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GPCRs in Stem Cell Function

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Abstract

Many tissues of the body cannot only repair themselves, but also self-renew, a property mainly due to stem cells and the various mechanisms that regulate their behavior. Stem cell biology is a relatively new field. While advances are slowly being realized, stem cells possess huge potential to ameliorate disease and counteract the aging process, causing its speculation as the next panacea. Amidst public pressure to advance rapidly to clinical trials, there is a need to understand the biology of stem cells and to support basic research programs. Without a proper comprehension of how cells and tissues are maintained during the adult life span, clinical trials are bound to fail. This review will cover the basic biology of stem cells, the various types of stem cells, their potential function, and the advantages and disadvantages to their use in medicine. We will next cover the role of G-protein coupled receptors in the regulation of stem cells and their potential in future clinical applications.

Keywords

GPCR; stem cell; embryonic; mesenchymal; pluripotent; cancer; cardiac; neural; induced pluripotent

I. Introduction

There are many organs or tissues in the body that are capable of self-renewal, such as the skin, liver, and epithelium. While the lifespan of fully differentiated cells is relatively short, these organs and tissues must contain some resident cells that can self-replicate and produce daughter cells capable of differentiation into the mature cells of that tissue. These multipotent cells are called adult stem cells that differ from embryonic stem cells (ESCs), which have an even greater potential for differentiation. However, ESCs cannot generate a functional organism.^{1,2}

There are also several different types of medical problems that could benefit from the use of stem cells to ameliorate or cure the defect. Type 1 diabetes mellitus in which the beta cells of the pancreas are destroyed or attacked can benefit from stem cells to repopulate the pancreas. There are various types of neurological diseases or injuries such as stroke, spinal cord injuries, Parkinson's disease or multiple sclerosis where particular types of neurons or glia have the potential to be replaced or regenerated. However, a major problem that has slowed their use in clinical settings is the immunological problems of rejection, mutations due to amplification of stem cells and aneuploidy.

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G-protein coupled receptors (GPCRs) are a large class of transmembrane (TM) receptors that transmit extracellular signals into cells by coupling to guanine nucleotide binding proteins (G proteins). They play a key role in many complex biological processes and development and our understanding of their role in stem cell regulation is expanding (Table 1). A potential benefit of using our own endogenous receptors to activate the stem cell process or to direct the differentiation of cells would avoid many of the current pitfalls in using stem cells, such as rapid *in vitro* differentiation and expansion or rejection in clinical applications.

GPCRs bind and regulate the effects of 80% of all hormones in the body, comprise 3-5% of the human genome, and account for about 20-50% of pharmaceuticals on the current market.³ While the receptors are conserved mostly in the TM domains, the ligands span a large range of diverse biological, from peptides to waves of light, and represent various evolutionary classes.⁴ They are composed of a single polypeptide containing seven regions of 20-28 hydrophobic amino acids that span the TM domains. The TM segments are α -helices oriented approximately perpendicular to the cell surface as shown in the crystal structure of rhodopsin.⁵ The amino terminus is located on the extracellular side of the membrane and contains several glycosylation sites. The carboxy terminus is located on the intracellular side and contains sites for phosphorylation, which are used in the regulation of receptor desensitization and internalization. Three intracellular and three extracellular loops link the TM domains and may contain ligand-binding sites. Many GPCRs also have a highly conserved disulfide bond between the cysteines in the second and third extracellular loops. This bond is needed for proper folding of the protein and the regulation of the high affinity binding site.⁶

The GPCRs bind a ligand on the extracellular side and trigger conformational changes, causing the intracellular loops to bind and activate the heterotrimeric G-protein. Once the G-protein is activated, it dissociates from the receptor and its subunits (α and $\beta\gamma$) to amplify a second messenger response mediated by effector molecules such as phospholipases, enzymes, or channels. GPCRs may also signal through non-G-protein mediated events that involve scaffolding proteins, transactivation of tyrosine kinase receptors, and/or G-protein receptor kinases that regulate the GPCR signal (reviewed in Ref. 7).

II. Types of Stem Cells

A. Embryonic Stem Cells

ESCs are pluripotent cells derived from the blastocyst of an early-stage embryo, generally about 4-5 days after fertilization. Their isolation in 1981 by Sir Martin J. Evans and Matthew Kaufman and independently by Gail R. Martin has contributed extensively to our knowledge of pluripotency and differentiation of stem cells.^{8,9} In 1998, James Thomson reported the first successful cultivation of human ESC (hESC) lines.¹⁰ Isolation of these cells results in the destruction of the embryo, which is the subject of intense ethical debate. The term pluripotent indicates that the stem cell is capable of differentiation into cells from the ectoderm, endoderm, and mesoderm, i.e., the three germinal layers in the body. Pluripotency results in the potential to culture approximately 220 different cell types present in the body. Feeder cells provide factors that are necessary to prevent the ESC from differentiating in culture, such as leukemic inhibitory factor and bone morphogenetic proteins.

While the potential to generate unlimited cell types is ground breaking, disadvantages of ESC therapy include the potential to form tumors upon transplantation (called teratomas). There is also the problem of immune-compatibility when the ESCs are derived from a different genetic background than the patient who receives them. While immunosuppressive

drugs can be used to minimize these problems, they are not perfect. Thus, the field of induced pluripotent stem cells has developed to solve immune-compatibility, where adult cells from the same person can be forced to switch their lineage or become pluripotent through nuclear reprogramming.

B. Induced Pluripotent Stem Cells

For human treatment, there is a need for patient-specific or patient-matched ESCs. These cells can then be grafted into the genetically identical host. Many groups use induced pluripotent stem cells (iPS cells) in their research in addition to hESCs, as this research faces fewer ethical issues. iPS cells are generated from adult (somatic) tissue; furthermore, they are easier to use in research.^{11,12}

Somatic cells can be reprogrammed to a pluripotent state through a variety of methods. Originally, pluripotent cells were created by injecting the nucleus of an adult cell into an enucleated oocyte.^{13,14} This leads to reprogramming of the somatic cell nucleus by the host cell. After several cell divisions, the reprogrammed cell forms a blastocyst, which is now genetically matched with the nuclear donor and can be used to obtain stem cells. The cell is not genetically identical to the donor because of the mitochondrial DNA, which is genetically identical to the recipient and present in the oocyte cytoplasm. This process of forming iPS cells also known, as somatic cell nuclear transfer, can achieve the greatest level of induced pluripotency, but is an inefficient process and potentially damaging to the cell. Instead of using oocytes, ESCs can be used to reprogram somatic cells but this method is even more inefficient and leads to higher rates of tetraploidy.¹⁵

The iPS cell field advanced in 2006, when Takahashi and Yamanaka found that retroviral transduction of only four genes was sufficient to induce pluripotency in somatic cells.¹¹ A combination of four regulatory proteins (Oct3/4, Sox2, Myc, and Klf4) can convert fibroblasts into ES-like cells with the ability to differentiate into diverse cell types, although the rate of conversion is still very low. The goal of patient-specific iPS cell therapy is to isolate somatic cells from diseased patients, reprogram and differentiate them to replace diseased cells, and successfully transplant them back into the patient without immune problems.¹⁶⁻¹⁸ Since retroviral delivery integrates the vector into the cell's genome, there is still a potential to induce tumorigenesis with this method.

Some of the more successful studies demonstrating the potential of iPS cells to treat disorders include rescue of sickle cell anemia in mice with a genetically corrected β -globin locus¹⁹ and in a patient whose skin fibroblasts were used to generate patient-specific iPS cells.²⁰ Parkinson's disease was improved in a rat model using iPS cells²¹ and injection of undifferentiated iPS cells into ischemic cardiac muscle induced repair.²² In humans, patient-specific iPS cells from Fanconi anemia patients corrected the genetic defect.²³ Of course, the major failures of iPS cell therapy in clinical applications or animal models are not outlined here, but serve as a cautionary note that more studies need to be performed before the use of iPS cells becomes accepted as a major treatment option.

C. Adult Stem Cells

In addition to iPS cells, adult stem cells can be used without ethical controversy. Furthermore, adult stem cells are readily available from several organs and tissues. These cells can be isolated from the patient and differentiated *in vitro* or differentiated *in vivo* through pharmacological agents, thus avoiding immune system incompatibility problems. However, the differentiation potential of adult stem cells is limited to cell-types within the boundaries of its endogenous tissue. For example, neural stem cells (NSCs) cannot be

induced to form cardiomyocytes or visa versa. The following adult stem cell categories are discussed separately.

1. HEMATOPOIETIC STEM CELLS—The most extensively studied adult stem cell system is that of hematopoietic stem cells in the bone marrow and in the umbilical cord blood.²⁴ Hematopoietic stem cells differentiate into only myeloid and lymphoid lineages, but there are controversial studies suggesting that they can differentiate into non-hematopoietic lineages.^{25,26} Transplantation studies to reverse disease were first performed using hematopoietic stem cells and are the most widely available stem cell therapy,^{27,28} with allogenic hematopoietic stem cell transplantation a common treatment for bone marrow failure. Hematopoietic stem cell transplantation has been successful because they do not need to be expanded prior to transplantation and no complex structures or organs need to be regenerated. Patients with leukemia, for example, can be irradiated or treated with chemotherapy to destroy the cancerous cells, as well as the rest of their hematopoietic cells. The patient is then transfused with healthy, non-cancerous hematopoietic stem cells which repopulate the bone marrow.²⁹ Of course, this type of treatment can produce immune rejection, but with careful tissue matching and the use of immunosuppressive drugs, the difficulties can be reduced to acceptable levels.

When the cancer is due to a mutation in only a particular type of blood cell, it is possible to treat patients with their own hematopoietic stem cells. In this case, a sample of the bone marrow is isolated before irradiation and sorted to obtain a pure population of cancer-free hematopoietic stem cells that are then transfused back into the patient. However, this autologous technique is not without problems, as patients may develop engraftment syndrome, typically characterized by fever, rash and pulmonary edema.³⁰

Umbilical cord blood was first used as a source of hematopoietic stem cell transplantation in 1989.³¹ Since then, umbilical cord blood has increasingly become a popular alternative to bone marrow, especially in children with hematological malignancies³² as they require less blood. An alternative source of hematopoietic stem cells is the placenta, which has up to ten times the number of stem cells as umbilical cord blood.³³

2. MESENCHYMAL OR STROMAL STEM CELLS—Mesenchymal stem cells (MSCs) are capable of differentiating into various mesodermal lineages such as adipocytes, fibroblasts, osteoblasts, neuronal cells, and myocytes.³⁴ Technically, they are stromal-like cells characterized by adherence in culture and a specific set of Cluster of Differentiation (CD) cell-surface antigens such as CD105+ /CD73+ /CD90+ /CD45– /CD34– /CD11b– or CD14– /CD19– or CD79α– /HLA– DR1–.³⁵ If the above conditions are not met completely, the term “mesenchymal stromal cells” or “MSC-like” is used.³⁶ MSCs have the advantage of possessing antimicrobial activity³⁷ and can produce trophic factors that promote anti-inflammatory effects and healing.³⁴ Furthermore, MSCs lack major histocompatibility complex (MHC)-II molecules and show only minimal MHC-I expression that renders them allogeneic and immunosuppressive.³⁸⁻⁴⁰ MSCs have the extraordinary ability to migrate toward injuries,⁴¹ and it is postulated that the MSC microenvironment involves similar mechanisms with wound healing.⁴² While MSCs reside in many different organs and tissues, they are the most abundant in the bone marrow and umbilical cord blood, but are also found in the amniotic fluid and placenta, contributing to their ease of collection and storage. Since their ability to self-renew has only recently been shown, it is debatable whether MSCs are true stem cells or a pool of partially differentiated progenitors.^{43,44}

3. CARDIAC STEM CELLS—There are various stem cell populations that have been proposed to repair the damaged heart. MSCs derived from the bone marrow can home into the heart and establish a temporary niche to aid in the regeneration of myocardium.⁴⁵⁻⁴⁷

Bone-marrow derived MSCs also have been shown to improve cardiac performance after injury.^{48,49}

However, there is considerable evidence for the existence of an actual population of residing cardiac adult stem cells. Substantial heart muscle regeneration can take place after resectioning of the heart,^{50,51} that triggers myocyte dedifferentiation and proliferation.⁵² Using various surface markers and transcription factors, most cardiac stem cells have been described to be Sca-1 and c-kit positive and to efflux Hoechst 33342 dye, although no marker is specific to any kind of stem cell.⁵²⁻⁵⁴ A population of residing cardiac stem cells which are negative for CD31, an antigen common to bone marrow cells, has been described that can form beating myocytes and acquire an adult phenotype when associated with differentiated myocytes.⁵⁵ Cardiac stem cells reside in clusters within niches in the heart that provide a microenvironment to maintain stemness.⁵⁶ Niches are normally located in areas with low wall stress (low blood flow) and hemodynamic load.

When cultured, cardiac stem cells can form free-floating aggregates called cardiospheres. Cardiospheres are heterogeneous cells that express c-kit in a fraction of the cells near the center of the sphere, as well as CD105 in the outer layers (bone marrow mesenchymal marker).³⁵ Connexin 43 is expressed within the highly dividing cells and differentiating cells of the sphere.⁵⁷ Cells from these cardiospheres can be differentiated into myocytes when co-cultured with neonatal myocytes.⁵⁸

4. NEURAL STEM CELLS—Along with heart tissue, the brain and spinal cord have little capacity to self-repair. However, in certain parts of the brain, stem cells continually produce new neurons and glia, although this occurs at naturally low rates in humans. A classic example of adult neurogenesis is the rapid turnover that occurs in songbirds as they learn a new song each breeding season.⁵⁹ Evidence for NSCs was first shown by the development of floating balls in culture called neurospheres from dissociated adult mammalian brain cells. These cells can differentiate into neurons and glia when plated onto adherent surfaces with the appropriate serum and/or growth factors.⁶⁰ Depending upon the culture conditions and factors added to the media, NSCs can be maintained in the stem cell state or induced into neurons, glia or a mixture of the two.

NSCs are defined by their ability to self-renew and are multipotent and able to differentiate into three major cell types in the CNS: neurons, astrocytes, and oligodendrocytes (reviewed in Ref. 61). The particular cell type of the resident adult NSC that can generate new neurons and glia is still debated and includes multi-ciliated ependymal cells,⁶² subependymal cells,⁶³ or a special type of astrocytic cell termed radial glia.⁶⁴ The astrocyte radial glia theory is the most popular, in which NSCs in the subventricular zone (SVZ) represent a subset of slowly dividing glial fibrillary acidic protein (GFAP)+ astrocytic cells with radial projections. These radial glia can proliferate to generate rapidly dividing cells called transient amplifying progenitors (TAP) cells. TAP cells are identified by being GFAP⁻ but Dlx2⁺. The distal-less homeobox gene (Dlx2) is a transcription factor that regulates the fate determination of interneurons and oligodendrocytes in embryogenesis but is pro-neural in adult neurogenesis.⁶⁵ TAP cells divide to generate migrating neuroblasts that are distinguished by the neuronal marker polysialylated neural cell adhesion molecule (PSA-NCAM).⁶⁶⁻⁶⁸

The SVZ and the subgranular zone (SGZ) of the hippocampus are the two major regions in the adult CNS capable of active adult neurogenesis. The neurons in the SVZ are generated continuously and migrate to the olfactory bulb where they mature into at least five different types of interneurons as well as a minor population of oligodendrocytes.⁶⁹ In a similar but distinct manner, residing and quiescent NSCs in the SGZ are a population of radial astrocytic-like cells containing GFAP, Sox2, and Nestin markers.⁷⁰ These stem cell bodies

are located at the SGZ with their radial processes projecting through the granular cell layer and shorter tangential processes extending along the border of the hilus and granule cell layer.⁷¹ Similar to TAP cells, NSCs in the SGZ generate actively dividing nonradial transient amplifying progenitors. These cells generate neuroblasts that are doublecortin (DCX)⁺ and migrate into the dentate granule cell layer where they mature,⁷² a process that takes about 28 days.⁷³ New granule neurons in the dentate gyrus are continuously generated locally⁷¹ and may survive for at least 2 years.⁷⁴ Adult neurogenesis has also been reported in the neocortex and striatum, but at much lower rates.⁷⁵⁻⁷⁷

NSCs, derived from fetal tissue, are currently the only practical source for human neural cell transplantation in their ability to be grafted into the adult brain and integrate to match their new location.⁷⁸ The initial pre-clinical studies using transplanted exogenously generated brain cells were performed in a Parkinson's disease animal model in the early 1970s⁷⁹ and then in very limited human clinical trials in the early 1990s⁸⁰⁻⁸¹ with reasonable success. A larger-scale trial of 40 Parkinson's disease patients in 2001 found that transplanted dopamine neurons from embryonic fetal tissue survive in the human brain, but only patients under the age of 60 showed clinical improvement over a three year time period.⁸² While the potential to form tumors is reduced in NSC transplantation compared to ESCs in animal models, the risk may not be so limited in humans and there is a need for caution.⁸³ Since then, NSCs have been used in successful transplantation studies on animals to repair the spinal cord,⁸⁴⁻⁸⁵ and to treat multiple sclerosis,⁸⁶⁻⁸⁷ Huntington's disease,⁸⁸ and stroke.⁸⁹⁻⁹¹

The first FDA-approved clinical trial for purified NSCs was performed in 2009 to treat neuronal ceroid lipofuscinosis (NCL, Batten's disease) (<http://www.stemcellsinc.com/clinicaltrials/clinicaltrials.html>). Human NSCs were transplanted into 6 patients with either infantile or late infantile NCL. Patients were immunosuppressed for 12 months after transplantation. This phase I trial showed a favorable safety profile with transplanted cells, which exhibited survival long-term; however, patients are still being tracked for outcomes. Other phase I trials using purified NSCs are ongoing for Pelizaeus-Merzbacher Disease (a myelination disorder) and for chronic spinal cord injury at the same company (StemCells, Inc.). Future areas of research may involve the utilization of endogenous mechanisms that activate adult neurogenesis after various brain injuries.⁹²

5. CANCER STEM CELLS—Cancer stem cells were first described in 1994 when a cell type of low abundance (0.1% of cells) derived from human acute myeloid leukemia caused cancer to develop in nude mice.⁹³⁻⁹⁵ Today, cancer stem cells are derived from most types of hematopoietic cancers including various types of myeloid cancers⁹⁶⁻⁹⁸ and acute lymphoblastic leukemia.⁹⁹ They are also found in solid tumors of the brain,¹⁰⁰⁻¹⁰¹ breast,¹⁰²⁻¹⁰³ lung,¹⁰⁴⁻¹⁰⁵ prostate,¹⁰⁶ and more recently in liver¹⁰⁷⁻¹⁰⁸ and colon.¹⁰⁹⁻¹¹⁰ The presence of cancer stem cells has important implications for treatment, as current therapies may abolish the bulk of tumor cells but miss the cancer stem cells leading to tumor reformation. Therefore, future treatments specifically targeting cancer stem cells may be more successful.

The origin of cancer stem cells is still being debated, particularly whether they represent a stromal stem cell, which has undergone some sort of malignant change, or whether they are differentiated cells that return to a "stem" state as part of the malignant transformation process.¹¹¹⁻¹¹³

Each cancer stem cell has unique phenotypic features and niche environment that may prove to complicate future targeted therapies.^{96,97,114-116} However, they do share some common cell-surface markers such as CD34⁺, CD38⁻, and Lin⁻.^{93,95,97,99} Cancer and neural stem cells share the markers CD133, CD15/Lex/Sea-1, and CD34.^{100,117} Tumor-suppressor

genes are commonly lacking in many cancer stem cells which may provide their ability to self-renew.¹¹⁸⁻¹²⁰

III. GPCRs in Embryonic Stem Cells

Since GPCRs play a major role in development,¹²¹ it stands to reason that they may also be a major regulator of stem cells (Table 1). Signal transduction pathways that are activated during stem cell renewal and differentiation are shared, cross-activated or synergistic with GPCR stimulation (reviewed in Ref. 122). The first study to define a major role for GPCRs in stem cell function performed a real-time PCR-based expression profile of 343 different GPCRs in mouse ESCs.¹²³ Of these, 161 GPCRs were expressed in undifferentiated ESC at a low level, 30 GPCRs were moderately expressed, and 7 were highly expressed. Many GPCRs display a dramatic difference in expression when the cells differentiate.¹²³

A. Proteinase-Activated Receptors

Using a GPCR-specific real time PCR microarray as described in the above studies, two GPCRs from the proteinase-activated receptor family (PAR), F2r (PAR1) and F2rl1, were highly expressed in ESCs¹²³ and shown in knockout (KO) mice studies to play an important role in early embryonic development.¹²⁴ Another important GPCR differentially expressed in ESCs was GPR125, an orphan adhesion-type GPCR¹²³ that was identified as a marker of germ-line progenitors.¹²⁵

B. G_i-Coupled Receptors

The role of G_i-coupled signaling in pluripotent stem cells is largely unknown, but it has been implicated in the maintenance of pluripotency¹²⁶ and directed differentiation¹²⁷ of hESCs. The G_s-coupled pathway and the generation of cAMP have been suggested to regulate ESC self-renewal¹²⁸ and maintenance of ESC pluripotency.¹²³

The secreted factors produced by ESCs may also provide insight into the production of therapeutics for the regeneration and repair of damaged tissues. Direct activation of endogenous progenitor cells as pluripotent hESCs can secrete paracrine signals associated with embryogenesis. Conditioned media from these stem cells was found to induce karyokinesis, cytokinesis, and proliferation in mono- and binucleated cardiomyocytes through the RhoA/Rho-associated kinases (ROCK) 1 and 2 pathways.¹²⁹ While RhoA is a small G-protein, it serves as an effector of numerous GPCRs such as thrombin, sphingosine-1-phosphate (S1P) and lysophosphatidic acid (LPA)¹³⁰ to regulate proliferation, migration, and survival.

C. Sphingosine-1-Phosphate and Lysophosphatidic Acid Receptors

Particular lipids acting through the binding and activation of GPCRs have been shown to regulate various aspects of ESC biology. Such lipids include the phospholipids S1P and LPA. Both LPA and S1P are positive regulators of ESC maintenance through either ERK or Ca²⁺ mediated pathways.^{131,132} Mouse ESCs express receptor subtypes LPAR_{1-3,5} and S1PR₁₋₅^{126,132} while hESCs express LPAR₁₋₅ and S1PR₁₋₃¹³³⁻¹³⁵. The LPA signaling pathway that effects mouse ES maintenance is the ERK activation of *c-fos*.¹³⁶ S1P activation of mouse ESC also signals through ERK, likely mediated by S1PR₅¹³¹ utilizing G_i-, PKC-, and c-Src-dependent mechanisms. While the ERK signaling pathway is implicated in mouse ESC proliferation, there is also evidence for a role in its differentiation by the suppression of ERK signals.^{137,138} The signaling pathway in hESCs is similar to mouse ESCs and involves G_i- and ERK-dependent mechanisms¹²⁶ resulting in the inhibition of apoptosis and activation of pro-survival signals.

D. Cannabinoid Receptors

The cannabinoid receptors CB₁ and CB₂ are activated by three major groups of ligands: endocannabinoids, plant cannabinoids, and synthetic cannabinoids. Mammalian endocannabinoids include four known compounds anandamide (AEA), anandamide derivatives (2-arachidonoylglycerol (2-AG) and noladin ether), virodhamine, and N-arachidonoyl-dopamine.¹³⁹ CB₁ and CB₂ are likely involved in mouse ESC survival since specific receptor antagonists AM251 and AM630 promoted cell death.¹⁴⁰ While mouse ESC express low levels of CB₁ and CB₂, they express high levels of 2-AG and moderate levels of AEA agonists.

E. Wnt/Frizzled

Wnt signaling has been implicated in ESC self-renewal;¹⁴¹ however, its exact role in ESC biology remains to be clarified. Wnt proteins bind to receptors of the atypical GPCR frizzled and low-density-lysoprotein-related-protein5/6 (LRP) families on the cell surface. Wnt binding to its receptors induces the nuclear translocation of β -catenin that acts as a transcriptional cofactor of lymphoid enhancer binding factor1 to modify gene transcription (referred to as the “canonical” Wnt pathway). Wnt can also signal in non-canonical pathways independent of β -catenin, leading to either small G protein activation and cytoskeletal changes or through Ca²⁺ signaling with activation of heterotrimeric G-proteins, causing various cell responses.¹⁴² hESCs have been shown to express members of the frizzled (FZD) receptor family (FZD1, FZD3, FZD4, FZD5, FZD6) and the secreted frizzled related protein (SFRP) family (SFRP1, SFRP2, FRZB, SFRP4), encoding soluble Wnt antagonists.¹⁴³ Adding recombinant Wnt3a stimulated hESC proliferation, but after 4-5 days of treatment, hESCs that survived were undifferentiated and few could self-renew. β -catenin-mediated transcriptional activation in the canonical Wnt pathway was minimal in undifferentiated hESCs, but was greatly upregulated during differentiation induced by Wnt.¹⁴⁴ Thus, canonical Wnt/ β -catenin activation does not suffice to maintain the undifferentiated and pluripotent state of hES cells. In contrast, some studies suggest that Wnt3a or the GSK-3 inhibitor, 6-bromindirubin-3'-oxime, maintains hESCs in the undifferentiated state and maintains pluripotency.¹⁴⁵ The Wnt receptor FZD7 has also been identified as important for hESC maintenance and self-renewal. FZD7 mRNA levels have been found to be 200-fold higher in hESCs compared to differentiated cell types and knockdown of FZD7 induces dramatic changes in the morphology of ESC colonies with a loss of OCT-4 expression, an ESC-specific transcription factor.¹⁴⁶

F. mGlu Metabotropic

The mGlu5 metabotropic GPCR supports self-renewal in mouse ES cells.^{147,148} Different mGlu receptor subtypes, mGlu3 and mGlu5 receptors in particular, are found in neural ESCs.¹⁴⁹ Differentiation of ESCs into embryoid bodies is associated with the induction of mGlu4 receptors, the activation of which drives cell differentiation towards the mesoderm and endoderm lineages.¹⁴⁹ Mouse ESCs expressed mGlu5 receptors throughout the entire differentiation period into neurons. Antagonists of mGlu5 receptors accelerated the appearance of the neuronal marker, β -tubulin, and increased the number of cells expressing glutamate decarboxylase-65/67, a marker of GABAergic neurons.¹⁵⁰ Other GPCRs are also found in ESCs but there are few studies to indicate their functional role. CXCR4 is expressed in mouse ES cells¹⁵¹ and endothelin receptors in hESCs.^{152,153}

IV. GPCRs in Induced Pluripotent Stem Cells

A. G_i-Coupled Receptors

Reprogramming adult somatic cells to create induced pluripotent stem cells requires dramatic morphological and organizational cell changes. Interestingly, human pluripotent

colonies form a flat uniform monolayer, while mouse pluripotent colonies form thicker, multilayered colonies.¹⁵⁴ Since pluripotent colony morphology correlates closely with the maintenance of pluripotency, the mechanisms by which these colonies form and organize may be important for controlling somatic cell reprogramming. It was found that inhibition of G_i-coupled receptor signaling via pertussis toxin retracted stem cell colonies inward, adopting a dense multi-layered conformation without affecting proliferation, survival or its pluripotency. Activation of G_s-receptor coupled signaling with cholera toxin did not affect the colony morphology.¹⁵⁵ These results suggest that GPCRs play a role in some aspects of somatic cell reprogramming.

V. GPCRs in Adult Stem Cells

A. GPCRs In Hematopoietic Stem Cells

1. G_s-COUPLED RECEPTORS—Hematopoietic stem and progenitor cells change location during development and circulate in mammals throughout life, moving into and out of the bloodstream to engage bone marrow niches in sequential steps of homing, engraftment and retention. Hematopoietic stem cell engraftment of bone marrow in fetal development is dependent upon the activity of G_s.¹⁵⁶ Hematopoietic stem cells from adult G_s KO mice do not home or engraft in the bone marrow despite their ability to differentiate and undergo chemotaxis, suggesting that pharmacological targeting of this pathway may lead to improved transplantation efficiency.¹⁵⁶

2. CHEMOKINE RECEPTORS—Chemokines are small pro-inflammatory chemoattractant cytokines that bind to specific GPCRs present on plasma membranes of target cells and are major regulators of cell trafficking. Stromal-derived factor-1 (SDF-1 or CXCL12), an alpha-chemokine that binds to CXCR4, plays an important and unique role in the mobilization and homing of hematopoietic stem cells.^{157,158} CXCR4 modulates transendothelial migration of hematopoietic stem cells and progenitors into the blood stream.¹⁵⁹ Drugs that block CXCR4 cause hematopoietic stem cells to move into the circulation. SDF-1 and its receptor CXCR4, are involved in regulation of migration, survival, and development of multiple cell types, including human hematopoietic CD34⁺/CD38⁻/low and stromal STRO-1⁺ stem cells.¹⁶⁰ The homing function of SDF-1 that primes or sensitizes cell chemotaxis to a concentration gradient is dependent on the cholesterol content in the membrane and the incorporation of both CXCR4 and Rac-1 in lipid rafts facilitated by GTP binding.¹⁶¹

3. SPHINGOSINE-1-PHOSPHATE AND LYSOPHOSPHATIDIC ACID

RECEPTORS—In addition, other cytokines such as granulocyte colony-stimulating factor act partially through the modulation of SDF-1/CXCR4 signaling to induce hematopoietic stem cell mobilization in the bone.¹⁵⁸ SDF-1 can also cross-talk with other GPCR signaling pathways, including S1P and LPA. For instance, S1P-activation of S1PR₃ leads to the transactivation of CXCR4 in human endothelial progenitor cells to stimulate their functional capacity.¹⁶² Both S1P and LPA also synergistically enhance the chemotactic migratory response of the hematopoietic stem cells to SDF-1.¹⁶³⁻¹⁶⁵

4. WNT/FRIZZLED—The Wnt pathway has also been implicated as a self-renewal and growth signal in hematopoietic stem cells.^{166,167} Purified Wnt3a protein induced self-renewal of hematopoietic stem cells.¹⁶⁷ Overexpression of activated β -catenin expands the pool of hematopoietic stem cells in long-term cultures by both phenotype and function. Ectopic expression of axin or a frizzled ligand-binding domain, which are inhibitors of the Wnt signaling, leads to inhibition of hematopoietic stem cell growth *in vitro* and *in vivo*. In

addition, activation of Wnt signaling induces increased expression of HoxB4 and Notch1, genes previously implicated in self-renewal of hematopoietic stem cells.¹⁶⁶

5. CYSTEINYL-LEUKOTRIENE RECEPTOR—Hematopoietic stem cells express other GPCRs such as the leukotriene D4 receptor, cysteinyl-leukotriene (cysLT) receptor cysLT1¹⁶⁸ and lysophospholipid receptors such as S1PR₁ that differentially regulate chemotaxis, adhesion, and proliferation.¹⁶⁹ The co-activation of both G_q and G_i by cysLT1 results in stronger proliferation of hematopoietic stem cells than stimulation of G_i by SDF-1 or S1P alone.¹⁶⁹

B. GPCRs in Mesenchymal Stem Cells

1. SPHINGOSINE-1-PHOSPHATE AND LYSOPHOSPHOTIDIC ACID

RECEPTORS—Both LPA and S1P play a role in mesenchymal/stromal stem cell recruitment and differentiation. Both GPCR systems inhibit human MSC migration; an effect linked to Rho activation.¹⁷⁰ However, they stimulate murine MSC migration through Rho/ERK signals.^{171,172} LPA exerts potent anti-apoptotic effects on MSCs through LPAR₁ and the activation of G_i/ERK/PI3K/Akt signaling pathway.¹⁷³ S1P1 induces differentiation of MSC to smooth muscle cells¹⁷⁴ with S1P1 receptor KO mice lacking vascular maturation caused by a deficiency of MSC-derived-vascular smooth muscle cells and pericytes,¹⁷⁵ causing them to be embryonically lethal. LPA also stimulates migration of osteoblast precursors through ERK phosphorylation¹⁷⁶ and either promotes or inhibits osteogenic differentiation of human MSCs by LPAR₁ and LPAR₄, respectively.¹⁷⁷

2. PROSTAGLANDIN RECEPTOR—Prostaglandins are lipids belonging to the eicosanoid family and act through receptor isoforms EP₁₋₄. Studies have demonstrated the importance of prostaglandins on hematopoietic stem/progenitor cells and on MSCs.^{178,179} For example, PGE₂ acts as an MSC survival factor through the binding of its receptor EP₄ and has anti-apoptotic effect on MSCs that correlates with intracellular S1P synthesis.¹⁸⁰

C. GPCRs in Cardiac Stem Cells

Circulating mobilized bone marrow stem cells can be recruited to damaged myocardial tissue, and this homing behavior is suggested to play a key role for myocardial repair and regeneration.¹⁸¹ However, there is increasing evidence that an endogenous, self-maintained pool of resident progenitor cells in the myocardium exists.^{50,51,182,183}

1. WNT/FRIZZLED—Multiple signaling pathways, acting in both stimulatory and inhibitory fashions, can act to regulate cardiomyogenesis. These signals include Wnts and Wnt inhibitors.¹⁸⁴ During development, there is evidence that β -catenin-dependent Wnt signaling negatively regulates cardiac differentiation, and that the inhibition of the Wnt pathway is required for cardiac specification.¹⁸⁴⁻¹⁸⁷ On the other hand, β -catenin-independent Wnt signaling is thought to enhance myocardial differentiation.¹⁸⁸ Cardiac progenitor cell migration is mediated via Wnt3A-RhoA activity.¹⁸⁹

2. APELIN RECEPTOR—*Grn* encodes the G protein-coupled receptor Agtr1b (APJ receptor). Agtr1b with its ligand, apelin, is a known regulator of heart field formation.¹⁹⁰ Reduced or excess Agtr1b or apelin function caused deficiency of cardiac precursors and, subsequently, the heart tissue.¹⁹⁰ In Apelin-deficiency, the cardiac precursors converged inefficiently to the heart fields and showed ectopic distribution, whereas cardiac precursors overexpressing Apelin exhibited abnormal morphology and rostral migration. Ectopic expression of apelin results in the complete absence of cardiomyocytes.¹⁹¹ Zebrafish *grinch* (*grn*) mutants form a reduced number of myocardial progenitor cells, which results in a profound deficit in cardiomyocyte numbers in the most severe cases.¹⁹¹

D. GPCRs In Neural Stem Cells

The role of GPCRs in regulating various aspects of adult neurogenesis and gliogenesis is extensive (reviewed in Ref. 192). The types of GPCRs that regulate adult neurogenesis and/or gliogenesis include adrenergics, cannabinoids, chemokines (SD1/CXCR4), dopamine, LPA and sphingosine, opioids, PACAP, various peptide hormones, neuropeptide Y (NPY), purinergic, and various GPCR-coupled effectors (phospholipase C- β 1, phospholipase A2, phosphodiesterase-4D). The major GPCR systems will be briefly summarized here.

1. α_1 -ADRENERGIC RECEPTORS—Previous studies indicate that α_1 -adrenergic receptors (ARs) increase proliferation of embryonic neuroepithelial cells, suggesting that this subtype might influence adult progenitor proliferation.¹⁹³ α_1 -ARs stimulate proliferation,¹⁹⁴ migration,¹⁹⁵ and protect against stress-induced death of mouse embryonic brain-derived neural progenitor cells through a caspase 3/7-independent mechanism *in vitro*.¹⁹⁶ We showed that α_{1A} -AR subtype stimulation increased neurogenesis and gliogenesis in adult mice *in vivo* and may be developmentally-dependent as embryonic and neonatal neurospheres differentiated into neurons while adult neurospheres differentiated into glia.¹⁹⁷

2. α_2 -ADRENERGIC RECEPTORS—In the adult, the α_2 -AR agonists clonidine and guanabenz decreased proliferation of hippocampal progenitors with no effect on survival or differentiation.¹⁹⁸ Adult hippocampal progenitors *in vitro* expressed all the α_2 -AR subtypes that decrease neurosphere frequency and BrdU incorporation, effects that are blocked with the α_2 -AR antagonist yohimbine. In contrast, another study reported enhanced progenitor survival following long duration treatment with the α_2 -AR antagonist dexefaroxan.¹⁹⁹

3. CANNABINOID RECEPTORS—The proliferation of embryonic and adult rodent NSCs is significantly increased by administration of endogenous anandamide (e.g., AEA) and synthetic agonists (e.g., HU210) for the CB₁ and/or CB₂ receptors, respectively.²⁰⁰⁻²⁰² Consistent with these results, CB₁ KO mice show impaired proliferation via BrdU incorporation studies of embryonic and adult neural stem/progenitors. A reverse effect is observed in fatty acid amide hydrolase (*Faah*) KO mice, which lack the ability to degrade endogenous AEA.²⁰⁰⁻²⁰⁴ CB₁ KO mice also demonstrated cortical lamination that was significantly perturbed, suggesting that CB₁ is involved in migration of neural progenitors.²⁰⁵ Concomitantly, cortical neuronal migration is accelerated with HU 210, an agonist of CB₁ and CB₂ or URB 597, a fatty acid amide hydrolase inhibitor in a cortical slice culture.²⁰⁵ PI3K/Akt phosphorylation is also thought to be involved with neuronal survival functions of endocannabinoids²⁰⁶⁻²⁰⁷ through inhibition of glutamate neurotoxicity.²⁰⁷ Cannabinoid-induced adult neurogenesis mechanisms include opposing the anti-neurogenic effect of neuronal nitric oxide.²⁰⁸

4. CHEMOKINE RECEPTORS—Developing granule cells of the cerebellum and hippocampus express SDF-1 and CXCR4²⁰⁹⁻²¹² and their CXCR4 signaling is suggested to be involved in the maintenance of the proliferative state and for their migration properties.²¹³⁻²¹⁴ Consistent with this hypothesis, SDF-1 and CXCR4 KO mice show severe malformation in the cerebellum and hippocampus, owing to impaired proliferation and migration of granule progenitor cells.^{209-210,215-216} SDF-1/CXCR4 regulates the cell cycle of early granule progenitor cells to help them survive in a quiescent, but proliferative-ready state.²¹⁷ Chemokines mediate progenitor proliferation through activation of Akt and concomitant inactivation of FOXO3, a fork head family of transcription factor regulating the cell cycle.²¹⁸ There is also evidence for involvement of Shp2 tyrosine phosphatase, a downstream signal of CXCR4, in migration and differentiation of cerebellar granule

progenitors, as conditional KO of Shp2 displays cerebellar malformation and explants from these mice fail to migrate in response to SDF-1.²¹⁹

CXCR4 is also suggested to be involved in adult neurogenesis.²²⁰⁻²²² There is a loss of mitotic, nestin+, and doublecortin+ cells in the dentate gyrus when a CXCR4 antagonist, AMD3100, is infused.²²² SDF-1 can also modulate gamma-aminobutyric acid (GABA)-induced maturation of granule progenitor cells and is co-released with GABA from interneurons onto immature neural progenitor cells.²²¹

5. DOPAMINE RECEPTORS—Ciliary neurotrophic factor (CNTF) regulation of adult neurogenesis in mice is mediated by the D2 receptor.²²³ Systemic treatment of either normal or dopamine-depleted rats with the D2-like agonists ropinirole or 7-OH-DPAT significantly increased precursor cell proliferation in the SVZ,²²⁴⁻²²⁶ resulting in increased differentiation of neurons. Destruction of dopaminergic neurons in the substantia nigra and ventral tegmental areas reduced the number of proliferating neural precursors in the SVZ by 40%.²²⁷

6. ENDOTHELIN RECEPTORS—Migration of cortical progenitor cells is inhibited by ET-1 through ET_B signaling coupled to G_q and the JNK pathway.²²⁸ Evidence also suggests a role of ET-1 signaling in gliogenesis,²²⁹ as oligodendrocyte progenitors express ET receptors.²²⁹ Since ET is released by astrocytes, it is possible that oligodendrocyte progenitor cell (OPC) differentiation is regulated through paracrine effects.

7. SPHINGOSINE-1-PHOSPHATE AND LYSOPHOSPHOTIDIC ACID RECEPTORS—LPA stimulates the differentiation of mouse cortical neuroblasts²³⁰ and the proliferation and differentiation of rat NSCs to cholinergic neurons.²³¹ In contrast, in hESC-derived neurospheres which expressed all five of the LPA receptors, LPA specifically inhibits the differentiation of NSCs toward neurons, without affecting proliferation, whereas it maintains the differentiation of NSCs toward astrocytes.¹³⁵ In humans, hESC-derived NSCs express S1P_{1,3} where they stimulate proliferation and growth.^{135,232} LPA₁ and S1P_{5>1-3} are expressed in embryonic and adult rodent OPCs,²³³⁻²³⁶ while fetal human OPCs express higher levels of S1P₁.²³⁷ Both LPA and S1P act on OPCs and inhibit oligodendrocyte maturation.²³³⁻²³⁴ S1P₅ is preferentially expressed in OPCs and is responsible for inhibition of their migration in rodents.²³⁵ In human OPCs, S1P_{5,3} agonism (FTY720) first inhibits differentiation via process retraction, but then stimulates this function and increases cell survival in a S1P₁-ERK-dependent manner.²³⁷⁻²³⁸

8. OPIOID RECEPTORS—Endogenous opioids and synthetic agonists acting on the mu-opioid receptors (MORs), delta-opioid receptors (DORs) and kappa-opioid receptors (KORs) have been shown to affect proliferation and differentiation in various embryonic neural cell types. In embryonic NSCs, stimulation of DORs using SNC80 promoted neuronal differentiation through PI3K/PKC/CAMKII/MEK, but MOR agonism (DAMGO) or KOR stimulation (U50,488H) had no effect.²³⁹ In ESCs derived from a mouse blastocyst, both MOR and KOR stimulation promoted proliferation and differentiation into neural progenitors through ERK.²⁴⁰ MOR and KOR functionality was found in neural progenitor-derived oligodendrocytes and induced their differentiation via ERK and p38.²⁴¹ Agonism of these opioid receptors inhibited the differentiation of both astrocytes and neurons in retinoic acid-induced neural progenitors.²⁴¹ Opiates acting on the adult SVZ and SGZ inhibit progenitor proliferation, maturation, and survival,²⁴²⁻²⁴⁶ and alter the progenitor cell cycle.^{243,247,248}

9. VASOACTIVE INTESTINAL POLYPEPTIDE RECEPTORS—Vasoactive intestinal polypeptide (VIP) is a peptide neurotransmitter released by GABAergic interneurons in the dentate gyrus. VIP and its receptors (VPAC₁ and VPAC₂) are expressed in developing and adult dentate gyrus. VIP shortens the cell cycle of embryonic neuroepithelial cells²⁴⁹ and promotes neuronal differentiation of embryonic hippocampal neurons in culture.²⁵⁰ Adult *Vipr2*^{-/-} mice showed reduced progenitor survival and SGZ neurogenesis.²⁵¹

10. NEUROPEPTIDE Y RECEPTORS—NPY is a 36-amino-acid peptide that belongs to a family of peptides that include pancreatic polypeptide and peptide YY. NPY is secreted by the hypothalamus and is widely distributed in the central and peripheral nervous systems. In an autocrine or paracrine fashion, NPY increases proliferation in the SVZ through an ERK pathway, but does not affect the self-renewal of NSCs.²⁵² Using KO mice *in vivo* or cell culture with specific NPY receptor agonists and antagonists, the neuroproliferative and neuronal differentiating effects of NPY in the SVZ and SGZ were found to be mediated by the Y1 receptor subtype through ERK mediated signaling.²⁵³⁻²⁵⁷

11. PURINERGIC RECEPTORS—P2Y receptors are a family of purinergic GPCRs stimulated by nucleotides such as ATP, ADP, UTP, UDP and UDP-glucose. ATP-mediated purinergic signaling through the P2Y1 receptor has been associated with developmental neurogenesis.²⁵⁸ In human NSCs, ATP and P2 receptors have been identified as a mitogen for v-myc immortalized neural progenitor cells through calcium release and PI3K.²⁵⁹ Embryonic neural progenitors expressed P2Y purinergic receptors and release ATP themselves in burst events to mobilize intracellular calcium and increase progenitor proliferation. Receptor antagonists suppressed proliferation and permitted differentiation into neurons and glia *in vitro*, while subsequent removal of purinergic inhibition restored progenitor cell expansion.²⁶⁰ Neurospheres isolated from P2Y1 deficient mice exhibited reduced proliferation.²⁶¹

12. WNT/FRIZZLED RECEPTORS—Wnt receptors are also implicated in adult hippocampal neurogenesis by regulating proliferation and differentiation.²⁶²⁻²⁶⁵ The Wnt3 receptor is expressed and β -catenin pathway is active in the hippocampal SGZ. Overexpression of Wnt3 is sufficient to increase neurogenesis in the SGZ *in vitro* and *in vivo*. By contrast, blockade of Wnt signaling abolishes neurogenesis almost completely *in vivo*.²⁶² LRP6 mutant mice had reduced production and proliferation of dentate granule neurons and abnormalities in the radial glial scaffolding in the dentate gyrus.²⁶³ In a series of *in vitro* studies utilizing adult mouse neurospheres, both Wnt3 and Wnt5a promoted neurogenesis, but only the Wnt5a neurogenesis was blocked by PKC inhibition.²⁶⁴

VI. GPCRs In Cancer Stem Cells

A. GPCRs in Glia Tumors

1. PURINERGIC RECEPTORS—Glioblastoma is the most common and aggressive tumor in the brain and possibly characterized by having a cancer stem cell subpopulation essential for tumor survival.²⁶⁶ The purinergic system plays an important role in glioma cell growth, since adenosine triphosphate (ATP) can regulate proliferation²⁶⁷⁻²⁶⁸ in these cell types. Nucleotide receptor-mediated cell communication is controlled by ecto-nucleotidases, which hydrolyze ATP into adenosine in the extracellular space. Changes in extracellular ATP degradation by the use of these exogenous nucleotidases dramatically alters the size of gliomas in rats and human glioma cultures.²⁶⁹⁻²⁷⁰ Several purinergic receptor mRNAs were differently expressed in tumor spheres containing markers for cancer stem cells when compared to a cell monolayer not containing spheres or the cancer markers. Treatment of human gliomas U87 or U343 as well as rat C6 gliomas with 100 μ M of ATP reduced the

number of tumor spheres as well as the expression of cancer stem cell markers.²⁷⁰ The differential expression of purinergic receptors in tumor spheres and the effect of ATP in reducing the number of tumor spheres suggest that the purinergic system affects cancer stem cell biology and that ATP may be a potential agonist for differentiation therapy.

2. Akt/PI3K—Downstream signaling pathways of GPCRs such as Ras/Raf/MAPK and Akt/PI3K promote cell survival, cell proliferation, cell migration, and angiogenesis in glial tumor cells. AKT/PI3K activation through loss of phosphatase and tensin homolog (PTEN) in combination with constitutively active epidermal growth factor receptor (EGFR) signaling has been shown to induce glial tumor formation.²⁷¹ In addition, glioma stem cells appear to be more dependent on AKT signaling than non-stem glioma cells²⁷² suggesting that AKT inhibition may be used to target the stem cell population in brain tumors. Several inhibitors of mammalian target of rapamycin (mTOR), which constitutes a downstream target of PI3K, are currently being evaluated in phase II clinical trials in patients with malignant glioma.²⁷³⁻²⁷⁵

B. GPCRs in Breast Cancer Stem Cells

1. MELATONIN RECEPTORS—Breast carcinoma is the most common cancer form among women worldwide, registering about one third of all new cancer cases each year.²⁷⁶ Melatonin suppresses breast cancer cell proliferation by inhibiting the upregulation of estrogen-induced cyclin D1 via its G-protein-coupled receptor MT1.²⁷⁷ Melatonin can downregulate estrogen receptor alpha (ER- α) and blocks the binding of estrogen receptor complexes that induce transcriptional activity on genes that regulate cell growth, proliferation and survival.²⁷⁸ The cancer suppression effects of melatonin are mediated through its G_{i2} G-protein signaling pathway and the MT1 receptor.²⁷⁹ The oxidated form of melatonin, 6-hydroxymelatonin, has also been shown to bind selectively to MT1 and have antioxidant properties.²⁸⁰⁻²⁸¹ Some isoforms of sulfotransferase (SULT), such as SULT1, show a high affinity for 6-hydroxymelatonin in breast cancer cells,²⁸² and SULT1 expression is elevated in malignant breast tissue.²⁸³⁻²⁸⁴ The enzyme SULT1E1 catalyzes the conjugation of estrogens to sulfate residues, protecting breast cancer cells from excessive estrogenic actions.²⁷⁸ Since melatonin can increase the expression of SULT1 isoforms,²⁷⁸ the anti-proliferative effects of MT1 receptor stimulation may also act through this mechanism.

2. CHEMOKINE RECEPTORS—While the effects of SDF-1/ CXCR4 are well known in hematopoietic stem cells, CXCR4 is expressed on several tumor cells that may metastasize to the organs that secrete/express SDF-1 (e.g., bones, lymph nodes, lung, and liver).²⁸⁵⁻²⁸⁶ SDF-1 exerts pleiotropic effects regulating processes essential to tumor metastasis, such as locomotion of malignant cells, their chemoattraction and adhesion, as well as playing an important role in tumor vascularization.

VII. Summary

Stem cells have the potential to be artificially manipulated into various cell types and transplanted into tissues for the treatment of multiple diseases. Hematopoietic stem cells can be transfused into leukemia patients or NSCs can be grafted into the adult brain to produce partial repair. However, ethical issues still need to be resolved in their use and their cell biology still needs to be explored to determine the best course of treatment.

Each type of stem cell has a restricted range of potential differentiated cell types. ESCs can differentiate into any cell type in the body, but they are still restricted because they cannot form trophoblasts that are crucial in formation of many structures needed to complete an organ or organism. The use of patient-specific and inducible stem cells have great promise

for tissue repair and avoids the problems of immune rejection since the cells contain the patients' genome.

Stem cells, besides their direct therapeutic potential, also hold great promise as a tool in drug discovery. Large homogenous populations of differentiated cells can be cultured and used to test the effects of chemical libraries in search of new drugs. Improving upon nature's mechanism of tissue repair is a discovery with great promise, providing an exciting time in science and medicine. The role of GPCRs in this process and our ever expanding knowledge of their roles in stem cell functions will make this promise a reality.

Abbreviations

2-AG	2-arachidonoylglycerol
7-OH-DPAT	7-Hydroxy- <i>N,N</i> -dipropyl-2-aminotetralin
AEA	N-arachidonylethanolamide
AKT	protein kinase B
AR	adrenergic receptor
ATP	adenosine triphosphate
BrdU	bromodeoxyuridine
CB	cannabinoid
CD	cluster of differentiation
CNS	central nervous system
CNTF	ciliary neurotrophic factor
CXCR	C-X-C chemokine receptor
cysLT	cysteinyl leukotriene
DAMGO	[D-Ala ² ,N-Me-Phe ⁴ ,Gly ⁵ -ol]-enkephalin
DCX	doublecortin
Dlx2	distal-less homeobox gene
DOR	delta-opioid receptor
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
ERK	extracellular signal-regulated kinase
ESC	embryonic stem cell
ET	endothelin
FZD	frizzled
GABA	gamma-aminobutyric acid
GFAP	glial fibrillary acidic protein
GPCR	G protein-coupled receptor
hESC	human embryonic stem cell
iPS	induced pluripotent stem

KO	knockout
KOR	kappa-opioid receptor
LPA	lysophosphatidic acid
mGlu	metabotropic glutamate receptor
MHC	major histocompatibility complex
MOR	mu-opioid receptors
MSC	mesenchymal stem cell
MT	melatonin
NCL	neuronal ceroid lipofuscinosis
NE	norepinephrine
NPY	neuropeptide Y
NSC	neural stem cell
OPC	oligodendrocyte progenitor cell
PAR	proteinase-activated receptor
PGE2	prostaglandin E2
PI3K	phosphoinositide-3-kinase
PKC	protein kinase C
PSA-NCAM	polysialylated neural cell adhesion molecule
RMS	rostral migratory stream
S1P	sphingosine-1-phosphate
SDF-1	stromal derived factor 1
SFRP	secreted frizzled-related protein
SGZ	subgranular zone
SULT	sulfotransferase
SVZ	subventricular zone
TAP	transient amplifying progenitor
TM	transmembrane
VIP	vasoactive intestinal polypeptide

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Tables 1

GPCRs and Roles in Stem Cell Regulation

Type of stem cell	Type of GPCR	Function
Embryonic stem cells	PAR1	Embryonic development (124)
	GPR125	Germ-line progenitor marker (125)
	Gi-coupled	Maintains pluripotency (126)
	S1P, LPA	Directs differentiation (137-138); ESC maintenance (131-132)
	CB ₁₋₂	ESC survival (140)
	WNT	ES self-renewal (141); hES proliferation (143); differentiation (144); maintains pluripotency (145)
	mGlu4,5	ES self-renewal (147-148); embryoid body differentiation (149); GABAergic differentiation (150)
Induced Pluripotent Stem cells	Gi-coupled	PTX affected stem cell colony retraction in reprogramming (154)
	Gs-coupled	Gs activation with cholera toxin does not affect stem cell morphology (155)
Hematopoietic stem cells	Gs	KO mice do not home or engraft in bone marrow (156)
	SDF-1 (CXCL12)	Regulates homing function (157-158)
	CXCR4	Modulates transendothelial migration (159); Migration, survival, development of multiple cell types (160)
	S1P	With LPA synergistically enhances homing function of SDF-1 (162-165)
	WNT	Self-renewal and growth (166-167)
Mesenchymal/Stromal stem cells	S1P, LPA	Inhibit human MSC migration (170) but increasing murine MSC migration (171-172); Differentiates MSC to smooth muscle (174)
	S1P	Anti-apoptotic (173)
	S1P	Anti-apoptotic (173)
	LPA	Stimulates osteoblast precursor migration (176)
Cardiac stem cells	WNT	Inhibition required for differentiation (184-187)
	WNT-3A	Migration of progenitor cells via RhoA (189)
	Agtr1b (Apelin)	Required for heart field formation (190); Required for progenitor cell formation (190)
Neural stem cells	α_1 -adrenergic	Increase proliferation of embryonic neuroepithelial cells (193); Stimulate proliferation, migration and protection of mouse embryonic neural progenitors (194-196); Increases both mouse neurogenesis and gliogenesis <i>in vitro</i> and <i>in vivo</i> that is developmentally dependent (197)
	α_2 -adrenergic	Decreased proliferation of hippocampal progenitors (198); Enhanced progenitor survival with antagonist dexefaroxan (199)
	CB	Proliferates embryonic and adult NSCs (200-202); CB1 involved in migration of neural progenitors (205); Neuronal survival (206-207)
	CXCR4	Proliferation and migration of hippocampal granule progenitors (213-214); Differentiation and migration of cerebellar granule progenitors (215-219); Adult neurogenesis (220-222)
	Dopamine	Adult neurogenesis (223); SVZ precursor proliferation (224-227)
	Endothelin	Inhibits cortical progenitor cell migration (228); Regulates gliogenesis (229); OPC differentiation (229)

Type of stem cell	Type of GPCR	Function
	LPA	Proliferates and differentiates rat NSCs to cholinergic neurons (230-231); In hESCs, inhibits neuronal but promotes astrocyte differentiation (135); Both S1P and LPA Inhibit oligodendrocyte maturation (233-234)
	S1P	Inhibits migration of OPCs in rodents (Novgorodow et al., 2007)
	Opioids	DOR promotes neuronal differentiation in embryonic NSC (239); MOR and KOR promotes proliferation and differentiation into neurons in ESCs from mouse blastocyst (240); MOR and KOR induced oligodendrocyte progenitor differentiation (241); Decrease adult SGZ proliferation, maturation and survival (242-246)
	VIP	Promotes neuronal differentiation of embryonic hippocampal progenitors in culture (250); KO mice show reduced progenitor survival and SGZ neurogenesis (251)
	NPY	Increases proliferation in SVZ (252); NPY1 regulates neuroproliferation and differentiation in SVZ and SGZ through ERK (253-257)
	P2Y	P2Y1 associated with developmental neurogenesis (258); ATP and P2 proliferate v-myc immortalized neural progenitors (259); proliferate embryonic neural progenitors and P2Y inhibition permitted differentiation into neurons and glia (260)
	WNT	Regulates adult hippocampal neurogenesis via proliferation and differentiation (262-265)
Cancer stem cells	Purinergic	Regulates proliferation of glioma cell growth via CSC subpopulation (267-268); alters size of gliomas in rats and human cultures (269-270); ATP reduces number of tumor spheres and expression of cancer cell markers (270)
	Second messengers	AKT/PI3K reduces glial tumor formation (271); glioma stem cells sensitive to AKT signaling than non-stem glioma cells (272)
	Melatonin	MT1 suppresses breast cancer cell proliferation (277-278); MT1 cancer suppression via Gi2 signaling (279); increases expression of SUL1 which protects against excessive estrogen actions (278)
	CXCR4	Suggested to promote metastasis to organs that secrete SDF-1 (285-286)