G protein signaling controls the differentiation of multiple cell lineages

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Running title: GPCR signaling in cellular differentiation

Key words: G protein-coupled receptor, differentiation, neuron, blood, adipocyte, smooth muscle cell, osteoblast.

Abstract

G protein-coupled receptors (GPCRs) detect a great diversity of extracellular stimuli ranging from hormonal peptides, chemokines, neurotransmitters, lipids, nucleotides, amino acids, biogenic amines to ions. G protein-coupled pathways regulate a rich collection of biological processes involved in normal physiological function of the body as well as in pathological progression of diseases. In addition to their function in post-mitotic steady-state tissues, GPCRs have been implicated in the differentiation of stem cells and tissue specific progenitor cells during development. Examples of these include the functions of nucleotides and neuropeptides in neuronal differentiation and axon growth, chemokines in lymphocyte differentiation and activation, and other GPCR-mediated processes in the differentiation of adipocytes, osteoblasts and smooth muscle cells. This review summarizes the recent advances in our understanding of the importance of GPCR-linked signaling cascades in the differentiation of different cell lineages.

GPCR signaling

The heptahelical G protein-coupled receptors (GPCRs) represent the largest family of receptors in human, mouse, worm and fly. Based on the human genome information, there may be as many as 700-800 human GPCRs [1]. Signaling by GPCRs mediates perception of light, tastes and odorants. Moreover, members of the GPCR family regulate intercellular signaling machineries by sensing extracellular signals such as hormones, neurotransmitters, chemokines and ions. As a consequence of their extreme diversity, GPCRs regulate a huge collection of physiological processes. Accordingly, more than 30% of our current pharmaceutical agents act as agonists or antagonists of GPCRs [1].

GPCRs transduce extracellular signals through the activation of heterotrimeric G proteins. Agonist activation of GPCRs induces conformational change on the receptors, which subsequently serve as guanine nucleotide exchange factors (GEFs) and induce the exchange of GDP to GTP on the $G\alpha$ subunits of heterotrimeric G proteins. GTP binding and activation of four classes of G proteins lead to stimulation of complex intracellular signaling networks. These include stimulation of adenylyl cyclase and production of cyclic AMP (cAMP) by $G\alpha_s$, repression of cAMP production but stimulation of Src tyrosine kinase by members of activated G_i proteins, activation of phospholipase $C\beta$ (PLC β) and subsequent release of intracellular Ca^{2+} by G_q family, and RhoGEF activation by $G_{12/13}$ [2-4]. Numerous studies have focused on the function of G protein signaling in differentiated, post-mitotic cells. Prominent examples include the function and regulation of neurotransmitter receptors in the

nervous system [5], chemokines in leukocyte attraction and cancer [6], and a variety of GPCRs in the regulation of smooth and cardiac muscle contraction [7]. These areas have been extensively covered by other reviews and will not be elaborated in this article. Instead, we set out to assimilate our current knowledge of the function of G protein-linked signaling pathways in cellular differentiation, and discuss the molecular basis of such regulation in different cell lineages.

The functions of G protein signaling in neuronal differentiation

Neuronal differentiation and survival are coordinated by receptor tyrosine kinases and GPCR signaling pathways. Nerve growth factor (NGF) signals through TrkA and downstream ERK and Akt to promote neuronal survival and differentiation [8]. NGF-induced neuronal survival and differentiation can be potentiated by activating the G_q -coupled purinergic P2Y2 receptor [9, 10]. ATP γ S-treated PC12 cells and dorsal root ganglion (DRG) neurons exhibited enhanced neurite formation, whereas genetic- or siRNA-mediated depletion of P2Y2 receptors abolished this effect [9]. Binding of ATP to P2Y2 activates G_q and PLC- β , leading to the production of inositol 1,4,5-trisphosphate (IP_3) and rapid Ca^{2+} influx. Increase in Ca^{2+} concentration activates Ca^{2+} -calmodulin-dependent protein kinase (CaMK) and calcineurin, and their downstream transcription factors including brain-derived neurotrophic factor (BDNF) and myocyte enhancer factor 2A (MEF2A) that are important for neuronal development [11]. Interestingly, P2Y2 also appears to transactivate TrkA receptor to induce neuronal differentiation, as it colocalizes with TrkA and enhances NGF

sensitivity [9]. Src family kinase (SFK) has been reported to mediate P2Y2 activation on TrkA receptor [12]. P2Y2 and TrkA synergistically prevent neuronal apoptosis in a similar mechanism [10]. Importantly, activation of purinergic receptors transmits complex communication between neurons and glials in adult brain [13]. It is not surprising to uncover that the same signaling mechanism is utilized during the developmental process of the nervous system.

Signaling through $G_{i/o}$ -coupled muscarinic acetylcholine receptors appears to cooperate with NGF in activating the PI3K/Akt pathway and promoting neuronal survival [14-16]. Treatment of PC12 cells with pertussis toxin (PTX; which inactivates $G_{i/o}$) reduced NGF-induced Akt phosphorylation, but has no effect on the Akt response elicited by epidermal growth factor (EGF). The induction of PI3K/Akt pathway by NGF is in part mediated through the $G\beta\gamma$ subunit released from activated $G_{i/o}$, which promotes the phosphorylation of translation regulator tuberlin to enhance neuronal survival [14, 15]. In a similar manner, $G_{i/o}$ mediates part of the NGF-induced p38 and JNK phosphorylations, which are required for neuronal differentiation [17, 18]. These examples suggest bidirectional crosstalk between GPCR and NGF signaling in the regulation of neuronal survival and differentiation.

In addition to nucleotide signaling, neuropeptides act through their GPCRs to regulate neuronal differentiation and brain development. Pituitary adenylyl cyclase-activating polypeptide (PACAP) and vasoactive intestinal peptide (VIP) act via VPAC1 and VPAC2 GPCRs [19]. Activation of VPAC1 and VPAC2 induces G_s -mediated cAMP production, as well as G_q -mediated IP_3 production and calcium

mobilization [20]. The effect of PACAP in neuronal cell proliferation remains controversial and is likely dependent on the receptor isoform expressed on target neurons [21]. On the other hand, PACAP and VIP potently induce differentiation of embryonic stem (ES) cells into neuronal lineage. Treatment of ES cells with PACAP or VIP induced neuronal marker expression, neurite outgrowth, and initiation of electrophysiological activity [22].

After early differentiation of stem cells into neuronal lineage, guidance of growing axons to specific location and formation of complicated neuronal networks represents one important aspect of neuronal differentiation. Growing axons navigate by sensing chemical cues at their growth cones and modulate intracellular cyclic nucleotide and Ca^{2+} levels to determine the direction of axon growth. Chemokines play an important role in either attracting or repulsing growth cones. For instance, the chemokine stromal-derived factor 1 (SDF-1) acts on its cognate receptor CXCR4 and guides axon growth [23]. When applied to isolated cerebellar granule cells, both SDF-1 and baclofen (an agonist of GABA_B receptor) showed marked repulsive turning of growth cones [23]. Both SDF-1 and baclofen signals through G_i to reduce intracellular cAMP, which is required for neuronal axon attraction [24]. Importantly, targeted disruption of either SDF-1 or CXCR4 in mice resulted in defective hematopoiesis and cerebellar neuronal migration, suggesting important functions of the chemokine SDF-1 signaling in both blood and brain development [25]. Unlike SDF-1, PACAP induces growth cone attraction by activating adenylyl cyclase and elevating local cAMP levels in neurons [26]. When cells were treated with forskolin to induce global cAMP increase,

or with PKA inhibitor, PACAP-induced growth cone attraction was completely abolished, suggesting the importance of locally induced cAMP/PKA activity on neurite outgrowth [26].

GPCR agonists function in an orchestrated way with other factors in neuronal axon guidance. For instance, netrins are bifunctional guidance cues that attract several classes of axons but repel others. The outcome of netrin signaling depends on the receptor repertoire present on target cells that transduces the netrin signal [27]. When bound with netrins, members of the Deleted in colorectal cancer (DCC) family mediate axon attraction by activating voltage-dependent Ca^{2+} channels (VDCCs) including L-type Ca^{2+} channels (LCCs), whereas DCC/UNC5 complex leads to axon repulsion [27]. Importantly, the cyclic nucleotide profile in the growth cone determines the outcome of netrin stimulation. At a high ratio of cAMP to cGMP, netrin signals axon attraction by inducing Ca^{2+} influx, whereas a low ratio of cAMP to cGMP inhibits Ca^{2+} influx and induces axon repulsion [24]. The regulation of cAMP by SDF-1, GABA and PACAP, and that of cGMP by nitric oxide (NO) signaling [28], converge at the point of netrin-induced Ca^{2+} influx. It is also noteworthy that UNC5H2, a member of UNC5 family, binds to activated (i.e., GTP-bound) $\text{G}\alpha_{i2}$ with its cytoplasmic domain [29]. The significance of such interaction remains to be addressed, but it may regulate cAMP production and modulate axon path-finding. Taken together, the intertwining signaling network of netrin, SDF-1 and PACAP indicates extensive crosstalk between the guidance cues in order to direct precise targeting of neuronal connections. The signaling processes regulating neuronal

differentiation and axonal guidance are summarized in Fig. 1. In general, agonist-bound GPCRs induce neuronal differentiation via various intracellular messenger systems. These signaling events orchestrate with other chemical stimuli in determining neuronal cell lineage specification, neurite outgrowth and axonal guidance.

Signaling through GPCRs also controls the differentiation of glial cells that provide support and nutrition to neurons in the nervous system. For instance, signaling through SDF-1/CXCR4 induces the differentiation of rat oligodendrocyte precursor cells into mature oligodendrocytes [30], in addition to its function in neuronal axon growth guidance. PACAP, which has been introduced above for its stimulation on neuronal differentiation, also induces astrocyte differentiation [31]. These examples indicate the important roles for G protein-mediated signaling in differentiation of both neurons and their supportive cells, and implicate therapeutic potential of GPCR-targeted medicines in the treatment of neurological disorders during development and aging.

The function of G protein signaling in blood cell differentiation

GPCR ligands such as chemokines are critical players in immune responses to foreign invasion, tissue damage and repair, and cancer. Supportive evidence has been well-summarized in other reviews and are beyond the scope of this article [6]. In addition to their function in regulating mature blood system, GPCRs mediate the development of blood cells by regulating their proliferation and differentiation [32]. A

recently identified orphan GPCR, Gpr48/LGR4 appeared to be required for definitive erythropoiesis in mice. Gpr48^{-/-} fetuses displayed transient anemia during midgestation and were defective in red blood cell development [33]. Deletion of Gpr48 reduced cell proliferation in fetal liver, with concomitant reduction in the levels of c-Myc and cyclin D1 [33]. Gpr48 signals through the G α_s -cAMP pathway [34]. Defective cAMP-PKA signaling leads to reduction in the level of ATF4 transcription factor [33], which is required for definitive erythropoiesis as evidenced by its deletion in mice [35]. Unfortunately, the ligand that activates Gpr48 remains to be identified.

The complement system plays an important role in innate immune response and adaptive immunity. Locally produced C5a and C3a anaphylatoxins stimulate T cell proliferation and differentiation through their specific receptors (C5aR and C3aR), and the downstream PI3K γ -Akt pathway [36]. Blockade of the interaction between C5a/C3a and their receptors resulted in defective expression of MHC class II and costimulatory molecules in T cells, along with significantly reduced T cell responses. Whereas addition of C5a or C3a to T cells stimulated with CD80^{-/-}CD86^{-/-} or CD40^{-/-} antigen presenting cells (APC) restored T cell activation [36], thus illustrating an important role of GPCR signaling in collaboration with CD28 costimulation in T cell activation and differentiation.

Peripheral cannabinoid receptor CB2 promotes myeloid leukemia [37]. The proto-oncogene CB2 is inserted with a viral sequence in murine retrovirally induced leukemias [37]. CB2 is normally expressed in B lymphocytes, but not in normal bone marrow immature myeloid precursor cells. Over-expression of CB2 in myeloid

precursor cells blocked neutrophilic differentiation [37, 38]. Administration of MEK inhibitors, and to a lesser extent, a PI3K inhibitor, reversed the inhibition of neutrophilic differentiation by CB2 [38]. Engagement of CB2 by its ligand leads to activation of $G\alpha_i$ and downstream MEK-ERK pathway, and reduction in cAMP level. Whereas ERK pathway is mainly responsible for suppressing neutrophilic differentiation, down-regulation of cAMP is important for migration induction by the CB2 ligand 2-arachidonoylglycerol (2-AG) [39].

In general, signaling through GPCRs mediates differentiation and migration of multiple lineages of blood cells. Given the great diversity of GPCRs and intracellular signaling events, it is anticipated that additional contributions by G protein-mediated signaling processes to blood development will be uncovered in the future.

GPCRs in the differentiation of smooth muscle cells, adipocytes and osteoblasts

GPCRs are also implicated in the differentiation of smooth muscle cells, osteoblasts and adipocytes. Smooth muscle cell (SMC) differentiation is regulated by a complex network of signaling processes. Serum response factor (SRF) is required for SMC differentiation by transcribing SMC-specific genes [40]. RhoA was demonstrated to modulate SRF-dependent transcription and promote SMC differentiation in a cell-based study [41]. In SMC and 10T1/2 fibroblasts, sphingosine 1-phosphate (S1P), acting through its cognate GPCR-RhoA pathway, was shown to promote SMC differentiation by facilitating the nuclear localization of myocardin-related transcription factor-A (MRTF-A), a cofactor of SRF [42]. S1P also

leads to phosphorylation and activation of Elk-1 by ERK [42]. In mesenchymal stem cells, sphingosylphosphorylcholine (SPC) was found to promote SMC differentiation through a $G_{i/o}$ -ERK pathway and in a TGF- β dependent manner [43]. Similarly, when treated with TGF- β , together with GPCR ligands endothelin, lysophosphatidic acid and carbachol, neural progenitor cells isolated from embryonic cerebral cortex differentiate into smooth muscle cells *in vitro*, as evidenced by the expression of α -smooth muscle actin and induction of smooth muscle cell morphology [44]. Inhibition of RhoA abolished endothelin-induced SMC differentiation, suggesting that the intracellular signaling mechanism might mimic that of S1P-induced SMC differentiation [42, 44].

Adipocytes comprise an important energy store in animals. They accumulate triacylglycerols during energy excess and release them during fasting. Therefore regulation of adipocyte differentiation and lipid accumulation is important for the maintenance of life, as well as for understanding human abnormalities like obesity. Adipocytes differentiate in response to extracellular signals including fatty acids and peptides. GPCR41 and GPCR43 recognize short chain fatty acids acetate and propionate as their ligands [45]. Short chain fatty acids have been reported to stimulate the production of leptin in adipocytes through GPCR41- G_i pathway [46]. However in a separate study, GPCR43, but not GPCR41, was found to be expressed in mouse adipose tissues [47]. Mice fed with high-fat diets up-regulated GPCR43, PPAR- γ 2 and leptin mRNA in adipose tissues. Furthermore, GPCR43 is up-regulated during adipocyte differentiation, and treatment of 3T3-L1 cells with propionate or

acetate enhanced adipocyte differentiation [47]. The controversy among the expression patterns of GPCR41 and GPCR43, and their role in leptin secretion by adipocytes remain to be clarified [45-47]. In addition to fatty acid signaling, peptide ligands also play a critical role in leptin regulation. For example, endothelin-1 (ET-1) was shown to stimulate leptin production and lipolysis in adipocytes, mainly through activating endothelin-A receptor [48-50]. Melanocortin (ACTH and α -MSH) stimulates cAMP production and inhibits leptin secretion in 3T3-L1 adipocytes [51]. ET-1 also represses the expression, but stimulates acute release, of adiponectin, a molecule that has potent insulin-sensitizing effects [52]. Taken together, signaling through a variety of GPCR ligands regulates adipose tissue differentiation and energy metabolism in a coordinated manner.

Osteoblasts construct and maintain animal bone architecture during development and throughout the life span. Along with bone morphogenetic protein (BMP) and Wnt signaling, G protein-coupled signaling processes play an important role in early differentiation of osteoblasts. For instance, in addition to its function in maintaining calcium homeostasis, parathyroid hormone (PTH) regulates bone remodeling by acting on osteoblasts and their progenitor cells [53, 54]. Intermittent treatment of rodents with PTH increased osteoblast number and bone formation [55]. PTH induces osteoblast specific osteocalcin expression, and increases the rate of bone formation, in part by inducing BMP and Wnt signaling pathways [54]. PTH also induces autocrine IGF-1 production in a cAMP dependent manner in osteoblasts cultured *in vitro* [56]. Prostaglandin (PG) E2 and F2 α also recruit and activate osteoblasts *in vivo*, and

stimulate proliferation and differentiation of osteoblasts *in vitro* [57, 58]. Signaling through PTH-G_s/G_q, PGF2 α -G_q, and lysophosphatidic acid-G_i/G_q transactivates EGF receptors and stimulates downstream ERK kinase that is required for osteoblast proliferation and differentiation [59]. These findings highlight the crucial roles of GPCRs in osteoblast differentiation and bone maintenance.

Conclusions

GPCRs and their signaling targets function in diverse systems and stages of life, such as homeostasis, neuronal sensory and signal transmission, and as reviewed in this article, differentiation of various cell lineages during development and adult life. The important functions of GPCRs in cellular differentiation are summarized in Table 1. Despite of the complex ligand-receptor combinations function on various cell types, they recruit common intracellular signaling pathways to control the differentiation process. For instance, cAMP-PKA signaling is required for the differentiation of neurons [26], red blood cells [33], neutrophils [37, 38], and osteoblasts [56]. Activation of ERK MAPK promotes the differentiation of neurons [9], smooth muscle cells [42], and osteoblasts [59], but inhibits neutrophilic differentiation [38]. Adding onto the recruitment of common signaling process for the differentiation of different cell lineages, the same ligand-receptor axis functions during different stages of cell differentiation, as in the case of SDF-1 in promoting differentiation of ES cells into neurons [22], and later on regulating axonal guidance in “more differentiated” neurons [26]. Cellular differentiation often involves concomitant withdrawal from cell

cycle and entry of senescence. G protein-coupled pathways have also been suggested to play important roles in controlling cellular proliferation [60], suggesting the pluripotency of GPCRs and their downstream signaling molecules during cellular proliferation and differentiation.

In most cases, GPCRs do not signal alone in cell differentiation, but orchestrate with other families of receptors and intracellular pathways. An example of such is in the guidance of neuronal axon growth, where ligands of GPCRs (SDF-1, PACAP) regulate intracellular cAMP levels in growth cones, and determine the outcome of netrin signaling on growth cone movement [23-27, 61, 62]. The crosstalk of GPCR and other signaling networks apparently contributes to the precise temporal and spatial regulation of the differentiation process.

Increasing knowledge has been deposited into the daunting pool of GPCR signaling database owing to the efforts of the science community. These include the findings that demonstrate the localization of GPCRs and their signaling components in lipid rafts and caveolae [63], dimerization of GPCRs [64], and trans-activation of receptor tyrosine kinases by GPCRs [65]. In the case of cell differentiation, examples of novel GPCR signaling mechanism include transactivation of TrkA by purinergic P2Y2 receptor [12], and mediation of partial NGF signaling by $G_{i/o}$ proteins [14, 17, 18]. With new discoveries in the signaling mechanisms of GPCRs and novel ligands for orphan receptors, we will undoubtedly appreciate the significance of G protein-linked signaling in the differentiation of various cell lineages and development of tissues and organs.

Acknowledgements

This work was supported in part by grants from the Research Grants Council of Hong Kong (HKUST 6420/05M, 6443/06M and 1/06C), the University Grants Committee (AoE/B-15/01), and the Hong Kong Jockey Club.

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Table 1. Function of GPCRs in cellular differentiation

Cell lineage	Ligand	Receptor	Signaling components	Function	References
Neuron	ATP/UTP	P2Y2	SFK-TrkA-ERK G _q -IP ₃ -Ca ²⁺	Survival, differentiation	[9, 10, 12]
Neuron	PACAP/ VIP	VPAC	cAMP, Ca ²⁺	Differentiation, axon guidance	[22, 26]
Neuron	SDF-1	CXCR4	cAMP, Ca ²⁺	Axon guidance	[23, 25, 66]
Erythrocyte		Gpr48	cAMP, PKA, ATF4	Definitive erythropoiesis	[33-35]
T lymphocyte	C5a, C3a	C5aR, C3aR	PI3K γ , Akt	Differentiation, activation	[36]
Neutrophil	2-AG	Cb2	G _i , cAMP, ERK, HSP90	Anti-differentiation, migration	[37-39, 67]
Smooth muscle cell	S1P	Edg	RhoA, ERK, MRTF-A, Elk-1	Proliferation, differentiation	[41, 42]
Smooth muscle cell	SPC		G _{i/o} , ERK	Differentiation	[43]
Adipocyte	Short fatty acids	GPCR41/ 43	G _i	Differentiation, leptin production	[45, 47]
Adipocyte	ET-1	ET _A	ERK, Akt	Leptin production, lipolysis, anti-differentiation	[48-50]
Adipocyte	Melanocortin	MC2R/MC5R	cAMP	Inhibit leptin secretion	[51]
Osteoblast	PTH	PTH1R	cAMP, ERK, BMP, Wnt	Differentiation, anti-proliferation	[54, 68]
Osteoblast	PGF2 α	PGF2Ar	G _q , EGFR, ERK	Proliferation, differentiation	[59, 69]

Figure 1. Regulation of neuronal differentiation and axon guidance. Neuronal differentiation is orchestrated by crosstalk between different extracellular ligand-induced signals. $G_{i/o}$ mediates part of the NGF-induced MAPK and Akt activation. Nucleotide-activated purinergic receptor P2Y₂ potentiates NGF signaling through SFK, and induces Ca^{2+} signaling in a G_q -dependent manner. Netrin activates DCC to induce Ca^{2+} influx through LCC. PACAP and VIP induce neuronal differentiation by activating cAMP and Ca^{2+} mediated pathways. PACAP, SDF-1 and baclofen (GABA) signal through their cognate GPCRs to regulate intracellular cAMP levels, which in turn determine the outcome of netrin signaling on LCC.