

Dedifferentiation: A New Approach in Stem Cell Research

SA CAI, XIAOBING FU, AND ZHIYONG SHENG

Dedifferentiation is an important biological phenomenon whereby cells regress from a specialized function to a simpler state reminiscent of stem cells. Stem cells are self-renewing cells capable of giving rise to differentiated cells when supplied with the appropriate factors. Stem cells that are derived by dedifferentiation of one's own cells could be a new resource for regenerative medicine, one that poses no risk of genetic incompatibility or immune rejection and provokes fewer ethical debates than the use of stem cells derived from embryonic tissue. Until now, it has not been quite clear why some differentiated cell types can dedifferentiate and proliferate, whereas others cannot. A better understanding of the mechanisms involved in dedifferentiation may enable scientists to control and possibly alter the plasticity of the differentiated state, which may lead to benefits not only in stem cell research but also in regenerative medicine and even tumor biology. If so, dedifferentiation will offer an ethically acceptable alternative route to obtain an abundant source of stem cells. Dedifferentiation is likely to become a new focus of stem cell research. Here we compile recent advances in this emerging but significant research, highlighting its central concepts, research findings, possible signaling pathways, and potential applications.

Keywords: cells, stem cells, dedifferentiation, regeneration, signaling pathways

Repair and regeneration are universal phenomena in the biological world, but the capacity for regeneration varies considerably among species. Some invertebrates, such as earthworms, readily regenerate two new and genetically identical individuals when they are cut in half. Amphibians such as newts can regenerate whole limbs, retinas, eye lenses, spinal cords, and tails, as well as upper and lower jaws. Both invertebrates and amphibians replace lost or damaged organs and tissues with new ones that are identical in structure or function to the original, in a process called complete regeneration. For mammals, the situation is apparently different. In most instances, mammals are capable only of incomplete regeneration: Although the function of the organ may be recovered, the structure will not be restored after part of the tissue has been damaged within a specific organ.

Two cellular mechanisms are involved in endogenous regeneration: morphallaxis, or renewal of lost tissue, and epimorphosis, or regeneration of a piece of an organism by way of cell proliferation on the cut surface. Both necessitate the participation of stem cells or other progenitor cells. During morphallaxis, the remaining undifferentiated cells simply migrate to the site and differentiate into the specialized cells, with little cellular proliferation. Epimorphosis, however, requires preexisting stem cells or dedifferentiation-generated progenitor cells to proliferate, differentiate, and finally replace the lost cells. The process by which the hydrozoan cnidarian *Hydra* regenerates a severed tentacle illustrates morphallaxis. The urodele amphibians, in contrast,

uniquely use a cellular dedifferentiation mechanism to regenerate a variety of tissues.

For mammals, epimorphic regeneration is largely limited by an irreversible differentiation process, although it has been demonstrated that stem cells are activated during the regeneration of muscles, bones, epithelia, and some other tissues (Ding and Schultz 2004). Because it is difficult for mammals, especially humans, to repair and regenerate damaged and diseased tissues, organ transplantation has been developed in recent years to replace damaged or diseased organs. But the limited number of donors of compatible organs and the host-versus-graft reaction are still hurdles that prevent the widespread use of this therapeutic measure. Therefore, three strategies have been put forward to improve the regenerative capacity of mammals: stem cell transplantation, cell-seeded scaffold embedding (tissue engineering technology), and local induction of dedifferentiation in mature cells (Odelberg 2002).

Sa Cai works at the Wound Healing and Cell Biology Laboratory, Institute of Basic Medical Science, Trauma Center of Postgraduate Medical College, Chinese PLA General Hospital, Beijing 100853, People's Republic of China, and at the Burns Institute at the Chinese PLA General Hospital. Xiaobing Fu (e-mail: fuxiaobing@vip.sina.com) is a professor at the Wound Healing and Cell Biology Laboratory at the Chinese PLA General Hospital; he also works at the Burns Institute. Zhiyong Sheng is also with the Burns Institute. © 2007 American Institute of Biological Sciences.

Stem cells are self-renewing, nondifferentiated cells with the ability to differentiate into a number of specialized phenotypes in response to appropriate signals (Körbling and Estrov 2003). They are found in embryonic and adult tissues, and thus are traditionally classified as embryonic stem cells and adult stem cells, also known as tissue-specific stem cells. Because of their great potential for differentiation, stem cells have attracted much attention as a therapeutic measure for the treatment of several conditions, including cardiovascular diseases, neurodegenerative diseases, musculoskeletal diseases, diabetes, and aging. There are still many obstacles to overcome, however, before stem cells can be used in cell replacement therapy. Among them are a lack of stable and efficient techniques to induce a specified differentiation, the limited sources for stem cells, immune rejection, and ethical issues, to name a few. It is imperative to find a clinically useful and ethically acceptable way to obtain stem cells.

Most of the problems associated with stem cells could be averted if the process of dedifferentiation could be induced. Obtaining cells able to multiply and develop into a specific tissue would have advantages over cell transplantation and cell-seeded scaffolds (Odelberg 2002). First, the employment of endogenous cells during this process should preclude immune rejection. Second, the remaining cells of the damaged tissues, or the surviving cells adjacent to the injury, may undergo dedifferentiation; these dedifferentiated cells may have been primed to redifferentiate into the appropriate cell types, as occurs during regeneration in newts (Davenport 2005). Third, the use of the cells of patients themselves instead of embryonic stem cells to regenerate damaged tissues is less likely to provoke ethical disputes. Thus, dedifferentiation might be an ethically acceptable route for reaping an abundant source of stem cells, without the risk of genetic incompatibility and tissue rejection. In this overview, we compile recent advances in the research on dedifferentiation of mature lineage-committed cells into stem cells or progenitor cells, highlighting the important biological phenomena and their possible mechanisms.

What is dedifferentiation?

A single multicellular organism consists of a variety of different cell types. All of these cells are derived from a zygote through serial processes of division and differentiation. During the early development of an organism, a certain cell type divides, and then its descendants gradually form stable differences in morphology, structure, and function, finally producing numerous different cell phenotypes. This process, differentiation, is the fundamental basis for development. During animal development, cells become progressively constrained to a certain cell type, and the capacity to respond to a variety of differentiation signals declines after each generation. In other words, the process of differentiation gradually reduces the potential of a cell. In the final stage of differentiation, the cell is thought to stop dividing permanently.

There is now much evidence demonstrating the remarkable ability of some differentiated cells to convert into a

completely different phenotype. The conversion of one cellular phenotype to another is referred to as *transdifferentiation* (Li et al. 2005). The most commonly accepted examples of transdifferentiation are limb regeneration in amphibia, and the conversion of pigment epithelia into lens and neural retinal cells (Eisenberg and Eisenberg 2003). In fact, the transdifferentiation process undergoes two phases in succession. In the first phase, the differentiated cells revert to cells with an apparent stem cell or progenitor cell phenotype. In the second phase, these dedifferentiated cells redifferentiate into the new, differentiated cell phenotypes. Other instances of transdifferentiation include the conversion of pancreatic cells to hepatocytes (Shen et al. 2003) and of vascular endothelium to smooth muscle (Frid et al. 2002). All of these experimental findings point to a dedifferentiation–redifferentiation model rather than to direct transdifferentiation.

Dedifferentiation is a process by which cells develop in reverse, from a more differentiated to a less differentiated state. The phenomenon can be observed at the levels of gene, protein, morphology, and function. At the genetic level, the cell undergoes reversion from a differentiated cell gene expression profile to a progenitor cell gene expression profile. During the dedifferentiation process, development-related gene activity is repressed, and genes that keep the cell in the undifferentiated state are activated. Dedifferentiation can also be observed at the protein level, as evidenced by the up-regulation of progenitor cell–related proteins and the down-regulation of differentiated cell–related proteins. During dedifferentiation, cells also experience morphological changes: Compared with mature cells, dedifferentiated cells are smaller, have fewer organelles, and have a higher karyoplasmic ratio. Finally, at the functional level, the cell regains the capacity to proliferate, which means that a postmitotic cell can reenter the cell cycle. Meanwhile, mature cells or lineage-committed cells might become multipotent or pluripotent progenitor cells with the potential of differentiating into a variety of cell types.

Changes at all four levels occur during dedifferentiation. Although many researchers have declared that they have observed the phenomenon of dedifferentiation, their findings were based on just one or two of the manifestations listed above. A definitive, well-accepted criterion for dedifferentiation has yet to be established. However, in the quest to understand the dedifferentiation process in a variety of cell types and species, researchers are identifying elements that may turn out to be common to the process and may, in the future, serve as a definitive marker for dedifferentiation.

Dedifferentiation in animal lives

Dedifferentiation can be observed in different tissues and organs of plants (Krikorian et al. 1981, Zhao et al. 2001, Grafi 2004), invertebrates, amphibians, and animals, including humans (table 1).

Dedifferentiation in amphibians. As stated earlier, some amphibians have the ability to regrow damaged organs and lost body parts, such as a limb or tail (Davenport 2005). It has been

Table 1. Representative examples of dedifferentiation in various organisms.

| Taxon | Cell type | Example of dedifferentiation | Stimuli | Phenomena | Signaling mechanisms |
|-----------|------------|---|--|--|--|
| Plant | Mesophyll | Cells of tobacco leaves | Removal of cell wall | Pluripotency, reentry into S phase, capacity for redifferentiation | Two phases of chromatin decondensation |
| Amphibian | Various | Regeneration of newt limb, jaw, tail, retina, lens, and spinal cord | Amputation, removal, injury | Loss of the characteristics of origin, production of blastema, expression of stem cell markers, redifferentiation to reconstitute lost organs | NA |
| Mammal | Myoblast | C2C12 mouse cells, adult human myoblasts | Extracellular signals (serum, limb regeneration extract, reversine, CNTF) | Down-regulation of MRFs, reentry into cell cycle, formation of mononucleated cells, presentation of new phenotypes | p44/p42 MAPK |
| Mammal | Neuron | Schwann cells, oligodendrocyte precursor cells | Injury, extracellular signals | Acquisition of pluripotency, expression of neural stem cells, redifferentiation to different cell types | ERK MAPK |
| Mammal | Renal | RPTC | Ischemia/reperfusion oxidant injury | Undergoing EMT, expression of mesenchymal cell marker, acquisition of dedifferentiated phenotype (elongated morphology) | p38 MAPK |
| Mammal | Epithelial | Corneal and skin epithelium | Recombination of epithelium and dermis (corneal epithelium), healing wound (skin epithelium) | Acquisition of limbal basal cell mesenchymal cell marker, loss of the expression of corneal-specific keratin, redifferentiation to form hair follicle and epidermis, appearance of stem cell-like islands in the suprabasal layer, expression of stem cell markers | Wnts, BMP/Noggin |
| Mammal | Germ | Sertoli cells | Heat treatment | Acquisition of undifferentiated phenotype, expression of ck18, cessation of spermatogenic activity | ERK MAPK |

BMP, bone morphogenetic protein; CNTF, ciliary neurotrophic factor; EMT, epithelial–mesenchymal transition; ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase; MRF, myogenic regulatory factor; NA, not applicable; RPTC, renal proximal tubule cell.

confirmed that this process involves the phenotypic reversion of fully differentiated cells, proceeding through dedifferentiation, proliferation, and redifferentiation to form the required tissue or organ. After limb amputation in the newt, for example, epithelial cells begin to migrate and gradually form a mature apical epithelium cap (AEC; McGann et al. 2001). The internal stump cells underlying the AEC lose their tissue characteristics and dedifferentiate in response to undefined signals. The dedifferentiated cells proliferate for a short time to form the blastema—a mass of progenitor or pluripotent cells—and then redifferentiate to build a replica of the lost part of the limb. In addition, after tail amputation, the ependymal tube, which resembles a developing neural tube, expresses neural stem cell markers that are undetectable in a normal urodele spinal cord. This up-regulation of neural stem cell markers suggests the occurrence of a dedifferentiation process in the spinal cord in response to injury (Walder et al. 2003). The same process is also found in the regeneration of the salamander jaw (Ghosh et al. 1994). During regeneration of the newt retina or lens after its removal, the pigment epithelial cells of the retina or dorsal iris, respectively, lose their characteristic pigmentation and dedifferentiate to form nonpigmented precursor cells, which in turn redifferentiate to rebuild the lost tissues (Kodama and Eguchi 1995, Ito et al. 1999).

Dedifferentiation in mammals. A growing number of studies indicate that when exposed to appropriate signals, certain

mammalian cell types can be induced to dedifferentiate to progenitor cells and generate different types of functional cells for the repair of damaged tissues.

Dedifferentiation in myoblasts. Adult skeletal myoblasts are lineage-committed cells that either are self-renewing or differentiate into multinucleated myotubes (Jankowski et al. 2002, Tamaki et al. 2003). Myoblast fate is mainly dependent on muscle differentiation proteins known as myogenic regulatory factors. Serum stimulation was previously found to induce newt myotubes, but not their mammalian counterparts, to reenter the cell cycle.

In a study by Velloso and colleagues (2001), researchers grew newt myoblasts and mouse myoblasts together, and induced them to differentiate to form myotubes. During the process, some of the differentiating mouse cells fused with differentiating newt cells to become hybrid myotubes. This culture mixture containing hybrid cells was then stimulated by serum. After induction, the newt nuclei began to synthesize DNA as expected. Simultaneously, some of the mouse nuclei also initiated DNA synthesis, which indicated that newt myotubes produced some factor that made the mouse myotube nuclei competent to respond to serum. When mouse myotubes were treated with an extract derived from the regenerating limbs of newts, the myotubes would dedifferentiate, as evidenced by their ability to reenter the cell cycle, exhibit a reduction in muscle differentiation proteins, and cleave to form smaller myotubes or mononucleated cells (McGann et al.

2001). Subjecting the extract to a variety of chemical or physical treatments, such as lipid removal, freeze–thaw cycling, boiling, and trypsin digestion, indicated that the dedifferentiation signal within the extract is a protein.

Using myotubes derived from the C2C12 mouse cell line, Odelberg and colleagues (2000) found that as yet unidentified serum factors could reduce nuclear muscle proteins to undetectable levels, and that most of the myotubes cleaved to produce either smaller multinucleated myotubes or proliferating mononucleated cells. Furthermore, the myotube-derived multipotent cells could be induced to redifferentiate into cells expressing chondrogenic, adipogenic, myogenic, and osteogenic markers. These data suggest that mammalian myotubes can dedifferentiate when stimulated with appropriate factors, as evidenced by reentry into the cell cycle, reduction of differentiation proteins, morphological change, or acquisition of multipotency.

The identification of small molecules that induce dedifferentiation of somatic cells would help to elucidate the molecular mechanism of this process and may finally allow researchers to regenerate tissues *in vivo* (Chen et al. 2004). It was previously considered a breakthrough in the study of dedifferentiation when it was demonstrated that treatment of mouse myotubes with the microtubule-binding purine known as myoseverin would induce the myotubes to cleave to form mononucleated cells, which could proliferate and redifferentiate into myotubes (Rosania et al. 2000, Duckmanton et al. 2005). But other researchers held different opinions about this conclusion. They suspected that the effect of myoseverin was most likely the result of cytoskeleton remodeling, not true dedifferentiation. They also believed that the bona fide dedifferentiation-inducing molecule should allow the lineage-reversed myoblasts to regain multipotency and to acquire the ability to differentiate into multiple cell lineages under appropriate induction conditions. On this premise, Chen and colleagues (2004) performed experiments that employed a different approach. They treated C2C12 cells with a library of 50,000 discrete small molecules to induce dedifferentiation, and then transferred these cells either to a medium containing osteogenesis-inducing substances or to a medium containing adipogenesis-inducing substances. In the end, they discovered a 2,6-disubstituted purine that could induce myogenic lineage-committed cells to become multipotent cells with the capacity to proliferate and redifferentiate into bone and fat cells. They named the molecule “reversine” because of its ability to reverse terminally differentiated cells to progenitor cells, which in turn were able to redifferentiate into different cell types.

Besides the many investigations conducted on rodent myoblasts, researchers have now conducted studies on the dedifferentiation of human skeletal myoblasts. Ciliary neurotrophic factor (CNTF) was previously found to play an important role in regulating many processes within the nervous system. Recent studies indicate that the CNTF receptor can be highly expressed in skeletal muscle. Chen and colleagues (2005) investigated the direct effect of CNTF on

skeletal myoblasts of the adult human and, surprisingly, found that CNTF induced the myogenic lineage-committed myoblasts to dedifferentiate into multipotent progenitor cells. These dedifferentiated cells not only could proliferate for more than 20 passages without the expression of myogenic-specific factors but also could differentiate into new phenotypes, mainly neurons, glial cells, smooth muscle cells, and adipocytes. These findings are of extraordinary importance for regeneration research, suggesting that these molecules might be used for therapeutic applications in the future. Furthermore, the formation of multipotent progenitor cells from differentiated cells can provide helpful information to elucidate the molecular mechanism of dedifferentiation in the process of regeneration.

Dedifferentiation in neurons. Many cell types that are produced continuously, such as some epithelial cells and hematopoietic cells, come from a slowly dividing population of stem cells, which give rise to rapidly dividing precursor cells and then undergo terminal differentiation. Other cell types, however, such as Schwann cells, tend to divide infrequently. Following specific challenges, such as injury, Schwann cells undergo a different process to produce new cells, perhaps involving dedifferentiation.

Schwann cells belong to a regenerative cell type. Following nerve injury, a differentiated, myelinating Schwann cell can dedifferentiate, regain the potential to proliferate, and then redifferentiate during the repair process. Harrisingh and colleagues (2004) found that sustained activation of Ras/Raf/ERK (extracellular signal-regulated kinase) signaling triggered the dedifferentiation of Schwann cells, which was indicated by switching off the characteristic proteins of differentiated Schwann cells (e.g., peripheral myelin protein 22 and myelin basic protein) and reducing the levels of transcriptional factors that regulate the expression of Schwann cell genes. Kondo and Raff (2000) also showed that certain extracellular signals could induce oligodendrocyte precursor cells to revert to multipotential neural stem cells, which could self-renew and give rise to neurons and astrocytes, as well as to oligodendrocytes. These findings have important implications for the understanding of the nerve regenerative process.

Dedifferentiation in renal cells. In contrast to the heart or brain, the kidney can completely recover from an ischemic or toxic insult resulting in cell death. When the kidney recovers from ischemia or reperfusion injury, it relies on a sequence of events that include epithelial cell spreading, and possibly migration, to cover the exposed areas of the basement membrane, followed by cell dedifferentiation, proliferation, and redifferentiation (Thadhani et al. 1996). Following injury, cells in the mature kidney begin to dedifferentiate, as evidenced by the production of vimentin, a protein that is typically expressed in the mesenchymal cells that give rise to the kidney cells (Witzgall et al. 1994). Likewise, the neural cell adhesion molecule, which is expressed in metanephric mesenchyme but not in mature kidneys, is abundantly expressed in kidney cells after injury (Abbate et al. 1999). After exposure to hydrogen peroxide stimulation, the surviving kidney cells

acquired a dedifferentiated phenotype (i.e., elongated in morphology with expression of vimentin), and the epidermal growth factor (EGF) receptor and p38 were activated (Zhuang et al. 2005). In addition, adhesion molecules, such as integrins (Bokel and Brown 2002, Zuk and Matlin 2002), as well as cytokines and chemokines, including tumor necrosis factor alpha, interleukin 1 beta (IL-1 β) (Chung 2001), and transforming growth factor beta (TGF- β) (Humes et al. 1993), may play important roles in the regulation of the repair process of migration–dedifferentiation–redifferentiation. Better understanding of all the mediators and mechanisms involved in dedifferentiation and proliferation of the renal tubule epithelial cells will lead to novel approaches to therapies designed to facilitate the recovery of renal histology and function in humans.

Dedifferentiation in epithelial cells. In ocular tissue, corneal stem cells and differentiated epithelial cells are located in different sites, with stem cells in the limbus forming a ring of tissue around the central cornea, and epithelial cells in the central area. The stem cells proliferate, migrate centripetally, and differentiate into the terminal corneal epithelial cells that replace the cells shed in the central cornea. When Pearton and colleagues (2004, 2005) performed the recombination between wild-type mouse embryonic dermis and adult rabbit central corneal epithelium, they found that the differentiated cells of the corneal epithelium dedifferentiated and reverted to a limbal basal cell phenotype. This process was confirmed to be triggered by dermal developmental signals, such as Wnt/ β -catenin and BMP (bone morphogenetic protein)/Noggin signaling.

In our laboratory, we have done a significant amount of work in dedifferentiation and regeneration. This work includes a relatively complete system for the isolation, culture, and identification of certain stem cells. We have also induced these stem cells to differentiate into many cell types, both *in vitro* and *in vivo*, and explored the possibility of enhancing the healing and regeneration of skin injury by autotransplantation (Fang et al. 2004, Fu et al. 2004). We have obtained evidence of the dedifferentiation of epithelial cells to stem cells or stem cell–like cells *in vivo* (Fu et al. 2001a, 2001b). It is possible that recombinant human epidermal growth factor serves as a dedifferentiation signal, inducing cell reversion to form stem cell–like islands. We obtained further evidence to demonstrate this dedifferentiation phenomenon. Human split-thickness epidermal sheets devoid of basal cells were transplanted onto full-thickness skin wounds in immunodeficient BALB/c nude mice. Immunohistochemical examination of the skin grafts showed that a significant number of cells were positive for stem cell markers in the suprabasal layer after transplantation. The results collectively indicated that some of the differentiating cells in grafted epidermal sheets might have dedifferentiated into stem cells or stem cell–like cells, and the result offered us new evidence of and insights into the dedifferentiation of human epidermal cells.

Dedifferentiation in germ cells. Although induction of dedifferentiation could be useful in tissue regeneration, it

might lead to some pathological processes. Under certain situations, then, blockage of dedifferentiation might be beneficial in preventing pathological events.

Mature Sertoli cells are the primary supportive cells of seminiferous epithelium and play an important role in regulating the spermatogenic process (de Kretser et al. 1998). Sertoli cells with immature characteristics have been found in the adult human testes in some pathological conditions associated with impaired spermatogenesis (Steger et al. 1996, 1999, Maymon et al. 2002). Cytokeratin-18 (CK-18) is expressed only in immature Sertoli cells and is normally lost at puberty (Stosiek et al. 1990). Zhang and colleagues (2004) demonstrated in the rhesus monkey that cryptorchid testis could induce expression of CK-18 in Sertoli cells coincidentally with a cessation of spermatogenic activity, which indicated that body temperature was capable of inducing adult Sertoli cells to revert to a dedifferentiated immature state and of destroying their supportive role in normal spermatogenesis. To confirm this finding, they did further studies and found that local testicular heat treatment of an adult monkey at 43 degrees Celsius ($^{\circ}$ C) was capable of activating re-expression of CK-18 in Sertoli cells. The heat treatment of the primary Sertoli cell culture isolated from the adult monkey testis at 43 $^{\circ}$ C could also induce CK-18 reexpression. These results from *in vivo* and *in vitro* experiments demonstrated that heat treatment of adult monkey Sertoli cells was capable of inducing a reversible change in the Sertoli cells from an adult differentiated state to an immature dedifferentiated state (Zhang et al. 2006).

Signaling pathways involved in dedifferentiation

Although the signaling pathways involved in dedifferentiation have not been completely identified, increasing evidence has shown that the following signaling pathways may play some critical role in these important phenomena (figure 1).

MAPK signaling pathway. Mitogen-activated protein kinases (MAPKs) are serine/threonine kinases that transmit signals from extracellular stimuli to multiple substrates involved in cell growth, differentiation, and apoptosis. Three major subfamilies of MAPKs have been identified: ERK, c-Jun N-terminal kinase (JNK), and p38. Most likely, ERK is highly responsive to mitogen stimulation (e.g., by growth factors), while JNK and p38 are activated by a variety of genotoxic stresses, including cell cycle arrest, DNA repair, and apoptosis (Roux and Blenis 2004). Renal tubular cell dedifferentiation, namely, epithelial–mesenchymal transition (EMT), is thought to be a prerequisite for regenerative proliferation and migration after renal injury. The signaling mechanism of EMT induced by TGF- β and the EGF receptor involves p38, and inhibition of p38 activity can prevent EMT. In addition, ERK activity in differentiated chondrocytes is dramatically increased during the course of dedifferentiation. The process is blocked after the activation of ERK has been inhibited (Yoon et al. 2002). Likewise, ERK signaling pathway activation correlates with dedifferentiation of smooth muscle cells.

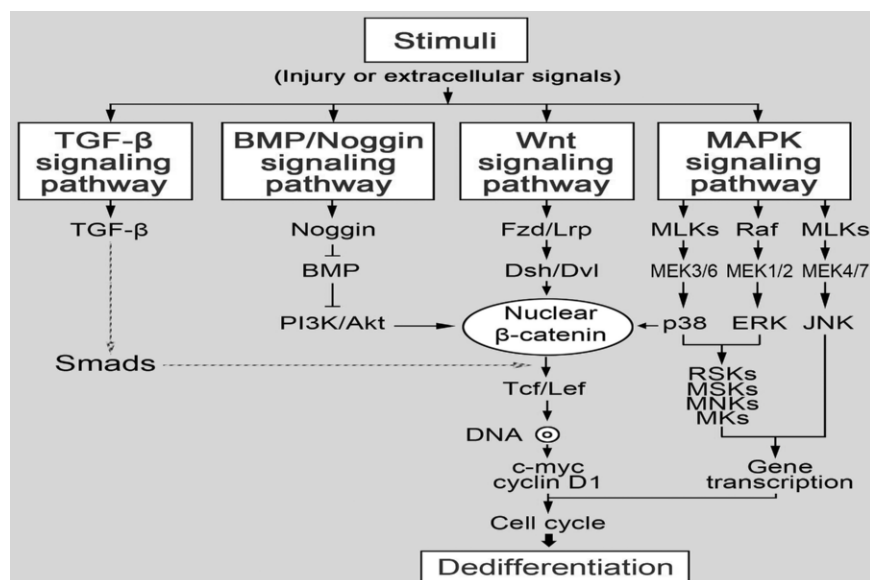


Figure 1. Candidate extrinsic signaling pathways that regulate dedifferentiation.

Pretreatment of smooth muscle cells with ERK inhibitor suppresses the ERK cascade, abolishes the down-regulation of desmin, and leads to cell cycle arrest (Vogel et al. 2006). The JNK-mediated c-Jun phosphorylation pathway is also required for IL-1 β -induced dedifferentiation of articular chondrocytes (Hwang et al. 2005).

Wnt/ β -catenin signaling pathway. Beta-catenin, a component of the cadherin cell adhesion complex, plays an important role in E-cadherin-mediated cell–cell adhesion and is an important intermediate in the Wnt signaling pathway. It has been conclusively demonstrated to regulate dedifferentiation. In the absence of Wnt signaling, β -catenin is phosphorylated by functional interaction with glycogen synthase kinase 3 beta (GSK-3 β) and subsequently targeted to degradation by the ubiquitin-proteasome system (Polakis 1997). Activation of the Wnt pathway inhibits GSK-3 β activity and thus induces stabilization of β -catenin, causing translocation of β -catenin to the nuclei, where its association with T-cell factor/lymphocyte enhancer factor (Tcf/Lef) causes transcriptional activation of target genes (Clevers and van de Wetering 1997). Accumulation of β -catenin and subsequent stimulation of Tcf/Lef transcriptional activity causes dedifferentiation of articular chondrocytes (Hwang et al. 2005). In a primary culture of articular chondrocytes, IL-1 β induces expression of β -catenin and Wnt-7 α , and Wnt-7 α induces dedifferentiation of articular chondrocytes by stimulating the transcriptional activity of β -catenin (Hwang et al. 2004). In addition, β -catenin plays a direct role in the dedifferentiation observed in thyroid cancer and is the key signaling intermediate involved in the EMT (Zhuang et al. 2005).

Other signaling pathways. Brawley and Matunis (2004) found that in the *Drosophila* male germ line, local activation of the Janus kinase–signal transducer and activator of transcription

(Jak-STAT) pathway maintained stem cells, whereas germ-line stem cells lacking Jak-STAT signaling differentiated into spermatogonia without self-renewal. So through conditional manipulation of Jak-STAT signaling, spermatogonia that are undergoing limited mitotic (transit-amplifying) divisions can repopulate the niche and revert to stem cell identity. Other signaling pathways involved in dedifferentiation include Notch signaling reactivation in dedifferentiated pancreatic cells (Jensen et al. 2005), BMP/Noggin signaling (Pearton et al. 2005), and other unidentified signaling in the stem cell niche (Moore and Lemischka 2006). Although several signal mechanisms have been found to play important roles in dedifferentiation and regeneration, they are members of a regulatory network with multiple feedback loops. We believe that dedifferentiation and regeneration are more

complicated than previously thought, and that numerous inducing factors and signal pathways coordinate mutually to promote these processes.

Conclusions

It has long been thought that once mammalian cells are committed to a specific lineage, they can no longer change their fate, and thus they become so-called terminally differentiated cells. But recent studies suggest that differentiated mammalian cells can be induced to assume new fates under appropriate conditions, a phenomenon termed *dedifferentiation*. By better understanding the biology of stem cells, researchers may be able to manipulate pluripotent stem cells to produce a great variety of cell types. Even more exciting is the prospect that the induction of dedifferentiation may make it possible to produce pluripotent stem cells from an individual's terminally differentiated cells, including cells that can be harvested using noninvasive procedures. These cells could then be expanded and transplanted back into the injured organ in the hope of inducing a regenerative response.

Much work needs to be done, however, before cellular dedifferentiation can be used for therapeutic purposes. First, it seems that different cell types undergo different dedifferentiation processes, and so far, regardless of the cell line, a stable and efficient dedifferentiation-inducing model has not been developed. If such a model system can be established, the study of dedifferentiation will become easier and its manipulation more efficient. Although we have established a human sheet transplantation model in immunodeficient BALB/c nude mice, which could be used to observe the dedifferentiation of human epidermal cells and to collect dedifferentiated cells, its stability and effectiveness still need to be evaluated.

Second, an important direction for future research in chemical biology is the derivation of compounds that could

affect cellular differentiation or its reversal (Kim et al. 2004). The discovery of reversine seems to be a breakthrough in that a small-molecule treatment might possibly transform one cell type into another. However, though reversing the differentiation of C2C12 cells is a fascinating development, reversing the differentiation of a cell obtained directly from muscle tissue of a human poses a much greater challenge. Besides, there are other questions to be answered in regard to reversine: Is it effective only on myoblasts, or can it act on other cell types? Can reversine change adipocytes or bone cells into muscle cells? All in all, the search for small-molecule dedifferentiation of other cell types will probably keep scientists in this field busy for many years to come.

Third, scientists have always been puzzled about the mechanism of dedifferentiation. Although recent published papers suggest that under certain circumstances, some induction signals might be sufficient to induce dedifferentiation of different cell types, many questions have not been adequately answered. The genes that control cellular dedifferentiation have not yet been completely identified, and the genes that have been shown to induce cellular dedifferentiation in cultured cells need to be further tested in animals to determine whether they function similarly *in vivo*. In addition, it is not clear what the fate of dedifferentiated cells would be. Would they respond to endogenous cues in the host tissue and redifferentiate into the appropriate cell types, or would differentiation signals also need to be supplied externally? Tight control of the dedifferentiation gene's expression will be required so that it can be incited to initiate the dedifferentiation process and subsequently terminated so that redifferentiation can take place. Therefore, if researchers identify which genes or signaling pathways act as the key factors during this process, we can potentially control the whole dedifferentiation process at will; the process of dedifferentiation can easily be turned on and off by activating or closing the expression of certain genes. When it comes to regeneration, however, the situation may not be so simple: To induce endogenous regeneration, it may be necessary that all the genes controlling dedifferentiation, redifferentiation, and patterning be expressed at the appropriate levels in the correct temporal and spatial context (Odelberg 2002). If so, a complete understanding of the molecular mechanisms that control the regenerative process will be necessary.

Finally, the purpose of basic medical research is mainly to apply research results in the clinical setting. The study of dedifferentiation is not an exception. Until now, it has not been clear whether these dedifferentiation-derived progenitor cells, or stem cell-like cells, could perform the same functions as embryonic or adult stem cells isolated from the body. Do the induced stem cells contain the same potentiality as native stem cells, and can they differentiate into a wide variety of cell types?

Dedifferentiation is likely to become a new focus in stem cell research because of its importance in fields such as stem cell biology, regenerative medicine, cancer research, and aging. We hope the foregoing and future studies in dedifferentiation will bring us closer to achieving the ultimate

goal of enhancing endogenous regenerative processes in mammals. But there is still a long way to go before these hopes are realized.

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