditions including cardiovascular disease, inflammation, immunosuppression, and tumorigenesis, due in part to the persistent release of stress hormones and catecholamines. Intriguingly, the chronic activation of Gαs-coupled β2-adrenoreceptors by β-adrenergic catecholamines has been found to trigger DNA damage, which may underlie some of these effects³¹⁰. Specifically, recruitment and activation of β-arrestin-1 (*ARRB1*) by activated β2-adrenoreceptors was found to facilitate the AKT-mediated activation of MDM2³¹⁰ (Figure 1). Concomitant with the function of β-arrestin-1 as a molecular scaffold facilitating the binding of MDM2 to p53, this molecular mechanism altogether leads to the suppression of p53 levels and subsequent accumulation of DNA damage³¹⁰. This finding coupled with observations that activation of the Gαs/PKA signalling axis can lead to DNA damage via the generation of ROS, highlights the complex interrelationship between GPCR, G-protein and β-arrestin-mediated cellular dynamics controlling DNA damage and integrity³¹⁰.

The tumor suppressive capacity of GPCRs has also been linked to DNA protective effects. For example, inactivating polymorphisms in the melanocortin 1 receptor (*MC1R*) have been found to promote melanoma through an ultraviolet radiation independent mechanism³¹¹ (Figure 1). Indeed, disruptive germline variant alleles in the *MC1R* gene, collectively referred to as R alleles, are associated with significantly increased somatic mutation burden in melanoma³¹². Aligned with this, activation of MC1R by α-melanocyte-stimulating hormone (α-MSH) has an antioxidant effect by promoting the phosphorylation and stabilization of p53, and an increase in expression of base excision repair enzymes³¹³. In another case, *ADGRB1*, also known as BAI1, has been found to have potent antiangiogenic and antitumorigenic functions, particularly in brain tumors³¹⁴. Strikingly, BAI1 has been shown to prevent MDM2-mediated p53 degradation, and BAI1 loss leads to a significant decrease in p53 levels⁹⁸. The protective effect of BAI1 on p53 degradation is a remarkable illustration of the diverse roles that GPCRs can play in tumorigenesis (Figure 1).

GPCRs and Cell Death Resistance

Parallel to driving cancer initiation, GPCR and G-protein-driven signalling has been found to contribute to resistance to cell death, particularly driving chemoresistance in a broad range of cancers (Figure 1). One of the most outstanding examples has been in BCCs (see G α s and SMO sections above), which are driven by overactive hedgehog (HH) signalling through the SMO GPCR^{76,79,315}. Vismodegib, is an FDA-approved SMO inhibitor that is used in the treatment of advanced BCC; however, acquired resistance is a common challenge³¹⁶. Interestingly, while many cancer types are able to subvert multiple signalling pathways to maintain cancer progression in spite of targeted therapies, BCC have been found to be exclusively dependent on HH signalling for growth. As such, reactivation of HH signalling is a significant driver for BCC drug resistance³¹⁷. HH pathway inhibitors are under current investigation to combat BCC resistance to vismodegib.

GPCR signalling has also been found to drive resistance in melanoma. In an open reading frame (ORF) screen designed to assess drivers of resistance to MAPK inhibition in a BRAF V600E mutant melanoma cell line, GPCRs emerged as the highest ranked class of resistance-driving proteins³¹⁸. GPCR signalling-related proteins, such as guanine exchange factors (GEFs) RASGRP2-4 and VAV1 were also found to be potent effectors of resistance³¹⁸. Similarly, genome-scale approaches to determining drivers of resistance to BRAF inhibition in melanoma revealed that 4 of the top 10 hits were GPCRs: *GPR35*, *LPAR1*, *LPAR5*, and *P2RY8*³¹⁹.

Interestingly, both RGS proteins and G β γ subunits have been implicated as drivers of drug resistance to cancer therapy (Figure 1). Loss of RGS6 has been found to significantly impair doxorubicin-mediated cell death *in vitro*, and *in vivo*³²⁰. Similarly, loss of RGS10 and RGS17 have been found to contribute to chemoresistance in ovarian cancer³²¹. Mutations in G β proteins have also been found to be broad and potent drivers of drug resistance among several cancer types. In comparative whole exome sequencing of drug-sensitive and drug-resistant ETV6-ABL1-positive leukaemia cells, *GNB1* was among the most significantly mutated genes²¹². In particular, mutation of *GNB1* at K89 was found to drive resistance to tyrosine-kinase inhibitors independent of the targeted kinase by induction of PI3K and MAPK signalling. Outstandingly, in several different *in vitro* models, ectopic expression of patient-derived G β mutants but not wild-type G β , rendered cells resistant to targeted kinase inhibitors, including imatinib, nilotinib, and ruxolitinib, highlighting the potent effect of G β mutations²¹³. The broad induction of resistance to kinase inhibitors mediated by G β mutations may represent a previously underappreciated mechanism of

resistance that has yet to be fully explored clinically and mechanistically and could provide novel strategies for the treatment of drug-resistant cancers.

GPCRs and Deregulation of Cellular Energetics

Metabolic reprogramming, from the enzyme level to the global proteome, occurs in cancer to meet the metabolic demands underlying unrestrained cell growth. GPCRs are critical regulators of numerous components of normal metabolism, including glucose and glucagon homeostasis, insulin regulation, and lipogenesis, as well as being key players in adaptive metabolic mechanisms. Moreover, many metabolic products and by-products are the cognate ligands for GPCRs. As such, various metabolite-binding GPCRs play a significant role in tumor promotion through both autocrine and paracrine mechanisms that contribute to control tumor energetic dynamics⁵².

The lactate-binding GPR81 has been found to be significantly upregulated in numerous cancer types and to be a crucial driver of tumor growth and metastasis with further roles modulating the tumor microenvironment in angiogenesis and immune evasion^{322,323}. Similarly GPR91, which binds succinate, has significant roles in modulation of the tumor-immune microenvironment, as well as angiogenesis, together with its role in cellular metabolism³²⁴⁻³²⁶ (Figure 1).

Among the most common metabolic abnormalities in cancer, *de novo* nucleotide biosynthesis is often upregulated in order to support the metabolic demands associated with increased DNA and RNA synthesis. Strikingly, activation of the α 2A-adrenergic receptor (*ADRA2A*), and more broadly, Gai-mediated signalling has been found to regulate purinosome formation and downstream purine biosynthesis, representing an exciting mechanism by which GPCR-mediated signalling may promote aberrant cell proliferation³²⁷ (Figure 1). A by-product of injury, hypoxia, and cell death, the release of the nucleoside adenosine into the tumor microenvironment by the breakdown of adenosine triphosphate (ATP) is also a potent signalling regulator that plays a role in multiple physiological and pathological processes. There are four subtypes of adenosine-binding GPCRs with distinct expression patterns and G protein-binding specificities. $A_{2A}R$ (*ADORA2A*) and $A_{2B}R$ (*ADORA2B*) in particular, are well-known to mediate an anti-inflammatory and immunosuppressive effect thus diminishing the antitumor immune response, and highlighting the complex interplay between GPCR-mediated signalling between cancer hallmarks (see above)³²⁸.

Conclusions and Perspectives

GPCRs and their coupled heterotrimeric G proteins are central to a diverse array of cellular processes, including the activation of numerous signalling and transcriptional networks. Since the original discovery that G protein-mediated signalling has the potential to induce cell transformation, dysfunctional G protein-mediated signalling has been increasingly tied to cancer initiation and progression. Both mutation and aberrant expression are molecular mechanisms that contribute to subverting the normal function of GPCRs and heterotrimeric G proteins to conferring pro-oncogenic capabilities on them. However, given that GPCR signalling is centrally embedded in normal physiology, cancer cells can also modulate the function of GPCRs to promote an immune suppressive and a pro-oncogenic state through dysregulated paracrine and autocrine (oncocrine) or even compensatory signalling mechanisms.

While hyperactive GPCR and heterotrimeric G protein-driven signalling has often been found to be pro-oncogenic, a growing body of literature is supporting a paradigm of the cell context specific nature of such signalling pathways. Loss-of-function mutations or copy number loss of genes GPCRs and heterotrimeric G proteins have unveiled the tumor suppressive roles that these protein families play in certain cancer types, highlighting the complexity of G protein-driven signalling and the significant impact of cellular states in the integration and output of these signalling events. Further insight into the relationship between cell type lineage and the functional duality of GPCR signalling outcomes will likely reveal unanticipated mechanisms driving cancer, and potentially novel vulnerabilities that can be targeted therapeutically. Moreover, while GPCRs and their coupled heterotrimeric G proteins have been primarily studied from a signalling perspective, their central role in diverse cellular functions strongly suggests their involvement in broader processes such as the regulation of epigenetic networks. Future work defining these nodes of connectivity will likely expand our understanding of GPCRs in cancer even further.

Within the complex network of cancer signalling, numerous opportunities arise for GPCRs to be used for therapeutic intervention. Interfering with GPCR activity using both pharmacological inhibitors and biologics, encompassing antagonists, inverse agonists, allosteric modulators, in addition to antibody-based therapies, can inform novel therapeutic strategies. Specifically, positive and negative allosteric modulators (PAMs and NAMs, respectively), will likely emerge as pharmacological avenues for cancer prevention and treatment, as they may dial up or down the signalling capacity of GPCRs that are aberrantly modulated in cancer rather than directly stimulating or inhibiting them, or competing for their natural ligands. Guided towards the development of precision therapeutic approaches, more comprehensive characterization of dysregulated GPCR expression and signalling coupled with the diverse modes of action of these

inhibitors will likely reveal context-specific dependencies that can provide opportunities to rationally target GPCRs for cancer therapies.

Recent studies investigating the range and depth of biased GPCR signalling, and the potential impact of GPCR mutations in G protein-coupling selectivity, will likely also enable the development of novel approaches exploiting the tremendous potential of GPCRs as signal transducers to modulate signalling in cancer and other diseases. Moreover, advances and emerging techniques in the field of gene editing may also add to the repertoire of mechanisms by which we can modulate GPCR activity.

Given the large proportion of FDA-approved drugs that target GPCRs, there is mounting evidence supporting the utility of repurposing existing drugs to block or modulate GPCR-mediated oncogenic signalling circuitries, either alone or as adjuvant therapies. Regarding the latter, drug resistance has remained a major challenge in the era of precision medicine. GPCR modulators administered along with conventional anticancer agents may allow abrogating the initiation of compensatory signalling mechanisms and resultant drug resistance. Similarly, the dynamic influx of immune cells to the tumor, which leads to a highly infiltrative “hot” tumour or a poorly infiltrative “cold” tumour, is in part dependent on the chemokine gene signature of the tumour itself, which may provide a powerful prognostic tool to predict therapeutic response rates in the clinic. Harnessing the immunomodulatory power of GPCRs or their ability to regulate tumour-immune interactions could revolutionize current immunotherapies and result in durable clinical responses while preventing tumour relapse. Investigation exploring combining GPCR-targeted therapies with existing chemo- and immunotherapies either for the treatment of cancer, or as cancer preventative strategies are currently ongoing. Ultimately, just as we gain a clearer understanding of the complex mechanisms underlying cancer initiation and progression, so too will the role of GPCRs as cancer drivers and therapeutic targets become more important.

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Author Contributions

N.A. performed the data analysis, N.A. and J.S.G wrote and edited the manuscript.

Figure Legends

Figure 1. The Roles of GPCRs and heterotrimeric G Proteins in the Hallmarks of Cancer.

Highlighted are representative G proteins and GPCRs with well-established roles as drivers of each cancer promoting hallmark. The centrality of GPCR signalling to cellular processes within and outside the tumor microenvironment through dysregulated oncocrine signalling networks promotes the development and progression of cancer. GPCRs are in black, heterotrimeric G proteins are in blue, and signalling regulators (RGS and β-arrestin) are in purple. Refer to the text for details on each gene. Figure adapted from Hanahan and Weinberg, *Cell*, 2011²² and modified from SMART (Servier Medical Art), licensed under a Creative Common Attribution 3.0 Generic License.

Figure 2. Gα Mutation Distributions Across TCGA PanCancer Atlas Studies.

Lollipop plot depicting the non-synonymous mutation spectrum of A) GNAQ, B) GNA11, C) GNAS, and D) GNA13 across TCGA PanCancer studies. Missense mutations are depicted in green, and truncating mutations are depicted in black along the gene body. OncoKB annotated hotspot mutations are depicted in red below each gene. Pie charts represent distribution of cancer types where selected hotspots have been found. The data shown here are based upon data generated by the TCGA Research Network. All data was downloaded from cBio Portal^{130,131}.

Table 1. Mutation frequencies of heterotrimeric G protein families in patients across TCGA PanCancer Atlas studies.

Frequencies (left column) and number (right column) of patients carrying a non-synonymous mutation in one or more heterotrimeric G protein subunits in each indicated family. Cumulative mutation frequencies and absolute number of patients carrying a mutation in one or more G proteins in each indicated family across all 10,437 patients in all studies are listed in the last row. Number of heterotrimeric G proteins considered in each family are listed with each family name. The data shown here are based upon data generated by the TCGA Research Network. All data was downloaded from cBio Portal^{130,131}.

Table S1: Mutation frequencies of GPCRs by gene in patients across TCGA PanCancer Atlas Studies.

(Sheet 1) Frequencies represent percentage of patients carrying a non-synonymous mutation in each respective GPCR gene. Cumulative mutation frequencies of each gene across all 10,437 patients in all cohorts are listed in the last column. (Sheet 2) Enumeration of mutation frequencies in Sheet 1, depicting the number of patients carrying a mutation in each GPCR gene. Number of patients in each study are listed above with the study abbreviation. Absolute number of patients carrying a mutation in each GPCR gene across all 10,437 patients in all cohorts are listed in the last column. The data shown here are based upon data generated by the TCGA Research Network. All data was downloaded from cBio Portal^{130,131}.

Table S2: Compiled mutation frequencies of GPCR classes in patients across TCGA PanCancer Atlas Studies.

(Sheet 1) Frequencies represent percentage of patients in each study carrying a non-synonymous mutation in one or more GPCRs in each indicated class. Cumulative mutation frequencies of each class across all 10,437 patients in all cohorts are listed in the last row. Number of GPCRs considered in each family are listed with the family name. (Sheet 2) Enumeration of mutation frequencies in Sheet 1, depicting the number of patients carrying a mutation in one or more GPCRs in each indicated class. Absolute number of patients carrying a mutation in each indicated GPCR class across 10,437 patients in all cohorts are listed in the last row. The data shown here are based upon data generated by the TCGA Research Network. All data was downloaded from cBio Portal^{130,131}.

Table S3: MutSig2CV q-values for GPCR and heterotrimeric G protein genes across TCGA PanCancer Atlas studies.

(Sheet 1) MutSig2CV q-values for GPCR genes. (Sheet 2) MutSig2CV q-values for heterotrimeric G protein genes. Significantly mutated genes ($q \leq 0.1$) are labeled in red. Genes for which MutSig2CV q-values were not calculated are listed as NC (not calculated). (Sheet 3) DOI links to data analysis hosted on FireBrowse. All data were downloaded from the Broad Institute GDAC

FireBrowse (<http://firebrowse.org/>) and generated by the Broad Institute TCGA Genome Data Analysis Center (2016): Mutation Analysis (MutSig 2CV v3.1), Broad Institute of MIT and Harvard.

Table S4: Mutation frequencies of heterotrimeric G proteins by gene in patients across TCGA PanCancer Atlas Studies.

(Sheet 1) Frequencies represent percentage of patients carrying a non-synonymous mutation in each respective heterotrimeric G protein gene. Cumulative mutation frequencies of each gene across all 10,437 patients in all cohorts are listed in the last row. (Sheet 2) Enumeration of mutation frequencies in Sheet 1, depicting the number of patients carrying a mutation in each heterotrimeric G protein gene. Absolute number of patients carrying a mutation in each heterotrimeric G protein gene across all 10,437 patients in all cohorts are listed in the last row. The data shown here are based upon data generated by the TCGA Research Network. All data was downloaded from cBio Portal^{130,131}.

Table S5: Compiled mutation frequencies of heterotrimeric G protein families in patients across TCGA PanCancer Atlas studies.

(Sheet 1) Frequencies represent percentage of patients in each study carrying a non-synonymous mutation in one or more heterotrimeric G proteins in each indicated family. Cumulative mutation frequencies of each family across all 10,437 patients in all cohorts are listed in the last row. Number of heterotrimeric G proteins considered in each family are listed with the family name. (Sheet 2) Enumeration of mutation frequencies in Sheet 1, depicting the number of patients carrying a mutation in one or more heterotrimeric G proteins in each indicated family. Absolute number of patients carrying a mutation in each indicated heterotrimeric G protein family across 10,437 patients in all cohorts are listed in the last row. The data shown here are based upon data generated by the TCGA Research Network. All data was downloaded from cBio Portal^{130,131}.

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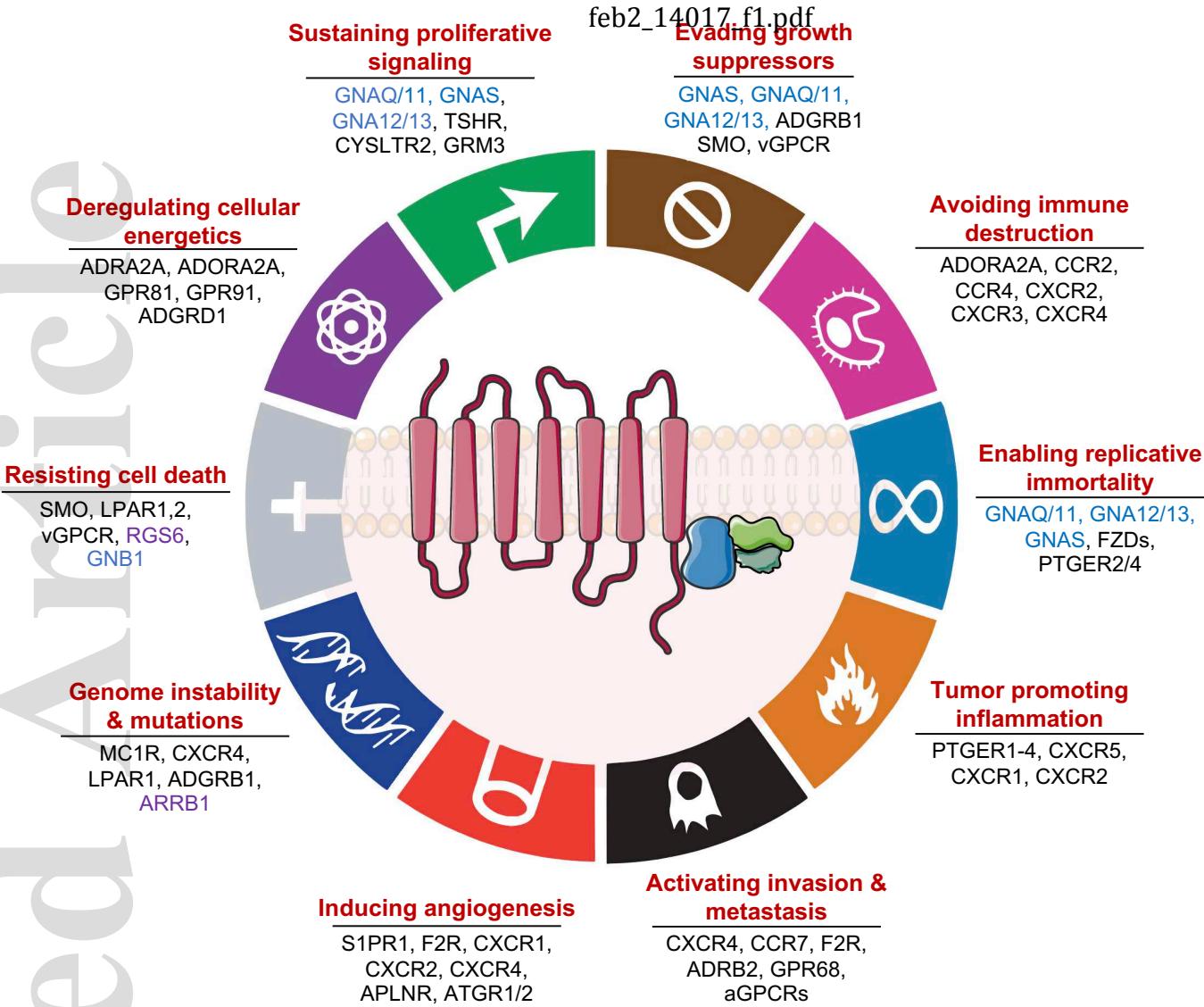
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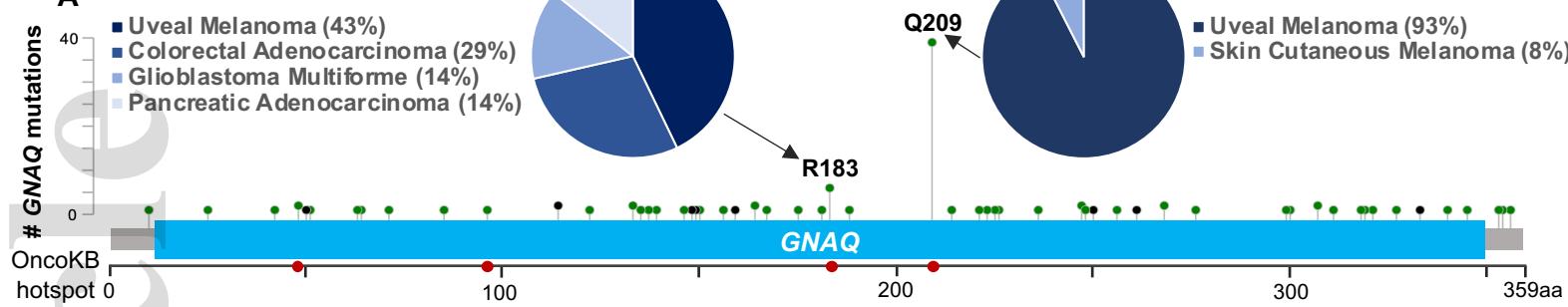
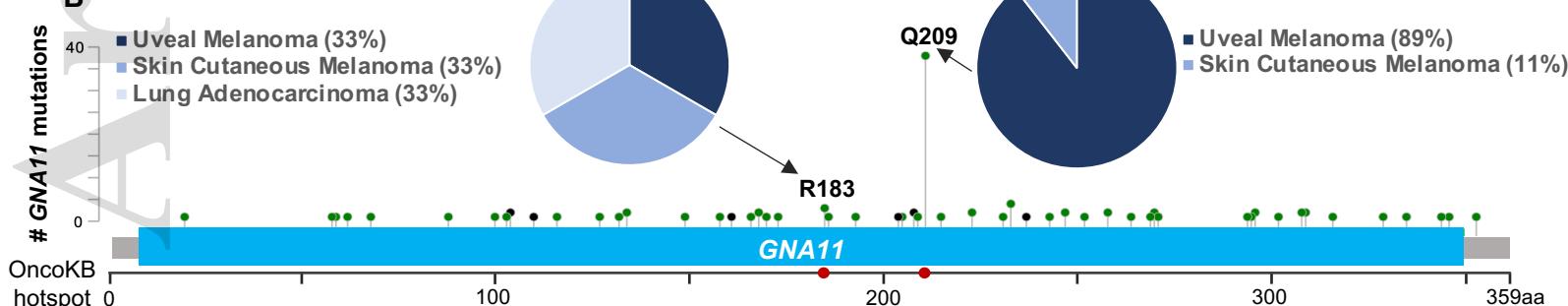
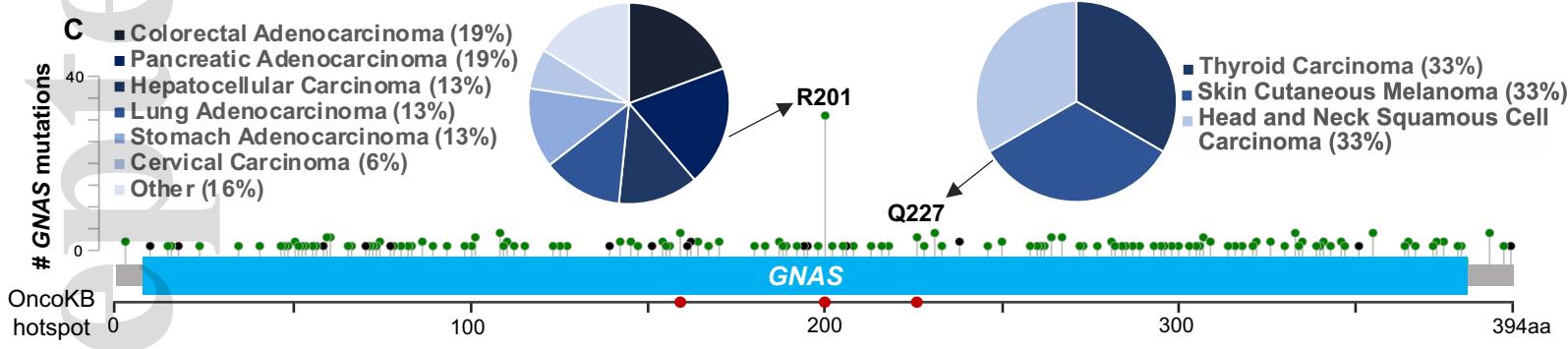
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| Heterotrimeric G Protein Family Mutation Frequencies | | | | | | | | | | | | | | | | |
|--|--|--------------|------------|--------------------|------|---------------------|------|--------------------|------|----------------------|------|-----------------|------|-------------------|------|-----|
| | TCGA PanCancer Atlas Studies | Abbreviation | Study size | G α q (n=4) | | G α 12 (n=2) | | G α s (n=2) | | G α i/o (n=8) | | G β (n=5) | | G γ (n=12) | | |
| | | | | % | # | % | # | % | # | % | # | % | # | | | |
| CNS | Glioblastoma multiforme | GBM | 397 | 0.76 | 3 | 0.50 | 2 | 1.01 | 4 | 1.76 | 7 | 0.50 | 2 | 1.01 | 4 | |
| | Brain Lower Grade Glioma | LGG | 512 | 0.78 | 4 | 0.00 | 0 | 0.39 | 2 | 1.37 | 7 | 0.39 | 2 | 0.20 | 1 | |
| Head and Neck | Head and Neck squamous cell carcinoma | HNSC | 515 | 1.75 | 9 | 0.19 | 1 | 3.30 | 17 | 3.50 | 18 | 3.11 | 16 | 1.17 | 6 | |
| | Thyroid carcinoma | THCA | 490 | 0.00 | 0 | 0.00 | 0 | 1.22 | 6 | 0.20 | 1 | 0.00 | 0 | 0.41 | 2 | |
| Endocrine | Adrenocortical carcinoma | ACC | 91 | 0.00 | 0 | 0.00 | 0 | 6.59 | 6 | 2.20 | 2 | 2.20 | 2 | 0.00 | 0 | |
| | Pheochromocytoma and Paraganglioma | PCPG | 178 | 0.00 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 | 0 | |
| Lung | Thymoma | THYM | 123 | 0.00 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 | 0 | 0.81 | 1 | 0.00 | 0 | |
| | Lung adenocarcinoma | LUAD | 566 | 1.94 | 11 | 1.24 | 7 | 4.77 | 27 | 4.77 | 27 | 2.83 | 16 | 4.06 | 23 | |
| Breast | Lung squamous cell carcinoma | LUSC | 484 | 2.27 | 11 | 1.45 | 7 | 3.10 | 15 | 5.37 | 26 | 2.69 | 13 | 1.86 | 9 | |
| | Breast invasive carcinoma | BRCA | 1066 | 1.22 | 13 | 0.56 | 6 | 1.03 | 11 | 1.69 | 18 | 1.03 | 11 | 0.28 | 3 | |
| Gastrointestinal | Esophageal carcinoma | ESCA | 182 | 1.10 | 2 | 0.00 | 0 | 5.49 | 10 | 2.20 | 4 | 1.10 | 2 | 1.10 | 2 | |
| | Stomach adenocarcinoma | STAD | 436 | 4.13 | 18 | 1.61 | 7 | 5.96 | 26 | 8.49 | 37 | 3.67 | 16 | 6.19 | 27 | |
| Genito-urinary | Colon adenocarcinoma | COAD | 534 | 5.06 | 27 | 2.06 | 11 | 5.24 | 28 | 7.68 | 41 | 5.62 | 30 | 4.31 | 23 | |
| | Cholangiocarcinoma | CHOL | 36 | 2.78 | 1 | 2.78 | 1 | 0.00 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 | 0 | |
| Genito-urinary | Pancreatic adenocarcinoma | PAAD | 179 | 1.12 | 2 | 0.00 | 0 | 5.03 | 9 | 1.12 | 2 | 1.68 | 3 | 0.00 | 0 | |
| | Liver hepatocellular carcinoma | LIHC | 366 | 1.09 | 4 | 1.09 | 4 | 1.64 | 6 | 3.28 | 12 | 2.19 | 8 | 0.55 | 2 | |
| Genito-urinary | Bladder Urothelial Carcinoma | BLCA | 410 | 2.20 | 9 | 3.66 | 15 | 3.41 | 14 | 6.34 | 26 | 3.90 | 16 | 2.93 | 12 | |
| | Kidney Chromophobe | KICH | 65 | 0.00 | 0 | 0.00 | 0 | 0.00 | 0 | 1.54 | 1 | 1.54 | 1 | 0.00 | 0 | |
| Reproductive | Kidney renal clear cell carcinoma | KIRC | 402 | 0.50 | 2 | 1.00 | 4 | 0.75 | 3 | 1.49 | 6 | 1.24 | 5 | 0.50 | 2 | |
| | Kidney renal papillary cell carcinoma | KIRP | 276 | 0.36 | 1 | 0.72 | 2 | 1.09 | 3 | 2.90 | 8 | 0.72 | 2 | 0.72 | 2 | |
| Reproductive | Cervical squamous cell carcinoma and endocervical adenocarcinoma | CESC | 291 | 3.44 | 10 | 1.37 | 4 | 4.81 | 14 | 5.50 | 16 | 3.09 | 9 | 0.69 | 2 | |
| | Uterine Corpus Endometrial Carcinoma | UCEC | 517 | 8.12 | 42 | 6.00 | 31 | 9.67 | 50 | 14.12 | 73 | 8.90 | 46 | 7.35 | 38 | |
| Melanoma | Uterine Carcinosarcoma | UCS | 57 | 1.75 | 1 | 0.00 | 0 | 3.51 | 2 | 3.51 | 2 | 1.75 | 1 | 0.00 | 0 | |
| | Ovarian serous cystadenocarcinoma | OV | 523 | 1.34 | 7 | 0.38 | 2 | 1.91 | 10 | 1.72 | 9 | 0.96 | 5 | 0.38 | 2 | |
| Hematological | Prostate adenocarcinoma | PRAD | 494 | 0.20 | 1 | 0.40 | 2 | 0.20 | 1 | 1.82 | 9 | 0.81 | 4 | 0.40 | 2 | |
| | Testicular Germ Cell Tumors | TGCT | 145 | 0.69 | 1 | 0.00 | 0 | 0.69 | 1 | 0.69 | 1 | 0.00 | 0 | 0.00 | 0 | |
| Other | Skin Cutaneous Melanoma | SKCM | 440 | 10.68 | 47 | 1.14 | 5 | 7.73 | 34 | 12.50 | 55 | 8.86 | 39 | 3.64 | 16 | |
| | Uveal Melanoma | UVM | 80 | 92.50 | 74 | 0.00 | 0 | 0.00 | 0 | 0.00 | 0 | 3.75 | 3 | 0.00 | 0 | |
| Hematological | Diffuse Large B-cell Lymphoma | DLBC | 41 | 0.00 | 0 | 7.32 | 3 | 0.00 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 | 0 | |
| | Acute Myeloid Leukemia | LAML | 200 | 1.00 | 2 | 0.00 | 0 | 0.50 | 1 | 0.50 | 1 | 1.00 | 2 | 0.50 | 1 | |
| Other | Mesothelioma | MESO | 86 | 0.00 | 0 | 0.00 | 0 | 2.33 | 2 | 1.16 | 1 | 0.00 | 0 | 1.16 | 1 | |
| | Sarcoma | SARC | 255 | 0.39 | 1 | 0.00 | 0 | 1.57 | 4 | 1.96 | 5 | 0.78 | 2 | 0.39 | 1 | |
| Cumulative | | | | 10437 | 2.90 | 303 | 1.09 | 114 | 2.93 | 306 | 3.98 | 415 | 2.46 | 257 | 1.73 | 181 |

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