Deubiquitinating enzymes in cancer stem cells: functions and targeted inhibition for cancer therapy

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The ability of cancers to evade conventional treatments, such as chemotherapy and radiation therapy, has been attributed to a subpopulation of cancer stem cells (CSCs). CSCs are regulated by mechanisms similar to those that regulate normal stem cells (NSCs), including processes involving ubiquitination and deubiquitination enzymes (DUBs) that regulate the expression of various factors, such as Notch, Wnt, Sonic Hedgehog (Shh), and Hippo. In this review, we discuss the roles of various DUBs involved in the regulation of core stem cell transcription factors and CSC-related proteins that are implicated in the modulation of cellular processes and carcinogenesis. In addition, we discuss the various DUB inhibitors that have been designed to target processes relevant to cancer and CSC maintenance.

Introduction

Stem cells are defined as cells that have the unique ability to self-renew or to differentiate into any mature tissue type [1] and can be categorized into two types: embryonic stem cells (ESCs) and adult stem cells (ASCs). ESCs are pluripotent in nature, whereas ASCs tend to differentiate into the different cell types of the source tissue.

CSCs have very similar characteristics to ASCs because they also have the ability to self-renew and generate tumor cells indefinitely. Stem cells are able to generate common and more restricted progenitor cells that eventually differentiate into mature cell types that constitute a particular tissue, whereas CSCs show aggressive self-renewal properties and enormous proliferative potential [1] (Fig. 1).

Oncogenic mutations in ASCs can explain the initiation and formation of cancers in tissues such as intestine, skin, or other specialized systems [2]. Dedifferentiation of highly differentiated cells because of mutations can also give rise to CSCs that have acquired the ability to self-renew indefinitely (Note S1 in the supplemental information online) [3]. Studies indicate that signaling pathways associated with normal stem cell development, such as Notch, Wnt, and Shh, also regulate CSCs, and that dysregulation of these pathways leads to cancer. The precise regulation of these pathways by the ubiquitination or deubiquitination activities of regulatory proteins is crucial to the proper execution of developmental programs, and manipulation of this regulation can promote cancer by altering stem cell properties [4]. Multiple cellular pathways are involved in the induction and maintenance of stemness of stem cells, among which regulation by the ubiquitin (Ub)-proteasome system has a major role [5].

Ubiquitination and deubiquitination

The Ub-proteasome system is the fundamental regulatory mechanism of protein stability, quality, and abundance. It was first discovered in 1980 by Avram Hershko and Aaron Ciechanover, who were awarded the Nobel Prize in 2004 [6]. Ubiquitination is a post-translational modification process by which a highly conserved 76-amino acid protein, Ub, is covalently conjugated to a lysine residue of a substrate protein through a cascade of enzymatic reactions [7].

The first enzyme in the process, E1 (Ub-activating), activates Ub in the presence of ATP, forming a thioester bond between the

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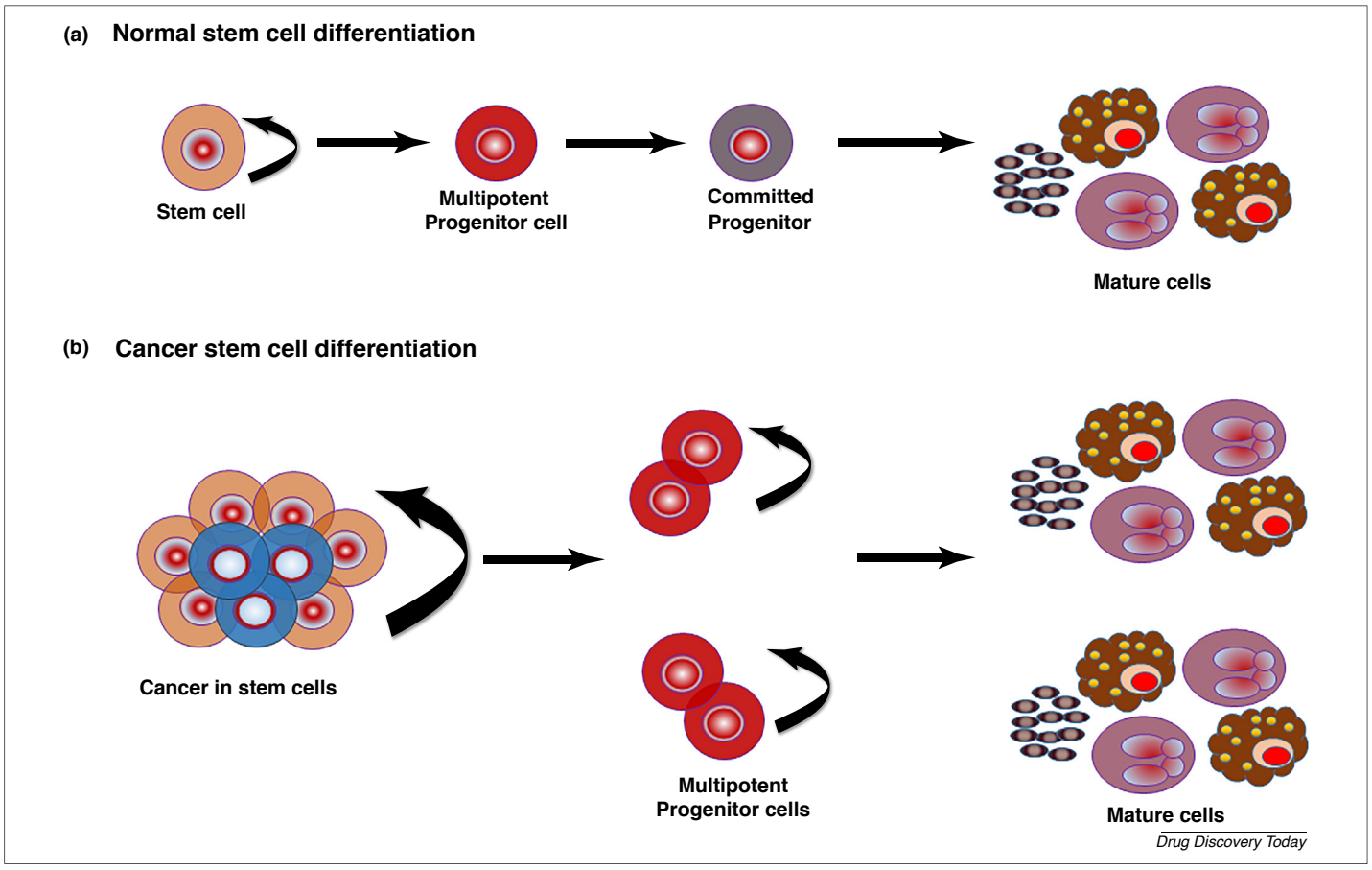

FIGURE 1

Normal versus cancer stem cell differentiation. **(a)** Differentiation of normal stem cells into highly specialized mature cells. **(b)** Differentiation of cancer stem cells acts as a seed for tumorigenesis.

C-terminal glycine of Ub and the active site cysteine of the E1 enzyme. The activated Ub is transferred to the cysteine residue of the second enzyme, E2 (Ub-conjugating). E3 (Ub ligase) is responsible for the transfer of Ub to the target substrate protein either directly from E2 or via a thioester intermediate between Ub and E3 [8] (Fig. 2).

Ubiquitination can occur repetitively on the same substrate, either at additional sites (multi-monoubiquitination) or as poly-ubiquitination, in which the first added Ub serves as the 'acceptor' for additional Ub molecules at one of the ε-NH2 lysine groups or, less frequently, at the α-NH2 group of the acceptor Ub [9].

The ubiquitination process can be reversed by specialized enzymes known as DUBs. DUBs oppose the action of E3 ligases by cleaving the isopeptide linkage between the amino group of lysine and the C-terminal glycine residues of Ub. Analysis of the human genome has identified approximately 100 functional DUBs [10], which have been divided according to active site homology into six broad classes: Ub-specific proteases (USPs), Ub C-terminal hydrolases (UCHs), ovarian tumor proteases (OTUs), Machado–Joseph disease protein domain proteases, JAMM/MPN domain-associated metallopeptidases (JAMMs), and monocyte chemotactic protein-induced proteins (MCPIPs) [11].

Within the classes of DUB, USPs are highly diversified and have >50 members, including the major ubiquitinase E3 ligase [12]. Many studies have reported mutations in USPs involved in multiple biological processes and frequent alteration of USPs in CSCs, indicating an association between mutations and/or changes in expression levels of USPs and tumor progression. However, the role of many USPs in cancer and CSC biology have remained largely unexplored [11]. Here, we review the role of DUBs relevant to CSCs, the various small molecules that have been developed to inhibit DUB activity, and the implications for CSC-targeted therapy.

DUBs associated with CSC signaling pathways and their properties

CSCs and NSCs show a striking resemblance in molecular phenotypes [13]. CSC properties, such as self-renewal, differentiation, and proliferation, are regulated by several signaling pathways, including (i) Hedgehog pathway, which maintains cell polarity and stemness by driving the expression of various stemness-related genes, including *Sox2*, *Oct4*, and *Bmi*; (ii) Wnt/β-catenin signaling, which has a role in tissue self-renewal, cell fate determination, and tumorigenesis; (iii) Notch pathway, which maintains cell stemness; (iv) Hippo pathway, which is involved in cell regeneration and tumorigenesis; [10] (v) TGF-β/BMP signaling, which has a role in cell differentiation, proliferation, survival, and motility [14]; and (vi) EGFR signaling, which maintains cell stemness and provides therapeutic resistance in numerous cancer types, including tumors in the lung, colon, breast, brain, head and neck [15] (Table S1 in the supplemental information online).

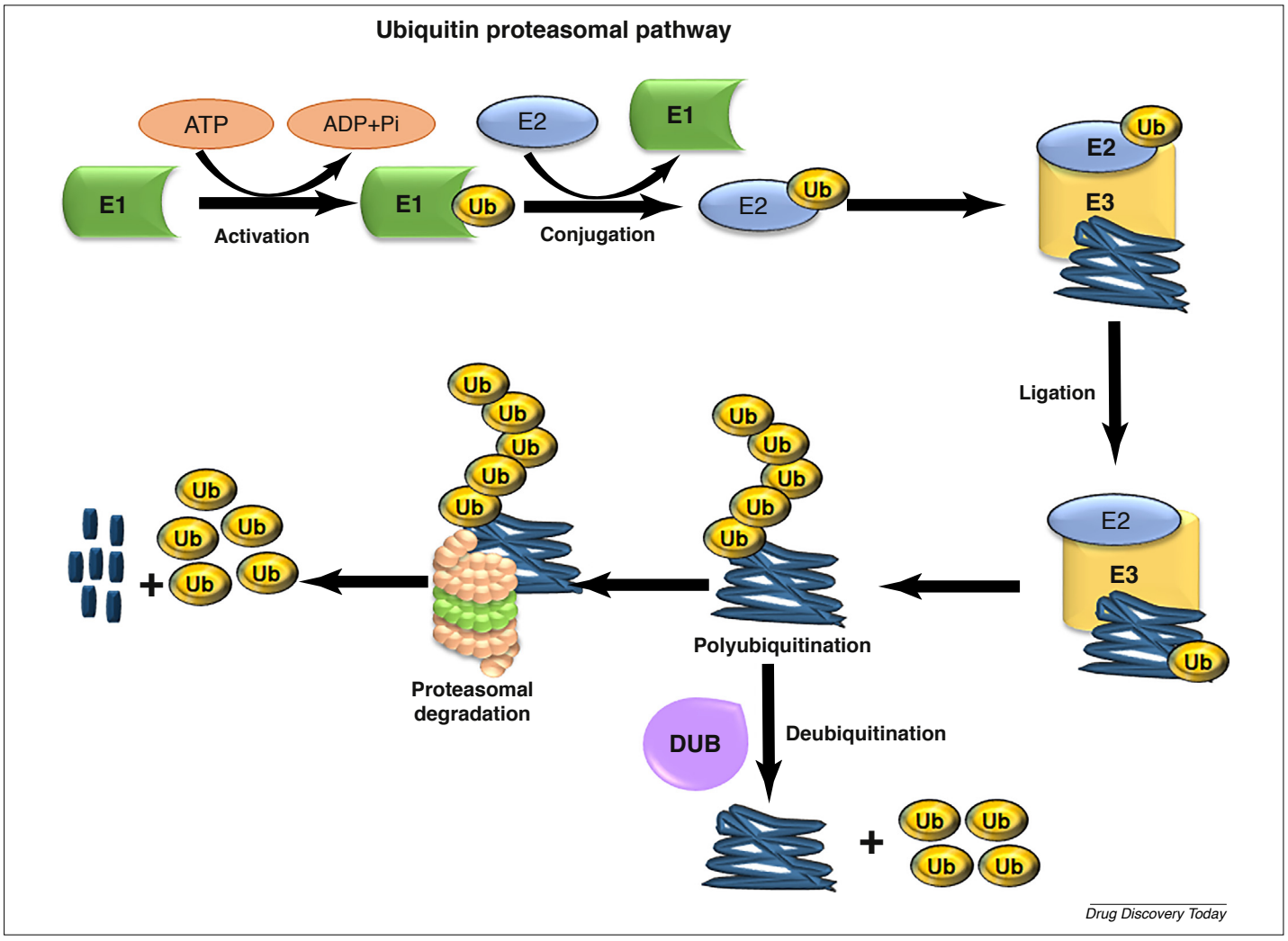

FIGURE 2

Ubiquitin (Ub) proteasomal pathway. Three enzymes are responsible for binding ubiquitin molecules to protein substrates: Ub-activating enzyme E1, Ub-conjugating enzyme E2, and Ub ligase E3. K-48-linked polyubiquitinated proteins are targeted by the proteasomal complex for protein degradation. Deubiquitination enzymes (DUBs) are mainly involved in the recycling of Ub molecules and also counteract Ub-protein ligase activity.

CSC properties are regulated by epithelial–mesenchymal transition (EMT)-inducing transcriptional factors (EIFs), such as Snail and Twist (Note S2 in the supplemental information online), inhibitors of differentiation (IDs); epigenetic modifiers, such as polycomb factors Bmi1 and Ezh2, histone lysine-specific demethylase1 (LSD1)/lysine (K)-specific demethylase 1A (KDM1A) and Sirt1; in addition to other CSC-related factors, such as c-Met and repressor element 1 silencing transcription factor (REST) [16].

Growing evidence suggests that many DUBs stabilize several CSC-associated transcriptional factors, such as Oct4, c-myc, Klf4, Sox2, Lin28,and Nanog, which are known as the Yamanaka factors that generate induced pluripotent stem cells (iPSCs) (Note S3 in the supplemental information online). Some of these transcriptional factors are considered biomarkers for CSCs, as summarized in Table S1 in the supplemental information online. A detailed review of important DUBs that are involved in the regulation of CSC self-renewal, proliferation and differentiation is provided below.

USP1

USP1 belongs to the EIF family of DUBs, which is involved in the DNA damage response by regulating DNA repair mechanisms and is also associated with the Fanconi anemia pathway [17]. USP1 is activated by formation of a complex with USP1-associated factor 1 (UAF1) and deubiquitinates two crucial DNA repair proteins, PCNA-Ub and FANCD2-Ub [18]. USP1 deubiquitinates polycomb repressive complex1 (PRC1), an important epigenetic modifier in stem cell development and maintenance [19]. Studies revealed that ID proteins are a substrate for USP1 in osteosarcomas and glioblastomas [20,21]. USP1 deubiquitinates and stabilizes the ID1, ID2, and ID3 proteins to preserve a mesenchymal stem cell (MSC) program in osteosarcoma [21].

USP2a

USP2a, an isoform of USP2, is an androgen-regulated DUB that deubiquitinates anti-apoptotic proteins fatty acid synthase, Mdm2, and MdmX (also known as Mdm4) [22,23]. USP2a stabilizes Mdm4 and facilitates the p53-mediated intrinsic apoptotic

pathway in glioblastoma [24]. TP53 is an important tumor suppressor gene encoding the protein p53, which regulates the cell cycle and is known as 'the guardian of the genome' [25]. Studies have shown that overexpression of USP2a leads to the degradation of p53 and increased cellular levels of Mdm2 and/or MdmX, whereas suppression of USP2a has the opposite effect [24]. Furthermore, studies on USP2a suggested an involvement in apoptotic pathways by stabilizing receptor-interacting protein 1 (RIP1) [26]. In prostate cancer, USP2a is generally overexpressed and its functional inactivation has been shown to enhance the apoptosis of cancer cells [27]. The Aurora-A protein, which is important for cell proliferation, has been shown to be deubiquitinated and stabilized by USP2a [28]. USP2a has also been found to be involved in cell cycle regulation and tumor progression by interacting with, and stabilizing cyclin A1 in bladder cancer cells [29].

USP4

USP4 has important roles in cancers by targeting proteins such as adenosine receptor acid-sensing ion channel 2A (ADORA 2A), tripartite motif 21 (TRIM 21), tumor necrosis factor (TNF)-receptor-associated factor 2 (TRAF), and TRAF6 [30]. USP4 shuttles between the nucleus and cytoplasm of the cell and is also involved in maintaining operational fidelity in the endoplasmic reticulum. USP4 has been shown to inhibit TNFα-induced cancer cell migration and breast cancer cell growth through upregulation of programmed cell death 4 (PCD4) [31]. USP4 also has an important role in the EMT of lung cancers, which is associated with acquirement and enhancement of stemness [32].

USP7

USP7, also known as Herpes virus-associated Ub-specific protease (HAUSP), is the key regulator of p53 activity; it deubiquitinates and stabilizes both, p53 and Mdm2, thus providing additional control over p53 regulation [33]. USP7 has a major role in the maintenance and differentiation of stem cells by stabilizing REST proteins, thereby preventing Skp1–Cullin-1–F-box beta and transducin repeat-containing E3 Ub protein ligase (SCFβ-TRCP)-mediated ubiquitination [34]. USP7 also deubiquitinates PRC1 and regulates LSD1/KDM1A, which has a role in embryonic development [35]. USP7, together with USP15, contributes to brain CSC maintenance by the reversal of stem cell transcription factor-REST activity [36].

USP9X

USP9X, also known as Fat facet in mouse (FAM), is an X-linked Ub-specific protease that is an essential component of the TGF-β signaling pathway. Monoubiquitination of the transcription factor Smad4 at K-519 is counteracted by USP9X, thereby impeding its activity [37]. USP9X is involved in the regulation of MAPK- and ASK1-mediated signaling in cancers [38]. USP9X stabilizes the MCL1 protein, which is normally expressed at low levels as a result of its rapid turnover by Ub ligases. [39]. USP9X interacts with Sox2 in glioblastoma cells [40] and has a role in the EMT of liver cells [41].

USP11

USP11 is capable of deubiquitinating IκBα *in vitro* [42]. Activation of NF-κB requires the Ub-mediated degradation of IκBα. Knockdown of USP11 enhances NF-κB activation by TNFα-induced ubiquitination of IκBα [42]. USP11 is responsible for deubiquitinating the type I TGF-β receptor, thus regulating TGF-β signaling [43]. TGF-β signaling is enhanced by the interaction of USP11 with SMAD7 [44]. The USP11 protein has also been associated with the BRCA2 protein involved in DNA damage repair systems [44]. In addition, USP11 directly deubiquitinates p53 and enhances its stabilization [45].

USP14

USP14 is a histidine and cysteine domain-containing DUB belonging to the Ub-specific processing (UBP) protease family [46]. USP14 is found in the cytoplasm of cells and cleaves Ub from precursors and ubiquitinated proteins [46]. USP14 is thought to regulate the degradation of proteins in neurons [47]. USP14 negatively regulates prion protein degradation [48] and has been shown to have an important role in the EMT of gastric cancers [49]. USP14 functions as a regulator of the Wnt signaling pathway by deubiquitinating the disheveled (dvl) protein, which is subsequently phosphorylated in response to Wnt, whereas knockdown of Usp14 prevents activation of downstream Wnt/β-catenin signaling [50]. The function of USP14 is thought to be complex because it also appears to be essential for the development of neuromuscular junctions and maintenance of synaptic Ub levels [51,52].

USP21

USP21 associates with microtubules and centrosomes, suggesting a role in the cell cycle [53]. USP21 catalyzes the hydrolysis of ubiquitinated H2A, thus activating transcription [54]. It also influences the Hedgehog signaling pathway by stabilizing glioma-associated oncogene 1 (GLI1) [55]. USP21 binds to the promoter region of IL-8 and mediates the transcriptional initiation required for stem cell-like properties in human renal cell carcinoma [56]. In addition, USP21 deubiquitinates Nanog and its absence affects the efficiency of somatic cell reprogramming [57].

USP22

USP22 positively regulates the histone deacetylase Sirt1, resulting in suppression of p53 function by reducing p53 acetylation [58]. Histone 2A (H2A) and histone 2B (H2B) complexes that are important in gene activation or silencing are regulated by USP22 in a similar manner to USP12 and USP46 [59]. USP22 directly influences the transcription of Sox2, which is essential for ESC differentiation, by binding to the Sox2 promoter region and promoting the lineage-specific differentiation of ESCs [60]. In addition, USP22 regulates LSD1/KDM1A in CSCs [35] and c-myc in breast cancer [61], thus augmenting its tumorigenic function. USP22 also has an important role in the EMT of pancreatic cancers [62].

USP28

USP28 has a crucial role in regulating Chk2-p53-PUMA signaling pathways and is involved in DNA damage-induced apoptosis in response to double-strand breaks [63,64]. USP28 also regulates cancer signaling; for example, USP28 stabilizes the oncogenic transcription factor c-myc by counteracting the action of a Skp1–Cullin-1–F-box complex containing Fbw7 as the F-box protein, the (SCF^{Fbw7}) Ub ligase complex [65]. USP28 antagonizes the Ub-dependent degradation of c-myc, as well as c-Jun and Notch, by targeting the Ub ligase Fbw7, thereby stabilizing the HIF-1α

transcription factor [66]. USP28 also stabilizes and regulates LSD1/KDM1A by preventing the ubiquitination of LSD1 [67].

USP44

USP44 is an important mitotic spindle checkpoint regulator that stabilizes the APC inhibitor Mad2-Cdc20 complex, thereby preventing the premature activation of APC [68]. Cells with high levels of USP44 tend to undergo aneuploidization and exhibit errors in chromosome separation [69]. Additionally, USP44 deubiquitinates H2B, which is essential for ESC differentiation [70]. USP44 expression in ESCs was found to be associated with the POU domain class 5 transcription factor 1 (*Pou5f1*), *Nanog*, and *Sox2* genes through complex interactions between several different epigenetic factors [71].

USP54

USP54 is overexpressed in intestinal stem cells and colorectal CSCs [72]. Silencing of USP54 in colorectal cancer cells decreases their proliferation, invasiveness, and colony-forming capacity, as well as their tumorigenicity, when injected into immunodeficient mice [72].

A20

A20 exhibits both DUB and E3 ligase activity [73]. It is also known as TNFα-induced protein 3 (TNFAIP3), and was shown to be responsible for negative regulation of the NF-κB transcription factor by removing K-63 linked polyubiquitin chains on RIP1 [74] and TRAF6 [73]. Through its inhibitory role in the NF-κB pathway, A20 acts as a tumor suppressor in lymphoid malignancies [74,75].

UCHL1

UCHL1 is overexpressed in lung adenocarcinomas linked to smokers compared with nonsmokers and was speculated to be an early marker of the transformation process of normal lung epithelium [76]. By contrast, the *UCHL1* gene was found to be silenced through methylation of CpG islands in the gene promoter in giant cell tumors in bone [77]. UCHL1 has an important role in the EMT of prostate cancers [78].

UCHL5

UCHL5 forms a reversible association with the proteasome by binding to the 26S proteasome via the Admr1 receptor in the 19S RP base complex; this enhances the isopeptidase activity of the enzyme [79] and displaces the crossover loop at the UCH-domain active site, allowing substrate entry [80]. Rpn11/POH1 is another important DUB associated with the 19S regulatory particle of the proteasome (Note S4 in the supplemental information online). UCHL5 expression has been proposed as a novel prognostic marker in lymph node-positive rectal cancer [81]. UCHL5 also promotes the degradation of specific substrates, such as nitric oxide synthase (NOS) and IκBα [82].

OTUB1

OTUB1 belongs to a family of DUBs that contain the ovarian tumor-OTU domain [83]. Overexpression of OTUB1 stabilizes and activates p53 by suppressing MDM2-mediated p53 ubiquitination [84]. Monoubiquitination of OTUB1 was found to suppress UbcH5 activity, thereby stabilizing p53 [85]. OTUB1 negatively regulates estrogen receptor (ER)-α-mediated transcription through deubiquitination of ERα [86], virus-induced type I IFN induction, and antiviral responses by deubiquitinating TRAF3 and TRAF6 [87]. OTUB1 has also been found to have an important role during the EMT of colorectal cancer [88].

CYLD

CYLD germline mutation is associated with the development of multiple skin tumors of the head and neck that occur in familial cylindromatosis [89]. CYLD mutant human cylindroma tumors demonstrate a hyperactive Wnt signaling pathway resulting from enhanced K-63 linked ubiquitination of the Wnt pathway protein, Dvl [90]. CYLD deubiquitinates K-63 linked polyubiquitin chains on Bcl-3, a proto-oncogene [91]. CYLD is also involved in the regulation of NF-κB activation by inhibiting NF-κB-mediated activation and deubiquitinating TNF receptors, such as TRAF2 and TRAF6 [92].

DUB inhibitors

DUBs have been proposed as an advantageous therapeutic target for cancer and other diseases because they have the ability to modulate protein fate in a specific and selective manner; they can alter a key aspect in the pathological fate of a cell, for example directing the cell toward recovery or death [93]. DUBs that regulate oncogenic proteins can be targeted by compounds that inhibit their activity via UPS degradation, whereas DUBs that regulate tumor suppressor proteins could be targeted for activation by decreasing their UPS degradation, thereby inhibiting oncogenic processes. Extensive research has been carried out to design DUB inhibitors because they are easier to design and develop than are enzyme activators using competitive inhibition and substrate modeling [93], as summarized in Table 1.

DUB inhibitors in CSC-targeted therapy

Multiple strategies have been proposed to target CSCs and their niche, including targeting specific surface markers, inhibition of drug-efflux pumps, manipulating miRNA expression, adjusting micro-environmental signaling, induction of apoptosis, differentiation of CSCs and, more lately, targeting DUBs using inhibitors. Reactive oxygen species (ROS) levels in CSCs are low compared with other cell types and studies have shown an inverse relationship between ROS levels and DUB activity in CSCs; therefore, targeting the hyperactivated DUBs in CSCs will be valuable in the treatment of tumors. Multiple DUBs, including Psmd14, USP16, and USP44 in ESCs [70,94] and USP3 and CYLD in HSCs [95,96], are potential targets for DUB inhibitors.

Studies have shown that downregulation of A20 expression in germline stem cells impairs their survival and growth *in vitro* and decreases tumorigenicity in mice bearing human glioma xenografts [97]. Several DUBs have been shown to regulate the EMT pathway in cancer cells [98] and, because of its relationship with cancer stemness, these DUBs should also be regarded as candidates for CSC-targeted therapy. Several DUB inhibitors that are being considered as potential candidates for CSC-targeted therapy are described below.

Pimozide (an antipsychotic drug) is a USP1-specific inhibitor and has been used to target radiation resistance and CSC-mediated

TABLE 1

Summary of DUB inhibitors[a]

Compound	Activity
Pimozide	USP1
ML323	USP1
VLX1570	USP14, UCHL5
LDN-57444	UCHL1
TCID	UCHL3, UCHL1
GW7647	USP1/UAF1
EOAI3402143 (G9)	USP9X, USP5, USP24
Vialinin A	USP5/IsoT, USP4
P5091	USP7, USP47
Cpd 14	USP7 and USP47
P22077	USP7/USP47
HBX 41,108	USP7
HBX-19,818	USP7
HBX-28,258	USP7
HBX 90,397	USP8
IU1	USP14
Isatin O-acyl oxime derivatives	UCHL1
LDN91946	UCHL1
LS1	UCHL3
NSC112200, NSC267309	TRABID/ZRANB1
PR-619	Broad-spectrum DUB inhibitor
12Δ-PGJ2	UCHL3
15-Deoxy-Δ12,14-prostaglandin J2	UCHL1
G5	Inhibition of cellular Ub-AMC cleavage
F6 (NSC 632839)	Inhibition of cellular Ub-AMC cleavage, USP2, USP7, SENP2 deSUMOylase
WP-1130	USP5/USP9x/USP14/UCHL1/UCHL5
b-AP15 (WO2013058691)	USP14/UCHL5
AM146, RA-9,and RA-14	USP2a/USP2b/USP5/USP8
Eeyarestatin 1	Ataxin-3
Curcumin	Accumulation of polyubiquitin, contains α,β unsaturated ketones
AC17 (curcumin analog)	USP14, UCH-L5
Betulinic acid	Broad-spectrum DUB inhibition
Gambogic acid	Accumulation of polyubiquitin, contains α, β unsaturated ketones
WO2013030218	USP7
Auranofin	USP14
PX-478 (WO/2005/007828)	DUBs associated to HIF-1α
Tricyclic heterocyclics (WO2011094545)	USP14
Azepan-4-ones (US20140370528A1)	UCHL5, USP14
6-Amino-pyrimidines (WO201405952A3)	USP1/UAF1

[a] References to DUB inhibitors can be found in Table S2 in the supplemental information online.

tumor resistance. Pimozide targets USP1 in osteosarcoma and glioblastoma cells [99,100].

ML323 is an inhibitor of the USP1/UAF1 deubiquitinase complex. ML323 has been shown to potentiate cisplatin cytotoxicity in NSCLC and osteosarcoma cells [101].

PX-478 or melphalan N-oxide {S-2-amino-3-[4′-N,N-bis(2-chloroethyl)amino] phenyl propionic acid N-oxide dihydrochloride} is a small-molecular-weight anticancer agent that inhibits DUBs associated with HIF-1α [102]. PX-478 also decreases the expression of downstream targets genes, such as vascular endothelial growth factor (*VEGF*), and inhibits HIF-1α transactivation in several cancerous cell lines [103]. PX-478 has been described as a valuable CSC-targeted therapeutic drug for its downregulation of HIF-1α signaling, which is often hyperactivated in the hypoxic niche of CSCs [104].

C527 was identified as a USP1 inhibitor that promotes the degradation of the ID1 protein, which has a central role in keeping cells in an immature state. C527 is also responsible for the concurrent upregulation of p21 in mouse osteosarcoma cells, thereby increasing erythroid differentiation of leukemic cells [105].

PR619 is a small-molecule DUB inhibitor that acts as a non-selective reversible inhibitor of DUBs. PR619 is a cell-permeable pyridinamine class broad-spectrum DUB inhibitor the known targets of which include ATXN3, BAP1, JOSD2, OTUD5, UCH-L1, UCH-L3, UCH-L5/UCH37, and USPs – 1, 2, 4, 5, 7, 8, 9X, 10, 14, 15, 16, 19, 20, 22, 24, 28, 47, and 48. PR619 treatment results in the upregulation of K-48- and K-63-linked polyUb chains [106].

P5091 has been shown to inhibit tumor growth by inhibiting USP7 and USP47 and is well tolerated in animals [107]. P5091 induces apoptosis of multiple myeloma cells that are resistant to bortezomib, a 20S proteasome inhibitor. It targets USP7 and USP47 of neural, glioblastoma, and multiple myeloma cells [34,35,108].

P22077, an analog of the recently discovered DUB inhibitor P5091, is an inhibitor of USP7 and its closely related DUB USP47. It inhibits neuroblastoma growth by inducing p53-mediated apoptosis [109].

WP-1130 is a small-molecule compound with Janus-activated kinase 2 (JAK2) kinase inhibitory activity that inhibits several DUBs of USP and UCH subclasses, such as USP5, USP9X, USP14, USP15, USP37, and UCHL1, in several CSC types, including liver and breast cancer [110,111]. WP-1130 rapidly induces ubiquitination of Bcr-Abl, resulting in its relocalization from the cytoplasm into aggresomes and resulting in the loss of Bcr-Abl oncogenic signaling [112]. Exposure of cells to WP-1130 results in the downregulation of the anti-apoptotic protein MCL1. This is expected to be because of inhibition of USP9X expression in many tumors, including hematological malignancies [113]. WP-1130 combined with bortezomib showed antitumor activity in a mantle cell lymphoma animal model [114].

b-AP15 (WO2013058691) is an inhibitor of USP14 and UCHL5 associated with 19S RP that has been found to inhibit the progression of tumors in certain human cancers as well as mouse cancer models of lung, colon, breast, and head and neck carcinomas [115]. b-AP15 was identified together with cathepsin-D and p53 in a cell-based screen of compounds that induce apoptosis [116]. Studies have shown that co-inhibition of both USP14 and UCHL5 using RNA interference leads to strong accumulation of proteasomal substrates and loss of cell viability [117]. Studies in animal models showed that b-AP15 has considerable activity against multiple myelomas and solid tumors [117].

VLX1570 is an analog of b-AP15 that shows higher potency and improved solubility. VLX1570 is an inhibitor of USP14 and UCHL5 with apoptosis-inducing and antineoplastic activities [117]. Increased expression of USP14 in multiple myeloma cells was associated with elevated sensitivity to proteasome DUB inhibition by VLX1570 [118]. VLX1570 was approved in a Phase 1/2 clinical trial in combination with dexamethasone (NCT02372240) by the US Food and Drug Administration (FDA) in 2017 [118].

LDN-57444 is an isatin *O*-acyl oxime reported to selectively, competitively, and reversibly inhibit UCHL1 [119]. LDN-57444 is an active site directed inhibitor that has been shown to increase proliferation of the H1299 lung tumor cell line expressing UCHL1 [119]. Studies conducted in SK-N-SH neuroblastoma cells showed that LDN-57444 caused an increase in the levels of polyubiquitinated proteins

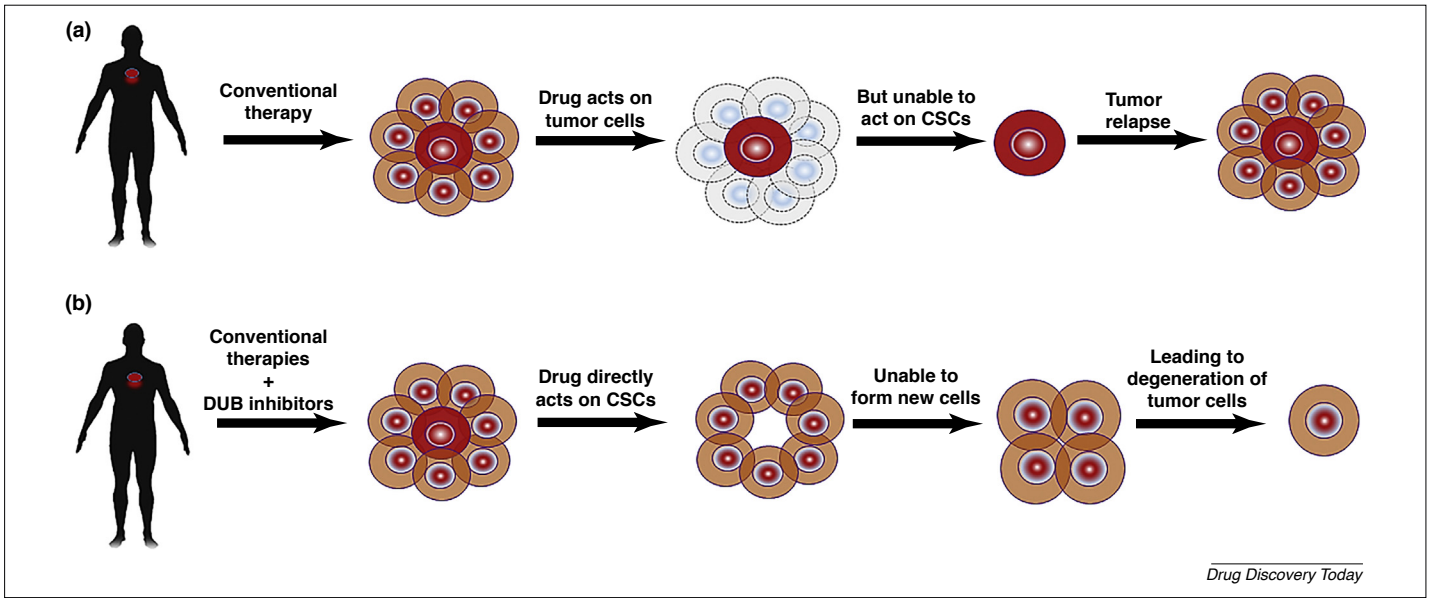

FIGURE 3

Cancer therapy strategies. (a) Conventional therapies, such as radiation and chemotherapy, target only the cancerous cells and not the cancer stem cells (CSCs) that lead to relapse. (b) CSC-targeted therapies, similar to conventional therapy plus deubiquitination enzyme (DUB) inhibitors, lead to tumor regression.

and induced endoplasmic reticulum stress-related apoptosis [120]. LDN-57444 also targets UCHL3 in prostate cancer [121].

TCID is a potent, selective, and cell-permeable inhibitor of UCHL3 that is responsible for the removal of Ub from polypeptides and the regulation of cellular Ub levels [119]. TCID targets UCHL3 and UCHL5 of multiple myeloma cells [117].

Concluding remarks

Targeting enzymes upstream of the proteasome in the UPS system is known to have adverse effects; targeting E1 leads to cell cycle arrest and targeting E2 impairs development [122]. By contrast, DUBs have sparring functions; they prevent substrate proteins from being degraded by the proteasome and might be responsible for protecting the stemness of CSCs as well as NSCs; in addition, they are key regulators of processes such as signal transduction, proliferation, and apoptosis. DUB inhibitors are an important part of the pharmaceutical armamentarium by targeting the most obstreperous DUBs that regulate diverse CSC-related proteins. This approach to targeting cancer can counter the inevitable problems faced in cancer therapy such as drug resistance and disease recurrence (Fig. 3). Further research should be undertaken to understand the natural regulatory mechanisms in cells to identify candidate pathways and targets required for the regulation of DUB activity and expression that could be pharmacologically modified.

DUBs represent a complex system for regulating cellular activities and the outcome of targeting these DUBs in combination with conventional cancer therapies is currently being investigated by companies such as Progenra [123]. Therefore, further research to better understand the roles of the DUBs being targeted should be undertaken so that intelligently crafted targeted clinical regimens can be designed for different cancers. The ability of DUB-targeted therapies to benefit other diseases and conditions in addition to cancers might also be worthy of further research.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.drudis.2018.05.035.

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