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G Protein-Coupled Receptors: Undervalued Targets for Cancer Therapy

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Abstract

Despite the G protein-coupled receptors (GPCRs) being the largest family of signalling proteins at the surface of cells, their potential to be targeted in cancer therapy is still under-utilised. This review highlights the contribution of these receptors to the process of oncogenesis and points to some likely challenges that might be encountered in targeting them. The GPCR-signalling pathways are often complex and can be tissue-specific. Cancer cells hijack these communication networks to their proliferative advantage. The role of selected GPCRs in the different hallmarks of cancer is examined to highlight the complexity of targeting these receptors for therapeutic benefit. Our increasing knowledge of the mechanisms governing the molecular functions of GPCRs may help to identify new targets to treat specific types of cancers.

Keywords: G protein-coupled receptor, Cancer, signalling pathways, Signal transduction, Cancer hallmarks, Targeting receptors.

المستقبلات المقترنة ببروتين G أهدافًا لم تقدر بقيمتها الحقيقية في معالجه السرطان

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الخلاصة

على الرغم من ان البروتينات المقترنه مع بروتين) G والتي تدعى بمستقبلات (GPCR هي اكبر عائله من بروتينات الاشاره المتواجده على سطح الخلايا، الا ان امكانيه استهدافها في علاج السرطان لاتزال غير مستغله. تسلط هذه المراجعه الضوء على مساهمه هذه المستقبلات في عمليه تكوين الورم السرطاني وتشير الى بعض التحديات المحتمله التي قد نواجهها في استهدافها. غالبا ماتكون مسارات اشارات هذه المستقبلات معقده بعض الشيء ويمكن ان تكون لها خصوصياتها في بعض الانسجة. تختطف الخلايا السرطانيه شبكات الاتصال هذه لمصلحتها التكاثرية. في هذه المراجعه تم فحص دور بعضا من هذه المستقبلات في السمات المميزه للسرطان لادراك مدى تعقيد عمليه الاستهداف. قد تساعد معرفتنا المتزايدة حول الآليات التي تحكم الوضائف الجزيئية لهذه المستقبلات في تحديد اهداف جديده لعلاج انواع معينه من السرطانات

الكلمات المفتاحية : مستقبلات بروتينG ، السرطان، مسارات الاشارات، نقل الإشارة، السمات المميزة للسرطان، استهداف المستقبلات

Introduction

The guanine nucleotide-binding protein coupled receptors (GPCRs) constitute the largest family of receptors on the surface of human cells that are involved in signal sensing and transmission. These receptors can detect signals in the extracellular environment in the form of chemical molecules to which they bind, undergo conformational changes and pass the message to the inside of the cell for action and outcome. There are around 800 such receptors in humans with half of that number responding to exogenous ligands such as odour molecules and photons which are concerned with physiological functions like smell and vision [(1,2)]. The remaining number of receptors has endogenous ligands, hence referred to sometimes as endoGPCRs, and their number is estimated to be 401 [(3)]. These G protein-coupled receptors with endogenous ligands will be the focus of our review as some could be targeted for therapeutic interventions including the treatment of cancer.

The endogenous ligands include small molecules, peptides, hormones and neurotransmitters [(4,5)]. One GPCR can interact with more than one ligand. Moreover, one ligand can interact with more than one GPCR to create complex and diverse signalling pathways.

Signals received by GPCRs and relayed to the inside of the cell will regulate a multitude of physiological processes such as behaviour, mood, taste, smell, blood pressure, immune response, neurotransmission, metabolism, cell growth and differentiation [(6,7)]. These important receptors also mediate the effects of around 34% of all the drugs currently on the market [(1,8)] (Table 1.)

The GPCRs are also known as seven-transmembrane domain receptors (7-TM) because they contain sequence stretches in the shape of α-helices that transverse the bilayer membrane of the cells seven times and exhibit a high degree of calculated hydrophobicity [(9,10)].

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Table 1. Some common drugs targeting various G protein-coupled receptors (GPCRs)[114,115]

Common drugs	GPCR targeted	Condition
Alfuzosin, Terazosin	α1-adrenergic	benign prostate hyperplasia and hypertension
Bisoprolol, Betaxolol, Clonidine	α2-adrenergic	hypertension
Atenolol, Metoprolol	β1-adrenergic	hypertension
Nadolol, Albuterol	β2-adrenergic	asthma
Atropine	all muscarinic receptors M1, M2, M3, M4 and M5	bradycardia, hypersalivation, bronchial secretions and poisoning with cholinergic drugs
Tolterodine	M1,M2,M3,M4 and M5	overactive bladder
Scopolamine	M1	motion sickness, diarrhoea
Cetirizine, Loratadine	Histamine H1	allergies
Cimetidine, Ranitidine	Histamine H2	heartburns and gastrointestinal ulcers
Trazodone	5-hydroxytryptamine (serotonin) 5-HT, 5-HT1B	depression and anxiety
Sumatriptan	5-HT1D	migraine
Codeine, Fentanyl	mu	pain
Oxycodone	mu, kappa	pain
Misoprostol	prostaglandin E2	gastric ulcers
Montelukast	CysLT1	asthma
Haloperidol, Olanzapine	Dopamine D2	schizophrenia
Pramipexol, Ropinirole	Dopamine D2	Parkinson's disease, restless leg syndrome
Metoclopramide	Dopamine D2	nausea and vomiting

Other less widely used names include serpentine receptors, G protein-linked receptors and heptahelical receptors. The seven α-helices are each made up of 23±5 residues and connected by three extracellular and three intracellular loops of varying residue numbers (Figure 1.).

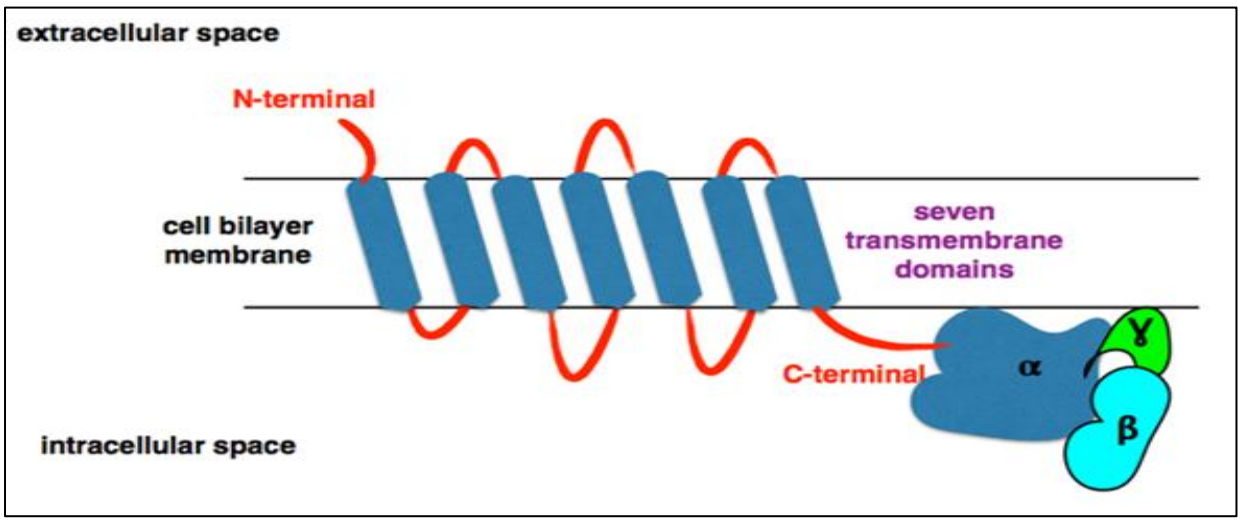

Figure 1. Depiction of the general structure of a typical G protein-coupled receptor showing the 7 transmembrane α-helices spanning the cell membrane and linked to the heterotrimeric G protein.

Each receptor has an extracellular amino-terminal and an intracellular carboxyl domain [11].

For a receptor to be classified as a GPCR it will have to satisfy two main requirements. The first is the presence of the seven-transmembrane structure and the second is the ability to interact with a guanine nucleotide-binding protein (G-protein) and together they make up the main features of GPCRs. The crystal structure of a representative GPCR is illustrated in Figure 2.

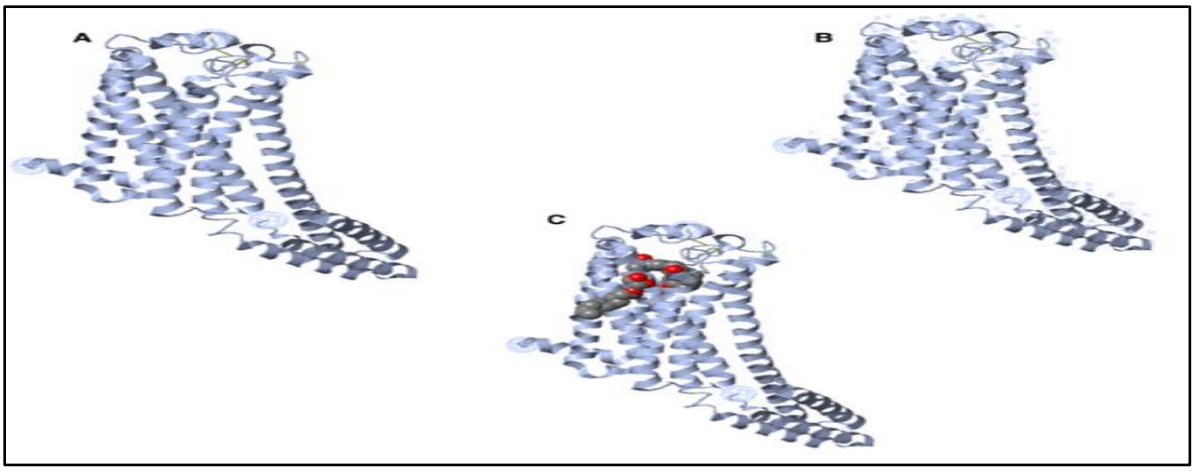

Figure 2. The crystal structure of the human Lysophosphatidic acid receptor 1 (A) illustrating the 7 transmembrane nature of these receptors, (B) with its constituents amino acids represented by their international one letter codes A-Alanine R-Arginine N-Asparagine D-Aspartic acid C-Cysteine E-Glutamic acid Q-Glutamine G-Glycine H-Histidine I-Isoleucine L-Leucine K-Lysine M-Methionine F-Phenylalanine P-Proline S-Serine T-Threonine W-Tryptophan Y-Tyrosine and V-Valine (C) The receptor being occupied by a ligand, shown as balls, called ONO-3080573. Baskets in the diagram indicate missing amino acids. **The structure was constructed using the visualisation software given in reference 110.**

There are more than 120 GPCRs with unknown ligands and these are termed orphan receptors [(12)]. Both orphan receptors and well-characterised receptors have been the subjects of several studies including targeting them for cancer therapy.

The G proteins signalling partners of the GPCRs are heterotrimeric proteins tethered to the inside of the cytoplasmic membrane. The three members of the heterotrimer are given the symbols α, β, and ϒ with 21 Gα proteins, 6 Gβ proteins and 12 Gϒ proteins in humans [(10,13,)]. The α member (i.e. Gα) being the largest of the trimers and itself is subdivided into four main subclasses based on their primary sequence similarity $G\alpha_s$, $G\alpha_i$, $G\alpha_q$ and $G\alpha_{12}$. Despite the large number and diversity of the heptahelical transmembrane receptors, they interact with a relatively small number of G proteins inside the cells (Figure 1.).

There are currently two classification systems for the GPCRs [(14)]. The first system is based on their sequence identity and classifies the human GPCRs into four main classes and these are:

- Class A (or 1) which are called *Rhodopsin*.
- Class B (or 2) which in turn is subdivided into B1 (*Secretins*) and B2 (*Adhesions*).
- Class C (or 3) which are called *Metabotropics/Glutamates*.
- Class F (or 6) which are called *Frizzleds/Smoothenes*.

Classes D (or 4) and E (or 5) are not being relevant for humans. The second system of classification is called the GRAFS system which is based on the phylogenetic origin of the receptor [(15)]. According to the GRAFS system, the human GPCR superfamily can be classified into five main families. These five families are the *Glutamate* family (22 members), the *Rhodopsin* family (701 members), the *Adhesion* family (33 members), the *Frizzled/taste2* family (11 members) and the *Secretin* family (15 members). The *Rhodopsin* family of GPCRs, as can be seen, is by far the largest group and includes receptors for neuropeptide Y, oxytocin, endothelin, melatonin, several olfactory receptors and many more. The GPCRdb (a G protein-coupled receptor databases website- see reference 3) lists 401 endoGPCRs (2*89 class A Rhodopsin, 15 class B1 Secretin, 33 class B2 Adhesion, 11 class F* Frizzled, 25 class T *Taste* and 6 others [(3)]. Of these 401 endoGPCRs 227 (56%) have not been targeted, 107 (27%) have been targeted with established drugs and 67 (17%) targeted in clinical trials. As can be seen, there is still an enormous potential to exploit the remaining, un-targeted, GPCRs for therapeutic benefit including the treatment of cancer.

Signalling mechanism

The signalling pathway detailed below is the commonly observed mechanism although alternative routes were reported.

In response to agonist ligand binding to the extracellular domains, GPCR undergoes ligand-specific conformational changes leading to the recruitment and activation of heterotrimeric G-proteins composed of three subunits namely α, β and ϒ [(16,17,18)]. Antagonist ligands are thought to block the conformational change in the receptor and hence stop the signal transduction [(19)]. In the inactive basal state, the α subunit of the heterotrimer is tethered to the inside of the plasma membrane and bound to Guanosine diphosphate (GDP) and the other two subunits of the G protein complex. Activation of this complex by its partner GPCR stimulates the exchange of guanosine triphosphate (GTP) instead of guanosine diphosphate (GDP) by the α subunit

(Gα) of the G-protein [20,21,22,23]. The GTP-bound α subunit will then dissociate from the complex leaving behind the β and ɣ subunits bound together as a dimer. Both the GTP bound Gα and the remaining bound β and ɣ subunits (Gβɣ) will then go on to modulate several signalling pathways by interacting with several downstream effector molecules [24]. The baseline signalling pathway of a GPCR is shown in Figure 3.

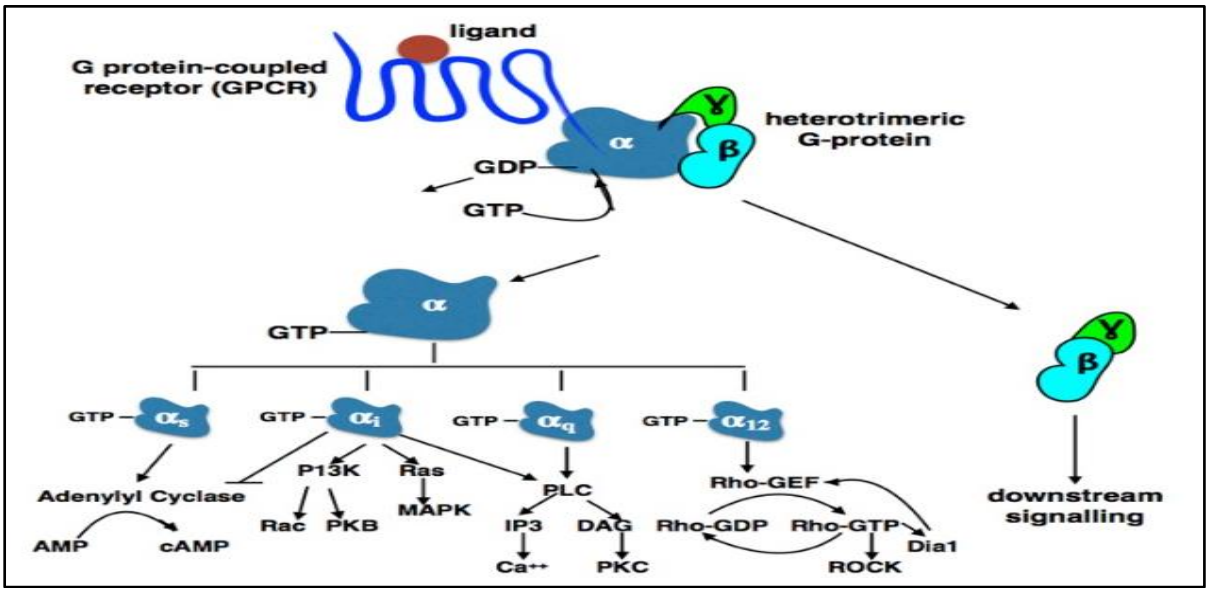

Figure 3. Baseline signalling pathways of GPCRs. GDP-guanosine diphosphate, GTP-guanosine triphosphate, AMP-adenosine monophosphate, cAMP-cyclic adenosine monophosphate, PI3K-phosphatidylinositol-3-kinase, Rac- a member of the Rho family of GTPases, PKB- protein kinase B also known as Akt., Ras- family of small GTPases proteins, MAPK- mitogen-activated protein kinase, PLC-phospholipase C, IP3-ionositol triphosphate (second messenger), DAG-diacylglycerol (second messenger), PKC-protein kinase C, Rho-Ras homologous protein, GEF-guanine nucleotide exchange factor, Dia1-a forming protein that elongates unbranched actin, ROCK-Rho-associated protein kinase. → (activation) ⊥ (inhibition)

Generally, the type of G-protein recruited by the receptor dictates the signalling pathway. Some GPCRs, such as the receptors for Lysophosphatidic acid (LPA), can couple with both G_i and G_q subtypes thus triggering dual signalling cascades [25] and the thyroid-stimulating hormone receptor (TSHR) can couple to all four subtypes of the G protein activating multiple pathways [26,27,28,29]. Other GPCRs can couple to only one G protein subtype e.g. the receptors for Sphingosine-1-phosphate (S1P) as will be discussed later.

The GPCRs can adopt different active conformational states depending on the nature of the ligand and are thus able to select a specific signalling pathway, a phenomenon known as biased signalling [26]. A biased ligand can selectively lead to an active receptor state for one particular signalling pathway out of many.

During the last two decades, it became apparent that certain GPCRs can also trans-activate Receptor Tyrosine Kinases (RTKs) such as the Epidermal Growth Factor Receptor (EGFR) through either ligand-dependent or ligand-independent mechanisms [30,31]. In the ligand-dependent transactivation, ligand precursors for RTKs are generated following the activation of GPCRs. However, in the ligand-independent transactivation, the cross-talk between GPCR and RTK creates a complex to trigger an RTK downstream signalling through activated G-protein. Cross-talk between acetylcholine muscarinic receptors (mAChRs) and EGFR or platelet derived growth factor receptor (PDGFR) lead to the activation of mitogenic pathways. Also, several GPCR ligands like LPA and Bradykinin can trans-activate EGFR[32,33].

Physiologically, the activation of the GPCRs must be followed by the removal of the signal when it is not required. Gα has an intrinsic guanosine triphosphatase (GTPase) activity that is capable of hydrolysing GTP back to GDP, aided by other proteins, and returning this subunit to its inactive state of being bound to GDP. This GDP-bound Gα will then re-associate with Gβɣ to form the heterotrimeric G-protein once again thus terminating the signal. The ligand-occupied receptor itself will normally undergo desensitisation and internalisation mediated by phosphorylation with G protein-coupled receptor kinases (GRKs). The phosphorylation facilitates the recruitment of a small family of proteins called β-arrestins to the receptor where it could sterically hinder its conformation and aids the dissociation from the heterotrimeric G-protein. Thus β-arrestins, via their interaction with Clathrin and the adapter protein AP-

2, target the receptor for vesicle-mediated endocytosis [31].

Beta (β)-arrestins themselves are also capable of initiating a signalling cascade independent of G-proteins thus creating an alternative (biased) signalling pathway and illustrating the complex role that β-arrestins play not only in the desensitisation of GPCR but also in initiating an independent signalling pathway [34].

GPCR signalling is diverse and not only dependent on the GPCR itself but also on the type of G-protein they couple with as well as the ligand, the strength and duration of the signal and the cellular environment. Over the past few decades, GPCRs became the target of a variety of drugs from a wide range of clinical applications as can be seen in Table 2 and Figure 4.

Table 2. Range of drugs targeting selected G protein-coupled receptors (GPCRs)[112-115]

GPCR	**Drugs and other molecules that target the listed GPCR**
Thyroid-stimulating hormone receptor (TSHR)	Ergoloid Thyroid-stimulating hormone (TSH) Thyrotropin alpha Thyrotropin
Smoothened (SMO)	Carbozantinib Fluocinonide Halcinonide Sonidegib Vismodegib
Lysophosphatidic acid receptor 1 (LPAR1)	None currently
Frizzled (FZD)	None currently
Melanocortin 1 receptor (MC1R)	Adrenocorticotropic hormone (ACTH) Afamelanotide
Chemokine receptor 4 (CXCR4)	Plerixafor
Chemokine receptor 5 (CCR5)	Maraviroc Vicriviroc Leronlimab
α2A-adrenergic receptor (ADRA2A)	Adrenaline Aminosalicylic Acid Amitriptyline Amoxapine Amphetamine Apomorphine Apraclonidine Aripiprazole Aspirin Atropine Benzphetamine Bethanidine Brimonidine Bromocriptin Cabergoline Carvedilol Chlorpheniramine Chlorpromazine Clonidine Clozapine Cortisone Acetate Desipramine Dexmedetomidine Dicyclomine Dihydroergotamine Diphenoxylate Dipivefrin Dobutamine Doxepin Dronedarone Droxidopa Echothiophate Ephedra Epinastine Epinephrine Ergoloid mesylate Ergotamine Fenoldopam Guanabenz Guanfacine Hydrocortisone Phosphoric Acid Levonordefrin Lisuride Lofexidine Loxapine lurasidone Maprotiline Mechlorethamine Mephentermine Methamphetamine Methohexital Methotrimeprazine Methyldopa Mirtazapine Moxonidine Naphazoline Nefazodone Noradrenaline Nortriptyline Olanzapine Oxycodone Oxymetazoline Paliperidone Paramethadione Pergolide Phenoxybenzamine Phentolamine Phenylpropanolamin Pramipexole Prazosin Propericiazine Pseudoephedrine Quetiapine Quinidine Reserpine Risperidone Ropinirole Spironolactone Sulfasalazine Terguride Tizanidine Tolazoline Trazodone Triamcinolone Acetonide Trihexyphenidyl Trimipramine Xylometazoline Yohimbine Ziprasidone Zuclopenthixol
Sphingosine-1-phosphate receptor 1 (S1PR1)	Fingolimod Asfotase Alfa
Adhesion G protein-coupled receptors (aGPCR)	None currently

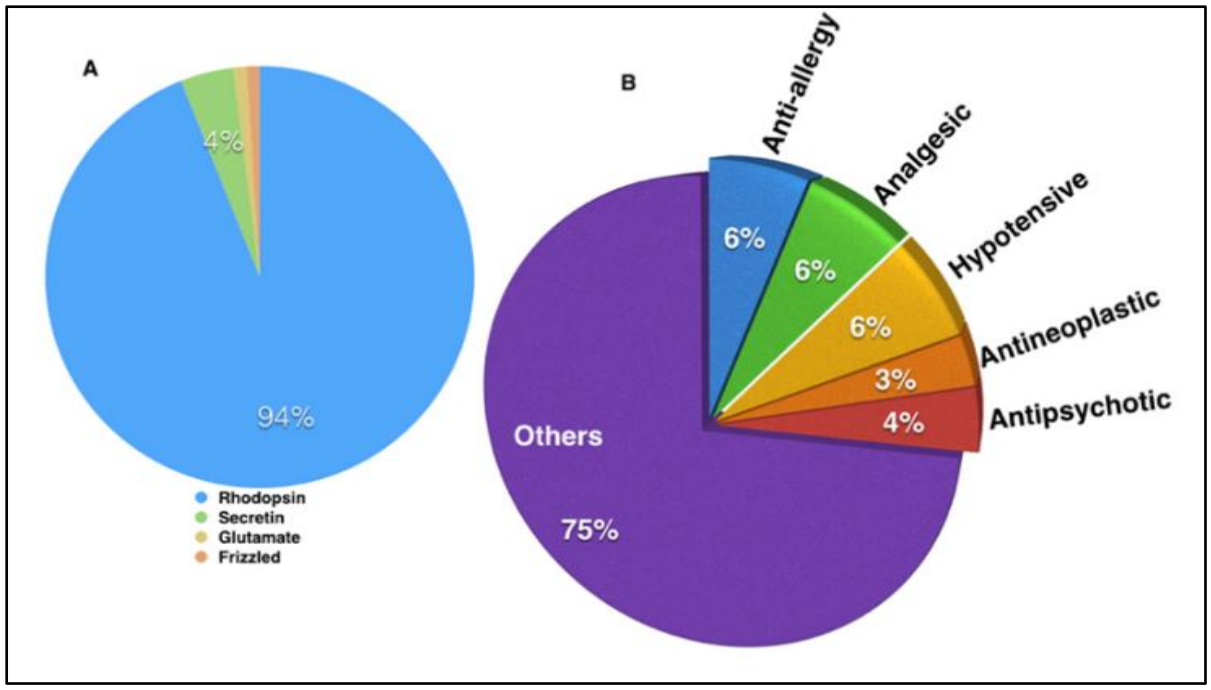

Figure 4. (A) The relative percentages of the four major subfamilies of G protein-coupled receptors. (B) The percentages of G protein-coupled receptors that target selected classes of conditions.Data were extracted from references 3 and 111.

However, targeting these receptors for the treatment of cancer appears to be lagging partly due to the complexity of this disease and the GPCRs' signalling pathways. Due to this complexity, we will restrict our discussion in this review to the targeting of the G protein-coupled receptors themselves rather than the other components of the signalling pathways and how that might affect aspects related to the initiation and progression of cancer.

G protein-coupled receptors and cancer

Enormous progress has been made since the discovery last century that certain viruses can hijack cellular genes, incorporate them into their genome and subsequently transfer them back to the host upon re-infection and potentially cause tumours [(35)]. We understand now that several viruses possess genes encoding GPCRs sharing a degree of homology to the human cytokine receptors with several substitutions conferring constitutive activity and enhanced coupling to G proteins. Viral genomes also express agonists and antagonists to cellular GPCRs to promote their survival and replication [(36)]. These ligands can modulate host cellular immune response and resistance to death thus promoting tumorigenesis. Kaposi sarcoma-associated herpesvirus (KSHV) encodes a GPCR that resemble cytokine receptors CXCR and CXCR2 and the human cytomegalovirus (HCMV) expresses at least four GPCRs. Moreover, all herpesviruses of the β and ϒ families have hijacked GPCR genes, almost certainly from a previous infection, and adapted them to their use [(37)]. These genes are then expressed via cellular machinery, following viral integration with host DNA, to subjugate the cell to their advantage.

It is now recognised that cancer usurps a subset of normal cellular genes and derails their function, through various genetic or epigenetic alterations. Many genes participate in cell growth, division, differentiation, programmed cell death, immune function and many more tasks that are exquisitely balanced to keep an overall check and control [(38,39,40)]. However, once that balance is disturbed, e.g. because of a mutation in a relevant gene, tumorigenesis can be initiated and then progressed upon the accumulation of further deleterious mutations. A study in 1986 represented early evidence of the involvement of GPCRs in oncogenesis [(41)]. A GPCR termed MAS1 was shown to induce the formation of foci when expressed in NIH3T3 fibroblasts (mouse cell line employed widely in research) and lead to the formation of tumours in the nude mouse. This suggested an oncogenic potential for the GPCR encoded by *the MAS1* gene that is not directly linked to viral infection. Subsequent work showed that the amount of ligand available and its binding specificity were key determinants of the oncogenic potential of the receptor [(37,41)]. Later on, it was demonstrated that mutations play a key role in turning other GPCRs oncogenic. Moreover, the discovery that some GPCRs are over-expressed in a wide range of different cancers highlighted the important role that these receptors play in the initiation and progression of cancer [(42,43)].

Altered expressions and mutations are now thought to play a pivotal role in converting a normally functioning GPCR into being cancerous. Over-expression of GPCRs or their autocrine/paracrine activation by an agonist, released by the tumour or the surrounding stromal cells, represents the most frequent tactic used by tumour cells to stimulate GPCR signalling to their advantage [44,45,46].

Although many G protein-coupled receptors have been implicated in carcinogenesis when they acquire an oncogenic potential, only a few have been targeted and exploited for the treatment of cancers leaving enormous opportunities for future drug discoveries [47]. Numerous studies linked aberrant GPCR activation or expression to different types of tumours. Tumour initiation, progression and metastasis involve many signalling networks mediated by GPCRs. Dysfunctional GPCRs signalling pathways and mutations in GPCR genes can be relevant for tumorigenesis. The understanding of the activation and signalling pathways of G protein-coupled receptors is fundamental to the design of targeted drugs for cancer [48,49].

The publication of the six hallmarks of cancer in the year 2000, and the later revision and extension in the year 2001 to ten hallmarks, has provided scientists and molecular biologists with a framework and a context to understand the process of carcinogenesis [50,51]. GPCRs impact these hallmarks with considerable overlap of the individual mechanisms involved due to the wide range of effects they exert and the network signalling cascades cross-talks. It is with that in mind that the following sections of this review will try to examine the role of selected GPCR players in the ten hallmarks of cancer.

GPCRs and sustaining proliferation

Cells have to maintain communication for the multicellular organism to have overall control over tissue architecture and function. Cells are required to grow, divide and increase in numbers when needed, such as during development, wound healing and to replace lost cells and required to stop when these processes are no longer necessary. To do that, individual cells communicate using chemical signals carrying the instructions to proceed through the cell cycle and proliferate. Cancer cells are capable of deregulating these chemical signals and divide out of the strict overall control [51]. A few GPCRs are known to be deregulated in various cancer types and the example that I have selected here is the thyroid-stimulating hormone receptor (TSHR) [52,53].

The TSHR is composed of 764 amino acids and encoded by a gene (*TSHR* gene) on chromosome 14. The receptor belongs to the *Rhodopsin* family of GPCRs with over half of its secondary structure located in the N-terminal domain and housing several cognate recognition sites for the thyroid - stimulating hormone (TSH). TSHR is predominantly expressed by thyroid cells and there is an estimated 5000 such receptors per cell. It responds to ligand binding by coupling to either Gαs or Gαq of the heterotrimeric G protein. Activation of Gαs stimulates adenylyl cyclase and cAMP/protein kinase A (PKA) pathway with their well-established mitogenic effects. Whereas activation of Gαq results in the stimulation of protein kinase B (PKB) and the mitogen-activated protein kinase (MAPK) pathway. The net resulting downstream effect is to increase thyroid hormone production.

The current strategy in reducing the mitogenic effect of TSH through TSHR signalling is to induce a state of systemic hyperthyroidism by the administration of Levothyroxine [53]. Hyperthyroidism will suppress the endogenous production of TSH and hence reduces its tumorigenic effect on the thyroid cells. However, systemic hyperthyroidism is associated with accelerated bone loss and cardiac side effects and alternative strategies are needed to reduce the mitogenic impact of TSH on thyroid cells. The alternative approach in treating certain thyroid cancers might be in the form of the selective targeting of TSHR using small molecule inhibitors to block its signalling. Several small molecules have been identified and trialled recently but none so far have been found to achieve sufficient specificity and further work is ongoing.

Other GPCRs involved in sustaining and promoting cancerous growth include the cysteinyl leukotriene receptor 2 (CYSLTR2) and the metabotropic glutamate receptor 3 (GRM3). The CYSLTR2 was shown to be associated with the development and progression of melanoma [54] while GRM3 was implicated in B-cell tumour apoptosis [55].

GPCRs and evading suppression

The growth signals driving cells to proliferate have to be attenuated to prevent the continuous uncontrolled division and ensure homeostasis. Defects in these negative feedback mechanisms can tip the balance in favour of the proliferative signals. G proteins, for example, possess the exchange of GTP for GDP as their negative feedback switch to terminate the transduction of signalling activated by ligand binding to their appropriate coupled 7-transmembrane protein. The GPCRs themselves can act as a growth suppressor and mutations disabling that function could lead to cancers as in the case illustrated here by a receptor called Smoothened (SMO).

Smoothened is an atypical GPCR with a central role in transducing the famous Hedgehog (Hh) signalling. It is one of the *Frizzled* family of receptors and consists of 787 amino acid residues [56]. The involvement of SMO in the Hedgehog signal

transduction pathways has been the subject of several studies [57,58,59,60]. In its canonical role, the activation of Hedgehog starts when another protein called Patched (PTCH) releases its suppressive action on SMO. This allows SMO to activate a complex signalling cascade that leads to the transcription of oncogenes and the development of cancer [60,61]. On the other hand, when Patched is repressing the action of SMO, target oncogenes are not transcribed. As can be seen, this signalling cascade does not involve the coupling with G proteins hence it was labelled atypical GPCR. However, in a separate non-canonical cascade SMO associates with heterotrimeric proteins and stimulates Rac1 (a member of the Rho family of GTPases) and RhoA (a GTPase protein A) leading to enhanced migration of fibroblasts and the consequent influence on the tumorigenesis process [61].

Mutations in SMO that increase its activity are associated with a higher incidence of basal cell carcinomas (BCC) and medulloblastomas. Therefore, targeting SMO by inhibiting its activity appears to be an attractive therapeutic option. Vismodegib became the first SMO antagonist to be marketed for the treatment of BCC [62,63] (Table 3.). Acquired resistance to Vismodegib action can be problematic if patients develop mutations in motifs of the receptor that bind to the drug. SMO also plays an important role in another hallmark of cancer namely resisting cell death.

Table 3. Drugs targeting G protein-coupled receptors (GPCRs) for the treatment of cancer[111,114,115]

Drug	GPCR target	Indications
Degarelix (Firmagon®)	GnRH1 class A- Rhodopsin	Advanced hormone-dependent prostate cancer
Lanreotide (Somatuline Autogel®)	SSR2 class A- Rhodopsin	Acromegaly Neuroendocrine tumours
Mogamulizumab (Poteligeo®)	CCR4 class A- Rhodopsin	Mycosis fungioides (MF) Sezary syndrome (SS)
Plerixafor (Mozobil®)	CXCR4 class A- Rhodopsin	Lymphoma Multiple Myeloma
Sonidegib (Odomzo®)	Frizzled class F- frizzled	Basal cell carcinoma
Vismodegib (Erivedge®)	SMO class F-frizzled	Basal cell carcinoma

GPCRs in resisting cell death

The balance between the number of cells dividing and dying at any one time is intricately controlled by programs of cell death, the most important of which is called apoptosis. Apoptosis machinery is present in a latent form in all cells and triggered by a variety of stimuli such as DNA damage, hypoxia and signal imbalances. Many GPCRs play a role in apoptosis among them are the receptors for Lysophosphatidic acid (LPA).

The Lysophosphatidic acids (LPAs) are simple lipids involved in virtually all of the hallmarks of cancer. The receptor for LPA (i.e. LPAR) was first identified and cloned back in 1996. There are currently six LPARs numbered 1 to 6 ranging in amino acid content from between 344 to 372 and the most important of them is LPAR1. An important challenge in targeting LPAR1 in cancer therapy is the complex array of heterotrimeric G proteins it can couple with to give rise to various outcomes. LPAR1 can couple with three types of Gα proteins namely $G\alpha_i$, $G\alpha_q$ and $G\alpha_{12}$ which initiates downstream signalling through PLC, MAPK and Rho (see Figure 3.). Its activation leads to several cellular responses including proliferation, migration, Ca^{++} mobilisation and adenylyl cyclase inhibition [64,65]. The role of LPA in protecting epithelial cells from programmed death and apoptosis induced by Cisplatin in cervical cancer cells has been investigated [66].

Although targeting LPA signalling appears to be an attractive option for cancer treatment, it is unlikely that this approach will be effective as a monotherapy. Previous studies showed that LPA is a very strong inducer of cell migration, invasion and metastasis but a weak stimulant of proliferation. Growth factors such as epidermal growth factor (EGF) and insulin-like growth factor (IGF) possess strong cell-proliferative effects but weak chemotactic activity. Therefore, a combination of these two approaches, i.e. targeting LPAR1 and a growth factor, may represent an effective treatment strategy.

GPCRs in enabling cell immortality

Normal cells can replicate an essentially limited number of times and it is defined by two main barriers which are hard-wired into our cells to prevent unnecessary proliferation. The first barrier is senescence, which is usually an irreversible entry into a non-proliferative but viable state, and the second is cell crisis leading to cell death. Cancer

cells, however, have to overcome these barriers to continue to replicate and emerge as "immortal" cells. An example of immortal cells that are commonly used in laboratories is HeLa cells which were first isolated from the cervical cancer of a woman in 1951 and are still replicating after passing through so many rounds of cell divisions [67].

The Wnt (a symbol derived originally from wingless integration) signalling pathway is a major player in the enhancement of cell proliferation thus increasing the chance for cells to break the restrictions imposed by the two barriers mentioned earlier [68,69,70]. *Frizzled* receptors (FZDs) are a family of proteins that serve as receptors for the Wnt ligand proteins and as such, they play a crucial role in cell proliferation [71]. The FZDs family of receptors consists of 10 members numbered 1-10. Their cognate ligands, the Wnt group of proteins, consist of 19 members. Each FDZ receptor can interact with several Wnt ligands to activate multiple downstream pathways including the canonical Wnt/β-catenin cascade. It is possible that dimerization of the Frizzled receptor may lead to Wnt signalling through the β-catenin path rather than the non-canonical signalling alternatives [71,72,73]. Members of the *Frizzled* receptors contain amino acids ranging in number from 500 to 700 depending on the subfamily member. They are considered a novel class of GPCRs even though there is a lack of experimental evidence of their interactions with G proteins. Nevertheless, there are ample indirect evidence and bioinformatics predictions pointing to the existence of such interactions. In the FZD/β-catenin pathway, Wnt stimulation of FZD receptor results in the activation of another protein calls Dishevelled (DVL) leading to the inhibition of the constitutively active glycogen-synthase kinase 3 (GSK3) within a destructive complex consisting of adenomatous polyposis coli (APC) and Axin which regulates the phosphorylation and destruction of β-catenin. The spared β- catenin is then translocated to the nucleus to aid the transcription of genes involved in cell proliferation [74].

Over-expression of FZDs is often encountered in various cancers pointing to their involvement in carcinogenesis. Therefore, targeting FZD receptors will be a potential approach for cancer treatment. Targeting an aberrantly over-expressed FZD at the receptor level may enhance the therapeutic advantages in Wnt-driven cancers. Approaches to target FZD receptors can include small molecules, short peptides and antibodies (Table 3.). However, the safety of these molecules needs to be carefully assessed as there are still moderate expression levels of FZDs in non-cancerous tissues.

GPCRs and genome instability

Genome instability is one of the enabling characteristics of cancer cells to thrive and proliferate. Through the deregulation of genes collectively referred to as "caretakers", neoplastic cells can facilitate their growth and proliferation. To counteract this, our body is endowed with detection and maintenance systems capable of correcting the vast majority of DNA damage. One of these important DNA-repair mechanisms is nucleotide excision repair (NER). Our GPCR example representing this hallmark is the Melanocortin1 receptor, MC1R, which is actively involved in the enhancement of NER function [75]. The Melanocortin receptors are a subclass of the *Rhodopsin* family of GPCRs. There are five Melanocortin receptors namely MC1R, MC2R, MC3R, MC4R and MC5R. We will focus on the rest of this discussion on MC1R. MC1R is expressed in melanocytes and leukocytes where its activation promotes UV resistance and anti-inflammatory action in these two types of cells respectively. This receptor is a relatively small protein consisting of 317 amino acid residues and is highly polymorphic. Its normal physiological function is to regulate the skin pigmentation and UV-damage responses. Loss of MC1R function is associated with having fair and UV-sensitive skin with higher melanoma risk [76].

Following activation with Melanocortin, MC1R will be coupled to $G\alpha_s$ and when this G-protein is activated and dissociated from the heterotrimer, it stimulates adenylyl cyclase activity which leaves ATP to generate c-AMP (see Figure 3.). Increased levels of c-AMP in melanocytes can lead to a host of other events including the increased Melanin synthesis and the acceleration of NER activity which together with the enhanced anti-oxidant defences can provide improved resistance to UV injury [75].

Inherited mutations in the *MC1R* gene are common among fair-skinned populations making them more UV-light sensitive and increase their risk of acquiring melanoma [76]. Improved MC1R signalling can be achieved through the manipulation of c-AMP levels in the skin using pharmacological agents such as the topical application of Forskolin (adenylyl cyclase activator) or Rolipram (a phosphodiesterase inhibitor) for the removal of photoproducts induced by UV light action on the skin. While these interventions are effective, they lack specificity against melanocytes and therefore may induce off-target side effects. Targeting MC1R with a degree of specificity to influence its c-AMP signalling cascade can be fruitful in the fight against melanoma, human cancer that harbours the most mutations [76].

GPCRs in avoiding immune destruction

Our immune system is primed to attack and destroy cancerous cells as they start to make variant proteins which can be interpreted as non-self-due to the acquisition of mutations. Therefore, for a tumour to thrive and grow it must be able to overcome the strict immune surveillance imposed by our bodies. Cancer cells can achieve that in numerous ways and one of those is through altering the expression of GPCRs with relevant function in the immune system [(77)]. Our understanding of the immune system has grown fast in the last three decades through the discovery of chemokine receptors and their crucial role in homeostasis. These receptors cause immune cells, notably leukocytes, to migrate and seek their cognate ligands (chemokine) in a process called chemotaxis [(77,78)]. Cancer cells can hijack the function of chemokine for their purpose.

Tumours consist not only of the mass of cancer cells but also many non-malignant types of cells called stromal cells (or simply stroma). Many of these stromal cells are leukocytes making up what is known as leukocyte infiltrate. The identity and number of cells in the leukocyte infiltrate is governed by the chemokine secreted by either the stromal or the tumour cells. There are about 48 distinct chemokines and 19 chemokine receptors in humans. An important member of these chemokines is CXCL12 which acts as a specific ligand for the receptor CXCR4. This receptor, CXCR4, is a GPCR consisting of 352 amino acids and a member of the *Rhodopsin* family. Signalling through this receptor plays an important role in the initiation and progression of cancers through the activation of several downstream signalling pathways [(79,80)]. CXCR4, of course, has the additional role of acting as a co-receptor for the human immunodeficiency virus (HIV) but that function is beyond the scope of this review. Like many GPCRs, CXCR4 can signal through G protein-dependent or G protein-independent pathways. However, the G protein-dependent pathway appears to delineate the majority of the biological outcomes through the activation of $G\alpha_i$. The activated $G\alpha_i$ can inhibit adenylyl cyclase as well as activates the Src (short for sarcoma) family of tyrosine kinases while the $G\beta\gamma$ dimer activates phospholipase C (PLC) and phosphatidylinositol-3-kinase (PI3K) leading to cell migration [(81)]. CXCR4 is frequently found over-expressed in many cancers such as those of the brain, breast, colon, rectum, lung, prostate, ovary, skin and leukaemia.

Several inhibitors which target the CXCL12/CXCR4 axis and attenuate the growth and progression of tumours have been trialled [(82)]. These include small molecules, small peptides and antibodies. Plerixafor is a small molecule inhibitor of the binding of CXCL12 to CXCR4 which is currently on the market for the treatment of non-Hodgkin's lymphoma and multiple myeloma (Tablet 3.). The resistance of some cancer types to the emerging immunotherapies may be due, in part, to the CXCL12/CXCR4 signalling and targeting this axis may enhance the effectiveness of these treatments.

GPCRs in promoting inflammation

The dense infiltration of many tumours with immune cells which mirrors inflammatory conditions has long been observed. The initial immune cells recruitment might be to fight nascent cancer cells, however, the continuous presence of inflammatory signals can have unintended consequences. Chronic inflammation can enhance tumorigenesis, and represents a further hallmark and capability for cancers to progress.

The C-C chemokine receptor type 5 (CCR5) is a 352 amino acid chemokine GPCR receptor and gained fame when it was discovered to be acting as another receptor for the entry of the human immunodeficiency virus (HIV) into cells. It binds multiple ligands including CCL3, CCL4, CCL5, CCL8, CCL11, CCL13 and CCL16. The binding of ligands to CCR5 induces conformational changes leading to the coupling of $G\alpha_i$-heterotrimer and the subsequent dissociation of the trimer. $G\alpha_i$ inhibits adenylyl cyclase while $G\beta\gamma$ subset activates phospholipase C (PLC) which leads to the rapid increase in the cytoplasmic calcium ions, Ca^{++} [(83)]. $G\beta\gamma$ also activates protein kinase C (PKC) and the expression of several genes implicated in inflammation [(84,85)]. Additional pathways induced by CCR5 can also lead to cell survival, cell proliferation and immune cell differentiation.

Owing to the prominent role that CCR5 plays in HIV infection, a few of the drugs that were developed to target that disease have been re-purposed for investigating their deployment in the treatment of cancer e.g. Maraviroc, Vicriviroc and the humanised monoclonal anti-CCR5 antibody Leronlimab (see Table 2.). Additionally, metastatic liver cells secrete CCL5, an important ligand for CCR5. This ligand appears to possess tumour-enhancing effects on cancer cells as well as their associated macrophages. Blocking CCL5 using an antagonist such as Maraviroc leads to the subsidence of the tumour-promoting inflammation in patients with colorectal cancer [(85)].

Mediators such as prostaglandins PGs can be another driving force behind carcinogenesis and presenting a further link between GPCRs and inflammation [(86)]. This pathway may start with a type of prostaglandin; The PG2 is the most common one, which is generated by the action of cyclooxygenase enzyme (COX2) on arachidonic acid. PG2 then binds to its cognate G protein-coupled receptor EP1-4 to initiate the inflammation signalling pathway. Several studies associated the increased expression of PG2 with gastrointestinal cancers. PG2 and COX2 can also drive the

expression of chemokines that participate in the process of forming new blood vessels (angiogenesis) and can further promote cancer progression through that route. Exploiting the role of GPCRs in chronic inflammation to control tumorigenesis can be challenging due to the complex interplay of several other mediators in this process [84,85].

GPCRs in de-regulating metabolism

Cancer cells are characterised by their ability to re-program their utilisation of glucose even in the presence of adequate oxygen supply. Normal cells, in the presence of oxygen, break down glucose to pyruvate by glycolysis and transport most of that pyruvate to the mitochondria to generate adenosine triphosphate (ATP). Under anaerobic conditions, however, little pyruvate is transported to the mitochondria and most of it is converted to lactate in the cytoplasm. Cancer cells exhibit what is known as the "Warburg effect" in that they can limit their energy metabolism largely to glycolysis, even in the presence of oxygen, despite this route being around 18 fold less efficient in generating energy. It is thought that this re-programming allows the diversion of the much-needed biosynthesis intermediates to the fast-growing and dividing cancer cells.

The GPCRs play an important role as regulators of several aspects of metabolism [87]. Some metabolic products are often cognate ligands for GPCRs and their binding plays a significant role in cancer development. GPR51 and GPR91 are G protein-coupled receptors, for lactate and succinate respectively that participate in many of the hallmarks of cancer and are found to be elevated in several cancer types [88]. The liver is the crucial organ of metabolism and over 50 GPCRs are predicted to be expressed in mouse liver [87]. However, our knowledge of the function of these GPCRs is still rudimentary.

The α2A-adrenergic receptor (ADRA2A) is 465 amino acids GPCR that has adrenaline and noradrenaline as its cognate ligands and is widely targeted by other drugs and molecules (see Table 2.). It couples primarily with $G\alpha_i$ and is associated with several functions including those of the central nervous system, cardiovascular system, insulin secretion and lipolysis [89]. The ADRA2A receptor has been investigated with cervical cancer and was found to be significantly down-regulated [90]. Elevated expression of ADRA2A in cervical cancers was demonstrated to suppress cell proliferation and promote senescence and apoptosis through the inhibition of PI3K/PKB/mTOR (mechanistic target of rapamycin) axis[90]. Here we have an example where an increase in the GPCR activity leads to the suppression of cancer. Interventions to elevate the targeted expression of ADRA2A might be worthy of investigation in certain types of cancers.

GPCRs in angiogenesis

The need for cells to be supplied with nutrients and oxygen and for the removal of their waste metabolic products obliges them to reside close to a capillary blood vessel (within about 1 mm). Accordingly, solid tumours cannot grow to more than the head of an average size matchstick without an adequate supply of blood. The formation of a new blood vessel requires the degradation of the extracellular matrix, the increased permeability of the cells, the disruption of cell adhesion and the proliferation and migration of endothelial cells towards the site where the new blood vessel is being constructed. The major mediators of these processes are the vascular endothelial growth factor (VEGF) and the fibroblast growth factor (FGF). However, GPCR agonists and signalling pathways have also been documented to have a role in angiogenesis [91].

The S1PR1 is a Sphingosine-1-phosphate receptor and a member of the GPCR superfamily. The receptor and its ligand, S1P a bioactive lipid, have been shown to have wide biological functions including proliferation, angiogenesis and migration. S1PR1 consists of 382 amino acid residues and signals primarily through $G\alpha_i$ to affect downstream targets including Ras/ERK (extracellular-signal-regulated kinase) and Rac/PAK (p21 activated kinase) [92]. Transgenic S1PR1-null mice result in embryonic lethality due to deficient formation of blood vessels indicating the essential role this GPCR plays in vascularisation.

Several investigations have pointed to the different functions of S1PRs in both pro-and anti-survival signalling depending on the cancer cell type e.g. activating S1PR1 in glioblastoma leads to a decrease in proliferation while activation in T-lymphoblastic lymphoma is linked to increased progression [93]. Consequently, it becomes necessary to consider the type of cancer tissue involved when targeting this receptor for optimum effect.

Release of chemokines such as CCL2, CCL3 and CCL5 by cancer cells and the consequent signalling through their GPCRs can act on stromal cells to stimulate the production of matrix metalloproteinases (MMPs) to degrade matrix proteins and aid vascularisation [91].

Targeting GPCRs, particularly in combination with VEGF, can be an effective strategy to starve tumours of their increasing need for nutrients.

GPCRs in activating metastasis

Mortality from cancer is largely due to the spread of the primary tumour to other sites and as high as 90% of cancer patients die because of metastasis rather than original cancer. It becomes obvious to see why successful intervention in this step of carcinogenesis can be very rewarding. The invasion-metastasis cascade is a complex multistep process involving local invasion, entry of cancer cells into the blood or the lymph vessels (intravasation), transit through these vessels, extravasation and colonisation of the new site [(94)].

GPCRs are active participants in the various steps of the metastatic process. Among these is a subfamily of 33 human receptors called *Adhesion* G protein-coupled receptors (aGPCRs). They form the second largest subfamily of GPCRs, after the *Rhodopsin* subfamily, with a large number of them still being orphan receptors. Emerging evidence suggests that they play a significant role in the adhesion of cells to the extracellular matrix and therefore particularly implicated in the metastasis hallmark of cancer. Several signalling pathways have been reported for aGPCRs that are cell and receptor-specific. The adhesion G protein-coupled receptors are uniquely identified by their large N-terminal fragment (NTF) which gives them the flexibility of containing several domains [(95,96)].

Most aGPCRs include a GPCR proteolytic site (GPS) located in the first transmembrane loop. Proteolytic cleavage at this site divides the receptor into two fragments NTF and a carboxylic terminal fragment (CTF). Signalling can occur through the NTF, through coupling with G proteins mediated by CTF or through one of several other alternative pathways [(95)]. This makes the targeting of aGPCRs for cancer treatment challenging with our limited current knowledge. De-orphansing these receptors could be the first step in improving our understanding of the mechanisms and targets of their signal transduction. There are currently no approved drugs targeting any of the aGPCRs [(96,97,98)].

Chemokines and chemokine receptors are often aberrantly expressed at the primary site of the tumour and correspondingly at its most preferred metastatic site. For breast cancer, for example, studies have found that the G protein-chemokine receptors CXCR4 and CCR7 are over-expressed at the primary site and their ligands (CXC12 and CCL21 respectively) were also over-expressed at main metastatic sites such as the bones and lungs. CXCR4 is widely expressed in a range of cancer types and interaction with its ligand CXCL12 (also referred to as stromal-derived factor 1, SDF1) initiates several signalling cascades leading to responses such as immune cell mobilisation, cell survival, proliferation and metastasis. Tumour cells are guided by their CXCR4 that they express towards the concentration gradient of its ligand CXCL12 which is released by organs that serve as a metastatic site for the primary tumour. Activated CXCR4 signalling was also noticed with therapy resistance in the treatment of breast, colon and pancreatic cancers. In basal cell and squamous cell carcinomas, enhanced CXCR4 interaction with its ligand also contributes to tumour progression. Targeting the CXCR4-CXCL12 axis was fruitful and led to the marketing of an inhibitor called Plerixafor which was shown to be effective against various types of cancers including non-Hodgkin's lymphoma and multiple myeloma [(99,100)] (refer to Table 3)

Landscape of mutations in GPCRs for investigative targeting

Sequencing of tumour DNA often reveals the presence of several mutations compared to its background normal tissue. Not all of the observed mutations are participants in the initiation or progression of cancer. Those that are the driving force behind tumorigenesis are termed "driver mutations' and the remaining gene alterations are neutral, as far as the neoplastic process is concerned, and are called "passenger mutations". Despite a large number of GPCRs and the variety of biological functions they perform, it has been difficult to predict the consequences of some of the observed mutations in G protein-coupled receptors[(101,102,103)]. A tissue-specific pattern of mutations is a further complicating factor in the overall biological function of GPCRs. Furthermore, GPCRs copy number variation (CNV) could dramatically alter their expression thus creating a potential complexity in driving oncogenesis [(104)]. However, recent advances in the fields of cancer genomics and bioinformatics have provided us with tools that we could utilise to identify mutated genes and assess their biological significance. Using these modern bioinformatics methods, a few studies highlighted the presence of clusters of mutations particularly near the cytoplasmic loop of helix 6 in TSHR (thyroid-stimulating hormone receptor) and the DRY motif mediating the inactive conformation of class A GPCRs [(105,106)].

The *Adhesion* family of GPCRs is highly mutated in several cancer types. Their N-terminal end mediates adhesion to matrix proteins. A member of this family, GPR98, has the highest number of amino acids residues among all GPCRs and is mutated in around 45% of melanomas. Other studies linked mutations and over-expression of other members of the adhesion family of GPCRs to different types of cancers [(101,102)].

The *Glutamate* family, which binds glutamate as a ligand, and the *Taste r*eceptors families of GPCRs are tasked with detecting nutrient availability and when sensing nutrient deprivation they activate the autophagy process thus enabling the survival of cancer cells. Therefore, disruption of these GPCRs could lead, albeit indirectly, to tumour

survival. A subfamily of glutamate, namely GRMs were found to be increasingly implicated in melanomas [103].

Mutations in hormone GPCRs such as GPER1 (G protein oestrogen receptor 1), FSHR (follicle-stimulating hormone receptor), LHR (luteinising hormone receptor), GNHRH (gonadotropin-releasing hormone receptor) and TSHR (thyroid-stimulating hormone receptor) have all been connected with cancer initiation and progression. Indeed TSHR, which was already mentioned above, was found to be highly implicated in many thyroid conditions including thyroid adenomas. Oestrogen binding to GPER1 activates a canonical cell growth signalling pathway called EGFR/MAPK to initiate transcriptional activation of growth signals in multiple tissue types.

Mutations in Smoothened (SMO), mentioned before with evading suppression, was found to be a possible contributing cause of basal cell carcinoma. Mutations in the *Frizzled* family of GPCRs (FZDs) were also found to be implicated in various cancer types [72]. The Frizzled receptor normally mediates another canonical signalling pathway that includes the Wnt and the β-catenin. As a family of GPCRs, the *Frizzled* family is collectively mutated in over 15% of colon adenocarcinomas [72,105].

The human genome is endowed with a remarkable ability to maintain its integrity against replication errors and a variety of internal and external insults. Complex systems are in place to identify and correct the vast majority of errors resulting from DNA damage before the cell is released through to cell division. Nevertheless, a minority of mistakes may pass the various checks and barriers unnoticed to end up replicating out of control. A few GPCR signalling pathways participate in the response to DNA damage surveillance and repair. One of these GPCRs is the receptor for Lysophosphatidic acid LPA (bio-active potent mitogen lipid) which was mentioned earlier in connection with resisting programmed cell death. This receptor, LPAR, was found to be aberrantly expressed and mutated in several types of cancers suggesting its involvement in a growth advantage for cancer cells. The LPA receptors can couple to several G proteins subtypes which might explain their wide range of effects. Their cognate molecules can establish an autocrine loop that further fuels their signalling actions. LPA also promotes stimulatory effects on different types of tumours by trans-activating EGFR and triggering a functional cross-talk between its ligands and EGFR signalling. Various isoforms of LPAR such as LPAR1 and LPAR2 have been shown to mitigate DNA damage induced by chemotherapy and radiation treatment particularly in the intestinal epithelium. This tissue consists of relatively fast replicating cells making them more vulnerable to DNA damage from chemotherapy and radiation and leading to the frequently encountered sickness and vomiting. Although the LPAs are rapidly degraded in the gastrointestinal tract, and hence cannot be administered as such orally, synthetic stable derivatives and/or formulations could be investigated for delivery to protect against the devastating side effects of the widely employed chemotherapy and radiation treatment for cancer [64,65].

The CXCR4 mentioned previously in connection with metastasis, has another fascinating role in tumour suppression. It was found that blocking the signalling of CXCR4 with a small peptide antagonist promotes ovarian cancer cell death through weakening the checkpoints for DNA damage thus precipitating cell crisis and death [107].

The intricate balance between cell division on the one hand and cell death on the other keep the cell number of an organism under unified overall control. Cancer develops as a result of tipping the balance in favour of cell division with fewer cells dying compared to those produced by cell division. Facilitating the death of cancerous cells is a mainstay strategy for treating this disease. GPCR signalling has been implicated in driving the resistance to some cancer therapy. Basal cell carcinoma (BCC), for instance, results from an overactive Hedgehog signalling through the Smoothened (SMO) GPCR axis. An inhibitor of SMO was found and marketed under the name Vismodegib. However, resistance to this therapy soon developed and appears to be common. It is thought that BCC largely relies on this signalling pathway for its survival and this over-reliance can drive the evolution of BCC into further activation of the Hedgehog signalling leading to the observed resistance to Vismodegib. Other GPCRs were also implicated in treatment resistance to melanomas [62,63].

Hyperactive GPCRs are often behind the initiation and progression of cancer highlighting their proto-oncogenic characteristics (eight out of the ten GPCRs featured in Figure 5 are proto-oncogene in that sense). However, a growing body of evidence points to a tumour-suppressive role of some GPCRs in certain types of cancer. An orphan GPCR called GPR 56 inhibited prostate cancer and melanoma progression and its expression were inversely correlated with malignancies of latter cancer suggesting a tumour-suppressor role for this orphan receptor. S1PR1 is another example of a GPCR that can mediate both proliferative and anti-proliferative effects on growing cancer cells.

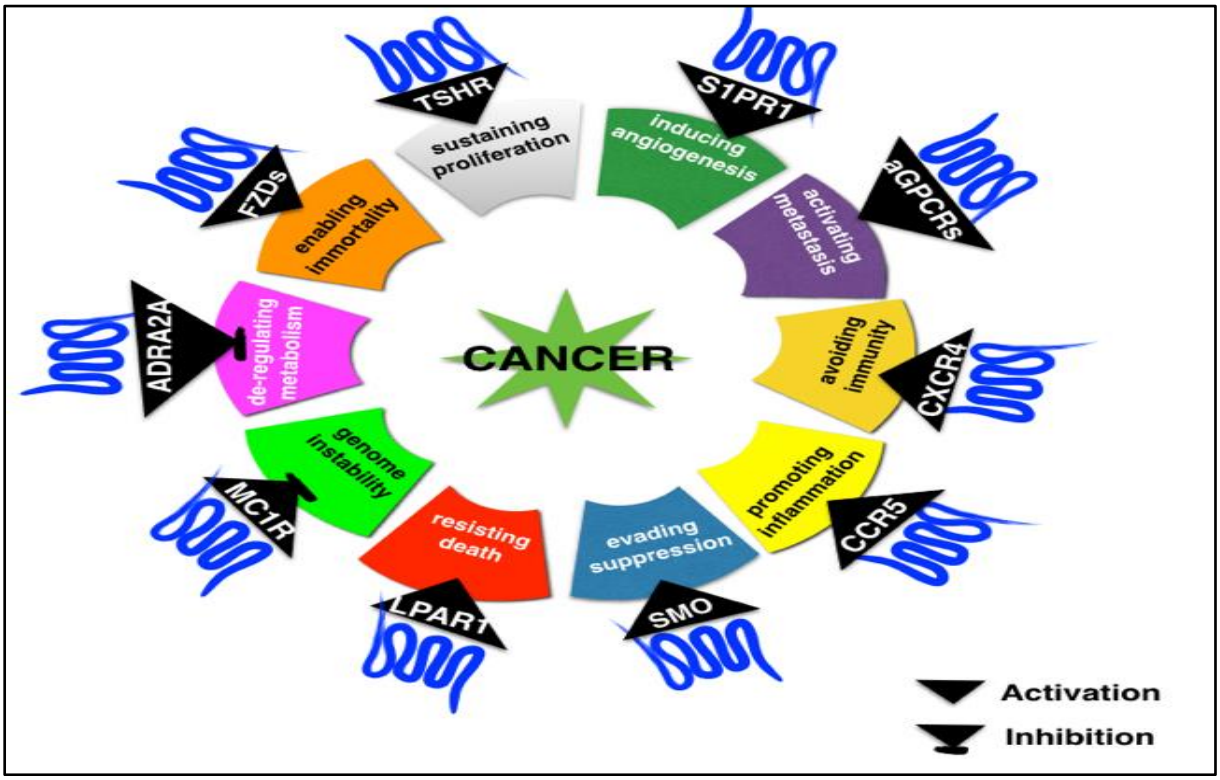

Figure 5. Depiction of the influence of selected GPCRs on the various ten hallmarks of cancer.(TSHR-thyroid-stimulating hormone receptor, S1PR1-Sphingosine-1-phosphate receptor 1, aGPCRs-adhesion G protein-coupled receptors, CXCR4-chemokine receptor type 4, CCR5-chemokine receptor type 5, SMO-Smoothened, LPAR1-Lysophosphatidic acid receptor 1, MC1R-Melanocortin 1 receptor, ADRA2A-alpha-2A adrenergic receptor, and FZDs-Frizzled receptors).

The GPCRs offer significant opportunities to intervene in their signalling functions for the treatment of cancer using small molecules or biologics [108,109]. Further comprehensive studies of how these GPCRs are de-regulated should reveal more targets for rational drug design. Repurposing existing drugs to target GPCRs for the treatment of cancer is a further investigative avenue to follow particularly given a large number of such drugs available on the market for indications other than cancer.

Concluding Remarks

The large number of the different types of GPCRs available and the variety of oncogenic signalling pathways they participate in offering a great opportunity for therapeutic intervention for the treatment of cancer. The tissue-specific and complex signal transduction network must be carefully investigated and assessed when targeting GPCRs to arrive at the best outcome for the patient. Future studies might expand our knowledge regarding the molecular functions of GPCRs and de-orphanise the significant number of these receptors that remain with unknown cognate ligands.

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