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Mini Review

G Protein-Coupled Receptor Systems and Their Role in Cellular Senescence

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ABSTRACT

Aging is a complex biological process that is inevitable for nearly all organisms. Aging is the strongest risk factor for development of multiple neurodegenerative disorders, cancer and cardiovascular disorders. Age-related disease conditions are mainly caused by the progressive degradation of the integrity of communication systems within and between organs. This is in part mediated by, *i*) decreased efficiency of receptor signaling systems and *ii*) an increasing inability to cope with stress leading to apoptosis and cellular senescence. Cellular senescence is a natural process during embryonic development, more recently it has been shown to be also involved in the development of aging disorders and is now considered one of the major hallmarks of aging. G-protein-coupled receptors (GPCRs) comprise a superfamily of integral membrane receptors that are responsible for cell signaling events involved in nearly every physiological process. Recent advances in the molecular understanding of GPCR signaling complexity have expanded their therapeutic capacity tremendously. Emerging data now suggests the involvement of GPCRs and their associated proteins in the development of cellular senescence. With the proven efficacy of therapeutic GPCR targeting, it is reasonable to now consider GPCRs as potential platforms to control cellular senescence and the consequently, age-related disorders.

Contents

Abbreviations: G protein-coupled receptors, (GPCRs); G protein-coupled receptor kinase interacting protein 2, (GIT2); Hutchinson–Gilford progeria syndrome, (HGPS); stress-induced premature senescence, (SIPS); Ataxia telangiectasia mutated, (ATM); tumor suppressor protein 53, (p53); cyclin-dependent kinase inhibitor 1, (cdkn1A/p21); retinoblastoma, (RB); cyclin-dependent kinase 2, (CDK2); tumor suppressor gene PTEN, (PTEN); transcription factor E2F3, (E2F3); senescence associated secretory phenotype, (SASP); nuclear factor kappa-light-chain-enhancer of activated B cells, (NF-κβ); mitogen-activated protein kinase, (MAPK); transmembrane, (TM); active state, (R*); inactive state, (R); G protein-coupled receptor kinase, (GRK); protein kinases, (PK); Lysophosphatidic acid, (LPA); endothelial cell differentiation gene, (Edg); purinergic receptors family, (P2Y); renin-angiotensin system, (RAS); Angiotensin II, (Ang II); angiotensin type 1 receptor, (AT1R); angiotensin type 2 receptor, (AT2R); vascular smooth muscle cells, (VSMC); AT1R blockers, (ARB); beta2-adrenergic receptor, (β_2AR); ADP-ribosylation factor GTPase-activating protein, (Arf-GAP); latent semantic indexing, (LSI); Relaxin family receptor 3, (RXFP3); Regulator of G-protein signaling, (RGS).

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1. Introduction

As cells and tissues grow old their replicative capacity diminishes with time. This intrinsic cellular process was first demonstrated by Hayflick and Moorhead [1] and was termed '*replicative senescence*'. Subsequent research has demonstrated that cellular attrition and damage, primarily to replicating DNA and chromosomal telomeric caps, lead to the generation of non-replicative senescent cells and tissues [2]. Cellular senescence has subsequently been shown to be a potentially important contributor to pathological aging and age-related disease. Hence it is imperative, given the increasing global burden of age-related diseases [3–5], to enhance our current understanding of cellular senescent signaling mechanisms and how to interdict them. Current research into the molecular mechanisms of senescence has attempted to transfer *in cellulo* findings to actual *in vivo* situations [6–8]. In living organisms cellular senescence is a now recognized as protective homeostatic response engendered to prevent the propagation of age-related cellular damage and neoplastic transformation. In times of cellular stress, associated with proliferative stress as well as DNA damage, senescent responses can be induced prematurely in actively dividing cells both *in cellulo* and *in vivo*. Pro-senescent stimuli include the aberrant expression/function of oncogenes and DNA damage caused by exposure to ionizing radiation and reactive oxygen species (ROS). Consequently, the integrity of the senescence program can have an active impact on cellular stress responses, cancer development and treatment outcomes. As senescence can contribute both to protective and disruptive cellular activities the therapeutic targeting of this program requires a highly nuanced approach to avoid indiscriminately targeting cell types (senescent or non-senescent) that may be acting in a protective manner. Despite these potential targeting issues effective therapeutic targeting of the pathological aging process still represents an exciting new concept in preventative medicine as pathological aging mechanisms likely underpin the etiology of *almost* every disease [9–11]. The generation of remedial strategies that can slow down aging-related cellular damage process may help prevent the development of multiple neurodegenerative, metabolic and DNA damage-related (*e.g.* cancer) diseases.

Since their discovery heptahelical G protein-coupled receptors (GPCRs) have become firmly established as perhaps the most effective therapeutic drug target in molecular biology and physiology [9]. Hence, almost 40% of the current drugs approved by the FDA target GPCRs [10] and there are currently over 300 new ongoing clinical trials targeting GPCRs. The majority of current GPCR-targeting drugs were developed with the concept that G protein-dependent signaling pathways were the sole source of information transduction emanating from GPCRs. Considerable research, into alternative non-G protein-dependent signaling modalities, in the past two decades however has suggested that new drugs can be developed to exploit these alternative GPCR signaling modalities [11–14]. The discovery and appreciation of alternative GPCR signaling pathways will aid in the creation of an expanded pharmacopeia that will refine and enhance the efficacy profile(s) of future GPCR-focused therapeutic agents. In this review, we intend to generate a more nuanced insight into how to GPCRs can be exploited to target the process of cell senescence to help treat age-related disorders.

2. Aging as a Multifactorial Biological Process

Aging is a systemic, hyper-complex process broadly characterized by the gradual functional decline in the body's ability over time to manage biological stress leading to an increased risk of cellular damage, disease and eventual mortality. Advancing age is perhaps the major risk factor for several of the most prevalent human pathologies, *e.g.* cancer [15,16], type 2 diabetes [17], dementia [18] and cardiovascular diseases [19,20]. It has become evident in recent years that highly complex physiological systems function as complex integrated networks rather than simplistic linear molecular signal transduction cascades [21,22]. Biological signaling networks associated with the aging process are, at the most basic level, likely to be composed of clusters of self-reinforcing protein-protein interactions (PPIs). However these PPIs do not all share the same degree of network connectivity, or 'betweenness' [23,24], and thus within the lattice there are trophic factors that may not exert individual biological functions, but orchestrate the functional coordination of others. Highly intricate and multi-level PPI networks are supported and integrated by these trophic factors (termed 'keystones' or 'hubs') that can then effectively regulate multiple signaling cascades, complex transcriptional responses and cell-cell communication systems to control aging trajectories from the single cell to the whole somatic level [25]. Thus, the higher the degree of interconnectivity of a protein in these networks, the more biologically important – for a given task in a specific paradigm - it may be considered to be [26,27]. Our research, using a rational combination of primary biological data with cutting-edge natural language processing (NLP)-based informatics has been able to identify one such network controlling factor in the aging paradigm, *i.e.* the G protein-coupled receptor kinase interacting protein 2 (GIT2) [28–32]. Therefore rather than needing to consider the plethora of potential factors involved in aging as individual therapeutic intervention points, this network-based deconvolution of aging presents the possibility of regulating such network controllers as a feasible mechanism to attenuate age-related damage [33,34].

Although a fully comprehensive understanding of the biopathological etiologies of aging remains largely unknown, López-Otín et al. identified and categorized so called 'cellular and molecular hallmarks' of aging [6]. For that, they considered nine tentative hallmarks that fulfilled different criteria. These nine features described in [6] are: genomic instability, telomere attrition, epigenetic alterations, deregulated nutrient sensing, loss of proteostasis, cellular senescence, mitochondrial dysfunction, stem cell exhaustion, and altered intercellular communication. However, McHugh et al. provided a more recent classification of such hallmarks in [35] by considering the causes of age-associated damage, the responses to this damage as well as the

consequences of these responses, which are thus, the culprits of aging. By doing so, they were able to conclude that senescence should be considered as one of the central hallmarks of aging. This hypothesis should be considered reasonable if we consider that the process of aging is nothing but the reduced capability to confront biological stress with years, mainly provoked by the degradation of receptor signaling, endocrine feedback and immune system functionality [39]. With regards to this final aspect of aging, senescent cells are normally destroyed by the immune system, allowing for the regeneration of tissues [36,37]. It is clear that in advanced age this anti-senescent process can become dysfunctional, resulting in the accumulation of such hazardous, senescent cells that can induce age-related disorders through the release of pro-inflammatory factors such as interleukin-6 [38–40]. Given this, targeting senescent cells has been now proposed as a potential approach to overcome aging, or more specifically, age-related diseases. In 2015 Zhu and colleagues identified and validated the use of senolytics, a novel class of drugs possessing the capacity to selectively kill senescent cells [41]. They were able to demonstrate *in vivo* that through the combination of desatinib and quercetin (agents targeting key nodes of pro-survival networks in senescent cells), typical aging symptoms such as cardiac dysfunction were improved in aged mice five days after a single dose administration. Furthermore, the healthspan of progeroid Ercc1$^{-/\Delta}$ mice was expanded after periodic treatment with these senolytics [41]. Since this discovery, several groups have been working on the identification of new senolytic therapies including synthetic small molecules and natural products that can exert anti-aging effects [42–44]. Recently Ovadya et al. [45] treated Prf1 knockout mice (Prf1, a protein required for the clearance of senescent cells) with the experimental agent ABT-737 that pushes senescent cells toward apoptosis. Thus, they were able to decrease the number of senescent cells in the treated mice and their inflammation was reduced. This specific research team expanded their work by employing a mouse model of Hutchinson–Gilford progeria syndrome (HGPS), a disease recognized as an exemplar of accelerated aging due to senescent cell formation accumulation linked to nuclear envelope anomalies. Treatment of these mice with ABT-737 alleviated this deleterious age-related phenotype and increased median survival age. In general, the identification of trophic levels of network control as well as the characteristic pathological aging hallmarks enables the theorization of aging in research and therefore facilitates the prioritization of intervention targets in the aging process. Promising candidates for targeting aging, or more specifically, the network controllers to influence the degree of presentation of the aging hallmarks, have recently been shown to include GPCRs [46,47].

3. Cellular Senescence in the Aging Paradigm

As previously described, cell senescence is one of the hallmarks of aging and could therefore contribute to age-related dysfunction and chronic sterile inflammation [48]. Cellular senescence was originally described as cell cycle arrest caused by a the limited proliferative capacity of normal human somatic cells, referred to as replicative senescence [1]. These cells are essentially arrested in either the G1 or G2/M phase of the cell cycle [49]. With increasing age and the progression of age-related pathology, the abundance of senescent cells increases with multiple tissues [50–54]. During that aging process, repair systems for intracellular and extracellular stress becomes substantially impaired [55]. Due to these aging-associated alterations, the immune system is less able to cope with stress and the clearance of senescent cells, resulting in the accumulation of these cells in aged tissues [56]. A multitude of mechanisms have already been proposed to explain cellular senescence under which telomere shortening occurs after extensive cell division events. Extensive recent research however has demonstrated that a similar phenotype of cellular senescence can be induced by intrinsic and/or extrinsic stress leading to cell cycle arrest, often referred to as stress-induced premature senescence (SIPS) [57]. These deleterious insults include DNA damage, mitochondrial dysfunction, activation of certain oncogenes and inflammatory responses [58,59], all of which are classically associated with the pathological aging process [48]. One of the most proximal effects in this pro-senescent pathway involves the activation of the oncogenic p53 pathway [60] (Fig. 1). Ataxia telangiectasia mutated (ATM), a DNA damage sensor protein kinase, is rapidly recruited (where it then auto-activates) to the sites of damage and stabilizes the tumor suppressor protein 53 (p53) and induces the upregulation of the p53 transcriptional target p21 (Cdkn1a) [60]. This upregulation of p21 prevents the activation of the retinoblastoma (RB) tumor suppressor family members through cyclin-dependent kinase 2 (CDK2), leading to the arrest of the cell cycle [60]. This pathway can be further activated by DNA damage-independent expression of the p53 stabilizer p19Arf (p14 in humans) [61], loss of the tumor suppressor PTEN [61], overexpression of the S-phase transcription factor E2F3 [62] and, surprisingly oncogenic Ras expression in human mammary epithelial cells [60]. While p21 and p19-based cell cycle arrest mechanisms are well characterized pro-senescent systems, additional mechanisms that can prevent effective cell cycle progression are also found in senescent tissues, *e.g.* through the CDK4-and CDK6-mediated inactivation of RB

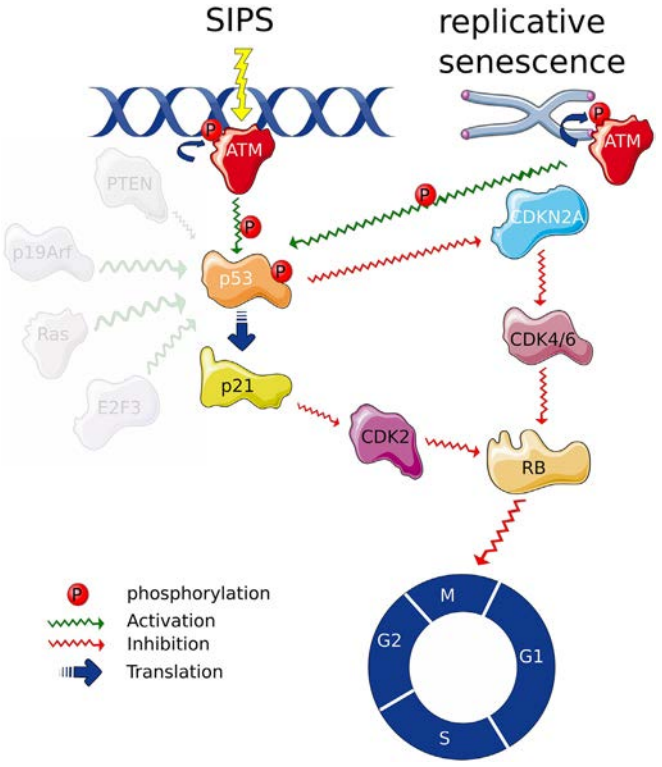

Fig. 1. Mechanisms of cellular senescence. Two different types of cellular senescence exist, namely replicative senescence and stress-induced premature senescence (SIPS). SIPS can be induced by a wide variety of stressors, *e.g.* DNA damage, mitochondrial dysfunction and inflammation. In the presence of DNA damage, Ataxia telangiectasia mutated (ATM) is recruited to the sites of damage. This DNA association induces ATM autophosphorylation, ATM phosphorylation of subsequent additional targets as well as activation of p53. This activation causes the upregulation of the p53 transcriptional target (p21). This upregulation results in the inhibition of Rb in a CDK2 dependent manner, leading to cell cycle arrest in either the G1 or G2/M phase. p53 can be furthermore activated in a non-DNA damage dependent manner through loss of the tumor suppressor gene PTEN, stabilization of p19Arf, expression of the oncogenic gene Ras or overexpression of the S-phase transcription factor E2F3. Another mechanism of inducing senescence is through the activation of CDKN2A causing inhibition of the Rb family members in CDK4 or CDK6 dependent manner.

by CDKN2A (also known as INK4A and ARF; (Fig. 1)) [63]. Demonstrating the potential for molecular complexity in senescence, these mechanisms can either act alone or in combination with each other [48]. Adding an additional layer of nuance to this pro-aging paradigm the speed of senescence progression is also variable, *e.g.* the progression from early to full senescence can take days to weeks [37].

Senescence-mediated cell cycle arrest is furthermore accompanied by phenotypic cellular changes, such as enlarged morphology, reorganization of chromatin, changes in gene expression, secretion of a broad variety of pro-inflammatory cytokines [59]. This latter secretory activity of the senescent cells is described as the senescence associated secretory phenotype, or SASP [64,65]. These proteins include, inflammatory cytokines, chemokines and growth factors, which can propagate the stress response and communicate in a deleterious manner with the neighboring cells. The creation of this SASP is largely initiated by nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κβ) and stress-activated p38 mitogen-activated protein kinase (MAPK) signaling and often maintained by interleukin Iα, a factor secreted by early stage senescent cells [57,66].

4. GPCR Signaling Complexity and Selective Efficacy Profiles

G protein-coupled receptors are hydrophobic proteins characterized by their 7 transmembrane (TM) α-helical regions and are thus also referred to as heptahelical receptors. They comprise a superfamily of integral membrane receptors, with approximately 800 known human GPCRs (https://www.guidetopharmacology.org), that are responsible for cell signaling events controlling nearly every physiological process known [67]. GPCRs act as molecular sensors for an unprecedentedly diverse range of stimuli, *e.g.* photons, lipids, carbohydrates, amines, small chemical mediators and complex proteins. GPCRs help sustain cellular functionality *via* the sensation of environmental cues and also develop tolerance/resistance against stressful conditions. The ability to sense and translate the functional effects of a plethora of cellular stimuli has made GPCRs perhaps the most important clinical drug target to date, with nearly 40% off all pharmacopeia targeting these receptors [67]. To effectively develop therapeutic agents exploiting the huge diversity of GPCRs, generic mechanistic models of GPCRs were constructed and deployed. One of the first conceptual models of GPCR function by Paul Leff, *i.e.* the 'two state' receptor signaling model, proposed that the receptor can exist in the active (R*) state or the inactive (R) state in an equal equilibrium [68]. In this theory of GPCR signaling (Fig. 2A), binding of the receptor ligand induces a conformational change from the R to the R* state, which affects the interaction with the heterotrimeric guanine nucleotide-binding proteins (G-proteins) [69–71]. GPCRs themselves can be considered as functional guanine nucleotide exchange factors, leading to the dissociation into α-subunits bound to GTP (guanosine triphosphate) and βγ-subunits. This allows the stimulation of multiple downstream effectors and the initiation of intracellular signaling responses unique to the GPCR *via* both the Gα and Gβγ units [67]. GPCR signaling is subsequently terminated by phosphorylation of the intracellular loops or the C-terminal tail of the receptor by G protein-coupled receptor kinases (GRKs) and/or second messenger-dependent protein kinases (PK) such as PKA or PKC. This phosphorylation results in the recruitment of β-arrestin, which inhibits further G-protein signaling [72] and causes the internalization of the receptor [73–75]. The internalized receptor will then either be recycled back to the plasma membrane or is targeted for lysosomal degradation [76]. This dogma of G protein-biased GPCR signaling however was broken with the groundbreaking discovery of a distinct non-G protein dependent GPCR signaling paradigm through β-arrestin [13]. Despite a considerable body of evidence suggesting the presence of non-G protein-dependent β-arresting signaling paradigms [80–85] it is still debatable whether it is possible for GPCRs to solely signal through β-arrestin in the complete absence of G protein involvement [86,87]. The concept of two state transition from inactive R to active R* does indeed occur and varies in kinetics for all GPCRs [77] but has now been superseded by the identification of pluridimensional active state transitions [70,78,79]. In the absence of conformational selecting ligands these diverse R* states are likely promoted and stabilized by a selective coterie of interactomic partners [70,80]. Thus rather than signaling diversity being induced by sequential effector promiscuity linked to an unitary receptor (Fig. 2B), diversity is likely generated by the creation of an ensemble of stable receptor complexes referred to as 'receptorsomes' [70,81,82]. Therefore, the diversity of GPCR signaling is likely a function of the cells capacity to generate an ensemble of stable receptorsomes (without ligand stabilization) that define a diverse set of pre-organized signaling outcomes (Fig. 2C) [11,83]. Since the discovery of non-G protein dependent signaling, more than 100 GPCR interacting proteins have been discovered [80,84]. Many of these proteins have the same scaffolding ability as β-arrestin [14]. In recent years, considerable research has demonstrated that the development of GPCR-targeting ligands possessing a bias toward β-arrestin signaling can generate distinct and beneficial therapeutic outcomes [11,12] *via* the generation of specific efficacy signatures *in vivo* compared to ligands primarily employing G protein-dependent pathways [11,12,85–87]. The implications of ligand bias for drug discovery are substantial. The ability of synthetic ligands to bias GPCR signaling output suggests that it may be possible to rationally design drugs that activate beneficial downstream signals while suppressing signals that contribute to adverse side effects [88]. In addition to this, GPCRs were historically considered to only respond to ligand activation on the plasma membrane and the remaining GPCRs in intracellular vesicles were thought to constitute a passive, non-signaling 'reserve'. This view of GPCRs still holds true, in part, especially for rapid G-protein dependent signaling induced by ligand activation of cell surface plasma membrane receptors. However, it is now clear that receptors also have the ability to signal from intracellular membranes, not just at the plasma membrane [89–91]. This new capacity of GPCR signaling suggests that receptors can also be activated by additional stimuli from inside the cell. It is therefore clear that GPCR signaling is much more complex than previously thought. This also implicates that receptors could play important cellular roles in processes linked to aging, such as oxidative stress, DNA damage [46] and perhaps even cellular senescence [14].

5. Functional and Physical Intersections Between GPCR Systems and Senescence Signaling Pathways

Considering the previously described importance of GPCR-targeting ligands in providing crucial therapeutic outcomes, it is reasonable to consider this ubiquitous signaling system as potential regulators in controlling senescent pathways. In the following sections we shall describe the multiple loci of molecular intersection between GPCR signaling and cell senescence pathways, in doing so we will highlight potential therapeutic strategies for future GPCR-mediated control of this pro-aging paradigm.

5.1. Heptahelical GPCRs and Cell Senescence

5.1.1. Lysophosphatidic Acid Receptor

The Lysophosphatidic acid, also known as LPA, is one of the simplest glycerophospholipids consisting of a glycerol backbone that is commonly ester-linked to an acyl chain of varied length and saturation and connected to a phosphate head group [92]. LPA can be found in a wide range of organisms from prokaryotes to eukaryotes and even in all mammalian cells and tissues [93]. Named after their sequence of molecular discovery, six different LPA receptors (LPA_{1-6}) have been reported to date [94]. These seven-transmembrane GPCRs bind different variants of LPA with varying affinities and couple to at least one of the four G-proteins ($G_{12/13}$, $G_{q/11}$, $G_{i/o}$, G_s), activating specific heterotrimeric

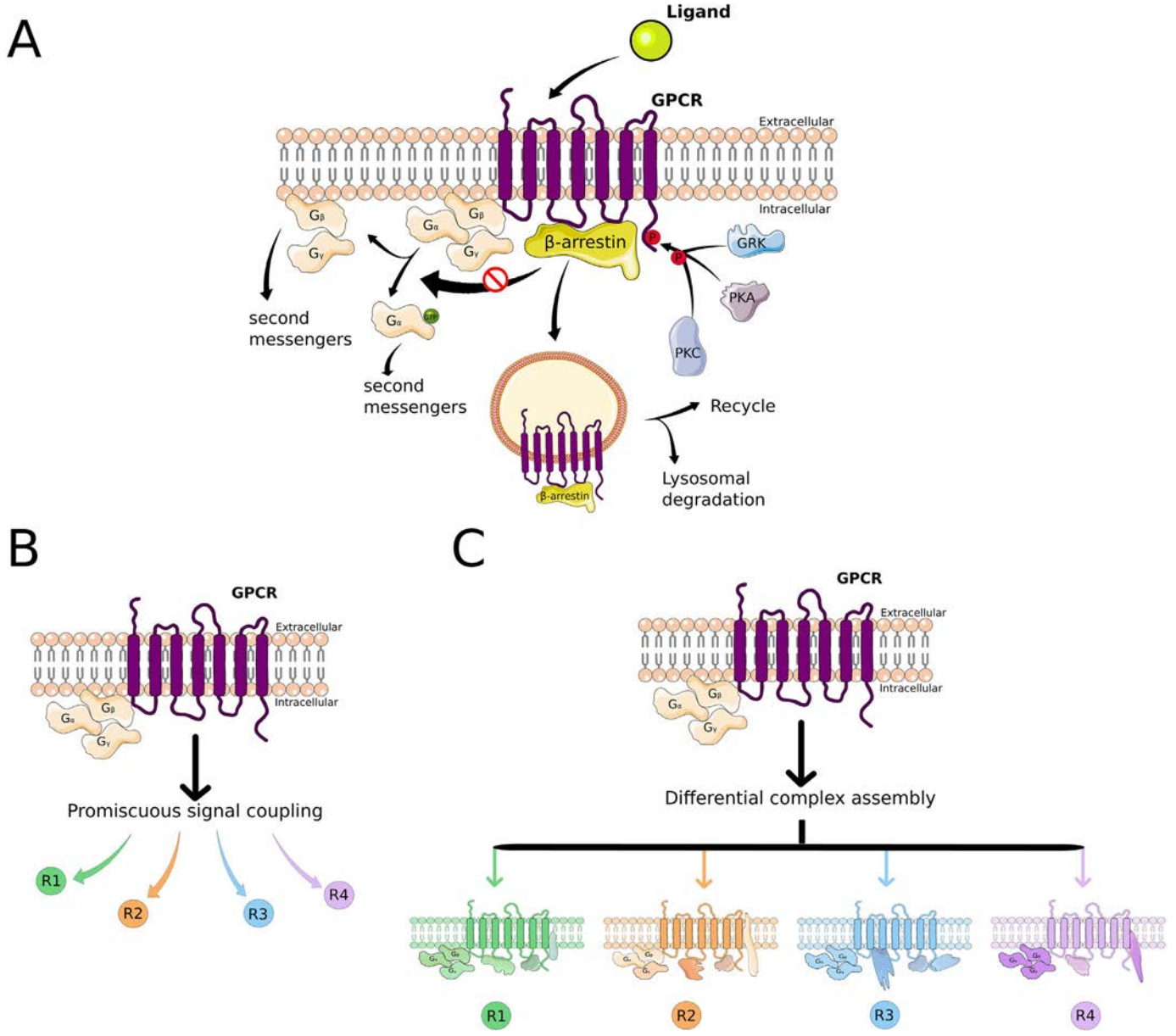

Fig. 2. GPCR signaling systems. (A) Ligand binding causes receptor activation resulting in the stimulation of associated G proteins. This activation causes dissociation in to $G_{\beta\gamma}$-subunits and the G_{α}-subunits bound to GTP. Subsequent second messengers can be activated through both the $G_{\beta\gamma}$-subunits and the G_{α}-subunits. Termination of G-protein signaling is initiated through a GRK or PK dependent phosphorylation of the C-terminal tail of the GPCR. This phosphorylation results in the recruitment of β-arrestin to the GPCR, following internalization of the receptor. The internalized receptor can then be either recycled back to the plasma membrane or undergo lysosomal degradation. (B) The classical view of GPCR signaling diversity suggests that differential signaling outcomes are induced by sequential effector promiscuity linked to a single stimulated receptor. (C) Recent insights in GPCR signaling indicated that diversity is likely generated through the formation of stable receptor complexes. These different receptor complexes then define a diverse set of pre-organized signaling outcomes.

G-protein pathways. These pathways include among others those initiated by some well-known mediators as Ras, Rho, Rac, Akt, MAPK and PKC. The resultant activation of these LPA signaling cascades influences major cellular processes as proliferation, differentiation, migration, adhesion, apoptosis and morphology of a range of cell types during development [95–97]. Three of these six receptors (LPA_{1-3}, also known as vzg-1/Edg2, Edg4 and Edg7, respectively) are members of the endothelial cell differentiation gene (Edg) family [98]. The first one was successful cloned in 1996 by Hecht et al. [99] and based on that finding the two subsequent receptors were identified by sequence similarity [100]. On the other hand, the last three receptors LPA4 (P2Y9/GPR23), LPA5 (GPR92/93) and LPA6 (P2Y5) belong to the purinergic receptors family (P2Y) and are thus considered found to be 'non-Edg' LPA receptors [101–103].

As we have discussed in the previous section, GPCR-signaling is now considered to be a multi-state, multi-site signaling paradigm. This is especially true for the LPA signaling system, *i.e.* LPA is not only present in the extracellular domain but also possesses signaling functions in the intracellular domain, leading to activation of both cell surface and intracellular domain signaling cascades [104]. LPA can thus perform the traditional activity of GPCR stimulation through plasma membrane LPA receptors but also *via* intracellular activity. For that, LPA is produced enzymatically from intracellular organelles such as mitochondria and the endoplasmic reticulum and can entrain distinct internal LPA receptor signaling cascades. For the latter, LPA plays an important role as

intermediate for the synthesis of other glycerolipids. One example is the degradation of LPA into monoacylglycerol (MAG) by lipid phosphate phosphatase enzymes which can exist intracellularly as well [105]. MAG can be then further rephosphorylated by monoacylglycerol kinase and finally take part in another round of LPA signaling [106].

Across multiple studies, LPA has already been reported to be a potential cross-section point between GPCRs-signaling pathways and several age-related disorders [93]. One example of that has been the implication of LPA in cancer. As previously mentioned, some LPA signaling cascades result in cell proliferation, migration, survival or growth, which are characteristic cellular processes dysregulated by cancer as well as senescence [107–110]. LPA was first identified to induce *in vitro* tumor cell invasion in 1993 [111]. Further studies have shown that different aspects of tumor progression are enhanced primarily through LPA_2 and LPA_3 signaling, as the activation of ovarian cancer cells and their protection from apoptosis [112] or the increase in expression of metastasis and invasion mediators [93,113,114]. On the contrary, LPA1 has been reported to act as a negative regulator in ovarian cancer [115]. These findings suggest that targeting LPA signaling might be a crucial key for cancer treatment associated with senescent mechanisms, *e.g. via* p53, of cell cycle dysfunction [116,117].

Within the aged population, cardiovascular-related complications remain the leading cause of death [19], with atherosclerosis as the underlying pathophysiological cause for the vast majority of these type of diseases [118]. Cardiovascular complications of aging are strongly influenced by both vascular and cardiac senescent processes [119–122]. LPA signaling has been observed to influence disease progression in different ways, such as accumulation within the thrombogenic core of atherosclerotic plaques [123] involvement in inflammatory cytokine release [124] as well as LDL uptake for plaque formation [92]. However, potential cardioprotective effects *via* LPA signaling have emerged as well [125,126]. This controversy suggests that the beneficial or harmful action of LPA is potentially dependent on several factors such as the expression profile of receptor subtypes and their connection to different signaling pathways in target cells, as previously proposed by Siess [124]. Other age-related disorders that have appeared to be influenced by LPA signaling are obesity [127], bone disorders [128], autoimmune disorders [92], infertility [129] and others. Therefore, it is not a surprise that several studies have proven that this phospholipid induces senescence as well, by using different approaches and testing different receptors [116,130]. Although the functional link between LPA receptors and cellular senescence may not be as strong at the present time as with other GPCR systems detailed in subsequent sections, the potential of pharmacological interventions controlling LPA signaling to attenuate senescence-associated loss of function should be investigated further as already suggested by Kanehira and colleagues [141]. Thus effective molecular targeting of LPA receptor systems could result in the creation of a potential approach to therapeutically modulate the aging process, which possesses senescence as one of its key hallmarks [9].

5.1.2. Angiotensin II Receptor

The renin-angiotensin system (RAS) is a synchronized hormonal cascade in the control of cardiovascular, renal, and adrenal function that governs arterial pressure as well as body fluid and electrolyte balance [131]. The active peptide and thus, major effector of this RAS system has been proven to be the Angiotensin II (Ang II). This octapeptide is a potent but labile vasoconstrictor that raises blood pressure [132] and has therefore multiple functions in regulating cardiovascular dynamics. Recent research has demonstrated that GPCR interacting proteins can control the output of the Ang II ligand-receptor system. Ang II can functionally interact in a selective manner with two major cell surface GPCRs, the angiotensin type 1 (AT1R) and angiotensin type 2 receptors (AT2R). However, most of the well-characterized Ang II functions are triggered by stimulation of the AT1R [131]. As already reviewed in [133] and briefly described in [46], Ang II is closely associated to the senescence of vascular cells, which is not surprising at all, since vascular senescence plays a critical role in age-related cardiovascular diseases.

There is increasing evidence thanks to several studies, that Ang II promotes vascular senescence *via* AT1R-coupled signaling mechanisms [134–136]. On the one hand, Ang II binding to the AT1 receptor has been demonstrated to induce two types of senescence in human vascular smooth muscle cells (VSMC), namely replicative senescence after a 30 days Ang II stimulation compared to a dose-dependent increase in premature VSMC senescence after 24 h stimulation [137]. ATR1-dependent signaling not only appears to regulate senescence in humans, but also in rodents. Interestingly, AT1R blockers (ARB) inhibit Ang II-induced vascular cell senescence, therefore showing an anti-aging effect. In their review regarding the functional link between AT1R-coupled signaling and senescence [133], Min and co-workers highlighted all the recently discovered signaling molecules involved in coupling those, being some examples the activation of NAD(P)H oxidase that finally may activate many signaling molecules as tyrosine kinases, MAPKs and transcription factors, as well as Ras, a small GTP-binding oncoprotein, that is characterized by its ability to activate several effector proteins, including Raf-1, Pl_3K and Ral-GDS.

However, the role of AT2R activation in vascular senescence has been an enigma for a considerable period of time after its discovery. In recent years a significant body of information has been gathered about this receptor and among others, it has been shown to generate the opposite signaling output as previously described for AT1R, thus, functionally antagonizing AT1R-mediated vascular senescence [138]. In this study, Min et al. [144] showed that deleting the AT2R enhances vascular senescence through methyl methanesulfonate 2 (MM2) inhibition. It has also been demonstrated that non-G-protein interacting proteins can condition the Ang II GPCRs as well and thus, regulate this complex signaling system in order to achieve cellular senescence control. One factor that has been shown to attenuate the ability of the AT1 receptor to induce vascular senescence is the AT1R-interacting protein (ATRAP) [133]. On the other hand, the AT2R-interacting protein (ATIP) is a further example of a protein that prevents vascular senescence by binding to the AT2 receptor [139]. As can be inferred from these studies, targeting these GPCRs results in a tractable approach to regulate vascular senescence. Min and co-workers [133] have already suggested several targets for the treatment of vascular aging and age-related vascular diseases in their review. Furthermore, possible ways of therapeutic interventions of Ang II for targeting hypertension have been considered already [140,141] as well as neuropathic and inflammatory pain [142]. Even studies on targeting prostate cancer reveal that receptor blockers (ARBs) have the potential to inhibit the growth of various cancer cells and tumors through the AT1R [143]. Interestingly, a further intersection between GPCRs and senescence may be zinc-based signaling. There is evidence that Ang II-induced senescence can be promoted by downregulation of several zinc transporters [155]. Alterations in zinc homeostasis also have been shown to result in accelerated senescence in primary endothelial cells [156]. Furthermore Zhao and co-workers (2015) demonstrated that zinc, in addition to Ang II overload elevated ROS levels resulting in the potentiation of cell senescence. To test this proposal this research team investigated the role of Nox1 (NADPH oxidase 1) in this paradigm. Hence Zhao et al. [157] downregulated Nox1 using siRNA and were able to effectively prevent Ang II and zinc-induced senescence. In addition to these findings it has been also shown that Nox1 activity in platelets can be regulated by GPCR agonists [158] suggesting that this is another possible nexus with which to control zinc and Ang II-induced cellular senescence. Zinc signaling has been linked to thus been linked to GPCR activity in several studies, from

GPCR-based sensation of extracellular zinc levels [159] to disturbed GPCR signaling in the absence of zinc transporters [160]. Hence, mice lacking the zinc transporter Slc39a14/Zip14 possessed a disturbed GPCR signaling capacity in their growth plates, pituitary gland and liver which resulted in growth retardation and impaired gluconeogenesis [160]. These findings taken together suggest that zinc-mediated senescence is strongly linked to GPCR signaling and thus presents a novel aspect effective targeting of senolytic agents.

6. β-Arrestin Family GPCR Interacting Proteins

GPCR desensitization and intracellular trafficking is strongly controlled by the β-arrestin family of proteins. β-arrestins are a small family of cytosolic proteins compromising four members: arrestin 1 and arrestin 4 are visual arrestins whereas β-arrestin 1 and β-arrestin 2 (also known as arrestin 2 and arrestin 3) are non-visual arrestins [14]. Desensitization of the receptor after ligand stimulation is performed by binding of arrestin (β-arrestin 1 or 2), which sterically inhibits G-protein activation. The receptor is subsequently internalized through an arrestin-dependent clathrin-coated pit formation, followed by recycling to the plasma membrane, or in times of excessive stimulation, lysosomal degradation [71]. However this is not the only function of β-arrestins, Luttrell et al. discovered in 1999 the involvement of β-arrestin in the formation of receptorsomes leading to the recruitment of c-Src to the plasma membrane [13]. Subsequently to this finding, a variety of signaling effectors have been demonstrated to bind β-arrestins [80,144]. These factors include: E3 ubiquitin ligases, phosphodiesterases and transcriptional activators. More recent research revealed that activation of the beta2-adrenergic receptor (β_2AR) recruited β-arrestins causing the activation of the E3 ubiquitin ligase MDM2 [46]. This activation promotes binding of MDM2 and p53 resulting in the degradation of p53 [145]. Given the involvement of p53 in the activation of the cellular senescence pathway, it is interesting to note that β-arrestin signaling pathways can functionally interact with cellular senescence programs [60]. Instead of activating the cellular senescence pathway, stimulation of β-arrestin signaling paradigms promotes apoptosis [146]. In this context, further work has demonstrated that β-arrestin1-associated pathways may hold promise for the treatment of B-lineage acute lymphoblastic leukemia (B-ALL LICs) [147]. Liu et al. (2017) demonstrated that molecular depletion of β-arrestin1 extended the population doubling time and the percentage of senile cells (signatures of cellular senescence) of B-ALL LICs. β-arrestin1 deletion also enhanced the expression of proteins (CBX1 – chromobox1, HIRA – Histone Cell Cycle Regulator) and genes (p53, p16) related to senescence in leukemic Reh cells and B-ALL-LICs-derived leukemic mice. Hence, loss of β-arrestin1 expression induced senescence of Reh cells through control of the hTERT-telomerase-telomere axis. Importantly, depletion of β-arrestin1 decreased the binding of Sp1 to hTERT promoter at the region of −28 to −36 bp. β-arrestin1 cellular depletion reduced the interaction of P300 (Histone acetyltransferase p300) with Sp1, reducing Sp1 binding to hTERT promoter, downregulate hTERT transcription, decreased telomerase activity, shortened telomere length with the eventual promotion of Reh cell senescence. β-arrestin1 is known to be a scaffold of GPCR signaling and was shown to translocate to the nucleus in response to GPCR activation facilitating histone acetylation and gene transcription [148], thus demonstrating a potential mechanism by which GPCRs may control cellular senescence. While β-arrestin has long been considered as a signaling adaptor for GPCRs, recent research has demonstrated that GPCR-independent activation of β-Arrestin signaling is apparent as well. Hence β-Arrestin 2 is able to activate c-Jun N-terminal Kinase (JNK) without prior upstream GPCR ligand engagement [149,150]. In this paradigm the stimulation of JNK entrains the subsequent activation of FOXO4 leading to cellular senescence [151]. Taken together, considering the GPCR-dependent β-arrestin signaling and given the involvement of senescence in age-related disorders, augmenting the capacity of GPCRs to signaling through β-arrestin might be an important mechanism by which to control cellular senescence in a variety of physiological systems.

6.1. G Protein-Coupled Receptor Kinases (GRKs) and Associated Proteins

GPCRs can respond to an unparalleled diversity of extracellular stimuli and are involved in almost every physiological process, including cellular senescence [67]. For the majority of GPCR systems, following receptor stimulation there is a reflexive interaction of GRKs with the activated state of the receptor. GRKs are a class of serine/threonine kinases that phosphorylate the receptor on the intracellular loops or the carboxyl-terminus. This phosphorylation can promote the association with β-arrestin, which inactivates the receptor and facilitates internalization. This is canonical process has been classified as homologous desensitization of the receptor. Second messenger-dependent kinases such as PKA or PKC are also able to desensitize the receptor, a distinct process termed heterologous desensitization [152].

GRKs are multifunctional proteins that in addition to their catalytic activity can furthermore function as a scaffolding protein for multiple signaling factors involved in cell signaling and sub-cellular vesicle trafficking independent of phosphorylation events [153]. It is therefore unsurprising that these proteins may also be involved in cellular senescence as they can link GPCRs to multiple signaling systems. The GRK family proteins consists out of seven different types of GRK, *i.e.* GRK1 to 7, which share 60–70% sequence homology and are all involved in kinase activity [153]. This superfamily is furthermore organized in three different subfamilies: *(i)* the rhodopsin kinases comprising GRK1 and GRK7, *(ii)* the β-adrenergic receptor kinases comprising GRK2 and GRK3 and lastly *(iii)* the GRK4 subfamily comprising GRK4, GRK5 and GRK6 [153,154]. With the respect to the involvement of GRKs in cellular senescence, it was first noted that both GRK2 and GRK5 are involved in cell cycle regulation [155,156]. GRK2, ubiquitously expressed in mammalian tissues, was shown to be important in the progression of the cell cycle [155] During normal cell cycle progression, GRK2 expression levels gradually decline during progression of the G2 cell cycle phase [155]. More recently it was shown that increased levels of GRK2 causes increased levels of p53 phosphorylation leading to cell cycle arrest and thus potentially inducing cellular senescence [157]. However, the precise role of GRK2 in cellular senescence has not yet been investigated. It is interestingly to note that GRK5 has also been shown to be involved in cell cycle progression [156]. In contrast to GRK2, it is shown that decreased levels of GRK5 causes cell cycle arrest in the G2/M phase of the cell cycle [156]. In contrast, the upregulation of GRK5 can lead to the inhibition of the cell senescence-controlling p53 protein leading to tumorigenesis. The regulation of GRK5 expression is therefore a promising in the treatment of multiple age-related diseases [158]. Given these findings, one inhibitor has been recently described, amlexanox. Amlexanox binds the active site of GRK5 by mimicking the adenine of ATP, resulting in the inhibition of GRK5 leading to cell cycle arrest and potentially apoptosis. However complete cell cycle arrest and induction of senescence by GRK5 has not been established. In contrast to GRK5 and GRK2, is has been shown that overexpression of GRK4 induces cellular senescence *via* a $p16^{INK4A}$ mechanism [159]. The specific regulatory pathway of cellular senescence in response to overexpression of GRK4 needs however to be further investigated. As previously mentioned, GRK and GRK-interacting proteins are involved in other signaling functions aside from receptor

desensitization. One such protein involved in cellular senescence is GIT2, an ADP-ribosylation factor GTPase-activating protein (Arf-GAP) and a G protein-coupled receptor interacting protein [29,160,161]. GIT2 was originally identified as a keystone protein in aging through latent semantic indexing (LSI) in the hypothalamus of aging rats [29]. This involvement of GIT2 in the aging process was further demonstrated by the upregulation in both human and primate hypothalamic tissue [29] and in the presence of hydrogen peroxide [32]. Additionally, it was found that GIT2 was strongly involved in DNA damage repair, through recruitment of ATM to the sites of damage to start the DNA damage repair process [32]. In this age-controlling paradigm it was demonstrated that GIT2 possessed a near irreversible association with the senescence regulator p53 [121]. Investigation of GIT2 knockout (GIT2KO) mice revealed age-accelerated levels of H2AX-associated DNA damage in cortical tissues compared to age-matched controls [31]. Genomic downregulation of GIT2 has been furthermore associated with the creation of SASP-like phenotype [31], *via* the increase of expression of pro-senescent factors such as Cdkn2c and Ehmt2 [31,162–164]. It is therefore possible to suggest that GIT2 may be a novel GPCR-associated bridging factor between DNA damage [46] and cellular senescence. Given the involvement of DNA damage and cellular senescence in multiple age-related disorders, GIT2 might represent a potential target for the treatment of age-related disorders. However, GIT2 is a scaffolding protein and therefore not a classical drug target, such as receptors, kinases, phosphatases and ion channels [22]. As previously discussed, GPCRs can be used to control expression of multiple downstream signaling proteins [88]. Therefore, GPCRs could be used to target the expression profile of GIT2 and its associated signaling factors. In order to do so, it was imperative to find a GPCR that is able to control the expression of this scaffolding protein. GIT2KO samples were analyzed to find a consistently regulated GPCR across different tissues [31,33,34]. This showed a consistently down-regulated GPCR, the RXFP3 in both central nervous and peripheral endocrine tissues. This penetrant GPCR-signaling protein relationship suggests that this coherent system may represent a crucial future target for age-related disorders.

6.2. Regulator of G Protein Signaling (RGS) Proteins

Given our current appreciation of the intricate complexity of GPCR signaling, multiple layers of regulation and feedback are needed to contend with the multidimensional efficacy signaling profiles. Aside from the activity regulation mechanisms of GRKs and β-arrestins, there exists a third generic mechanism common to many GPCR, namely the RGS proteins [165]. All RGS proteins interact with the active GTP-bound Gα subunits, which induces a conformational change leading to accelerated GTP hydrolysis [166]. Currently there are more than twenty mammalian RGS proteins known, which are further divided into eight different subfamilies based on their sequence homology [167,168]. Given their pivotal roles in duration of GPCR activation, they might be important for regulation of cellular senescence. An interesting possibility arises from the observation that RGS10 is highly involved in aging and multiple age-related disorders [169]. RGS10 is one of the smallest RGS proteins, belonging to the D/R12 subfamily. RGS10 is highly expressed in the brain, thymus and lymph nodes [170]. RGS10 is a negative regulator of the transcription factor NF-κβ leading to alterations in the immune system [170,171]. It has been shown that genomic diminution of RGS10 increases the immune responses [170]. Given the involvement of the immune system in the clearance of senescent cells, RGS10 represents an actionable therapeutic target for the treatment of age-related disorders.

6.3. Functional Intersection of the GPCR-Senescence System

In recent years, cellular senescence has become more important in the development of age-related disorders. Given that GPCRs could control protein expression to connect and coordinate multiple responses to multiple stressors, these systems might show a strong functional intersection with cellular senescence as already suggested previously. To illustrate directly this potential functional intersection between GPCRs and cellular senescence, we generated two protein lists in an unbiased manner (Fig. 3A). We employed BioGrid (https://thebiogrid.org/) to

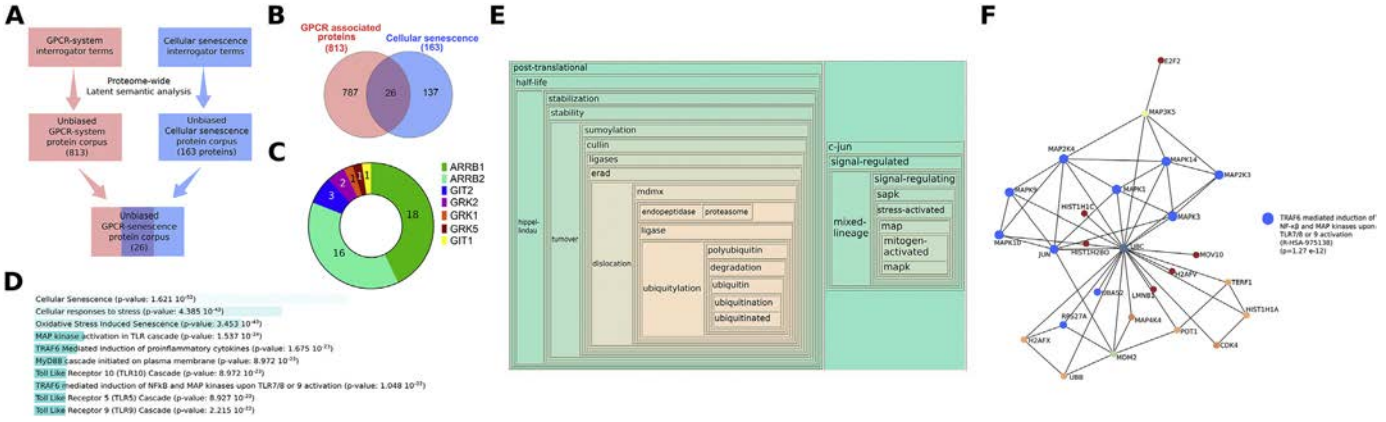

Fig. 3. Functional intersection of the GPCR-senescence system. (A) Using the BioGrid database (https://thebiogrid.org/) the interacting proteins of several GPCR associated proteins were extracted and assembled into one GPCR-system protein list (red). A cellular senescence associated protein list (blue) was generated using the Reactome Pathway database (https://reactome.org/). (B) Here we show the number of overlapping proteins between the GPCR-system protein (red) list and the cellular senescence associated protein lists (blue). (C) The distribution of the 26 GPCR-senescence overlapping proteins originating from the different GPCR-associated factors are visualized in the donut plot. (D) Next, Enrichr (http://amp.pharm.mssm.edu/Enrichr/) was used to perform pathway analysis of these 26 overlapping proteins, showing cellular senescence as the most enriched pathway in this dataset. Probability (P)-values for each significantly populated signaling pathway are shown in the parentheses. (E) The proteins common to GPCR-associated proteins and cellular senescence were used for further analysis using Textrous! (https://textrous.irp.nia.nih.gov/), which employs latent semantic indexing to achieve a highly data-dependent unbiased appreciation of our data. The biomedical terms semantically associated with the 26-protein input dataset are organized into an agglomerative hierarchical cloud in which the strongest associations are denoted by increased font size and green-to-red color intensity. (F) Lastly we assessed the potential physical relationship with the NetworkAnalyst platform (https://www.networkanalyst.ca/). This platform uses protein-protein interaction analysis by using data extracted from the IMEx consortium (The International Molecular Exchange Consortium) consortium (https://www.imexconsortium.org/). A generic, zero-order, network was created using the 26 overlapping proteins. This data shows that the network is centered upon ubiquitin C (UBC) and is furthermore strongly associated with TRAF6-associated SASP functionality.

extract interacting proteins of GPCR-associated proteins (Table S1) and then employed the Reactome pathway database (https://reactome.org/) to generate a comprehensive list of senescence-associated proteins (Table S2). In order to investigate the functional crossovers between these unbiased protein lists, we first scrutinized the amount of overlapping proteins using InteractiVenn (http://www.interactivenn.net/). This indicated an overlap of 26 proteins, shown in Fig. 3B. Next, we investigated how these overlapping protein identifications are distributed among the data linked to our GPCR-associated proteins (Fig. 3C: Table S2). This analysis revealed that currently, perhaps the most important loci of GPCR-Senescence interactions are associated with the β-arrestins. The next most strongly associated GPCR-Senescence interaction factor was GIT2, suggesting that this new non-G protein dependent signaling effector may hold considerable promise for future biased ligand therapy. To assess the functional intersections of these two systems – denoted by these 26 overlapping proteins - we performed Reactome 2019 pathway analysis of this data set using the Enrichr (http://amp.pharm.mssm.edu/Enrichr: Fig. 3D). In this analysis the most significantly enriched pathway was 'Cellular Senescence', followed by 'Cellular Responses to Stress' and 'Oxidative Stress Induced Senescence'. To generate an unbiased and nuanced appreciation of the functional ramifications of this intersecting dataset, we applied a natural language processing-based analytical workflow to these 26 factors. Using the web-based natural language processing Textrous! Application [172] the specific scientific words associated with these overlapping proteins are displayed in an agglomerative hierarchical wordcloud (Fig. 3E). These words were obtained from gene-word document matrices assembled using all PubMed PMC Abstracts, all OMIM entries and all entries in the Jackson Laboratories Mammalian Phenotypes database. This wordcloud shows two different subclouds, *i.e.* stress-activated signaling terms and ubiquitination related terms. Demonstrating the validity of our informatic text analysis approach there is considerable evidence demonstrating a profound link between stress-activated protein kinase (SAPK) pathways, cellular senescent activity [173–175] and GPCR functionality [176–178]. During the development of cellular senescence, the degradation by the ubiquitin-proteasome pathway of selective proteins is regularly engaged [179]. Hence, multiple researchers have shown that ubiquitination of key factors regulating cell senescence are critically linked to pathophysiological aging processes [180–182]. In addition to our classical pathway and natural language processing interrogations of the 26 common factors we further assessed their potential physical relationship to each other using PPI analysis using data extracted from the IMEx (The International Molecular Exchange Consortium) consortium (https://www.imexconsortium.org/) within the NetworkAnalyst platform (https://www.networkanalyst.ca/: [183]). A zero order generic PPI network was created using the 26 GPCR-Senescence interacting factors (Fig. 3F). This self-organized network centered upon ubiquitin C (UBC) and was strongly associated with TRAF6-associated SASP functionality (indicated by the multiple highlighted blue components of the network). It is interesting to note that TRAF6 (TNF receptor-associated factor 6) is a pro-senescence factor linked with SAPK signaling [184–186], receptor functionality [176,187–189] as well as β-arrestin [190,191] and GIT2 signaling [192]. Such findings mechanistically suggest a tight functional synergy between the domains of cell senescence and GPCR activity as *i*) SAPK signaling can be entrained through GPCR systems and *ii*) both GIT2 and β-arrestin are functional GPCR-associated signaling adaptors [14,146]. These signaling factors therefore represent a definite active bridge (and potential drug target(s)) between GPCR and cell senescence systems. Therefore, using diverse unbiased analyses of publicly available biomedical text *corpi* and curated databases, we have shown that these two systems might be functionally interconnected. A better understanding of this functional intersection may create a novel series of drug-based strategies to regulate cellular senescence in the aging process. Therapeutic targeting of the aging process represents an exciting new concept in preventative medicine as pathological aging mechanisms, including cellular senescence, may underpin the etiology of almost every disease [6,7]. The generation of remedial strategies that can slow down the damaged-related aging process might slow down or even prevent several age-related disorders.

7. GPCR-Based Intervention for Cell Senescence Control

Since their discovery in the early 1970s, GPCRs as a pharmacological intervention platform have demonstrated an unparalleled ability to functionally contribute to the current pharmacopeia. Hence, almost half of the currently employed drugs either target, directly or indirectly, GPCR signaling systems. Much of this drug development was performed using simplistic models of GPCR activity [68,193]. Subsequent advances in receptor theory [194–196] as well as the introduction of the receptorsome concept [70,197,198] have now transformed our understanding of this crucial therapeutic system. However, given this advance in knowledge there is a current lag in the deployment of these insights into therapeutic design [14,88,199,200]. It is therefore clear that a concerted effort is now required to enhance the capacity to exploit these flexible GPCR systems in the context of controlling the expression of proteins involved in cell senescence, *e.g.* cdkn2a, p18, p21, p53 and Psma5 [201–203]. Given the broad spectrum of factors associated with the creation of a senescent phenotype it is likely that an enhanced appreciation of how GPCR signaling controls transcription and protein translation, via non-G protein signaling paradigms [11,12,204–206]. Given the functional distinctions between G protein and non-G protein signaling it is important to factor into this anti-senescence drug discovery program the concept that non-G protein-dependent signaling paradigms, *e.g.* β-arrestin or GIT2, may be highly context sensitive and thus may only be present in certain pathophysiological conditions such as metabolic stress or DNA damage. Thus, the identification of cellular signaling phenotypes that promote the switching of biases between G protein and non-G protein signaling paradigms will be critical for developing agents capable of controlling pro-senescent networks. It is interesting to note from this standpoint that two most prominent signaling factors in the realm of non-G protein signaling, *i.e.* β-arrestin [14,206] and GIT2 [22] can both be considered to be highly connected 'keystone' or 'hub' proteins [29]. Therefore, rather than considering cell signaling paradigms as loosely connected linear cascades, it is vital to redefine our working concepts of cell signaling to a more interactome-based paradigm [172,207,208]. Hence, it is vital for the rational development of future anti-senescent GPCR-agents that our appreciation of the context-dependence of β-arrestin- or GIT2-dependent receptorsome functionality be enhanced. Given the relative novelty of the concept of controlling cell senescence *via* GPCR systems there are already several excellent studies that are demonstrating an important spearhead in this venture, linked to receptor-mediated control of macular degeneration [209] GRK-associated cell senescence functional profiling [159].

In this review we have highlighted the potential of targeting senescence in order to overcome age-related diseases. For that, GPCRs have arisen as promising candidates, since they are capable of modulating the activity of proteins involved in signaling pathways that are directly or indirectly involved in cell senescence and consequently in aging. Therefore, these receptors have become crucial regulators of surely almost every process of cell maintenance and survival thereby evolving into excellent effectors for controlling those processes. By rationally exploiting their advantageous properties *via* novel drug design, we could enable the direct therapeutic treatment of senescent-related factors and thus avoid the development into more acute phases of certain age-related disorders or even prevent the appearance of such diseases. Given the well documented nature of GPCR-based signal transduction – coupled to this new functional intersection with pro-aging senescent mechanisms the rational generation of a combinatorial database linking

these two signaling domains would represent an important tool for future therapeutic discovery and development.

Author Contributions

Writing, P.S.-O, H.L. and S.M.; Reviewing & Editing, J.v.G., J.O.H., B.M. and S.M.; Visualization, P.S.-O, H.L. and S.M.; Supervision, S.M.; Project Administration, S.M.; Funding Acquisition, J.v.G., J.O.H. and S.M.

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Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.csbj.2019.08.005.

References

[1] Hayflick L, Moorhead PS. The serial cultivation of human diploid cell strains. Exp Cell Res 1961;25:585–621.
[2] Childs BG, et al. Cellular senescence in aging and age-related disease: from mechanisms to therapy. Nat Med 2015;21(12):1424–35.
[3] Abdelmohsen K, et al. Growth inhibition by miR-519 via multiple p21-inducing pathways. Mol Cell Biol 2012;32(13):2530–48.
[4] Munoz-Espin D, Serrano M. Cellular senescence: from physiology to pathology. Nat Rev Mol Cell Biol 2014;15(7):482–96.
[5] Howcroft TK, et al. The role of inflammation in age-related disease. Aging (Albany NY) 2013;5(1):84–93.
[6] Lopez-Otin C, et al. The hallmarks of aging. Cell 2013;153(6):1194–217.
[7] Nkuipou-Kenfack E, et al. Proteome analysis in the assessment of ageing. Ageing Res Rev 2014;18:74–85.
[8] Colman RJ, et al. Caloric restriction delays disease onset and mortality in rhesus monkeys. Science 2009;325(5937):201–4.
[9] Lefkowitz RJ, Haber E, O'Hara D. Identification of the cardiac beta-adrenergic receptor protein: solubilization and purification by affinity chromatography. Proc Natl Acad Sci U S A 1972;69(10):2828–32.
[10] Hauser AS, et al. Trends in GPCR drug discovery: new agents, targets and indications. Nat Rev Drug Discov 2017;16(12):829–42.
[11] Maudsley S, et al. Informatic deconvolution of biased GPCR signaling mechanisms from in vivo pharmacological experimentation. Methods 2016;92:51–63.
[12] Maudsley S, et al. Delineation of a conserved arrestin-biased signaling repertoire in vivo. Mol Pharmacol 2015;87(4):706–17.
[13] Luttrell LM, et al. Beta-arrestin-dependent formation of beta2 adrenergic receptor-Src protein kinase complexes. Science 1999;283(5402):655–61.
[14] van Gastel J, et al. beta-Arrestin based receptor signaling paradigms: potential therapeutic targets for complex age-related disorders. Front Pharmacol 2018;9:1369.
[15] Zinger A, Cho WC, Ben-Yehuda A. Cancer and aging - the inflammatory connection. Aging Dis 2017;8(5):611–27.
[16] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. Ca-a Cancer J Clin 2015;65(1):5–29.
[17] Gunasekaran U, Gannon M. Type 2 diabetes and the aging pancreatic beta cell. Aging (Albany NY) 2011;3(6):565–75.
[18] Stephan Y, et al. Subjective age and risk of incident dementia: evidence from the National Health and aging trends survey. J Psychiatr Res 2018;100:1–4.
[19] North BJ, Sinclair DA. The intersection between aging and cardiovascular disease. Circ Res 2012;110(8):1097–108.
[20] Steenman M, Lande G. Cardiac aging and heart disease in humans. Biophys Rev 2017;9(2):131–7.
[21] Barabasi AL, Oltvai ZN. Network biology: understanding the cell's functional organization. Nat Rev Genet 2004;5(2):101–13.
[22] van Gastel J, et al. GIT2-a keystone in ageing and age-related disease. Ageing Res Rev 2018;43:46–63.
[23] Kocbek S, Kim JD. Exploring biomedical ontology mappings with graph theory methods. PeerJ 2017;5:e2990.
[24] Zhao X, Liu ZP. Analysis of topological parameters of complex disease genes reveals the importance of location in a biomolecular network. Genes (Basel) 2019;**10**(2).
[25] Fraser HB. Modularity and evolutionary constraint on proteins. Nat Genet 2005;37(4):351–2.
[26] Albert R, Jeong H, Barabasi AL. Error and attack tolerance of complex networks. Nature 2000;406(6794):378–82.
[27] Jeong H, et al. Lethality and centrality in protein networks. Nature 2001;411(6833):41–2.
[28] Chadwick W, et al. Minimal peroxide exposure of neuronal cells induces multifaceted adaptive responses. PLoS One 2010;5(12):e14352.
[29] Chadwick W, et al. GIT2 acts as a potential keystone protein in functional hypothalamic networks associated with age-related phenotypic changes in rats. PLoS One 2012;7(5):e36975.
[30] Martin B, et al. GIT2 acts as a systems-level coordinator of neurometabolic activity and pathophysiological aging. Front Endocrinol (Lausanne) 2015;6:191.
[31] Siddiqui S, et al. Genomic deletion of GIT2 induces a premature age-related thymic dysfunction and systemic immune system disruption. Aging (Albany NY) 2017;9(3):706–40.
[32] Lu D, et al. Nuclear GIT2 is an ATM substrate and promotes DNA repair. Mol Cell Biol 2015;35(7):1081–96.
[33] Jaana Van Gastel JJ, Etienne Harmonie, Azmi Abdelkrim, Maudsley Stuart. The synergistic GIT2-RXFP3 system in the brain and its importance in age-related disorders. Front Aging Neurosci 2016:8.
[34] Van Gastel JHJ, Leysen H, Luttrell LM, Lee M-HM, Azmi A, Janssens J, et al. The RXFP3-GIT2 signaling system represents a potential multidimensional therapeutic target in age-related disorders. FASEB J 2018;32(1).
[35] McHugh D, Gil J. Senescence and aging: causes, consequences, and therapeutic avenues. J Cell Biol 2018;217(1):65–77.
[36] Vitlic A, Lord JM, Phillips AC. Stress, ageing and their influence on functional, cellular and molecular aspects of the immune system. Age (Dordr) 2014;36(3):9631.
[37] van Deursen JM. The role of senescent cells in ageing. Nature 2014;509(7501):439–46.
[38] Rolt A, Nair A, Cox LS. Optimisation of a screening platform for determining IL-6 inflammatory signalling in the senescence-associated secretory phenotype (SASP). Biogerontology 2019;20:359–71. https://doi.org/10.1007/s10522-019-09796-4 (PMID: 30741380).
[39] Mosteiro L, et al. Senescence promotes in vivo reprogramming through p16(INK)(4a) and IL-6. Aging Cell 2018;17(2).
[40] Mendelsohn AR, Larrick JW. Mitochondrial-derived peptides exacerbate senescence. Rejuvenation Res 2018;21(4):369–73.
[41] Zhu Y, et al. The Achilles' heel of senescent cells: from transcriptome to senolytic drugs. Aging Cell 2015;14(4):644–58.
[42] Kirkland JL, et al. The clinical potential of senolytic drugs. J Am Geriatr Soc 2017;65(10):2297–301.
[43] Fuhrmann-Stroissnigg H, et al. Identification of HSP90 inhibitors as a novel class of senolytics. Nat Commun 2017;8(1):422.
[44] Chang J, et al. Clearance of senescent cells by ABT263 rejuvenates aged hematopoietic stem cells in mice. Nat Med 2016;22(1):78–83.
[45] Ovadya Y, et al. Impaired immune surveillance accelerates accumulation of senescent cells and aging. Nat Commun 2018;9(1):5435.
[46] Leysen H, et al. G Protein-coupled receptor systems as crucial regulators of dna damage response processes. Int J Mol Sci 2018;19(10).
[47] Zhao J, et al. G protein-coupled receptors (GPCRs) in Alzheimer's disease: a focus on BACE1 related GPCRs. Front Aging Neurosci 2016;8:58.
[48] Tchkonia T, et al. Cellular senescence and the senescent secretory phenotype: therapeutic opportunities. J Clin Invest 2013;123(3):966–72.
[49] Gire V, Dulic V. Senescence from G2 arrest, revisited. Cell Cycle 2015;14(3):297–304.
[50] Burton DG. Cellular senescence, ageing and disease. Age (Dordr) 2009;31(1):1–9.
[51] Ohtani N, et al. Real-time in vivo imaging of p16gene expression: a new approach to study senescence stress signaling in living animals. Cell Div 2010;5:1.
[52] Herbig U, et al. Cellular senescence in aging primates. Science 2006;311(5765):1257.
[53] Waaijer ME, et al. The number of p16INK4a positive cells in human skin reflects biological age. Aging Cell 2012;11(4):722–5.
[54] Campisi J, et al. Cellular senescence: a link between cancer and age-related degenerative disease? Semin Cancer Biol 2011;21(6):354–9.
[55] Gorbunova V, et al. Changes in DNA repair during aging. Nucleic Acids Res 2007;35(22):7466–74.
[56] Denkinger MD, et al. HSC aging and senescent immune remodeling. Trends Immunol 2015;36(12):815–24.
[57] He S, Sharpless NE. Senescence in health and disease. Cell 2017;169(6):1000–11.
[58] Wei W, Ji S. Cellular senescence: molecular mechanisms and pathogenicity. J Cell Physiol 2018;233(12):9121–35.
[59] Watanabe S, et al. Impact of senescence-associated secretory phenotype and its potential as a therapeutic target for senescence-associated diseases. Cancer Sci 2017;108(4):563–9.
[60] Qian Y, Chen X. Senescence regulation by the p53 protein family. Methods Mol Biol 2013;965:37–61.
[61] Chen Z, et al. Differential p53-independent outcomes of p19(Arf) loss in oncogenesis. Sci Signal 2009;2(84):ra44.

[62] Lazzerini Denchi E, et al. Deregulated E2F activity induces hyperplasia and senescence-like features in the mouse pituitary gland. Mol Cell Biol 2005;25(7):2660–72.
[63] Mirzayans R, et al. Role of p16(INK4A) in replicative senescence and DNA damage-induced premature senescence in p53-deficient human cells. Biochem Res Int 2012;2012:951574.
[64] Zhang B, et al. The senescence-associated secretory phenotype is potentiated by feedforward regulatory mechanisms involving Zscan4 and TAK1. Nat Commun 2018;9(1):1723.
[65] Andriani GA, et al. Whole chromosome instability induces senescence and promotes SASP. Sci Rep 2016;6:35218.
[66] Byun HO, et al. From cell senescence to age-related diseases: differential mechanisms of action of senescence-associated secretory phenotypes. BMB Rep 2015;48(10):549–58.
[67] Tuteja N. Signaling through G protein coupled receptors. Plant Signal Behav 2009;4(10):942–7.
[68] Leff P. The two-state model of receptor activation. Trends Pharmacol Sci 1995;16(3):89–97.
[69] Luttrell LM, Gesty-Palmer D. Beyond desensitization: physiological relevance of arrestin-dependent signaling. Pharmacol Rev 2010;62(2):305–30.
[70] Maudsley S, Martin B, Luttrell LM. The origins of diversity and specificity in g protein-coupled receptor signaling. J Pharmacol Exp Ther 2005;314(2):485–94.
[71] Shenoy SK, Lefkowitz RJ. Beta-Arrestin-mediated receptor trafficking and signal transduction. Trends Pharmacol Sci 2011;32(9):521–33.
[72] Freedman NJ, Lefkowitz RJ. Desensitization of G protein-coupled receptors. Recent Prog Horm Res 1996;51:319–51 [discussion 352-3].
[73] Ferguson SS, et al. Role of beta-arrestin in mediating agonist-promoted G protein-coupled receptor internalization. Science 1996;271(5247):363–6.
[74] Goodman Jr OB, et al. Beta-arrestin acts as a clathrin adaptor in endocytosis of the beta2-adrenergic receptor. Nature 1996;383(6599):447–50.
[75] Zhang J, et al. Dynamin and beta-arrestin reveal distinct mechanisms for G protein-coupled receptor internalization. J Biol Chem 1996;271(31):18302–5.
[76] Yu SS, Lefkowitz RJ, Hausdorff WP. Beta-adrenergic receptor sequestration. A potential mechanism of receptor resensitization. J Biol Chem 1993;268(1):337–41.
[77] Lefkowitz RJ, et al. Constitutive activity of receptors coupled to guanine nucleotide regulatory proteins. Trends Pharmacol Sci 1993;14(8):303–7.
[78] Costa-Neto CM, Parreiras ESLT, Bouvier M. A pluridimensional view of biased agonism. Mol Pharmacol 2016;90(5):587–95.
[79] Maudsley S, et al. Functional signaling biases in G protein-coupled receptors: game theory and receptor dynamics. Mini Rev Med Chem 2012;12(9):831–40.
[80] Magalhaes AC, Dunn H, Ferguson SS. Regulation of GPCR activity, trafficking and localization by GPCR-interacting proteins. Br J Pharmacol 2012;165(6):1717–36.
[81] Kenakin T. Drug efficacy at G protein-coupled receptors. Annu Rev Pharmacol Toxicol 2002;42:349–79.
[82] Vaidehi N, Kenakin T. The role of conformational ensembles of seven transmembrane receptors in functional selectivity. Curr Opin Pharmacol 2010;10(6):775–81.
[83] Sleno R, Hebert TE. Shaky ground - the nature of metastable GPCR signalling complexes. Neuropharmacology 2019;152:4–14. https://doi.org/10.1016/j.neuropharm.2019.01.018.
[84] Bockaert J, et al. GPCR interacting proteins (GIPs) in the nervous system: roles in physiology and pathologies. Annu Rev Pharmacol Toxicol 2010;50:89–109.
[85] Ibrahim IA, Kurose H. Beta-arrestin-mediated signaling improves the efficacy of therapeutics. J Pharmacol Sci 2012;118(4):408–12.
[86] Violin JD, Lefkowitz RJ. Beta-arrestin-biased ligands at seven-transmembrane receptors. Trends Pharmacol Sci 2007;28(8):416–22.
[87] Bologna Z, et al. Biased G protein-coupled receptor signaling: new player in modulating physiology and pathology. Biomol Ther (Seoul) 2017;25(1):12–25.
[88] Luttrell LM, Maudsley S, Gesty-Palmer D. Translating in vitro ligand bias into in vivo efficacy. Cell Signal 2018;41:46–55.
[89] Irannejad R, von Zastrow M. GPCR signaling along the endocytic pathway. Curr Opin Cell Biol 2014;27:109–16.
[90] Staubert C, Schoneberg T. GPCR signaling from intracellular membranes - a novel concept. Bioessays 2017;39(12).
[91] Estelles A, et al. Exosome nanovesicles displaying G protein-coupled receptors for drug discovery. Int J Nanomedicine 2007;2(4):751–60.
[92] Yung YC, Stoddard NC, Chun J. LPA receptor signaling: pharmacology, physiology, and pathophysiology. J Lipid Res 2014;55(7):1192–214.
[93] Lin ME, Herr DR, Chun J. Lysophosphatidic acid (LPA) receptors: signaling properties and disease relevance. Prostaglandins Other Lipid Mediat 2010;91(3–4):130–8.
[94] Kihara Y, et al. Lysophospholipid receptor nomenclature review: IUPHAR review 8. Br J Pharmacol 2014;171(15):3575–94.
[95] Takuwa Y, Takuwa N, Sugimoto N. The Edg family G protein-coupled receptors for lysophospholipids: their signaling properties and biological activities. J Biochem 2002;131(6):767–71.
[96] Dubin AE, Herr DR, Chun J. Diversity of lysophosphatidic acid receptor-mediated intracellular calcium signaling in early cortical neurogenesis. J Neurosci 2010;30(21):7300–9.
[97] Stoddard NC, Chun J. Promising pharmacological directions in the world of lysophosphatidic acid signaling. Biomol Ther (Seoul) 2015;23(1):1–11.
[98] An S, et al. Characterization of a novel subtype of human G protein-coupled receptor for lysophosphatidic acid. J Biol Chem 1998;273(14):7906–10.
[99] Hecht JH, et al. Ventricular zone gene-1 (vzg-1) encodes a lysophosphatidic acid receptor expressed in neurogenic regions of the developing cerebral cortex. J Cell Biol 1996;135(4):1071–83.
[100] Fukushima N, et al. Lysophospholipid receptors. Annu Rev Pharmacol Toxicol 2001;41:507–34.
[101] Noguchi K, Ishii S, Shimizu T. Identification of p2y9/GPR23 as a novel G protein-coupled receptor for lysophosphatidic acid, structurally distant from the Edg family. J Biol Chem 2003;278(28):25600–6.
[102] Lee CW, et al. GPR92 as a new G12/13- and Gq-coupled lysophosphatidic acid receptor that increases cAMP, LPA5. J Biol Chem 2006;281(33):23589–97.
[103] Pasternack SM, et al. G protein-coupled receptor P2Y5 and its ligand LPA are involved in maintenance of human hair growth. Nat Genet 2008;40(3):329–34.
[104] Sheng X, et al. Lysophosphatidic acid signalling in development. Development 2015;142(8):1390–5.
[105] Brindley DN, Pilquil C. Lipid phosphate phosphatases and signaling. J Lipid Res 2009;50(Suppl):S225–30.
[106] Pages C, et al. Lysophosphatidic acid synthesis and release. Prostaglandins Other Lipid Mediat 2001;64(1–4):1–10.
[107] Evans BA, et al. Ligand-directed signalling at beta-adrenoceptors. Br J Pharmacol 2010;159(5):1022–38.
[108] Gonzalez LC, et al. Premature aging/senescence in cancer cells facing therapy: good or bad? Biogerontology 2016;17(1):71–87.
[109] Haronikova L, et al. The p53 mRNA: an integral part of the cellular stress response. Nucleic Acids Res 2019;47(7):3257–71.
[110] Lujambio A, Banito A. Functional screening to identify senescence regulators in cancer. Curr Opin Genet Dev 2019;54:17–24.
[111] Imamura F, et al. Induction of in vitro tumor cell invasion of cellular monolayers by lysophosphatidic acid or phospholipase D. Biochem Biophys Res Commun 1993;193(2):497–503.
[112] Xu Y, et al. Lysophospholipids activate ovarian and breast cancer cells. Biochem J 1995;309(Pt 3):933–40.
[113] Pustilnik TB, et al. Lysophosphatidic acid induces urokinase secretion by ovarian cancer cells. Clin Cancer Res 1999;5(11):3704–10.
[114] Mills GB, Moolenaar WH. The emerging role of lysophosphatidic acid in cancer. Nat Rev Cancer 2003;3(8):582–91.
[115] Murph MM, et al. Sharpening the edges of understanding the structure/function of the LPA1 receptor: expression in cancer and mechanisms of regulation. Biochim Biophys Acta 2008;1781(9):547–57.
[116] Kortlever RM, et al. Suppression of the p53-dependent replicative senescence response by lysophosphatidic acid signaling. Mol Cancer Res 2008;6(9):1452–60.
[117] Kanehira M, et al. An lysophosphatidic acid receptors 1 and 3 Axis governs cellular senescence of mesenchymal stromal cells and promotes growth and vascularization of multiple myeloma. Stem Cells 2017;35(3):739–53.
[118] Head T, Daunert S, Goldschmidt-Clermont PJ. The aging risk and atherosclerosis: a fresh look at arterial homeostasis. Front Genet 2017;8:216.
[119] Garrido AM, Bennett M. Assessment and consequences of cell senescence in atherosclerosis. Curr Opin Lipidol 2016;27(5):431–8.
[120] Childs BG, et al. Senescent intimal foam cells are deleterious at all stages of atherosclerosis. Science 2016;354(6311):472–7.
[121] Cesselli D, et al. Cardiac cell senescence and redox Signaling. Front Cardiovasc Med 2017;4:38.
[122] Cianflone E, et al. Adult cardiac stem cell aging: a reversible stochastic phenomenon? Oxid Med Cell Longev 2019;2019:5813147.
[123] Siess W, et al. Lysophosphatidic acid mediates the rapid activation of platelets and endothelial cells by mildly oxidized low density lipoprotein and accumulates in human atherosclerotic lesions. Proc Natl Acad Sci U S A 1999;96(12):6931–6.
[124] Siess W. Athero- and thrombogenic actions of lysophosphatidic acid and sphingosine-1-phosphate. Biochim Biophys Acta 2002;1582(1–3):204–15.
[125] Karliner JS, et al. The lysophospholipids sphingosine-1-phosphate and lysophosphatidic acid enhance survival during hypoxia in neonatal rat cardiac myocytes. J Mol Cell Cardiol 2001;33(9):1713–7.
[126] Li ZG, et al. Influence of acetylsalicylate on plasma lysophosphatidic acid level in patients with ischemic cerebral vascular diseases. Neurol Res 2008;30(4):366–9.
[127] D'Souza K, Paramel GV, Kienesberger PC. Lysophosphatidic acid signaling in obesity and insulin resistance. Nutrients 2018;10(4).
[128] Sims SM, et al. Lysophosphatidic acid: a potential mediator of osteoblast-osteoclast signaling in bone. Biochim Biophys Acta 2013;1831(1):109–16.
[129] Ye X, Chun J. Lysophosphatidic acid (LPA) signaling in vertebrate reproduction. Trends Endocrinol Metab 2010;21(1):17–24.
[130] Kanehira M, et al. Targeting lysophosphatidic acid signaling retards culture-associated senescence of human marrow stromal cells. PLoS One 2012;7(2):e32185.
[131] Carey RM, Siragy HM. Newly recognized components of the renin-angiotensin system: potential roles in cardiovascular and renal regulation. Endocr Rev 2003;24(3):261–71.
[132] Fyhrquist F, Metsarinne K, Tikkanen I. Role of angiotensin II in blood pressure regulation and in the pathophysiology of cardiovascular disorders. J Hum Hypertens 1995;9(Suppl. 5):S19–24.
[133] Min LJ, et al. Signaling mechanisms of angiotensin II in regulating vascular senescence. Ageing Res Rev 2009;8(2):113–21.
[134] Kunieda T, et al. Angiotensin II induces premature senescence of vascular smooth muscle cells and accelerates the development of atherosclerosis via a p21-dependent pathway. Circulation 2006;114(9):953–60.

[135] Min LJ, et al. Cross-talk between aldosterone and angiotensin II in vascular smooth muscle cell senescence. Cardiovasc Res 2007;76(3):506–16.
[136] Basso N, et al. Protective effect of the inhibition of the renin-angiotensin system on aging. Regul Pept 2005;128(3):247–52.
[137] Herbert KE, et al. Angiotensin II-mediated oxidative DNA damage accelerates cellular senescence in cultured human vascular smooth muscle cells via telomere-dependent and independent pathways. Circ Res 2008;102(2):201–8.
[138] Min LJ, et al. Angiotensin II type 2 receptor deletion enhances vascular senescence by methyl methanesulfonate sensitive 2 inhibition. Hypertension 2008;51(5):1339–44.
[139] Min LJ, et al. Angiotensin II type 2 receptor-interacting protein prevents vascular senescence. J Am Soc Hypertens 2012;6(3):179–84.
[140] Dasgupta C, Zhang L. Angiotensin II receptors and drug discovery in cardiovascular disease. Drug Discov Today 2011;16(1–2):22–34.
[141] Papadopoulos DP, Papademetriou V. Targeting angiotensin II type I receptors to reduce the risk of stroke in patients with hypertension. Expert Opin Ther Targets 2006;10(2):231–7.
[142] Smith MT, Muralidharan A. Targeting angiotensin II type 2 receptor pathways to treat neuropathic pain and inflammatory pain. Expert Opin Ther Targets 2015;19(1):25–35.
[143] Uemura H, Ishiguro H, Kubota Y. Molecular targeting therapy with angiotensin II receptor blocker for prostatic cancer. Oncol Rev 2007;1(1):3–13.
[144] Peterson YK, Luttrell LM. The diverse roles of arrestin scaffolds in G protein-coupled receptor signaling. Pharmacol Rev 2017;69(3):256–97.
[145] Hara MR, et al. A stress response pathway regulates DNA damage through beta2-adrenoreceptors and beta-arrestin-1. Nature 2011;477(7364):349–53.
[146] Yin D, et al. Beta-Arrestin 2 promotes hepatocyte apoptosis by inhibiting Akt protein. J Biol Chem 2016;291(2):605–12.
[147] Liu S, et al. The cellular senescence of leukemia-initiating cells from acute lymphoblastic leukemia is postponed by beta-Arrestin1 binding with P300-Sp1 to regulate hTERT transcription. Cell Death Dis 2017;8(4):e2756.
[148] Kang J, et al. A nuclear function of beta-arrestin1 in GPCR signaling: regulation of histone acetylation and gene transcription. Cell 2005;123(5):833–47.
[149] Gurevich VV, Gurevich EV. Arrestin-mediated signaling: is there a controversy? World J Biol Chem 2018;9(3):25–35.
[150] Chen Q, et al. Structural basis of arrestin-3 activation and signaling. Nat Commun 2017;8(1):1427.
[151] Bourgeois B, Madl T. Regulation of cellular senescence via the FOXO4-p53 axis. FEBS Lett 2018;592(12):2083–97.
[152] Steele AD, et al. Interactions between opioid and chemokine receptors: heterologous desensitization. Cytokine Growth Factor Rev 2002;13(3):209–22.
[153] Hendrickx JO, et al. GRK5 - a functional bridge between cardiovascular and neurodegenerative disorders. Front Pharmacol 2018;9:1484.
[154] Premont RT, Inglese J, Lefkowitz RJ. Protein kinases that phosphorylate activated G protein-coupled receptors. FASEB J 1995;9(2):175–82.
[155] Penela P, et al. G protein-coupled receptor kinase 2 (GRK2) modulation and cell cycle progression. Proc Natl Acad Sci U S A 2010;107(3):1118–23.
[156] Michal AM, et al. G protein-coupled receptor kinase 5 is localized to centrosomes and regulates cell cycle progression. J Biol Chem 2012;287(9):6928–40.
[157] Wei Z, et al. Growth inhibition of human hepatocellular carcinoma cells by overexpression of G-protein-coupled receptor kinase 2. J Cell Physiol 2012;227(6):2371–7.
[158] Gambardella J, et al. Dual role of GRK5 in cancer development and progression. Transl Med UniSa 2016;14:28–37.
[159] Xiao P, et al. G protein-coupled receptor kinase 4-induced cellular senescence and its senescence-associated gene expression profiling. Exp Cell Res 2017;360(2):273–80.
[160] Premont RT, et al. The GIT family of ADP-ribosylation factor GTPase-activating proteins. Functional diversity of GIT2 through alternative splicing. J Biol Chem 2000;275(29):22373–80.
[161] Premont RT, et al. The GIT/PIX complex: an oligomeric assembly of GIT family ARF GTPase-activating proteins and PIX family Rac1/Cdc42 guanine nucleotide exchange factors. Cell Signal 2004;16(9):1001–11.
[162] Yuan Y, et al. A small-molecule probe of the histone methyltransferase G9a induces cellular senescence in pancreatic adenocarcinoma. ACS Chem Biol 2012;7(7):1152–7.
[163] Kim S, et al. Association between genetic variants in DNA and histone methylation and telomere length. PLoS One 2012;7(7):e40504.
[164] Bertolo A, et al. Autofluorescence is a reliable in vitro marker of cellular senescence in human mesenchymal stromal cells. Sci Rep 2019;9(1):2074.
[165] De Vries L, et al. The regulator of G protein signaling family. Annu Rev Pharmacol Toxicol 2000;40:235–71.
[166] Guan KL, Han M. A G-protein signaling network mediated by an RGS protein. Genes Dev 1999;13(14):1763–7.
[167] Stewart A, Fisher RA. Introduction: G protein-coupled receptors and RGS proteins. Prog Mol Biol Transl Sci 2015;133:1–11.
[168] Kach J, Sethakorn N, Dulin NO. A finer tuning of G-protein signaling through regulated control of RGS proteins. Am J Physiol Heart Circ Physiol 2012;303(1):H19–35.
[169] Kannarkat GT, et al. Age-related changes in regulator of G-protein signaling (RGS)-10 expression in peripheral and central immune cells may influence the risk for age-related degeneration. Neurobiol Aging 2015;36(5):1982–93.
[170] Lee JK, Tansey MG. Physiology of RGS10 in neurons and immune cells. Prog Mol Biol Transl Sci 2015;133:153–67.
[171] Lee JK, et al. Regulator of G-protein signaling-10 negatively regulates NF-kappaB in microglia and neuroprotects dopaminergic neurons in hemiparkinsonian rats. J Neurosci 2011;31(33):11879–88.
[172] Chen H, et al. Textrous!: extracting semantic textual meaning from gene sets. PLoS One 2013;8(4):e62665.
[173] Wada T, Penninger JM. Stress kinase MKK7: savior of cell cycle arrest and cellular senescence. Cell Cycle 2004;3(5):577–9.
[174] Kiran S, Oddi V, Ramakrishna G. Sirtuin 7 promotes cellular survival following genomic stress by attenuation of DNA damage, SAPK activation and p53 response. Exp Cell Res 2015;331(1):123–41.
[175] Senthil KK, et al. A steroid like phytochemical Antcin M is an anti-aging reagent that eliminates hyperglycemia-accelerated premature senescence in dermal fibroblasts by direct activation of Nrf2 and SIRT-1. Oncotarget 2016;7(39):62836–61.
[176] Davidson L, et al. Gonadotropin-releasing hormone-induced activation of diacylglycerol kinase-zeta and its association with active c-src. J Biol Chem 2004;279(12):11906–16.
[177] Chen J, et al. GPCR kinase 2-interacting protein-1 protects against ischemia-reperfusion injury of the spinal cord by modulating ASK1/JNK/p38 signaling. FASEB J 2018;32:6833–47. https://doi.org/10.1096/fj.201800548 (PMID: 29912587).
[178] Robinson JD, McDonald PH. The orexin 1 receptor modulates kappa opioid receptor function via a JNK-dependent mechanism. Cell Signal 2015;27(7):1449–56.
[179] Deschenes-Simard X, et al. Cellular senescence and protein degradation: breaking down cancer. Cell Cycle 2014;13(12):1840–58.
[180] Liu B, et al. MDM2-mediated degradation of WRN promotes cellular senescence in a p53-independent manner. Oncogene 2019;38:2501–15. https://doi.org/10.1038/s41388-018-0605-5 (PMID: 30532073).
[181] Mazzucco AE, et al. Genetic interrogation of replicative senescence uncovers a dual role for USP28 in coordinating the p53 and GATA4 branches of the senescence program. Genes Dev 2017;31(19):1933–8.
[182] Wang Z, Zhu WG, Xu X. Ubiquitin-like modifications in the DNA damage response. Mutat Res 2017;803-805:56–75.
[183] Xia J, Benner MJ, Hancock RE. NetworkAnalyst – integrative approaches for protein-protein interaction network analysis and visual exploration. Nucleic Acids Res 2014;42(Web Server issue):W167–74.
[184] Nishitoh H, et al. ASK1 is essential for JNK/SAPK activation by TRAF2. Mol Cell 1998;2(3):389–95.
[185] Mochida Y, et al. ASK1 inhibits interleukin-1-induced NF-kappa B activity through disruption of TRAF6-TAK1 interaction. J Biol Chem 2000;275(42):32747–52.
[186] Korchnak AC, et al. Cytokine-induced activation of mixed lineage kinase 3 requires TRAF2 and TRAF6. Cell Signal 2009;21(11):1620–5.
[187] Sun W, Yang J. Molecular basis of lysophosphatidic acid-induced NF-kappaB activation. Cell Signal 2010;22(12):1799–803.
[188] Hsiao HM, et al. Resolvin D1 attenuates polyinosinic-polycytidylic acid-induced inflammatory signaling in human airway epithelial cells via TAK1. J Immunol 2014;193(10):4980–7.
[189] Chadwick W, et al. Targeting TNF-alpha receptors for neurotherapeutics. Trends Neurosci 2008;31(10):504–11.
[190] Chen L, et al. beta-arrestin 2 negatively regulates NOD2 signalling pathway through association with TRAF6 in microglia after cerebral ischaemia/reperfusion injury. J Cell Mol Med 2019;23:3325–35. https://doi.org/10.1111/jcmm.14223.
[191] Xiao N, et al. SUMOylation attenuates human beta-arrestin 2 inhibition of IL-1R/TRAF6 signaling. J Biol Chem 2015;290(4):1927–35.
[192] Wei J, et al. The GTPase-activating protein GIT2 protects against colitis by negatively regulating toll-like receptor signaling. Proc Natl Acad Sci U S A 2014;111(24):8883–8.
[193] De Lean A, Stadel JM, Lefkowitz RJ. A ternary complex model explains the agonist-specific binding properties of the adenylate cyclase-coupled beta-adrenergic receptor. J Biol Chem 1980;255(15):7108–17.
[194] Samama P, et al. A mutation-induced activated state of the beta 2-adrenergic receptor. Extending the ternary complex model. J Biol Chem 1993;268(7):4625–36.
[195] Weiss JM, et al. The cubic ternary complex receptor-occupancy model. III. Resurrecting efficacy. J Theor Biol 1996;181(4):381–97.
[196] Kenakin T. Allosteric theory: taking therapeutic advantage of the malleable nature of GPCRs. Curr Neuropharmacol 2007;5(3):149–56.
[197] Bockaert J, et al. GPCR-GIP networks: a first step in the discovery of new therapeutic drugs? Curr Opin Drug Discov Devel 2004;7(5):649–57.
[198] Gurevich VV, Gurevich EV. GPCR monomers and oligomers: it takes all kinds. Trends Neurosci 2008;31(2):74–81.
[199] Luttrell LM, Maudsley S, Bohn LM. Fulfilling the promise of "biased" G protein-coupled receptor agonism. Mol Pharmacol 2015;88(3):579–88.
[200] Rodriguez-Espigares I, et al. Challenges and opportunities in drug discovery of biased ligands. Methods Mol Biol 2018;1705:321–34.
[201] Ozcan S, et al. Unbiased analysis of senescence associated secretory phenotype (SASP) to identify common components following different genotoxic stresses. Aging (Albany NY) 2016;8(7):1316–29.
[202] Al-Khalaf HH, Aboussekhra A. p16 controls p53 protein expression through miR-dependent destabilization of MDM2. Mol Cancer Res 2018;16(8):1299–308.

[203] Hudgins AD, et al. Age- and tissue-specific expression of senescence biomarkers in mice. Front Genet 2018;9:59.
[204] Gesty-Palmer D, et al. Beta-arrestin-selective G protein-coupled receptor agonists engender unique biological efficacy in vivo. Mol Endocrinol 2013;27(2):296–314.
[205] Luttrell LM, et al. Transcriptomic characterization of signaling pathways associated with osteoblastic differentiation of MC-3T3E1 cells. PLoS One 2019;14(1):e0204197.
[206] Luttrell LM, et al. Manifold roles of beta-arrestins in GPCR signaling elucidated with siRNA and CRISPR/Cas9. Sci Signal 2018;11(549).
[207] Mann M. Fifteen years of stable isotope Labeling by amino acids in cell culture (SILAC). Methods Mol Biol 2014;1188:1–7.
[208] Kohli P, et al. Label-free quantitative proteomic analysis of the YAP/TAZ interactome. Am J Physiol Cell Physiol 2014;306(9):C805–18.
[209] Luu J, Palczewski K. Human aging and disease: lessons from age-related macular degeneration. Proc Natl Acad Sci U S A 2018;115(12):2866–72.