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REVIEW ARTICLE

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GPCRs and fibroblast heterogeneity in fibroblast-associated diseases

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Abstract

G protein-coupled receptors (GPCRs) are the largest and most diverse class of signaling receptors. GPCRs regulate many functions in the human body and have earned the title of "most targeted receptors". About one-third of the commercially available drugs for various diseases target the GPCRs. Fibroblasts lay the architectural skeleton of the body, and play a key role in supporting the growth, maintenance, and repair of almost all tissues by responding to the cellular cues via diverse and intricate GPCR signaling pathways. This review discusses the

Abbreviations: A2B, adenosine receptor; ACE, angiotensin-converting enzyme; ALK5, activin receptor-like kinase 5; AngII, angiotensin II; apCAFs, antigen-presenting CAFs; ATR1, angiotensin II receptor 1; B2-AR, beta-2-adrenergic receptor; CAFs, cancer-associated fibroblasts; cAMP, cyclic adenosine monophosphate; CB1, cannabinoid receptor 1; CB2, cannabinoid receptor 2; CFs, cardiac fibroblasts; CP, chronic pancreatitis; CRC, colorectal cancer; CREB, cAMP response element binding; CTGF, connective tissue growth factor; CVDs, cardiovascular diseases; CXCR3, C-X-C motif chemokine receptor 3; DUBs, deubiquitinating enzymes; ECM, extracellular matrix; ECs, endothelial cells; EDA-FN, extra domain A containing fibronectin; EMT, epithelial to mesenchymal; EP2, prostaglandin E receptor; Epac, exchange protein activated by cAMP; ERK, extracellular signal-related kinase; ET, endothelin; ETAR, endothelin receptor A; ETBR, endothelin receptor B; ETRs, endothelin receptors; FAP, fibroblast activation protein; FDA, Food and Drug Administration; Foxp1, forkhead box transcription factor P1; FSP, fibroblast-specific protein; GFAP, glial fibrillary acidic protein; GI, gastro-intestinal; GPCRs, G protein-coupled receptors; GPER1, G-protein coupled receptor; GPR40, G-protein coupled receptor 40; GRK2, GPCR kinase 2; HIF, hypoxia-inducible factor; HRT, hormone replacement therapy; HSCs, hepatic stellate cells; iCAF, inflammatory CAFs; IDO1, indoleamine 2, 3 dioxygenase 1; IL-11, interleukin 11; IL-1β, interleukin 1-β; IPF, idiopathic pulmonary fibrosis; JNK, c-Jun N-terminal kinase; KA, keratoacanthomas; lncRNA, long non-coding RNA; LPA, lysophosphatidic acid; mAchRs, muscarinic acetylcholine receptors; MAPK, mitogen-activated protein kinase; miRNA, microRNA; MRTF-A, myocardin-related transcription factor A; MSCs, mesenchymal stem cells; myCAF, myofibroblasts CAFs; NAFLD, non-alcoholic fatty liver disease; NLRP3, NOD-like receptor family pyrin domain containing 3; OC, oral contraceptives; ORs, olfactory receptors; PAR1, protease-activated receptor 1; PDAC, pancreatic ductal adenocarcinoma; PDGF, platelet-derived growth factor; PGE2, prostaglandin E2; PI3K, phosphoinositide-3-kinase; PKA, protein kinase A; PPAR-γ, peroxisome proliferator-activated receptor gamma; PSCs, pancreatic stellate cells; RA, retinoic acid; RAR-β, Retinoic acid receptor beta; ROCK, Rho-associated protein kinase; ROS, reactive oxygen species; S1PR1, sphingosine-1-phosphate receptor 1; SCC, squamous cell carcinoma; TAK1, TGF-β1-activated kinase 1; TAS2Rs, taste receptors; TAZ, taffazin; TGF-β1, transforming growth factor β1; TME, tumor microenvironment; VEGF, vascular endothelial growth factor; YAP, yes-associated protein; α-SMA, alpha smooth muscle actin; β-AR, beta-adrenergic receptors.

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dynamic architecture of the GPCRs and their intertwined signaling in pathological conditions such as idiopathic pulmonary fibrosis, cardiac fibrosis, pancreatic fibrosis, hepatic fibrosis, and cancer as opposed to the GPCR signaling of fibroblasts in physiological conditions. Understanding the dynamics of GPCR signaling in fibroblasts with disease progression can help in the recognition of the complex interplay of different GPCR subtypes in fibroblast-mediated diseases. This review highlights the importance of designing and adaptation of next-generation strategies such as GPCR-omics, focused target identification, polypharmacology, and effective personalized medicine approaches to achieve better therapeutic outcomes for fibrosis and fibrosis associated malignancies.

KEYWORDS

fibroblast behavior, fibrotic diseases, functional heterogeneity, GPCR signaling, pathophysiology, pharmacological targeting

1 | GPCRS—A MAJOR SIGNALING FAMILY

G-protein-coupled receptors (GPCRs) are a highly diversified class of membrane receptors in the human genome comprising more than 800 membrane receptors.[1] GPCRs contain a single polypeptide unit folded into a globular structure and are embedded in the plasma membrane via seven transmembrane domains.[2] Therefore, GPCRs are also known as the seven pass-transmembrane protein receptors.[3] GPCRs are critical regulators of various physiological processes and have been exploited as potential therapeutic targets for numerous diseases. Analogically, GPCRs act as a transmitter in communicating the input signals, i.e., the extracellular cues in the form of environmental stimulants like hormones, ions, photons, odorants, and neurotransmitters to a functional output in the form of intracellular signals.[4] This transmission of external stimuli is facilitated by one or more of the four major G proteins namely Gαi/o, Gαq/11, Gα12/13, and Gαs (Figure 1).[2]

Besides the four major G proteins, there are also small single subunit G-proteins such as the signaling protein Ras and the heterotrimeric GPCRs containing three different subunits—alpha, beta, and gamma subunits respectively.[5] The alpha and the gamma subunits are anchored to the plasma membrane via the lipid anchors while the beta subunit is attached to the gamma subunit. In the resting state, the GPCR is bound intrinsically to GDP, which is attached to the heterotrimeric subunits in the cytosolic domain. Upon stimulation, the GPCR behaves like an exchange factor mediating the release of GDP from the alpha subunit of the G-protein. The GDP is replaced by GTP and the GPCR is activated and undergoes conformational changes. The conformational changes as a result of the GTP bound Gα subunit lead to the dissociation of the heterotrimeric structure into heterodimeric Gβγ subunit and Gα subunit.[6] When the GTP is hydrolyzed to GDP, the subunit regains its original conformation to form the heterotrimeric structure. Each of the free subunits can interact with their downstream mediators and drive the second messenger signaling swiftly, thereby regulating cellular physiology (Figure 1). Thus, depending on the extracellular cues, the GPCRs can promptly act as rapid bimodal switches for their signaling.

2 | FIBROBLASTS IN NORMAL PHYSIOLOGY

Fibroblasts are the major active cellular components of the connective tissues. They are large spindle-shaped cells that synthesize the extracellular matrix (ECM) and collagen, laying the architectural framework of animal tissues while also contributing to the signaling niche through extracellular cues.[7] Fibroblasts that are of mesenchymal origin express the filamentous protein Vimentin, whereas those that are derived from epithelial cells upon epithelial to mesenchymal transition express fibroblast surface markers such as fibroblast activation protein (FAP) and fibroblast specific protein (FSP).[8,9] Fibroblasts actively participate in tissue homeostasis through wound repair and healing. Firboblast break down the fibrin clot and produce a protective cushion of the ECM to support the neighboring cells during wound healing and contraction.[10] Fibroblasts primarily originate as fibrocytes which are inactive mesenchymal cells and are primarily involved in supporting tissue maintenance and metabolism.[11] Following tissue injury, several cytokines, chemokines, and growth factors are released which promote maturation, differentiation,

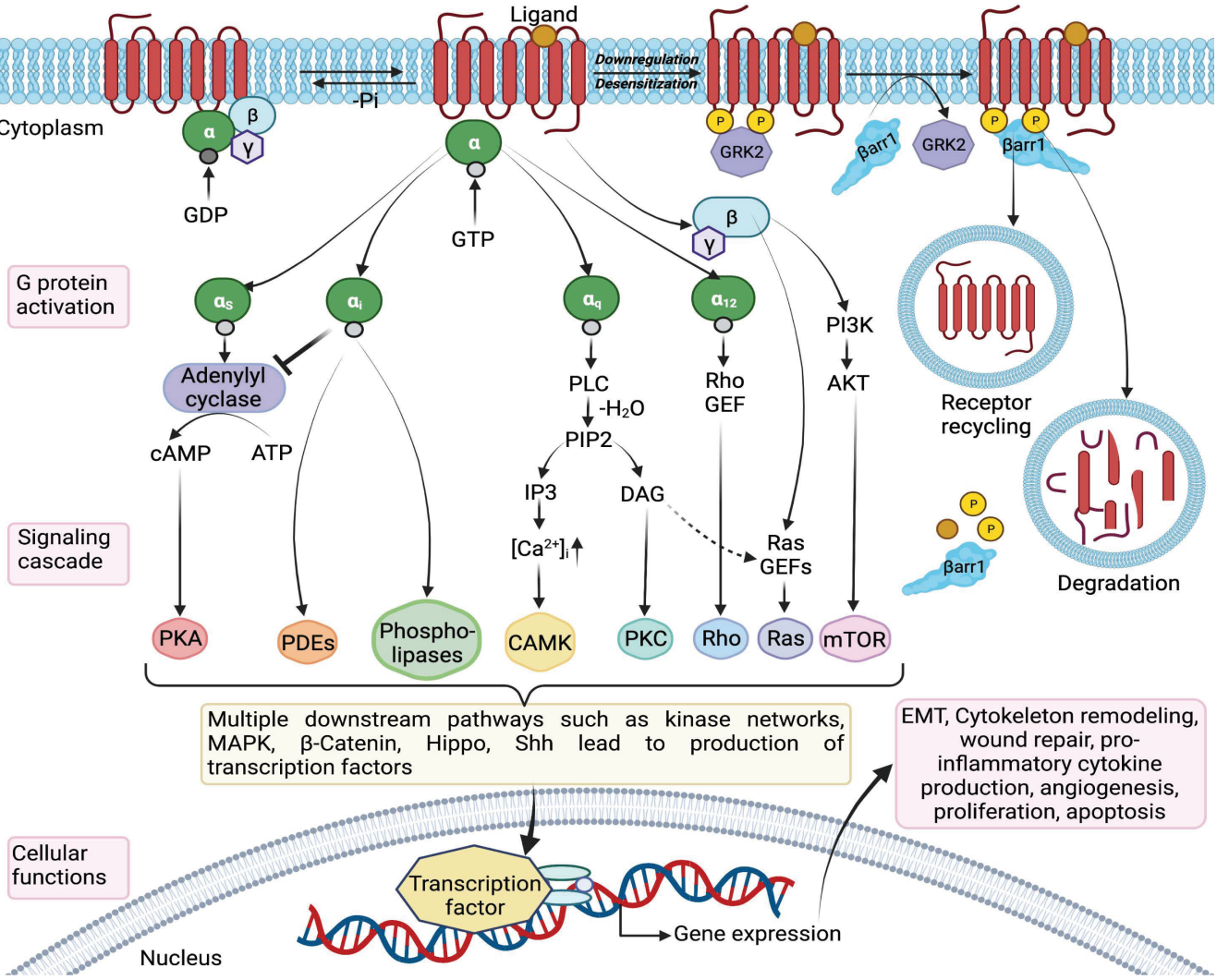

FIGURE 1 G-protein-coupled receptor (GPCR)-mediated signal transduction. In response to stimulus from external ligands, the G-protein activation controls several cellular functions. A multitude of downstream pathways that regulate crucial physiological processes, including wound healing, proliferation, apoptosis, angiogenesis, tumor growth, invasion, and metastasis, are governed by the four G-protein α subunits (Gs, Gi, Gq/11, and G12/13). Subsequently, the receptor desensitization and downregulation occur via the β-arrestin-GRK2 pathway to be either recycled or degraded. Figure generated using *BioRender*.

and activation of the fibrocytes to fibroblasts. The activated fibroblasts trigger the release of ECM proteins such as collagen, tenascin, laminin, and elastin leading to stromal remodeling to facilitate wound repair.[12] In addition, the malleable nature of fibroblasts in terms of their elasticity and plasticity, helps them migrate to the site of the wound and commit to their developmental fates. Under diseased conditions, dysregulated fibrogenesis leads to scarring, lesion formations, overgrowth, and hardening of tissues, resulting in excessive fibrosis and organ failure. Life-threatening diseases such as idiopathic pulmonary fibrosis, cardiovascular fibrosis, liver cirrhosis, pancreatitis, and cancers involve extensive tissue remodeling and fibrosis. Treatment strategies for these diseases focus, among others, on targeting the inflammatory signaling pathways to disrupt the fibroblast activating cues.[13] However, the mechanisms of autocrine self-activation of the heterogeneous fibroblasts under stressed conditions have not been completely elucidated, except for myofibroblast activation via a positive feedback loop. Myofibroblasts produced in the process of EMT from either resident mesenchymal cells, epithelial or endothelial cells, or those derived from bone-marrow stem cells have been widely reported to be the key mediators of the fibrosis.[13] Klingberg et al. have reported that transforming growth factor β1 (TGF-β1) and extra domain A containing fibronectin (EDA-FN) induce autocrine self-activation of myofibroblasts to progress in a positive feedback loop.[14] Additionally, paracrine activation of dermal and lung fibroblasts via signals received from classically activated (M1) macrophages, alternatively activated (M2) macrophages, and lymphocytes have also been studied.[15,16]

An extension of fibroblast subtypes and heterogeneity has recently emerged in the context of cancer-associated fibroblasts (CAFs). The heterogeneity in CAFs is not limited to the plastic nature of the fibroblasts but is even more complicated and extends to the organ and tissue level. Depending on the tumor microenvironment (TME) and the location of the tumor, e.g., in the lungs, pancreas, liver, and breasts, the markers expressed by the CAFs, their phenotype, genotype, and functionality vary. The complexity of the fibroblasts is rooted in the intricate downstream signaling pathways that perhaps lead to the activation of diverse transcriptional mechanisms that are different, though not independent of the inflammatory response. Thus, exploring the repertoire of complex molecular signatures in fibroblast heterogeneity and function, through the lenses of major GPCRs might help in identifying a converging downstream pathway or specific fibrosis-promoting targets.

3 | GPCR SIGNALING AND FIBROBLAST HETEROGENEITY IN PATHOLOGICAL STATES

3.1 | Idiopathic pulmonary fibrosis

Idiopathic pulmonary fibrosis (IPF) is an advanced and chronic lung disease marked by uncontrolled fibrogenesis leading to the accumulation of fibrous connective tissue. Fibrogenesis leads to lung damage and fibrotic scarring, compromising the lung function. Worsening pulmonary fibrosis eventually leads to respiratory distress and death. Over ~3 million people are affected by IPF worldwide, wherein 30 000 to 40 000 cases are reported in the United States annually, with the median overall life expectancy of patients around 4.5 years.[17-19] A wide number of GPCRs such as endothelin receptor A (ET_AR) and endothelin receptor B (ET_BR), lysophosphatidic acid (LPA), sphingosine, angiotensin, and G-protein-coupled receptor 40 (GPR40), and GPR84 have been implicated in IPF and are being studied in both preclinical and clinical studies.[5] Interestingly, these receptors have been found to drive the activation of pulmonary fibroblasts leading to fibrosis by specific activation of multiple subclasses of G proteins, such as Gαi/o, $G\alpha_{q/11}$, and $G\alpha_{12/13.}$[20] It has been found that profibrotic ligands such as LPA, sphingosine, endothelin, and serotonin activate these G subclass proteins to initiate multiple pathways such as Ras, mitogen-activated protein kinase (MAPK), Rho, and Rho-associated protein kinase (ROCK), phosphoinositide 3-kinase (PI3K), and AKT for modulating the actin cytoskeleton to form a stable F-actin assembly.[21] The important converging downstream targets of these pathways are the activation of the transcription factors such as myocardin-related transcription factor A (MRTF-A) and yes associated protein (YAP)/taffazin (TAZ) which mediate the transcription of the profibrotic genes: *COL1A*, *COL1A2*, *CTGF*, and A*CTA2*. In a normal physiological state, activation of the $G\alpha_s$ subunit leads to the production of cyclic adenosine monophosphate (cAMP) which acts as a negative regulator of fibroblastic growth. The transcriptional activation is facilitated mainly by exchange protein activated by cAMP (Epac)1/2, downstream protein kinase A (PKA), and cAMP response element-binding (CREB), resulting in destabilization of the F-actin assembly. Thereby, the GPCRs render an anti-fibrotic effect and protect against the pro-fibrotic effect of MRTF and YAP/TAZ.[22,23] But this mechanism is often suppressed in the fibroblasts of IPF patients. Huang et al. demonstrated that Epac-1 and PKA function as downstream targets of the prostaglandin E2 (PGE2) pathway, Epac-1 and PKA agonists prevent fibroblast proliferation via the action of small GTPase Rap1, mitigating collagen 1 expression, and exhibiting a collective anti-fibrotic activity.[24] However, a possible therapeutic design incorporating upregulation of the anti-fibrotic signaling pathways and simultaneous downregulation of fibrosis-promoting pathways remains unaccomplished. Emerging studies have shifted their focus to reverse lung scarring and lung fibrosis as a therapeutic approach to IPF.[25] Recently, Ng et al. identified that TGF-β-induced interleukin-11 (IL-11) autocrine signaling of fibroblasts leads to the translation of profibrotic proteins via the extracellular signal-regulated kinase (ERK) pathway. IL-11-specific therapeutic antibodies in a bleomycin-induced pulmonary fibrosis preclinical model, not only exerted anti-inflammatory effects but also arrested myofibroblast differentiation and cellular senescence of the lung, reversing lung fibrosis.[26] Uneomori et al. explored the role of relaxin, a growth factor, in inducing an ECM degrading phenotype in human lung fibroblasts. Relaxin inhibited the deposition of ECM and restricted TGF-β-mediated overexpression of pro-fibrotic factors like collagen type I, III, and fibronectin.[27] While exploring the heterogeneity of GPCRs that are potentially involved in IPF progression, rather peculiar receptors, namely bitter taste receptors (TAS2Rs) and olfactory receptors (ORs), whose expressions were thought to be restricted to the tongue and the nose have also been discovered. These GPCRs have recently been found on the human airway smooth muscle cells as well. Sharma et al. established the anti-fibrotic role of TAS2Rs in mice using TAS2R agonists which resulted in a significant decrease in collagen type 1 deposition, lung ECM remodeling, α-SMA expression along with inhibition of Smad2 phosphorylation, and activation of the pro-fibrotic cytokine

markers like TGF-β.[28,29] Overall, the versatility of the GPCRs to mediate signaling with the most unexpected dynamics makes them an exciting but challenging targets to treat IPF.

3.2 | Cardiac fibrosis

Heart diseases are the predominant cause of death in the United States; 1 in every 4 death is due to cardiovascular diseases (CVDs).[30] About 655,000 Americans die of heart disease each year and the overall annual financial burden of CVDs is $219 billion since 2014.[30-33] The expenses of dealing with health care services, medicines, treatments, loss of life, and decreased productivity as a result of increased CVDs are projected to rise to a whopping ~$918 billion by 2030.[31] Almost all clinical manifestations of heart diseases involve the activation of cardiac fibroblasts (CFs), leading to the myocardial remodeling of the ECM and the release of pro-fibrotic factors. Cardiac fibrosis causes thickening and loss of flexibility in the valves and myocardium of the heart leading to valvular dysfunction that hinders the functioning of the heart. There has been a never-ending debate around the origin of CFs. Recent lineage-tracing studies have delineated the diverse origin of CFs and proposed numerous precursors such as the resident fibroblasts, cells of vascular origin like the epithelial and epicardium, the perivascular cells, the hematopoietic bone marrow-derived progenitor cells, and the fibrocytes.[34] Physiologically, resident CFs are vital for the structural and mechanical protection of the heart to maintain its precise conductivity and rhythmicity by appropriately altering and modulating the cardiac collagen network. The role of resident CFs in guiding the functioning of the heart has recently come under substantial scrutiny. As the delineation of the CFs based on their origin, characteristics, and plasticity is evolving, it has been shown that the resident CFs indeed originate from the embryonic epicardium, and these epicardium-derived CFs are predominantly involved in inducing the fibrotic response of these fibroblasts.[35,36] Therefore, considerable efforts have been invested to identify the source of the activated fibroblasts and the exclusive sources of collagen production, to understand the long-term implications of their activation, and to unravel a crucial potential target for antifibrotic therapies. Depending on the origin and mode of activation of the CFs their action may vary, but they all result in cardiac fibrosis. Interestingly, the signaling events mediated by the activated CFs all begin and converge through the secretion of TGF-β. The activin receptor-like kinase (ALK5), also known as the type I TGF-β receptor primarily modulates the fibrotic properties of the TGF-β.[37] Canonically, Smad3 regulates the production of ECM proteins via TGF-β. It was also seen that the deletion of Smad3 not only blocks the epithelial-myofibroblast transition but also, inhibits the production of type 1 collagen both in-vivo and in-vitro. Moreover, other Smad proteins such as Smad7 negatively regulate TGF-β /Smad3-induced fibrogenesis (Figure 2).[38,39]

Non-canonically, TGF-β has also been speculated to act through the c-Jun N-terminal kinase (JNK) and p38 MAPK pathways (Figure 2). TGF-β-activated kinase 1 (TAK1) has been proven to be a key player in mediating TGF-β-induced fibrosis as TAK1 inhibition led to a decrease in the production of ECM proteins attenuating the expression of collagen, fibronectin, and α-SMA in the activated myofibroblasts.[40,41] Furthermore, an integral system such as the renin-angiotensin system, predominantly angiotensin II (AngII), contributes to the progression of cardiac fibrosis by inducing cell proliferation, migration, and synthesis of ECM proteins. The activation of the angiotensin system, particularly a GPCR angiotensin II receptor 1 (ATR1), mediates the production of TGF-β, which is involved in the development of cardiac hypertrophy and fibrosis. ATR1 is different from the other GPCRs that are generally involved in fibrosis; ATR1 demonstrates mechanical sensing abilities contributing to stretch-induced response and is activated during myocardial remodeling, consequently leading to chronic fibrosis.[42,43] ATR1 antagonists such as candesartan and losartan have been demonstrated to mitigate the effects of TGF-β-induced fibroblast activation and decrease secretion of inflammatory markers such as interleukin-6 (IL-6) and interleukin 1-β (IL-1β) thereby, protecting against myocardial hypertrophy and fibrosis in patients.[44-46]

The endothelin (ET) system has also been widely studied in the context of cardiac fibrosis. The ET-axis components are expressed by endothelial cells, smooth muscle cells, macrophages, CFs, and cardiomyocytes and regulate vasomotor tone via modulating vasoconstriction and vasodilation. Elevated levels of ET-1 ligand were observed in scarred tissues of aged cardiac fibrosis patients with myocardial infarction, which signals through the endothelin receptors (ETRs) belonging to the diverse superfamily of GPCRs.[47] Moreover, ET-1 has been demonstrated to promote EMT and Endo-MT, leading to the differentiation and activation of epithelial and endothelial cells, respectively to myofibroblasts.[47,48] Additionally, the activation of the ET_AR receptor and overstimulation by the ligand ET-1 has been associated with increased collagen production, cell proliferation, and α-SMA expression in the CFs. Intriguingly, ET-1 acts as a downstream mediator of TGF-β while also activating p38 MAPK through the non-canonical TGF-β signaling (Figure 2).[49,50]

A new modulator-forkhead box transcription factor P1 (Foxp1), found in the endothelial cells (ECs), has also been implicated in cardiac remodeling. Intriguingly, EC-Foxp1

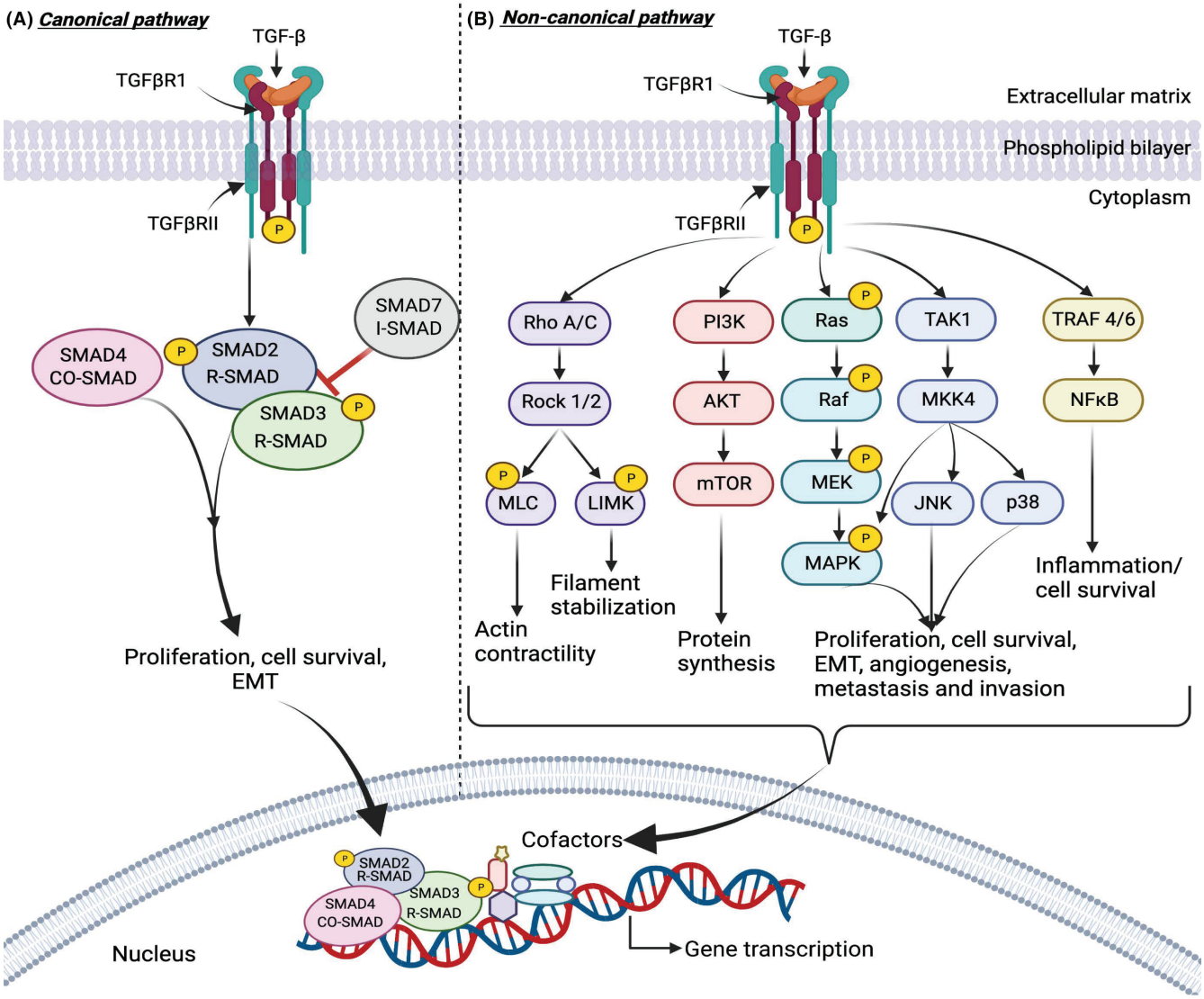

FIGURE 2 Canonical and non-canonical TGF-β-mediated signaling pathways. TGF-β signaling is mediated intracellularly by either a (A) canonical, SMAD2, and SMAD3-dependent pathway or various (B) non-canonical pathways that communicate in different ways, including the Rho-ROCK, MAP-kinase pathways via the Ras-Raf-MEK-ERK or JNK and p38, PI3K-AKT-mTOR, and NFκB. Proliferation, cell survival, epithelial-to-mesenchymal transition (EMT), and protein synthesis are a few examples of the several cellular processes that each signaling arm regulates. Figure generated using *BioRender*.

deletion augments TGF-β-induced ET-1 expression resulting in the reactivation of myocardial remodeling genes, increased cardiomyocyte size, and cardiac hypertrophy.[51] Foxp1 deletion leads to aggressive collagen deposition and extensive production of ECM proteins which was found to be otherwise restricted in the presence of EC-Foxp1.[51] Bosentan is a dual ETR antagonist, clinically approved for the treatment of group 1 pulmonary arterial hypertension, and has reportedly enhanced cardiac function while also attenuating myocardial infarction in rats.[52] With the advent of next-generation selective ET_AR antagonists like ambrisentan and darusentan, and dual ETR antagonist macitentan, the prospect of ET-axis targeting to diminish CF's profibrotic properties and resolving cardiac fibrosis with the goal of improved cardiac function appears to be a promising therapeutic approach.

Another key pathway that acts downstream of GPCRs such as the ATR1, and ETRs, and the TGF-β signaling pathway is the RhoA-MRTF-SRF signaling, which confers the CFs their pro-fibrotic properties (Figures 2 and 3). Importantly, this pathway is also implicated in IPF through similar mechanisms as discussed earlier. An increased expression of MRTF-A alone leads to the overexpression of α-SMA and conversion to the myofibroblast type, which is reversed upon the deletion of MRTF-A.[53]

It is noteworthy to mention that GPCRs are not only involved in the pathogenesis of the disease but are also manipulated by the body to fight and prevent the progression

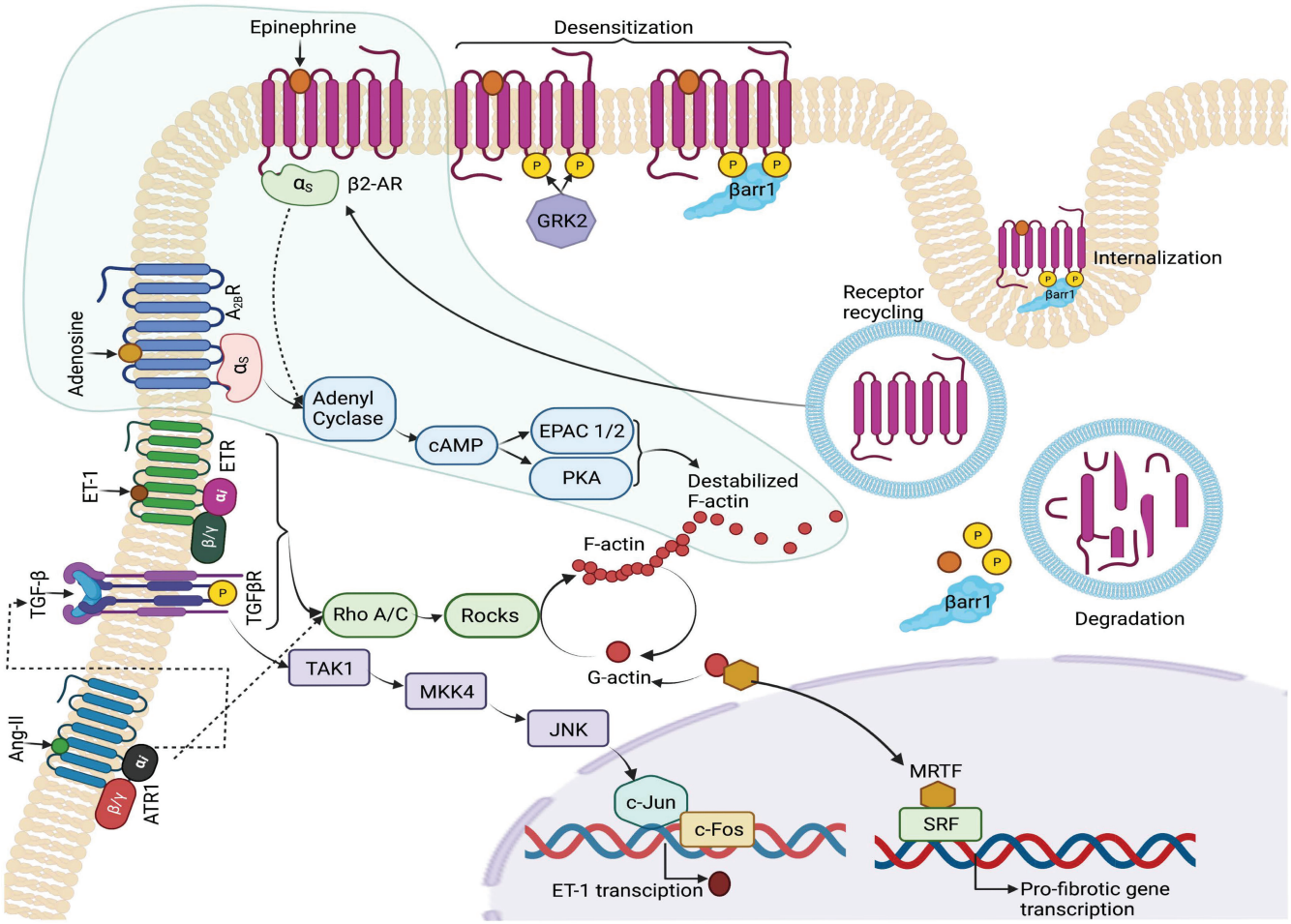

FIGURE 3 The interplay of GPCRs to activate pro-fibrotic pathways and deactivate the anti-fibrotic GPCR pathways driving active transcription of pro-fibrotic genes. The RhoA-MRTF-SRF signaling pathway is an important pathway that confers the cardiac fibroblasts (CF) their pro-fibrotic potential. Elements of this pathway such as downstream RhoA and SRF-mediated signaling are activated by TGF-β, AngII, and ET-1 signaling. The activation of the angiotensin II receptor 1 (ATR1) also mediates the production of TGF-β ligand which then activates the canonical and non-canonical TGF-β signaling. Additionally, TGF-β signaling increases ET-1 synthesis, which activates the genes involved in myocardial remodeling. Physiologically, GPCRs adenosine receptor (A2BR) and beta-2-adrenergic receptor (β2-AR) have been found to increase the cAMP levels resulting in the destabilization of the F-actin assembly, preventing nuclear translocation of transcription factors MRTF and SRF, inhibiting transcription of pro-fibrotic genes, myofibroblast activation and fibrosis progression. However, in the pathological condition, A2BR and β2-AR are desensitized and downregulated by GPCR kinase 2 (GRK2) supporting fibrotic response. GRK2 also acts as a scaffold protein for RhoA, further contributing to fibrosis progression. Figure generated using *BioRender*.

of the disease. Purinergic GPCRs like adenosine receptor (A_{2B}) with adenosine as their endogenous ligands were found to inhibit ET-1-induced α-SMA expression in the CFs and their conversion, activation, and proliferation to the myofibroblasts via the cAMP/EpacC/PI3K/Akt signaling pathway (Figure 3).[54] However, A2BR downregulation was observed in the hearts of human patients with chronic heart failure.[55] Additionally, the beta-2-adrenergic receptor (β_2-AR) has been reported to increase the cAMP levels that inhibits the TGF-β-stimulated myofibroblast activation and fibrosis progression (Figure 3). Under pathological conditions, GPCR kinase 2 (GRK2) via the β-arrestin pathway desensitizes the β_2-ARs and leads to their downregulation mediating the fibrotic response (Figure 3).[56] Apart from desensitization, GRK2 also acts as a scaffold protein for RhoA which contributes to fibrosis. The discovery of an amalgam of direct and indirect targets such as A_{2B}R, β_2-ARs, RhoA, GRK2, and β-arrestins has thus, provided an opportunity to exploit the complexity and intricacy of the GPCRs to carefully target them with agonists and antagonists to treat cardiac fibrosis.

Therapeutic targeting of TGF-β in heart diseases has been long pursued. TGF-β targeting is achieved through various approaches including ligand traps by anti-ligand (TGF-β) antibodies, antisense oligonucleotides, small molecule receptor kinase inhibitors (inhibit the TGF-β receptor ALK5), and the use of peptide aptamers that block downstream signaling through ALK5 by blocking

Smad signaling.[57] Although, these strategies inhibit fibroblast activation, remodeling, and collagen synthesis, in some patients no significant changes in cardiac function have been observed. Moreover, this treatment model has been seen to affect renal autophagy (as a cytoprotective mechanism) leading to renal failure, affecting the overall health of the patient, and further contributes to worse outcomes.[58] Mice treated with TGF-β blockers exhibited severe vascular and inflammatory defects, raising concerns about their use in humans.[59,60] Long-term exposure and high dosage of these blockers resulted in hemorrhagic lesions of heart valves and aortic aneurysms in rats and dogs.[61-63] High doses and persistent treatment with ligand trap GC-1008 leads to adverse effects such as skin lesions, non-malignant keratoacanthomas (KA), squamous cell carcinoma (SCC), gingival bleeding, and fatigue when used in malignant melanoma patients.[64,65] With therapeutic advancements, microRNA (miRNA), long non-coding (lncRNA), and deubiquitinating enzymes (DUBs) have been used to target the modulatory components of TGF-β signaling as safer alternatives.[66] Co-inhibition with dual targeting of ATR1 and ETRs using valsartan and bosentan has reportedly exhibited a synergistic effect in reducing TGF-β, α-SMA, and collagen IV expression protecting against renal fibrosis in unilateral ureteral obstructed mice.[67,68] This provides evidence that combination treatment with indirect blockers of TGF-β modulators might be a promising therapeutic approach to treat cardiac fibrosis.

3.3 | Pancreatic fibrosis

Pancreatic fibrosis is an established feature of chronic pancreatitis and desmoplastic pancreatic cancer. The condition is marked by abnormal activation of pancreatic stellate cells (PSCs) that leads to the formation and deposition of ECM disrupting pancreatic function. In the case of chronic pancreatitis (CP), pancreatic cancer, or pancreatic fibrosis, although, a fraction of PSCs is proven to be derived from the bone marrow, there is evidence of their hematopoietic and mesodermal origin.[69]

Under normal physiological conditions, the PSCs act as a reservoir to store vitamin A lipid droplets, and express markers such as glial fibrillary acidic protein (GFAP), vimentin, desmin, nestin, and retinoids that help them differentiate into the normal fibroblasts.[69] An injury to the pancreas promotes fibrogenesis mainly by exasperating the PSCs, mesenchymal stem cells (MSCs), ductal cells, and acinar cells. A sustained injury or inflammation to the pancreas causes the otherwise quiescent pancreatic stellate cells (PSCs) to get perpetually activated and lead to fibrosis. When activated through paracrine or autocrine mechanisms, the PSCs transform into α-SMA$^+$ myofibroblast-like cells that not only proliferate but migrate, and release ECM-forming components, pro-inflammatory cytokines, and chemokines subsequently aggravating the disease.[70] The release of these factors further leads to self-activation of the PSCs maintaining a positive feedback loop for their activation through GPCRs like ATR1, ETRs, frizzled receptors, lysophosphatidic acid receptor, and proton sensing GPCRs.[71,72] Following activation, the PSCs orchestrate various intracellular signaling mechanisms by secreting stimulatory and inhibitory signaling molecules including MAPK, PI3K, reactive oxygen species (ROS), and the nuclear receptor peroxisome proliferator-activated receptor-γ (PPAR-γ).[73] Upon activation, these signaling molecules mediate processes such as EMT, ECM remodeling, imbalanced redox homeostasis, as well as pancreatic inflammation.[70] Advanced stages of pancreatic fibrosis can progress to chronic pancreatitis and pancreatic cancer.[74] The activated PSCs mediate crosstalk with the cancer cells promoting their survival, proliferation, metastasis, invasion, angiogenesis, and consequent tumor growth thus, making it essential to delineate the specific role of these GPCRs in PSC activation.[75] The role of ATR1 and AngII in inducing pancreatic fibrosis has been well established by Kuno et al., where inhibitor of angiotensin-converting enzyme (ACE) lisinopril, ameliorated pancreatic inflammation and fibrosis by suppressing the expression of TGF-β1 mRNA, thus preventing activation of PSCs in-vivo.[76,77] This was further corroborated by a study published by Aoki et al. demonstrating that AngII promoted PSC proliferation by inhibiting Smad7 expression leading to an activated TGF-β pathway.[76,78] The role of ET-1 in mediating PSC activation via ERK pathways and the use of Rho kinase inhibitors in preventing ET-1-fostered PSC migration is also well known.[76] Moreover, the NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome leads to the production of IL-1β and activation of TGF-β pathway and plays a direct role in the activation of PSCs both in-vivo and in-vitro.[79] Interestingly, a GPCR sphingosine-1-phosphate receptor 1 (S1PR1) and its ligand sphingosine-1-phosphate have been found to increase the expression of NLRP3 inflammasome thus leading to PSC activation.[80] The Frizzled receptors have also been implicated in PSC activation by both canonical and non-canonical Wnt signaling pathways. Hu et al. have reported the role of secreted Dickkopf-1 protein which inhibits the canonical Wnt/β-catenin pathway preventing the nuclear translocation of β-catenin in PSCs and their subsequent activation.[81]

Interestingly, the use of retinoic acid (RA) to revert the activated PSCs to a quiescent state has emerged as an attractive therapeutic strategy for resolving pancreatic fibrosis. A corroborating study by Xiao et al. showed the role of RA as an inhibitor of PSC activation attenuating pancreatic

fibrosis via the Wnt/β-Catenin signaling pathway.[82,83] RA treatment inhibited PSC proliferation, decreased ECM formation, and nuclear translation of β-Catenin while also downregulating the expression of Wnt2, TGFRII, PDGFR, and collagen 1 in mice with chronic pancreatitis.[82] In addition, retinoic acid receptor beta (RAR-β) activation led to the downregulation of actomyosin (MLC-2) contractility thus hampering the mechanosensory ability of the PSCs and their ECM remodeling ability. As a result, the activated PSCs return to their vitamin A-storing quiescent state if the fibrotic injury is controlled.[83] Thus, the functional heterogeneity of the PSCs plays a significant role in orchestrating the intracellular signaling effects to modulate the antifibrotic pathways such as one mediated by the crucial retinoic acid receptors. As our understanding of PSCs and pancreatic fibrosis progresses, additional targetable molecules may be employed to prevent the profibrotic effects of these GPCRs.

3.4 | Liver fibrosis

Similar to organs like the lung, heart, and pancreas, liver fibrosis too occurs because of sustained injury or inflammation causing accumulation of ECM proteins and scar tissue formation. Repeated wound and damage to the liver leads to the development of liver cirrhosis, portal hypertension, hepatocellular carcinoma, and eventual organ failure. Liver cirrhosis is a late-stage fibrosis that results in approximately 1.3 million deaths per year globally and is the 11th most common cause of death worldwide.[84] Hepatic stellate cells (HSCs) are the key mediators of hepatic fibrosis.[85] They transdifferentiate from a quiescent to an activated state, depositing extensive ECM proteins, leading to the initiation, and progression of liver fibrosis. GPCRs are instrumental in promoting the fibrogenic potential of HSCs. GPCRs direct various pro-inflammatory and pro-fibrotic responses and modulate the activity of resident macrophages (Kupffer cells), and recruitment of bone marrow-derived monocytes, and myofibroblasts to accelerate fibrosis. Therefore, delineating the role of GPCRs in the pathophysiology of liver fibrosis is crucial to foster effective pharmacological intervention.

Kimura et al. have recently summarized the involvement of various GPCRs representing different families in HSC activation and proliferation in the non-alcoholic fatty liver disease (NAFLD).[86] Adding to the long list of such GPCRs, we tabulated some more GPCRs, and discussed their relevance in facilitating liver fibrosis based on the literature survey (Table 1). Contrasting functions of multiple GPCRs have emerged in the progression of hepatic fibrosis. Prostaglandin E receptor (EP2) was shown to increase the proliferation of LI90, a human HSC cell line in a platelet-derived growth factor (PDGF)-dependent manner whereas, another study reported suppression of the same GPCR in PDGF-induced proliferation in rat primary HSC line.[87,88] Cannabinoid receptors such as cannabinoid receptor 1 (CB1) further add to the complexity of the role of GPCRs in fibrosis. Studies by Dai et al. and Mallat et al. have emphasized the role of CB1 in HSC activation and their trans-differentiation to myofibroblasts to potentiate hepatic fibrosis, whereas Wang et al. suggest that deletion of CB1 in stellate cells of mice does not prevent fibrosis indicating that CB1 does not have a direct role in mediating hepatic fibrosis.[89-91] Moreover, the cannabinoid receptor 2 (CB2), a GPCR, functions opposite to CB1, and $CB2^{-/-}$ mice exhibited advanced fibrosis upon CCL4 treatment indicating that CB2 is anti-fibrotic.[92] C-X-C motif chemokine receptor 3 (CXCR3) is another GPCR, which functions ambiguously in mediating hepatic fibrosis. A widely accepted role of CXCR3 is in chronic liver inflammation where it promotes macrophage activation and release of factors that could potentially promote fibrosis.[93] The outcome and effect of CXCR3 activation in liver fibrosis are dictated by the interacting ligand. Wasmuth et al. demonstrated the anti-fibrotic role of CXCR3 when activated by CXCL9 in human and mice liver.[94] On the contrary, Singh et al. reported liver fibrosis progression upon activation of CXCR3 by intrahepatic CXCL10.[95] Other GPCRs, including the muscarinic acetylcholine receptors (mAchRs), have also been demonstrated to mediate variable effects during the progression of liver fibrosis. Luo et al. reported that the group I muscarinic acetylcholine receptors (M1, M3, and M5) are associated with hepatic injury, and the group II receptors (M2 and M4) activate the Nrf2/ARE pathway, protecting against oxidative stress-induced liver injury, preventing fibrosis via positive feedback regulation.[96] In contrast, studies from Morgan et al. and Khurana et al. suggest the pro-fibrotic role of M2, and the anti-fibrotic role of M3 receptors, respectively (Table 1).[97,98]

Therefore, over the years multiple facets of GPCRs have emerged indicating their involvement in both the promotion and prevention of hepatic fibrosis. Yet, the contribution of functionally plastic and dynamic classes of several other GPCRs in advancing the diseased state remains obscure.

4 | GPCRS IN CANCER AND GPCROmics

The functional diversity and implications of GPCRs in multiple non-oncological diseased conditions have been well established. However, the potential of these vast receptor families in modulating the TME is not elucidated. In addition, the contribution of the GPCRs to the

TABLE 1 G-protein-coupled receptors expressed in HSCs and their function in the development of hepatic fibrosis.

Receptor name	G protein family	Function in liver fibrosis	References
B1-adrenoreceptor (ADRB1)	G_s	Production of TGF-β, activation of HSCs being pro-fibrotic, modulates pro-inflammatory response by regulating the expression of TNF-α and IL6	[99]
B2-adrenoreceptor (ADRB2)	G_s	Knockout (KO) decreases fibrosis and HSC proliferation. It is also expressed on natural killer (NK) cells, T-cells, and Kupffer cells with possible involvement in pro-inflammatory response	[100]
B3-adrenoreceptor (ADRB3)	G_s	Potentially promotes activation of M2 macrophages and limits hepatocyte apoptosis	[101]
Dopamine receptor D1 (DRD1)	G_s	Expressed on liver mesenchymal cells (lMSCs). DRD1 agonist inhibits YAP/TAZ function and reverses the pro-fibrotic phenotype of lMSCs to fibrosis-resolving phenotype	[102]
Dopamine receptor D2 (DRD2)	G_s	DRD2 antagonism inhibits YAP/TAZ function in liver macrophages reducing $CTGF^{+}VCAM1^{+}$ vascular niche preventing fibrosis and promoting regeneration	[103]
Adenosine A2A receptor (A2-AR)	G_s, G_q	Induces TGF-β-mediated activation of HSCs with α-SMA upregulation. Also, augments TNF-α in Kupffer cells	[104]
Parathyroid hormone 1 receptor (PTH1R)	G_s	Hedgehog (Hh)-dependent activation of HSC leads to ECM deposition and liver fibrosis progress	[105]
Relaxin family peptide receptor 1 (RXFP1)	G_s, G_i	Agonist of the receptor reduces collagen content, and α-SMA expression, affecting ECM remodeling and cytokine signaling, exhibiting anti-fibrotic behavior	[106]
Relaxin family peptide receptor 2 (RXFP2)	G_s, G_i	Activation alleviates liver fibrosis by inducing intrahepatic nitric oxide (NO) leading to vasodilation	[107]
Prostaglandin E receptor (EP2)	G_s	Modulates expression of IL-1, TNF-α, ET-1 (pro-inflammatory and fibrotic) and enhances IL-10 and NO (anti-inflammatory) levels. Also inhibits collagen synthesis, proliferation, and trans-differentiation of HSCs[a]	[108]
Endothelin receptor type B (ETBR)	G_s, G_i, G_q	Upregulated in fibrotic condition and activation increases HSC contraction. The interplay of ETRs in the condition warrants exploration	[109]
Sphingosine-1- phosphate receptor 1 (S1PR1)	G_i	Leads to differentiation of bone marrow mesenchymal stem cells (BMSCs) to myofibroblasts, mediating liver fibrogenesis	[110]
Sphingosine-1- phosphate receptor 2 (S1PR2)	G_i, G_q, $G_{12/13}$	Promotes neutrophil activation and inflammation. Also, macrophage S1PR2 in the liver upregulates NLRP3 inflammasome inducing fibrogenesis	[110,111]
Sphingosine-1- phosphate receptor 3 (S1PR3)	G_i, G_q, $G_{12/13}$	Mediates bone marrow monocyte (BMM) motility and their activation fostering hepatic fibrosis	[110]
Sphingosine-1- phosphate receptor 4 (S1PR4)	G_i	Activation of NLRP3 inflammasome in hepatic macrophages stimulates the development of liver fibrosis	[110]
Cannabinoid receptor 1 (CB1)	G_i	Mediates HSC proliferation and is positively correlated to the expression of fibrosis-mediated genes ACTA2, TIMP-1, and MMP-13[a]	[92]
Cannabinoid receptor 2 (CB2)	G_i	Alleviates hepatic inflammation by upregulation of TNF-α, and IL-1β levels	[92]
C-C chemokine receptor type 2 (CCR2)	G_i	Strictly expressed on Kupffer cells and HSCs, CCR2 mediates the recruitment of hepatic macrophages supporting fibrogenic response	[112,113]
C-C chemokine receptor type 5 (CCR5)	G_i	Expressed by intrahepatic lymphocytes and HSCs, it is involved in the activation and proliferation of stellate cells	[112,113]
C-X-C motif chemokine receptor 3 (CXCR3)	G_i	Prevents fibrosis progression via its Th1-associated immune response. Also mediates the expression of hepatic pro-inflammatory cytokines, activation of NF-κB, and macrophage infiltration[a]	[93-95]

TABLE 1 (Continued)

Receptor name	G protein family	Function in liver fibrosis	References
C-X-C motif chemokine receptor 4 (CXCR4)	G_i	Facilitates activation and proliferation of HSC. Its targeting reduces the expression of α-SMA, TGF-β, and collagen I	[114]
Adenosine A3 receptor (A3-AR)	G_i	Mediates pro-fibrotic and pro-inflammatory response by regulation of the PI3K/NF-κB/Wnt/β-catenin signaling pathway	[115]
G protein-coupled estrogen receptor 1 (GPER)	G_i	Represses activation of HSCs and its autophagy mechanism. Also, decreases the expression of IL-6 by BMM, preventing fibrosis	[116,117]
G-protein-coupled bile acid receptor 91 (GPR91)	G_i	Succinate-induced activation leads to increased expression of α-SMA, TGF-β, and collagen I in HSCs	[118]
Neuropeptide Y receptor Y1 (Y1-R)	G_i	Activation induces phosphorylation of mTOR, p70S6K, and 4EBP1 in HSCs leading to fibrosis	[119]
Lysophosphatidic acid receptor 1 (LPAR1)	G_i	Enables activation and differentiation of HSCs into myofibroblast leading to actin rearrangement and proliferation while inhibiting HSC apoptosis	[120]
Smoothened receptor (SMO)	G_i	Transduces the Hedgehog pathway resulting in EMT of the HSC-derived myofibroblasts supporting their pro-fibrogenic phenotype	[121]
Frizzled receptor 2 (Fz2)	G_i	Leads to the expression of collagen I and TGF-β leading to the differentiation of HSCs into myofibroblast	[122]
C5a receptor (C5aR)	G_i	Activates HSCs to produce α-SMA, hyaluronic acid, and collagen IV while inhibiting apoptosis of TNF-α and ligand-induced HSC apoptosis	[123]
Apelin receptor	G_i	Leads to the expression of pro-fibrotic genes like α-SMA and collagen I via the ERK signaling pathway	[124]
M2 acetylcholine receptor (M2)	G_i	Induces HSC hyper-proliferation and upregulation of pro-fibrotic markers such as collagen I and TGF-β[a]	[97]
M3 acetylcholine receptor (M3)	G_q	Agonist-mediated activation alleviates HSC activation, collagen deposition, pro-fibrotic and pro-ECM markers, diminishing liver injury[a]	[98]
Angiotensin II type 1 receptor (AT1R)	G_q	Supports activation of HSCs and fibrosis via phosphorylation of JAK2 and following activation of RhoA/Rho-kinase	[125]
α1A-adrenoreceptor (ADRA1A)	G_q	Expressed by activated HSCs, upregulates secretion of NF-κB, inducing pro-inflammatory phenotype, and increased HSC contraction	[126]
Serotonin receptor 1B (5-HT1B)	G_q	Upregulated in activated HSCs requiring investigation into its specific role in fibrosis progression	[127]
Serotonin receptor 2A (5-HT2A)	G_q	Antagonists decreased the activation of HSCs, expression of pro-fibrotic markers, and inflammatory markers	[128]
Arginine vasopressin receptor 1A (AVPR1A)	G_q	Induces increase in intracellular calcium and enhanced MAPK activity mediating HSC proliferation and contraction	[129]
Endothelin receptor type A (ETAR)	G_q	Upregulated in activated HSCs expressing α-SMA. In-depth implications and associated signaling pathways need to be investigated	[130]
G protein-coupled receptor 55 (GPR55)	G_q	Leads to the activation of acetyl-coenzyme A carboxylase initiating HSC activation	[131]

[a]Indicates GPCRs with both pro and anti-fibrotic effects in hepatic fibrosis.

significant mutational burden in tumors is underestimated. A significant number of GPCRs are aberrantly overexpressed in solid tumors where more than 50 GPCRs are differentially expressed in multiple tumor types.[72] Most of these GPCRs have been the therapeutic targets of Food and Drug Administration (FDA) approved drugs for no cancer indication, but remain underexplored for cancer therapy as compard to kinase inhibitors. GPCRomic analysis is an emerging tool for GPCR identification and is largely based on mRNA studies by RNAseq, qPCR analysis, GPCR-specific microarrays, or hybridization-based DNA microarrays.[132] A recent study published by Li et al.

provides a pipeline to establish relationship between different omics data including genomics, transcriptomics, proteomics, and metabolomics, in the context of GPCRs pan-cancer.[133] Thus, it enables the discovery of potentially new or obscured GPCRs that are crucial to the pathophysiology of cancers.

Recent research has uncovered certain GPCRs to better understand their role in cancer etiology . CXCR2 which binds to CXCL8/IL-8 was found to associate with several signaling pathways involved in tumorigenesis, angiogenesis, proliferation, and metastasis in various cancers, including melanoma,[134] lung,[135] pancreatic,[136] gastric,[137] and ovarian[138] cancer. Furthermore, overexpression of protease-activated receptor 1 (PAR1) in various cancer types such as breast, lung, ovarian, and prostate has led to increased tumor invasiveness and metastasis.[139] On the other hand, blocking antibodies targeting PAR2 has been shown to mitigate tumor growth and metastasis in breast xenograft models.[140] The role of the neurotransmitters, adrenaline and noradrenaline in promoting tumor growth and metastasis through the beta-adrenergic receptors (β-AR) has also been studied.[141] Noradrenaline-mediated activation of β-AR in the stromal cells directly affects the tumor cells leading to the production of VEGF, IL-6, and matrix metalloproteases influencing the cancer survival.[142] Overexpression of alpha-1-beta adrenergic receptors in RAT-1 or NIH 3T3 fibroblasts renders them tumorigenic as demonstrated by their tumorigenic activity upon injection into a nude mouse.[143]

GPCRomics has helped in the identification of GPCRs that are often mutated and are the hotspots for accumulating DNA damage across different cancer types, particularly in solid tumors, such as adenocarcinomas, cervical, ovarian, breast, prostate, and bladder cancers.[144] Interestingly, a handful of GPCRs exert bidirectional effects on tumorigenesis in various cancer types exhibiting both pro-tumorigenic and anti-tumorigenic depending on TME characteristics, variable affinities of their ligands, and diverse implications of signaling pathways downstream.[145] For example, Sphingosine-1-phosphate receptor 5 (S1PR5) overexpression in colorectal cancer (CRC) cell lines promotes colon cancer proliferation and invasion via activation of NF-κB/indoleamine 2,3 dioxygenase 1 (IDO1) axis.[146] In contrast, in esophageal squamous cell carcinoma S1PR5 limits cell proliferation and invasion via the Ras/ERK, PI3K/Akt, and Rho/ROCK signaling pathways.[147] A couple of proton-sensing GPCRs such as GPR4, TDAG8 (GPR65), OGR1 (GPR68), and G2A (GPR132) also fall into this dual-functioning category as discussed by Sisignano et al.[148] The functioning of these GPCRs is highly context and TME-dependent, mandating strict context-dependent studies and careful evaluation of pharmacological strategies to eliminate possible off-target effects.

An exciting perspective toward studying cancers in the light of GPCRs is the aspect of sexual dimorphisms affecting the disease incidence and mortality. A keen interest has grown in the scientific community to explore the role of estrogen and estrogen-related GPCRs, like the G-protein-coupled estrogen receptor (GPER1) which is involved in the initiation and progression of gastrointestinal (GI) cancers.[145] It is particularly fascinating that hormone replacement therapy (HRT) or the use of oral contraceptives is positively associated with a lower risk of certain cancers such as CRC, endometrial, and ovarian cancer.[149-151] However, women who have undergone hysterectomy or oophorectomy were found to be increasingly susceptible to developing CRC.[145] A higher incidence of GI cancers is found in males as compared to females alluding to the protective role of estrogen against the development of some cancers.[152,153] GPER1-mediated estrogen signaling has been found to inhibit the proliferation of CRC, urinary bladder, and oral cancer cells.[154] Additionally, while GPER1 is not expressed in liver or liver tumors, its global silencing augmented hepatocarcinogenesis.[117] On the contrary, studies have also reported GPER1-mediated upregulation of connective tissue growth factor (CTGF), hypoxia-inducible factor (HIF), and vascular endothelial growth factor (VEGF) promoting CRC progression, invasion, and metastasis.[155,156] Estrogen-mediated GPER1 signaling was also found to promote proliferation of laryngeal cancer cells of squamous cell carcinoma and oral squamous cell carcinoma.[154] Epidemiological data showed a positive correlation between HRT and oral contraceptives (OC) with increased incidences of lung cancer, however, the results and risk associated with different HRT usage (ever/current/former vs never) are found to be inconsistent.[157] Furthermore, Wen et al. conducted a meta-analysis of a total of 22 studies comprising 911 194 participants including 17 329 patients to evaluate the correlation between lung cancer incidences and different HRT usage.[158] Overall, their results indicated that current HRT users and postmenopausal women with current HRT use had significantly decreased risk of lung cancer, whereas ever-HRT users correlated with decreased risk of lung cancer incidences.[158] Moreover, GPER1 activates chemotaxis and migration of mesothelioma, kidney cancer cells, as well as thyroid cancer cells.[159-161] Though, there has been invigorating research in exploring the mechanistic roles of GPCRs in dictating sexual dimorphisms of human cancers, significant efforts and further investigations are clearly warranted to elucidate the contribution of sex hormone signaling in the observed sexual dimorphisms of each cancer type.

In the context of PDAC progression, CAFs regulate the pro-fibrotic events promoting a highly desmoplastic and immunosuppressive TME leading to worse clinical outcomes.[162,163] In this framework, GPCRomics led to the discovery of GPR68 (a proton-sensing GPCR) in PDAC CAFs. Wiley et al. also reported that GPR68-positive PDAC CAFs led to increased production of IL-6 via $G\alpha_s$ signaling, promoting PDAC cell proliferation.[164,165] Since, IL-6 production in PDAC CAFs is strongly associated with the inflammatory CAFs (i.e., the iCAF subtype), this raises the question of the possible involvement of GPCRs in influencing the CAF subtypes (such as iCAF, myofibroblast CAF (myCAF), and antigen-presenting CAFs (apCAFs)) thereby, regulating the complex heterogeneity of CAFs in PDACs.

5 | CONCLUSIONS AND FUTURE PERSPECTIVES

Irrespective of the disease, the heterogeneity of the downstream intracellular signaling and functionality exhibited by the repertoire of the GPCR family of receptors is quite fascinating and is a promising avenue for designing precise therapeutics. This family of receptors exhibits an astounding and intricate interplay of downstream signaling molecules to mitigate the anti-fibrotic factors and reprogram the cell to a pro-fibrotic architecture in fibrotic diseases. Interestingly, this coordinated interplay also involves modulation of the pro-inflammatory response as the GPCRs are adept at recruiting immune cells such as activated macrophages, lymphocytes, and NK cells to facilitate fibrosis. The identification of numerous GPCRs that display comparable effects across a wide range of disorders has been substantially aided by technological developments and an improved understanding of the crosstalk involving GPCRs, allowing predictable target identification to achieve better treatment outcomes. Furthermore, emerging players like TAS2R could potentially drive the development of a personalized targeting strategy. As we dive deeper into discerning the complexities of GPCR-driven fibrosis, the contribution of resident cells, such as Kupffer cells, is being appreciated. Delineating the origin of activated fibroblasts such as the CFs, HSCs, and PSCs will not only help in understanding the course of progression of the implicated diseases but also facilitate elucidating their long-term health outcomes.

There is an impending question on the activation of various compensatory pathways when any one of the signaling molecules is targeted. Since the GPCRs are so entwined in rendering their effects on fibrosis, targeting one nodal pathway that results in fibrosis may not be sufficient. A common player contributing to fibrosis is the TGF-β pathway which can emerge as the most viable target to rescue ECM remodeling, fibrosis, and dysfunction. However, considering the side effects and other health implications, the multi-nodal or major node targeting of TGF-β is a significant challenge. Reprogramming the activated fibroblasts to their quiescent state, as achieved by the administration of RAs in PSCs, motivates the design of novel anti-fibrotic strategies. Studying the role of GPCR heterogeneity in driving fibrosis will therefore help identify potential targets to tweak the pro-fibrotic pathways to anti-fibrotic pathways.

The increased scientific awareness and innovations involving GPCRomics have laid a strong foundation for the elucidation of novel GPCRs and the GPCRs prone to mutations potentiating their application as prognostic markers of fibrotic diseases. Further, sex differences in the incidences of cancers have led to an increased appreciation of epigenetic factors, age distribution, ethnicity, geographical distribution, lifestyle, and clinical stage of cancer progression which could all dictate the regulation mediated by the sex hormone associated GPCRs. Understanding the in-depth mechanisms leading to sexual disparities in incidences and mortality of human cancers can tremendously benefit the evolution of such GPCR-mediated precision medicine. Moreover, fibroblasts, especially CAF, and their heterogeneity have emerged as a crucial component of the TME of solid tumors, where various CAF subtypes could exhibit exclusive functions through GPCRs. GPCR-specific identification and characterization of the CAFs could therefore potentiate personalized oncotherapy achieving better therapeutic outcomes. Overall, the GPCR family of receptors can open possible dimensions of targeted therapy in fibrotic diseases which can revolutionize their treatment in the future.

AUTHOR CONTRIBUTIONS

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REFERENCES

1. Yang YM, Kuen DS, Chung Y, Kurose H, Kim SG. Gα 12/13 signaling in metabolic diseases. *Exp Mol Med*. 2020;52(6):896-910.
2. Rosenbaum DM, Rasmussen SG, Kobilka BK. The structure and function of G-protein-coupled receptors. *Nature*. 2009;459(7245):356-363.
3. Trzaskowski B, Latek D, Yuan S, Ghoshdastider U, Debinski A, Filipek S. Action of molecular switches in GPCRs-theoretical and experimental studies. *Curr Med Chem*. 2012;19(8):1090-1109.
4. Djamin RS, Talman S, Schrauwen EJA, et al. Prevalence and abundance of selected genes conferring macrolide resistance genes in COPD patients during maintenance treatment with azithromycin. *Antimicrob Resist Infect Control*. 2020;9(1):1-8.
5. Haak AJ, Ducharme MT, Diaz Espinosa AM, Tschumperlin DJ. Targeting GPCR signaling for idiopathic pulmonary fibrosis therapies. *Trends Pharmacol Sci*. 2020;41(3):172-182.
6. Weis WI, Kobilka BK. The molecular basis of G protein–coupled receptor activation. *Annu Rev Biochem*. 2018;87:897-919.
7. Plikus MV, Wang X, Sinha S, et al. Fibroblasts: origins, definitions, and functions in health and disease. *Cell*. 2021;184(15):3852-3872.
8. Kidd ME, Shumaker DK, Ridge KM. The role of vimentin intermediate filaments in the progression of lung cancer. *Am J Respir Cell Mol Biol*. 2014;50(1):1-6.
9. Li M, Luan F, Zhao Y, et al. Epithelial-mesenchymal transition: an emerging target in tissue fibrosis. *Exp Biol Med*. 2016;241(1):1-13.
10. Bainbridge P. Wound healing and the role of fibroblasts. *J Wound Care*. 2013;22(8):407-412.
11. Reilkoff RA, Bucala R, Herzog EL. Fibrocytes: emerging effector cells in chronic inflammation. *Nat Rev Immunol*. 2011;11(6):427-435.
12. Frantz C, Stewart KM, Weaver VM. The extracellular matrix at a glance. *J Cell Sci*. 2010;123(24):4195-4200.
13. Wynn TA. Cellular and molecular mechanisms of fibrosis. *J Pathol*. 2008;214(2):199-210.
14. Klingberg F, Chau G, Walraven M, et al. The fibronectin ED-A domain enhances recruitment of latent TGF-β-binding protein-1 to the fibroblast matrix. *J Cell Sci*. 2018;131(5):jcs201293.
15. Kleaveland KR, Moore BB, Kim KK. Paracrine functions of fibrocytes to promote lung fibrosis. *Expert Rev Respir Med*. 2014;8(2):163-172.
16. Ploeger DT, Hosper NA, Schipper M, Koerts JA, de Rond S, Bank RA. Cell plasticity in wound healing: paracrine factors of M1/M2 polarized macrophages influence the phenotypical state of dermal fibroblasts. *Cell Commun Signal*. 2013;11(1):1-17.
17. Martinez FJ, Collard HR, Pardo A, et al. Idiopathic pulmonary fibrosis. *Nat Rev Dis Primers*. 2017;3:17074.
18. Nett RJ, Wood JM, Blackley DJ. Collecting occupational exposure data would strengthen idiopathic pulmonary fibrosis registries. *Am J Respir Crit Care Med*. 2020;201(4):495-496.
19. Kaunisto J, Salomaa ER, Hodgson U, et al. Demographics and survival of patients with idiopathic pulmonary fibrosis in the FinnishIPF registry. *ERJ Open Res*. 2019;5(3):00170-2018.
20. Flock T, Hauser AS, Lund N, Gloriam DE, Balaji S, Babu MM. Selectivity determinants of GPCR–G-protein binding. *Nature*. 2017;545(7654):317-322.
21. Maekawa M, Ishizaki T, Boku S, et al. Signaling from rho to the actin cytoskeleton through protein kinases ROCK and LIM-kinase. *Science*. 1999;285(5429):895-898.
22. Liu Y, Xu H, Geng Y, et al. Dibutyryl-cAMP attenuates pulmonary fibrosis by blocking myofibroblast differentiation via PKA/CREB/CBP signaling in rats with silicosis. *Respir Res*. 2017;18(1):1-11.
23. Liu X, Sun SQ, Ostrom RS. Fibrotic lung fibroblasts show blunted inhibition by cAMP due to deficient cAMP response element-binding protein phosphorylation. *J Pharmacol Exp Ther*. 2005;315(2):678-687.
24. Huang SK, Wettlaufer SH, Chung J, Peters-Golden M. Prostaglandin E2 inhibits specific lung fibroblast functions via selective actions of PKA and Epac-1. *Am J Respir Cell Mol Biol*. 2008;39(4):482-489.
25. Chioccioli M, Roy S, Newell R, et al. A lung targeted miR-29 mimic as a therapy for pulmonary fibrosis. *EBioMedicine*. 2022;85:104304.
26. Ng B, Cook SA, Schafer S. Interleukin-11 signaling underlies fibrosis, parenchymal dysfunction, and chronic inflammation of the airway. *Exp Mol Med*. 2020;52(12):1871-1878.
27. Unemori EN, Pickford LB, Salles AL, et al. Relaxin induces an extracellular matrix-degrading phenotype in human lung fibroblasts in vitro and inhibits lung fibrosis in a murine model in vivo. *J Clin Invest*. 1996;98(12):2739-2745.
28. Sharma P, Yi R, Nayak AP, et al. Bitter taste receptor agonists mitigate features of allergic asthma in mice. *Sci Rep*. 2017;7(1):1-14.
29. An SS, Liggett SB. Taste and smell GPCRs in the lung: evidence for a previously unrecognized widespread chemosensory system. *Cell Signal*. 2018;41:82-88.
30. Virani SS, Alonso A, Benjamin EJ, et al. Heart disease and stroke statistics—2020 update: a report from the American Heart Association. *Circulation*. 2020;141(9):e139-e596.

31. Mozaffarian D, Benjamin EJ, Go AS, et al. Heart disease and stroke statistics—2015 update: a report from the American Heart Association. *Circulation*. 2015;131(4):e29-e322.
32. Benjamin EJ, Muntner P, Alonso A, et al. Heart disease and stroke statistics—2019 update: a report from the American Heart Association. *Circulation*. 2019;139(10):e56-e528.
33. Benjamin EJ, Virani SS, Callaway CW, et al. Heart disease and stroke statistics—2018 update: a report from the American Heart Association. *Circulation*. 2018;137(12):e67-e492.
34. Travers JG, Kamal FA, Robbins J, Yutzey KE, Blaxall BC. Cardiac fibrosis: the fibroblast awakens. *Circ Res*. 2016;118(6):1021-1040.
35. Tallquist MD, Molkentin JD. Redefining the identity of cardiac fibroblasts. *Nat Rev Cardiol*. 2017;14(8):484-491.
36. Norris R, Borg TK, Butcher JT, Baudino TA, Banerjee I, Markwald RR. Neonatal and adult cardiovascular pathophysiological remodeling and repair. *Ann N Y Acad Sci*. 2008;1123(1):30-40.
37. Sridurongrit S, Larsson J, Schwartz R, Ruiz-Lozano P, Kaartinen V. Signaling via the Tgf-β type I receptor Alk5 in heart development. *Dev Biol*. 2008;322(1):208-218.
38. Saadat S, Noureddini M, Mahjoubin-Tehran M, et al. Pivotal role of TGF-β/Smad signaling in cardiac fibrosis: non-coding RNAs as effectual players. *Front Cardiovasc Med*. 2021;7:588347.
39. Yue Y, Meng K, Pu Y, Zhang X. Transforming growth factor beta (TGF-β) mediates cardiac fibrosis and induces diabetic cardiomyopathy. *Diabetes Res Clin Pract*. 2017;133:124-130.
40. Choi ME, Ding Y, Kim SI. TGF-β signaling via TAK1 pathway: role in kidney fibrosis. *Semin Nephrol*. 2012;32:244-252.
41. Landström M. The TAK1–TRAF6 signalling pathway. *Int J Biochem Cell Biol*. 2010;42(5):585-589.
42. Nakamura F, Tsukamoto I, Inoue S, Hashimoto K, Akagi M. Cyclic compressive loading activates angiotensin II type 1 receptor in articular chondrocytes and stimulates hypertrophic differentiation through a G-protein-dependent pathway. *FEBS Open Bio*. 2018;8(6):962-973.
43. Zou Y, Akazawa H, Qin Y, et al. Mechanical stress activates angiotensin II type 1 receptor without the involvement of angiotensin II. *Nat Cell Biol*. 2004;6(6):499-506.
44. Galie P, Russell MW, Westfall MV, Stegemann JP. Interstitial fluid flow and cyclic strain differentially regulate cardiac fibroblast activation via AT1R and TGF-β1. *Exp Cell Res*. 2012;318(1):75-84.
45. Duerrschmid C, Trial J, Wang Y, Entman ML, Haudek SB. Tumor necrosis factor: a mechanistic link between angiotensin-II–induced cardiac inflammation and fibrosis. *Circ Heart Fail*. 2015;8(2):352-361.
46. Wong CKS, Falkenham A, Myers T, Légaré JF. Connective tissue growth factor expression after angiotensin II exposure is dependent on transforming growth factor-β signaling via the canonical Smad-dependent pathway in hypertensive induced myocardial fibrosis. *J Renin Angiotensin Aldosterone Syst*. 2018;19(1):1470320318759358.
47. Wang X, Guo Z, Ding Z, et al. Endothelin-1 upregulation mediates aging-related cardiac fibrosis. *J Mol Cell Cardiol*. 2015;80:101-109.
48. Rodríguez-Pascual F, Busnadiego O, González-Santamaría J. The profibrotic role of endothelin-1: is the door still open for the treatment of fibrotic diseases? *Life Sci*. 2014;118(2):156-164.
49. Parichatikanond W, Luangmonkong T, Mangmool S, Kurose H. Therapeutic targets for the treatment of cardiac fibrosis and cancer: focusing on TGF-β signaling. *Front Cardiovasc Med*. 2020;7:34.
50. Ueyama T, Kawashima S, Sakoda T, et al. Endothelin-1 activates p38 mitogen-activated protein kinase via endothelin-A receptor in rat myocardial cells. *Mol Cell Biochem*. 1999;199(1):119-124.
51. Liu J, Zhuang T, Pi J, et al. Endothelial forkhead box transcription factor P1 regulates pathological cardiac remodeling through transforming growth factor-β1–endothelin-1 signal pathway. *Circulation*. 2019;140(8):665-680.
52. Clozel M, Salloukh H. Role of endothelin in fibrosis and anti-fibrotic potential of bosentan. *Ann MedAnn Med*. 2005;37(1):2-12.
53. Francisco J, Zhang Y, Jeong JI, et al. Blockade of fibroblast YAP attenuates cardiac fibrosis and dysfunction through MRTF-A inhibition. *JACC Basic Transl Sci*. 2020;5(9):931-945.
54. Phosri S, Arieyawong A, Bunrukchai K, et al. Stimulation of adenosine A2B receptor inhibits endothelin-1-induced cardiac fibroblast proliferation and α-smooth muscle actin synthesis through the cAMP/Epac/PI3K/Akt-signaling pathway. *Front Pharmacol*. 2017;8:428.
55. Vecchio EA, White PJ, May LT. Targeting adenosine receptors for the treatment of cardiac fibrosis. *Front Pharmacol*. 2017;8:243.
56. Gurevich VV, Gurevich EV. GPCR signaling regulation: the role of GRKs and arrestins. *Front Pharmacol*. 2019;10:125.
57. Connolly EC, Freimuth J, Akhurst RJ. Complexities of TGF-β targeted cancer therapy. *Int J Biol Sci*. 2012;8(7):964-978.
58. Ding Y, Kim S, Lee SY, Koo JK, Wang Z, Choi ME. Autophagy regulates TGF-β expression and suppresses kidney fibrosis induced by unilateral ureteral obstruction. *J Am Soc Nephrol*. 2014;25(12):2835-2846.
59. Kulkarni AB, Huh CG, Becker D, et al. Transforming growth factor beta 1 null mutation in mice causes excessive inflammatory response and early death. *Proc Natl Acad Sci*. 1993;90(2):770-774.
60. Shull MM, Ormsby I, Kier AB, et al. Targeted disruption of the mouse transforming growth factor-β1 gene results in multifocal inflammatory disease. *Nature*. 1992;359(6397):693-699.
61. Frazier K, Thomas R, Scicchitano M, et al. Inhibition of ALK5 signaling induces physeal dysplasia in rats. *Toxicol Pathol*. 2007;35(2):284-295.
62. Anderton MJ, Mellor HR, Bell A, et al. Induction of heart valve lesions by small-molecule ALK5 inhibitors. *Toxicol Pathol*. 2011;39(6):916-924.
63. Akhurst RJ. Targeting TGF-β signaling for therapeutic gain. *Cold Spring Harb Perspect Biol*. 2017;9(10):a022301.
64. Morris JC, Tan AR, Olencki TE, et al. Phase I study of GC1008 (fresolimumab): a human anti-transforming growth factor-beta (TGFβ) monoclonal antibody in patients with advanced malignant melanoma or renal cell carcinoma. *PLoS One*. 2014;9(3):e90353.
65. Lacouture ME, Morris JC, Lawrence DP, et al. Cutaneous keratoacanthomas/squamous cell carcinomas associated with neutralization of transforming growth factor β by the monoclonal antibody fresolimumab (GC1008). *Cancer Immunol Immunother*. 2015;64(4):437-446.

66. Huynh LK, Hipolito CJ, Ten Dijke P. A perspective on the development of TGF-β inhibitors for cancer treatment. *Biomolecules*. 2019;9(11):743.
67. Chang Y-K, Choi H, Jeong JY, Na KR, Lee KW, Choi DE. Co-inhibition of angiotensin II receptor and endothelin-1 attenuates renal injury in unilateral ureteral obstructed mice. *Kidney Blood Press Res*. 2016;41(4):450-459.
68. Komers R, Plotkin H. Dual inhibition of renin-angiotensin-aldosterone system and endothelin-1 in treatment of chronic kidney disease. *Am J Physiol Regul Integr Comp Physiol*. 2016;310(10):R877-R884.
69. Xue R, Jia K, Wang J, et al. A rising star in pancreatic diseases: pancreatic stellate cells. *Front Physiol*. 2018;9:754.
70. Jin G, Hong W, Guo Y, Bai Y, Chen B. Molecular mechanism of pancreatic stellate cells activation in chronic pancreatitis and pancreatic cancer. *J Cancer*. 2020;11(6):1505-1515.
71. Sriram K, Salmerón C, Wiley SZ, Insel PA. GPCRs in pancreatic adenocarcinoma: contributors to tumour biology and novel therapeutic targets. *Br J Pharmacol*. 2020;177(11):2434-2455.
72. Insel PA, Sriram K, Wiley SZ, et al. GPCRomics: GPCR expression in cancer cells and tumors identifies new, potential biomarkers and therapeutic targets. *Front Pharmacol*. 2018;9:431.
73. Masamune A, Shimosegawa T. Signal transduction in pancreatic stellate cells. *J Gastroenterol*. 2009;44(4):249-260.
74. Huang C, Iovanna J, Santofimia-Castaño P. Targeting fibrosis: the bridge that connects pancreatitis and pancreatic cancer. *Int J Mol Sci*. 2021;22(9):4970.
75. Masamune A, Watanabe T, Kikuta K, Shimosegawa T. Roles of pancreatic stellate cells in pancreatic inflammation and fibrosis. *Clin Gastroenterol Hepatol*. 2009;7(11):S48-S54.
76. Shimizu K. Mechanisms of pancreatic fibrosis and applications to the treatment of chronic pancreatitis. *J Gastroenterol*. 2008;43:823-832.
77. Kuno A, Yamada T, Masuda K, et al. Angiotensin-converting enzyme inhibitor attenuates pancreatic inflammation and fibrosis in male Wistar Bonn/Kobori rats. *Gastroenterology*. 2003;124(4):1010-1019.
78. Aoki H, Ohnishi H, Hama K, et al. Autocrine loop between TGF-β1 and IL-1β through Smad3-and ERK-dependent pathways in rat pancreatic stellate cells. *Am J Physiol Cell Physiol*. 2006;290(4):C1100-C1108.
79. Artlett CM. The role of the NLRP3 inflammasome in fibrosis. *Open Rheumatol J*. 2012;6(1):80-86.
80. Cui L, Li C, Zhang G, et al. S1P/S1PR2 promote pancreatic stellate cell activation and pancreatic fibrosis in chronic pancreatitis by regulating autophagy and the NLRP3 inflammasome. *Chem Biol Interact*. 2023;380:110541.
81. Hu Y, Wan R, Yu G, et al. Imbalance of Wnt/Dkk negative feedback promotes persistent activation of pancreatic stellate cells in chronic pancreatitis. *PLoS One*. 2014;9(4):e95145.
82. Xiao W, Jiang W, Shen J, et al. Retinoic acid ameliorates pancreatic fibrosis and inhibits the activation of pancreatic stellate cells in mice with experimental chronic pancreatitis via suppressing the Wnt/β-catenin signaling pathway. *PLoS One*. 2015;10(11):e0141462.
83. Matellan C, Lachowski D, Cortes E, et al. Retinoic acid receptor β modulates mechanosensing and invasion in pancreatic cancer cells via myosin light chain 2. *Oncogenesis*. 2023;12(1):23. doi:10.1038/s41389-023-00467-1
84. Cheemerla S, Balakrishnan M. Global epidemiology of chronic liver disease. *Clin Liver Dis (Hoboken)*. 2021;17(5):365-370.
85. Yi HS, Jeong WI. Interaction of hepatic stellate cells with diverse types of immune cells: foe or friend? *J Gastroenterol Hepatol*. 2013;28:99-104.
86. Kimura T, Singh S, Tanaka N, Umemura T. Role of G protein-coupled receptors in hepatic stellate cells and approaches to anti-fibrotic treatment of non-alcoholic fatty liver disease. *Front Endocrinol*. 2021;12:773432.
87. Hui AY, Cheng ASL, Chan HLY, et al. Effect of prostaglandin E2 and prostaglandin I2 on PDGF-induced proliferation of LI90, a human hepatic stellate cell line. *Prostaglandins Leukot Essent Fatty Acids*. 2004;71(5):329-333.
88. Koide S, Kobayashi Y, Oki Y, Nakamura H. Prostaglandin E2 inhibits platelet-derived growth factor-stimulated cell proliferation through a prostaglandin E receptor EP2 subtype in rat hepatic stellate cells. *Dig Dis Sci*. 2004;49(9):1394-1400.
89. Mallat A, Teixeira-Clerc F, Lotersztajn S. Cannabinoid signaling and liver therapeutics. *J Hepatol*. 2013;59(4):891-896.
90. Dai E, Zhang L, Ye L, et al. Hepatic expression of cannabinoid receptors CB1 and CB2 correlate with fibrogenesis in patients with chronic hepatitis B. *Int J Infect Dis*. 2017;59:124-130.
91. Wang S, Zhu Q, Liang G, et al. Cannabinoid receptor 1 signaling in hepatocytes and stellate cells does not contribute to NAFLD. *J Clin Invest*. 2021;131(22):e152242.
92. Patsenker E, Stoll M, Millonig G, et al. Cannabinoid receptor type I modulates alcohol-induced liver fibrosis. *Mol Med*. 2011;17(11):1285-1294.
93. Zhang X, Han J, Man K, et al. CXC chemokine receptor 3 promotes steatohepatitis in mice through mediating inflammatory cytokines, macrophages and autophagy. *J Hepatol*. 2016;64(1):160-170.
94. Wasmuth HE, Lammert F, Zaldivar MM, et al. Antifibrotic effects of CXCL9 and its receptor CXCR3 in livers of mice and humans. *Gastroenterology*. 2009;137(1):309-319. 319.e1-3.
95. Singh KP, Zerbato JM, Zhao W, et al. Intrahepatic CXCL10 is strongly associated with liver fibrosis in HIV-Hepatitis B co-infection. *PLoS pathogens*. 2020;16(9):e1008744.
96. Luo L, Zhang G, Mao L, et al. Group II muscarinic acetylcholine receptors attenuate hepatic injury via Nrf2/ARE pathway. *Toxicol Appl Pharmacol*. 2020;395:114978.
97. Morgan ML, Sigala B, Soeda J, et al. Acetylcholine induces fibrogenic effects via M2/M3 acetylcholine receptors in non-alcoholic steatohepatitis and in primary human hepatic stellate cells. *J Gastroenterol Hepatol*. 2016;31(2):475-483.
98. Khurana S, Jadeja R, Twaddell W, et al. Effects of modulating M3 muscarinic receptor activity on azoxymethane-induced liver injury in mice. *Biochem Pharmacol*. 2013;86(2):329-338.
99. Zapater P, Gómez-Hurtado I, Peiró G, et al. Beta-adrenergic receptor 1 selective antagonism inhibits norepinephrine-mediated TNF-alpha downregulation in experimental liver cirrhosis. *PLoS One*. 2012;7(8):e43371.
100. Lelou E, Corlu A, Nesseler N, et al. The role of catecholamines in pathophysiological liver processes. *Cell*. 2022;11(6):1021.
101. Fujisaka S, Usui I, Nawaz A, et al. M2 macrophages in metabolism. *Diabetol Int*. 2016;7(4):342-351.
102. Haak AJ, Kostallari E, Sicard D, et al. Selective YAP/TAZ inhibition in fibroblasts via dopamine receptor D1 agonism reverses fibrosis. *Sci Transl Med*. 2019;11(516):eaau6296.

103. Qing J, Ren Y, Zhang Y, et al. Dopamine receptor D2 antagonism normalizes profibrotic macrophage-endothelial crosstalk in non-alcoholic steatohepatitis. *J Hepatol*. 2022;76(2):394-406.
104. Schwinghammer UA, Melkonyan MM, Hunanyan L, et al. α2-adrenergic receptor in liver fibrosis: implications for the adrenoblocker mesedin. *Cell*. 2020;9(2):456.
105. He S, Tang J, Diao N, et al. Parathyroid hormone-related protein activates HSCs via hedgehog signalling during liver fibrosis development. *Artif Cells Nanomed Biotechnol*. 2019;47(1):1984-1994.
106. Kaftanovskaya EM, Ng HH, Soula M, et al. Therapeutic effects of a small molecule agonist of the relaxin receptor ML290 in liver fibrosis. *FASEB J*. 2019;33(11):12435-12446.
107. Devaraj E. Relaxin in hepatic fibrosis: what is known and where to head? *Biochimie*. 2021;187:144-151.
108. Dieter P, Scheibe R, Bezugla Y, et al. The regulatory role of prostaglandin E2in liver (patho) physiology is controlled at its site of synthesis and its action on the receptors. *Comp Hepatol*. 2004;3 Suppl 1(Suppl 1):S35.
109. Örmeci N. Endothelins and liver cirrhosis. *Potal Hypertension Cirrhosis*. 2022;1(1):66-72.
110. Hong CH, Ko MS, Kim JH, et al. Sphingosine 1-phosphate receptor 4 promotes nonalcoholic steatohepatitis by activating NLRP3 inflammasome. *Cell Mol Gastroenterol Hepatol*. 2022;13(3):925-947.
111. Hou L, Yang L, Chang N, et al. Macrophage sphingosine 1-phosphate receptor 2 blockade attenuates liver inflammation and fibrogenesis triggered by NLRP3 inflammasome. *Front Immunol*. 2020;11:1149.
112. Bartneck M, Koppe C, Fech V, et al. Roles of CCR2 and CCR5 for hepatic macrophage polarization in mice with liver parenchymal cell-specific NEMO deletion. *Cell Mol Gastroenterol Hepatol*. 2021;11(2):327-347.
113. Lefere S, Devisscher L, Tacke F. Targeting CCR2/5 in the treatment of nonalcoholic steatohepatitis (NASH) and fibrosis: opportunities and challenges. *Expert Opin Investig Drugs*. 2020;29(2):89-92.
114. Ullah A, Chen G, Yibang Z, et al. A new approach based on CXCR4-targeted combination liposomes for the treatment of liver fibrosis. *Biomater Sci*. 2022;10(10):2650-2664.
115. Fishman P, Cohen S, Itzhak I, et al. The A3 adenosine receptor agonist, namodenoson, ameliorates non-alcoholic steatohepatitis in mice. *Int J Mol Med*. 2019;44(6):2256-2264.
116. Chen Y, Que R, Zhang N, Lin L, Zhou M, Li Y. Saikosaponin-d alleviates hepatic fibrosis through regulating GPER1/autophagy signaling. *Mol Biol Rep*. 2021;48(12):7853-7863.
117. Wei T, Chen W, Wen L, et al. G protein-coupled estrogen receptor deficiency accelerates liver tumorigenesis by enhancing inflammation and fibrosis. *Cancer Lett*. 2016;382(2):195-202.
118. Li YH, Woo SH, Choi DH, Cho EH. Succinate causes α-SMA production through GPR91 activation in hepatic stellate cells. *Biochem Biophys Res Commun*. 2015;463(4):853-858.
119. Dai W, Liu Y, Zhang Y, et al. Expression of neuropeptide Y is increased in an activated human HSC cell line. *Sci Rep*. 2019;9(1):1-11.
120. Kaffe E, Magkrioti C, Aidinis V. Deregulated lysophosphatidic acid metabolism and signaling in liver cancer. *Cancer*. 2019;11(11):1626.
121. Michelotti GA, Xie G, Swiderska M, et al. Smoothened is a master regulator of adult liver repair. *J Clin Invest*. 2013;123(6):2380-2394.
122. Duspara K, Bojanic K, Pejic JI, et al. Targeting the Wnt signaling pathway in liver fibrosis for drug options: an update. *J Clin Transl Hepatol*. 2021;9(6):960-971.
123. Xu R, Lin F, He J, et al. Complement 5a stimulates hepatic stellate cells in vitro, and is increased in the plasma of patients with chronic hepatitis B. *Immunology*. 2013;138(3):228-234.
124. Wang Y, Song J, Bian H, et al. Apelin promotes hepatic fibrosis through ERK signaling in LX-2 cells. *Mol Cell Biochem*. 2019;460(1):205-215.
125. Granzow M, Schierwagen R, Klein S, et al. Angiotensin-II type 1 receptor-mediated Janus kinase 2 activation induces liver fibrosis. *Hepatology*. 2014;60(1):334-348.
126. Sancho-Bru P, Bataller R, Colmenero J, et al. Norepinephrine induces calcium spikes and proinflammatory actions in human hepatic stellate cells. *Am J Physiol Gastrointest Liver Physiol*. 2006;291(5):G877-G884.
127. Middlemiss DN, Hutson PH. The 5-HT1B receptors. *Ann N Y Acad Sci*. 1990;600:132-147.
128. Kim DC, Jun DW, Kwon YI, et al. 5-HT 2A receptor antagonists inhibit hepatic stellate cell activation and facilitate apoptosis. *Liver Int*. 2013;33(4):535-543.
129. Bataller R, Nicolas JM, Gines P, et al. Arginine vasopressin induces contraction and stimulates growth of cultured human hepatic stellate cells. *Gastroenterology*. 1997;113(2):615-624.
130. Deng J, Huang Y, Tao R, et al. The expression of ETAR in liver cirrhosis and liver cancer. *Cancer Biol Ther*. 2017;18(9):723-729.
131. Fondevila MF, Fernandez U, Gonzalez-Rellan MJ, et al. The L-α-lysophosphatidylinositol/G protein–coupled receptor 55 system induces the development of nonalcoholic steatosis and steatohepatitis. *Hepatology*. 2021;73(2):606-624.
132. Insel PA, Sriram K, Gorr MW, et al. GPCRomics: an approach to discover GPCR drug targets. *Trends Pharmacol Sci*. 2019;40(6):378-387.
133. Li S, Chen X, Chen J, et al. Multi-omics integration analysis of GPCRs in pan-cancer to uncover inter-omics relationships and potential driver genes. *Comput Biol Med*. 2023;161:106988.
134. Balkwill F. Cancer and the chemokine network. *Nat Rev Cancer*. 2004;4(7):540-550.
135. Yanagawa J, Walser TC, Zhu LX, et al. Snail promotes CXCR2 ligand-dependent tumor progression in non-small cell lung carcinoma. *Clin Cancer Res*. 2009;15(22):6820-6829.
136. Matsuo Y, Raimondo M, Woodward TA, et al. CXC-chemokine/CXCR2 biological axis promotes angiogenesis in vitro and in vivo in pancreatic cancer. *Int J Cancer*. 2009;125(5):1027-1037.
137. Xu J, Zhang C, He Y, et al. Lymphatic endothelial cell-secreted CXCL1 stimulates lymphangiogenesis and metastasis of gastric cancer. *Int J Cancer*. 2012;130(4):787-797.
138. Agarwal A, Tressel SL, Kaimal R, et al. Identification of a metalloprotease-chemokine signaling system in the ovarian cancer microenvironment: implications for antiangiogenic therapy MMP1-PAR1 paracrine signaling through angiogenic chemokines. *Cancer Res*. 2010;70(14):5880-5890.
139. Hempel D, Sierko E, Wojtukiewicz MZ. Protease-activated receptors-biology and role in cancer. *Postepy Hig Med Dosw*. 2016;70:775-786.

140. Versteeg HH, Schaffner F, Kerver M, et al. Inhibition of tissue factor signaling suppresses tumor growth. *Blood*. 2008;111(1):190-199.
141. Thaker PH, Han LY, Kamat AA, et al. Chronic stress promotes tumor growth and angiogenesis in a mouse model of ovarian carcinoma. *Nat Med*. 2006;12(8):939-944.
142. Yang EV, Kim SJ, Donovan EL, et al. Norepinephrine upregulates VEGF, IL-8, and IL-6 expression in human melanoma tumor cell lines: implications for stress-related enhancement of tumor progression. *Brain Behav Immun*. 2009;23(2):267-275.
143. Shah N, Khurana S, Cheng K, Raufman JP. Muscarinic receptors and ligands in cancer. *Am J Physiol Cell Physiol*. 2009;296(2):C21-C232.
144. Sriram K, Moyung K, Corriden R, Carter H, Insel PA. GPCRs show widespread differential mRNA expression and frequent mutation and copy number variation in solid tumors. *PLoS Biol*. 2019;17(11):e3000434.
145. Zeng Z, Ma C, Chen K, et al. Roles of G protein-coupled receptors (GPCRs) in gastrointestinal cancers: focus on sphingosine 1-shosphate receptors, angiotensin II receptors, and estrogen-related GPCRs. *Cell*. 2021;10(11):2988.
146. Zhou H, Yin X, Bai F, Liu W, Jiang S, Zhao J. The role and mechanism of S1PR5 in colon cancer. *Cancer Manag Res*. 2020;12:4759-4775.
147. Hu W-M, Li L, Jing BQ, et al. Effect of S1P5 on proliferation and migration of human esophageal cancer cells. *World J Gastroenterol*. 2010;16(15):1859-1866.
148. Sisignano M, Fischer MJ, Geisslinger G. Proton-sensing GPCRs in health and disease. *Cell*. 2021;10(8):2050.
149. Burkman R, Schlesselman JJ, Zieman M. Safety concerns and health benefits associated with oral contraception. *Am J Obstet Gynecol*. 2004;190(4):S5-S22.
150. Bassuk SS, Manson JE. Oral contraceptives and menopausal hormone therapy: relative and attributable risks of cardiovascular disease, cancer, and other health outcomes. *Ann Epidemiol*. 2015;25(3):193-200.
151. Wentzensen N, de Gonzalez AB. The Pill's gestation: from birth control to cancer prevention. *Lancet Oncol*. 2015;16(9):1004-1006.
152. Abancens M, Bustos V, Harvey H, McBryan J, Harvey BJ. Sexual dimorphism in colon cancer. *Front Oncol*. 2020;10:607909.
153. Rawla P, Barsouk A. Epidemiology of gastric cancer: global trends, risk factors and prevention. *Prz Gastroenterol*. 2019;14(1):26-38.
154. Zheng D, Williams C, Vold JA, et al. Regulation of sex hormone receptors in sexual dimorphism of human cancers. *Cancer Lett*. 2018;438:24-31.
155. Gilligan LC, Rahman HP, Hewitt AM, et al. Estrogen activation by steroid sulfatase increases colorectal cancer proliferation via GPER. *J Clin Endocrinol Metabol*. 2017;102(12):4435-4447.
156. Bustos V, Nolan ÁM, Nijhuis A, et al. GPER mediates differential effects of estrogen on colon cancer cell proliferation and migration under normoxic and hypoxic conditions. *Oncotarget*. 2017;8(48):84258-84275.
157. Musial C, Zaucha R, Kuban-Jankowska A, et al. Plausible role of estrogens in pathogenesis, progression and therapy of lung cancer. *Int J Environ Res Public Health*. 2021;18(2):648.
158. Wen H, Lin X, Sun D. The association between different hormone replacement therapy use and the incidence of lung cancer: a systematic review and meta-analysis. *J Thorac Dis*. 2022;14(2):381-395.
159. Avino S, Marco PD, Cirillo F, et al. Stimulatory actions of IGF-I are mediated by IGF-IR cross-talk with GPER and DDR1 in mesothelioma and lung cancer cells. *Oncotarget*. 2016;7(33):52710-52728.
160. Tanaka Y, Sasaki M, Kaneuchi M, Fujimoto S, Dahiya R. Estrogen receptor alpha polymorphisms and renal cell carcinoma—a possible risk. *Mol Cell Endocrinol*. 2003;202(1–2):109-116.
161. Zhu P, Liao LY, Zhao TT, Mo XM, Chen GG, Liu ZM. GPER/ERK&AKT/NF-κB pathway is involved in cadmium-induced proliferation, invasion and migration of GPER-positive thyroid cancer cells. *Mol Cell Endocrinol*. 2017;442:68-80.
162. Nissen NI, Karsdal M, Willumsen N. Collagens and cancer associated fibroblasts in the reactive stroma and its relation to cancer biology. *J Exp Clin Cancer Res*. 2019;38(1):1-12.
163. Hu B, Wu C, Mao H, et al. Subpopulations of cancer-associated fibroblasts link the prognosis and metabolic features of pancreatic ductal adenocarcinoma. *Ann Transl Med*. 2022;10(5):262.
164. Wiley SZ, Sriram K, Liang W, et al. GPR68, a proton-sensing GPCR, mediates interaction of cancer-associated fibroblasts and cancer cells. *FASEB J*. 2018;32(3):1170-1183.
165. Wiley SZ, Sriram K, Salmerón C, Insel PA. GPR68: an emerging drug target in cancer. *Int J Mol Sci*. 2019;20(3):559.

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