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REVIEW

Deubiquitinases in cancer: new functions and therapeutic options

JM Fraile[1], V Quesada[1], D Rodríguez, JMP Freije and C López-Otín

Departamento de Bioquímica y Biología Molecular, Facultad de Medicina, Instituto Universitario de Oncología, Universidad de Oviedo, Oviedo, Spain

Deubiquitinases (DUBs) have fundamental roles in the ubiquitin system through their ability to specifically deconjugate ubiquitin from targeted proteins. The human genome encodes at least 98 DUBs, which can be grouped into 6 families, reflecting the need for specificity in their function. The activity of these enzymes affects the turnover rate, activation, recycling and localization of multiple proteins, which in turn is essential for cell homeostasis, protein stability and a wide range of signaling pathways. Consistent with this, altered DUB function has been related to several diseases, including cancer. Thus, multiple DUBs have been classified as oncogenes or tumor suppressors because of their regulatory functions on the activity of other proteins involved in tumor development. Therefore, recent studies have focused on pharmacological intervention on DUB activity as a rationale to search for novel anticancer drugs. This strategy may benefit from our current knowledge of the physiological regulatory mechanisms of these enzymes and the fact that growth of several tumors depends on the normal activity of certain DUBs. Further understanding of these processes may provide answers to multiple remaining questions on DUB functions and lead to the development of DUB-targeting strategies to expand the repertoire of molecular therapies against cancer.
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Proteases have fundamental roles in multiple biological and pathological processes, including cancer (Lopez-Otin and Bond, 2008; Mason and Joyce, 2011). These proteins were first characterized as a group of non-specific enzymes involved in protein catabolism. However, multiple studies performed over recent years have demonstrated that proteases carry out highly specific reactions of proteolytic processing on a wide variety of substrates and regulate many processes that are essential for cell life and death in all organisms (Lopez-Otin and Hunter, 2010). Consistent with the functional relevance of proteases in these processes, alterations in their structure or in the mechanisms controlling their spatiotemporal expression patterns and activities cause diverse pathologies such as arthritis, neurodegenerative alterations, cardiovascular diseases and cancer. Accordingly, many proteases are an important focus of attention for the pharmaceutical industry either as drug targets or as diagnostic and prognostic biomarkers (Turk, 2006; Drag and Salvesen, 2010).

The recent availability of the genome sequence of different organisms has facilitated the identification of their entire protease repertoire, which has been defined as degradome (Lopez-Otin and Overall, 2002). The human degradome consists of at least 569 proteases and homologs grouped into 5 catalytic classes: 194 metallo, 176 serine, 150 cysteine, 28 threonine and 21 aspartic proteases (Quesada *et al*., 2009). Interestingly, the mouse and rat degradomes are even more complex, with at least 644 and 629 members, respectively (Puente *et al*., 2003; Puente and Lopez-Otin, 2004). All these proteases have the common ability of catalyzing the hydrolysis of peptide bonds. Most of them cleave α-peptide bonds between naturally occurring amino acids, but there are some degradome members that perform slightly different chemical reactions. Among them, a large and growing group of proteases known as DUBs (deubiquitylating enzymes or deubiquitinases) have emerged as pivotal regulators of ubiquitin-mediated signaling pathways, because of their capacity to hydrolyze isopeptide bonds in ubiquitin protein conjugates (Reyes-Turcu *et al*., 2009; Komander *et al*., 2009a). After a brief introduction to the structural and enzymatic diversity of human DUBs and their regulatory mechanisms, this review will focus on the description of the functional complexity of these enzymes in tumor development and progression. Finally, we will discuss the growing relevance of DUBs as novel therapeutic targets in cancer.

The large and complex group of DUBs

The post-translational modification of cellular proteins through ubiquitylation is a dynamic and reversible process coordinated by the action of ubiquitylating and deubiquitylating enzymes. The conjugation of ubiquitin to proteins is catalyzed by the successive action of three enzymes: a ubiquitin-activating enzyme, E1; a ubiquitin-

Correspondence: Dr C López-Otín, Departamento de Bioquímica y Biología Molecular, Facultad de Medicina, Universidad de Oviedo, 33006 Oviedo, Spain.
E-mail: clo@uniovi.es
[1]These authors contributed equally to this work.
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conjugation enzyme, E2; and a ubiquitin ligase that transfers ubiquitin to a lysine residue or to the N terminus of the target protein (Hershko *et al.*, 1983). Ubiquitin can be conjugated to target proteins either as a monomer or as polyubiquitin chains that vary in length and linkage type. Depending on the lysine residue involved in the formation of the polyubiquitin chain, there are different kinds of ubiquitin linkages which have distinct physiological roles. Thus, Lys63-linked polyubiquitin chains and multiple mono-ubiquitin conjugation are preferentially involved in the lysosomal pathway, whereas Lys11-, Lys29- and Lys48-linked polyubiquitin chains target proteins for proteasome degradation (Ikeda and Dikic, 2008; Dammer *et al.*, 2011). Conversely, ubiquitin removal is catalyzed by DUBs, which specifically cleave the isopeptide bond between the ε-amino group of lysine side chains of target proteins and the C-terminal group of ubiquitin, or the peptidic bond between the α-amino group of the target protein and the C-terminal group of ubiquitin (Wilkinson, 1997). The human genome encodes at least 98 DUBs subdivided into 6 families based on sequence and structural similarity: ubiquitin-specific proteases (USPs), ubiquitin carboxy-terminal hydrolases (UCHs), ovarian-tumor proteases (OTUs), Machado–Joseph disease protein domain proteases, JAMM/MPN domain-associated metallopeptidases (JAMMs) and the recently discovered monocyte chemotactic protein-induced protein (MCPIP) family (Reyes-Turcu *et al.*, 2009; Komander *et al.*, 2009a; Liang *et al.*, 2010). All of them are cysteine proteases with the exception of JAMMs, which belong to the catalytic class of metalloproteases (Figure 1, Supplementary Table 1 and http://degradome.uniovi.es).

The USPs constitute the largest DUB family described to date, with >50 members (Quesada *et al.*, 2004). All of them have highly conserved USP domains formed by three subdomains resembling the palm, thumb and fingers of a right hand (Hu *et al.*, 2002). The catalytic site is located between the first two subdomains, whereas the finger domain is responsible

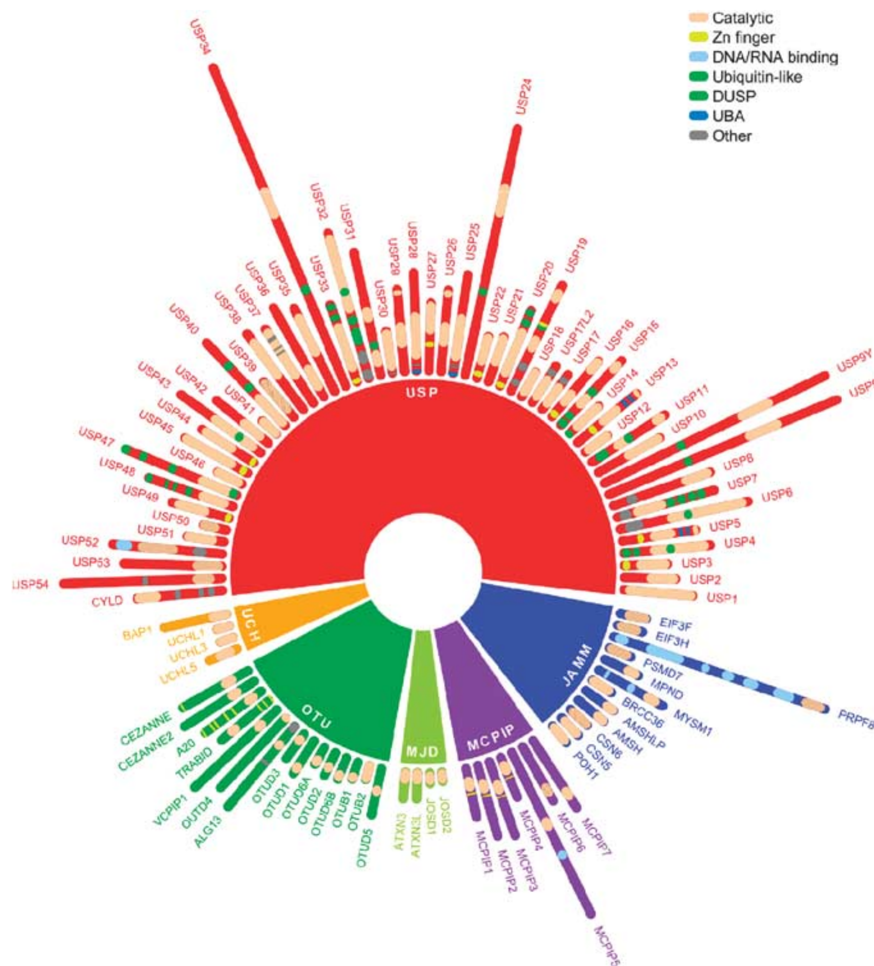

Figure 1 Classification of human DUBs. Human DUBs are classified into six families represented by different colors: USPs, UCHs, OTUs, MJDs, JAMMs and MCPIPs. The catalytic core domain of each individual enzyme is indicated in plain light red if the DUB is active and stripped if inactive. Additional common domains are also shown in different colors. Proteins are represented with their N termini oriented towards the center of the circle. The length of each DUB corresponds to the size of the protein in amino acids. USPs, ubiquitin-specific proteases; UCHs, ubiquitin carboxy-terminal hydrolases; OTUs, ovarian-tumor proteases; MJDs, Machado–Joseph disease protein domain proteases; JAMMs, JAMM/MPN domain-associated metallopeptidases; MCPIPs, monocyte chemotactic protein-induced proteins.

for interactions with distal ubiquitin. Only CYLD (cylindromatosis D), a DUB involved in the development of human cylindromatosis, lacks the finger domain (Komander *et al.*, 2008). Furthermore, many USPs exhibit additional domains and terminal extensions that have important roles in their activity and specificity. These domains include the B-box domain found in CYLD, the zinc-finger USP domain shared by USP3, USP5, USP39, USP44, USP45, USP49 and USP51, the ubiquitin-interacting motif located in USP25 and USP37, the ubiquitin-associated domain in USP5 and USP13, the domain in USPs (DUSP) present in USP4, USP11, USP15, USP20, USP33 and USP48, the exonuclease III domain found in USP52, as well as the ubiquitin-like domain that can be located both within and outside the catalytic domains of several USPs, such as USP4, USP7, USP14, USP32, USP47 and USP48 (Quesada *et al.*, 2004; Zhu *et al.*, 2007b; Komander *et al.*, 2009a) (Figure 1). Despite their relative structural diversity, most USPs share the common feature of undergoing conformational changes upon ubiquitin binding, which drives the transition from an inactive form to a catalytically active state.

Ubiquitin carboxy-terminal hydrolases were the first DUB family to be structurally characterized. These enzymes can only target small peptides from the C terminus of ubiquitin as they have a confined loop that precludes the processing of polyubiquitin chains and large folded proteins. There are four UCHs in humans: UCHL1, UCHL3, UCHL5/UCH37 and BAP1. The additional C-terminal extension present in UCHL5 facilitates the trimming of polyubiquitin chains from conjugated proteins, whereas that of BAP1 interacts with the N-terminal ring finger of BRCA1 (Jensen *et al.*, 1998; Koulich *et al.*, 2008).

The OTU domain was first identified in an ovarian tumor gene from *Drosophila melanogaster* by a bioinformatics approach (Makarova *et al.*, 2000). In 2003, some members of the OTU superfamily were then described as deubiquitylating enzymes (Balakirev *et al.*, 2003) with an active cysteine protease site and no sequence homology to any DUB known so far. We have annotated 15 protein-coding genes with OTU domains in the human genome which can be classified in 3 groups: otubains (OTUB1 and OTUB2), A20-like OTUs (A20/TNFAIP3; Cezanne, Cezanne 2, TRABID and VCPIP1) and OTUDs (OTUD1, OTUD2/YOD1, OTUD3, OTUD4, OTUD5, OTUD6A, OTUD6B and ALG13) (Quesada *et al.*, 2009). The OTU core domain is composed of five β-strands, situated between helical domains that vary in size among OTU family members (Reyes-Turcu *et al.*, 2009). Similar to USPs, the OTU core is accompanied by ancillary ubiquitin-binding domains such as A20-type Zn fingers in A20, NPl4-type Zn fingers in TRABID, ubiquitin-interacting motif in OTUD1 and OTUD5, as well as ubiquitin-associated domain in Cezanne (Komander *et al.*, 2008).

The Josephin family of DUBs is composed of four different members including ataxin-3 (ATXN3), which is mutated in spinocerebellar ataxia type 3 or Machado–Joseph disease (Nicastro *et al.*, 2005). This protein acts as a polyubiquitin chain-editing enzyme controlling protein folding and stability (Mao *et al.*, 2005). Furthermore, its ubiquitin hydrolase activity is essential for a normal lifespan, thereby demonstrating that ubiquitin chain editing contributes to longevity regulation (Kuhlbrodt *et al.*, 2011). The three remaining members of the Josephin family, namely ATXN3L, JOSD1 and JOSD2, also exhibit deubiquitylating activity. All of them have one cysteine and two histidine residues, which are highly conserved and form the catalytic triad. Apart from the Josephin domain, ATXN3 and ATXN3L have additional domains like ubiquitin-interacting motif, suggesting a possible interaction with two distal ubiquitins in a polymer (Burnett *et al.*, 2003).

The JAMMs form the only family of DUBs with zinc-metalloprotease activity, and their catalytic mechanism has been elucidated through the study of the crystal structures of AMSH-LP (associated molecule with SH3 domain-like protease), alone and in complex with a Lys63-linked diubiquitin (Sato *et al.*, 2008). The members of the AMSH family specifically cleave Lys63-linked polyubiquitin chains, thus facilitating vesicle trafficking and receptor recycling. The AMSH-LP DUB domain is composed of a JAMM core and two conserved insertions. Other JAMM proteases that have no specificity for Lys63-linked polyubiquitin lack the AMSH-specific inserts. Apart from AMSH-LP, the human genome encodes 11 additional JAMM proteins, 5 of which are catalytically inactive, whereas the rest of them have isopeptidase activity for ubiquitin or ubiquitin-like proteins: AMSH/STAMBP, BRCC36 (BRCA1/BRCA2-containing complex subunit 36), POH1/PSMD14 (26S proteasome-associated PAD1 homolog 1), MYSM1 (Myb-like with SWIRM and MPN domains 1), MPND (MPN domain-containing protein) and CSN5/JAB1 (COP9 signalosome subunit 5). The high degree of sequence conservation between POH1, AMSH and AMSH-LP suggests the existence of a common strategy for ubiquitin recognition and DUB catalysis in these JAMMs (Sato *et al.*, 2008).

The DUB group of isopeptidases has recently expanded after the description of a new domain in the MCPIP1 protein, which exhibits deubiquitylating activity (Liang *et al.*, 2010). The finding of this novel domain with DUB activity points to the existence of a sixth family of DUBs in the human genome, which according to our bioinformatic analysis, should be composed of at least seven members (Figure 1 and Supplementary Table 1). The founding member MCPIP1 contains a functional ubiquitin-associated domain at the N terminus that mediates its interaction with ubiquitylated proteins but is not required for DUB activity. There is then an N-terminal conserved region, a conserved CCCH-type zinc-finger domain in the middle region of the protein, and a Pro-rich domain at its C terminus. The N-terminal conserved region and CCCH zinc fingers are critical for MCPIP1 activity. Moreover, the catalytic domain contains the Cys and Asp boxes characteristic of cysteine proteases, but lacks the His box that is likely located outside the N-terminal conserved region (Liang *et al.*, 2010).

Enzymatic roles and regulation of DUBs

Deubiquitinases have fundamental roles in both ubiquitin homeostasis and protein stability control through enzymatic activities that can be classified into three different categories: ubiquitin precursor processing, ubiquitin deconjugation and editing of ubiquitin conjugates (Figure 2). Thus, DUBs contribute to the generation of free ubiquitin through their ability to process ubiquitin precursors consisting of either multiple ubiquitin copies or ubiquitin fusions to L40 and S27 ribosomal proteins (Pickart and Rose, 1985; Amerik and Hochstrasser, 2004). Moreover, DUBs can remove the ubiquitin chains from ubiquitylated proteins and rescue them from degradative pathways or lead to the reversion of ubiquitin signaling. As discussed above, the nature of the ubiquitin chain linkage defines the fate of the conjugated protein, mediating their lysosome-mediated recycling or their degradation in the proteasome. Hence, DUBs exhibit high versatility and enhance protein stability by preventing both lysosomal and proteasomal degradation. Interestingly, there are three proteasome-associated DUBs the activity of which directly removes ubiquitin from proteins targeted for degradation: UCHL5, USP14 and POH1 (Guterman and Glickman, 2004a; Koulich *et al*., 2008; Lee *et al*., 2010). Another level of protein stability regulation is shown by the interactions of DUBs with E3 ligases with self-ubiquitylating activity. USP8, USP19 and CYLD are examples of DUBs the activity of which can stabilize neuregulin receptor degradation protein 1, cyclin-dependent kinase inhibitor p27Kip1 (CDKN1B) and tumor necrosis factor (TNF) receptor-associated factor 2 (TRAF2) E3 ligases, respectively (Brummelkamp *et al*., 2003; Cao *et al*., 2007; Lu *et al*., 2009). Finally, some DUBs can trim ubiquitin chains conjugated to protein substrates and edit the type of ubiquitin signal (nondegradative vs degradative) harbored by them. This is the case of A20 that changes receptor-interacting serine-threonine kinase 1 (RIPK1) ubiquitylation status from Lys63- to Lys48-linked polyubiquitin and causes its degradation by the proteasome (Hymowitz and Wertz, 2010).

An additional level of control for DUB activities comes from the specificity for the type of ubiquitin chain linkages that are processed. Thus, structural and functional analyses have demonstrated that USPs and OTUs recognize either Lys48- or Lys63-linked polyubiquitin chains. For instance, USP14 processes Lys48-linked chains (Hu *et al*., 2005), whereas CYLD only efficiently cleaves Lys63 linkages and linear ubiquitin chains (Komander *et al*., 2009b). Similarly, some OTU family components such as OTUB1 and A20 hydrolyze Lys48-linked chains, whereas TRABID and OTUD5 have preference for Lys63 linkages (Virdee *et al*., 2010). Furthermore, Cezanne preferentially cleaves Lys11 over Lys48 and Lys63 linkages (Bremm *et al*., 2010), whereas JAMMs share the specificity for Lys63-linked polyubiquitin (McCullough *et al*., 2004; Sato *et al*., 2008; Cooper *et al*., 2009). Finally, the Josephin ATXN3-editing activity shows a restricted specificity for K63-linked chains (Winborn *et al*., 2008).

Owing to their pivotal roles in ubiquitin homeostasis and control of protein stability, DUB activities must be tightly regulated through a number of different mechanisms, including transcriptional control of gene expression, post-translational modifications, changes in subcellular localization and activation mediated by

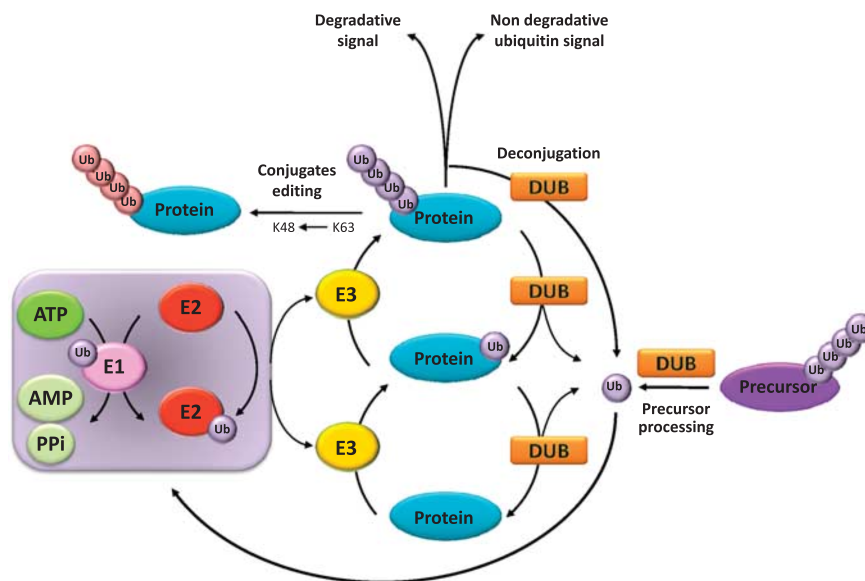

Figure 2 Enzymatic activities of DUBs. Schematic representation of the involvement of DUBs at different steps of the ubiquitylation pathway. Ubiquitin is conjugated by the action of three consecutive enzymes: ubiquitin-activating enzyme E1, ubiquitin-conjugating enzyme E2 and ubiquitin ligases E3. DUBs generate free ubiquitin through the processing of ubiquitin precursors maintaining ubiquitin homeostasis. Some DUBs can edit ubiquitin chains and thereby change ubiquitin signals. DUBs can also rescue proteins from degradation or can remove a nondegradative ubiquitin signal by ubiquitin deconjugation.

interacting proteins. Examples of transcriptional regulation are those affecting mouse *DUB-1*, *DUB-2* and *DUB-3*, which are induced by inflammatory cytokines (Jaster *et al*., 1999; Burrows *et al*., 2004; Baek, 2006), and *CYLD* that is induced by the activation of the nuclear factor-κB (NF-κB) and MAPK Kinase 3/6 (MKK3/6)-p38 pathways (Yoshida *et al*., 2005). In addition, many DUB functions are tightly controlled by post-translational modifications (Kessler and Edelmann, 2011). Thus, multiple DUBs undergo phosphorylation by protein kinases that can switch their activity on or off. For example, phosphorylation inhibits CYLD and USP8, while it activates A20, USP7, USP15, USP16, USP19, USP28, USP34 and USP37 (Lopez-Otin and Hunter, 2010; Huang *et al*., 2011). Ubiquitin and ubiquitin-like modifications can also modulate DUB activity, either positively in the case of ATXN3 activation by ubiquitylation (Todi *et al*., 2009) or negatively, as occurs with ubiquitylation of UCHL1 (Meray and Lansbury, 2007) and sumoylation of USP25 (Meulmeester *et al*., 2008). USP7, USP36 and DUB-1 are also ubiquitylated, although the relevance of this modification is still unknown (Baek *et al*., 2004; Kim *et al*., 2005; Lee *et al*., 2005). Reactive oxygen or nitrogen species can also post-translationally modify DUBs as illustrated by the hydrogen peroxide-mediated modification of Cezanne (Enesa *et al*., 2008a). Furthermore, some DUBs such as USP1 and ATXN3 are inactivated by autoproteolytic cleavage, whereas CYLD and A20 are inactivated through the action of other proteases (Huang *et al*., 2006; Mauri *et al*., 2006; Coornaert *et al*., 2008; Staal *et al*., 2011).

The activity of DUBs can also be modulated by changes in their subcellular localization, which contribute to facilitate their interaction with specific substrates. Targeting of USP30 to the mitochondria seems to influence the morphological properties of this organelle (Nakamura and Hirose, 2008), whereas USP36 nucleolar localization regulates its structure and function (Endo *et al*., 2009). In addition, the presence of accessory ubiquitin-binding domains in DUBs contributes to regulate the activity and specificity of several family members such as USP8, USP25, A20, OTUD5, ATXN3, AMSH and UCHL5 (Komander *et al*., 2009a). Another mechanism of regulation found in several USPs derives from the presence of a ubiquitin-like domain within their catalytic region, as occurs in the case of the partial self-inhibition of USP4 activity (Luna-Vargas *et al*., 2011). Other DUBs, like UCHL1, UCHL3, OTU1, USP1, USP5, USP7, USP12, USP14 and USP46 undergo allosteric conformational changes upon interaction with ubiquitin or other proteins, which cause an increase in their catalytic rate (Reyes-Turcu *et al*., 2009; Komander *et al*., 2009a). Finally, the regulation and specificity of DUB-mediated deubiquitylation largely depends on the association of DUBs with their protein partners (Ventii and Wilkinson, 2008). In fact, USP1 interacts with U2 small nuclear ribonucleoparticle auxilliary factor 1 having a role in DNA damage repair. Similarly, several DUBs must be incorporated within large macromolecular complexes, such as the 26S proteasome or the COP9 signalosome, to become active (Guterman and Glickman, 2004b; Adler *et al*., 2008). In a global proteomics approach, using tandem affinity-based pull-outs and bioinformatics for the high confidence identification of interacting proteins of 75 DUBs, Sowa *et al*. (2009) uncovered a large landscape of 774 putatively associated proteins. In this scenario, protein–protein interactions and complex formation add new layers of complexity in the regulation of DUB functions, yet to be explored.

Functional roles of DUBs in cancer

The wide functional diversity of DUBs has a profound impact on the regulation of multiple biological processes such as cell-cycle control, DNA repair, chromatin remodeling and several signaling pathways that are frequently altered in cancer (Hussain *et al*., 2009; Reyes-Turcu *et al*., 2009; Komander *et al*., 2009a) (Figure 3 and Supplementary Table 2). As a consequence, different DUB functions are directly linked to the development of neoplastic diseases.

Cell-cycle regulation

The relevance of DUBs in cell-cycle progression is underscored by the fact that several family members are integral components of the core cell-cycle machinery and cell-cycle checkpoints. Functional analyses have revealed the importance of USP28 in regulating the stability of c-Myc, a central modulator of cell growth, proliferation and apoptosis (Popov *et al*., 2007). Other DUBs such as CYLD, USP13, USP37, USP39 and USP44 are crucial regulators of events occurring in mitosis. Thus, CYLD is required for timely entry into mitosis through the regulation of polo-like kinase 1 (Stegmeier *et al*., 2007b). Moreover, USP13 is recruited by the ubiquitin-recognition protein Ufd1 to counteract anaphase-promoting complex (APC/C)-Cdh1-mediated ubiquitylation of Skp2, resulting in accumulation of the cyclin-dependent kinase inhibitor p27 and a concomitant cell-cycle delay (Chen *et al*., 2011). Another DUB that also antagonizes APC/C-Cdh1 function is USP37 which, after being activated by CDK2, deubiquitylates cyclin A and promotes S-phase entry (Huang *et al*., 2011). Furthermore, USP39 controls levels of Aurora B kinase and is essential for mitotic spindle checkpoint integrity (van Leuken *et al*., 2008), whereas USP44 prevents the premature activation of APC/C by stabilizing the APC/C-inhibitory Mad2–Cdc20 complex through deubiquitylation (Stegmeier *et al*., 2007a). In contrast, USP50 is involved in the G_2/M checkpoint and acts as a regulator of HSP90-dependent Wee1 stability to repress entry into mitosis (Aressy *et al*., 2010). Furthermore, USP17L2 deubiquitylates and stabilizes Cdc25A promoting oncogenic transformation (Pereg *et al*., 2010). Moreover, USP2 directly interacts with cyclin D1 thereby promoting its stabilization (Shan *et al*., 2009) and USP19 regulates cell proliferation in a cell context-dependent manner through both

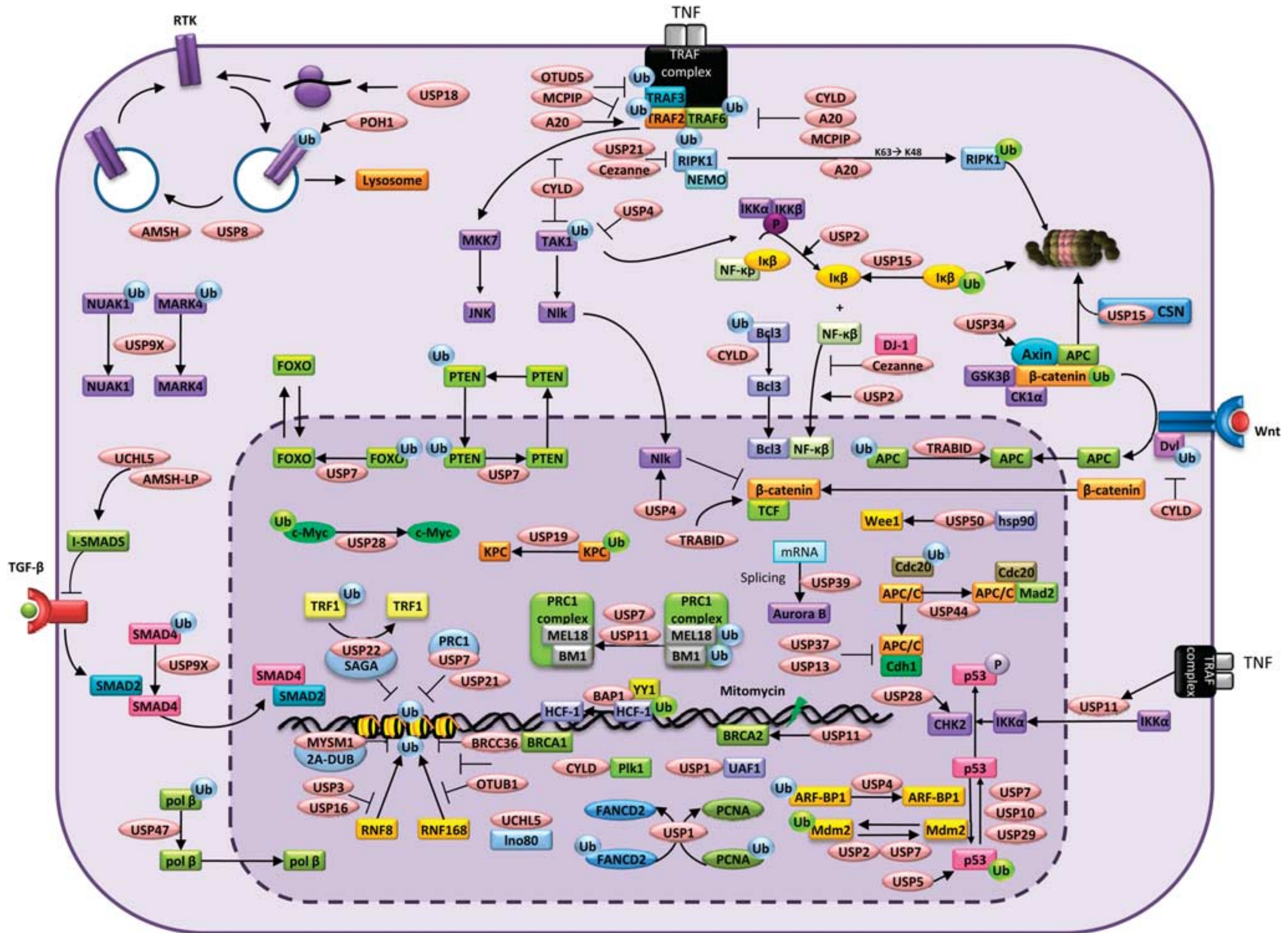

Figure 3 Overview of the different roles of DUBs in cancer. Examples of DUBs (pink boxes) involved in distinct cellular pathways. Ubiquitin in green corresponds to Lys48-linked chains targeting proteins to the proteasome, whereas blue ubiquitin indicates non-Lys48-linked chains. USP7, USP11, USP13, USP19, USP37, USP39, USP44, USP50 and BAP1 are involved in cell-cycle progression. USP1, USP3, USP11, USP16, USP28, USP47, BRCC36 and OTUB1 have important roles in DNA damage repair. USP3, USP7, USP16, USP21, USP22, UCHL5, MYSM1, BAP1 and BRCC36 participate in chromatin remodeling by deubiquitylating histones or other chromatin-related substrates. USP2, USP4, USP5, USP7, USP10 and USP29 intervene in p53 regulation. A20, Cezanne, OTUD5, CYLD, USP2, USP4, USP11, USP15 and USP21 participate in NF-κB signaling. USP8, USP18, AMSH and POH1 interfere in receptor tyrosine kinase trafficking. USP4, USP15, USP34 and TRABID are associated with Wnt signaling. Finally, USP9X, AMSH-LP and UCHL5 are involved in the regulation of the TGF-β pathway.

E3 ligase KPC1-dependent and KPC1-independent mechanisms (Lu *et al*., 2011). Similarly, USP7 is essential in cell proliferation and differentiation, through its regulatory activity on phosphatase and tensin homolog and FOXO localization (van der Horst *et al*., 2006; Song *et al*., 2008). Furthermore, CSN5 is a modulator of the mammalian cell cycle, preventing senescence and the proper progression of the somatic cell cycle (Yoshida *et al*., 2010). Finally, BAP1 forms complexes with the transcription factors Yin Yang 1 (YY1) and host cell factor 1, and influences cell-cycle progression at G_1/S by co-regulating transcription from host cell factor 1/E2F-responsive promoters (Yu *et al*., 2010; Eletr and Wilkinson, 2011).

DNA damage repair

The link between genetic damage repair and cancer development is illustrated by the increased tumor rates reported for those disorders associated with deficient DNA repair mechanisms, such as Fanconi's anemia. In this respect, the deubiquitylation of Fanconi's anemia protein (FANCD2) by USP1 and the subsequent stabilization of CHK1 are critical in DNA damage repair (Nijman *et al*., 2005; Guervilly *et al*., 2011). Moreover, USP1 modulates proliferating cell nuclear antigen ubiquitylation, a safeguard factor against error-prone DNA translesion synthesis that is ubiquitylated in response to genotoxic stress (Huang *et al*., 2006). As discussed above, USP1 forms a complex with U2 small nuclear ribonucleoparticle auxilliary factor 1 and promotes double-strand break repair through homologous recombination (Murai *et al*., 2011). There are other DUBs implicated in the regulation of DNA repair. Thus, BRCC36, USP3, USP16 and OTUB1 participate in the control of the RNF8/168 pathway of double-strand breaks repair (Al-Hakim *et al*., 2010), USP11 in

the cellular response to mitomycin C-induced DNA damage within the BRCA2 pathway (Schoenfeld *et al.*, 2004), and USP28 in the CHK2-p53-PUMA pathway (Zhang *et al.*, 2006a). Finally, recent studies have identified USP47 as the enzyme responsible for the deubiquitylation of the base excision repair DNA polymerase (Polβ), thus having an important role in DNA repair regulation and genome integrity maintenance (Parsons *et al.*, 2011).

Chromatin remodeling

Several DUBs interact with histones, mainly H2A and H2B, the post-translational modifications of which regulate chromatin structure dynamics and gene transcription, which are processes that are frequently altered in cancer. There are at least seven DUBs that can deubiquitylate histones: USP3, USP7, USP16, USP21, USP22, MYSM1 and BRCC36 (Joo *et al.*, 2007; Clague *et al.*, 2008; Atanassov *et al.*, 2011). All of them deubiquitylate both H2A and H2B, although H2A is more preferentially targeted (Zhang, 2003). MYSM1, USP7, USP22 and BRCC36 are part of the 2A-DUB, polycomb-repressive complex 1, SAGA and BRCA1-A multisubunit complexes, respectively (Zhu *et al.*, 2007a; Zhang *et al.*, 2008; Feng *et al.*, 2010; Maertens *et al.*, 2010). Nevertheless, USP3 and USP16 have not been found in any of these complexes, suggesting that their chromatin-regulatory mechanisms may be different. Apart from histones, gene expression can be modulated by deubiquitylation of other chromatin-related substrates. Thus, USP22 regulates the protein stability of telomeric-repeat binding factor 1 (Atanassov *et al.*, 2009), whereas USP7 and USP11 deubiquitylate MEL18 and BMI, two chromatin-bound polycomb-repressive complex 1 complex components that affect the transcriptional regulation of $p16^{INK4a}$ (Maertens *et al.*, 2010). Moreover, UCHL5, the activation of which requires association with the proteasome, also interacts with the human lno80 chromatin-remodeling complex (Yao *et al.*, 2008). Finally, and as discussed above, BAP1 deubiquitylates the chromatin-associated protein host cell factor 1, which modulates transcription by linking histone-modifying enzymes to a subset of transcription factors (Eletr and Wilkinson, 2011).

Signaling pathways

The importance of focusing on pathways rather than on individual genes altered in cancer has become increasingly recognized (Vogelstein and Kinzler, 2004). Thus, mutations in individual genes involved in the same cancer-relevant pathway have been described in many tumor types and are known to have similar functional effects, offering a broader range of drug targets (Forbes *et al.*, 2011). Some pathways are recurrently altered in many types of cancer, as those involving p53, NF-κB, receptor tyrosine kinases (RTKs), Wnt and transforming growth factor-β (TGF-β), which are profoundly influenced by the activity of DUBs.

p53 is a tumor suppressor critically involved in maintaining cellular homeostasis and frequently mutated in most tumor types (Harris and Levine, 2005). So far, USP2, USP4, USP5, USP7, USP10 and USP29 have been described to participate in p53 regulation. USP7 is involved in the dynamic control of the p53-MDM2 pathway by regulating the stability of both p53 and MDM2, a ubiquitin ligase that also contributes to the maintenance of p53 ubiquitylation levels (Brooks *et al.*, 2007; Kon *et al.*, 2010). Hence, *USP7* can be considered as an oncogene or a tumor suppressor depending on whether it predominantly deubiquitylates MDM2 or p53, respectively. Other studies have reported a similar role for USP2 in the stabilization of MDM2, although this DUB, unlike USP7, does not deubiquitylate p53 (Stevenson *et al.*, 2007). USP10 also takes part in the regulation of p53 localization and stability, but in contrast to USP2 and USP7, it does not interact with MDM2 (Yuan *et al.*, 2010). Interestingly, USP10 can stabilize both mutated and wild-type p53, thus having a dual role in tumorigenesis depending on p53 status. Similarly, USP5 has been proposed to regulate p53 activity, based on the evidence that USP5 knockdown causes the accumulation of nuclear p53 and an increase in p53 transcriptional activity (Dayal *et al.*, 2009). Furthermore, USP29 deubiquitylates and stabilizes p53 in response to oxidative stress (Liu *et al.*, 2011). Very recently, USP4 has been reported to promote p53 degradation through deubiquitylation and stabilization of the ubiquitin ligase ARF-BP1 (Zhang *et al.*, 2011b). On this basis, together with the finding that *Usp4*-deficient murine embryonic fibroblasts (MEFs) exhibit retarded growth, premature senescence, hyperactive DNA damage checkpoints and resistance to oncogenic transformation, it has been proposed that *USP4* is a potential oncogene (Zhang *et al.*, 2011b).

Nuclear factor-κB is one of the most important modulators of innate and adaptive immune responses, which are frequently deregulated and constitutively activated in cancer (Prasad *et al.*, 2010). Several DUBs such as A20 and CYLD act as tumor suppressors through their ability to downregulate NF-κB signaling by acting on several components of the pathway (Harhaj and Dixit, 2011). Both of them control levels of ubiquitin linked to TRAF6. CYLD is also involved in the deubiquitylation of TGF-β-activated kinase 1 (Reiley *et al.*, 2007), B-cell CLL/lymphoma 3 (Bcl-3) (Massoumi *et al.*, 2006) and mitogen-activated protein kinases (Reiley *et al.*, 2004), whereas A20 promotes the degradation of TRAF2 in lysosomes by means of its own E3 ligase activity (Li *et al.*, 2009). A20 also promotes the proteasomal degradation of RIPK1 through Lys48 polyubiquitylation, whereas its OTU domain removes Lys63-linked ubiquitin chains of RIPK1, leading to the downregulation of NF-κB signaling (Wertz *et al.*, 2004). USP21 inhibits NF-κB activation by regulating ubiquitin levels of RIPK1 (Xu *et al.*, 2010). Similarly, Cezanne suppresses NF-κB nuclear translocation and transcriptional activity by deubiquitylating RIPK1 signaling intermediaries and interacting with DJ-1 (Enesa *et al.*, 2008b; McNally *et al.*, 2011). In addition, OTUD5 deubiquitylates

TRAF3 resulting in diminished type I interferon and interleukin-10 responses (Gonzalez-Navajas *et al*., 2010). Furthermore, USP15 stabilizes IκBα by inhibiting its degradation by the proteasome (Schweitzer *et al*., 2007) and USP11 interacts with IκB kinase α, an inhibitor of NF-κB, upon induction by TNFα (Yamaguchi *et al*., 2007). More recently, USP2 has also been identified as a modulator of TNFα-induced NF-κB signaling, being required for Iκb phosphorylation, nuclear translocation of NF-κB and expression of NF-κB-dependent target genes (Metzig *et al*., 2011). USP4 also has a critical role in the downregulation of TNFα-induced NF-κB activation through deubiquitylation of TGF-β-activated kinase 1 (Fan *et al*., 2011). Finally, MCPIP1 negatively regulates c-Jun N-terminal kinase and NF-κB activity through deubiquitylation of TRAF2, TRAF3 and TRAF6, thus having an essential role in inflammatory signaling (Liang *et al*., 2010).

The relevance of RTKs in cancer is reflected by multiple abnormalities in RTK-dependent pathways that have been found in several human tumor types (Mosesson *et al*., 2008). In fact, numerous oncogenic mechanisms are known to interfere with RTK internalization. There are at least four DUBs—USP8, USP18, AMSH and POH1—that affect the trafficking of RTKs like epidermal growth factor receptor (EGFR), Met and ErbB2. USP8 has an important role in the stabilization of RTKs through deubiquitylation, allowing their recycling to the plasma membrane (Niendorf *et al*., 2007), although other studies have suggested that USP8 might promote degradation of RTKs (Alwan and van Leeuwen, 2007). In addition, the endosome-associated AMSH (also known as STAMBP) promotes EGFR recycling at the expense of lysosomal sorting (McCullough *et al*., 2004; Clague and Urbe, 2006). USP18 has been identified in a recent RNA interference screen as a new regulator of EGFR synthesis by modulating its translation (Duex and Sorkin, 2009). Further studies have shown that USP18 regulates EGFR expression and cancer cell survival due to transcriptional activation and mRNA stabilization of miR-7 host genes (Duex *et al*., 2011). Finally, another RNA interference screen has identified POH1 as a regulator of ubiquitylated ErbB2 levels, although it is not involved in its turnover (Liu *et al*., 2009).

The Wnt signaling pathway is essential for control of embryonic development and is frequently activated in cancer (Klaus and Birchmeier, 2008). At least five DUBs are associated with this pathway: CYLD, USP4, USP15, USP34 and TRABID. Thus, CYLD acts as a negative regulator of Wnt signaling and β-catenin activation by deubiquitylating the cytoplasmic effector Dishevelled (Dvl) (Tauriello *et al*., 2010). USP4 negatively modulates Wnt signaling through interaction with Nemo-like kinase (Zhao *et al*., 2009) and USP15 promotes β-catenin degradation by stabilizing adenomatous polyposis coli (APC), a negative regulator of Wnt-mediated transcription (Huang *et al*., 2009). Conversely, USP34 acts as a positive regulator of Wnt signaling by hindering β-catenin-dependent transcription (Lui *et al*., 2011). Furthermore, TRABID is a DUB critically involved in T-cell factor (TCF)-mediated transcription of *Wnt* genes that also shows the ability to deubiquitylate APC (Tran *et al*., 2008).

Transforming growth factor-β is a multifunctional protein that has a dual role in oncogenesis acting as an antiproliferative factor at early stages and promoting epithelial-to-mesenchymal transition at later stages (Pardali and Moustakas, 2007). So far, three DUBs have been reported to be involved in the regulation of this pathway: USP9X, AMSH-LP and UCHL5. USP9X positively regulates TGF-β signaling by deubiquitylating SMAD4 and promoting its association with SMAD2 (Dupont *et al*., 2009). USP9X also deubiquitylates the AMPK-related kinases NUAK1 and MARK4, implicated in the regulation of cell polarity and proliferation, and modulates their phosphorylation and activation by LKB1 (Al-Hakim *et al*., 2008). Finally, AMSH-LP and UCHL5 potentiate TGF-β responses through their interaction with inhibitory I-SMADs (Ibarrola *et al*., 2004; Wicks *et al*., 2005).

Other functional roles for DUBs in cancer

Deubiquitinases may also have additional and important roles distinct from those described above in different steps of cancer progression, such as epithelial-to-mesenchymal transition, cell migration or apoptosis (Supplementary Table 2). For instance, UCHL1, which has been described as a potent oncogene (Hussain *et al*., 2010), regulates prostate cancer progression and metastasis by inducing epithelial-to-mesenchymal transition (Jang *et al*., 2011). USP17 is induced by chemokines and has a critical role in cell migration through the regulation of the subcellular localization of GTPases that are essential for cell motility (de la Vega *et al*., 2011). CYLD has also been suggested to influence cell migration through the GTPase Rac1 (Gao *et al*., 2010). On the other hand, DUBs have dual and complex roles in the regulation of apoptotic processes, either promoting (USP2, USP7, USP8, USP9X, USP15, USP16, USP17, USP28, USP41, CYLD, UCHL1, A20 and ATXN3) or suppressing apoptosis (USP2, USP9X, USP18, UCHL3 and A20) (Vucic *et al*., 2011; Ramakrishna *et al*., 2011a). In an example of these dual functions, USP2 rescues prostate cancer cells from apoptosis by stabilizing fatty-acid synthase (Graner *et al*., 2004), and deubiquitylates and stabilizes the truncated form of the apoptosis-inducing factor AIF, thus promoting cell death (Oh *et al*., 2011). Similarly, USP9X deubiquitylates and stabilizes MCL1 leading to cell survival (Schwickart *et al*., 2010), but also promotes apoptosis by stabilizing apoptosis signal-regulating kinase 1 (Nagai *et al*., 2009). Furthermore, USP17 deubiquitylates SDS3 regulating its histone deacetylase activity and leading to apoptosis in cervical carcinoma cells (Ramakrishna *et al*., 2011b). In addition, the downregulation of *CYLD* expression is involved in the apoptotic resistance of human hepatocellular carcinoma cells (Urbanik *et al*., 2011). Interestingly, although

A20 is widely considered as a tumor-suppressor DUB promoting cell death, it also acts as a potent prosurvival gene by inhibiting apoptosis in certain malignancies such as gliomas and breast carcinomas (Vendrell *et al*., 2007). Moreover, in this regard, A20-binding inhibitors have been described to have a critical role in the inhibition of NF-κB and apoptosis signaling (Verstrepen *et al*., 2009). Notably, USP20 and USP33 are implicated in Von Hippel–Lindau's syndrome (VHL), a familial cancer syndrome caused by germline mutations of the *VHL* gene that predispose to various benign and malignant tumors (Li *et al*., 2002a, b). USP20 stabilizes hypoxia-inducible factor through deubiquitylation, thereby inhibiting the degradation promoted by the E3 ubiquitin ligase VHL (Li *et al*., 2005). The actual function of USP33 is still unknown, but some of the disease-causing mutations in VHL block its binding to USP33 suggesting a possible role for this enzyme in VHL protein regulation. Finally, the recent identification of multiple candidate DUB substrates and interacting proteins (Sowa *et al*., 2009) will likely unveil novel DUB functions in cancer that could help in the identification of additional therapeutic targets.

Genetic or functional alterations of DUBs in cancer

The finding of several mutated DUBs acting as oncogenes (*USP6* and *USP28*) or tumor suppressors (*CYLD*, *A20* and *BAP1*), together with the frequent changes in the expression levels of multiple DUB family members in malignant tumors, highlights the relevance of these enzymes in cancer development (Supplementary Table 2). Further studies have shown that several DUBs may function either as oncogenes or tumor suppressors depending on the cellular context. In fact, there are DUBs with dual roles as pro-tumorigenic and anti-tumorigenic enzymes depending on the target affected by their regulation, as discussed above for USP7, CYLD and A20 (Vendrell *et al*., 2007; Stegmeier *et al*., 2007b; Kon *et al*., 2010).

Mutations in *CYLD* have been described in familiar cylindromatosis, in which patients show a predisposition to develop multiple head and neck skin tumors (Saggar *et al*., 2008). The generation of mutant mice deficient in *CYLD* has confirmed the tumor-suppressor role of this DUB because of its ability to enhance NK-κB activity (Zhang *et al*., 2006b). In the case of *A20*, chromosomal deletions and inactivating mutations have been found in several lymphoma subtypes (Honma *et al*., 2009; Novak *et al*., 2009), whereas point mutations and deletions in *BAP1* have been described in breast and lung cancer (Jensen *et al*., 1998; Harbour *et al*., 2010). Furthermore, *BAP1*-inactivating mutations have been identified in 84% of metastasizing uveal melanomas (Harbour *et al*., 2010) and in 23% of malignant pleural mesotheliomas (Bott *et al*., 2011). Regarding DUBs with oncogenic roles, somatic mutations in *USP6* and *USP28* have been found in different human malignancies. Thus, chromosomal rearrangements that create a fusion gene in which the osteoblast cadherin 11 gene (*CDH11*) promoter region causes the overexpression of *USP6*—also known as *Tre2*—turn this DUB into an oncogene associated with neoplastic aneurismal bone cysts (Oliveira *et al*., 2004). As for *USP28*, mutations in this gene have been reported in cases of lobular breast cancer (Shah *et al*., 2009).

In addition, a number of DUBs are linked to cancer through changes in their expression patterns (Supplementary Table 2). For instance, *JOSD1*, *CSN5*, *UCHL1* and *USP9X* are significantly overexpressed in nonsmall cell lung carcinomas, whereas *USP10*, *USP11*, *USP22*, *USP48* and *CSN5* are upregulated in malignant melanoma. USP10, USP11 and USP22 levels are associated with a more aggressive and invasive phenotype (Luise *et al*., 2011) and may represent useful markers for diagnostic/prognostic evaluation or as new therapeutic targets. Similarly, *USP22* is overexpressed in colorectal carcinomas and is part of a set of marker genes that can be used as predictors of metastatic potential and therapeutic outcome in human cancer (Glinsky, 2005). Furthermore, *USP1* expression correlates with initial steps of transformation in gastric cancer (Luise *et al*., 2011), whereas *USP2* is overexpressed in ovarian and prostate carcinomas and is associated with lesions of poor prognosis (Yang *et al*., 2007). Moreover, overexpression of *USP2* protects prostate cancer cells from apoptosis and confers them resistance to chemotherapeutic agents by reducing p53 stability (Priolo *et al*., 2006). Conversely, *USP2* expression is downregulated in breast carcinomas, suggesting that this DUB may have pro- or antitumor properties that are exerted in a time- and tissue-specific manner (Metzig *et al*., 2011). In addition, *USP7* overexpression in prostate cancer has also been associated with tumor aggressiveness (Song *et al*., 2008). Interestingly, *USP4*, originally characterized as an oncogene by its ability to transform NIH3T3 cells (Gupta *et al*., 1994), maps at 3p21.3, a locus frequently rearranged in human tumor cells. This gene is overexpressed in several types of human cancer (Gray *et al*., 1995; Zhang *et al*., 2011b), and downregulated in small cell lung cancer cell lines (Frederick *et al*., 1998). Moreover, high levels of USP17 have been identified in primary lung, colon, esophagus and cervix tumor biopsies (McFarlane *et al*., 2010). Furthermore, *USP15* is downregulated in paclitaxel-resistant ovarian cancer (Xu *et al*., 2009), and *CYLD* in melanoma and other malignant tumors (Hellerbrand *et al*., 2007; Jenner *et al*., 2007; Massoumi *et al*., 2009; Gilbert *et al*., 2011). Finally, *A20* expression is also downregulated in some types of lymphoma (Durkop *et al*., 2003).

Targeting DUBs in cancer

The therapeutic efficacy of the proteasome inhibitor Bortezomib for treatment of multiple myeloma and mantle cell lymphoma points to the ubiquitin–proteasome system as a promising target for the development

of new anticancer strategies (Navon and Ciechanover, 2009; Crawford *et al.*, 2011). As DUBs are key effectors of the ubiquitin–proteasome system, and having in mind their multiple and diverse biological roles, these proteases are emerging as attractive druggable targets in cancer. However, to our knowledge, no DUB inhibitors have successfully entered clinical trials yet, which in part may be due to the fact that most DUBs are cysteine proteases, a group that is difficult to target when compared with proteolytic enzymes belonging to other catalytic classes (Turk, 2006). Historically, cysteine proteases have been less amenable for inhibition by small-molecule drugs than metalloproteases because of the lack of specificity, poor bioavailability and metabolic instability of these drugs. Moreover, irreversible inhibitors capable of alkylating the active site cysteine are in general reactive to other nucleophiles, especially other thiols (Daviet and Colland, 2008). Accordingly, several members of the JAMM metalloprotease family of DUBs, such as POH1, CSN5, AMSH and BRCC36, have been proposed as alternative candidates for drug targeting in cancer (Bedford *et al.*, 2011).

Despite these difficulties, recent studies have evaluated different possibilities for targeting DUBs in cancer (Daviet and Colland, 2008; Colland, 2010). The proposed therapies are based on blocking either DUB activity through specific inhibitors or DUB gene transcription by targeting extracellular factors, signal-transduction pathways or nuclear factors that activate expression of these genes. To date, several studies with oncogenic DUBs have led to the first attempts of therapeutic applications involving active-site inhibitors. The first DUB inhibitors with these characteristics were cyclopentenone prostaglandins, which induce cellular accumulation of polyubiquitylated proteins and cause p53-dependent apoptosis in colon cancer cells (Mullally and Fitzpatrick, 2002). Moreover, potent irreversible inhibitors of DUBs like UbaI (ubiquitin aldehyde) or UbVS (ubiquitin vinyl sulfone) have been characterized and used as a research tool for resolving three-dimensional structures of DUBs and for detecting their enzymatic activities. However, the high molecular mass and relative lack of specificity of these inhibitors have precluded their use as therapeutic agents (Love *et al.*, 2007). Nevertheless, there are small-molecule inhibitors against UCHs and USPs that could be useful as anticancer drugs (Supplementary Table 2). Thus, UCHL1 was the first DUB reported to be neutralized by small-molecule inhibitors, which belong to two different classes: isatin *O*-acyl oximes and 3-amino-2-oxo-7*H*-thieno[2,3-*b*]pyridin-6-one derivatives (Liu *et al.*, 2003; Colland, 2010). The treatment of lung carcinoma cell lines with these inhibitors promotes cell proliferation, which confirms the antiproliferative role of UCHL1 in these cells (Liu *et al.*, 2003). In the case of UCHL3, small-molecule inhibitors have been identified by virtual screening using crystal structure data for this enzyme and a virtual compound library (Hirayama *et al.*, 2007). There are three competitive inhibitors against UCHL3 with similar dihydro-pyrrole skeletons and several isatin derivatives identified in the previous high-throughput screening of UCHL1 inhibitors (Liu *et al.*, 2003). Regarding USPs, 2-cyano-pyrimidine and triazine derivatives have been identified as inhibitors of USP2 (Guedat and Colland, 2007), whereas a small-molecule compound that inhibits USP7 (HBX 41,108) stabilizes and activates p53 in a nongenotoxic manner, inhibiting cancer cell growth (Colland *et al.*, 2009). There is another USP7 selective inhibitor (P022077), which has been recently characterized using a fluorescence-based multiplex assay (Tian *et al.*, 2011). Moreover, another high-throughput screening has highlighted 9-oxo-9 H-indeno[1,2-b]pyrazine-2,3-dicarbonitrile and analogs as potent and selective inhibitors of USP8 with antiproliferative and pro-apoptotic activities in various cancer cell lines (Colombo *et al.*, 2010). In addition, HBX 90,397 and HBX 90,659 are two small-molecule inhibitors of USP8, which also inhibit cancer cell growth (Guedat and Colland, 2007; Daviet and Colland, 2008). In the case of USP14, a small-molecule inhibitor called IU1 that enhances the degradation of several proteasomal substrates has recently been identified (Lee *et al.*, 2010). Finally, recent studies have demonstrated that WP1130, a partly selective DUB inhibitor originally identified in a screening for JAK2 kinase inhibitors, downregulates antiapoptotic and upregulates proapoptotic proteins by blocking the DUB activity of USP5, USP9X, USP14 and UCHL5 (Kapuria *et al.*, 2010; Sun *et al.*, 2011).

Apart from using active-site small-molecule inhibitors, there are other ways of blocking the tumor-promoting activity of oncogenic DUBs, such as those based in interfering with their mechanisms of regulation. Thus, a challenge to effectively target DUBs in cancer should be to identify the regulatory mechanisms that increase DUB synthesis and activity, a difficult task if we consider that DUBs are part of complex regulatory cascades and signal-transduction pathways. Further understanding of these regulatory mechanisms could clarify possible functions of DUBs as critical players in tumor development and maintenance, even without undergoing activating mutations. This could lead to the identification of DUBs as *bona fide* components of 'non-oncogene addiction' events, which are related to the exacerbated dependence of tumors on the normal function of certain genes which are not mutated oncogenes (Solimini *et al.*, 2007; Freije *et al.*, 2011). Consistent with this possibility, USP2 inhibition has been described as an effective approach to induce growth suppression in cancer cells with addiction to cyclin D1 expression (Shan *et al.*, 2009). Moreover, the disruption of USP9X and MCL1 interaction through gemcitabine sensitizes cells to ABT-737 treatment inducing caspase-dependent apoptosis (Zhang *et al.*, 2011a). The finding of non-oncogene addiction to DUBs in cancer would provide new potential therapeutic targets within this group of enzymes, the inhibition of which could reverse the oncogenic phenotype without affecting the viability of normal cells at a significant degree.

In the case of tumor-protective DUBs, like CYLD, A20 or BAP1, effective cancer therapeutics should be

aimed at increasing those DUB activities that are lost during tumor progression. Recombinant forms of protective DUBs might have potential as tumor-suppressor agents, although the high doses that would be necessary to achieve therapeutic effects and the pharmacokinetic problems linked to their administration may represent serious limitations. Furthermore, it could also be possible to upregulate the expression of protective DUBs through the use of agents with the ability to reverse those modifications that result in the decreased expression of these enzymes. For example, the finding of promoter hypermethylation as the basis for loss of expression of most tumor-suppressor genes in cancer provides a good opportunity to use DNA demethylating drugs that can reactivate their expression. This could be the case of *UCHL1*, in which silencing by promoter methylation has been observed in some cancer cell lines and primary tumors (Yu *et al*., 2008). Moreover, *A20* is also inactivated by promoter methylation in several lymphomas (Honma *et al*., 2009). Another interesting approach in this regard is the use of exogenous compounds with the ability to compensate the loss of function of tumor-suppressor DUBs inactivated in cancer. Thus, the use of anti-inflammatory drugs restoring the negative regulation of the NF-κB pathway, which is lost through *CYLD* mutations, has been proposed as an effective therapeutic intervention in cylindromatosis patients (Brummelkamp *et al*., 2003). Furthermore, inhibition of the dysregulated tropomyosin kinase TRK has also been proposed as a strategy to treat tumors with loss of CYLD (Rajan *et al*., 2011). In a different approach, it has been recently suggested that downregulation of Snail (a *CYLD* repressor) through inhibition of its activator GLI1 could reverse the dramatically reduced expression of *CYLD* in basal cell carcinomas (Kuphal *et al*., 2011). Finally, exploiting concepts such as synthetic lethality could facilitate the identification of novel therapeutic targets in the DUB family. Synthetic lethality describes the relationship between two genes, whereby the loss of either gene is compatible with cell viability, but loss or inhibition of both genes results in cell death. Accordingly, targeting a DUB gene that is synthetic lethal to a cancer-relevant mutation in an oncogene or in a tumor-suppressor gene should lead to cancer cell death without affecting the viability of normal cells (Hartwell *et al*., 1997; Kaelin, 2005; Rehman *et al*., 2010; Chan and Giaccia, 2011).

Although the increasing number of strategies for DUB inhibition or modulation has led to a growing interest on these enzymes as targets for the development of new therapies, targeting of DUBs is at relatively early stages. As already shown for other proteases, efficient DUB inhibition needs a more comprehensive understanding of all DUB substrates and regulatory functions. Individual DUBs can influence distinct pathways through different substrates. It has been already shown that some DUBs can have dual roles in cancer, detrimental or protective, depending on the functional context in which these specific DUBs act. Therefore, care should be taken not to affect protective DUB functions upon therapeutic inhibition, to avoid undesired side effects. Clear understanding of this complex enzymatic system by using large-scale genetic and proteomic approaches, and carefully designed *in vitro* and *in vivo* model systems preceding clinical trials will be necessary for developing efficient DUB-based anticancer therapies.

Conclusions and perspectives

The availability of the complete genome sequences of different organisms has recently allowed the identification of their entire complement of DUBs and the establishment of novel insights into their evolutionary diversification. The group portrait of human DUBs has revealed that, beyond a shared core domain, they exhibit a wide structural and functional complexity to participate in multiple biological processes including cancer. In this review, we have curated the census of human DUBs and annotated a total of 98 members (Figure 1 and Supplementary Table 1), including the 7 members of a new DUB family, uncovered after the recent finding of a domain with deubiquitylase activity in MCPIP1 protein (Liang *et al*., 2010). Nevertheless, this number may not be definitive and could be increased in the near future with the discovery of new DUBs with structural features distinct from those defining the currently known families. Consistent with this large and still growing complexity of DUBs, the evidence discussed above brings a broad spectrum of DUB functions to light, including those occurring at several stages of cancer development and progression. In fact, there is a growing list of human malignancies in which several DUBs are mutated and behave as oncogenes or tumor suppressors. In addition, many DUBs show profound changes in their expression levels in different malignant tumors. On this basis, together with the recent success of clinically targeting the ubiquitin proteasome system in cancer, DUBs emerge as appealing targets in the development of new specific therapies against human malignancies. So far, no DUB-targeted strategies have reached clinical trials and many challenges remain before translating this information into clinical benefits for cancer patients. Thus, further understanding of the catalytic activity of DUBs, as well as their regulation and substrate specificity will be required for the development of DUB inhibitors useful as anticancer drugs. Furthermore, the assignment of oncogenic or tumor-suppressive roles to certain DUBs is still dependent on the cellular context and further analysis will be required for functional and clinical validation of DUBs as drug targets. Finally, the generation of gain- or loss-of-function animal models for selected family members will likely contribute to clarify the relative relevance of individual DUBs and their alterations in the tumorigenic progress. Hopefully, the knowledge derived from all these studies will provide new insights into the multiple questions still open in relation to DUBs and may lead to the introduction of DUB-targeting

strategies as an essential component of molecular therapies against cancer.

Conflict of interest

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the Oncogene website (http://www.nature.com/onc)