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Review article

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Deubiquitinases in cancer

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Abstract

Ubiquitination is an essential regulator of most, if not all, signalling pathways, and defects in cellular signalling are central to cancer initiation, progression and, eventually, metastasis. The attachment of ubiquitin signals by E3 ubiquitin ligases is directly opposed by the action of approximately 100 deubiquitinating enzymes (DUBs) in humans. Together, DUBs and E3 ligases coordinate ubiquitin signalling by providing selectivity for different substrates and/or ubiquitin signals. The balance between ubiquitination and deubiquitination is exquisitely controlled to ensure properly coordinated proteostasis and response to cellular stimuli and stressors. Not surprisingly, then, DUBs have been associated with all hallmarks of cancer. These relationships are often complex and multifaceted, highlighted by the implication of multiple DUBs in certain hallmarks and by the impact of individual DUBs on multiple cancer-associated pathways, sometimes with contrasting cancer-promoting and cancer-inhibiting activities, depending on context and tumour type. Although it is still understudied, the ever-growing knowledge of DUB function in cancer physiology will eventually identify DUBs that warrant specific inhibition or activation, both of which are now feasible. An integrated appreciation of the physiological consequences of DUB modulation in relevant cancer models will eventually lead to the identification of patient populations that will most likely benefit from DUB-targeted therapies.

Sections

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Introduction

Post-translational modifications with ubiquitin influence every cellular process through either proteolytic or non-proteolytic mechanisms (reviewed elsewhere[1,2]). During the process of ubiquitination, a cascade of E1 (ubiquitin-activating), E2 (ubiquitin-conjugating) and E3 (ubiquitin ligase) enzymes act sequentially to covalently attach a ubiquitin molecule to a substrate, with substrate selection provided by >600 E3 ubiquitin ligase enzymes. Substrate monoubiquitination (ubiquitination at a single lysine (Lys) residue) is the simplest yet most highly abundant ubiquitin signal in cells. Monoubiquitin can be further ubiquitinated at eight amine attachment points within ubiquitin itself (seven Lys residues, and the amino-terminal methionine (Met)) to form ubiquitin chains that are homotypic (single linkage type) or heterotypic (mixed linkage types, or branched polyubiquitin architectures)[2,3]. Moreover, ubiquitin can also be phosphorylated and acetylated, constituting yet again distinct ubiquitin signals[4]. Finally, recent insights have also broadened the substrate range of ubiquitination, revealing that in addition to Lys residues, also serine (Ser) and threonine (Thr) residues in proteins, hydroxy groups in glycans and ADP-ribose, and hydroxy and amino groups in lipids can be ubiquitinated[3]. Indeed, it is currently not clear just how expansive the ubiquitin system is.

The plethora of ubiquitin signals provides cells with a versatile switchboard to relay highly sophisticated signalling. Indeed, E3 ligases (sometimes in conjunction with additional modifying enzymes) purposefully shape ubiquitin signals[5], whereas ubiquitin receptors relay appropriate cellular responses[6]. Those responses also vary greatly as ubiquitination affects fundamental cell biological pathways, including replication, transcription, splicing, translation, localization and trafficking, aggregate and condensate formation, autophagy and more[1–3].

The majority of ubiquitination events signal for imminent degradation of a substrate via the proteasome, lysosome or autophagosome[7]. Proteasomal degradation is triggered by several chain types, including Lys48, Lys11, Lys29 and Lys6 polyubiquitination, and also by more complex ubiquitin architectures such as branched chains[2,3]. These ubiquitin signals are abundant and delivered or directly recognized by ubiquitin receptors on the proteasome or upstream unfoldases such as the AAA+ ATPase valosin-containing protein (VCP) (also known as p97 or CDC48)[8]. Proteins at the plasma membrane are principally degraded via the lysosome following internalization. Ubiquitination, primarily Lys63, directs membrane proteins for lysosomal degradation through the endosomal sorting complex required for transport (ESCRT) pathway. ESCRT proteins mediate the formation of multivesicular bodies (MVBs) that encapsulate the internalized cargo and which are then directed to fuse with lysosomes for substrate degradation by acidic proteases[9]. Selective autophagy involves the capture and degradation of cytosolic protein aggregates or organelles, including mitochondria, ribosomes and peroxisomes. Ubiquitination of the cargo triggers recruitment of selective adaptor proteins that promote formation of a double-membrane autophagosome. Fusion of the autophagosome with a lysosome facilitates cargo degradation and recycling[10].

However, ubiquitin signals also regulate more nuanced 'signalling' outcomes than target degradation. For example, some chain types, most notably Met1-linked chains, often provide interaction platforms to initiate protein complex formation to provide directionality in signal transduction[11–13]. Furthermore, chain linkage specific ubiquitin binding domains (UBDs) in scores of proteins find and respond to specific ubiquitination events. Additionally, specialized ubiquitin signals such as Met1-linked chains or Ser65-phosphoubiquitin are maintained at low, barely detectable, levels at steady state, but are amplified for acute signalling[14,15].

The complex landscape of ubiquitination is counteracted by ~100 specialist proteases in humans, the deubiquitinases or deubiquitinating enzymes (DUBs). Six of the seven known DUB families in humans comprise distinct cysteine (Cys) protease folds, and one family comprises a zinc metalloproteinase fold[16] (Fig. 1). DUBs specifically cleave the isopeptide bonds between ubiquitin and Lys side chains in the ubiquitinated substrate and only rarely cleave peptide bonds (Box 1). Although all DUBs target the same substrate, ubiquitin, they are capable of proficiently dealing with the above-described multitude of ubiquitin signals added to thousands of proteins. DUBs typically either identify a ubiquitin signal (for example, chain type) in a target-agnostic manner or identify a target protein in a ubiquitin signal-agnostic manner (Box 1), although a scenario in which both the target and the specific ubiquitin signal are recognized cannot be excluded. The observed substrate or signal specificity is typically provided by bipartite interactions that appropriately coordinate the scissile bond for cleavage[16–18]. Similar to the essential duality between kinases and phosphatases, a fine balancing act between the opposing E3 ligase and DUB factions is fundamental for coordinated proteostasis and cell signalling. Given their fundamental roles in various signalling pathways[19], it is imperative that DUB activity is tightly controlled through localization, post-translational modifications, conformational transitions or binding of adaptor proteins to facilitate substrate binding and ensure a peptidase competent configuration[20] (Box 1).

DUBs have been linked to each of the recognized hallmarks and enabling characteristics of cancer[21–23] (Tables 1 and 2). We have attempted to reconcile this substantial body of literature in Supplementary Table 1, selecting papers that provided evidence of DUB–substrate interaction and evidence of modulation of ubiquitin on the substrate by the DUB. In preparing this resource, we became aware that many reports remain one-off studies that lack independent validation and that many earlier studies would benefit from improved next-generation tools and approaches. Moreover, whereas ubiquitin and DUB research is generally rich in mechanistic and structural studies, ubiquitin and DUB-focused cell biological, cell signalling and physiological analysis somewhat lags behind. For example, the use of animal models in DUB research is comparatively limited. On the contrary, ubiquitin and DUB biochemistry efforts have embedded numerous DUBs in key protein complexes, including the proteasome, the VCP complex, the Spt–Ada–Gcn5 acetyltransferase (SAGA) complex and the COP9 signalosome (CSN), or have identified fundamental cellular 'house-keeping' roles for some of the most conserved and abundant DUBs. These central roles and associations for key enzymes (some of them listed in Box 2) are often insufficiently considered when, for example, genetic screens link DUBs with certain cancer phenotypes. The effect of DUBs in cancer also often appears to be highly context dependent. Numerous DUBs are tumour promoting in one context and cancer setting, yet tumour suppressive in a different cancer setting, and these contrasting effects have obvious implications for targeting specific DUBs as a cancer therapy.

It is nonetheless clear that modulating DUB activity is an emerging therapeutic avenue to limit cancer growth, or to improve response to chemotherapy and immunotherapy, and we pay specific attention to those DUBs that have emerged as clear therapeutic targets. The ongoing effort to develop potent and highly specific DUB inhibitors for a small number of enzymes, together with new biological validation methods, will vastly improve the quality and impact of

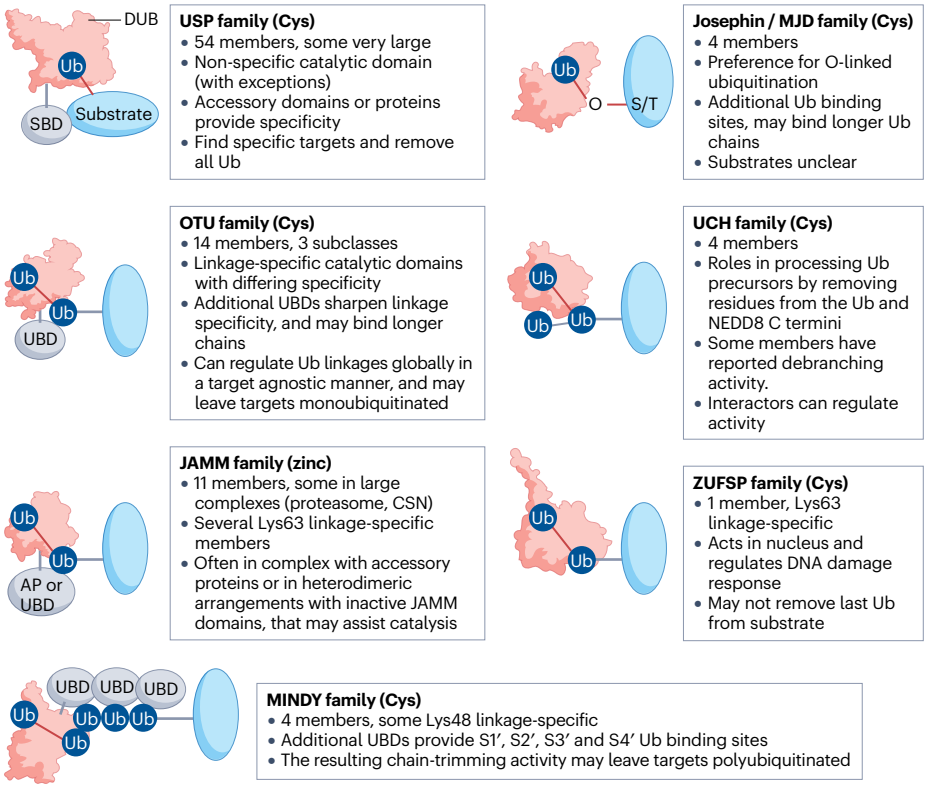


Fig. 1 | DUB classes and features. Deubiquitinating enzymes (DUBs) delete or edit ubiquitin chains to modulate the effect on target substrates, which is dependent on the DUB, context and substrate, as well as process ubiquitin chains to maintain an unconjugated ubiquitin pool. Approximately 100 human DUBs are sub-divided into seven different classes based on their structural fold. For more details and domain annotations see refs. 16,17,273. Some DUBs achieve an increased substrate range by their interactions with adaptor proteins, through different isoforms that determine context-dependent targets and via numerous forms of post-translational regulation[17]. 'Cys' denotes Cys proteases, whereas 'zinc' denotes metalloproteinase folds. Red lines indicate the scissile isopeptide bonds between substrates and ubiquitin. 'Ub' spheres are shown to illustrate binding sites and are not drawn to scale. AP, adaptor protein; CSN, COP9 signalosome; JAMM, JAB1/MPN/MOV34 DUB; MINDY, motif interacting with ubiquitin (MIU)-containing novel DUB; MJD, Machado Joseph disease; NEDD8, neural precursor cell expressed, developmentally downregulated 8; OTU, ovarian tumour protease; S′, substrate ubiquitin binding site, providing the ubiquitinated lysine (Lys); SBD, substrate binding domain; Ser/Thr, serine/threonine; Ub, ubiquitin; UBD, ubiquitin binding domain; UCH, ubiquitin carboxy-terminal hydrolase; USP, ubiquitin specific protease; ZUFSP, zinc finger-containing ubiquitin peptidase 1.

DUB studies. Therefore, we end the Review with a brief discussion of the first highly selective DUB inhibitors that will improve research toolkits and enhance the understanding of the specific contextual biological role of each of these DUBs, ostensibly leading to better outcomes for patients.

Regulation of cancer targets by DUBs

Principally, DUBs can regulate their targets directly or indirectly, and the suggested mode of regulation requires scrutiny, as indirect regulation implicates potential pleiotropic effects with implications for potential therapeutic targeting. For DUBs that directly regulate a target of interest, the DUB needs to bind and deubiquitinate the target protein. Although this typically leads to stabilization of the target, the removal of non-degradative ubiquitin signals can also alter target conformation and interactions. For example, OTULIN-mediated removal of Met1-linked ubiquitin influences recruitment of adaptor proteins to the tumour necrosis factor (TNF) receptor 1 (TNFR1) complex to dictate downstream nuclear factor-κB (NF-κB) signalling[24]. However, the investigation of ubiquitinated substrates is often confounded by the difficulty in unveiling endogenous ubiquitination as opposed to that driven by artificial overexpression of ubiquitin enzymes.

Likely prevalent are indirect modes of DUBs regulating cancer genes. DUBs can mediate the regulation of E3 ligase complexes or degron-inducing signalling cascades, thereby causing a global imbalance in ubiquitination landscapes in cells that affects many ubiquitinated proteins and not just one specific target. Yet DUBs can also influence translation and transcription as ubiquitination affects gene expression at various levels, for example due to dysregulated epigenetic mechanisms or dysregulated transcriptional activation of oncogenes[25].

A prime example of a DUB that has far-reaching influence on cellular processes is ubiquitin carboxyl-terminal hydrolase 22 (USP22), encoded by a gene that has been designated a 'death-from-cancer' gene, part of a signature of genes identified across multiple cancer types that is predictive of cancer aggressiveness and poor patient response to treatment[26]. USP22 has been implicated in many cancer settings, including through the regulation of nuclear proteins such as MYC, its degrader F-box/WD repeat-containing protein 7 (FBXW7),

telomeric repeat-binding factor 1 (TRF1) (part of the shelterin complex involved in telomere length maintenance), transcriptional repressor far upstream element (FUSE)-binding protein 1 (FBP1), the cell cycle inhibitor p21 (WAF1) and proteins involved in DNA damage repair, such as XPC, as well as through the regulation of cytosolic targets, including programmed cell death protein 1 ligand 1 (PDL1) and mTOR (all discussed further below). However, biochemically, USP22 as part of the chromatin-associated SAGA complex (Box 2) primarily serves to deubiquitinate histones H2A and H2B[27]. Although it is theoretically possible that the SAGA complex deubiquitinates all of the predicted nuclear substrates directly with some degree of specificity, while also localizing to the cytosol to target, for example, membrane-bound proteins such as PDL1, we believe this is a somewhat unlikely scenario; although USP22 could perhaps form novel non-SAGA complexes. Therefore, the simplest explanation to understand the impact of USP22 on cancer-related pathways and substrates is likely an indirect effect through regulating epigenetic marks on chromatin thereby affecting transcription, and so the validation of USP22 targets should at the very least also involve examination of mRNA transcripts to test this indirect mechanism. Although we try to comprehensively list all reported roles

Box 1

Principles and mechanisms of DUB activity

Principles

As intracellular proteases, deubiquitinating enzymes (DUBs) are specialized towards regulating ubiquitin (and sometimes ubiquitin-like (Ubl)) signals, which are abundant and complex. The ~100 DUBs oppose >600 E3 ubiquitin ligases and >50,000 ubiquitination sites detected at steady state in cells, resulting in a modification system that is both dynamic and plastic. To effectively modulate this system, DUBs provide both generalist and specialist enzyme activity towards ubiquitin modifications.

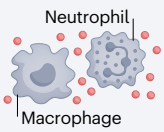
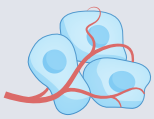
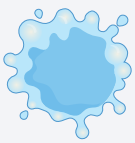
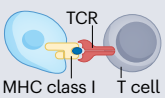
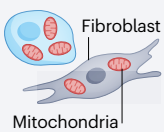
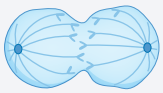
- Ubiquitin signal-specific, substrate-agnostic DUBs show strong chain linkage specificity, are directed towards branches in ubiquitin chains or preferentially remove oxyester-linked ubiquitination. These enzymes can globally regulate abundance of certain types of ubiquitin signals. A good example is OTULIN, which is highly specific for methionine 1 (Met1)-linked polyubiquitin, and which keeps this inflammatory chain type barely detectable in cells under homeostatic conditions. A third of all DUBs are estimated to act in a ubiquitin signal-specific but target-agnostic fashion. Moreover, many DUBs are linked to defined cellular contexts, by virtue of their localization or their coordination as part of large cellular machines such as the proteasome, the AAA+ ATPase valosin-containing protein (VCP) complex, the ribosome, transcriptional complexes such as Spt–Ada–Gcn5 acetyltransferase (SAGA) and E3 ligase complexes such as linear ubiquitin chain assembly complex (LUBAC). The activity of these enzymes within complexes can be ubiquitin signal-specific and target-agnostic, but is generally regulated by the cell biological context or by the parent complexes themselves.
- Ubiquitin signal-agnostic, substrate-specific DUBs, including many of the ubiquitin specific protease (USP) family enzymes (Fig. 1), recognize their substrate protein and keep it ubiquitin-free, thereby reversing the effects of E3 ligases on specific cellular substrates. This can result in direct stabilization of substrates, with direct implications for diseases, including cancer. Substrate-specific DUB activity can also result in removal of epigenetic marks on chromatin, diversion of protein trafficking and ubiquitin signalling outputs or destabilization of protein complexes, all of which can be disease relevant when deregulated.

Mechanisms

DUBs have at least one ubiquitin binding site (S1; see also Fig. 1) that drives the interaction of the DUB with ubiquitin. S1 orientates the carboxy terminus of a ubiquitin molecule into the DUB active site where the scissile bond between the C-terminal glycine of ubiquitin and commonly a lysine (Lys) residue on a substrate or another ubiquitin molecule is hydrolysed (reviewed elsewhere[16,264]). Some DUBs are in an inhibited conformation in the absence of bound ubiquitin, with conformational switching facilitating catalytic activity. Maintenance of inactivity sometimes involves an inhibitory loop that occludes the DUB active site. Loop reorientation, sometimes triggered by post-translational modifications or cofactor engagement, promotes an open conformation to facilitate ubiquitin binding. Although there are structural variations, most DUBs catalyse bond cleavage through a catalytic Cys-His-Asp/Asn/Glu triad. Additional ubiquitin binding sites bind ubiquitins proximal or distal to the S1-bound ubiquitin and provide binding surfaces to coordinate ligase-selective ubiquitin chain binding to determine DUB specificity. Additional regulatory domains within DUBs also influence their activity and specificity. For example, USP7 bears a TRAF-like domain that is unique amongst USP family enzymes, which together with five Ubl domains determines substrate recognition and activity. In addition, the C terminus of USP7, far removed from the catalytic domain by several hundred amino acids, binds back and allosterically activates USP7 cleavage activity[274].

Given their defined roles in cellular signal transduction, a vast number of DUB regulatory mechanisms have been uncovered, and in addition to transcriptional and translational regulation, DUBs are known to be phosphorylated, ubiquitinated, SUMOylated, oxidized and allosterically activated by binding partners or substrates. We refer the reader to other reviews for further insights into DUB mechanisms and regulation[16,17]. Moreover, certain DUBs also exhibit functions independent of their catalytic activity. For example, OTU domain-containing protein 4 (OTUD4) has a phosphorylatable switch that governs catalytic activity, but also has a scaffolding role in coordinating responses to alkylating DNA damage that is independent of its catalytic activity[275,276]. Understanding the molecular control of DUB levels and/or activity, and resolving non-catalytic functions, will certainly reveal more nuanced roles of DUBs in signalling networks, including in cancer.

Table 1 | DUBs in the cancer hallmarks

Cancer hallmark	DUBs	Regulation
Genomic instability and mutation 	USP1, USP3, USP7, USP8, USP9X, USP10, USP11, USP13, USP14, USP15, USP17, USP19, USP26, USP28, USP36, USP37, USP38, USP48, BRCC26, CSN5, OTUB1, OTUB2, UCHL3	↑
	USP20, USP21, USP29, USP47, USP51, BAP1, VCIP135	↓
Sustained proliferative signalling 	USP1, USP2A, USP4, USP5, USP7, USP8, USP9X, USP10, USP12, USP13, USP14, USP15, USP16, USP18, USP19, USP20, USP21, USP22, USP25, USP26, USP28, USP32, USP34, USP35, USP37, USP38, USP47, A20, AMSH, ataxin 3, ataxin 3-like, MINDY1, OTUB1, OTUD5, OTUD7B, UCHL1	↑
	USP11, USP46, USP49, BAP1, CYLD, OTUD3	↓
Tumour-promoting inflammation 	USP4, USP6, USP7, USP10, USP11, USP22, USP38, OTUD5, POH1, TRABID	↑
	USP25, A20, CYLD, MYSM1	↓
Inducing angiogenesis 	USP10, USP39, BAP1, OTULIN	↑
Resisting cell death 	USP2A, USP3, USP4, USP8, USP9X, USP11, USP13, USP14, USP17, USP19, USP21, USP22, USP28, USP30, USP33, USP35, USP40, USP47, BRCC36, CYLD, JOSD1, OTUB1, OTUD5, PSMD7, UCHL1	↑
	USP7, USP10, USP15, USP27X, USP29, USP39, BAP1, OTUD1	↓
Avoiding immune destruction 	USP7, USP9X, USP14, USP15, USP18, USP20, USP22, USP24, USP35, CSN5, CYLD, OTUB1	↑
	USP12	↓
Deregulating cellular energetics 	USP7, USP9X, USP14, USP17, USP20, USP21, USP22, USP28, USP29, USP37, USP44, A20, BAP1, JOSD2, OTUB2, OTUD6A, OTUD7B	↑
	USP10, USP30, USP48, USP49, USP53	↓
Evading growth suppressors 	USP1, USP2, USP3, USP6, USP7, USP9X, USP10, USP11, USP12, USP15, USP20, USP21, USP37, OTUD5	↑
	USP28, BAP1	↓
Enabling replicative immortality 	USP1, USP2A, USP2, USP4, USP5, USP6, USP7, USP9X, USP10, USP13, USP14, USP15, USP16, USP17, USP20, USP21, USP22, USP25, USP27, USP28, USP33, USP34, USP35, USP36, USP37, USP38, USP39, USP42, USP43, USP46, USP47, USP48, USP52, CSN5, OTUB1, OTUD6A, OTUD7B, POH1, UCHL3	↑
	USP11, USP44, BAP1, CYLD, OTUD1	↓

See Supplementary Table 1 for details and references. AMSH, associated molecule with the SH3 domain of STAM; BAP1, BRCA1-associated protein 1; BRCC36, BRCA1/BRCA2-containing complex subunit 36; CSN5, COP9 signalosome complex subunit 5; DUB, deubiquitinating enzyme; JOSD, Josephin domain-containing protein; MINDY, MINDY, motif interacting with ubiquitin (MIU)-containing novel DUB family; MYSM1, Myb-like, SWIRM and MPN domain-containing protein 1; OTUB, OTU domain-containing ubiquitin aldehyde-binding protein; OTUD, OTU domain-containing protein; TRABID, TRAF-binding domain-containing protein (also known as ZRANB1); UCHL, ubiquitin carboxyl-terminal hydrolase isozyme L; USP, ubiquitin carboxyl-terminal hydrolase.

of DUBs associated with cancer, we also highlight obvious instances of apparent discordance between biochemical, molecular and cell biological studies and proposed roles in cancer regulation.

Cancer-causing DUB mutations

Cancer-associated mutations in DUBs are relatively rare and predominantly found in cancers that have high mutational load, such as melanoma and lung carcinoma[28]. However, there are a few notable exceptions. For example, A20 is frequently inactivated by somatic mutations and/or deletions in haematological cancers[29,30]. Additionally, genetic linkage analyses and sequencing of a region on chromosome 3p21 identified inherited mutations in the ubiquitin carboxy-terminal hydrolase (UCH) domain-containing DUB BRCA1-associated protein 1 (BAP1)[31–33]. Moreover, BAP1 tumour predisposition syndrome is a hereditary condition characterized by germ-line mutations in BAP1 and is associated with the development of various benign and malignant tumours, mainly clear cell renal cell carcinoma, bladder cancer, mesothelioma, cutaneous melanoma and basal cell carcinoma[31–35]. Sporadic inactivating mutations in BAP1 have also been identified in uveal melanoma, which always metastasize from the eye, with more than 90% of patients succumbing to the disease within 2 years[35]. Furthermore, more than 100 different mutations have been found in the DUB CYLD, which causes familial cylindromatosis, a condition that results in typically benign skin tumours[36].

Although mutations are relatively rare, many cancers do exhibit aberrant expression of DUBs based on measures of mRNA or protein that correlates with disease prognosis, suggesting that during transformation more subtle dysregulated expression of DUBs can provide a selective advantage. Dysregulated control of DUB activity through post-translational modifications and induced protein–protein interactions is also observed in cancers[37,38]. For example, USP25 exists in an equilibrium between an auto-inhibited tetrameric form and an active dimeric form, with the active form selectively enriched in cancers, including breast cancer and non-small cell lung cancer[39]. Moreover, dysregulated expression or activity of DUBs can have opposing effects on disease progression dependent on the tissue and/or stage of disease[40].

DUBs are also observed in chromosomal translocation events, fusing a protein stabilizing entity such as a USP domain to a proto-oncogene, resulting in deregulated oncoprotein abundance. One of the

first DUB fusions to be identified as an oncogene arises from the fusion of TBC (TRE2–Bub2–Cdc16; a GTPase regulating domain) to the catalytic domain of USP6 (ref. 41). The Gene Fusion Gene Annotation Database (FusionGDB) lists hundreds of instances in which DUBs are fused with other proteins and oncogenes. However, the direct role of these fusions in cancer progression has not been fully explored.

DUBs in the hallmarks of cancer

The pathways and processes leading to cancer have been most succinctly annotated in the papers on cancer hallmarks[21,23]. Although we appreciate that segregation of the cancer hallmarks as described by Hanahan and Weinberg[21,23] is not straightforward as they are often directly connected or interdependent, we attempt to discuss DUBs that have been linked to each specific hallmark and highlight compelling examples for each based on evidence for target engagement, genetic deletion in animal models and human data (Supplementary Table 1).

DUBs in sustained proliferative signalling

Few cellular processes exemplify the importance of finely balanced control of ubiquitination and deubiquitination better than the tightly orchestrated regulation of the cell cycle. The irreversible transition through the various phases of the cell cycle must be exquisitely coordinated to prevent deregulated cell cycle progression leading to the accumulation of DNA damage, chromosomal instability and proliferation that underpins tumour initiation and progression[42]. Ubiquitination of checkpoint effector proteins, the cyclins and the cyclin-dependent kinases (CDKs), and multiple positive and negative regulators, to control their levels or to reversibly control their function, is essential to provide the exquisite temporal control of the cell cycle. Many of the proteins that regulate the cell cycle are targeted for ubiquitination by the multisubunit E3 ubiquitin ligase complexes, the S-phase kinase-associated protein 1 (SKP1)–Cullin–F-box (SCF) complex and the anaphase-promoting complex/cyclosome (APC/C) complex[43]. Whereas SCF ubiquitination is typically associated with Lys48 polyubiquitination (its main associated E2 enzyme, CDC34, is Lys48 specific (ref. 44)), the human APC/C complex catalyses branched Lys11-linked and Lys48-linked polyubiquitination, which ensures rapid proteasomal degradation[2,45]. Lys11-specific OTU domain-containing protein 7B (OTUD7B; also known as Cezanne) is reported to deubiquitinate key cell cycle regulators, including Aurora A and cyclin B, to impact cell cycle control in some cancer types[46] (Table 1; see Supplementary Table 1). USP44 has also been implicated in regulating the spindle checkpoint (see below)[47]. Other Lys11-specific and Lys48-specific OTU family DUBs may also play a role[48].

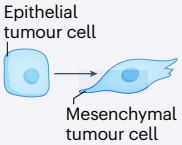
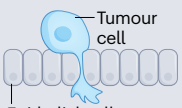
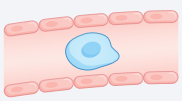
DUBs and positive cell cycle regulators. Various DUBs are implicated in the turnover of cyclins, either directly or by regulating proteins that control the activity of CDKs, to control cell cycle progression. Such DUBs can either have oncogenic or tumour-suppressive potential depending on the substrate and how that substrate is affected by ubiquitination.

Cyclin D and cyclin E are critical regulators of the G1–S transition through their interaction with CDK2, CDK4 and CDK6, and their levels are limited in non-transformed cells by proteasomal turnover by the SCF complex. USP2, USP4, USP15 and USP17 (also known as DUB3) were identified from in vitro DUB assays to remove monoubiquitin from cyclin D1 (ref. 49). Of these, however, only USP2 was found to deubiquitinate cyclin D1 in cellular assays and consequently regulate cyclin D1-dependent cell cycle progression in cancer cells[49].

USP7 is overexpressed in various cancer types and is also implicated in regulating cell cycle progression. Specific USP7 inhibitors were found to promote uncontrolled CDK1–cyclin B activity resulting in premature mitosis, DNA damage and cellular toxicity, implicating USP7 in the suppression of CDK1 activity throughout the cell cycle[50]. The effect of USP7 inhibition was mediated, in part, through enhanced nuclear translocation of protein phosphatase 2A (PP2A) that promoted removal of inhibitory phosphorylation on CDK1 (ref. 50). Another fascinating implication of this study is that ubiquitin modifications may determine the specificity of the interactions between CDKs and cyclins. USP7, and also USP10, have broad influence on cell cycle progression due to their ability to target NMYC and MYC, respectively (see below), and therefore indirectly promote the expression of MYC target genes that promote cell cycle progression, including cyclins and checkpoint kinases. Hence, both USP7 and USP10 have emerged as therapeutic targets[51,52].

Relatively little is known about DUBs in the transition from G2 to mitosis. A short hairpin RNA (shRNA)-based screen identified an essential role for USP44 in the spindle checkpoint by stabilizing the MAD2–CDC20 complex that inhibits the APC/C complex[47]. Consequently, USP44 suppressed tumorigenesis in mice with USP44-deficient mice prone to spontaneous lung tumours, with low expression correlating with poor prognosis in human lung cancer[53]. Independent short interfering RNA (siRNA)-based screens identified USP35 and USP39 as positive regulators of mitosis whose individual deletion caused

Table 2 | DUB involvement in EMT, invasion and metastasis

Cancer-related process	DUBs	Regulation
EMT 	USP3, USP4, USP5, USP7, USP11, USP14, USP17, USP27X, USP29, USP36, USP37, USP42, USP46, USP47, A20, CSN5, OTUB1, OTUD4, UCHL1	↑
Invasion 	USP2A, USP3, USP4, USP6, USP13, USP15, USP16, USP17, USP21, USP22, USP27X, USP28, USP38, USP39, USP42, USP43, USP49, USP51, CSN5, POH1, TRABID, UCHL1, UCHL3	↑
	USP9X, USP53	↓
Migration 	USP3, USP4, USP8, USP9X, USP17, USP22, USP27X, USP29, USP37, USP45, UCHL1	↑
	USP11, USP53	↓
Metastatic dissemination 	USP1, USP4, USP7, USP10, USP11, USP20, USP21, USP24, USP26, USP33, A20, ataxin 3, CSN5, OTUB2, UCHL1	↑
	OTUD1, TRABID	↓

See Supplementary Table 1 for details and references. CSN5, COP9 signalosome complex subunit 5; DUB, deubiquitinating enzyme; EMT, epithelial-to-mesenchymal transition; OTUB, OTU domain-containing ubiquitin aldehyde-binding protein; OTUD, OTU domain-containing protein; TRABID, TRAF-binding domain-containing protein (also known as ZRANB1); UCHL, ubiquitin carboxyl-terminal hydrolase isozyme L; USP, ubiquitin carboxyl-terminal hydrolase.

Box 2

DUBs with pervasive impact on multiple cancer hallmarks

Certain deubiquitinating enzymes (DUBs) have been implicated in impacting multiple cancer hallmarks either through pleiotropic effects as an indirect consequence of integral roles in large protein machineries with fundamental homeostatic functions or by directly targeting multiple substrates in different cellular pathways.

The proteasome DUBs RPN11, RPN8, USP14 and UCHL5

At least three DUBs have been associated with the proteasome[277]. The JAB1/MPN/MOV34 DUB (JAMM) family enzyme RPN11 cooperates with the pseudo-DUB RPN8 (which lacks deubiquitinase activity) in guiding and timing ubiquitinated substrate entry into the proteolytic 20S core particle[278]. More peripherally associated with the proteasome are ubiquitin carboxyl-terminal hydrolase 14 (USP14) and ubiquitin carboxyl-terminal hydrolase isozyme L5 (UCHL5; also known as UCH37), which pre-process substrates at the 19S regulatory subunit[279,280]. UCHL5 requires conformational activation by other proteasome subunits, but also exists in extra-proteasomal complexes[281].

VCP-associated DUBs OTUD2, VCIP135 and ataxin 3

Two OTU family DUBs, OTU domain-containing protein 2 (OTUD2; also known as YOD1)[282] and VCIP135 (ref. 283), and also the Machado Joseph disease (MJD) family DUB ataxin 3 (ref. 284), have been associated with the ubiquitin-dependent AAA+ ATPase valosin-containing protein (VCP). They bind to this complex via dedicated interaction domains, and presumably serve to pre-process or post-process ubiquitin signals on VCP clients.

USP22 in the SAGA complex

USP22, which is encoded by a 'death-from-cancer' gene[26], is an integral part of the Spt–Ada–Gcn5 acetyltransferase (SAGA) complex that controls histone acetylation, and in doing so deubiquitinates histones H2A and H2B (ref. 27). Alternative SAGA complexes may use USP27X or USP51 (ref. 285). Extensive, conserved interactions with additional SAGA components suggest that USP22 requires other components to perform roles outside the SAGA complex[286]. The function of USP22 in the SAGA complex likely underpins most, if not all, of its influence on multiple cancer-associated pathways[287]. Yet USP22 has been implicated in the regulation of cytosolic targets, including programmed cell death protein 1 ligand 1 (PDL1), receptor-interacting serine/threonine-protein kinase 3 (RIPK3) and mTOR[99,146,288]. However, given its prominent association with the nuclear SAGA complex, the mechanism by which USP22 exits the nucleus to recognize and deubiquitinate cytoplasmic substrates is unclear.

Rheostat DUBs USP5 and OTUB1

USP5 and OTU domain-containing ubiquitin aldehyde-binding protein 1 (OTUB1) are highly abundant DUBs in all cells and organisms, reflecting their roles as rheostats that read cellular ubiquitin and ubiquitin-charged E2 enzyme concentrations, respectively. USP5 recognizes and disassembles free polyubiquitin chains, prevents their build-up and replenishes the free ubiquitin pool; without USP5, cells experience 'ubiquitin stress' due to the lack of unattached 'free' ubiquitin. OTUB1 is a small enzyme that via an amino-terminal helix binds to ubiquitin-conjugating E2 enzymes. Uncharged E2 enzymes activate OTUB1 to hydrolyse Lys48 polyubiquitin, to replenish the ubiquitin pool. Charged E2 enzymes place their ubiquitin in the S1′ site of OTUB1, thereby inhibiting OTUB1 (refs. 289,290). Hence, OTUB1 balances the integrity of the ubiquitin system by ensuring sufficiently high concentrations of charged E2 enzymes are available for E3 ligase reactions.

USP7

USP7 is touted as a potential anticancer drug target as it is overexpressed in diverse cancer types and is implicated in targeting regulators of multiple cancer hallmarks. These include promotion of cell survival signalling through regulation of the tumour suppressor PTEN[89] and activation of WNT–β-catenin signalling[291], and its regulation of cell cycle progression and genome integrity[242]. However, although often pro-oncogenic, USP7 has also been implicated to be tumour suppressive in certain contexts, hence the consequence of modulating its activity in cancer is unclear. This is highlighted by its influence on the tumour suppressor p53. Initially USP7 overexpression was found to deubiquitinate and stabilize p53 to limit tumour growth[63]. However, through its regulation of the E3 ubiquitin ligase MDM2, USP7 deletion can also stabilize p53 (ref. 62). Hence, variations in USP7 expression differentially effect p53 to indirectly impact the expression of p53 target genes to influence cancer development and progression.

pre-mitotic arrest[54,55]. Both USP35 and USP39 were found to promote levels of Aurora B kinase, an effector of chromosome segregation during cytokinesis[54,55], but they do so by different mechanisms. Whereas USP35 deubiquitinates Aurora B kinase to prevent its proteasomal degradation[54], USP39, a pseudo-DUB that lacks catalytic residues, acts via its scaffolding role in the splicing machinery, and regulates Aurora B kinase mRNA processing[55]. Although both USP35 and USP39 have been shown to promote tumorigenesis, as they are implicated in regulating various cancer-associated pathways (see below), the contribution of their influence on cell cycle progression to their oncogenic activity is unclear.

DUBs and cell cycle inhibitors. DUBs also regulate cell cycle checkpoints to effect cell cycle progression. For example, USP19 is reported to control, albeit indirectly, key negative regulators such as the CDK inhibitors p27 (KIP1) and p21. USP19 stabilizes the E3 ubiquitin ligase KPC1, which ubiquitinates p27 to promote its proteasomal turnover[56]. Hence, genetic ablation of USP19 led to increased p27 levels and perturbed cell cycle progression through G1 (ref. 56). The ability of USP19 to promote cell proliferation conflicts with USP19 being downregulated in various cancer types, including renal cell carcinoma, in which its low levels correlate with poorer outcomes; these latter findings may reflect the role of USP19 in controlling ERK signalling (see below)[57].

Activation of checkpoint kinase 1 (CHK1) promotes cell cycle arrest and is a major hub for regulation by ubiquitin signalling, and again USP7 and USP37 reportedly deubiquitinate and stabilize CHK1 (ref. 58). USP9X, USP20, USP28 and USP29 similarly promote CHK1 activity, but do so by deubiquitinating the CHK1 activator claspin to inhibit the S–G2 transition[59]. Another alternative mechanism was proposed for USP3, whereby it counteracts Lys63 polyubiquitination of CHK1, which is required for its association with chromatin[60].

Contrasting with its potential to promote cell cycle progression, USP7 is also implicated to stabilize the key tumour suppressors, and cell cycle inhibitors, p53 and RB, either directly via deubiquitination of these proteins or indirectly via the oncogenic E3 ubiquitin ligase and negative regulator of p53, MDM2 (refs. 61–63). Hence, as will become clear, USP7 is implicated in multiple cancer types, and multiple cancer hallmarks, with multifactorial and context-dependent roles in tumorigenesis (Table 1 and Box 2; see Supplementary Table 1). Likewise, USP10 has also been implicated in regulating p53 by counteracting MDM2-mediated ubiquitination and reversing MDM2-induced p53 nuclear export[64], portending a tumour-suppressive role for USP10. So, although their common roles in regulating oncogenic proteins, including MYC (see below), highlight the potential value of inhibiting USP7 and USP10 as a clinical strategy, their context-dependent tumour suppressor functions remain a potential challenge for therapeutic targeting.

MYC — a key oncogenic target of DUBs. Given its oncogenic potential and its key role as an arbiter of cell cycle control through its promotion of expression of positive cell cycle regulators and its antagonism of cell cycle inhibitors[65], there has been substantial interest in identifying ubiquitin mechanisms that regulate MYC[66]. Numerous DUBs are implicated in modulating MYC, including USP10, USP13, USP16, USP17, USP22, USP28, USP29, USP36, USP37, USP38 and OTU domain-containing ubiquitin aldehyde-binding protein 1 (OTUB1) (see Supplementary Table 1). A possible explanation for the plethora of DUBs implicated in directly removing ubiquitin from MYC is their tissue-specific expression. However, the impact of these DUBs on MYC is likely much more intricate. Notably, USP28 (the most extensively studied DUB in this context), USP36 and USP38 all interact with and regulate FBXW7 (refs. 67–69). FBXW7 is a component of the SCF ubiquitin ligase complex, where FBXW7 recognizes and facilitates the degradation of specific substrates, including MYC[70]. Under homeostatic conditions, FBXW7 is subject to autocatalytic ubiquitination by the SCF complex, leading to its degradation. USP28 deubiquitinates and stabilizes FBXW7, thereby promoting the ubiquitination and degradation of FBXW7 substrates. However, when USP28 is overexpressed it also deubiquitinates and stabilizes FBXW7 substrates directly, including MYC. USP28 overexpression in glioma correlates with poor survival, and USP28 silencing limits the growth of subcutaneous glioblastoma xenografts in mice via destabilization of MYC[71]. Conversely, complete loss of USP28 expression leads to ubiquitination and degradation of FBXW7, resulting in the stabilization of MYC. Thus, the expression level of USP28 in tumours dictates its tumour-promoting or tumour-suppressive activities, as elegantly demonstrated by the work of Popov, Eilers and colleagues[67,72].

USP37 is upregulated in lung, colorectal and breast cancers, and is highly expressed in breast cancer stem cells where it was proposed to promote stemness[73]. Its expression correlates with MYC expression in lung cancer and it has been reported to bind and deubiquitinate MYC[74]. However, USP37 may also antagonize APC/C-driven proteasomal degradation of cyclin A and, hence, promote S-phase entry[75]. The activity of USP37 is regulated throughout the cell cycle by CDK2-mediated phosphorylation and USP37 itself is Lys11 polyubiquitinated by APC/C[75]. Hence, the putative oncogenic activity of USP37 is not restricted to modulating MYC.

USP13 has been shown to counteract F-box/LRR-repeat protein 14 (FBXL14)-mediated ubiquitination of MYC in the context of glioblastoma[76]. USP13 expression is enriched in glioma stem cells, where it interacts with and stabilizes MYC. Consequently, shRNA-mediated knockdown of USP13 significantly limited growth of glioma xenografts in mice[76]. A complication with USP13 is that despite its having an intact catalytic domain, its biochemical activity is low and often seems to be inactive in catalytic assays. Hence, additional layers of regulation or distinct ubiquitin signals likely play a role in USP13-regulated processes.

USP22 is also proposed to directly deubiquitinate and stabilize MYC to promote cell proliferation in breast cancer cell lines[77]. However, in addition, the function of USP22 as part of the SAGA complex is critical for the transcriptional activity of oncogenic MYC; hence, deletion of USP22 blocks the transcription of MYC target genes and MYC-induced transformation in vivo[78]. Similarly, USP10 was found to deubiquitinate and stabilize the MYC repressor sirtuin 6 (SIRT6), thereby antagonizing the transcriptional activity of MYC[79]. USP10 is an abundant and widely expressed DUB with many proposed protein targets and cancer associations[80], and so, similar to USP22 with its fundamental role in the SAGA complex, further work is needed to test for direct or indirect roles of USP22 and USP10 in regulating MYC.

As most earlier studies commonly utilized siRNA techniques resulting in partial reduction of expression rather than complete knockout, it remains unclear which of these DUBs directly act on MYC itself or exert their effects indirectly. DUBs that stabilize MYC would seem excellent candidates for small molecule inhibitors. For example, USP28 has been the focus of several drug discovery efforts, and small molecule inhibitors targeting USP28, and its close orthologue USP25, greatly diminish MYC levels in mouse models of squamous non-small cell lung cancers[81]. However, the aforementioned DUBs affect multiple substrates besides MYC, some of which are involved in tumorigenesis or tumour suppression depending on the cellular context[82,83]. Hence, although DUBs that stabilize MYC would on face value seem to be good therapeutic targets, these complexities need to be fully resolved and considered for each DUB.

USP7 has also been reported to bind, deubiquitinate and stabilize NMYC to promote neuroblastoma progression[84]. Specifically, USP7 expression correlated with poor prognosis and small molecule inhibition of USP7 limited neuroblastoma xenograft growth in mice[84]. Regulation of NMYC by USP7 has similarly been implicated in small cell lung cancer[85].

DUBs in growth factor and PI3K signalling. The PI3K pathway is responsible for the generation of potent lipid-based second messenger signalling responses triggered after most cell growth stimuli and transduced via RAS GTPase and protein kinase signalling processes. As a result, enhanced PI3K signalling is commonly associated with cancer[86].

PTEN is a tumour suppressor that reverses the action of PI3K and plays key roles in cell cycle progression, metabolism and cell survival[87]. PTEN is a major hub for ubiquitin-mediated control in cancer with several DUBs implicated in regulating its levels and transcriptional activity[88]. USP7 is implicated in removing monoubiquitin from PTEN, to promote its nuclear exclusion and, in turn, limiting the tumour

suppressor function of PTEN[89]. Hence, USP7 overexpression in human prostate cancer correlates with PTEN nuclear exclusion in patient samples[89]. Conversely, USP11, which is itself transcriptionally activated by PTEN, deubiquitinates PTEN to stabilize it and promote tumour suppression, with low USP11 expression observed in patient samples of breast cancer and prostate cancer[90].

USP13 and OTUD3 also modulate PI3K signalling by stabilizing PTEN[91,92], thereby limiting downstream pro-tumorigenic AKT signalling in cancer types such as breast cancer[91], oral squamous cell carcinoma[93] and bladder cancer[94]. Moreover, loss-of-function mutations or downregulated expression of USP13 and OTUD3 have been identified in these cancer types. Nevertheless, although USP11 and USP13 are tumour-suppressive in these contexts, USP11 has been shown to enhance oncogenic translation and proliferation in diffuse large B cell lymphoma by deubiquitinating eukaryotic translation initiation factor 4B (eIF4B)[95] and USP13 overexpression confers resistance to platinum-based therapies in ovarian cancer due to its role in the DNA damage response[96]. These two examples once again highlight the complex, context-dependent roles of DUBs in cancer.

A further key regulator of growth factor signalling is the mTOR pathway. mTOR is a Ser/Thr kinase and master regulator of glucose, amino acid and lipid metabolism that is frequently elevated in cancers[97]. OTUD5 was identified as a positive regulator of mTOR signalling from a customized DUB shRNA screen[98]. OTUD5 deubiquitinates and stabilizes the SCF E3 ligase adaptor β-transducin repeat-containing protein 1 (β-TrCP1; also known as FBXW1A), leading to the degradation of DEP domain-containing mTOR-interacting protein (DEPTOR), an inhibitor of mTOR complex 1 (mTORC1) and mTORC2. Consequently, OTUD5 depletion was found to inhibit proliferation in cancer cell lines that harbour activating mutations in proteins involved in mTOR signalling[98]. USP22 is also reported to regulate mTOR signalling in colorectal cancer, but, conflicting with its 'death-from-cancer' gene status, USP22 suppressed mTOR and hence inhibited colorectal cancer growth in the *Apc*^{Min} mouse model of colorectal cancer[99]. CRISPR–Cas9-mediated deletion of USP22 in colorectal cancer cell lines increased growth in vitro and tumorigenic capacity in vivo[99]. mTORC1 is also regulated by the Lys63-specific DUB associated molecule with the SH3 domain of STAM (AMSH)-like protease (AMSHLP; also known as STAMBPL1), which removes Lys63 ubiquitin chains from sestrin2, a positive regulator of mTORC1 (ref. 100). Thus, AMSHLP silencing limited the growth of colorectal cancer xenografts in mice, suggesting AMSHLP might be a novel anticancer target[100].

OTUD7B has also been reported to regulate mTOR signalling by removing Lys63 polyubiquitin from MLST8, a component of mTORC2, to promote complex formation and growth factor signalling[101]. Consequently, OTUD7B depletion limited growth factor signalling and tumour growth in an in vivo model of KRAS-driven lung cancer[101]. However, it should be noted that this reported activity of OTUD7B conflicts with its strong biochemical preference for Lys11-linked polyubiquitin chains[102].

USP28, in addition to regulating MYC, is also implicated in negatively regulating MAPK signalling by promoting FBXW7-dependent degradation of BRAF[83]. Dysregulated BRAF is a major driver of sustained proliferative signalling in cancer types such as melanoma, and for this reason is now inhibited in the clinic with targeted therapies. Downregulation of USP28 in melanoma correlates with poor prognosis and promotes resistance to BRAF inhibition[83]. However, other DUBs also regulate BRAF signalling, such as USP9X (indirectly via the transcription factor SRY-Box 2 (SOX2))[103].

DUBs in replicative immortality

Maintenance of telomere length is a distinguishing feature of immortalized, transformed cancer cells[104]. Ubiquitin signalling plays a central role in modifying the behaviour or levels of proteins that maintain telomere length. The main mechanism to maintain telomere length is upregulated telomerase activity, with 90% of cancers exhibiting telomerase activity[105]. However, 10–15% of cancers use a telomerase-independent mechanism to lengthen telomeres, known as alternative lengthening of telomeres (ALT)[106]. The multi-protein shelterin complex comprising TRF1, TRF2, RAP1, TRF1-interacting nuclear factor 2 (TIN2), TPP1 and protection of telomeres protein 1 (POT1) binds to and protects telomeric DNA and is important for both mechanisms of telomere replication. Thus, mutation or upregulation of its key components is implicated in multiple cancer types, and downregulation of their expression, or inhibition of their function, is an emerging anticancer strategy.

For example, TRF1 is essential for tumour growth in tumour-prone p53-deficient mice and CDKN2A-deficient mice[107], and its genetic ablation limits tumour growth in xenograft models of small cell lung cancer and glioblastoma[107–109]. TRF1 is ubiquitinated by the E3 ubiquitin ligases RLIM, FBX4 and β-TrCP1 that drive its turnover. USP22, through its interaction with the SAGA complex (Box 2), deubiquitinates TRP1 at telomeres to stabilize the protein and promote telomere maintenance[110]. This activity of USP22 possibly contributes to the correlation between high USP22 expression and poor outcome in a broad spectrum of cancer types[26]. USP7 similarly deubiquitinates components of the shelterin complex, TPP1 and POT1, thereby preventing their proteasomal degradation, with the regulation of POT1 exclusive to ALT^{+} cancers[111,112].

DUBs in the evasion of growth suppression

Contact inhibition due to engagement of cell surface adhesion molecules or growth factor receptors is a major regulatory mechanism of growth suppression that goes awry particularly in solid cancers[113,114]. A major pathway controlling contact inhibition is the Hippo–Yes-associated protein (YAP) pathway[115], and several DUBs have been implicated in controlling Hippo signalling. USP7 and USP10 were first identified to influence Hippo signalling in *Drosophila* with both DUBs deubiquitinating the transcriptional co-activator Yorkie (homologue of mammalian YAP) that promotes cell proliferation and inhibits apoptosis[116,117]. USP7 and USP10 were subsequently shown to similarly target and stabilize YAP1, with expression of these DUBs correlating with YAP1 levels in tumour samples from patients with hepatocellular carcinoma and promoting progression of hepatocellular carcinoma in xenograft mouse models[117,118]. USP47 is similarly implicated in regulating YAP1 levels in colorectal cancer cells and its expression is elevated in tumour samples from patients, although direct evidence that USP47 promotes colorectal cancer growth in vivo is currently lacking[119].

The SCF complex with its adaptor SKP2, although typically catalysing Lys48 polyubiquitination, has been reported to mediate Lys63 polyubiquitination of YAP to promote its nuclear translocation and transcriptional activity. Hence, removal of these polyubiquitin chains by the Lys63-specific DUB OTUD1 inhibits the transcriptional activity of YAP[120]. Analysis of The Cancer Genome Atlas (TCGA) database also indicates that low OTUD1 expression correlates with poor prognosis in various cancer types, including cervical cancer and prostate cancer. Why multiple DUBs control Hippo signalling via an apparently redundant mechanism, that is, by deubiquitinating YAP1, is currently unclear, but may reflect the importance of controlling this pathway and/or tissue-specific regulation.

DUBs in the regulation of cell death

The coordinated demise of cells during development and tissue homeostasis is key to life, whereas its deregulation is key to tumorigenesis[121]. Research over the past decade has highlighted a key role for ubiquitin signalling, including regulation by DUBs, in the control of extrinsic (death receptor-mediated) apoptosis triggered by the ligation of cell surface receptors of the TNF receptor superfamily (reviewed elsewhere[122]). However, TNF cytokine signalling balances the processes of cell death and non-lethal inflammatory signalling, and the involved DUBs typically regulate both processes. We will discuss DUBs in inflammatory TNF signalling in more detail below, and we also note that this area has been extensively reviewed elsewhere[123].

Intrinsic (mitochondrial) apoptosis is triggered by various stimuli, including growth factor deprivation, DNA damage, matrix detachment and chemotherapeutic agents, and is controlled by proteins of the BCL-2 family. The complex interplay between the three factions of this family (pro-survival, BH3-only and effector proteins) ultimately determines the integrity of the mitochondrial outer membrane and the release of apoptogenic factors, including cytochrome *c*[124]. This triggers the activity of proteolytic caspases that coordinate the end-stage events of apoptosis. Overexpression or amplification of pro-survival BCL-2 proteins promotes cancer development and is a major determinant of responses to chemotherapy[121]. Hence, pro-survival BCL-2 proteins are emerging as important targets to inhibit for the treatment of cancer, and highly specific inhibitory drugs termed BH3 mimetics, such as venetoclax, are now successful agents in the clinic[125].

Modulating ubiquitin-dependent turnover or activation of BCL-2 family proteins has considerable therapeutic potential. For example, various DUBs, including USP13, USP9X, OTUD1 and USP17, have been reported to counter ubiquitination of the pro-survival protein MCL1 by E3 ubiquitin ligases, including HUWE1 (also known as ARF-BP1), MARCHF5 (also known as MARCH5 and RNF153) and Parkin[126–128]. Deubiquitination of MCL1 by DUBs, including USP9X, USP13 and OTUD1, stabilizes this otherwise short-lived protein, and promotes resistance to radiotherapy and chemotherapy, including resistance to BH3-mimetic drugs, in both in vitro and in vivo models[129–133].

Similarly, the pro-apoptotic BH3-only proteins such as BIM (also known as BCL2L11) are subject to multiple post-translational control mechanisms, including ubiquitination. The DUB USP27X was found to interact with and stabilize BIM. As a potential consequence, USP27X expression promoted cell death in non-small cell lung cancer cells in the absence of overt stimuli and also exacerbated cell death following treatment with the epidermal growth factor receptor (EGFR) inhibitor gefitinib[134].

BAX and BAK are two critical apoptosis effectors, and ubiquitination by E3 ligases, including Parkin and IBR domain-containing protein 2 (IBRDC2), has been suggested to limit their apoptotic activity either by degradative or non-degradative mechanisms[135–137]. Whether ubiquitination of BAX or BAK is countered by specific DUBs is less clear. The mitochondrial DUB USP30, which counters the E3 ligase activity of Parkin in mitophagy[138], has been reported to impair apoptosis mediated by BAX or BAK[139,140]. However, this is in apparent conflict with Parkin-mediated ubiquitination of BAX or BAK as anti-apoptotic[135], suggesting deubiquitination of a pro-survival protein rather than BAX or BAK.

In most circumstances, mitochondrial damage by BAX and/or BAK signals death for the cell. However, the kinetics of caspase activation can be modulated by ubiquitin-dependent turnover of apoptogenic factors released from mitochondria. For example, deubiquitination of X-linked inhibitor of apoptosis (XIAP), which inhibits caspase 3 and caspase 7, by USP11 is implicated in promoting breast cancer tumorigenesis[141].

The suppression of other cell death modalities besides apoptosis is also emerging as an important mediator of tumorigenesis and is regulated by DUBs. Ferroptosis is a form of iron-dependent cell death characterized by lipid peroxidation[142]. USP35 has been shown to limit ferroptosis in lung cancer by targeting and stabilizing the iron exporter ferroportin[143]. In contrast, OTUB1 has been proposed to promote ferroptosis by stabilizing the ferroptosis regulator solute carrier family 7 member 11 (SLC7A11), with OTUB1 deletion promoting the growth of subcutaneous bladder carcinoma xenografts[144]; although the role of OTUB1 in maintaining free ubiquitin will also influence associated phenotypes (Box 2).

It is clear that DUBs target multiple checkpoints to modulate extrinsic and intrinsic apoptosis, as well as other cell death pathways relevant to tumour progression. Considering the clinical success of cancer drugs regulating apoptosis, these enzymes may represent an untapped resource of new therapeutic opportunities.

DUBs in immune evasion

A hallmark of cancers is their ability to evade destruction by the immune system[21]. This ability has been the topic of intense research with ground-breaking developments in the clinic to modulate the immune response to tumours through strategies that include blocking immune inhibitory checkpoint interactions between cytotoxic T lymphocyte-associated antigen 4 (CTLA4) and programmed cell death protein 1 (PD1) and their respective ligands CD80 and PDL1 (ref. 145).

Numerous DUBs have been implicated in the immune evasion of tumours. USP22, COP9 signalosome complex subunit 5 (CSN5; also known as JAB1), OTUB1, USP5 and USP9X have been proposed to deubiquitinate and stabilize PDL1 on the surface of various cancer cell types, including hepatocellular carcinoma, pancreatic cancer, oral squamous cell carcinoma and breast cancer, thereby promoting immune evasion[146–150]. However, these DUBs also influence other cancer-associated phenotypes such as cell proliferation (USP22) and cell death (USP9X), so it remains unclear as to what extent immune evasion contributes to the pro-tumorigenic effect of these DUBs in specific tumours. Moreover, USP22 (Box 2) and CSN5 are part of the SAGA or COP9 signalosome (CSN) complexes, respectively, and USP5 and OTUB1 have key roles in maintaining homeostasis of ubiquitin and E2 ligases, complicating interpretation of their proposed direct roles in regulating cell surface immune receptors. Nevertheless, OTUB1 was reported to remove Lys48-linked ubiquitin from the PDL1 intracellular domain to inhibit its degradation via the ER-associated degradation (ERAD) pathway[151]. Consistent with this, OTUB1 expression correlates with PDL1 levels in breast cancer[148].

As well as having a tumour cell-intrinsic role, multiple DUBs influence immune evasion by playing more general roles in T cell activation and function (see 'DUBs in tumour-promoting inflammatory signalling') (see Supplementary Table 1). Nevertheless, the role of DUBs in immune-related processes during tumour development and progression remains largely underexplored.

DUBs in angiogenesis

Various DUBs play important roles in vascular biology and are implicated in diseases such as atherosclerosis[152], and hence may play a role

in angiogenesis in cancers. Hypoxia-inducible factor 1α (HIF1α) is a master transcriptional regulator of the response to hypoxia and drives the transcription of various genes encoding molecules involved in angiogenesis, including vascular endothelial growth factor (VEGF), as well as genes encoding molecules involved in migration and metabolism[153]. HIF1α is constitutively ubiquitinated and degraded in normoxia (normal levels of oxygen) by the E3 ubiquitin ligases von Hippel–Lindau protein (pVHL) and hypoxia-associated factor (HAF), but can be stabilized through the activity of various DUBs, including USP20 (ref. 154), USP7 (ref. 155), USP19 (ref. 156) and USP28 (ref. 157), to trigger its transcriptional activity[154].

The Lys11-specific DUB OTUD7B also regulates the HIF1α transcription factor in response to hypoxia[158,159]. Interestingly, an additional layer of regulation of this DUB exists via oxygen-dependent hydroxylation of asparagine (Asn) in the ubiquitin-associated (UBA)-like domain, which inhibits ubiquitin binding[160]. The induced expression and activity of OTUD7B in low oxygen conditions in turn regulates the levels of HIF1α, but, intriguingly, HIF1α degradation is independent of the proteasome, and possibly involves chaperone-mediated autophagy[161].

DUBs also regulate angiogenesis in alternative ways. Loss of the Met1-specific DUB OTULIN causes embryonic lethality in mice with defects in blood vessel sprouting, which was initially linked to dysregulated WNT signalling[162]. Interestingly, OTULIN also counteracts the polyubiquitination of activin receptor-like kinase 1 (ALK1), which promotes its kinase activity and influences SMAD1-mediated and SMAD5-mediated signalling and angiogenesis[163]. As OTULIN also regulates NF-κB activation and inflammatory signalling[164,165], the contribution of the pro-angiogenic function of OTULIN to cancer progression remains unresolved.

DUBs in cellular energetics

Cancer cell transformation and tumour progression involve a necessary metabolic adaptation to oxidative stress, as highlighted by the Warburg effect – a transition to aerobic glycolysis in the presence of oxygen that is a feature of numerous solid cancers[166]. To identify sensitivities to such stress, Harris et al.[167] performed a genome-wide CRISPR–Cas9 library screen and found that depletion of DUBs, in particular USP7 and ubiquitin carboxyl-terminal hydrolase isozyme L5 (UCHL5), sensitized cancer cells to apoptosis triggered by glutathione (GSH) depletion. Consistent with this genetic screen, a chemical library screen identified broad-spectrum DUB inhibitors, including WP1130 (ref. 168), BAY-11-7082 (ref. 169), MI-2, EERI[170] and PR-619 (ref. 171), as sensitizing breast cancer cells to oxidative stress[167]. The more potent effect of pharmacological inhibition compared with genetic depletion of specific DUBs that was observed suggests some degree of redundancy between DUBs or may be attributed to the numerous off-target effects anticipated with these first-generation DUB inhibitors (for example, BAY-11-7082 inhibits inhibitor of NF-κB kinase (IKK)[172] and MI-2 inhibits the paracaspase MALT1 (ref. 173)). However, these data do suggest that DUB activity is a potential 'Achilles heel' of cancer cells under conditions of oxidative stress.

USP7 may also indirectly regulate the metabolic response to hypoxia and low glucose levels and sensitize cells to low glucose stress by removing Lys63-ubiquitination from SIRT7 to limit its histone deacetylase activity[174]. SIRT7 activity is linked to tumour development and progression as it relieves contact inhibition and promotes anchorage-independent growth[175], but also promotes the expression of key enzymes in gluconeogenesis, such as glucose-6-phosphatase catalytic subunit[174]. In addition, USP7 may also influence the forkhead box protein O1 (FOXO1)-mediated transcription of gluconeogenesis genes, suggesting that USP7 has a multi-layered role in glucose metabolism[176].

In conflict with its tumour-suppressive function in stabilizing PTEN, USP13 is elevated in certain cancer types, including ovarian cancer and lung cancer. USP13 was reported to deubiquitinate and stabilize oxoglutarate dehydrogenase and ATP citrate lyase, enzymes involved in glutaminolysis and lipid synthesis, thereby activating ovarian cancer cell metabolism[177]. Conversely, shRNA-mediated depletion of USP13 has been shown to impair glutaminolysis and mitochondrial oxidative phosphorylation in ovarian cancer cells to limit cancer cell survival and tumour growth in mice[177]; although reduced expression of another USP13 substrate, the pro-survival protein MCL1, may also contribute to the impaired cell survival in this context.

OTUB2 has recently been shown to promote aerobic glycolysis and, in doing so, support tumour growth in colorectal cancer xenografts in mice[178]. In this case, OTUB2 opposed the ubiquitination of mitochondrial pyruvate kinase M2 (PKM2) by the E3 ubiquitin ligase Parkin, to shift cellular metabolism towards glycolysis[178]. However, given the general lack of specificity observed for OTUB2, and that Parkin is considered auto-inhibited in the absence of mitochondrial damage[179], the mechanism remains to be confirmed.

USP2 has also been implicated in mitochondrial homeostasis with *Usp2* deletion in mouse myoblasts promoting mitochondrial fragmentation and mitochondrial dysfunction, leading to elevated levels of reactive oxygen species (ROS)[180]. However, it is currently unclear whether the effect of USP2 on mitochondria contributes to its function in cancer progression.

DUBs in invasion and metastasis

Epithelial-to-mesenchymal transition (EMT) is a reversible, dynamic process that was first identified to regulate the body plan through differentiation of multiple tissues leading to structured organ development[181]. This same process has been co-opted by cancer cells to invade surrounding tissues, eventually driving an oncogenic signalling cascade that permits the development of metastases[181]. Furthermore, changes in cellular plasticity driven by EMT transcriptional programmes lead to the development of drug-tolerant states and therapy resistance[182]. Several signalling pathways, including the WNT and transforming growth factor-β (TGFβ) pathways, are known regulators of EMT primarily through the regulation of EMT activating transcription factors (EMT-TFs), most notably SMAD, ZEB, TWIST and SNAIL[181].

Unsurprisingly, the EMT-TFs within these central oncogenic pathways exploit ubiquitination and DUBs to regulate their dynamics, and several DUBs have been reported to regulate invasion and metastasis via EMT-TFs (Table 2). For example, USP17 functions downstream of CDK4, CDK6 and interleukin 6 (IL-6) to deubiquitinate and stabilize SNAIL1, promoting EMT and breast cancer metastasis[183,184]. Similarly, TWIST is a substrate of USP2, and also of the Lys29-specific and Lys33-specific OTU family DUB TRAF-binding domain-containing protein (TRABID; also known as ZRANB1). However, TWIST deubiquitination by these different enzymes leads to distinct paths of cancer progression. USP2 deubiquitinates and stabilizes TWIST1 regulating cancer cell stemness, resulting in a sustained cancer stem cell population and reducing sensitivity to the chemotherapeutic drug doxorubicin in mouse models of triple-negative breast cancer[185]. In contrast, and counter-intuitively, TRABID targets TWIST1 for degradation. TRABID deubiquitination of non-degradative chains enhances the binding of TWIST to the degradative cullin E3 ligase adaptor β-TrCP1. Consistently,

TRABID was found to limit the growth and metastasis of hepatocellular carcinoma in mice[186].

TGFβ signalling is commonly dysregulated in cancers and is implicated in various cancer-associated phenotypes, including metastasis and immunosuppression[187]. However, therapeutic regimens utilizing TGFβ inhibitors are being approached with caution due to associated toxicities[188]. The regulation of the TGFβ signalling axis mediated by upstream TGFβ receptors (TGFβRs) is predominantly controlled by distinct endocytic pathways that compartmentalize receptor complexes to dictate receptor signalling or receptor turnover[189]. Although numerous ubiquitin ligases have been demonstrated to regulate TGFβR ubiquitination, in terms of receptor degradation, the most well studied is the SMAD ubiquitination regulatory factor 2 (SMURF2) E3 ubiquitin ligase in concert with the scaffold protein, and transcriptional target of TGFβ signalling, SMAD7 (ref. 187). Through this negative feedback loop, transcriptionally induced SMAD7 binds to the TGFβR complex permitting SMURF2 to ubiquitinate the TGFβR complex targeting it for proteasomal degradation[190]. Numerous DUBs have been shown to regulate various components of this complex, including USP2A, USP4, USP8, USP11, USP15, USP19 and UCHL5, all of which seem to stabilize the receptors to enhance downstream TGFβ signalling[191,192]. Although functional redundancy or cell-specific expression may contribute to the large number of DUBs that affect TGFβR stability, DUBs likely also associate with specific ubiquitin mediator complexes, or act within specific endocytic compartments, to regulate distinct phases of TGFβR internalization and compartmentalization.

Utilizing proteomics or functional genetic screens, three independent studies found that the DUBs USP4, USP11 and USP15 can deubiquitinate and stabilize TGFβR1 resulting in increased TGFβ transcriptional responses[191,193,194]. Interestingly, ten Dijke and colleagues[191,195] demonstrated that USP4 could also form homomeric and heteromeric complexes with USP11, USP15 and USP19, but whereas USP4 seems to directly bind and deubiquitinate the TGFβR1, USP11 and USP15 require the presence of the scaffold protein SMAD7 to enable deubiquitination. Nevertheless, TGFβR stability and internalization is nuanced and intrinsic signalling is dependent on TGFβ signalling intensity. At high concentrations of TGFβ, USP15 is unable to bind the SMAD7–SMURF2 complex allowing SMURF2-mediated ubiquitination and degradation of the TGFβR, whereas at low TGFβ levels, USP15 activity supersedes SMURF2 activity to promote TGFβ signalling[195]. USP4 and USP15 are overexpressed in numerous tumour types, including glioblastoma, ovarian cancer and breast cancer[193]. Promisingly, genetic inhibition of USP4 or USP15 mitigated TGFβ-induced oncogenesis and metastasis in mouse and zebrafish models, highlighting the potential of USP4 or USP15 inhibitors as an alternative approach to target TGFβ-mediated oncogenic responses[191,193]. However, the lack of selectivity of current inhibitors targeting structurally similar USP4, USP11 and USP15 remains a challenge[196].

An elegant in vivo DUB-targeted shRNA library screen identified OTUD1 as a suppressor of breast cancer metastasis[197]. Similar to USP4, OTUD1 was found to also directly interact with the TGFβR complex. But rather than deubiquitinate the TGFβR directly, OTUD1 was proposed to remove Lys33 polyubiquitin from SMAD7 to enhance the recruitment of SMURF2 and degradation of TGFβR1 via the proteasome[197]. Although the mechanism is somewhat at odds with the preferential cleavage of Lys63-linked chains by OTUD1 (ref. 198), reduced expression of OTUD1 and its negative regulation of pro-metastatic TGFβ signalling was suggested to be prognostic for poor outcome in breast cancer[197].

DUBs in enabling characteristics of cancer

DUBs in tumour-promoting inflammatory signalling

Dysregulated inflammatory signalling is associated with the development and progression of certain cancer types such as colorectal cancer[199]. Inflammation is also finely balanced with cell death signalling, often involving the same multi-protein complexes. Extrinsic apoptosis triggered by death receptor signalling or TNF involves receptor multimerization as a platform for recruitment of signalling proteins, including receptor-interacting serine/threonine-protein kinase 1 (RIPK1) and E3 ligases such as TNF receptor-associated factor 2 (TRAF2), TRAF5 and cellular inhibitor of apoptosis protein 1 (cIAP1) and cIAP2 (reviewed elsewhere[200–202]). These E3 ligases determine the outcome of signalling through the TNF receptor, either promoting cell death via the activation of initiator caspases, caspase 8 or caspase 10, or promoting pro-inflammatory NF-κB signalling, which involves Lys63 polyubiquitination of complex constituents NF-κB essential modulator (NEMO), TRAF2 and TRAF6, and downstream activation of kinases, including the TGFβ-activated kinase (TAK1) complex and the IKK complex. A further E3 ligase complex triggers pro-inflammatory pathways. The linear ubiquitin chain assembly complex (LUBAC) comprising haeme-oxidized IRP2 ubiquitin ligase 1 (HOIL1; also known as RBCK1), HOIL1-interacting protein (HOIP; also known as RNF31) and Sharpin is recruited to limit pro-death signalling by decorating substrates with distinct additional Met1-linked polyubiquitin signals. As a result, the pro-inflammatory or pro-death outcome of TNF receptor engagement is determined by the ubiquitination status of RIPK1 and other complex components[200]. DUBs are powerful regulators of this signalling balance. Three DUBs have well-established roles in regulating cytokine and death receptor signalling, and loss of each can lead to cancer in distinct settings.

The USP family DUB CYLD is a negative regulator of NF-κB signalling and dictates survival in response to TNF receptor signalling through deubiquitination of TRAF2 (refs. 203–205). CYLD can also remove Lys63-linked polyubiquitin from RIPK1 to limit NF-κB activation and divert the cell towards death by promoting caspase 8 activation[203,206]. CYLD can directly bind LUBAC via the adaptor protein SPATA2 (refs. 207,208), and its Lys63 activity is activated by IKK-mediated phosphorylation[209], suggesting that it acts after IKK activation to limit kinase signalling. CYLD was one of the earliest DUBs to be annotated as a tumour suppressor, as it is mutated (likely through loss of function or hypomorphic variants) in familial cancer predisposition syndromes, including cylindromatosis, that predispose to head and neck tumours that, although commonly benign, can transform into malignancy[36]. *CYLD*$^{-/-}$ mice are predisposed to tumours of the skin and also colitis-induced colon cancer that are likely driven by dysregulated NF-κB signalling[210]. However, numerous other roles for CYLD have been identified. CYLD was reported to deubiquitinate polo-like kinases (PLKs) and regulate mitotic entry[47,211], and to have an evolutionarily older role in regulating the DNA damage response via p53 (ref. 212).

A more complicated case is the OTU family DUB A20, which binds via multiple zinc finger UBDs to the Lys63 and Met1 chains in receptor complexes, but biochemically prefers to cleave degradative Lys48 chains[213]. A20 is a potent negative regulator of NF-κB signalling and is strongly upregulated after TNFR1 stimulation[214], although a role for its deubiquitination activity in NF-κB signalling has been debated[215]. Similar to CYLD, A20 was identified as a tumour suppressor in various cancer types with gene deletion of A20 frequently found in Mantle cell lymphoma and diffuse large B cell lymphoma. Furthermore, A20 gene silencing impairs apoptosis of lymphoblastoid cells in vitro[216].

Glossary

Alternative lengthening of telomeres
(ALT). A mechanism to limit degradation of telomeres that is independent of telomerase.

Autophagosome
A double-membrane structure encapsulating autophagy cargo.

Autophagy
The clearance of organelles or protein aggregates through lysosomal degradation.

BH3 mimetics
Small molecules designed to mimic the activity of BH3-only proteins in binding pro-survival BCL-2 proteins.

Condensate formation
The membrane-less compartmentalization of biomolecules driven by changes in solubility.

Degron
A minimal protein signal for target protein degradation.

E3 ubiquitin ligase enzymes
Enzymes that catalyse the transfer of ubiquitin to substrates.

Fanconi anaemia DNA repair pathway
A multi-protein pathway to repair DNA inter-strand cross-links.

Glutaminolysis
The conversion of glutamine into carbon sources for the TCA cycle.

Hypomorphic variants
Amino acid substitutions that reduce, but do not block, protein function.

Isopeptide bonds
A type of covalent bond between the carboxyl group of one amino acid and the amino group of another.

Lipid peroxidation
The oxidative damage of lipids.

Lysosome
Membrane-bound organelles containing enzymes to breakdown biomolecules, including protein and nucleic acids.

Mitophagy
The autophagy of mitochondria.

Non-homologous recombination
An error-prone DNA double strand break repair mechanism.

Nucleotide excision repair
The bulk removal of mutagen-induced DNA lesions.

Peroxisomes
An organelle that performs metabolic reactions and detoxifies oxygen species.

Proteasome
A multisubunit machine for the selective degradation of proteins.

Scissile bond
A covalent bond that can be broken by an enzyme.

Spindle checkpoint
A complex that ensures proper segregation of duplicate chromosomes during mitosis or meiosis.

Synthetic lethality
Mutations or alterations in multiple genes that are lethal when in combination, but not alone.

Telomerase
An enzyme that adds repetitive sequence to telomeres to maintain telomere length at chromosome ends.

Translesion synthesis
DNA synthesis across a lesion to avoid DNA replication failure.

Unfoldases
Enzymes that promote protein unfolding.

Finally, the Met1-specific OTU family DUB OTULIN counteracts Met1 ubiquitination by the LUBAC complex to restrain inflammatory signalling and acts as a powerful gauge in determining the balance between cell death and inflammation[24,217]. OTULIN directly binds LUBAC via HOIP, but also forms additional complexes independent of LUBAC, for example with sorting nexin 27 (SNX27)[218]. Mutations in OTULIN in humans are associated with ORAS (OTULIN-related auto-inflammatory syndrome) that can be managed with the anti-TNF treatment infliximab[219]. However, liver-specific knockout of OTULIN in mice leads to striking, early-onset liver malignancies that can be partially rescued by treatment with the mTOR inhibitor rapamycin, but not through TNF receptor co-deletion, suggesting a new role for OTULIN (and Met1 signalling) in metabolism or cell energetics[164,220].

Apart from cytokine signalling, important pro-inflammatory signals are also triggered by damaged mitochondria through the release of mitochondrial DNA (mtDNA) or the generation of ROS that serve as danger-associated molecular patterns (DAMPs) to trigger cyclic GMP–AMP synthase (cGAS)–stimulator of interferon genes (STING) and nucleotide-binding oligomerization domain-like receptor P3 (NLRP3) inflammatory signalling[221–225]. Mitophagy is a key response mechanism to limit the potential pro-inflammatory effects of mitochondrial damage[226]. The E3 ubiquitin ligase Parkin mediates mitochondrial damage-induced mitophagy[227], and although commonly linked with early-onset Parkinson disease[228], Parkin is also implicated as a tumour suppressor in various cancer types, including colorectal cancer and glioblastoma[229–232], and *Parkin* knockout mice are prone to hepatocellular carcinoma[233]. The DUBs USP30 (refs. 138,234,235) and USP15 (ref. 208) counteract Parkin-mediated mitophagy and have been linked with certain cancer types[236,237], although their potential tumorigenic effect likely extends to the regulation of other pathways, including cell death. USP30 and USP15 are being targeted in drug discovery programmes to treat Parkinson disease[138,235,238]. It will be intriguing to test whether such drugs might prove useful to limit pro-tumorigenic inflammation in certain cancer types.

DUBs in genomic instability and mutation

Maintaining DNA integrity is essential to limit the accumulation of transforming mutations and restrain the emergence of neoplastic clones, whereas loss of function mutations in DNA repair genes are the major driver of genomic instability in inherited cancers[239]. DNA damage signalling and DNA repair rely heavily on ubiquitination[240]. For example, USP7 is considered a guardian of genome integrity, and low *USP7* mRNA expression correlates with genomic instability in the NCI-60 cancer cell line panel[241]. However, this correlation conflicts with the role of USP7 in deubiquitinating and stabilizing the oncogenic E3 ubiquitin ligase MDM2, that degrades the tumour suppressor p53. It is argued that USP7–MDM2 restrains p53 under basal conditions, but following DNA damage USP7 preferentially deubiquitinates and stabilizes p53 to unleash the p53 DNA damage response[63]. In addition, USP7 has been linked to non-p53 DNA damage repair pathways targeting multiple regulators of diverse repair pathways, including nucleotide excision repair, non-homologous recombination and translesion synthesis[242].

Similarly, USP1 regulates components of multiple DNA repair pathways, in particular translesion synthesis and the Fanconi anaemia DNA repair pathway mediated by Fanconi anaemia group D2 protein (FANCD2). USP1 recognizes the FANCI–FANCD2 heterodimer to remove monoubiquitin from FANCD2 to allow DNA lesion repair[243–245]. Consequently, knockdown of USP1 promotes genome instability and consequent apoptosis in multiple myeloma cells[246] and colorectal cancer cells[247]. USP1 is also implicated in deubiquitinating proliferating cell nuclear antigen (PCNA), which enables a switch to the use of more promiscuous DNA polymerases and the promotion of translesion synthesis[248].

Base-excision repair is another repair process that is regulated by ubiquitination. Amongst its other roles in cancer-associated phenotypes, USP47 was found to deubiquitinate nascent DNA polymerase-β (Polβ) in the cytosol to limit its proteolytic degradation[249]. This promotes the functioning of Polβ in the nucleus as a key mediator of base-excision repair[250]. Furthermore, USP47 deletion, or inhibition with the DUB inhibitor P22077 (that inhibits USP47 and also USP7 (ref. 251)), was found to sensitize chronic myeloid leukaemia in vivo to tyrosine kinase inhibition through inhibition of USP47-mediated DNA repair[252]. The function of USP47 in limiting mutagenesis and maintaining genome integrity is somewhat discordant with its amplified expression in various cancer types such as breast cancer, lung cancer and even chronic myeloid leukaemia, but this amplification may indicate a driving role in tumorigenesis that may be exploitable.

Both USP22 and USP28 are implicated in regulating DNA damage responses. USP22 has also been linked to the DNA damage response in prostate cancer through its deubiquitination of the nucleotide excision repair factor XPC[253], adding to the multiple influences USP22 exerts on cell proliferation and cancer-associated phenotypes[78]. USP28 meanwhile plays a key role in the response to DNA double strand breaks by deubiquitinating key cell cycle control proteins, including claspin, p53-binding protein 1 (53BP1) and CHK2, thereby stabilizing p53 (refs. 254,255). USP28 is also required for DNA damage-induced cell death by controlling the CHK2–p53–PUMA axis[255].

USP15 is highly expressed in haematopoietic cells and leukaemia and is thought to protect genome integrity and ensure the fidelity of haematopoiesis[256,257]. USP15 interacts with, and stabilizes, the DNA repair factor fused in sarcoma (FUS)[256]. Hence, deletion of USP15 promotes genotoxic stress limiting leukaemic cell proliferation in vitro[256] and implicates USP15 as a new target for selective inhibition to treat certain cancer types. Given its key role in normal haematopoiesis, and also its potential roles in other cellular pathways, such as negatively regulating mitophagy[235], its targeting in cancer may not be straightforward. However, using engineered ubiquitin variants, Teyra et al.[258] have recently revealed that targeting specific domains of USP15 is feasible, which may pave the way for small molecules that target specific USP15 substrates and therefore the potential to specifically inhibit oncogenic rather than homeostatic cellular processes.

Zinc finger-containing ubiquitin peptidase 1 (ZUP1; also known as ZUFSP) was characterized as the prototypical, and only, mammalian member of a new DUB class due to its unique protease fold, involving a ZUFSP domain and a ZHA domain to specifically bind and cleave Lys63-linked polyubiquitin[259] (Fig. 1). ZUP1 was found to localize to DNA lesions and play a key role in the maintenance of chromosomal stability that is dependent on its DUB activity[260,261]. As a consequence, cancer cell lines exhibit enhanced DNA damage upon siRNA-mediated silencing of ZUP1 (ref. 260). Hence, although its role in cancer progression remains poorly studied, inhibition of ZUP1 to promote chemotherapy-induced DNA damage may be a potential therapeutic strategy.

Targeting DUBs to treat cancer

The impact of DUBs in cancer is often complex, with many DUBs playing multiple, sometimes opposing, roles in distinct cellular contexts and pathways. So, is there a perfect target amongst the DUBs that deserves to be the focus of cancer drug discovery efforts? This question is not easily answered. It is clear from the literature that the DUBs most commonly associated with cancer, and most commonly heralded as worthy targets, are well-studied and broadly expressed enzymes for which tools are readily available. This bias in the literature may have obscured identification of low-abundance, specialist enzymes in certain cell types or tissues that are deregulated in specific cancer settings. Furthermore, most studies have focused on the oncogenic role of DUBs rather than DUBs that are associated with tumour suppression, which has limited understanding of the role of DUBs on the global ubiquitination patterns and their associated functions in cancer cells. Therefore, continuing to gain knowledge of the biology of DUBs is paramount, including DUBs with isoform-specific functions. For example, USP35 has two isoforms with distinct N termini, which either inhibit cell death induced by TNF or the broad-spectrum protein kinase inhibitor staurosporine, or promote endoplasmic reticulum (ER) stress-induced cell death[262].

Nonetheless, based on our analysis of the literature presented above (also see Supplementary Table 1), a handful of DUBs stand out for their broad and established roles in promoting cancer across multiple pathways (Fig. 2). Many other DUBs commonly cited as cancer targets may have tumour-promoting and tumour-suppressive roles dependent on context, which presents a higher bar to generate safe small molecule drugs.

Excitingly, DUB drug discovery has taken several leaps in recent years. The first generation of DUB-targeting compounds remain widely used but are generally non-specific[263]; therefore, we refrain from placing weight on data generated with such molecules. Much more interesting are recently developed, highly specific and potent small molecule inhibitors (Box 3). These compounds, although sometimes not yet optimized to become drugs, have already led to many insights into DUB biology, and will prove valuable tool compounds[264]. Most compounds (and efforts) target USP enzymes, and it is striking that even for the same enzyme, distinct pockets can be targeted with similar potency; it is believed that the specific DUB inhibitors mentioned in Box 3 all have non-overlapping binding sites[52]. DUBs in general seemingly outperform many target classes as specificity is clearly achievable. A caveat to this is that many human USP enzymes arose from gene duplication events, hence achieving inhibitor specificity for these structurally similar enzymes has proven challenging[265]. It is also clear that specific compounds will unlock further insights into DUB mechanisms and reveal the intrinsic plasticity of these highly dynamic enzymes[198,266].

The most advanced effort to translate a DUB inhibitor into the clinic to treat cancer is that of KSQ Therapeutics, whose first-in-class, highly selective, potent (IC_{50} of 11 nM assessed by the ubiquitin-rhodamine assay; Andrew Wylie, personal communication) oral USP1 inhibitor KSQ-4279 has now entered phase I clinical trials as a monotherapy or in combination with poly(ADP-ribose) polymerase (PARP) inhibitors or chemotherapy, for the treatment of advanced solid tumours[267]. Born from the concept of synthetic lethality, which gave rise to the development of PARP inhibitors for the treatment of solid tumours harbouring BRCA1 or BRCA2 mutations, USP1 regulates replication

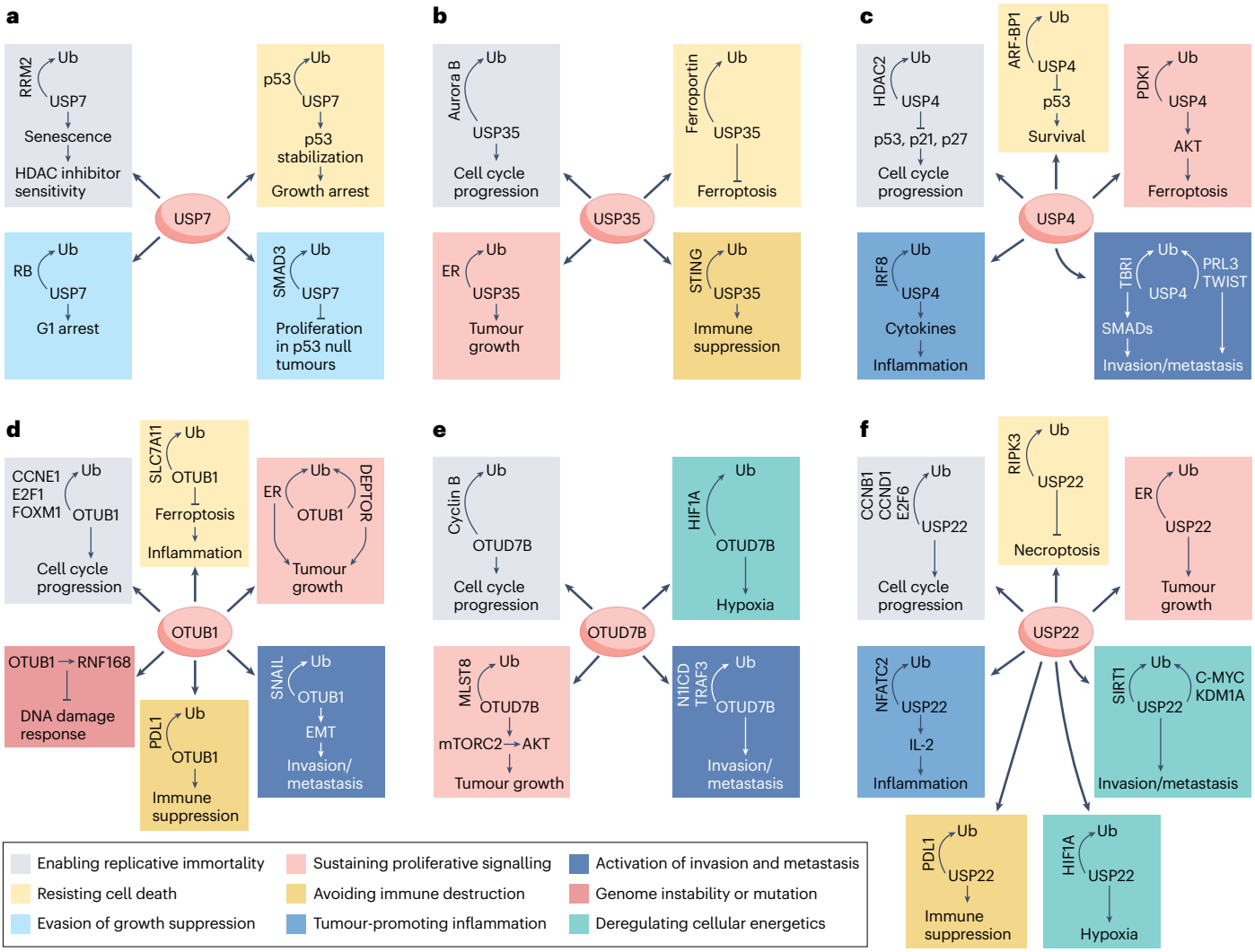


Fig. 2 | DUB targets in cancer. The role of deubiquitinating enzymes (DUBs) in cancer development and progression is multifaceted and highly context dependent. Most DUBs exhibit either tumour suppressor or oncogenic activities depending on the specific cellular context and tumour type. Nevertheless, despite the relative infancy of the understanding of DUB biology in cancer, several DUBs have emerged as notable candidates for further preclinical research based on their distinct characteristics and apparent ability to promote tumorigenesis through various cancer hallmarks. An example of a DUB with context-dependent anti-tumorigenic or pro-tumorigenic roles is ubiquitin carboxyl-terminal hydrolase 7 (USP7). USP7 for the most part acts in an oncogenic fashion. This has led to the development of several USP7-specific inhibitors, with both USP1 and USP7 at the forefront of DUB inhibitor design for implementation as a potential anticancer treatment. However, as with the majority of DUBs, USP7 can also act as a tumour suppressor potentially limiting the utility of USP7 inhibitors. The DUBs presented in this figure (USP7 (**a**), USP35 (**b**), USP4 (**c**), OTUB1 (**d**), OTUD7B (**e**) and USP22 (**f**)) hold potential as targets for the development of new cancer therapies. DEPTOR, DEP domain-containing mTOR-interacting protein; EMT, epithelial-to-mesenchymal transition; ER, oestrogen receptor; HDAC, histone deacetylase; HIF1α, hypoxia-inducible factor 1α; IRF8, interferon regulatory factor 8; IL-2, interleukin-2; mTORC2, mTOR complex 2; OTUB, OTU domain-containing ubiquitin aldehyde-binding protein; OTUD, OTU domain-containing protein; PDL1, programmed cell death protein 1 ligand 1; RIPK3, receptor-interacting serine/threonine-protein kinase 3; SIRT1, sirtuin 1; SLC7A11, solute carrier family 7 member 11; TRAF6, tumour necrosis factor (TNF) receptor-associated factor 6; Ub, ubiquitin.

and DNA repair through deubiquitination of PCNA[268] and the Fanconi anaemia complex[269], respectively, and so USP1 inhibition should promote the death of DNA-damaged cancer cells. Preclinical studies with KSQ-4279 support this synergy with durable anti-tumorigenic affects in orthotopic ovarian xenografts when treated with PARP inhibitor olaparib and KSQ-4279, but not olaparib alone (Andrew Wylie, personal communication). Clearly, the results of this trial will be eagerly anticipated by a broad field of researchers, and positive outcomes will be excellent news for patients, but may also supercharge the hunt for new therapies targeting other DUBs.

As well as translating antagonists with specificity against certain DUBs, there is recent excitement in the field for harnessing DUBs with the development of heterobifunctional chimeric molecules that bring together a DUB with a non-cognate substrate to promote target

Box 3

Specific DUB inhibitors

Numerous deubiquitinating enzyme (DUB) inhibitors have been described; however, to date, few have achieved sufficient specificity to enable meaningful association of a DUB to a particular signalling context. In a comprehensive study of commonly used DUB inhibitors against a large panel of recombinant DUBs[263], it became clear that first-generation, often covalent, compounds should be considered pan-DUB inhibitors, and that papers using these compounds require re-evaluation.

Nonetheless, the last few years have seen numerous highly specific, potent, non-covalent DUB inhibitors entering the literature, and in two cases the clinic. In these cases, compound specificity has been comprehensively tested and molecularly understood, and has been used to validate known, or uncover new, DUB function(s) (see chemical structures below).

KSQ-4279

KSQ-4279 is a highly specific and potent inhibitor of ubiquitin carboxyl-terminal hydrolase 1 (USP1) developed by KSQ Therapeutics and is currently in phase I clinical studies as a single agent and in combination with poly(ADP-ribose) polymerase (PARP) inhibitors, against advanced solid tumours harbouring BRCA1 or BRCA2 mutations or other homologous recombination deficiencies[267].

FT671

FT671 is a highly specific, nanomolar USP7 inhibitor. Crystal structures explained how FT671 and related compounds selectively exploit specific, dynamic features of the ubiquitin binding site of USP7. Multiple USP7-specific inhibitors have been reported, targeting similar, yet distinct, binding pockets specific to USP7 (ref. 52). Proteomics-based profiling has resolved a library of USP7 substrates, which provides an opportunity to validate USP7 inhibitors[292,293].

FT709

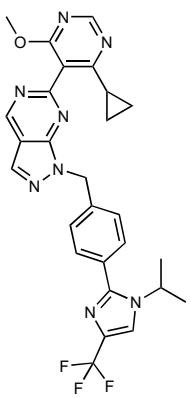
FT709 is a highly selective, nanomolar USP9X-specific compound. USP9X has been associated with multiple cancer-relevant pathways, in part due to the use of the pan-DUB inhibitor WP1130 (ref. 263). However, inhibition of USP9X activity with FT709, consistent with USP9X genetic deletion, supported a role for USP9X in ribosomal stalling that probably contributes to its importance in cancer cells[294] (see also Supplementary Table 1).

FT206

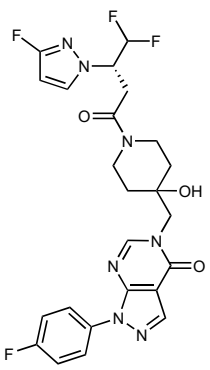
FT206 is a highly selective inhibitor of USP25 and USP28, two paralogous human DUBs of high structural and sequence similarity[265], but with non-overlapping functions and localization[265]. Genetic and compound studies in mouse models of squamous non-small cell lung cancer have revealed the importance of the USP28–MYC axis in tumour progression and suggest efficacy of USP28 inhibition[81]. Other anticancer mechanisms of USP28 inhibition have also been shown[295,296].

VLX1570

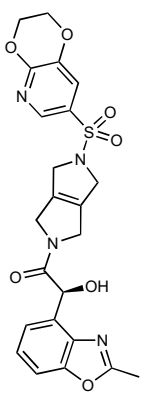
VLX1570 inhibits the proteasome-associated DUB USP14, while also having weaker affinity for ubiquitin carboxyl-terminal hydrolase isozyme L5 (UCHL5)[297], potentially presenting an alternative for treating patients with multiple myeloma whose cancers have become resistant to proteasome inhibitors such as bortezomib or carfilzomib. However, a phase I clinical dose-escalation study was terminated early owing to the death of two patients from severe adverse effects[298]. Recent evidence indicates that VLX1570 may also cause non-specific protein aggregation that could contribute to its broad cellular toxicity[299].



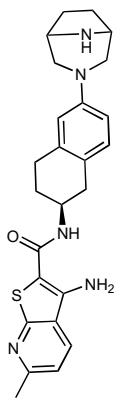
KSQ-4279
USP1-specific
IC_{50}: 11 nM



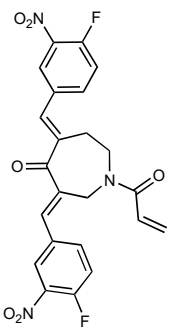
FT671
USP7-specific inhibitor
IC_{50}: 52 nM



FT709
USP9x-specific inhibitor
IC_{50}: 82 nM



FT206
USP25- and USP28-specific inhibitor
EC_{50}(cell) = 300 nM



VLX1570
Proteasomal DUB inhibitor
IC_{50} =10 μM (in vitro), 1 μM (cell)

deubiquitination, starting the field of 'targeted protein stabilization' (TPS)[270]. The new modalities, termed deubiquitinase-targeting chimeras (DUBTACs), enhancement-targeting chimeras (ENTACs) or survival-targeting chimeras (SURTACs), were shown in proof-of-concept experiments to stabilize specific targets, for example via OTUB1-mediated Lys48-deubiquitination, proteins, including ΔF508-cystic fibrosis transmembrane conductance regulator (CFTR) and the tumour suppressor WEE1 (ref. 271). OTUB1 has since been exploited in targeted stabilizers of tumour suppressor transcription factors, including FOXO3A, p53 and interferon regulatory factor 3 (IRF3)[272]. Although the physiological and therapeutic relevance has not yet been established, TPS is a new frontier for promoting or modulating function of an extensive pool of cancer targets and may overcome the challenges inherent in developing small molecule functional agonists.

Conclusions

Over recent years, there has been a major expansion in the understanding of the complexities of ubiquitin signalling and the role of the varied DUBs in its control. With the advent of new and improved tools, including inhibitory small molecules with clearly defined specificity and selectivity, we are now able to define, and in certain circumstances redefine, the role of specific DUBs in signalling networks, which include those associated with cancer. With the already available proof of concept that specific DUB inhibition is achievable, it seems only a matter of time until the DUB field produces its equivalent of imatinib, which has transformed the treatment of leukaemia, to set the pharma industry abuzz.

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Author contributions

The authors contributed equally to all aspects of the article.

Competing interests

G.D. and D.K. are employees of the Walter and Eliza Hall Institute of Medical Research, which receives milestone payments for Venclexta (venetoclax). D.K. is founder and shareholder of Entact Bio. P.J.A.E. declares no competing interests.

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