Review

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Epigenetic regulation in stem cell development, cell fate conversion, and reprogramming

Abstract: Stem cells are identified classically by an *in vivo* transplantation assay plus additional characterization, such as marker analysis, linage-tracing and *in vitro*/*ex vivo* differentiation assays. Stem cell lines have been derived, *in vitro*, from adult tissues, the inner cell mass (ICM), epiblast, and male germ stem cells, providing intriguing insight into stem cell biology, plasticity, heterogeneity, metastable state, and the pivotal point at which stem cells irreversibly differentiate to non-stem cells. During the past decade, strategies for manipulating cell fate have revolutionized our understanding about the basic concept of cell differentiation: stem cell lines can be established by introducing transcription factors, as with the case for iPSCs, revealing some of the molecular interplay of key factors during the course of phenotypic changes. In addition to de-differentiation approaches for establishing stem cells, another method has been developed whereby induced expression of certain transcription factors and/or micro RNAs artificially converts differentiated cells from one committed lineage to another; notably, these cells need not transit through a stem/progenitor state. The molecular cues guiding such cell fate conversion and reprogramming remain largely unknown. As differentiation and de-differentiation are directly linked to epigenetic changes, we overview cell fate decisions, and associated gene and epigenetic regulations.

Keywords: cell fate; epigenetics; reprogramming; stem cell.

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Introduction

In the years before iPSCs were developed, much research focused on harnessing the pluripotent potential of embryonic stem cells (ESCs) for regenerative medicine. Any stem cell therapy using established ESC lines, however, would require overcoming non-self, immune rejection by the host, so significant effort was put into finding ways to create ESC-like pluripotent cells from a patient's own tissues. In general, the approaches for garnering such patient-specific stem cells followed one of two approaches: find and isolate multi-potent stem cells that already exist in the patient's tissues; or render pluripotent stem cells from patient cells that have already differentiated.

Early research that focused on the first approach (finding multi-potent stem cells) yielded reports that, *in vivo*, stem cells reside in both embryonic and adult tissues (Figure 1 summarizes stem cells derived from different sources). Embryos offer not only ESCs, which are derived from inner cell mass (ICM) of blastocysts, but also epiblast stem cells (EpiSCs), which are pluripotent stem cells in post-implantation staged embryos (1, 2). Determining the best source of stem cells in adult tissues has been less straightforward. In 2002, Verfaillie et al. reported, that bone marrow offers a source of MAPCs (3); however most attempts to reproduce this work ended in failure. Meanwhile, two other papers reported identification of adult-type multi-potential stem cells, i.e. VSEL cells and Muse cells (4–6), but neither type has been widely utilized so far.

The second approach – reverting cells from differentiated tissue to a multi-potent state – led to a series of stem cell research breakthroughs. For example, the cloning of an entire organism by injecting a nucleus from one of its somatic cells into an enucleated, fertilized egg, was a technique originally demonstrated in *Xenopus* in 1962 by Gurdon (7). In higher vertebrates, the technique was first successfully extended to sheep, and later cattle and mice (8–10). Today, somatic cell cloning is an established de-differentiation method; and ESCs obtained from such cloned embryos are termed nuclear transfer (NT)-ESCs (11). This technique has been slow to gain popularity;

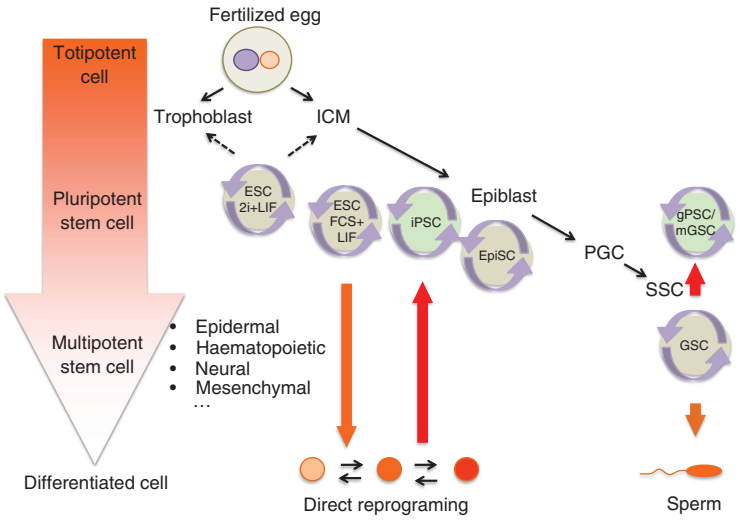

Figure 1 Schematic showing stem cell derivation from various cell types and developmental stages.
Pluripotent stem cells are generated from early embryos (ESCs and EpiSCs) and differentiated cells (iPSCs) and can give rise to three germ layers (ectoderm, mesoderm, and endoderm). gPSCs and mGSCs generated from testis also show ESC-like pluripotent properties (50, 51). Most tissue-specific stem cells found in adult tissues, including epidermal, haematopoietic, neural and mesenchymal stem cells, are already committed to one lineage and exhibit multi-potential. Meanwhile, GSCs can only give rise to sperm and therefore are monopotent. Direct reprogramming renders one differentiated cell population from another through the induction of transcription factors and micro RNAs (see text for details).

however, because the efficiency of obtaining viable offspring is low, the significant technical expertise is needed for even nominal success, and the equipment is costly. In 2006, stem cell biologists worldwide were astounded by Yamanaka's report that he had established iPSCs (12). This enormous breakthrough has since been widely employed not only for studies of regenerative medicine, but also studies of developmental biology and drug screening. Very recently a report claimed that a simple acid treatment could induce de-differentiation in various differentiated cells, rendering multi-potent STAP cells (13, 14). Ultimately, however, difficulties with reproducing the technique eventually led the authors to retract their claims.

Another method of generating desired cell types and tissues was termed 'trans-differentiation,' a technique that purported to allow lineage conversion without obliging cells to first de-differentiate to a stem cell state. Since the first report of trans-differentiation in 1998 (15), several groups reported physiological and post-injury cell conversion from one lineage to another, and an expanding list of publications using this conversion method meant that the term 'trans-differentiation' became widely accepted. The published literature now contains reports of adult bone marrow cells and haematopoietic stem cells generating liver, muscle, neuron and other cell types (16–20). Work with the trans-differentiation technique abruptly ended in 2002: the last rites for the technique were given when two groups independently showed trans-differentiation was a consequence of cell fusion between the transplanted cells and the host's resident cells (21, 22).

The essence of 'trans-differentiation' was revived, however, by Yamanaka's discovery of iPSCs (12). Yamanaka took a new approach – he induced transcription factors not normally expressed in differentiated cells, to acquire pluripotency. Like trans-differentiation, this transcription factor-induction method was capable of generating many different cell types, including cardiac muscle cells, neurons, hepatocytes, and blood cells (23). [It should be noted that, before iPSCs, pioneering work of the previous decade had already showed induction of transcription factors can switch cell fate (24–26)]. These cell fate alterations were directly linked to changes in gene expression profiles and epigenetic modifications that are only now beginning to be understood.

The classical definition of stem and progenitor cells

For different organs, the term 'stem cell' is still vaguely and inconsistently used. 'Stem cells' might mean highly purified, self-renewing cells or a mixture of stem cells plus progenitor cells, or undifferentiated cells. Functionally, stem cells are defined by whether or not they have the capacity for self-renewal. In addition, stem cells are generally

multi-potent, with a few exceptional lineages (e.g., male germ cells, which only produce sperm). Self-renewal activity can only be demonstrated by a transplantation assay, which confirms long-term reconstitution of tissue consisting of multiple cell types. (Serial transplantation of putative stem cells is an even more expedient way to prove a stem cell identity.) Still, long-term reconstitution of tissues by transplantation of putative stem cells has only been performed in a limited number of tissues, e.g., haematopoietic and male germ lineages, because the transplantation assay is not well developed for most adult stem cells. Therefore, alternative approaches are used, such as marker-based study, gene expression profiling, *in vitro* culture (including a colony assay in semiliquid culture), and ectopic transplantation. Stem cells cannot be distinguished by their appearance, so stem cell-specific markers have been intensively characterized for various types of adult stem cells. Markers allow prospective stem cells to be identified, the first step in isolating a stem cell population. Once a subpopulation that contains rapidly repopulating cells is isolated, these can be further characterized by gene expression profiling, which in turn facilitates enrichment of a stem cell population that should ideally be clonal.

Stem cells can be classified into two types: actual stem cells and potential stem cells (27). Potential stem cells are those already fated to differentiate, while still retaining stem cell activity. They are of interest because transplantation and environmental conditions might have the power to change potential stem cells into actual stem cells, suggesting the two types might be interchangeable, with both maintaining intrinsic, stem cell activity. Indeed, cell fate appears to fluctuate in both adult and ESCs, as evidenced by new and continually developing stem cell and lineage markers (28, 29). The existence of a 'metastable' state implies that different layers of status might reside within actual and potential categories.

The path to differentiation, however seemingly short, can be a one-way street. For example, daughter cells of stem cells can lack self-renewal activity but maintain multi-potential activity; these cells are classified either as transient amplifying (TA) cells or progenitor cells. Progenitor cells are physiologically unable to de-differentiate to stem cells, implying that insurmountable barriers can and do exist between two populations.

Self-renewing stem cells derived from different embryonic stages

The source of both ESCs and EpiSCs is the early embryo. Each cell type is from a different developmental stage and so offers its own unique qualities. In mice, ESCs are derived from the ICM of pre-implantation embryos and EpiSCs from the epiblast of post-implantation embryos (Figure 1). To illustrate the difference might require a short primer on early embryogenesis. Briefly, a fertilized egg undergoes cleavage to form blastomeres, which upon compaction form the morula stage embryo. Then the first differentiation process will take place giving rise to the blastocyst composed by the outer cells destined to form extraembryonic tissues and the ICM. The ICM produces the pluripotent epiblast, which will give rise to the embryonic proper and to the hypoblast, which will form extraembryonic tissues (the parietal and visceral endoderm).

Mouse ESCs (which, again, are from the pre-implantation ICM) bear self-renewal, pluripotency, and the capacity to form chimera, but generally will not populate the trophoblast lineage. When cultured in medium containing FCS plus added LIF, mouse ESCs become a heterogeneous mix of cells consisting of both uncommitted and developmentally advanced cells. In other words, ESCs cultured in FCS/LIF are possibly in a metastable state and can become either actual or potential stem cells. Meanwhile, ESCs cultivated in the presence of two inhibitors (2i), which target the ERK and GSK3 signaling pathways, plus added LIF are in a ground state of pluripotency (30, 31). In addition, when GFP expression is driven by a promoter of pluripotency marker genes (e.g., *Nanog*), GFP expression is more homogenous under 2i/LIF than FCS/LIF, suggesting that ESCs grown in 2i/LIF might be closer to actual stem cells. Even more interestingly, a subpopulation of the ESCs grown under 2i/LIF can differentiate into trophoblast and extraembryonic endoderm, suggesting that 2i/LIF support a totipotent state in ESCs (32).

Although both types of ESCs express a number of the same pluripotency markers (i.e., *Oct4*, *Nanog*, *Sox2*, *Esrrb*, *Klf4* and *Tbx3*), each cell type has distinct epigenetic features (33, 34). In 2i/LIF-grown ESCs, the global level of DNA methylation is low (35–37), a state generated by the DNA hydroxylase, Tet1/2, which converts 5-methylcytosine into 5-hydroxymethylcytosine, 5-formylcytosine, and 5-caroxylcytosine through three consecutive oxidation reactions (38). In addition, the DNA hypomethylation status is also maintained by repression of the *de novo* DNA methyltransferases, Dnmt3l and Dnmt3b, but not Dnmt3a, by PRDM14 (39, 40).

With regard to histone modifications, FCS/LIF-grown ESCs have so-called bivalent modifications at a number of developmental loci: here, silent promoters in pluripotent cells carry both active (H3K4me3) and repressive (H3K27me3) marks (41, 42). Although bivalent modification was initially considered typical for pluripotent stem

cells, it turns out that ESCs grown in 2i/LIF carry far fewer bivalent loci (approximately 1000 loci). Furthermore, in ESCs grown under 2i conditions, the polycomb complex (which catalyzes H3K27me3 methylation) does not play a central role in the gene expression silencing (43). Nevertheless, H3K27me3 is indeed critical in more developmentally advanced cells, including ESCs cultured in FCS/LIF. This apparent contradiction might have arisen because the terms 'actual/potential stem cells' and 'metastable state' were coined before more recent findings in epigenetic analysis. Increasingly, evidence suggests that various epigenetic modifications might determine the differences between actual and potential stem cells and the metastable state.

'One step away' from ESCs are EpiSCs that, as mentioned, come from the embryo epiblast post-implantation, rather than pre-implantation. EpiSCs are more differentiated than ESCs, but still retain stem cell activity and pluripotency. EpiSCs can be derived from ESCs by cultivation with activin A and basic fibroblast growth factor (bFGF) (1, 2); conversely, EpiSCs can be converted into ESCs by Klf4 expression (44). Although both ESCs and EpiSCs express pluripotent factors (e.g., Oct4, Nanog, and Sox2), clear phenotypic differences remain: EpiSCs express mesoderm and definitive endoderm transcripts. EpiSCs do not respond to LIF, so for self-renewal require bFGF and activin A (31). EpiSCs differentiate in 2i medium. In 'female' (XX) EpiSCs, one of the two X chromosomes is inactivated (whereas both are active in ESCs). Despite that both cell types form teratomas, EpiSCs do not contribute to normal tissues in mouse chimeras. These phenotypic differences reflect differences in the tissues from which the cell lines were originally derived, so even though both ESCs and EpiSCs are self-renewing stem cells that maintain the capability to differentiate into the three germ layers, ESCs grown in 2i/LIF are considered naïve pluripotent stem cells while EpiSCs are considered primed pluripotent stem cells.

In comparing naïve vs. primed pluripotent cells, recent RNA sequencing analysis identified genes expressed in each: naïve cells express *Esrrb*, *Zfp42*, *Dppa3* and *Klf4*, while primed cells express *Fgf5*, *Cer1* and *Lefty1* (34). Moreover, at the naïve to primed transition, pluripotency-enriched gene enhancers reorganize. Strikingly, RNA analysis also unveiled that fewer than 6% of the total transcripts differ significantly between the naïve and primed states. Digging deeper, however, showed this equivalent expression level in different states can be controlled by different cis-regulatory sequences. In naïve cells, naïve-dominant enhancers are heavily marked by H3K4me1 and H3K27ac; these marks only weakly label 'seed enhancers'. In primed cells, the seed enhancers acquire high levels of H3K4me1 and H3K27ac modifications, and then expand to form multi-enhancer clusters called 'super enhancers' (34). Super enhancers are key for maintaining cellular identity (45, 46). Even more interestingly, while the expression level of Oct4 is equivalent in ESCs and EpiSCs, two groups found that at the transition from ESCs to EpiSCs, Oct4 switches binding partners (33, 34). In naïve ESCs, Esrrb, Klf5 and Tcf3 interact with Oct4, whereas in EpiSCs, Otx2, Zic2 and Zfp281 interact with Oct4. This change probably withdraws Oct4 from naïve-specific enhancers, redirecting it to other enhancers, at loci implicated in the development of post-implantation epiblast. Despite these intriguing findings and the ever-expanding knowledge about the epigenetic characteristics of ESCs and EpiSCs, the molecular mechanisms that establish stemness cannot be fully explained.

Adult-type stem cells and progenitor cells

In contrast to the embryo-derived stem cells, various types of mammalian tissues harbor adult-type tissue-specific stem cells. One well-studied example is testis-specific spermatogonial stem cells (SSCs), which support a continued production of sperm through self-renewal and differentiation (47, 48). In the mouse, SSCs originate from primordial germ cells (PGCs) that develop in the genital ridges of embryos. After seminiferous cords are formed, the germ cells that reside inside the cords show different morphology from PGCs and are called gonocytes. After birth, gonocytes give rise to spermatogonia, including SSCs, a subpopulation of which is destined to differentiate and enter meiosis. Neonatal spermatogonia provide a source of cells for the long-term culture of spermatogonia *in vitro* (49). These cells, termed germline stem cells (GSCs) can be maintained for a few years without losing stem cell activity. Furthermore, a population of GSCs can de-differentiate and become ESC-like cells called mGSCs and gPSCs (50, 51) (Figure 1). Type A spermatogonia consist of single (A_s) cells, as well as two paired cells connected by an intercellular bridge (A_{pr}), or aligned cells of more than two interconnected cells (A_{al}) (52, 53) (Figure 2). This interconnection is a result of incomplete cytokinesis. The 'A_s model' proposed that only A_s cells are SSCs (54, 55); however, our recent data noted putative spermatogonial differentiation pivots at a dynamic, epigenetic switch point. This switch did not occur at the A_s to A_{pr} transition, but rather at the A_{al} to A_1 transition, a point at which the cells begin to express the spermatogonial differentiation marker Kit (56). Concomitant with the onset of Kit

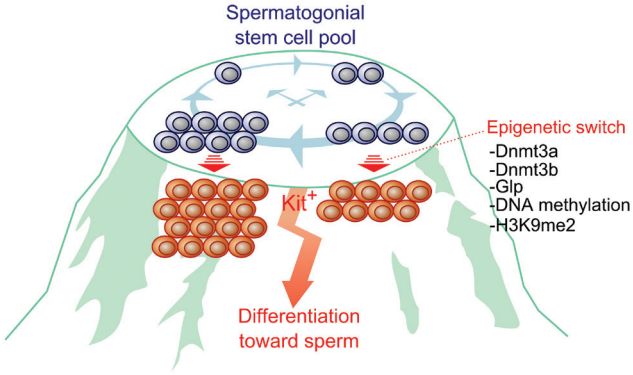

Figure 2 The epigenetic switch model for spermatogonial stem cell differentiation.
Type A spermatogonia consisting of A_s, A_{pr} and A_{al} cells exhibit equivalent epigenetic features and comprise a pool of stem cells (at the top of a flat-topped mountain in Figure 2) (56). Syncytial fragmentation supports continual interconversion between A_s and connected chains of spermatogonia, which contributes to maintenance of the stem cell pool (57). A major epigenetic switch triggers the cells to exit from the stem cell pool and differentiate (the cells leave the 'mountain top' on a one-way path to differentiation). As they emerge from the pool, differentiating spermatogonia display distinct epigenetic features (with high expression of Dnmt3a, 3b and Glp, as well as altered patterns of DNA methylation and H3K9me2) and express Kit (see text for details).

expression, the major DNA methyltransferases, Dnmt3a2 and Dnmt3b, and a component of histone H3K9 methylating complex, Glp, show a substantial increase. This is accompanied by the increase (as assessed by immunohistochemistry) in a global level of H3K9me2. These data suggest that A_s, A_{pr} and A_{al} spermatogonia comprise a stem cell pool and that the epigenetic switch triggers the exit from stemness (Figure 2). Consistent with this observation, another report suggests that connected chains of spermatogonia can also contribute to stem cell function through syncytial fragmentation (57).

These epigenetic properties of the male germ cell lineage might illuminate the nature of stem cells in other tissues. In skin epithelial tissues for example, bulge stem cells were initially believed to contribute to the entire epithelium during steady-state homeostasis in epidermal stem cells; however, updated lineage tracing and time-lapse experiments showed that stem cells are more heterogeneous and are detected in the hair follicle bulge, sebaceous gland, lower isthmus, and upper isthmus (58). Thus, the epidermis seems to be regionally compartmentalized into functional units that are maintained autonomously by independent stem cells. Although each type of stem cell might express different stem cell markers, they possibly share epigenetic modifications characteristic of stem cells, so this information about stem cells in the epidermis might enable us to identify stem cells in other tissues.

In fact, the data implicates epigenetic changes as a key feature of artificially generated stem cells also, as specific methods can reverse the *in vivo* differentiation process. For example early experiments in somatic nuclear cloning demonstrated that the genome in a differentiated nucleus can be effectively reprogrammed (59–61). Yamanaka's method for generating iPSCs – reprogramming by introducing four transcription factors: *Oct4*, *Sox2*, *Klf4* and *c-Myc* (OSKM) – put the tools for reprogramming into the hands of experimentalists (12). Nevertheless many questions remain surrounding the molecular mechanisms controlling this phenomenon.

Meanwhile, epigenomic analyses have highlighted important elements of efficient reprogramming. Efficient reprogramming can be blocked by the repressive histone H3K9 methylation (62–64). Accordingly, reducing levels of H3K9 methyltransferases or overexpressing H3K9 demethylases can improve the efficiency of generating iPSCs. These results suggest that, as cells lose pluripotency and differentiate, the levels of repressive histone marks such as H3K9me3 and H3K27me3 increase, extending the repressive genomic regions.

Histone modification comes into play because chromatin conformation must be properly remodeled for cells to acquire pluripotency. Remodeling is in part facilitated by the ATP-dependent, BAF chromatin-remodeling complex, which may also contribute to locus-specific epigenetic changes (65). Moreover, when Mbd3, a core component of the Mbd3/NuRD (nucleosome remodeling and acetylation) complex, was depleted, efficiency of iPSC generation by OSKM reached ~100% (66). Mbd3 likely silences genes encoding core pluripotency factors, so loss of Mbd3 activates these genes, rendering them into an active and open-chromatin state (with high levels of H3K4me3 and H3K27 acetylation, and low levels of H3K27me3).

DNA methylation serves as another barrier that must be overcome to achieve efficient reprogramming. Upon induction of OSKM, an elevated level of the Tet2 gene induces global hydroxylation of 5mC (67). This likely helps promoters of pluripotency genes, such as *Nanog* and *Esrrb*, to lose methylation and become activated. This feature provides another layer of epigenetic control, which acts in concert with the others (listed above) to regulate key reprogramming.

Direct reprogramming as a new de-differentiation approach

Reports of Yamanaka's method spurred research into a new type of trans-differentiation approach called direct

reprogramming, whereby fully differentiated cells might be converted to other cell types (Figure 1). By virtue of our accumulated knowledge about developmental biology, direct reprogramming was achieved through identification of key transcription factors as well as growth/differentiation factors found to guide pluripotent stem cells to become the desired *functional* differentiated cells. Direct reprogramming methods can be classified in three different types: trans-differentiation, within one lineage; cross over differentiation, from one lineage to another but within the same germ layer; or cross differentiation, from one germ layer to another.

A key series of studies by Graf's group showed that B cells, myeloid cells, erythroid cells and monocytes can convert into one another by first traversing an undifferentiated state within the blood lineage (25, 26, 68). This type of differentiation was coined 'trans-differentiation'.

An example of cells from the same germ layer crossing from one lineage to another is given by rat fibroblasts induced to express the transcription factors Gata4, Mef2c and Tbx5, which give rise to cardiac cells (69–71). In another combination, myocardin, Gata4, Hand2, Mef2c, and Tbx5, together with two micro RNAs miR-1 and miR-133, can also render cardiomyocytes from fibroblasts (72). Mouse fibroblasts can also be committed to a hemogenic program by induced expression of four transcription factors, Gata2, Gfi1b, cFos and Etv6 (73).

Finally, others have induced cells to switch from one germ layer to another. For example, Wernig's group showed that forcing expression of Brn2, Ascl1, and Myt1l induces mouse and human fibroblasts to become neurons (74, 75). Highly functional human liver cells, an endodermal lineage, have been generated from fibroblasts by induced expression of either Hnf4a/Hnf1a/Foxa3 with SV40 large T antigen, or Hnf1a/Hnf4a/Hnf6/Atf5/Prox1/Cebpa with siRNAs for p53 and c-Myc (76, 77). Direct reprogramming techniques are rapidly gaining popularity; however the details of chromatin architecture remain a black box, leaving many questions about how, as cells differentiate, expression profiles are reset and reprogrammed by a handful of transcription factors/micro RNAs. A key facet of direct reprogramming is that somatic cells are converted from one lineage to another without traversing a pluripotent state. Reprogramming differentiated cells to a dedifferentiated state (tissue-specific stem/progenitor cells) is a similar and even more attractive technique. This is because such cells can generate differentiated cells as well as different types of daughter cells, attributes more suitable when attempting to reconstitute entire tissues. Induced neural stem cells (iNSC) provide one example of stem cells generated by the combination of either Brn4, Sox2, Klf4, c-Myc and E47/Tcf3 (78) or Brn2, Sox2 and FoxG1 (79), but other examples of stem/progenitor cell generation from fibroblasts are, so far, very limited.

Expert opinion and outlook

As stem cell research is directly linked to regenerative medicine, recent progress in genetic manipulations and information about epigenetic landscapes and gene expression profiles provide resources for cell transplantation, drug screening and analyzing pathogenesis in rare diseases. The progress makes it possible for stem cell researches to step toward the practical use of reprogrammed cells in clinical fields. At the same time, it should be noted that corroborative evidence that supports the phenomena introduced here is not fully elucidated yet. Understanding physiological programming and artificial reprogramming, at the molecular level, is necessary before we can be confident in generating functional and safe materials for regenerative treatments. As yet, unresolved difficulties hinder transfer of experimental accomplishments from the bench to the bed. For example, any method introduced here needs a certain period of *in vitro* cell culture at the conversion step, so it remains difficult to circumvent culture-associated gene aberrations such as mutations, copy number variations and translocations.

Most de-differentiation experiments started with mouse embryonic fibroblasts, but fibroblasts obtained from adult or human tissues might yield different outcomes. Thus, functional integrity of induced cells must be strictly evaluated. For regenerative medicine applications, appropriate criteria must undoubtedly be set for the use of each tissue and/or cell type. Clearly, our efforts must and will continue to elucidate the corroborative molecular mechanisms of the cell fate decision.

Highlights

- Pluripotent stem cells can be harvested from live tissues and propagated under appropriate culture conditions.
- ESCs and EpiSCs are powerful tools for analyzing embryonic stem cell properties.
- Seed enhancers and Oct4 binding partners play pivotal roles in determining cellular identities of ESCs and EpiSCs.

- Induced expression of key transcription factors triggers cell fate conversion.
- Differentiated cells can be converted to pluripotent stem cells.
- Introducing particular combinations of transcription factors into differentiated cells can redirect cell fate.
- Future studies need to unveil the molecular mechanisms that determine cellular identity and cell fate.

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List of abbreviations

ATP	adenosine 5′-triphosphate
Dnmt	DNA methyltransferase
EpiSC	epiblast stem cell
ERK	extracellular signal-regulated kinase
ESC	embryonic stem cell
FCS	fetal calf serum
GFP	green fluorescent protein
gPSC	germline-derived pluripotent stem cell
GSC	germline stem cell
GSK3	glycogen synthase kinase 3
ICM	inner cell mass
iPSC	induced pluripotent stem cell
LIF	leukemia inhibitory factor
mGSC	multipotent germline stem cell
MAPC	multi-potent adult progenitor cell
Muse	multilineage-differentiating stress-enduring
PGC	primordial germ cell
STAP	stimulus-triggered acquisition of pluripotency
SSC	spermatogonial stem cell
VSEL	very small embryonic-like

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