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Regulation of stem cell pluripotency and differentiation by G protein coupled receptors

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ABSTRACT

Stem cell-based therapeutics have the potential to effectively treat many terminal and debilitating human diseases, but the mechanisms by which their growth and differentiation are regulated are incompletely defined. Recent data from multiple systems suggest major roles for G protein coupled receptor (GPCR) pathways in regulating stem cell function in vivo and in vitro. The goal of this review is to illustrate common ground between the growing field of stem cell therapeutics and the long-established field of G protein coupled receptor signaling. Herein, we briefly introduce basic stem cell biology and discuss how several conserved pathways regulate pluripotency and differentiation in mouse and human stem cells. We further discuss general mechanisms by which GPCR signaling may impact these pluripotency and differentiation pathways, and summarize specific examples of receptors from each of the major GPCR subfamilies that have been shown to regulate stem cell function. Finally, we discuss possible therapeutic implications of GPCR regulation of stem cell function.

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1. Introduction

Stem cell-based therapeutics have become a reality in the past decade, and are currently one of the fastest growing categories of novel therapeutics in clinical trials. In contrast, drugs that target G protein coupled receptors (GPCRs) are among the oldest and most established therapeutics. Recent studies defining the molecular pathways that govern stem cell function suggest unexpected intersections between these oldest and newest therapeutic classes. Several classic GPCR effector cascades impinge on the basic pathways that dictate whether stem cells remain in the pluripotent proliferative state or undergo differentiation. Thus, these receptors and their endogenous and exogenous ligands will critically impact the fate of stem cells. The purpose of this review is to highlight examples and potential mechanisms of GPCR regulation of stem cell function, with sufficient background to be accessible for both stem cell biologists and molecular pharmacologists. We will first describe basic stem cell biology in development and in vitro, and outline the critical pathways that define self-renewal and differentiation. We will then describe

Abbreviations: EB, Embryoid body; ES cells, Embyronic stem cells; FZD, Frizzled; GPCRs, G protein coupled receptors; GSK3, Glycogen synthase kinase 3; hATSCs, Human adipose tissue-derived mesenchymal stem cells; hESCs, Human embryonic stem cells; iMEF, Inactivated mouse embryonic fibroblast; iPSCs, Induced pluripotent stem cells; LPA, Lysophosphatidic acid; mESCs, Mouse embryonic stem cells; NPs, Neural progenitors; OPC, Oligodendrocyte progenitor cell; PACAP, Pituitary adenylate cyclase-activating peptide; S1P, Sphingosine 1-phosphate; SPC, Sphingosylphosphorylcholine; SDF-1α, Stromal cell-derived factor-1α; VIP, Vasoactive intestinal peptide.

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basic GPCR function and signaling cascades, and highlight examples from each GPCR family implicated in regulating stem cell function. Finally, we discuss therapeutic implications of GPCR regulation of stem cell function.

2. Stem cell biology

2.1. Stem cell classification

Stem cells, by definition, maintain the ability to proliferate and regenerate identical cells, but can also be stimulated to leave this self-renewal cycle and differentiate into specific cell types. These general criteria apply to diverse populations of cells; thus, "stem cells" is a general descriptive term, and is applied to cells from many different sources with many different functions and phenotypes. Stem cells can be further defined according to their potential lineages, i.e. the number of different cell types to which they may give rise, and according to their source. There are four categories of lineage potential: totipotent, pluripotent, multipotent or unipotent. Totipotent cells are formed following the fertilization of an oocyte and are capable of giving rise to any cell in the embryo and extraembyonic tissue. Cells that are capable of giving rise to all cells in the developing embryo are termed pluripotent. Specialized stem cell populations that may give rise to only limited cell types are multipotent, while cells that give rise to only one cell type are termed unipotent. Finally, cells eventually leave the cell cycle and undergo terminal differentiation, at which point they are termed post-mitotic. These cell populations can be further defined according to their source. Pluripotent cells derived from the inner cell mass of the blastocyst are commonly called "embryonic stem cells" (ESCs). It is now also clear that certain tissues harbor stem cell populations throughout fetal development into adulthood; these cells have been called "adult stem cells". Further, it has also been shown in recent years that 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 embryonic stem cells (Rodriguez-Pinilla et al., 2007; Chen et al., 2008; Jeter et al., 2009). Pluripotent stem cells have also been obtained using somatic cell nuclear transfer in both bovine and rodent systems (Cibelli et al., 1998; Wakayama et al., 2001). Finally, it is now possible to generate pluripotent cells through genetic manipulation, generating "induced pluripotent stem cells" (iPSCs) (Yu et al., 2007; Takayama et al., 2008). It is worth mentioning that the use and definitions of these terms are the subjects of ongoing debate, and the classifications of stem cell populations are likely to evolve as our understanding of the underlying biology is refined.

2.2. Stem cells in development

Stem cells are critical in development as cells transition from a totipotent state to being more highly differentiated through a series of tightly regulated signaling events. In humans, four to five days following fertilization the totipotent cells of the morula begin to specialize as they form the blastocyst. The blastocyst consists of the trophoblastic cells which surround the blastocoel cavity containing the inner cell mass. While the trophoblast gives rise to a portion of the extraembryonic tissue, the pluripotent inner cell mass is capable of giving rise to all cells in the embryo proper. As the embryo develops, the cells further specialize into precursor or multipotent stem cells that can be induced to generate one of the 200+ types of terminally differentiated cells which form the fully developed adult. For example, multipotent neural stem cells are committed to a neural lineage, but may give rise to neurons, astrocytes, or oligodendrocytes. These changes result from complex autocrine, paracrine, and endocrine signals which direct cells to their terminal fate. A select few cells do not reach senescence, but instead initiate self-renewal genes that allow the cell to retain a multipotent potential into adulthood (Morshead et al., 1994). These nascent stem cells can be triggered to expand and differentiate through poorly understood mechanisms.

2.3. Embryonic stem cells in culture

The complex regulation that occurs following fertilization in humans is inaccessible to researchers; thus, the molecular details that govern human pluripotent cells in vivo are poorly defined. The past decades have seen significant research efforts focused on defining the transitions between these distinct stages of differentiation and cell fates and the signaling mechanisms that determine whether a stem cell remains in a pluripotent/multipotent stage or undergoes differentiation. Recent advances in the *ex vivo* culturing of embryonic stem cells have provided a glimpse into the complex regulatory processes that are involved in stem cell maintenance and differentiation during human development. The ability to grow highly enriched populations under defined culture conditions has been an essential part of defining these transitions. Multiple genetic markers – typically transcription factors – have been identified that are exclusively associated with pluripotent cells (See Table 1 for partial list). Three markers in particular – Oct4, Nanog, and Sox2 – are expressed in both human embryonic stem cells (hESCs) and mouse embryonic stem cells (mESCs), and their expression is required for pluripotency. While pioneering studies in mESCs provided much of the groundwork for our understanding of stem cell biology and differentiation, more recent work in hESCs has revealed marked differences in the pluripotency pathways between the two species and make a strong case for verifying results from rodent systems in human systems.

2.3.1. Mouse embryonic stem cell pluripotency

The isolation of mouse embryonic stem cells in 1981 provided new opportunities for studying development. The inner cell mass was extracted and cultured on mitotically inactivated mouse

Table 1
Pluripotency and differentiation markers. Examples of markers indicating specific stages of pluripotency and differentiation are shown (not intended to be a comprehensive listing).

Pluripotency Markers		
Common	**Human**	**Mouse**
Pou5f1 (Oct 3/4)	SSEA3	SSEA1
Sox2	SSEA4	
Nanog	Tr–1–81	
Rex–1	Tra–1–60	
Alkaline Phosphatase		
Lineage markers		
Ectoderm	**Mesoderm**	**Endoderm**
N–cam	Brachyury	AFP
NeuroD	Mesp1	HNF–4
βIIItubulin	Runx1	GATA4
Multipotent Markers		
Neural	**Hematopoietic**	**Mesenchymal**
Sox2	CD31	CD73
Musashi	CD34	CD105
Pax6	CD43	Thy–1 (CD90)
FoxG1 (BF1)	CD38	CD166
Forse–1	Thy–1 (CD90)	
N–cad	CD133	
Notch–1	Flk1	
A2B5		
Noggin		
Vimetin		