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Review

The voyage of stem cell toward terminal differentiation: a brief overview

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Presently, worldwide attempts are being made to apply stem cells and stem cell-derived products to a wide range of clinical applications and for the development of cell-based therapies. In order to harness stem cells and manipulate them for therapeutic application, it is very important to understand the basic biology of stem cells and identify the factors that govern the dynamics of these cells in the body. Several signaling pathways have emerged as key regulators of stem cells. Some of these signaling pathways regulate the stem cell's proliferative capacity and therefore act as direct regulators of the stem cell, whereas others are involved in shaping and maintaining the stem cell niche and therefore act as indirect regulators of the stem cell. It is difficult to identify which signaling pathways critically affect the stem cell's behavior and which are important for maintaining the quiescent population. A stem cell receives different extrinsic signals compared with the bulk population and responds to them differently. In order to manipulate these adult cells for therapeutic approaches it is crucial to identify how signaling pathways regulate stem cells either directly by regulating proliferative status or indirectly by influencing the niche. The main challenge is to identify whether different factors provide diverse extrinsic signals to the stem cell and its daughter cell population, or whether there are intrinsic differences in stem cell and daughter cell populations that is reflected in their behavior. In this study, we will focus on the various aspects of stem cell biology and differentiation, as well as exploring the potential strategies to intervene the differentiation process in order to obtain the desired yield of cells applicable in regenerative medicine.

Keywords stem cells; differentiation; cell fate; regulation; signaling

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Introduction

The earliest event in embryonic development is the specification of three germ layers, i.e. ectoderm, mesoderm, and endoderm. This process requires the sequential activation of a large number of gene products in the stem cell. However, the exact switch by which a stem cell gets committed to a particular lineage is still a mystery [1]. Self-renewal capacity and pluripotency are the characteristic features of the stem cell, which open new avenues for their application in cell-based therapeutic strategies and tissue engineering. Self-renewal enables the extensive *ex vivo* (and *in vivo*) expansion of progenitor cells for a targeted tissue. This is a key feature for generating sufficient cells to meet the potential demand for tissue replacement. Pluripotency, or ability of the stem cell to differentiate into multiple cell types, allows for the possibility of generating tissues of multiple lineages from a single cell source. Initially, it is thought that in adult tissue systems progenitor cells of a particular tissue never cross their boundaries to differentiate into tissue cells of other type [2]. Recent studies on heterokaryon demonstrated that the differentiated state can be changed in the presence of appropriate regulatory molecules and under some selective conditions; these cells could be made to differentiate into a broad spectrum of cells [3]. This phenomenon of trans-differentiation described the conversion of cell to a cell of different type acquiring new cell fate, genetic repertoire and functions similar to the trans-differentiated cell type [4]. The concept that the identity of a somatic cell can be changed became a reality as lineage reprogramming was established. In 2006, Yamanaka and Takahashi [5] discovered how to 'reprogram' adult cells with specialized function (e.g. skin cells) in the laboratory, so that they behave like an embryonic stem cell. These cells called induced pluripotent cells or iPS cells were created by inducing the differentiated cells to express genes that are normally made in embryonic stem cells and that control the cell functions [5]. A major goal in cell biology is to maintain an intricate balance between stem cell renewal and the differentiated counterpart. The multi-lineage differentiation potential of stem cells is not only beneficial but is also a challenge as differentiation at the wrong time, place, or to an undesired cell type may lead to a harmful pathophysiological condition. To avoid such maladaptive responses, stem cells have evolved

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elaborate circuitry that triggers them to respond to differentiation cues only in an appropriate biological context. Various soluble factors (e.g. growth factors and cytokines) have been identified to play important roles in regulating stem cell differentiation [2]. Recent evidence demonstrated that the response to these stimuli is strongly modified by adhesive and mechanical cues, and that these micro-environmental factors may be used explicitly to control stem cell differentiation in their own right [6]. Despite rapid advances in the field of stem cell biology, the precise and the efficient differentiation into distinct cell types and tissues is still a major challenge. One of the major barriers to the successful stem cell therapy is to make the cells to behave in the desired way [7]. Our understanding of how stem cells are regulated to maintain their quiescent state or induced to differentiate is not fully elucidated. A number of transcription factors and signaling pathways have been identified that affect the differentiation process [8], much more still need to be identified. Thus a complete knowledge of regulatory mechanisms is mandatory to unwind the complex web of switches that push a naive stem cell into a more specialized state.

Biology of Stem Cells

'Stem cells' is a term to describe precursor cells that can form multiple tissue types of an organism or can give rise to all the three germ layers of an organism. Stem cells are undifferentiated, highly specialized kinds of cell types having the ability to renew itself, found in different tissue or organ. Stem cells are capable of dividing for long period of time and furnish different cell types with specific functions [9].

Stem cells are classified into two categories on the basis of their origin and their functional properties. One is the embryonic stem cell (ESC), whereas the other is the adult stem cells. ESCs are often confused with embryonic germ (EG) cells. The cells derived from the various sources differ in the ability to transform into cells of various lineages, and on this basis they can be classified into five different types which are described in **Table 1**.

'Human ESCs (hESCs)' are formed in the blastocyst phase of development; i.e. a 4- or 5-day-old human embryo that consists of an inner cell mass called embryoblast and an outer cell mass called trophoblast. The outer cell mass becomes part of the placenta, and the inner cell mass is the source of embryonic stem cells. These cells are totipotent cells having the potential to develop into any cell type in the body including the embryonic and extraembryonic structures [10].

'Human EG cells' are derived from primordial germ line cells in early fetal tissue. EG cells are capable of forming the three germ layers that make all the specific organs of the body, but their range of potential fates is relatively limited as compared with ESCs [11].

Table 1 Classification of stem cells derived from various sources based on their differentiation potential

Type of stem cells	Developmental potency	Examples
Totipotent	Ability to differentiate into all cell types and a functional organism	Zygote and the first few cells that result from the division of the zygote
Pluripotent	Ability to differentiate into almost all cell types but cannot form a functional organism	ESCs
Multipotent	Ability to differentiate into a closely realted family of cells	Hematopoietic (adult) stem cells, MSCs, dental pulp stem cells, etc.
Oligopotent	Ability to differentiate into a few cell types	Lymphoid (adult) or myeloid stem cells
Unipotent	Ability to only produce cells of their own type, but have the property of self-renewal required to be labeled a stem cell	Muscle (adult) stem cells

'Human adult stem cells' are found in developed tissue, regardless of the age of the organism [12]. The most well-known example of this is the hematopoietic stem cells (HSCs) of blood and mesenchymal stem cells (MSC) required for the maintenance of bone [13], cartilage, and other tissues [12]. Adult stem cells are multipotent; their potency is poor as compared with that of the embryonic stem cells and EG cells [14]. However, these cells offer excellent potential for use of stem cells in clinics [12].

Self-regeneration is a critical feature of stem cells, because these cells are constantly subjected to physiological stresses that stimulate them toward the differentiation pathways resulting in their depletion [15]. For example, HSCs are needed under conditions of hypoxia to increase red blood cell numbers, or during infections to amplify granulocytes and macrophages [16]. Self-renewal ensures that sufficient numbers of stem cells are available to meet demands of the organism. Upon division, a stem cell gives rise to another stem cell which maintains the quiscent pool population and a daughter cell which finally gives rise to terminally differentiated cells [2]. The stem cells need appropriate inductive conditions in order to differentiate into specific cell lineages [17]. In this context, the stem cell intimately depends on its surrounding environment for maintaining its properties [18].

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Both intrinsic and extrinsic mechanisms cooperate stringently to maintain the balance between stem cell quiescence and proliferation [19]. The stem cells divide asymmetrically into a new stem cell (self-renewal, reserved) and a committed progenitor (differentiation, active) [20]. In the niche environment, active stem cells are the 'primed' sub-population that account for the generation of corresponding tissues, whereas quiescent stem cells function as a 'back-up' or 'reserved' sub-population and replace the damaged cells [21]. A complex loop of genetic determinants and signaling factors are involved in maintaining the delicate balance between MSC self-renewal and differentiation. MSCs from a variety of mammalian species express the embryonic stem cell gene markers including Oct-4 [22], c-Myc [23], Sox2 [24], rex-1 [25] others like nanog [26], klf4 [27], etc. A critical amount of Oct-3/4 is required to sustain stem cell self-renewal, and up- or down-regulation induces divergent developmental specificities [22]. A less than 2 fold increase in expression causes differentiation into primitive endoderm and mesoderm, whereas repression of Oct-3/4 induces loss of pluripotency and dedifferentiation to trophectoderm. c-Myc, in addition to being essential for proliferation of lineage-committed hematopoietic cell *in vivo*, plays an unexpected role in controlling the balance between HSC self-renewal and differentiation. A loss of c-Myc function leads to up-regulation of adhesion molecules which in turn, results in failure of differentiation, presumably by retention of HSCs in the bone marrow niche. Therefore increased c-Myc induces HSC differentiations [23].

Leukemia inhibitory factor (LIF) [28], fibroblast growth factors (FGFs) [29], and mammalian homologues of *Drosophila* wingless (Wnts) [30] are among some of the growth factors and cytokines, that have been observed to play a role in the maintenance of the balance between the 'stemness' and differentiation of MSCs. LIF, a pleiotropic cytokine 9, maintains the stem state [11]. *In vitro* studies have indicated that, higher concentrations of LIF stimulate self-renewal [31]. In the absence of LIF, ESC differentiation occurs at a similar rate in a chemically defined medium as it does in serum containing medium. FGF2 maintains the stemness by increasing viability in a cell-autonomous fashion [13]. Wnts may also regulate the MSC maintenance, as they are involved in the self-renewal of hematopoietic [32], neural [33], intestinal [34], and skin stem cells [14]. Wnt3a treatment increases adult MSC proliferation while inhibiting their osteogenic specification. The combination of these reciprocal backup systems provides a robust mechanism to ensure a high rate of physiological self-renewal as well as flexible damage repair.

Stem cells in health and disease

Both embryonic and adult stem cells are potential resource for investigating early developmental processes as well as assessing the therapeutic potential of these cells in numerous diseases models [35]. The most widespread application of human bone marrow stem cells is the replacement therapies—to replace diseased or degenerating tissues, or to replace cell populations, such as those of the hematopoietic system. Understanding the entire paradigm that regulates their self-renewal and differentiation can lead to new stem cell therapies for developmental defects and disease. In theory, bone marrow stem cells could provide an unlimited supply of specific cell types for transplantation and can be used as an impending cell source for generation of artificial functional tissue [36]. HSC-derived cardiomyocytes, neural precursors, and hematopoietic precursors have been transplanted into recipient animals. Although the analyses of the long-term outcome of such experiments are limited, the initial findings suggest that the transplanted cells were able to function normally in the host animal [37]. Allogenic bone marrow transplantation in osteogenesis imperfecta patients resulted in 1.5−2.0% engraftment of donor osteoblasts, thus demonstrating a potential therapeutic role of these cells [38]. These studies are paving the way for further applications of stromal cell system in the field of transplantation with respect to hematopoietic support, immuneregulation, and graft facilitation [39]. Stem cell therapy has been found useful to repair damaged spinal cords [40]; cure Crohn's [41], Alzheimer's and Parkinson's disease [42]; re-grow arteries around a blockage [43]; re-grow limbs [44]; replace failed kidneys [45], and hearts [46]; cure diabetes by replacing non-functional cells in the pancreas [47]; restore vision [48], and hearing [49]; treat leukemia and lymphoma that are non-responsive to normal therapy [50]; and treat brain cancer [51]. All these potential applications exploit the ability of selective differentiation of stem cells under predefined culture condition. Human hiPS cell-derived cardiomyocytes can also be valuable as a test system for evaluating the toxicity and efficacy of new medicines or chemicals [52]. The wide varieties of cell type and tissue that may develop from stem cells represent a biological system that mimics many of the complex interactions of the cells and tissues of the body, and hence provides an attractive and valuable screening tool [53]. This type of assay could have wide applications in the pharmaceutical, chemical, cosmetics, and agrochemical industries [53], providing new insights in the area of tissue engineering.

Harnessing the differentiation potential of stem cells

There are at least three essential requirements for practical use of stem cells in regenerative medicine: (i) the directed differentiation of stem cell to specific cell types, (ii) achieving high survival of the cells after transplantation, and (iii) prevention of undifferentiated stem cells that are prone to form teratomas or cancers [54]. A number of

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signal modulators including growth factor- and cytokine-driven pathways can govern the stemness and differentiation of stem cells. A number of studies have shown that stem cells (MSCs, HSCs ,or ESCs) can be induced to differentiate by any alteration in the niche environment by any changing the extrinsic or intrinsic factor [55]. Traditionally, ESCs can be made to differentiate via three fundamental methods, which include the formation of embryoid bodies (EBs) [56], culturing directly on stromal cells following by the differentiation [57] and differentiating ESCs in a monolayer on extracellular matrix proteins [58]. However, each method has its own advantages and complications. EBs can generate a three-dimensional structure which can further enhance cell–cell interaction, modulate certain developmental programs, whereas the same complexity might lead to the generation of certain cytokines to interfere with the signaling pathways [56]. Co-culturing of ESCs with osteopetrotic 9 (OP9) stromal cells provides the advantage of growth factors of OP9 stromal cells, but these same factors may lead to the differentiation of stem cells to undesired cell type [56]. Differentiation in monolayers on known substrates is one of the simplest protocols, but with this protocol the matrix proteins are critical. The different proteins can have a dramatic effect on the developing ESC [56]. The molecular mechanisms regulating stem cell differentiation is slowly being unraveled.

Chemicals and growth factors during differentiation

A number of growth factors/cytokines/transcription factors and synthetic chemicals (collectively called inducing agents) that have been reported to induce or regulate the differentiation of stem cells are shown in **Table 2**.

Apart from the differentiation factors mentioned above in **Table 2**, a plethora of molecules also are identified to have a role in modulating stem cell fate. These molecules offer a wider repertoire of choice for selective differentiation of stem cells which may be exploited for future application in therapy. IQ-1 (calmodulin binding motif) is a newly discovered molecule which prevents the differentiation of the stem cells and maintains the cell in quiescent state till required. Wnt pathway is known to have dichotomous effects on stem cells, i.e. both proliferative and differentiative [99]. This molecule can block one arm of Wnt-signaling pathway, while enhance the signal coming from the other arm of the Wnt pathway [100]. Other transcription factors like core-binding factor subunit alpha-1/runt-related transcription factor 2 (CBFA-1/RUNX2) and Osterix (Osx) are required for bone formation [101], while Sox9 is required for chondrogenesis [102]. There exists a reciprocal regulation between Sox9 and RUNX2, which may have some effect on MSC fate [103]. In human MSCs, *N*-formyl-methionine-leucine-phenylalanine promotes osteoblast

Table 2 Induction agents that regulate cellular differentiation

Induction agents	Expected cell fates	References
Basic fibroblast growth factor (BFGF), BMP-4, EGF, and RA	Ectodermal and mesodermal	[59–63]
TGF-β1, activin-A	Mesodermal	[61,64]
Hepatic growth factor, β neural growth factor	Ectoderm, mesoderm, and endoderm	[62,65]
Interleukin-3 (IL-3)	Macrophages, mast cells, or neutrophils	[66]
IL-6	Erythroid	[67]
RA, B-27 supplement, BFGF, and nerve growth factor	Neurogenic	[59,63,68,65]
myoD, myf6, TGF-β1, and FGF-8	Myogenic	[59,64,69]
Peroxisome proliferator-activated receptor-γ2, adipocyte protein 2 (ap2), iso-butyl-methyl-xanthine, and indomethacin	Adipogenic	[13,70,71]
Sox9, FGF-2, BMP-6, TGF-β1, and TGF-β3	Chondrogenic	[72–75]
TGF-β, FGF, PDGF, insulin-like growth factor, IL-1, CBFA-1, RUNX-2, transcriptional coactivator with PDZ-binding motif (TAZ), MSX2, Dlx-5, AP-1, osteopontin, dexamethasone, β-glycerol-phosphate, and ascorbate	Osteogenic	[12,76–82]
Vascular endothelial growth factor receptor-2, Von Willebrand factor, and vascular endothelial cadherin	Endothelial	[83–85]
TGF-β1 family, IGF-1, PDGF, FGF, oxytocin, erythropoietin, 5 azacytidine, ascorbic acid, RA, DMSO, and dynorphin-B	Cardiomyocyte	[86–96]
Hepatocyte growth factor (HGF), oncostatin M, dexamethasone, and insulin-transferrin selenium-X	Hepatogenic	[62,97,98]

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differentiation via the *N*-formyl peptide receptor 1-mediated signaling pathway [104].

Various chemicals have been demonstrated to modulate stem cell fate, e.g. rosiglitazone (promotes adipocytic differentiation of MSCs) [105], fluoxetine (stimulate hippocampal neurogenesis) [106], myoseverin (revert terminally differentiated myotubes into myoblasts), etc. [107]. Chemicals like KHS101, phosphoserine, chlamydocin, repsox, 5-azacytidine, Bix0129, valporic acid, etc. can switch stem cell lineage to a dramatic extent via up- or down-regulation of one or more signaling pathways [108]. Some other molecules including CHIR99021 (the activator of canonical Wnt signaling) [108], dorsomorphin (the inhibitor of bone morphogenetic protein, BMP, signaling), PD0325901 (the inhibitor of extracellular signal-regulated kinase, MEK, signaling) [109], Cyclopamine (the inhibitor of hedgehog, Hh, signaling) [110], XAV939 (the inhibitor of Wnt signaling) [111], and the activator like Hh-Ag1.2/1.3 [108], also play a role in directing the stem cell to a particular lineage.

Mechanical cues for differentiation

Apart from chemicals or growth factors, mechanical means also can differentiate the stem cell. Mechanical factors can also be used to control the fate of stem cell and direct the differentiation to desired cell type. The mechanical aspects have been largely ignored by stem cell biologists till date. Studies have shown that the physical properties of extracellular matrix can control stem cell fate toward osteogenesis or chondrogenesis, which depends on its mechanical strength [112]. Recently, it was reported that manipulating the membrane potential of cultured MSCs can influence their fate and differentiation [113]. Researchers from the University of Chicago found that the shape of the matrix can dictate the fate of stem cell [114]. They observed that on a flower shape matrix majority of cells turned into fat, and on a star shape they turned into bone. Since the star shape promotes a tense cytoskeleton in cells, which provides structural support for cells, whereas flower shape promotes a looser cytoskeleton [114]. These authors have also built a novel type of stem cell scaffold, which resembles an ultrafine carpet of 'microposts', hair-like projections made of the elastic polymer polydimethylsiloxane. It was possible to manipulate the rigidity of matrix by adjusting the height of the microposts [114]. Thus a number of diverse mechanisms operate together to regulate the final fate of stem cell. However, although the cell differentiation is the result of a complex orchestration of many signals from multiple signaling pathways, a single chemical/physical factor can alter the relative balance of these signals and direct the differentiation.

The complex crosstalk of signals in the ultimate decision of cell fate

The signaling environment is a part of natural mechanism regulating stem cell fate. Stem cell differentiation needs to be explored in the context of various interacting pathways which encompasses more than just stem cell fate control.

Wnt proteins represent a growing family of secreted signaling molecules that influence multiple developmental processes in both vertebrates and invertebrates. The founding member of this family, Int-1 (now called Wnt1), was identified as a proto-oncogene [115]. Wnts act by binding to two types of receptor molecules at the cell surface. One is the Frizzled family which is a seven-pass transmembrane protein family, containing a cysteine-rich extracellular domain that binds to Wnt proteins [116]. The second is a subset of the low-density lipoprotein receptor-related protein family [117], a single-pass transmembrane protein. Binding of Wnt proteins to their receptors leads to the stabilization and accumulation of β-catenin in the cytosol by inhibiting its phosphorylation by glycogen synthase kinase 3 beta (GSK-3β) [118]. β-Catenin then migrates to the nucleus, binding to members of the lymphoid enhancer-binding factor 1 (LEF)/T-cell factor (TCF) family of transcription factors which [119] represses gene transcription. Binding of β-catenin relieves this repression and allows LEF/TCF factors to induce the expression of appropriate target genes [120]. Wnt signaling pathway contributes to the regulation of stem cell self-renewal in the hematopoietic system. For example, Wnt5A results in increased progenitors in fetal liver and bone marrow-enriched [121] precursors.

BMPs belong to the transforming growth factor-β (TGF-β) superfamily. They are involved in the regulation of cell differentiation, proliferation, apoptosis, and development [122]. There are more than 20 BMPs which play important roles in regulation of stem cell properties; however, their functions are different in the different stem cell compartments. For example, in ESCs, BMP signaling is required for ESC self-renewal, but this is owing to its ability to block neural differentiation [123]. In addition to its ability to promote non-neural (mesoderm and trophoblast) differentiation [123] in MSCs, the BMP signal induces osteoblastic differentiation through the isoform Bmpr1b, but inhibits the differentiation through another isoform, Bmpr1a [124]. In intestinal stem cells, BMP signaling inhibits stem cell activation and expansion [125]. In HSCs, BMP signaling controls the niche size through Bmpr1a and restricts the stem cell number [124]. BMP functions through receptor-mediated intracellular signaling. There are two signaling pathways involved in BMP signal transduction [126]. The canonical BMP pathway is through receptor I mediated phosphorylation of Smad1, Smad5, or Smad8 (R-Smad). Two phosphorylated R-Smads form a

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heterotrimeric complex with a common Smad4 (co-Smad). The Smad heterotrimeric complex translocates into the nucleus and cooperates with other transcription factors to modulate target gene expression [126].

Another class of signaling molecules having important role in development is the receptor tyrosine kinases (RTKs). Some tyrosine kinases play crucial roles in self-renewal or cell fate decisions in ESCs and MSCs, while the role of the others remain to be investigated. Among all the RTKs, the FGF, epidermal growth factor (EGF), and platelet-derived growth factor (PDGF) receptors are important for regulating cell survival, proliferation, and differentiation in both embryonic and adult stem cells [127]. FGF2, also named bFGF (basic FGF), binds to members of the FGF RTK family. FGF2 supports self-renewal but its mechanism is still unclear [128,129]. FGF2 induces the development of ectodermal and mesodermal cells from hESCs, and can also support differentiation of hESC into neural lineages [129]. *In vitro* studies with bFGF showed that it increased human MSC proliferation potential, and helped to retain osteogenic, adipogenic, and chondrogenic differentiation potentials [130]. FGF induces proliferation through the mitogen-activated protein kinase (MAPK) cascade in various cell types. The TGF-β pathway is also important in MSC differentiation into the osteogenic and chondrogenic lineages [131]. The activin/nodal pathway, which signals through the TGF-β pathway, cooperates with FGF signaling in maintaining the pluripotency of embryonic stem cells [132].

EGFR signaling induces the proliferation, motility, and survival of MSCs. Two ligands, EGF and heparin-binding EGF, promote *ex vivo* expansion of MSCs without triggering the differentiation into any specific lineage. In ESC, the EGFR increases the level of glucose transporter 1, a major glucose transporter in inner cell mass (ICM) of blastocyst, hence help to meet the high energy demands of the cell required for its proliferation [133].

PDGFRs are all involved in lineage commitment of pre-differentiated ESCs and do not play a role in the self-renewal of ESCs. PDGF is an angiogenetic factor and stimulates blood vessel formation in EBs [134], and also can promote cardiogenic differentiation in murine ESCs (mESCs) [135]. During MSC differentiation, PDGF signaling is significant in adipogenesis and chondrogenesis [136].

The endoplasmic reticulum kinase (ERK) 1/2 pathway, also called p42/p44 MAPK pathway, can regulate different cellular responses in somatic cells. ERK1/2 activation promotes the differentiation of ESCs, whereas the suppression of ERK1/2 pathway enhances self-renewal [137]. mESCs have higher ERK1/2 activity when they undergo differentiation. In mESCs, the activation of ERK1/2 via gp130 is dependent on the phosphorylation of the SH2-domain-containing cytoplasmic tyrosine phosphatase, SHP2. Phosphorylation of Tyr757 in gp130 recruits SHP2, leading to its tyrosine phosphorylation in jasmonic acid kinase 1-dependent manner [138]. SHP2 then acts as an adaptor protein, associating with GRB2 (growth-factor-receptor-bound protein 2) and, thereby, activates the Ras/MEK (MAPK/ERK kinase)/ERK1/2 pathway. SHP2 may also associate with the scaffold protein GAB1 (GRB2-associated-binder protein 1), as has been demonstrated in other cell types, which may recruit phosphoinositol-3-kinase, although this interaction remains to be shown in ESCs [139]. Eliminating the SHP2-binding site from a chimaeric gp130 receptor in ESCs blocks the Ras/ERK1/2 pathway and increases self-renewal [140]. In MSCs ERK–MAPK signaling is significant in adipogenic, chondrogenic, and osteogenic differentiation [136].

Notch encodes a transmembrane receptor that is cleaved to release an intracellular domain (Nicd) directly involved in transcriptional control [141]. The Notch proteins, represented by four homologs in mammals (Notch1–Notch4), interact with a number of surface-bound or secreted ligands (Delta-like 1, Delta-like 3, Delta-like 4, Jagged 1, and Jagged 2) [142]. Notch receptors are activated upon ligand binding, which involves multiple cleavage events assisted by members of A Disintegrin and metalloprotease protease family and gamma secretase. Cleavage is followed by translocation of the intracellular domain on Notch to the nucleus, where it acts on downstream targets [143]. In general, Notch activation leads to transcriptional suppression of lineage-specific genes, inhibiting differentiation in response to inductive signals and leaving some progenitors uncommitted but competent to adopt alternative fates [144]. Notch receptors, Notch1 and Notch2, are expressed throughout stem cell maturation. The Notch pathway has been shown to promote neural differentiation in both human and mouse embryonic stem cells [145]. Notch in combination with EGFR pathway regulates stem cell number and self-renewal in neural stem cells [146]. Loss of EGFR signaling accelerates chondrocyte and osteoblast differentiation in mice, suggesting that EGFR signaling may negatively regulate bone cell differentiation [147]. The Hh pathway is another cascade that plays a pivotal role in HSC proliferation. The *Hh* gene is involved in various aspects of embryonic development such as left–right asymmetry, anterior–posterior patterning of the limb bud, and neural tube formation [148,149]. Sonic Hh promotes the proliferation of adult stem cells from various tissues, including primitive hematopoietic cells [150], mammary [151], and neural stem cells [152].

Members of the *Hox* homeobox gene family are among the first transcription factors to be implicated as critical regulators of lineage specification and stem cell development

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in a number of tissues, including the hematopoietic system [153]. Among these, the transcription factor *HOXB4* was first observed to express at high levels among human $CD34^{-}CD38^{-}$/lo$CD45RACD71^{-}$ bone marrow cells that were highly enriched in long-term culture-initiating cells, but were absent in more mature progenitor populations [154]. However expression of *HOXB4*-transduced HSCs had no effect on the overall HSC pool size which was observed to be maintained within normal limits [155]. There is also involvement of a number of other transcription regulators like Oct4, Sox2, nanog, etc. Among these, Oct4 is a major governing factor and master regulator of pluripotency which is rapidly silenced when a stem cell gets committed to a specific lineage [156]. Oct-4 is expressed at high levels in mouse in pre-implantation embryos and in ICM, embryonic carcinoma (EC), ESC, and embryonic germ (EG) cells, and is down-regulated in the majority of murine adult tissues excluding the germ line [157,158]. This gene is a key factor in controlling self-renewal of mESCs. The decrease or increase of Oct-4 expression in ESCs leads to differentiation into trophoblas/ endoderm and mesoderm, respectively [158]. Ingenuity pathways analysis to identify signaling pathways involved in trophoblast differentiation or function, leads to the discovery of many genes which are involved in Wnt/ β-catenin, ERK/MAPK, Nuclear factor κB, and calcium signaling pathways, further emphasizing their potential role in trophoblast development [125]. This work provides an *in vitro* functional genomic model to identify genes involved in the trophoblast development and differentiation. It also helps to understand the cellular and the molecular mechanisms involved in placental development and clinically relevant to fetal development, fertility, and maternal health [159]. Recent investigations have shown that amino acids can also regulate cellular processes by altering intracellular cell signaling pathways. The amino acid L-proline has been identified as a component which is required for ESC differentiation [160]. Some purinergic receptors have also been shown to play a crucial role in determining the phenotypic, proliferative, and differentiation aspects of committed cells [161]. Retinoic acid (RA) acts as a well-characterized inducer of differentiation by silencing Oct4 locus [158]. Some nuclear repressors like apoAI regulatory protein-1, COUP Transcription Factor 1, and germ cell nuclear factor (also referred to as Nr6a1) can also silence Oct4 [162]. In pluripotent ESCs, chromatin is in decondensed state, containing numerous active histones and a fraction of loosely bound chromatin proteins. As cells differentiate, chromatin assumes condensation, silencing histone marks, and structural chromatin proteins get stably associated with chromatin [163]. Histone modifications, associated with differentiation involve the loss of H3K4me3, H3K7, and H3K9 acetylation, at Oct4, whereas others associated with H3K9me2 and me3 heterochromatin, are gained via G9a histone methyltransferase-dependent manner [164]. DNA methyltransferases like DNMT3a/3b, are also recruited leading to CpG DNA methylation of Oct4 promoter via G9a. Thus Oct4 and other ESC-specific genes, undergo a stringent silencing mechanism to adopt a state characteristic of heterochromatin during differentiation [165]. Thus a complex interplay of many transcription factors and signal transduction pathways are involved in the differentiation of stem cells as shown in the **Fig. 1** .

All that glitters is not gold

The emerging multipotential stem cell area can also have many side effects and potential risks. The well known transcription factors that are essential during reprogramming and differentiation are also well-known carcinogens. Oct4 the master regulatory gene of reprogramming is the most potent carcinogen, myc is an oncogene and klf4 also contribute toward carcinogenesis [166]. Mice injected with these factors have been reported to show teratoma formation. Thus applications in the human recipients, are not totally risk free and this is the major hurdle in transformation of a laboratory-produced stem cell to a clinically applicable modality. Also, these applications carry the risk of genetic mutations, and the normal recipe of human genome thus might get contaminated with exogenous genetic material introduced by the viral vectors, chemicals, etc., which may carry the risk of many diseases and other abnormalities in the genotype and phenotype. These may accumulate in the genome leading to long-term disease effects that may be fatal for subsequent generations. A significant amount of research is still required to understand and predict the behavior of the stem cells during the therapeutic applications. Understanding the basic cell biology and physiology of the stem cell is very important before embarking on a widespread use of this potentially useful tool for the benefit of humanity. Extensive efforts and refined techniques are needed so that the risks of genetic mutations can be reduced to a minimum level and the contamination of the human genome can be avoided.

Perspectives

To increase the production efficiency of stem cells, the further exploration of the physiological, mechanical, and chemical factors regulating the differentiation and reprogramming process is required. Technologies need to be refined further to reduce the potent carcinogenic potential of the vectors/chemical compounds to a tolerable level or search for the natural alternatives to manipulate these cells. More effective animal models can be developed to mimic

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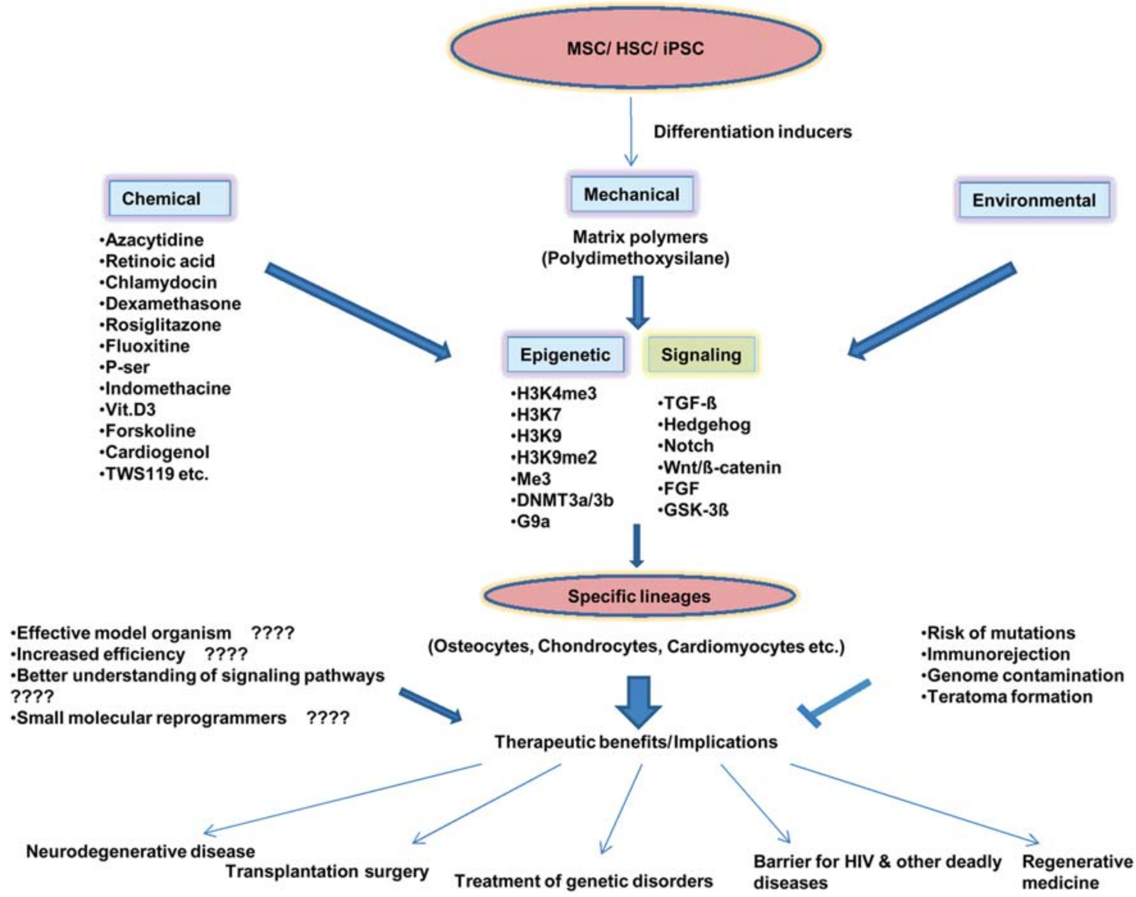

Figure 1 Deciding the stem cell fate HSC, MSC, and iPSC can be made to differentiate in culture via introduction of certain small molecules (chemical), via matrix/microgravity (physical) or spontaneously (environmental) due to some changes in their niche environment. These factors lead to chromatin changes causing acetylation/deacetylation of certain epigenetic factors. They also initiate a signaling cascade via different pathways like Wnt, notch, GSK-3β, etc., up- and down-regulation of certain transcription factors responsible for stemness like Oct3/4, c-Myc, nanog, etc. These changes make a cell committed to a specific lineage, e.g. osteo, chondro or adipogenic, etc. These cells can be further manipulated in clinic for various therapeutic purposes. However effective manipulation involves risk of mutation, immune rejection, and genome contamination. Lack of effective model organism for experimentation, low efficiency of stem cell expansion in culture and insufficient information about reprogramming factors/molecules are some of the bottlenecks in the path of resourceful implementation of this technology. These side effects can be reduced by having a thorough knowledge of the signaling pathway during stem cell differentiation.

human systems more efficiently, so that the effects produced in the animals can be studied over several generations before applying in humans. The sequence of activation of various transcription factors and pathways leading to differentiation is still not clear. The factors that control or regulate the decision of the stem cell to differentiate need to be deciphered. Present methods of differentiation rely on the application of growth factors, chemicals, or selective media that initiate the conversion of a subset of cells into the desired lineage or allow expansion of a particular subset of cells. However, unless we understand the mechanisms that operate to bring about the requisite changes in phenotype, we cannot have controlled differentiation. This will assure a better-quality control and make stem cell-derived therapies financially and logistically more viable. The time frame or the factors to determine when the differentiation process becomes irreversible is also unknown. All the factors which play a crucial role in stem cell fate need to be studied thoroughly in order to ensure the safety of the stem cell-based therapies.

The basic science of stem cell biology has been established, however, translating it into an effective medical treatment is a long and difficult process. It is possible that what looks promising in cultured cells may fail as a therapy in an animal model or when applied in human trials. Once therapies are tested in humans, ensuring patient safety becomes a critical issue and this means starting with very few people until the safety and side effects are better understood. The biology of stem cell modulation need further analysis and understanding in order to unravel the vague mysteries of stem cells and fully enjoy the benefits of this profitable area.

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