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G protein-coupled receptors in stem cell maintenance and somatic reprogramming to pluripotent or cancer stem cells

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Abstract

The G protein-coupled receptors (GPCRs) compose the third largest gene family in the human genome, representing more than 800 distinct genes and 3–5% of the human genome. GPCRs are divided into five distinct families: rhodopsin, secretin, adhesion, glutamate, and frizzled. They bind and regulate 80% of all hormones and account for 20–50% of the pharmaceuticals on the current market. Hundreds of GPCRs integrate and coordinate the functions of individual cells, mediating signaling between various organs. GPCRs are crucial players in tumor progression, adipogenesis, and inflammation. Several studies have also confirmed their central roles in embryonic development and stem cell maintenance. Recently, GPCRs have emerged as key players in the regulation of cell survival, proliferation, migration, and self-renewal in pluripotent (PSCs) and cancer stem cells (CSCs). Our study and other reports have revealed that the expression of many GPCRs is modulated during the generation of induced PSCs (iPSCs) or CSCs and during CSC sphere formation. These GPCRs may have crucial roles in the regulation of self-renewal and other biological properties of iPSCs and CSCs. This review addresses the current understanding of the role of GPCRs in stem cell maintenance and somatic reprogramming to PSCs or CSCs.

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Introduction

Many tissues of the body—for example, skin, liver, and epithelium—not only repair themselves but also self-renew, a property mainly found in stem cells (1). Embryonic stem cells (ESCs) have an even greater potential for self-renewal and differentiation. Recently, mouse and human fibroblasts were reprogrammed into pluripotent stem cells (PSCs) through the introduction of a diverse set of stem cell-related transcription factors including Oct4, Sox2, Klf4, and c-myc (2, 3). These induced PSCs (iPSCs) derived from somatic fibroblasts had genetic, epigenetic, and developmental features that were highly similar to those of ESCs. Although ESCs and iPSCs are considered unlimited cell sources for regenerative medicine, techniques for maintaining undifferentiated ESC or iPSCs remain inefficient, which results in inhomogeneous cell populations.

Tumor cells are assumed to include a population of cells responsible for the initiation of tumor development and growth and the capacity to metastasize and reoccur (4) . Because of their similarities with stem cells, these cells have been named cancer stem cells (CSCs). CSCs have properties such as self-renewal, heterogeneity, and resistance to apoptosis. CSCs likely arise from stem cells, and the transformation of normal stem cells into CSCs may be due to the accumulation of genetic modifications such as mutations in oncogenes, suppressor genes, and mismatch repair genes or epigenetic alterations such as abnormal methylation and histone modification (5) .

The factors that control the regulation of cell survival, proliferation, migration, and self-renewal in PSCs and CSCs are still emerging. Some evidence demonstrates the potent effects of various GPCR ligands on the biology of PSCs and CSCs. G protein-coupled receptors

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(GPCRs) are a large class of transmembrane (TM) receptors that conduct extracellular signals into cells by combining with guanine nucleotide-binding proteins (G proteins) and interacting with a diverse set of ligands, including biogenic amines, amino acids, ions, lipids, and peptides, as well as light, taste, and odor stimuli and coupling the presence of these signals to such fundamental cellular responses as growth, death, movement, transcription, and excitation (6) . They are by far the largest family of cell surface molecules, and they modulate key physiological functions, including neurotransmission, hormone and enzyme release, immune response, and blood pressure regulation. Their signaling converges on common downstream effectors and modulators, such as G proteins, arrestins, and GPCR kinases/G protein-coupled receptor kinases. Most GPCRs activate one or multiple Gα proteins, which can be subdivided into four major families: Gαi, Gα12, Gαs, and Gαq (7) . GPCRs act more as molecular regulators than on-off switches, so the engagement of different G proteins and the duration of signaling may differ not only among GPCRs but also for a given GPCR depending on the ligand and cellular environment (8) . Considerable evidence now exists that demonstrates the important roles of various GPCRs in the regulation of the biological properties of PCSs or CSCs.

Recently, we analyzed the expression profiles of GPCRs during somatic reprogramming to iPSCs or CSCs and during CSC sphere formation (Figure 1 and Table 1). More than 106 GPCRs were expressed exclusively in the PCSs or CSCs, whereas expression of 22 GPCRs were down-regulated during somatic reprogramming to iPSCs. Eighty-one GPCRs were differentially expressed during somatic reprogramming to iPSCs, and the expression of 195 GPCRs was up-regulated or down-regulated during somatic reprogramming to CSCs and sphere formation of CSCs. These data suggest that various GPCRs may have key roles in

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somatic reprogramming to iPSCs or CSCs and may be involved in the regulation of the specific self-renewal and other biological properties of PCSs or CSCs. Recently, considerable functional evidence has accumulated to support specific roles of GPCRs in somatic reprogramming or transformation to iPSCs or CSCs. In the following section, we review the general role of GPCR signaling pathways and the current understanding of the role of GPCRs in stem cell maintenance and somatic reprogramming to PCSs or CSCs.

General role of GPCR signaling pathways

GPCRs bind and regulate the effects of 80% of all hormones in the body and account for 20–50% of the pharmaceuticals on the current market (9). Members of the GPCR superfamily are structurally defined by an extracellular N-terminus, seven TM domains, and a cytosolic C-terminus (10). They compose the third largest gene family in the human genome, representing more than 800 distinct genes and 3–5% of the human genome. The superfamily has traditionally been divided into three major families—class A/rhodopsin-like receptors, class B/secretin-like receptors, and class C/glutamate-like receptors—and recent bioinformatics analyses have updated the phylogenetic characterization to five distinct families: glutamate, rhodopsin, adhesion, frizzled (FZD), and secretin (i.e., the GRAFS classification system) (Figure 2) (11, 12).

GPCR stimulation triggers the activation of heterotrimeric G proteins as guanosine triphosphate (GTP) replaces guanosine diphosphate on the Gα subunit, promoting its dissociation from the Gβγ subunits. Both α-GTP-bound and Gβγ subunit complexes then stimulate multiple downstream signaling cascades (7), including the rapid generation of

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multiple second messengers. For example, Gαs stimulates adenylyl cyclase, increasing the cytosolic levels of cyclic adenosine monophosphate (cAMP), whereas Gαi inhibits adenylyl cyclase and hence decreases cAMP levels (13) . Members of the Gαq family activate phospholipase-Cβ, which cleaves phosphatidylinositol-4, 5-bisphosphate into diacylglycerol and inositol 1,4,5-trisphosphate; the latter increases cytosolic calcium (14) . The targets of these diffusible second messengers include ion channels, calcium-sensitive enzymes, and kinases such as cAMP-dependent kinase, protein kinase C, cyclic guanosine monophosphate-dependent kinase, and calcium-calmodulin regulated kinases. Many of these kinases contribute to cancer progression and metastasis (15).

Although GPCRs can stimulate multiple diffusible second messenger-generating systems, their capacity to promote normal and aberrant cell proliferation often relies on the persistent activation of phosphatidylinositol 3′-kinase/protein kinase B, Ras and Rho GTPases, and mitogen-activated protein kinase cascades, thereby regulating the activity of nuclear transcription factors and co-activators such as JUN and FOS (16).

A more universal definition of the general systems through which GPCRs exert their numerous physiological and pathological roles is necessary to appreciate the overall implications for tumorigenesis. In particular, extensive cross talk and co-regulation may occur between GPCR- and tyrosine kinase growth factor receptor-initiated signaling pathways and through receptor transactivation. Therefore, the final biological outcome of GPCR activation is a result of the integration of the GPCR-initiated biochemical response networks in each cellular and environmental context (8). This systems-level understanding may provide a molecular framework for the development of novel approaches to therapeutic intervention in some of the most prevalent human diseases.

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GPCRs in stem cell maintenance

PCSs have great potential to aid in the understanding of the early development and treatment of human disease and tissue disturbances. We found that optimization of culture condition can lead to enhancement of pluripotency of stem cells (17, 18). Expression of some membrane proteins including GPCRs play roles in the regulation of cell morphology, polarity and the migration of stem cells (19). Extensive evidence suggests that GPCRs show dramatically different expressions when cells differentiate, but the roles of GPCRs in stem cells maintenance are poorly understood (20). The self-renewal and pluripotency of PCSs are regulated by several Gs- and Gi-coupled GPCR signaling pathways (21). Signaling mediated by the G proteins of the Gi subfamily affects the morphology and organization of iPSC colonies and may reflect signal contributions from multiple Gi-coupled receptors (20). Gs signaling in self-renewing and differentiating ESCs also reportedly promotes both proliferation and pluripotency (21), suggesting that G proteins have fundamental roles in stem cell pluripotency and differentiation, which indirectly implicates upstream GPCRs.

Evidence of GPCR regulatory roles in PCS differentiation is found in the dramatic changes in expression levels of GPCRs at distinct stages of differentiation. The results of comprehensive analysis of the changes in GPCR transcript expression during in vitro neural differentiation show that distinct GPCR genes are specifically expressed at each differentiation stage (22).

Considerable functional evidence has accumulated to support specific roles of distinct ligand-GPCR pairs in stem cell maintenance. Specific roles of GPCRs from each of the five families (glutamate, rhodopsin, adhesion, FZD, and secretin) are reviewed in the following section.

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154 Glutamate GPCR family

155 The glutamate family includes metabotropic glutamate (mGlu) receptors, gamma-
156 aminobutyric acid B (GABAB) receptors (GABAA receptors are ligand-gated ion channels,
157 and metabotropic GABAB receptors are GPCRs), taste receptors, and related orphan
158 receptors (12). Glutamate and GABA are the primary excitatory and inhibitory
159 neurotransmitters of the adult neuron, respectively, and therefore have obvious roles in
160 neuronal activity. Glutamate receptors that couple to G proteins are referred to as mGlu
161 receptors, and they have distinct effects on the cell growth and differentiation of ESCs. The
162 mGlu receptor family is composed of eight subtypes. mGlu 1 and 5 couple to Gq proteins,
163 whereas mGlu 2, 3, 4, 6, and 7 couple to Gi G proteins (23). The activation of mGlu 5
164 promotes self-renewal through inhibition of glycogen synthase kinase-3β (GSK-3β) and
165 leukemia inhibitory factor (LIF) activation of phosphatidylinositol 3′-kinase (24). During
166 ESC differentiation, mGlu5 expression decreases and mGlu4 expression is up-regulated (25) .
167 Moreover, activation of the mGlu3 receptor suppresses the differentiation of neural stem cells
168 via mitogen-activated protein kinase-dependent inhibition of bone morphogenetic protein
169 signaling (26).

170

171 Rhodopsin GPCR family

172 The rhodopsin family is the largest GPCR family, and its ligands include lipids, purines,
173 opsins, peptides, amines, and a number of orphan receptors for which the endogenous ligand
174 remains unidentified (12). The roles of cytokine, cannabinoid, and lysophospholipid receptors

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175 in the regulation of stem cell maintenance are described herein.

176 Chemokine receptors comprise a large subfamily of seven TM proteins that bind one or more
177 chemokines, which are small cytokines that typically have chemotactic activity for leukocytes
178 (27). The chemokine receptor CXCR4 and its ligand, stromal cell-derived factor-1α (also
179 known as CXCL12), have important roles in stem cell maintenance (28). CXCR4 is
180 expressed in mouse ESCs and is involved in the adhesion phase of blastocyst implantation. It
181 regulates the induction of proliferation, trafficking, locomotion, and adhesion of PCSs and
182 has a role in the homing of engrafted stem cells (29). CXCR4 also regulates the migration of
183 stem cells and has been identified as an interesting target for stem cell-based therapies for
184 multiple sclerosis.

185 The cannabinoid receptors CB1 and CB2 are also members of the rhodopsin family of
186 GPCRs and are activated by endogenous ligands that include anandamide, anandamide
187 derivatives (2-arachidonoyl glycerol and noladin ether), virodhamine, and N-
188 arachidonoyldopamine. CB1 is largely expressed in the central nervous system, whereas CB2
189 is mainly expressed in the immune system and hematopoietic cells (30). The
190 endocannabinoid system is expressed in ESCs in vitro and promotes ESC proliferation and
191 differentiation (31). Both CB1 and CB2 couple to Gi G proteins and have been implicated in
192 the regulation of protein kinase A and C, Raf-1, extracellular signal-regulated kinase (ERK),
193 c-Jun N-terminal kinase, p38, c-fos, and c-jun signaling and are likely involved in mouse
194 ESC survival ancillary to conventional LIF/gp130 signaling (32). Moreover, CB1 and CB2
195 receptors promote the differentiation of human glioma stem-like cells, which suggests a
196 possible anti-cancer role for cannabinoid receptors (33).

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The rhodopsin family of lysophospholipid receptors includes receptors for lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P). Both LPA and S1P are reportedly positive regulators of the self-renewal and pluripotency of PCSs (34). LPA may regulate mouse ESC maintenance via ERK activation of c-fos (35). Similarly, S1P treatment of mouse ESCs activates ERK. LPA also regulates the expression of the key pluripotency gene c-myc (36), which is implicated in ESC self-renewal and somatic cell reprogramming to iPSCs (2). The mode of action in human ESCs (hESCs) also involves Gi- and ERK-dependent mechanisms (37). LPA activates six LPA receptors (LPA1–6) and has also been identified as an agonist at other orphan GPCRs (PSP24, GPR87, and GPR35) as well as at receptors of the nuclear hormone peroxisome proliferator-activated receptor γ, although the physiological significance of these observations remains unclear (38). Specific LPA receptor expression has been described in bone marrow-derived stem cells. LPA1 and LPA2 are expressed in hematopoietic cells (39), and LPA1–3 are expressed in mesenchymal stromal cells (40).

S1P activates five S1P receptors (S1P1–5) (41). S1P receptors are expressed in hematopoietic as well as muscle progenitor cells and S1P2 mediates proliferation and survival. Expression studies in ESCs have revealed that LPA1–3 and 5 and S1P1–5 are expressed in ECSs and that LPA and S1P increase the expression of pluripotency genes and stimulate cell proliferation (42) through a Gi-ERK-dependent pathway (34).

Adhesion GPCR family

The expression of adhesion GPCRs during embryogenesis and their roles in development have been reported. Adhesion family GPCRs have adhesion motifs containing long N-termini

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that are often involved in protein-protein interactions and mediate signaling through both G protein-dependent and G protein-independent mechanisms (43). The cadherin/CELSR subgroup of adhesion receptors, Celsr1–3, have key roles in migration and proliferation during development (44). Other adhesion GPCRs are also associated with multipotency and differentiation. The orphan receptor GPR56 couples with Gα12 to induce Rho-mediated cytoskeletal changes, suggesting a role for GPR56 in neural development and differentiation (31). Another orphan adhesion receptor, GPR125, is expressed in undifferentiated spermatogonia in the testis and may be involved in the maintenance of multipotency in spermatogonial precursors (45).

Wnt/FZD GPCR family

The FZD family receptors are not traditionally classified as GPCRs, but they share the common structural characteristics of GPCRs and were recognized as an official class of GPCRs in 2005 by the International Union of Pharmacology (46, 47). They were originally identified in *Drosophila* and are highly conserved across species (48). FZDs are activated by the Wnt family, which comprises cysteine-rich lipoglycoproteins with fundamental functions in ontogeny and tissue homeostasis. The FZD family of receptors has 11 members, most of which are involved in embryonic development in a range of organisms. There are 19 mammalian Wnt genes, the products of which bind to the extracellular, N-terminal cysteine-rich domain that is common to all FZD receptors. Wnt signaling has been implicated in various stem cell systems, including ESC self-renewal. Activation of the Wnt pathway by wnt3a or the GSK-3 inhibitor 6-bromoindirubin-3′-oxime leads to self-renewal and

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pluripotency (49), whereas that by another GSK-3 inhibitor, LiCl, does not (50). Wnt3a may stimulate hESC proliferation, and the Wnt receptor FZD7 has also been identified as important for hESC maintenance (51). FZD7 messenger RNA levels in hESCs are higher than those in differentiated cell types, and FZD7 knockdown induces dramatic changes in the morphology of ESC colonies and a loss of octamer-binding transcription factor 4 expression, implying that FZD7 is an important ESC-specific surface marker and a potential regulator of ESC self-renewal. Wnt/FZD signaling represents an exciting area of research that will undoubtedly be critical in the use of pluripotent and other stem cells in translational applications.

Secretin GPCR family

The secretin family of GPCRs is named for the first receptor discovered in the family. These GPCRs form complexes with large polypeptide ligands and regulate a range of physiologic functions (11). Vasoactive intestinal polypeptide receptor 1 (VPAC1), VPAC2, and phosphatase of activated cells 1 (PAC1) receptors, which bind vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide, couple to Gs G proteins and increase cAMP levels, thereby regulating stem cell maintenance (52). The dynamic expression patterns of VPAC1/2 and PAC1 in the differentiation of ESCs suggest a role for these receptors in the regulation of stem cell pluripotency. These receptors also affect a number of signaling pathways in the context of differentiation.

GPCRs in somatic reprogramming to PCSs

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The generation of iPSCs has significant therapeutic promise due in part to the potential for generating stem cells from individual patients (53). Importantly, iPSCs provide an alternative to the use of human embryos, overcoming ethical issues. In addition, iPSC technology allows the use of patient-specific somatic cells to generate therapeutic iPS cells, thus overcoming the potential for immune rejection. The iPSC field advanced in 2006 when Takahashi and Yamanaka (2) found that retroviral transduction of only four genes—Sox2, Oct4, c-myc, and Klf4—was sufficient to induce pluripotency in somatic cells. Post-mitotic cells can be genetically manipulated to "de-differentiate" in vitro via expression of specific pluripotency genes, giving rise to PCSs. In human cells, reprogramming using the additional factors Nanog and Lin28 to replace c-myc and Klf4 dramatically reduces the time required to generate iPSCs (3). To avoid genetic modification and to improve the efficiency of iPSC generation and differentiation, iPSC production technology was advanced by techniques that avoided stable integration of foreign genetic material into the host genome (42, 53). Moreover, synthetic compounds (small molecules) such as valproic acid maintain or induce pluripotency, which enhances PCSs efficiency of cellular reprogramming or mimics the effects of iPSC-specific pluripotency genes during reprogramming (2, 3, 42). The goal of patient-specific iPSC therapy is to prepare somatic cells from patients, reprogram and differentiate them to replace diseased cells, and successfully transplant them back into the same patients without immune problems. Because pluripotent colony morphology associates closely with the maintenance of pluripotency, the mechanisms through which these colonies form and organize may be important for managing somatic cell reprogramming (54). Our results showed that 81 GPCRs are differentially expressed during somatic reprogramming to iPSCs (Figure 1B and Table 1). Of these GPCRs, several have confirmed roles in the regulation of

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self-renewal and other properties of PCSs. Several GPCRs have been proposed to have crucial roles in somatic reprogramming to PCSs.

Gi-coupled receptors

Somatic reprogramming to iPSCs requires dramatic morphological and organizational cell changes (55). Interestingly, the inhibition of Gi-coupled receptor signaling via pertussis toxin retracts stem cell colonies inward into a dense multilayered conformation without affecting proliferation, survival, or pluripotency (56). Activation of Gs-coupled receptor signaling with cholera toxin did not affect colony morphology, suggesting that Gi-coupled GPCRs may play a role in some aspects of somatic cell reprogramming to PSCs.

GPR125

We hypothesize that GPR125 is a potential marker for somatic reprogramming to PCSs. GPR125 is an orphan adhesion-type GPCR also known as germ-line progenitor marker (57) and tumor endothelial marker 5-like. GPR125 is found in proliferative adult spermatogonial progenitor cells and multipotent adult spermatogonial-derived stem cells but is down-regulated after differentiation (57). Members of the tumor endothelial marker family have been identified by searching for genes with elevated expression under tumor angiogenesis (58). A recent study revealed that GPCRs display dramatic differences in expression when cells differentiate (21). The authors generated a real-time polymerase chain reaction-based expression profile of 343 GPCRs in ESCs and revealed that 161 of them were expressed at

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low levels in undifferentiated ESCs, 30 were moderately expressed, and 7 were highly expressed. GPR125 was one of the important GPCRs that were differentially expressed in ESCs.

S1P and LPA receptors

Specific lipids regulate various features of ESCs through binding to GPCRs. Both S1P and LPA are positive modulators of ESC maintenance through ERK- and Ca^{2+}-mediated pathways (34, 36). Mouse ESCs express LPA and S1P receptor subtypes (36, 37). The LPA signaling pathway that affects mouse ESC maintenance is the ERK activation of c-fos (35). Similarly, S1P treatment of mouse ESCs activates ERK, likely mediated by S1P receptor 5 via Gi-, protein kinase C-, and c-Src-dependent mechanisms (34). Significantly, although the ERK signaling pathway is implicated in mouse ESC proliferation, the suppression of ERK signals has a confirmed role in cell differentiation (59). Finally, LPA regulates the expression of the key pluripotency gene c-myc, implying a role in self-renewal and somatic cell reprogramming to iPSCs (2, 60).

Wnt/FZD receptors

Signaling by WNT glycoproteins and their cognate FZD receptors affects pluripotency and differentiation in diverse ways (48). The binding of Wnt to its receptors induces the nuclear translocation of beta-catenin, which acts as a transcription factor of T-cell factor (TCF)/lymphoid enhancer binding factor to modify gene transcription in “canonical”

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pathways. Non-canonical pathways are independent of beta-catenin, leading to both small G protein activation and cytoskeletal changes or through Ca^{2+} signaling with activation of heterotrimeric G proteins, causing various cell responses (61). In particular, hESCs express members of the FZD7 receptor family and secrete FZD-related proteins encoding soluble Wnt antagonists (62). However, the effects of this pathway on the maintenance of pluripotency are subject to debate. Although there is evidence of direct involvement of Wnt signaling in the activation of pluripotency in both mouse ESCs and hESCs (50), additional evidence indicates specific roles for Wnt ligands and receptors in ESC specification/differentiation. The Wnt-activated transcription factor TCF3 binds to the promoters of pluripotency genes Sox2, Oct4, and Nanog. Knockdown of TCF3 increases the transcription of these genes, suggesting that Wnt/TCF is a negative regulator of pluripotency in mouse ESCs (63). Accordingly, signaling through Wnt pathways can induce pluripotency or differentiation, and the exact role of Wnt signaling in a given ESC population likely reflects the specific complement of Wnt ligands and receptors expressed in the population and the interactions among multiple signaling networks (49).

mGlu receptors

In mouse ESCs, mGlu5 activation promotes self-renewal through interaction with LIF signaling pathway (24, 64). Various mGlu receptor subtypes are involved in differentiation. Specifically, the differentiation of mouse ESCs into embryoid bodies is associated with the induction of mGlu4 receptors, which promote differentiation of GABAergic neurons (25, 65). Other GPCRs are found in ESCs, but very few studies have described their functional roles.

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GPCR signaling in somatic reprogramming to CSCs

CSCs were first described in 1994 when a cell type of low abundance derived from human acute myeloid leukemia caused cancer in mice (66). Many cancers include a population of tumor stem cells that de-differentiate to return to the proliferative state, often expressing the same genes that are “markers” in ESCs (20). The origin of CSCs is still being debated; particularly whether they represent a stromal stem cell that has undergone some sort of malignant change or whether they are differentiated cells that acquire stemness as part of the malignant somatic reprogramming or transformation process. According to the CSC hypothesis, only a small fraction of immature cellular intermediates is responsible for mediating tumor expansion, resistance, and metastases. Thus, CSCs are the tumor component that diffuses out of the organ, where it cannot be eliminated by surgical therapy and causes relapses resistant to chemotherapy and radiotherapy (67). The presence of CSCs has important implications for treatment, as current therapies may target the bulk of tumor cells but miss CSCs, resulting in tumor recurrence. Consequently, future treatments specifically targeting CSCs may be more effective.

Tumor-suppressor genes are commonly lacking in many CSCs, and this absence may provide the capacity for CSC self-renewal (67). FZD and chemokine receptors are among the receptors that collect signals within the environmental niche. Our results showed that 195 GPCRs are differentially expressed during transformation to CSCs or during CSC sphere formation (Figure 1C, 1D, and Table 1). Of these GPCRs, several have confirmed roles in the regulation of self-renewal and other CSC properties. Several GPCRs may have crucial roles

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372 in the transformation to CSCs.

373

374 Wnt/FZD receptors

375 The Wnt and FZD pathways are involved in various differentiation events during
376 embryonic development and tumor formation (68). During malignant progression, cancers
377 actively rearrange the extracellular matrix and tumor stroma to create suitable
378 microenvironments. In cancers, the Wnt and FZD pathways are typically associated with a
379 transition from epithelial to mesenchymal cellular stages as the tumor develops during
380 metastasis (69). Members of the FZD family activate upstream signaling that results in
381 epithelial-mesenchymal transition (67).

382

383 Chemokine receptors

384 The GPCR family of chemokine receptors is centrally linked to the organ-specific
385 metastasis of a number of cancers, in line with their normal immune cell function of directing
386 receptor-bearing leukocytes toward the sites of chemokine production. Similarly, tumor cells
387 abnormally expressing chemokine receptors can elect the migratory activity of chemokines,
388 facilitating metastasis to other organs (8). Recently, several studies have suggested that
389 chemokine receptors are relevant targets for the development of stem cell-based therapies
390 (70). CXCR4 is included among stem cell markers (71), and evidence of its importance in
391 tumor development is rapidly emerging (72). Recent studies have revealed that CXCR4
392 signaling has contrasting effects on normal and malignant breast stem cell activity,

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demonstrating that it specifically regulates breast CSC activities (73) . CXCR4 activates Rac1 through P-REX1, which plays a central role in metastasis in most types of breast cancer (34). CXCR4 can also couple to G12/13 in basal-like breast cancer cells, in which Ga13 protein expression is highly up-regulated, thus driving metastasis through a Ga12/13-RhoA-dependent mechanism (59) similar to that of LPA and PAR-1 receptors, all of which can be considered potential targets for metastasis prevention and treatment. Other chemokine receptors, including CCR7 and CCR10, have also been shown to have direct roles in the metastatic homing of cancer cells and cancer cell survival and growth (8). Chemokines may enhance cytokine-rich microenvironments and induce the release of matrix metalloproteases, which facilitate tumor cell survival, proliferation, and invasion. In addition, recent studies have demonstrated a role for GPR116, a member of the poorly characterized family of adhesion GPCRs, in the invasion and migration of breast cancer cells via a Gαq-RhoA/Rac1-dependent mechanism (74).

Melatonin receptors

Melatonin suppresses breast cancer cell proliferation by inhibiting the up-regulation of estrogen-induced cyclin D1 via its G protein-coupled melatonin receptor MT1 and down-regulating estrogen receptor alpha (75). 6-Hydroxymelatonin, an oxidated form of melatonin, has also been shown to bind selectively to MT1 and have antioxidant properties (76).

Purinergic receptors

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The purinergic system plays a key role in cell growth in glioblastoma, the most common and aggressive tumor of the brain, and may be characterized by a CSC subpopulation (77). Several purinergic receptor messenger RNAs have been differently expressed in tumor spheres containing markers for CSCs, suggesting that the purinergic system affects CSC biology.

Fatty acid-sensing GPCRs

Cancer cells or CSCs generate a supportive microenvironment by activating wound-healing, chemotherapy, and various stress response of normal cells (16). Conversely, stromal cells, such as cancer-associated fibroblasts or tumor-associated macrophages, promote tumor progression by secreting growth factors, chemokines, and pro-migratory extracellular matrix components (8). In particular, tumor-surrounding adipocytes exhibit a specific and activated phenotype; these cells have been designated cancer-associated adipocytes, and they contribute to the progression of various tumors by stimulating local and distant invasion (78). Several fatty acid-sensing GPCRs that are usually expressed in tumor microenvironments are involved in somatic reprogramming to CSCs. They bind and are activated by free fatty acids, lipid molecules, or both.

GPR35 was first described as being activated by kynurenic acid (an intermediate in tryptophan catabolism that has neurotransmitter activity as an anti-excitotoxic and anticonvulsant) but is most likely the receptor for 2-arachidonyl LPA. The emerging function of GPR35 demonstrates that it may be an important target in pain, heart disease, inflammatory bowel disease, cancer, and asthma. GPR35 is a member of the class A GPCR

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family and exists in at least two forms due to alternative splicing. The highest expression levels of GPR35 are observed in the stomach, small intestine, and colon.

GPR41 and GPR43 are activated by short-chain fatty acids such as propionic acid, butyric acid, and pentanoic acid. Both of these receptors are expressed at their highest levels in adipose tissue and immune cells. GPR41 and GPR43 activation is involved in adipogenesis and the production of leptin by adipose tissue. GPR81 (HCA1) is almost exclusively expressed in adipocytes and activated by the elevated blood lactate levels, which are apparently caused by intensive exercise. During intensive exercise, increased fatty acid release from adipocytes may be required for skeletal muscle energy generation. Adipocytes are another source of lactate, and the reduction of glycolytic pyruvate to lactate increases as a result of insulin-stimulated glucose uptake into these cells. Therefore, the normal function of lactate-induced stimulation of GPR81 is likely to contribute to the insulin-induced inhibition of adipocyte lipolysis. Indeed, GPR81 knockout mice show an associated decrease in insulin-mediated inhibition of lipolysis.

GPR120 is expressed in mature adipocytes but not in pre-adipocytes. Docosahexaenoic acid (DHA) stimulation of GPR120 in adipocyte precursor cells in culture increases insulin-sensitive glucose transporter 4 translocation to the cell surface, with a subsequent increase in glucose transport into the cells. The DHA-mediated effects on glucose uptake via GPR120 stimulation in adipocytes are independent of β-arrestin2.

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Summary

The molecular function of GPCR signaling pathways has been thoroughly studied for more than a century from the perspectives of basic science and therapeutic application. Despite this long history, new biological functions and applications for GPCRs continue to emerge. In this review, we addressed our current understanding of the role of GPCRs in stem cell maintenance and somatic reprogramming to PCSs or CSCs. Differential GPCRs make crucial role not only in tumor progression, adipogenesis, and inflammation but also in embryonic development and stem cell maintenance. Specific GPCRs also have key roles in somatic reprogramming to iPSCs and transformation to CSCs via regulation of cell survival, proliferation, migration, and self-renewal of PCSs and CSCs. Given the evidence discussed herein, the roles of differential GPCRs in stem cell maintenance and somatic reprogramming to PCSs or CSCs can be added to the growing list of GPCR functions. The significance of these functions may be manifested in multiple biological and therapeutic scenarios.

Acknowledgements

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478 **Figure legends**

479 Figure 1. Changes in G protein-coupled receptor (GPCR) expression in stem cell
480 maintenance and/or during somatic reprogramming to iPSCs or CSCs. The transcriptional
481 profile of the selected GPCR family was analyzed using high-throughput RNA sequencing.
482 (A) GPCRs showing up- or down-regulated expression during stem cell maintenance. (B)
483 GPCRs showing up- or down-regulated expression during somatic reprogramming to iPSCs.
484 (C) GPCRs showing up- or down-regulated expression during CSC sphere formation. (D)
485 GPCRs showing up- or down-regulated expression during malignant transformation (somatic
486 reprogramming) to CSCs.

487

488 Figure 2. Differential GPCRs and GPCR signaling, which may be involved in stem cell
489 maintenance and/or during somatic reprogramming to iPSCs or CSCs. (A) The GPCR
490 superfamily has traditionally been divided into three major families: class A/rhodopsin-like
491 receptors, class B/secretin-like receptors, and class C/glutamate-like receptors. Recent
492 bioinformatics analyses have updated the phylogenetic characterization to five distinct
493 families: glutamate, rhodopsin, adhesion, frizzled and secretin (GRAFS classification system).
494 (B) Various ligands bind GPCRs to stimulate various G proteins. GPCRs interact with
495 heterotrimeric G proteins composed of α, β, and γ subunits that are guanosine diphosphate
496 bound in the resting state. Most GPCRs activate one or multiple Gα proteins, which can be
497 subdivided into four major families: Gαi, Gα12, Gαs, and Gαq. Ultimately, the integration of
498 the functional activities of G protein-regulated signaling networks controls many cellular
499 functions, and the aberrant activity of G proteins and their downstream target molecules can

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contribute to various cellular mechanisms, including roles in stem cell maintenance and somatic reprogramming to iPSCs or CSCs. (B) Activation of pluripotency and differentiation pathways by GPCRs.

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Figure 1

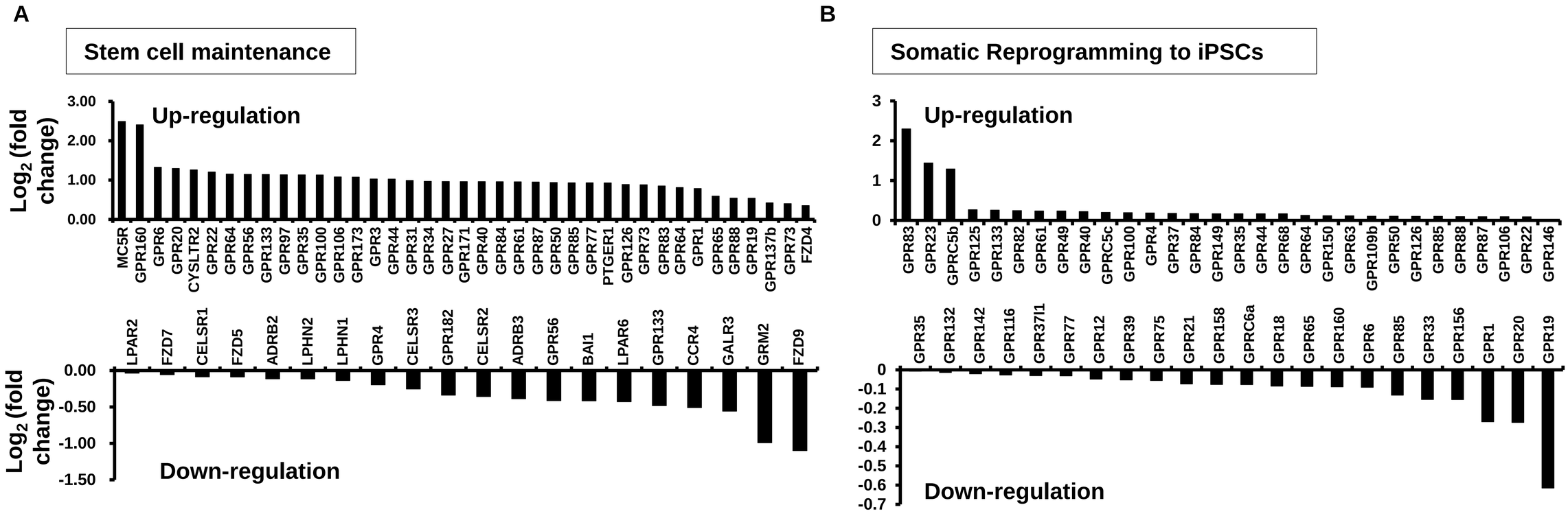
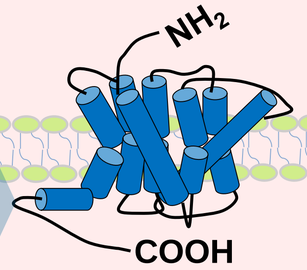

A
Stem cell maintenance
Up-regulation
Log2 (fold change)
3.00
2.00
1.00
0.00
MC5R GPR160 GPR6 GPR20 CYSLTR2 GPR22 GPR64 GPR56 GPR133 GPR97 GPR35 GPR100 GPR106 GPR173 GPR3 GPR44 GPR31 GPR34 GPR27 GPR171 GPR40 GPR84 GPR61 GPR87 GPR50 GPR85 GPR77 PTGER1 GPR126 GPR73 GPR83 GPR64 GPR1 GPR65 GPR88 GPR19 GPR137b GPR73 FZD4
LPAR2 FZD7 CELSR1 FZD5 ADRB2 LPHN2 LPHN1 GPR4 CELSR3 GPR182 CELSR2 ADRB3 GPR56 BAI1 LPAR6 GPR133 CCR4 GALR3 GRM2 FZD9
0.00
-0.50
-1.00
-1.50
Log2 (fold change)
Down-regulation
B
Somatic Reprogramming to iPSCs
Up-regulation
3
2
1
0
GPR83 GPR23 GPRC5b GPR125 GPR133 GPR82 GPR61 GPR49 GPR40 GPRC5c GPR100 GPR4 GPR37 GPR84 GPR149 GPR35 GPR44 GPR68 GPR64 GPR150 GPR63 GPR109b GPR50 GPR126 GPR85 GPR88 GPR87 GPR106 GPR22 GPR146
GPR35 GPR132 GPR142 GPR116 GPR37l1 GPR77 GPR12 GPR39 GPR75 GPR21 GPR158 GPRC6a GPR18 GPR65 GPR160 GPR6 GPR85 GPR33 GPR156 GPR1 GPR20 GPR19
0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
-0.7
Down-regulation
C
CSC Sphere formation
Up-regulation
Log2 (fold change)
8.00
6.00
4.00
2.00
0.00
VIPR1 FZD1 F2RL3 GPR68 GPR37 F2RL1 GIPR CCR10 EDNRa LGR4 CXCR4 NPY1r S1PR1 OPRL1 CCRL2 P2RY2 F2R LPAR5 GPR35 FZD8 FZD4 SSR4 MC1r GPR173 GPR137b GALT OPN3 LPAR1
GPR161 PTGER4 FZD2 GPR63 GPR75 CRHR1 FZD5 ADORA2b SSR2 GPR3 S1PR2 FZD7 HRH1 EDNRb GPR176 HTR7 F2RL2 SSR3 ADRB2
0.00
-0.50
-1.00
-1.50
-2.00
-2.50
Log2 (fold change)
Down-regulation
D
Somatic Reprogramming to CSCs
Up-regulation
5.00
4.00
3.00
2.00
1.00
0.00
AVPR1b BAI2 BAI3 BRS3 C5AR1 CCR2 CCR3 CCR4 CCR6 CCR7 CD97 CELSR1 CHRM1 CHRM2 CHRM4 CMKLR1 CNR1 CNR2 CRHR1 CRHR2 CX3CR1 CXCR1 CXCR2 CXCR3 CXCR4 CXCR5 CXCR6 CXCR7 EMR2 EMR3 F2R FZD10 FZD2 FZD3 FZD4 FZD5 GIPR GPR115 GPR12 GPR123 GPR125
P2RY10 P2RY11 P2RY6 PROKR1 PROKR2 PTAFR PTGDR PTGER1 RHO RRH RXFP1 RXFP2 RXFP4 S1PR1 S1PR2 S1PR4 S1PR5 SCTR SSTR1 SSTR2 SSTR3 SSTR4 SSTR5 TAAR1 TAAR2 TAAR3 TAAR5 TAAR6 TACR1 TACR2 TACR3 TBXA2r TRHR TSHR
0.00
-1.00
-2.00
-3.00
-4.00
-5.00
-6.00
Down-regulation

Figure 2

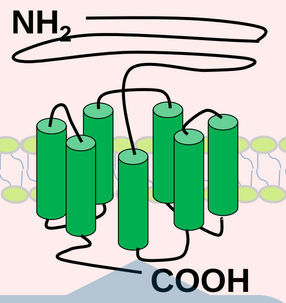
A

Stem Cell Maintenance
(A1 (2, GPR137b et al.), A2 (2, CXCR7 et al.), A5 (2, CYSLTR2 et al.), A8 (CMKLR1), A9 (2, GPR83 et al.), A10 (LGR4), A13 (4, MC5R et al.) A14 (PTGER), A15 (6, LPAR4 et al.), A17 (3, ADRB1 et al.), A18 (ADORA2b)
Somatic reprogramming to iPSCs
(A4 (2, GPR22 et al.), A5 (3, GPR100 et al.), A7 (GPR39), A8 (2, GPR44 et al.), A9 (4, GPR50 et al.), A10 (2, GPR49 et al.), A13 (3, GPR6 et al.), A15 (8, GPR4 et al.) A18 (9, GPR61 et al.), Orphan (14, GPR23 et al.)
CSC sphere formation
(A1 (2, CCRL2 et al.), A2 (2, CCR10 et al.), A4 (5, OPRL1 et al.), A5 (2, GALT et al.), A6 (GPR176), A7 (4, ENDRA et al.), A9 (2, NYP1R et al.), A10 (LGR4), A11 (P2RY2), A13 (6, S1PR1 et al.) A14 (PTGER4), A15 (7, F2RL3 et al.), A16 (OPN3), A17 (ADRB2), A18 (6, GPR173 et al.), A19 (HTR7)
Somatic reprogramming CSCs
(A1 (7, CCR2 et al.), A2 (13, CCR6 et al.), A3 (2, GPR15 et al.), A4 (6, GPR1 et al.), A5 (8, CYSLTP1 et al.), A6 (8, AVPR1b et al.), A7 (7, BRS3 et al.), A8 (6, C5AR1 et al.), A9 (9, GPR19 et al.), A10 (TSHR), A11 (15, FFAR1 et al.), A12 (5, GPR171 et al.), A13 (9, CNR1 et al.) A14 (8, PTGDR et al.), A15 (10, F2R et al.), A16 (3, RGR et al.), A17 (3, DRD1 et al.), A18 (17, CHRM1 et al.), A19 (4, HTR1D et al.)

Class A
(Rhodopsin-like receptor)

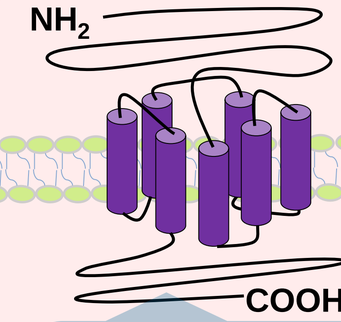


Class B
(Secretin-like receptor)



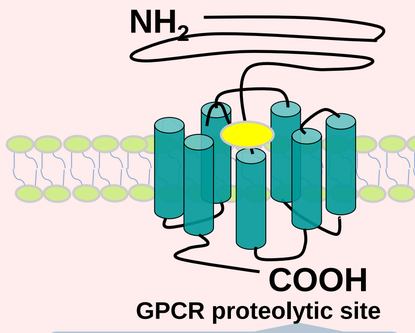
Stem Cell Maintenance
(B1 (GLP1R), B2 (11, GPR125 et al.))
Somatic reprogramming to iPSCs
(B2 (8, GPR133 et al.)
CSC sphere formation
(B1 (VIPR1),
Somatic reprogramming CSCs
(B1 (8, CRHR1 et al.), B2 (20, BAI2 et al.))

Class C
(Glutamate-like receptor)



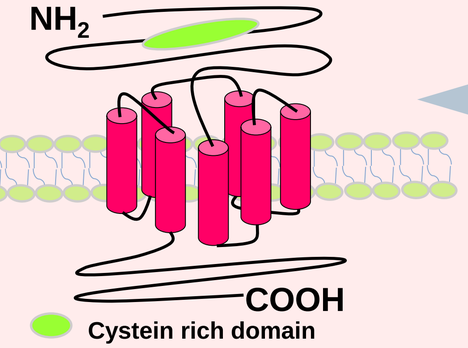
Stem Cell Maintenance
(GRM2),
Somatic reprogramming to iPSCs
(6, GPRC5b et al.)
Somatic reprogramming to CSCs
(8, GRM1 et al.)

Adhesion receptor

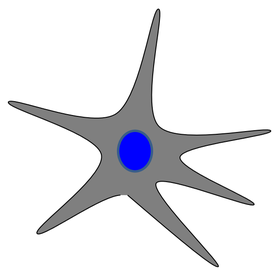


Stem Cell Maintenance
(GPR124, GPR126)

Frizzled receptor

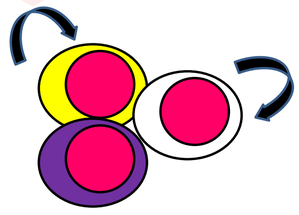


Somatic reprogramming CSCs
(9, FZD10 et al.),
CSC sphere formation
(7, FZD1 et al,)



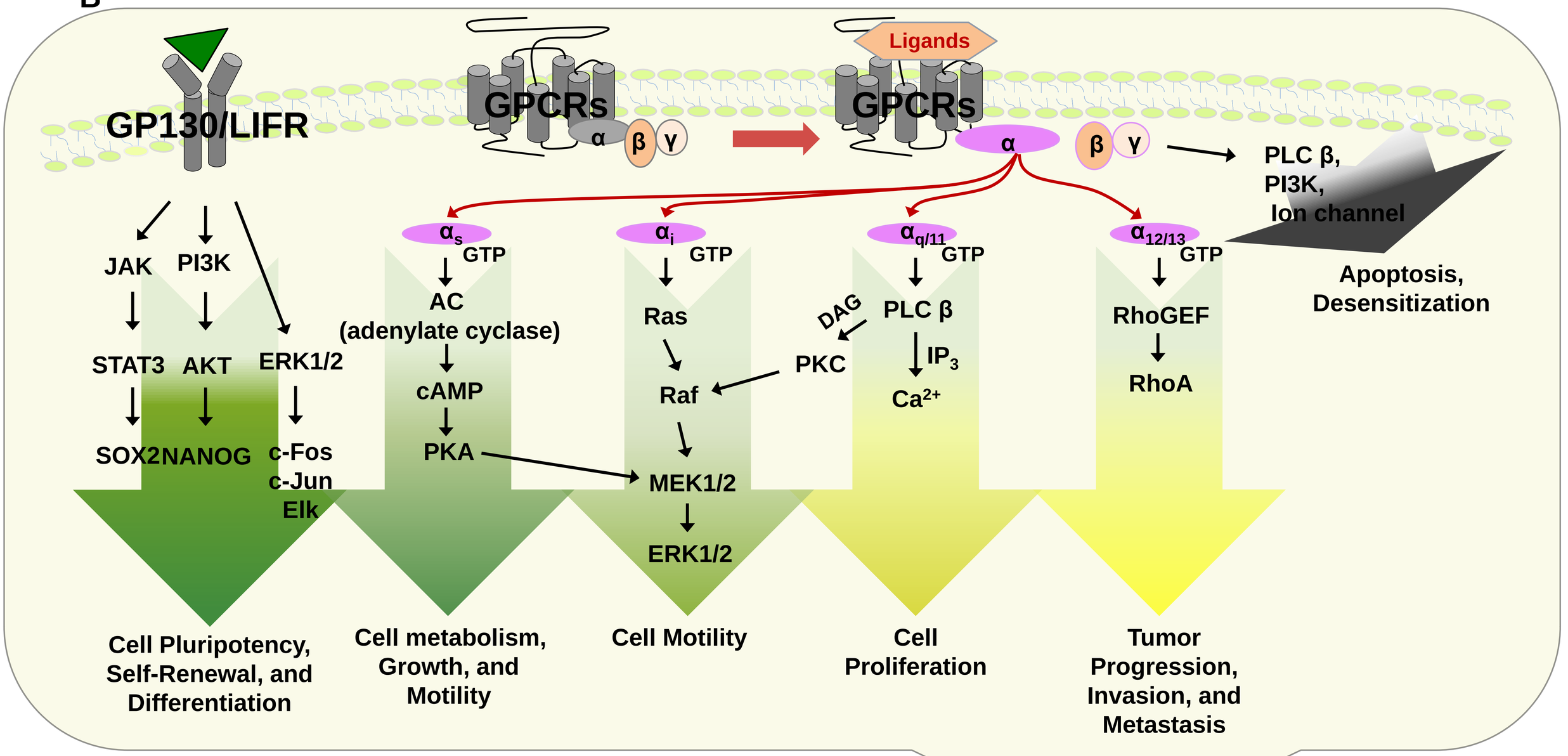
Somatic cells

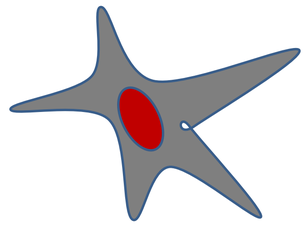
Self-renewal, Maintenance



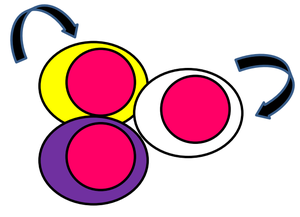
Reprogramming cells (iPSCs, CSCs)

Figure 2
B





Somatic cells



Reprogramming cells
(iPSCs, CSCs)

Stem Cell Maintenance
Somatic cell reprogramming to iPSCs
CSC sphere formation
Somatic cell reprogramming to CSCs