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Review

Regulation of pluripotency and differentiation by deubiquitinating enzymes

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Post-translational modifications (PTMs) of stemness-related proteins are essential for stem cell maintenance and differentiation. In stem cell self-renewal and differentiation, PTM of stemness-related proteins is tightly regulated because the modified proteins execute various stem cell fate choices. Ubiquitination and deubiquitination, which regulate protein turnover of several stemness-related proteins, must be carefully coordinated to ensure optimal embryonic stem cell maintenance and differentiation. Deubiquitinating enzymes (DUBs), which specifically disassemble ubiquitin chains, are a central component in the ubiquitin-proteasome pathway. These enzymes often control the balance between ubiquitination and deubiquitination. To maintain stemness and achieve efficient differentiation, the ubiquitination and deubiquitination molecular switches must operate in a balanced manner. Here we summarize the current information on DUBs, with a focus on their regulation of stem cell fate determination and deubiquitinase inhibition as a therapeutic strategy. Furthermore, we discuss the possibility of using DUBs with defined stem cell transcription factors to enhance cellular reprogramming efficiency and cell fate conversion. Our review provides new insight into DUB activity by emphasizing their cellular role in regulating stem cell fate. This role paves the way for future research focused on specific DUBs or deubiquitinated substrates as key regulators of pluripotency and stem cell differentiation.
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Facts

- Ubiquitination and deubiquitination of stemness-related proteins are well coordinated to ensure optimal embryonic stem cell maintenance and differentiation.
- Extensive research has been achieved on ubiquitination system in the maintenance of stem cell and differentiation. Deubiquitinating enzymes (DUBs)-mediated reversal of ubiquitination also has an equally critical role.
- Recent studies with USP7, USP9X, USP22, USP44, and Psmd14 have shown that DUBs are involved in maintaining stem cell pluripotency.
- First attempt to review the relationship between DUBs and stem cells, and suggesting DUBs as potential candidates for regulating stem cell fate determination and cellular reprogramming.

Open Questions

- What is the evidence to support the involvement of DUBs in stem cells?
- What is the role of DUBs in regulating stem cell fate determination?
- How can the DUBs be targeted to regulate stem cell pluripotency, differentiation, and cellular reprograming?

Embryonic stem cells (ESCs) that are derived from the inner cell mass (ICM) of the blastocyst can undergo unlimited self-renewal. Moreover, ESCs can be triggered to differentiate into all three embryonic germ layers: (a) ectoderm – skin and nerve; (b) mesoderm – bone, blood, and muscle; and (c) endoderm – gut and lung tissues. Human ESCs were first isolated by Thomson *et al.*[1] from the ICM of preimplantation blastocysts.

ESC self-renewal and differentiation are known to be regulated by a network of transcription factors including Oct3/4, Sox2, c-Myc, Klf4, and Nanog.[2,3] However, in addition to transcription-mediated regulation of ESC fate, recent studies have indicated that transcription-independent mechanisms also exist for controlling ESC fate. However, little information has been obtained regarding the role of post-transcriptional modifications (PTMs) in ESC maintenance and differentiation.

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Abbreviations: PTM, post-translational modification; DUB, deubiquitinating enzyme; ICM, inner cell mass; ESCs, embryonic stem cells; UCH, ubiquitin C-terminal hydrolase; USP, ubiquitin-specific protease; REST, repressor element 1-silencing transcription factor; Hes1, hairy and enhancer of split 1; iPSCs, induced pluripotent stem cells; EMT, epithelial – mesenchymal transition

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A few studies have indicated that PTMs such as phosphorylation and sumoylation contribute to the spatial and temporal regulation of pluripotency-associated transcriptional networks.[2,3] However, the roles of ubiquitin-proteasomal pathway-mediated protein turnover and the counterpart of this pathway, deubiquitination, on pluripotency-related transcriptional networks are poorly understood. Recent studies have provided strong evidence that deubiquitinating enzymes (DUBs) are important for the maintenance of stemness and stem cell differentiation.

Ubiquitin-Proteasome Pathway

Ubiquitination is a PTM in which ubiquitin is conjugated to a protein substrate, thereby regulating the stability and activity of the modified protein. For a ubiquitin molecule to be attached to a target protein, the sequential actions of three different classes of enzymes – E1 (ubiquitin activating enzymes), E2 (ubiquitin conjugating enzymes), and E3 (ubiquitin ligases) – are required. Initially, the ubiquitin molecule is activated by an E1 enzyme through an ATP-dependent reaction. This activation is then followed by conjugation via an E2 class enzyme, after which an E3 ubiquitin ligase transfers the ubiquitin molecule specifically to its target protein[4–6] (Figure 1).

Ubiquitin is a small and highly conserved 76-amino acid protein with a molecular weight of 8.5 kDa. The ubiquitin modification can be covalently attached to protein substrates as either a monomer or as a polymer.[7,8] The different ubiquitin modifications depend on the type of chain formed during the process.[9] Substrate proteins can be modified with mono-ubiquitin, multiple monoubiquitin (multi-ubiquitination), or a polyubiquitin chain (polyubiquitination) (Figure 2). During polyubiquitination, any of the seven lysine (K) residues (K6, K11, K27, K29, K33, K48, and K63) of ubiquitin can be utilized for the formation of ubiquitin – ubiquitin linkages, resulting in a sizeable chain increase with different configurations called polyubiquitin chains.[10]

Generally, protein substrates that undergo monoubiquitination are involved in DNA repair, vesicle sorting, receptor endocytosis, or signal transduction.[11–14] On the other hand, K6, K11, K29, and K48 polyubiquitin chains regulate protein stability. Specifically, K48 polyubiquitin chains target proteins for proteolysis through the 26S proteasome,[15–17] K11 polyubiquitin chains regulate endoplasmic reticulum-mediated degradation and cell cycle progression,[18–20] K63 polyubiquitin chains signal activation of the transcription factor nuclear factor-κB or regulate the DNA repair process,[21–24] and K29 chains are involved in lysosomal degradation.[25] However, the roles of other polyUb chains are not well understood, in spite of a few intriguing reports. K29 or K33 chains are found to bind AMPK-related kinases to regulate their enzymatic activity,[26] K6 chain linkages are induced by BRCA1/BARD1 E3 and speculated to be involved in the DNA repair process,[27,28] and K27 and K33 chains may be assembled by U-box-type E3 ligases during a stress response[29,30] (Figure 2). However, ubiquitination is also involved in diverse cellular functions such as transcriptional regulation, the immune response, apoptosis, cell cycle control, oncogenesis, embryonic development, preimplantation, and intracellular signaling pathways.[31]

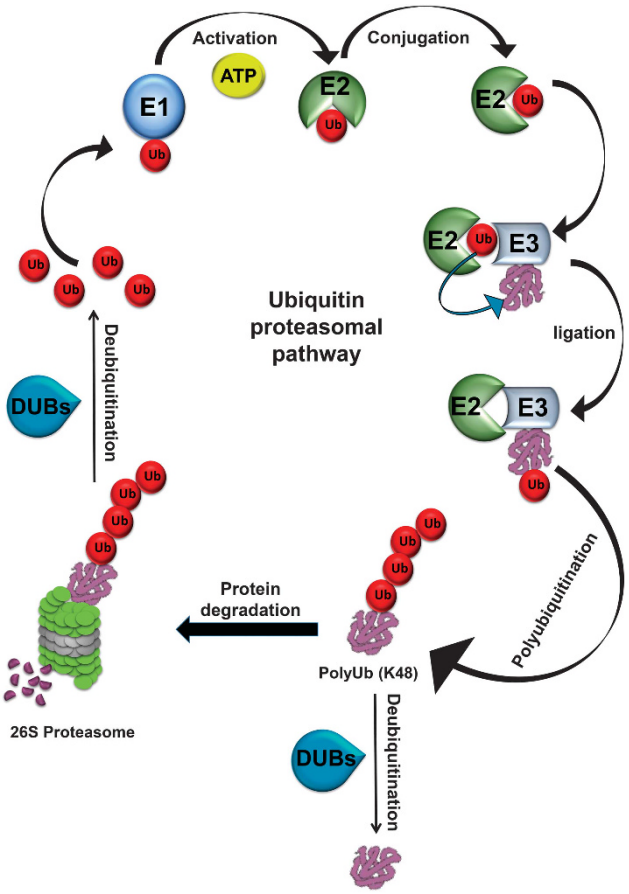

Figure 1 The ubiquitin proteasomal pathway. Ubiquitin molecule to be attached to a target protein, the sequential actions of three different classes of enzymes – E1 (ubiquitin-activating enzymes), E2 (ubiquitin-conjugating enzymes), and E3 (ubiquitin ligases) – promote the ligation of a ubiquitin molecule to the lysine (K) residues in the protein substrates. K48-linked polyubiquitination chain-attached protein substrates are targeted to the 26S proteasome for protein degradation. Ubiquitins are recycled by the action of DUBs through the ubiquitin-proteasome pathway

Deubiquitination

The DUBs comprise a class of proteases that cleave ubiquitin molecules from ubiquitin-conjugated protein substrates. Specifically, DUBs selectively cleave the isopeptide bond present at the ubiquitin C-terminus.[9,32] DUBs prevent proteasome-dependent and lysosome-dependent protein degradation because they counteract E3 ligase-mediated ubiquitination. Consequently, DUBs indirectly alter the activities and levels of their target proteins.

Deubiquitinating enzymes and their classifications. DUBs can be classified into six families: (i) ubiquitin C-terminal hydrolases (UCHs), (ii) ubiquitin-specific proteases (USPs), (iii) Jab1/Pab1/MPN domain-containing metallo-enzymes, (iv) otu-domain ubiquitin aldehyde-binding proteins, (v) Ataxin-3/Josephin proteases, and (vi) monocyte chemotactic protein-induced proteases. Of these families, the USP family is the largest. This family is comprised of more than 50 members, each of which contains conserved domains and catalytic sites.[3,4,33–37]

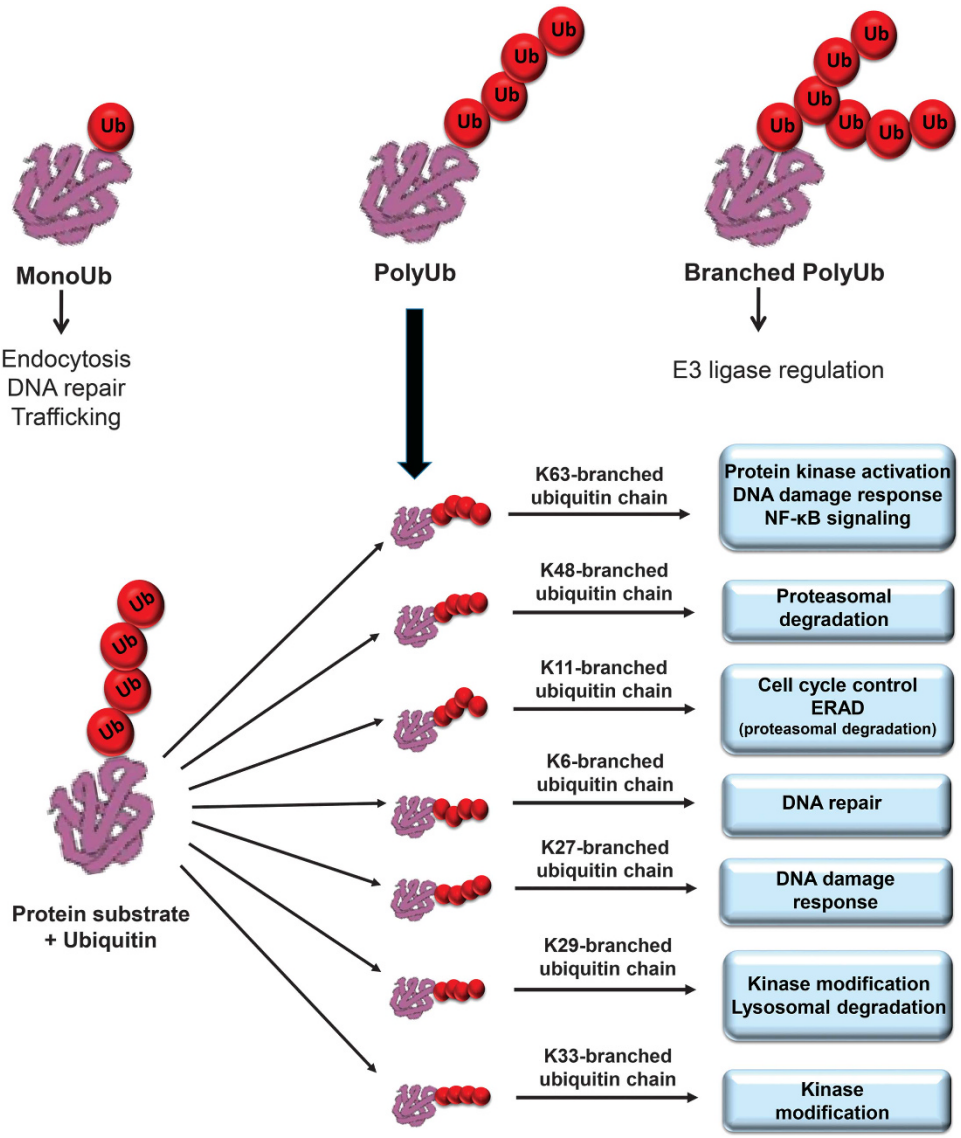

Figure 2 Different forms of polyubiquitination and their cellular functions. Protein substrates can be monoubiquitinated or polyubiquitinated. The attachment of ubiquitin molecules to one or more lysine (K) residues results in polyubiquitination. Different forms of polyubiquitin chains linked via K-residues on the protein substrate are implicated in diverse cellular functions

Deubiquitinating enzymes functions. DUBs have four distinct mechanisms of action: (i) processing of ubiquitin precursors/*de novo* ubiquitin synthesis, (ii) recycling of ubiquitin molecules during ubiquitination, (iii) cleavage of polyubiquitin chains, and (iv) reversal of ubiquitin conjugation.[4,38] Through these actions, DUBs are critical regulators of the proteasomal pathway.

DUBs regulate several cellular functions such as proteasome-dependent and lysosome-dependent proteolysis, gene expression, cell cycle progression, chromosome segregation, kinase activation, apoptosis, localization, DNA repair, spermatogenesis, and degradation of signaling intermediates.[3,4,36–39]

Deubiquitinating Enzymes in Stem Cells

All stem cells possess two defining characteristics, the ability to self-renew and the ability to differentiate. ESCs maintain high-genomic plasticity and can therefore enter any differentiation pathway. However, ESC differentiation is mainly regulated by the turnover of transcription factors such as Oct3/4, Sox2, Klf4, c-Myc, Nanog, LIN28, and Sall4. These transcription factors are master regulators of stem cell pluripotency.[3,40,41] A growing body of evidence supports the idea that UPSs are important for stem cell pluripotency and differentiation.[2,3,40] Achieving the appropriate UPS expression levels and subcellular localizations is critical for maintaining stem cell pluripotency.[40] Although UPSs have been reported to have a number of physiological functions related to ESC pluripotency, only limited information is available regarding DUB function in stem cell maintenance and differentiation. However, recent studies with USP7, USP9X, USP22, USP44, and Psmd14 have shown that DUBs are involved in maintaining stem cell pluripotency. We will now discuss the published

evidence and current knowledge regarding DUB function and the contribution of DUBs to stem cell maintenance and differentiation.

Ubiquitin-specific protease 7. Herpesvirus-associated ubiquitin-specific protease, also known as ubiquitin-specific protease 7 (USP7), was initially identified via its association with the viral protein ICP0 (herpes simplex virus type 1 regulatory protein) and was shown to regulate its stability.[42] USP7 was also found to regulate the transcriptional activity of Epstein – Barr nuclear antigen 1.[43] Although USP7 is involved in various cellular processes,[44] it was recently shown to prevent the degradation of repressor element 1-silencing transcription factor (REST) through its deubiquitinating activity, thereby facilitating the maintenance of neural stem/progenitor cells.[45] REST is a stem cell transcription factor whose protein level is altered during neural differentiation. REST is targeted for ubiquitin-dependent protein degradation via the $SCF^{\beta\text{-}TrCP}$ E3 ubiquitin ligase complex. USP7 interacts with and stabilizes REST by preventing $SCF^{\beta\text{-}TrCP}$-mediated ubiquitination, thus promoting the maintenance of stemness.[45]

Ubiquitin-specific protease 9X. USP9X is one of the largest members of the USP family and was originally identified in *Drosophila*; mutations in USP9X cause characteristic eye defects called fat facets.[46,47] In mammals, USP9 is known as FAM.[48] Several stemness-related genes are highly expressed in stem cells compared with their differentiated progeny. Of these genes, *USP9X* has been shown to be highly expressed in stem cells *in vivo*, including pre-implantation blastomere embryos and neural stem cells.[49–51] *USP9X* has also been identified in mouse and human stem cells, including ESCs, neural stem cells, neuronal progenitors, hematopoietic stem cells, and adult epidermal stem cells.[52,53] Although inhibition of *USP9X* in mouse ESCs did not affect their growth *in vitro*,[54] USP9X has been hypothesized to regulate the early differentiation of stem cells. Interestingly, USP9X has been found to regulate the mTOR pathway, thus controlling the proliferation and differentiation of muscle stem cells.[55] Although *USP9X* is highly expressed in neural stem cells, its expression in adult brain tissue is significantly decreased.[50,51] However, *USP9X* expression is maintained in the neural progenitors located in the adult neurogenic niches.[50,51] Thus, *USP9X* expression is critical for stem cell function.

Ubiquitin-specific protease 22. USP22 is a deubiquitinating subunit of the SAGA mDUB complex.[56] This enzyme has been reported to affect transcription by hydrolyzing the monoubiquitin molecules that are conjugated to uH2A and uH2B.[56–60] A number of studies have indicated that USP22 has an important role in tumorigenesis and tumor progression.[61–63] Indeed, *USP22* was initially reported as a member of an 11-gene 'death from cancer' gene expression signature characterized by high malignancy, metastatic dissemination, and resistance to therapy.[64,65] In addition to its role in tumorigenesis, USP22 also has a major role in stem cell function. The *USP22* locus has been shown to be actively transcribed in human ESCs and induced pluripotent stem cells (iPSCs).[66] Moreover, the histone H3 lysine 4 trimethyl epigenetic mark is recruited to the *USP22* promoter, which is co-occupied by the stemness factor *KLF4*, indicating its role in stem cell pluripotency and differentiation.[66]

Recent evidence has indicated that USP22 regulates core pluripotency factors, including c-Myc and Sox2.[56,67] USP22 was originally identified as an essential cofactor for the stem cell transcription factor Myc, thereby regulating transcription of *Myc* target genes.[56] Moreover, Sussman *et al.*[67] showed that USP22 is required for proper ESC differentiation into all three germ layers. During ESC differentiation, USP22 negatively regulates *Sox2* transcription in ESCs. Moreover, USP22 is located directly on the *Sox2* promoter, catalyzes H2B deubiquitination, and attenuates transcription of the *Sox2* locus.[67] Thus, USP22 has a pivotal role in differentiation by repressing *Sox2* in ESCs, which allows them to transition from a self-renewal state into lineage-specific differentiation pathways.

Usp22 is highly expressed in adult murine tissue and is also prominent at the early embryonic stages in the midbrain, forebrain, hindbrain, and dorsal root ganglia.[68] Usp22 has also been reported to be essential for embryonic development in mice. Mice with genetic ablation of *Usp22* exhibit early embryonic lethality at E10.5 of the postimplantation stage.[65] Recently, Kosinsky *et al.*[69] showed that *Usp22*-deficient mice displayed growth defects and reduced body weight. Furthermore, *Usp22*-deficient mice exhibited differentiation defects in the cells of the small intestine.[69] Hairy and enhancer of split 1 (Hes1) expression has been found to oscillate in mouse ESCs and in neural stem cells; this oscillation contributes to the maintenance of stem cell potency and differentiation fate.[70] Recently, Usp22 was found to stabilize Hes1 via deubiquitination. On the other hand, knockdown of *Usp22* shortened the half-life of Hes1 and triggered its rapid degradation, resulting in delayed auto-repression and dampened oscillation. In turn, neuronal differentiation was increased.[71] Thus, Usp22 has a critical role in neuronal differentiation in the developing brain.

Ubiquitin-specific protease 44. USP44 has been reported to be a critical regulator of the mitotic spindle checkpoint in differentiated cells. Specifically, USP44 deubiquitinates Cdc20 and regulates anaphase initiation during mitosis.[72] USP44 also mediates chromosome instability and aneuploidy.[73] Recently, several lines of evidence have indicated that USP44 is involved in stem cell differentiation. For example, genome-scale location analysis revealed that USP44 is a direct target of Oct4.[74] In addition, USP44 is localized in the nucleus, where it associates with chromatin;[72,75] moreover, USP44 is downregulated during ESC differentiation.[76]

Recently, Fuchs *et al.*[76] showed that USP44 directly regulates stem cell differentiation. Specifically, monoubiquitination of histone H2B on lysine 120 (H2Bub1) increases during differentiation of human and mouse ESCs; similar effects were observed in embryonic carcinoma cells. RNF20, an E3 ligase, regulates H2B ubiquitination during ESC differentiation. Genetic silencing of *USP44* during ESC differentiation also resulted in increased levels of H2Bub1, suggesting that USP44 is a negative regulator of H2B ubiquitination.[76] Thus, ESC differentiation requires that USP44 is expressed at an optimum level.

Psmd14. Psmd14 is a component of the 19S proteasome lid, which also includes Psmd3, Psmd6, Psmd7, Psmd11, Psmd12, and Psmd13.[77] Although Psmd14 is highly expressed in pluripotent ESCs, its expression decreases during differentiation. Buckley *et al*.[40] performed a UPS-targeted siRNA screen to identify essential genes required for stem cell maintenance. This screen identified two DUBs, *Psmd14* and *USP9X*. Depletion of *Psmd14* is accompanied by a significant decrease in the level of Oct4 and abnormal ESC morphology. Although knockdown of *Psmd14* did not affect the overall stoichiometry of the 26S proteasome, proteasome activity was impaired, as evidenced by the accumulation of both K48-linked and K63-linked polyubiquitinated proteins. Moreover, MEFs expressing *Psmd14*-targeting shRNA failed to reprogram and generate iPSCs.[40] Thus, Psmd14 is essential for the maintenance of ESC pluripotency and cellular reprogramming.

Other deubiquitinating enzymes. As discussed previously, USP7 interacts with REST, a stem cell transcription factor that regulates neuronal differentiation.[45] However, REST degradation is also regulated by other DUBs, including USP14 and USP15.[78,79] Treatment with IU-1, a USP14 inhibitor, resulted in decreased protein levels of REST in both *in vitro* and *in vivo* studies.[78] Moreover, USP15 was found to stabilize newly synthesized REST rather than pre-existing REST and a small fraction of USP15 was found associated with polysomes. Thus, USP15 has a critical role in controlling cell cycle oscillations by facilitating rapid replenishment of newly synthesized REST upon mitotic exit, which regulates the beginning of the next cell cycle.[79]

Deubiquitinating Enzymes as Targets for Therapeutics

Many studies have implicated DUBs in the pathogenesis of several human diseases such as neurological disorders, cancer, and infectious diseases.[80,81] Thus, DUBs are a key alternative target upstream of the proteasome ubiquitin conjugation/deconjugation system that can potentially generate more reliable, specific, and less toxic anticancer agents. In addition, recent advances in small-molecule-based inhibitors specifically targeting DUBs also make DUBs attractive therapeutic targets for antiviral and anticancer agents.[82–84]

Several DUBs are directly or indirectly involved in down-regulating or ablating oncogene products or, alternatively, upregulating or suppressing tumor suppressors (reviewed in Lim and Baek,[85] Pal and Donato[86]). Some specific examples of DUBs that are viable targets for anticancer therapy include USP2, USP4, USP7, USP9X, USP11, and USP15. USP2a is a isoform of USP2 that is involved in prostate cancer, and deubiquitinates and stabilizes fatty acid synthase.[87] In support of this oncogenic role, ectopic expression of *USP2a* in non-transformed cells promotes oncogenic signaling *in vitro* and *in vivo*.[88] Elevated expression of *USP7* resulting in tumor aggressiveness was reported in prostate cancer,[89] whereas the absence of *USP7* in nude mice led to a reduction in tumor size.[90] USP7 also inactivates several tumor suppressors including p53, FOXO4, and PTEN.[89,91–93] Several DUBs are involved in regulating TGFβ signaling. Inhibition of *USP4*, *USP11*, and USP15 attenuates TGFβ-mediated epithelial – mesenchymal transition (EMT) and invasion in breast cancer.[94–96] Similarly, USP9X alters levels of the monoubiquitinated protein Smad4 in cells through deubiquitination, thus regulating TGFβ-mediated EMT and invasion.[97] USP9X was also found to interact with and deubiquitinate SMURF1, a negative regulator of TGFβ/BMP signaling during tumor cell migration and invasion.[98,99] Furthermore, inhibition of *USP9X* leads to elevated SMURF1 protein levels resulting in SMURF1-dependent breast cancer cell motility.[99] Elevated expression of *USP9X* is reported in human follicular lymphoma and correlates with poor prognosis in multiple myeloma.[100] In addition, *USP9X* expression is necessary for the growth of glioblastomas and medulloblastomas.[101] Taken together, these findings indicate that DUBs function as cancer-associated proteases, and their unique biochemical structures allow them to be considered as potential targets for anticancer therapies.

Recently there has been extensive research on the development of small-molecule inhibitors to target DUBs. Because DUBs have critical role in regulating cellular homeostasis, proliferation, and survival,[36] they have been considered as anticancer targets.[85,86] Screening for compounds that modulate the UPS has been challenging research for pharmaceutical companies. Although there are strong similarities within the active-site cysteine and histidine boxes of several DUBs, the three-dimensional structure of each DUB has unique differences in accessibility to the catalytic pocket.[83] Since the approval of the proteasome inhibitors bortezomib and carfilzomib for the treatment of hematological malignancies,[102] there has been great interest in targeting the deubiquitination process upstream of the proteasome in cancer therapy. Auronofin (Aur) is an inhibitor of the proteasome-associated deubiquitinases UCHL5 and USP14, but not the 20S proteasome, that leads to Aur-induced cytotoxicity. Thus, Aur is more effective in inhibiting tumor growth *in vivo* and induces cytotoxicity in cancer cells from acute myeloid leukemia patient samples.[103] Along with cancer, the applicability of DUB inhibitors is being examined in other therapeutic areas including neurodegeneration and infectious disease.[82,83,104,105] Several partial and specific inhibitors against USPs have been developed and utilized in research. So far, many DUB inhibitors have been found to be effective against USP family members such as USP1, USP2, USP5, USP7, USP8, USP9x, USP10, USP11, USP13, USP14, USP20, USP30, and USP37, as well as UCH family members such as UCHL1, UCHL3, and UCHL5.[82–84]

The tumor suppressor p53 acts as a master regulator of stem cell and iPSC differentiation in response to genomic damage.[106–108] Thus, targeting DUBs specific to p53 might be an alternative method to regulate stem cell differentiation. In response to DNA damage, p53 represses several stemness-related and iPSCs-associated genes including *Oct4*, *Sox2*, *Nanog*, *Sal4*, *Esrb*, *Utf1*, *Prdm14*, *n-Myc*, and *c-Myc* which signifies that p53 mediates transcriptional repression during ES cell differentiation and somatic cell reprogramming.[109] USP10 deubiquitinates and stabilizes both wild-type and mutant p53, and reverses MDM2-mediated nuclear transport and degradation of p53.[110] In addition, USP10 was found to antagonize *c-Myc* transcription by deubiquitinating SIRT6 and NEMO.[111] Considering the importance of p53 and c-Myc in the process of reprogramming somatic cells to iPSCs, USP10

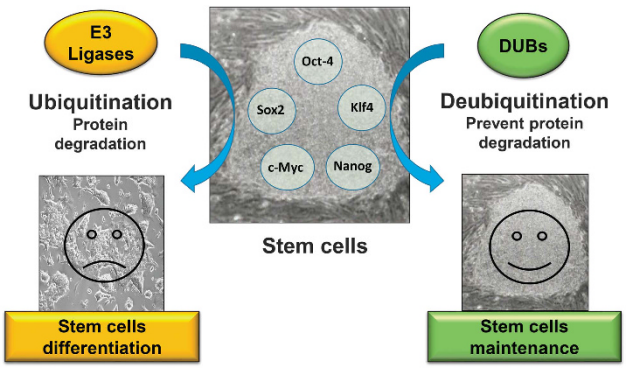

Figure 3 Roles of ubiquitination and deubiquitination in regulating stem cell differentiation and pluripotency. Ubiquitination of core stem cell transcription factors by E3 ligases drives stem cell differentiation. Deubiquitination of the core stem cell transcription factors by DUBs mediates stem pluripotency

might be a prime target during iPSCs generation. USP15 was shown to deubiquitinate and stabilize MDM2, which impacts both p53 and the T-cell transcription factor NFATc.[112] Thus, USP15 is a potential candidate for targeting and analyzing the p53-mediated regulation of genome stability in stem cells. The biological functions associated with *USP7* silencing and the links between USP7 and p53/Mdm2[91,92] strongly suggest that small-molecule inhibitor-mediated targeting of USP7 could be a highly useful therapy for the regulation of stem cell pluripotency and differentiation.

Conclusions

The determination of cell fate is exquisitely controlled by transcription factors that regulate stem cell maintenance and differentiation. Perturbation in activation of core stem cell pathways leads to transformation, whereas deficiencies in these cellular mechanisms drives to degenerative conditions. Stem cell fate results from a delicate balance between ubiquitination and deubiquitination. For instance, E3 ligases catalyze the ubiquitination of stemness-related proteins, thereby driving stem cell differentiation, whereas DUBs stabilize stemness-related proteins, thus preventing stem cell differentiation (Figure 3).

A growing body of evidence indicates that stemness-related proteins, which regulate self-renewal and stem cell maintenance, are ubiquitinated by E3 ligases.[3,40] Although much attention has been given to the function of the ubiquitination system in the maintenance of pluripotency, DUB-mediated reversal of ubiquitination also has an equally critical role. DUBs are usually present in large multi-protein complexes that function directly or indirectly in stem cell pluripotency, differentiation, and reprogramming (Figure 4). For instance, Boyer *et al.*[74] performed a genome-scale location analysis that implicated several DUBs in the transcriptional regulation of human ESCs via their binding to the promoter regions of Yamanaka factors such as *Oct4*, *Sox2*, and *Nanog*. Specifically, USP7 and USP44 bind to the *Oct4* promoter; USP7, USP25, USP44, and USP49 bind to the *Sox2* promoter; and USP3, USP7, USP10, USP16, USP37, and USP44 bind to the *Nanog* promoter (Table 1). However, the mechanism(s) by which DUBs regulate Oct4, Sox2, and Nanog, and the role of

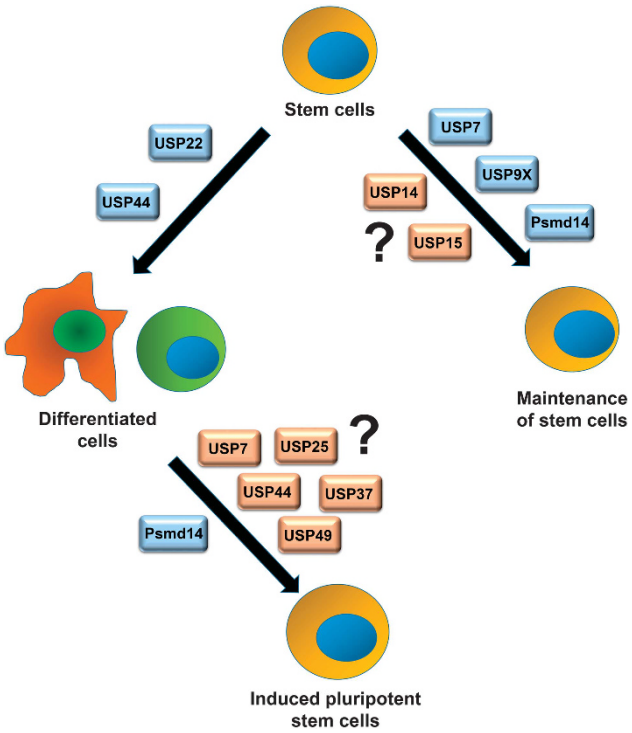

Figure 4 Roles of DUBs in regulating stem cell differentiation, pluripotency, and cellular reprogramming. Reported DUBs involved in fate determination of stem cells are colored in blue and predicted DUBs are colored in orange

Table 1 Deubiquitinating enzymes binding to Oct4, Sox2, and Nanog promoter region

Oct4	*Sox2*	*Nanog*
USP7	USP7	USP3
USP44	USP25	USP7
	USP37	USP10
	USP44	USP16
	USP49	USP37
		USP44

USP, ubiquitin-specific protease

DUBs in stem cell differentiation and cellular reprogramming have not yet been elucidated.

All work to this point indicates that Yamanaka factors are specifically deubiquitinated by multiple DUBs. Thus, reprogramming efficiency could be improved by screening for specific DUBs that regulate the levels of stem cell transcription factors. Moreover, reprogramming could be optimized by overexpressing a combination of DUBs that stabilize stemness-related proteins and certain Yamanaka factors, which could theoretically improve the efficiency of generating iPSCs. Therefore, screens for specific DUBs that catalyze the cleavage of ubiquitin moieties from Yamanaka factors promise to shed light on the molecular mechanisms that determine the cell fate of ESCs. In addition, screening specific DUBs for defined factors involved in the direct conversion of fibroblasts to functional neurons,[113] melanocytes,[114] endothelial cells,[115] astrocytes,[116] osteoblasts,[117] hepatocytes,[118] and so

on, might have significant implications on research studies and improve the efficiency of cell fate conversion. Further detailed mapping of the cross talk between ubiquitination and deubiquitination in the context of the regulation of ESC function, pluripotency, and differentiation will also be important. We anticipate that these studies will open up exciting new avenues for future research and could also initiate the development of DUB-targeted treatment approaches for various human disorders.

Conflict of Interest

The authors declare no conflict of interest.

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