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USP17-mediated de-ubiquitination and cancer: clients cluster around the cell cycle

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Abstract

Eukaryotic cells perform a range of complex processes, some essential for life, others specific to cell type, all of which are governed by post-translational modifications of proteins. Among the repertoire of dynamic protein modifications, ubiquitination is arguably the most arcane and profound due to its complexity. Ubiquitin conjugation consists of three main steps, the last of which involves a multitude of target-specific

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ubiquitin ligases that conjugate a range of ubiquitination patterns to protein substrates with diverse outcomes. In contrast, ubiquitin removal is catalysed by a relatively small number of de-ubiquitinating enzymes (DUBs), which can also display target specificity and impact decisively on cell function. Here we review the current knowledge of the intriguing ubiquitin-specific protease 17 (USP17) family of DUBs, which are expressed from a highly copy number variable gene that has been implicated in multiple cancers, although available evidence points to conflicting roles in cell proliferation and survival. We show that key USP17 substrates populate two pathways that drive cell cycle progression and that USP17 activity serves to promote one pathway but inhibit the other. We propose that this arrangement enables USP17 to stimulate or inhibit proliferation depending on the mitogenic pathway that predominates in any given cell and may partially explain evidence pointing to both oncogenic and tumour suppressor properties of USP17.

Keywords:
USP17
Ubiquitin
Proliferation
Mitogen induction
Signalling pathways
Cancer

1.1. Introduction

Ubiquitin is a protein with numerous regulatory functions in eukaryotes. Its covalent linkage as a post-translational modification to other proteins, known as ubiquitination (or ubiquitylation), promotes a multitude of downstream signalling effects. Ubiquitin is enzymatically conjugated to target proteins *via* lysine residues with conjugation to serine, threonine, cysteine or the amino-terminal methionine also known (McClellan et al., 2019). Ubiquitin-ubiquitin linkages can also be formed intermolecularly between the carboxyl-terminal glycine and any of the seven internal lysines within ubiquitin (K6, K11, K27, K29, K33, K48 and K63) or to the amino-terminus to form polyubiquitin chains (Vijaykumar et al., 1987) (Figure 1). K48- and K63- linked polyubiquitin chains are the most studied and well-

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characterised linkage types; their conjugation to substrates is associated with proteasomal degradation and cell signalling pathways, respectively (Haglund & Dikic, 2005). Understanding the information imparted by an ubiquitination event and how it is read (and hence unravelling the 'ubiquitin code'), is highly complex due to the variety and dynamic nature of ubiquitin modifications. The network giving rise to the ubiquitin code consists of *writers* (ubiquitination enzymes- E1, E2 and E3 ligases), *readers* (proteins capable of recognising ubiquitin marks) and the focus of this review, *erasers* (de-ubiquitinating enzymes) (Komander & Rape, 2012).

De-ubiquitinating enzymes (DUBs) remove ubiquitin from target proteins. There are approximately 100 DUBs in the human genome, organised into eight families. These families are ubiquitin-specific proteases (USPs), ubiquitin carboxyl-terminal hydrolases (UCHs), ovarian tumour proteases (OTUs), Josephin domain-containing proteins (MJDs), JAB1/MPN/MOV34 metalloenzymes (JAMMs), the motif interacting with ubiquitin-containing novel DUB family (MINDY), monocyte chemotactic protein-induced protein (MCPIPs), and the Zn-finger and UFSP domain protein (ZUFSP) (Komander et al., 2009; Abdul Rehman et al., 2016; Kolattukudy & Niu, 2012; Haahr et al., 2018; Hermanns et al., 2018; Hewings et al., 2018). DUB activity is crucial for nearly all cellular functions: the importance of control over ubiquitination events is such that they often act as oncoproteins and tumour suppressors in cancer development (Hussain et al., 2009). Individual DUBs have specific functions and substrates, proceeding either through direct interaction with the target proteins harbouring ubiquitin modifications or through interaction with polyubiquitin chains, which can also determine whether chain cleavage occurs from the proximal or distal end (Mevissen & Komander, 2017). Ubiquitin specific proteases (USPs) are the largest family of DUBs, comprising over half of the known DUBs in humans. They are all cysteine proteases and are defined by a USP domain with a conserved catalytic triad (Komander et al., 2009). The USP domain adopts a conserved structure consisting of three domains – an array of β-sheets known as the fingers, a β-sheet core termed the palm and an α-helical thumb domain, which together create a binding pocket for ubiquitin between the palm and thumb regions and at the tip of the fingers, first shown in USP7 (HAUSP) (Hu et al., 2002). This review is focussed on the USP family member USP17, with a particular emphasis on its involvement in cell cycle control and proliferation and its importance in various cancers.

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1.2. Overview of USP17

USP17, also referred to as DUB3, describes a number of very similar proteins expressed from a multigene family, consisting of multiple gene copies in blocks of tandem repeats that are highly copy-number variable (Alkan et al., 2009; Burrows et al., 2010). *USP17* genes were discovered in mice and named *Dub1*, *Dub1a, Dub2* and *Dub2A*; their expression was found to be induced in response to cytokines such as interleukin 2 (IL-2) and IL-3 (Y. Zhu et al., 1996; Y. Zhu et al., 1997; Baek et al., 2001; Baek et al., 2004). These genes, and other members identified as part of the same DUB sub-family, are localised to the central region of chromosome 7 in mice, presumably as a result of tandem duplication events (Y. Zhu et al., 1997; Burrows et al., 2010). A tandemly repeated mega-satellite sequence (named RS447) on human chromosome 4 was found to harbour an open reading frame (ORF) with high homology to both murine *Dub1* and *Dub2* (Gondo et al., 1998). This was later found to be an intron-less gene encoding an active de-ubiquitinating enzyme that was named *USP17* (Saitoh et al., 2000).

The discovery of multiple genomic sequences on human chromosomes 4 and 8 with homology to the murine *Dub* genes led to the cloning of a de-ubiquitinating enzyme with similar cytokine inducibility, that was named *DUB3* (Burrows et al., 2004). *USP17/DUB3* genes reside within RS447, which has a copy number varying from 20-103, mostly on chromosome 4 (4p16.1) with some also present on chromosome 8 (8p23.1) (Okada et al., 2002). Multiple other *USP17*-like genes were later identified spread across chromosomes 4 and 8 as part of this highly polymorphic mega-satellite repeat (Burrows et al., 2005). Some RS447 repeats on chromosome 8 are also embedded in the beta-defensin gene cluster, an additional copy-number variable region within the human genome which is likely to further influence the variation in *USP17*-like gene copy number (Burrows et al., 2010). *USP17* genes are absent from amphibian and avian genomes, indicating late acquisition in vertebrate evolution (Burrows et al., 2010).

The ancestral sequence of *DUB3/USP17* has gone through independent ancestral gene duplication events in all species it has been studied in (including human, mouse, rat, dog, cow and chimpanzee), probably due to it originating in an inherently unstable genomic region prior to speciation in mammals, although some duplication events in humans and

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chimpanzees appear to have occurred before their divergence (Burrows et al., 2010). Next-generation sequencing of three human genomes gave a *USP17/DUB3* copy number of between 122-186, highlighting the genetic variability and complexity of these genes (Alkan et al., 2009). Due to this genetic complexity and their inherent similarity, it is currently not clear which of the *USP17*-like genes identified in the NCBI database are expressed in a given cellular context. Whether different USP17 sequences have different substrate specificities, and the level of redundancy between them is also unclear, warranting further study.

1.3. Domain structure and localisation of USP17-like proteins

Due to their high sequence similarity and for convenience, USP17-like proteins will be referred to collectively as USP17. There are obvious caveats to this, as the canonical reference sequence (USP17L2) can vary in primary amino acid sequence from other USP17-like proteins annotated in the NCBI database by as much as ~8%, although without a more detailed understanding of individual USP17-like expression patterns and specificities, this review aims to provide a framework of the general substrates and functions of USP17-like proteins. USP17 possesses the characteristic USP domain that defines this family of DUBs with the catalytic triad of cysteine (C89), histidine (H334) and aspartate (D350) separated into a Cys box (C89) and His box (H334 and D350) respectively (Hjortland & Mesecar, 2016) (Figure 2). However, USP17 lacks domains present in some other USPs, such as ubiquitin-like (UBL) and ubiquitin-associated (UBA) domains (Komander et al., 2009). Human USP17 possesses two carboxyl-terminal hyaluronan (and RNA) binding motifs (HABMs), which are not present in murine Dub3 family members (Shin et al., 2006) (Figure 3). Hyaluronan is a non-sulphated glycosaminoglycan that is a component of the extracellular matrix, but can also be intracellular, where it associates with microtubules (Evanko & Wight, 1999). Additionally, the USP17 carboxyl-terminus contains two tumour necrosis factor receptor-associated factor 2 (TRAF2) binding motifs (amino acids 428-431 and 480-484) (Lu et al., 2018).

Several examples illustrate that interaction regions vary among USP17 substrates: while TRAF2, SETD8 and SIN3A histone deacetylase corepressor complex component (SDS3) interact with the carboxyl-terminus of USP17 downstream of the USP domain, ELK-1, bromodomain-containing protein 4 (BRD4) and myeloid cell leukaemia 1 (MCL-1) bind to

the amino terminal region (which includes the catalytic USP domain) (Ramakrishna et al., 2011; Lu et al., 2018; Fukuura et al., 2019; Ducker et al., 2019; Jin et al., 2018; Xiaowei Wu et al., 2019). In the above examples, USP17 displays direct interaction with target proteins, which can occur in the absence of ubiquitin modification. However, the molecular basis of how USP17 recognises its specific substrates is not well understood and requires further characterisation.

Evidence of USP17 localisation within the cell is varied and could in part reflect the use of different sequences for ectopic expression or differential endogenous expression depending on the cell line/tissue. USP17 ectopically expressed in HeLa cells localised predominantly within the nucleus, particularly abundant within the nucleoli, similarly to hyaluronan (Evanko & Wight, 1999; Shin et al., 2006). In U2OS and NIH-3T3 cells, ectopically expressed USP17 was largely and exclusively localised to the nucleus respectively, and subcellular fractionation of HEK293T cells identified some USP17 associated with chromatin (Delgado-Diaz et al., 2014; Van der Laan et al., 2013). Other studies found that USP17 co-localised with substrates in the cytoplasm of HEK293T cells and TOV-21G ovarian cancer cells (X. Chen et al., 2019; Xiaowei Wu et al., 2019). Endogenous USP17 was reported to be distributed throughout HeLa cells, to co-localise with client proteins at the endoplasmic reticulum (ER) and to be particularly abundant in the nucleus (Burrows et al., 2009; Ramakrishna et al., 2011). The Human Protein Atlas records USP17L2 localisation as cytoplasmic and membranous in several tissues (Uhlén et al., 2015; https://www.proteinatlas.org/ENSG00000223443-USP17L2).

1.4. Polyubiquitin chain specificity of USP17

USP17 has been shown to hydrolyse polyubiquitin chains, with no established specificity over chain topology *in vivo*. This is in line with other USPs, which generally lack particular linkage preferences (Mevissen & Komander, 2017). Most commonly, USP17 is associated with the disassembly of well-studied and abundant K48-linked polyubiquitin conjugates, thereby protecting its client proteins from proteasomal degradation (Xiaowei Wu et al., 2019)(Q. Zhang et al., 2019). However, it has also been shown to target non-proteolytic K63-linked polyubiquitin chains, in addition to mono-ubiquitinated substrates (Burrows et al., 2009; Ramakrishna et al., 2011; Delgado-Diaz et al., 2014; Ducker et al.,

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2019). The list of USP17 clients continues to grow (Table 1). All clients highlighted in the table have been demonstrated to be de-ubiquitinated by USP17, with catalytic activity shown to be important for ascribed impacts on cell signalling. Despite this, it is possible that USP17 could also have ubiquitin-independent functions.

In vitro de-ubiquitination assays demonstrated that USP17 exhibits high catalytic efficiency ($k_{cat}/K_m = 1500$ [x10^3] $M^{-1}sec^{-1}$) compared to other well-studied DUBs such as USP7, and is monomeric in solution. The same study found that USP17 can effectively hydrolyse various di-ubiquitin (K11, K33, K48 and K63) and tetra-ubiquitin chains (K11, K48 and K63), while only partially processing other di-ubiquitin substrates (K6, K27 and K29), and having no effect on linear di-ubiquitin (Hjortland & Mesecar, 2016). It is worth noting that overexpression of this highly active DUB has been shown to deplete global cellular polyubiquitin levels and therefore any substrates identified as a USP17 client through overexpression studies should always be confirmed with reciprocal knock-down experiments (R. Chen et al., 2010).

1.5. Control over USP17 expression and activity

Expression of a subset of *USP17* gene repeats is under the control of mitogens. Cytokines and chemokines (IL-4, IL-6, IL-8, stromal cell-derived factor 1 [SDF-1]) as well as mitogens such as epidermal growth factor (EGF) and the tumour promoter 12-O-tetradecanoylphorbol-13-acetate are all able to stimulate USP17 expression (Burrows et al., 2004; Yiwei Lin et al., 2017; de la Vega et al., 2011; Jaworski et al., 2014; Ducker et al., 2019). Net transcription of *USP17* genes is regulated in a cell cycle-dependent manner, with higher levels of expression during late G1, and also on entry into G2/M (McFarlane et al., 2010). *USP17* transcripts were found to be particularly abundant in heart, liver and pancreatic tissue (Shin et al., 2006). However, low tissue specificity of USP17L2 is reported in the Human Protein Atlas (Uhlén et al., 2015).

The steroid hormone receptor oestrogen-related receptor β (Esrrb) is implicated in regulating murine Dub3 expression, which is important in self-renewal of mouse embryonic stem cells (mESCs). Essrb directly targets an identified *Dub3* gene promoter through three consensus binding sites to promote its expression in these cells (van der Laan et al., 2013). This is enhanced by nuclear coactivator 1 (Ncoa1) as its cell-cycle dependent expression

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potentiates Essrb-mediated *Dub3* transcription in mESCs (Van Der Laan et al., 2014). Regulation of expression is also mediated through the nuclear receptor corepressor 2 (NCOR2)- histone deacetylase 10 (HDAC10) complex, which transcriptionally suppresses *USP17* gene expression in prostate cancer cells (Jin et al., 2018). O-6-methylguanine-DNA methyltransferase (MGMT) is an activator of *USP17* transcription in ovarian cancer cell lines (Xiaowei Wu et al., 2019). Analysis of an illustrative *USP17* gene promoter also identified binding sites for hypoxia-inducible factor 1 (HIF-1), signal transducer and activator of transcription 3 (STAT3), STAT6 and nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB), suggesting these could also regulate *USP17* expression (Lu et al., 2018). Alongside this, it was discovered that USP17 activity is partially regulated through phosphorylation by Cyclin-dependent kinase 4/6 (CDK4/6). Phosphorylation at S41 appears to be important for USP17 catalytic activity, as a S41A substitution impaired substrate de-ubiquitination (T. Liu et al., 2017).

2.1. Roles of USP17 in the regulation of cell cycle progression, proliferation and apoptosis

Modulation of USP17 expression levels, either by ectopic expression or shRNA mediated down-regulation, led to cell cycle arrest and induction of apoptosis in a variety of cellular contexts (Burrows et al., 2004; Shin et al., 2006; Pereg et al., 2010; McFarlane et al., 2010; Ducker et al., 2019; B. Hu et al., 2019; Baohai et al., 2019). Reports of unsuccessful attempts to express USP17 stably in some cell lines are also in agreement with evidence that constitutive USP17 expression can halt proliferation and induce apoptosis (Mehić et al., 2017). In line with this, USP17 has an array of substrates through which it regulates the cell cycle at multiple points, displaying extensive control over cell cycle progression.

Impact on growth factor signalling: Mitogen signalling through RAS is classically mediated through the well characterised extracellular signal-regulated kinase (ERK)/ mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K)/protein kinase B (PKB) pathways. USP17 controls RAS activation through the removal of K63-linked polyubiquitin chains from RAS-converting enzyme 1 (RCE1), independent of the ubiquitin proteasome system (UPS) (Burrows et al., 2009). Prior to plasma membrane translocation,

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RAS must be modified at the ER by addition of a farnesyl lipid moiety to its carboxyl-terminus CAAX motif (C= modified cysteine) in a process termed prenylation (Casey et al., 1989). RCE1 then removes the -AAX carboxy-terminal to the prenylated cysteine by proteolysis to allow methylation of the carboxyl group of the modified cysteine and trafficking of RAS to the membrane (Boyartchuk, 1997; Otto et al., 1999; Choy et al., 1999). When overexpressed, USP17 abrogated RCE1 catalytic activity through de-ubiquitination, thus preventing RAS localisation to the plasma membrane and inhibiting ERK signalling (Burrows et al., 2009). This affected both H-RAS and N-RAS, but not K-RAS, possibly because despite also being processed by RCE1, some K-RAS is nonetheless membrane-localised even in RCE-null cells (De La Vega et al., 2010; Brunet et al., 2004). Depletion of USP17 also impaired RAS translocation to the plasma membrane and reduced MEK and ERK phosphorylation, showing that USP17 is essential for RAS localisation and downstream signalling (McFarlane et al., 2010).

The cysteine protease asparaginyl endopeptidase (AEP) is polyubiquitinated by K63-linked chains through TRAF6 E3 ligase activity, leading to AEP/TRAF6/heat shock protein 90 (HSP90α) complex formation and intracellular AEP stabilisation (Yingying Lin et al., 2014). USP17 de-ubiquitinates AEP, which downregulates AEP protein levels (Yingying Lin et al., 2014; X. Chen et al., 2019). Evidence has pointed to AEP involvement in the ERK cascade through interaction with the scaffold protein RAS GTPase-activating-like protein (IQGAP1) (Roy et al., 2005; Cui et al., 2017). AEP knockout in gastric cancer cell lines led to increased levels of IQGAP1 and decreased ERK phosphorylation, possibly because in the absence of AEP, IQGAP1 cannot be degraded, unbalancing the stoichiometric ratios with its clients and hence disrupting ERK signalling (Cui et al., 2017). Therefore, through downregulation of AEP protein levels, USP17 negatively regulates ERK phosphorylation, and accordingly USP17 knockdown stimulated proliferation in breast cancer cell line MCF-7 (X. Chen et al., 2019). However, it should be noted that a separate study yielded the opposite result in the same cell line whereby USP17 depletion retarded cell proliferation (Fukuura et al., 2019). AEP de-ubiquitination represents another point of control for USP17 over the ERK cascade alongside RCE1, which both involve removal of regulatory K63-polyubiquitin chains.

Impact on regulators of cell cycle progression: USP17 depletion interfered with G1-S phase transition in HeLa cells (McFarlane et al., 2010). The phosphatase cell division cycle

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25A (CDC25A) is a crucial mitotic regulator that acts to de-phosphorylate CDKs and drive forward the cell cycle at both G1/S and G2/M transitions (Shen & Huang, 2012). CDC25A abundance is tightly controlled through the UPS (Mailand et al., 2000). USP17 can de-ubiquitinate and stabilise CDC25A to maintain steady-state levels of CDC25A in concert with the UPS (Pereg et al., 2010). USP17 depletion in U2OS cells retarded cell cycle progression at both G1/S and G2/M, the latter illustrated by an increase in inhibitory phosphorylation on CDK1 and a reduction in associated CDK1/Cyclin B1 activity (Pereg et al., 2010). Conversely, USP17 overexpression in U2OS cells led to mitotic defects and triggered apoptosis (Pereg et al., 2010). However, another study found that ectopic expression of USP17 increased cell proliferation in this cell line, although it is unclear which USP17 sequences were used in each study (C. Song et al., 2017). The activation of CDK2 in G1/S and CDK1 in G2/M through interaction with Cyclin A are further examples of crucial events in the cell cycle (Pagano et al., 1992). Cyclin A levels are also under the control of the UPS (Geley et al., 2001). Through de-ubiquitination by USP17, Cyclin A is protected from UPS processing, promoting G1/S transition. USP17-depleted A549 cells were less able to proceed to S-phase and showed reduced proliferation, both of which could be partially rescued by Cyclin A expression (B. Hu et al., 2019).

The DNA damage-induced G2/M arrest of the cell cycle is achieved by blocking Cyclin B/CDK1 activity, through activating checkpoint kinase 1 (CHK1) and CHK2, which phosphorylate, inactivate and target CDC25 phosphatases for degradation, and through stabilisation and accumulation of p53, which upregulates target genes such as *CDKN1A* (encoding Cyclin dependent kinase inhibitor 1A or p21) to aid in Cyclin A/CDK1 and Cyclin B/ CDK1 inhibition (Harper & Elledge, 2007). The transcription factor deleted in oesophageal cancer 1 (DEC1) is stabilised through de-ubiquitination by USP17 in response to genotoxic stress, allowing it to interact with and stabilise p53, which then promotes the G2 DNA damage checkpoint (Thin et al., 2007)(Kim et al., 2014). By preventing DEC1 turnover, ectopic USP17 expression can prolong the G2 checkpoint following DNA damage (Kim et al., 2014).

In addition to G2, CDC25 phosphatases are key targets of the DNA-damage response (DDR) checkpoint machinery at G1 and intra-S checkpoints, where CDC25A degradation is promoted to halt cell cycle progression (Mailand et al., 2000; Donzelli & Draetta, 2003). In mESCs, it has been demonstrated that Dub3 is expressed at high levels, resulting in a relaxed

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G1/S checkpoint due to high Cdc25a levels, with a large proportion of cells in S-phase (Van der Laan et al., 2013). Thus, through de-ubiquitination and stabilisation of CDC25A, USP17 regulates the DNA damage checkpoint at multiple points. High Dub3 levels in mESCs also help to maintain stemness, and during neural conversion *Dub3* transcription is downregulated alongside *Essrb*, leading to destabilisation of Cdc25a (Van der Laan et al., 2013). In accordance with this, knockdown of either Dub3 or Cdc25a induces spontaneous differentiation in these cells, linking pluripotency to the cell cycle through Dub3 activity (Van der Laan et al., 2013). To our knowledge it remains to be confirmed whether USP17 has similar functions in human ESCs.

Impact on proliferation and apoptosis-associated transcription factors: In response to mitogens, ERK phosphorylates E-twenty six (ETS) transcription factor ELK-1 to promote cell proliferation (Gille et al., 1992)(Gualdrini et al., 2016). Our own work has shown that mitogen induction of USP17 expression results in the removal of mono-ubiquitin from the DNA-binding ETS domain of ELK-1 and its subsequent de-repression (Ducker et al., 2019). ELK-1 de-ubiquitination occurs alongside phosphorylation by active ERK, potentiating ELK-1 transactivation and transcription of immediate early gene (IEG) targets (such as *CFOS*) and downstream *CCND1* (encoding Cyclin D1), promoting cell-cycle entry. Knockdown of USP17 in HEK293T cells retarded cell proliferation, which could be partially rescued by expression of a hypo-ubiquitinated ELK-1 lysine mutant, displaying increased transcriptional activity compared to wild-type ELK-1 due to the unavailability of a repressive mono-ubiquitin conjugation (Ducker et al., 2019). This suggests USP17 is an indirect regulator of Cyclin D1 expression, which in turn can form a complex with CDK4/6 and phosphorylate USP17 to positively regulate its activity (T. Liu et al., 2017). In line with this, USP17 knockdown in oral squamous cell carcinoma (OSCC) and prostate cancer cells reduced Cyclin D1 levels (F. Luo et al., 2020; Baohai et al., 2019). Moreover, given that USP17 also regulates RAS localisation through de-ubiquitination of RCE1, this gives another point through which USP17 exerts control over the RAS-RAF-MEK-ERK-ELK-1 axis to promote IEG expression and drive forward G1 (Burrows et al., 2009; McFarlane et al., 2010).

The transcriptional activators yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ) are downstream effectors of the Hippo pathway and act to promote proliferation in conjunction with transcriptional enhanced associate

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domain (TEAD) transcription factors, with targets such as *BIRC5* (encoding cellular inhibitor of apoptosis protein 1 [cIAP1]) (Zhao et al., 2008). YAP and TAZ activities are suppressed through phosphorylation by Hippo pathway large tumour suppressor kinases (LATS1/2), which promotes their cytoplasmic translocation and proteasomal degradation of YAP (Zhao et al., 2010; C. Y. Liu et al., 2010). This occurs through a kinase cascade, whereby upstream mammalian sterile 20-like kinases (MST1/2) are activated through phosphorylation (at T183/ T180 in MST1/MST2) and homodimerisation, and proceed to phosphorylate LATS1/2, activating them and potentiating their activity towards YAP/TAZ, leading to anti-proliferative and pro-apoptotic outcomes (Praskova et al., 2004). Angiomotin (AMOT) proteins bind to YAP/TAZ to suppress their transcriptional functions, in addition to scaffolding interactions between YAP and itchy E3 ubiquitin protein ligase (ITCH) to facilitate YAP turnover (Chan et al., 2011; Adler et al., 2013). LATS kinases and AMOT are themselves regulated by the UPS following ubiquitination by ITCH (Salah et al., 2011; C. Wang et al., 2012). A shRNA based screen of 94 DUBs performed in HEK293T cells revealed that USP17 could modulate YAP/TAZ activity (H. T. Nguyen et al., 2016). Further examination confirmed that USP17 was able to stabilise LATS1/2, AMOT and ITCH through de-ubiquitination, thereby promoting ITCH-mediated ubiquitination and degradation of YAP and downregulation of pro-proliferative YAP/TAZ activity (H. T. Nguyen et al., 2017). USP17 therefore regulates Hippo signalling, antagonising the proteasomal processing of multiple pathway components to promote the proteasomal degradation of YAP.

The anti-proliferative role of USP17 in Hippo signalling contrasts with its positive role in ERK signalling and may be explained by the cross-talk between these integrated pathways. The scaffolding protein RAS-association domain family 1 isoform A (RASSF1A) interacts with MST1/2 and prevents their de-phosphorylation by protein phosphatase 2 (PP2A), promoting downstream apoptotic induction (Guo et al., 2011). Mutant K-RAS can interact with RASSF1A to promote MST2 activation (Matallanas et al., 2011). RAF-1 can also interact with MST2, which suppresses the activity of both Hippo and ERK pathways, preventing RASSF1A-MST2 and RAS-RAF-1 interactions respectively (Matallanas et al., 2007; Romano et al., 2014). This is enhanced by phosphorylation of RAF-1 at S259, which can be mediated by LATS1 kinase activity through negative feedback on ERK and Hippo signalling (Dhillon et al., 2002; Romano et al., 2014). MST2 is also subject to inhibitory phosphorylation by PKB (at T117 and T384) in response to mitogens, promoting RAF-1

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interaction and antagonising homodimerisation and RASSF1A binding (Romano et al., 2010). AEP has been shown to be important for PKB activation in prostate cancer cells, implicating another USP17 substrate in Hippo pathway regulation, additional to its aforementioned role in the ERK cascade (W. Zhu et al., 2016). Whether cell fate in response to signalling is apoptotic or proliferative seems to be governed by a fine-tuned balance of cellular switches and cross-talk between these pathways (L. K. Nguyen et al., 2015). USP17 regulates the stability of multiple Hippo effectors, as well as controlling AEP levels, RAS localisation and downstream activity of the ERK responder ELK-1. This positions USP17 as an arbiter of proliferative versus apoptotic cell fate and illustrates the extensive integration of the ERK and Hippo pathways (Figures 4 and 5).

Impact on chromatin, chromatin modifiers and transcriptional machinery: USP17 has been shown to remove mono-ubiquitin from histone H2AX, which has important implications in the DDR (Delgado-Diaz et al., 2014). Mono-ubiquitination of histone H2AX (following phosphorylation) by ubiquitin ligase ring finger protein 8 (RNF8) and amplification of this signal by the E2-E3 pair ubiquitin-conjugating enzyme E2 13 (UBC13)-RNF168 precedes recruitment of p53-binding protein 1 (53BP1) and breast cancer gene (BRCA) at sites of DNA damage, which facilitates repair by non-homologous end joining or homologous recombination (Huen et al., 2007; Doil et al., 2009). Through de-ubiquitination of H2AX, USP17 influences DDR by counteracting RNF8 and RNF168 activity and inhibiting RNF168 recruitment to DNA lesions (Delgado-Diaz et al., 2014). Through competing E3 ligase and DUB activity, a balance of H2AX ubiquitination levels is maintained to enable a correct DDR, with de-ubiquitination allowing recovery following lesion repair (Delgado-Diaz et al., 2014). This illustrates a role of USP17 in maintaining genomic integrity.

The histone methyltransferase SETD8 acts as an epigenetic writer through mono-methylation of histone H4K20 (Nishioka et al., 2002). SETD8 methylation of the *CDKN1A* gene represses its transcription and inhibits induction of cellular senescence (Shih et al., 2017). SETD8 levels are controlled by the UPS, and various E3 ligases have been shown to target it for ubiquitination (S. Wu et al., 2010; Centore et al., 2010; Yin et al., 2008). However, USP17 removes polyubiquitin chains from SETD8 to antagonise its proteasomal degradation. Accordingly, USP17 knockdown in MCF7 cells led to a reduction in H4K20 methylation and increased p21 levels, causing G1 arrest and induction of apoptosis (Fukuura

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et al., 2019). Depletion of USP17 in HeLa cells also gave rise to p21 stabilisation (McFarlane et al., 2010). USP17 therefore opposes cellular senescence through stabilisation of SETD8 and downstream downregulation of p21, promoting CDK activity and cell proliferation.

Through deacetylation of the amino-terminal extension of core histones, HDACs negatively regulate transcription (Gallinari et al., 2007). USP17 de-ubiquitinates SDS3, a critical subunit of the SIN3A-HDAC 1/2 co-repressor complex, binding to SDS3 through its carboxyl-terminal region. Through K63-linked polyubiquitin chain removal, USP17 negatively regulates SDS3-associated HDAC activity, leading to disrupted proliferation and apoptotic induction in HeLa cells overexpressing USP17, which could be partially rescued with concomitant SDS3 depletion (Ramakrishna et al., 2011). USP17 is further involved in regulation of HDAC activity, by protecting HDAC2 from UPS processing through removal of K48-linked polyubiquitin chains (H. Song et al., 2015). USP17 HABMs have been implicated in the induction of apoptosis through interaction with intracellular hyaluronan. Overexpression of full-length USP17 induced apoptosis in HeLa and endometrial cancer HEC-1A cells, whereas a USP17 variant lacking the HABM-containing carboxyl-terminus did not (Shin et al., 2006). SDS3 also contains HABMs, and co-expression of full-length SDS3 and USP17 promoted apoptosis in HeLa cells, whereas HABM-deleted versions of SDS3 and USP17 did not, implying that hyaluronan mediates the USP17 and SDS3 interaction and their influence on apoptosis (Ramakrishna et al., 2012).

During initiation of RNA polymerase II (RNAPII) transcription, positive transcription elongation factor b (P-TEFb or CDK9/Cyclin T) is recruited to phosphorylate serine 2 within the carboxyl-terminal domain (CTD) repeats of RNAPII and promote elongation. BRD4, a member of the bromodomain and extra-terminal domain (BET) protein family, can mediate P-TEFb recruitment through its epigenetic "reading" of acetylated chromatin (Moon et al., 2005). BRD4 abundance is controlled by the UPS following its polyubiquitination by the E3 ligase speckle-type POZ protein (SPOP) (Dai et al., 2017). USP17 de-ubiquitinates and stabilises BRD4, which binds to the *NCOR2* gene promoter and positively regulates its expression in prostate cancer cell lines. NCOR2 forms a complex with HDAC10 and represses transcription of *USP17* genes (Jin et al., 2018). In this way, USP17 acts indirectly to control its own expression, representing another example of USP17 regulation of HDAC activity. By protecting BRD4 from degradation, USP17 also indirectly regulates the histone methyltransferase Enhancer of zeste homolog-2 (EZH2) in OSCC cells (F. Luo et al., 2020).

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EZH2 is a component of the polycomb repressor complex 2 (PRC2) that is essential for cell proliferation, and its expression is upregulated through transcriptional activity of the oncogene MYC in a BRD4-dependent manner (Bracken et al., 2003; Xinchao Wu et al., 2016). Attenuated proliferation and increased apoptosis in the OSCC cell line HSC-2 following USP17 depletion could be rescued by ectopic EZH2 expression (F. Luo et al., 2020).

Impact on pro-survival proteins: Pro-apoptotic functions of USP17 have been established, notably in regard to its HABMs and through co-operation with its client SDS3. However, depletion of USP17 in ovarian cancer cell line OVCA433 triggered apoptosis (Xiaowei Wu et al., 2019). This was due to K48-linked polyubiquitin chain removal from the anti-apoptotic protein MCL1 by USP17, protecting it from proteasomal degradation (Xiaowei Wu et al., 2019). MCL1 is a member of the B-cell lymphoma-leukaemia 2 (BCL-2) family that promotes cell viability while not impacting proliferation, and has a short half-life (T. Yang et al., 1996). Elevated levels of MGMT activates *USP17* transcription, which stabilises MCL1 and promotes cell survival (Xiaowei Wu et al., 2019). As with the contradictory roles of USP17 in promoting or dampening proliferative signalling, anti- and pro-apoptotic substrates of USP17 likely display differential outputs depending on pathways targeted. Due to the paucity of evidence regarding how USP17 mediates client-specific interactions at the molecular level, further investigation is required to greater understand how this is achieved, and how this drives downstream signalling.

2.2. Roles of USP17 in cell migration

In addition to its impact on cell fate, USP17 has been shown to have important functions in cell migration, including the regulation of several motility-associated RAS homologue (RHO) GTPases. RHOA, CDC42 and RAS-related C3 botulinum toxin substrate (RAC) are all mis-localised following USP17 knockdown, which inhibits both amoeboid and mesenchymal cellular migration through impaired cytoskeletal rearrangements affecting actin and tubulin polymers (de la Vega et al., 2011). Following chemokine stimulation, USP17 expression is induced and all three GTPases are transported from the cytoplasm to the plasma membrane to mediate their activation and downstream signalling, which is blunted in USP17-depleted cells (de la Vega et al., 2011). As with RAS, it is likely that RCE1 is

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involved in this process, as RHOA has also been shown to be processed similarly (Fueller et al., 2006).

Epithelial-mesenchymal transition (EMT) is a reversible differentiation essential for various developmental and wound healing processes, whereby epithelial cells lose their polarity and cell-cell adhesion to become migratory mesenchymal cells (Kalluri & Weinberg, 2009). Central to this process is the zinc-finger transcription factor SNAIL1, which represses transcription of *CDH1*, the gene encoding E-cadherin, as well as other targets (Batlle et al., 2000). E-cadherin is a calcium-dependent adhesion protein, located at points of cell-cell contact on the surfaces of epithelial cells known as adherens junctions (AJs), and E-cadherin loss promotes the destabilisation of AJs and cell phenotype changes (Thiery, 2002). SNAIL1 is an unstable protein that is readily degraded by the UPS, and multiple E3 ligases have been implicated in its polyubiquitination (B. P. Zhou et al., 2004; Zheng et al., 2014; Viñas-Castells et al., 2010). USP17 has been shown to de-ubiquitinate and stabilise SNAIL1, and in line with this, overexpression of USP17 in luminal breast cancer cell lines MCF7 and T47D promoted downregulation of epithelial markers (e.g. E-cadherin), upregulation of mesenchymal markers (e.g. N-cadherin) and induced morphological changes consistent with EMT (Y. Wu et al., 2017; T. Liu et al., 2017). USP17 depletion in MDA-MB-231 breast cancer cells inhibited their migration while having no impact on proliferation, an effect that could be rescued with SNAIL1 overexpression (T. Liu et al., 2017).

Phosphorylation of USP17 by CDK4/6 appears to be important for stabilisation of SNAIL1, as USP17-depleted MDA-MB-231 cells exhibited lower SNAIL1 protein levels and migratory potential, both of which could be rescued with concomitant expression of wild-type USP17 and phospho-mimetic S41D mutant, but not phosphorylation null S41A mutant (T. Liu et al., 2017). Interestingly, SNAIL1-mediated down-regulation of E-cadherin involves recruitment of other *bona fide* USP17 substrates SDS3 and HDAC2 to deacetylate the *CDH1* promoter, implying that USP17 has multiple control points along this axis (Peinado et al., 2004). In a similar manner, USP17 can protect another member of the SNAIL family, SLUG (SNAIL2), and the transcription factor twist-related factor 1 (TWIST) from proteasomal degradation, both of which are also labile proteins important in EMT (Yiwei Lin et al., 2017). TWIST upregulates protease activated receptor 1 (PAR1), an inhibitor of LATS1/2 kinases, suppressing the Hippo signalling pathway (Y. Wang et al., 2016). The USP17 client AEP can promote cell migration following secretion through extracellular matrix degradation and

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remodelling, and is also thought to be involved in regulating TWIST expression, as its depletion significantly reduced *TWIST* mRNA levels in gastric cancer cells (Cui et al., 2016).

Mothers against decapentaplegic homolog 4 (SMAD4) associates with the receptor-mediated SMAD2/3 (R-SMADs) to form an active transcriptional complex following transforming growth factor β (TGF-β) signalling. The TGF-β/SMAD signalling pathway plays important roles in a multitude of cellular processes, and has also been shown to be involved in EMT (H. Yang et al., 2015; Zeng et al., 2016). The SMAD complex can recruit the catalytic IκB kinase (IKK) subunit IKKα to both the *SNAI1* and *SNAI2* genes promoters (encoding SNAIL1 and SLUG respectively) and activate their transcription (Brandl et al., 2010). Another study found that SMAD3 and SMAD4 could form a transcriptional repression complex with SNAIL1 and promote EMT (Vincent et al., 2009). USP17 was shown to protect SMAD4 from UPS processing through de-ubiquitination (C. Song et al., 2017). USP17 depletion in osteosarcoma cell line MG-63 caused upregulation and downregulation of epithelial (e.g. E-cadherin) and mesenchymal (e.g. N-cadherin) markers respectively, while SMAD4 co-expression could reverse these trends (C. Song et al., 2017). Excess hyaluronan production from hyaluronan synthase 2 (HAS2) can also positively regulate EMT through upregulation of TGF-β and induction of *SNAI1* and *TWIST* (Chanmee et al., 2014). HAS2 represents another stabilisation target of USP17 through de-ubiquitination, adding another EMT-linked client to its repertoire (Mehić et al., 2017). USP17 therefore regulates EMT at multiple points through the de-ubiquitination of various clients, promoting migratory cellular outputs.

2.3. USP17 as an oncogene

USP17 is upregulated in a range of cancers at mRNA and protein levels (McFarlane et al., 2010). This could be due to the abundance of cytokines in tumour tissue, which stimulate USP17 expression. For example, in lung cancer tissue, USP17 expression increased alongside higher abundances of inflammatory mediators such as IL-1β, IL-6 and IL-8, which are secreted by macrophages in the tumour microenvironment (Lu et al., 2018). Further amplification of *USP17* genes (located on chromosome 8) has also been reported in plasma and tumour tissue from breast cancer patients, which was not observed in healthy plasma

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controls (Shaw et al., 2012). Elevated USP17 levels can have various impacts on cancer development and progression.

Cancer cell proliferation and tumorigenesis: There is inherent complexity in the relationship between USP17 expression and cancer, illustrated by a variety of effects of USP17 overexpression or depletion on cell proliferation depending on cellular context (Table 2). Depletion of USP17 has been shown to retard tumour growth in mouse xenograft models using NSCLC, prostate cancer, ovarian cancer and OSCC-derived cell lines (S. Zhang et al., 2016; Jin et al., 2018; Baohai et al., 2019; Xiaowei Wu et al., 2019; F. Luo et al., 2020). Upregulated USP17 expression can promote cell proliferation by stabilising mitotic regulators such as CDC25A and Cyclin A, and inhibit apoptosis induction by stabilising MCL1 (Pereg et al., 2010; B. Hu et al., 2019; Xiaowei Wu et al., 2019). Overexpression of CDC25A is common in human cancers, and USP17 expression correlates positively with that of CDC25A in primary human breast tumours (Pereg et al., 2010). This is also the case in epithelial ovarian cancers, where high expression of USP17 was also associated with shortened mean patient survival time (B. Zhou et al., 2015). Expression of USP17 and Cyclin A were strongly correlated in clinical NSCLC tissue, and both were found to be more abundant in cancerous versus regular adjacent lung tissue (B. Hu et al., 2019).

However, constitutive USP17 expression halts cell cycle progression, which can stall mitosis, but also causes aberrant DNA replication and DDR, compromising genome integrity (Pereg et al., 2010). This places a selection pressure on cells, which overcome this through oncogene induction and transformation to malignant clones, no longer tethered to cell cycle checkpoint control. As an example of this, USP17 expression was sufficient to transform NIH-3T3 cells to promote anchorage-independent proliferation, which was enhanced in combination with co-expression of active H-RAS (Pereg et al., 2010). Ectopic expression of USP17 in NSCLC cell lines also increased their tumorigenesis, while depletion significantly reduced this (Lu et al., 2018; S. Zhang et al., 2016; McCann et al., 2018).

Chemoresistance: Notably, USP17 targets nuclear factor (erythroid-derived 2)-like 2 (NRF2), MCL1 and BRD4 play roles in promoting treatment resistance in tumours. The homeostatic regulator and transcription factor NRF2 is protected from proteasomal degradation by USP17 de-ubiquitination, potentiating its transcriptional activity and driving

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chemoresistance in colon cancer cells. Knockout of NRF2 or knockdown of USP17 could sensitize HCT116 cells to treatment with the chemotherapy drug paclitaxel, which could be reversed by NRF2 expression (Q. Zhang et al., 2019). Consistent with this, colorectal cancer tissues showed a strong correlation between NRF2 and USP17 levels, and both were more abundant in a range of colorectal cancer cell lines compared to the normal colon cell line FHC and HEK293T cells (Q. Zhang et al., 2019).

HDAC inhibitors such as Entinostat, Mocetinostat and Abexinostat drive cancer cell apoptosis induction, decreasing MCL-1 levels (Torres-Adorno et al., 2017). However, in ovarian cancer cells, HDAC inhibitors can stimulate MGMT expression, which upregulates USP17 expression, and in turn stabilises MCL1, inhibiting the apoptotic cascade (Xiaowei Wu et al., 2019). MGMT, USP17 and MCL1 levels were positively correlated in ovarian cancer samples, and all displayed higher expression in chemoresistant tumours when compared to those more responsive to treatment (Xiaowei Wu et al., 2019).

BRD4 activity in carcinomas can be targeted by the BET (and MYC) inhibitor JQ1, although JQ1 can also upregulate USP17 expression (Borbely et al., 2015). In prostate cancer cells, increased USP17 levels following BET inhibitor treatment promotes the stabilisation of BRD4, causing treatment resistance (Jin et al., 2018). In castration resistant prostate cancer, genomic deletion of the negative regulator of *USP17* transcription NCOR2 can occur (Robinson et al., 2015). This removes BRD4 control over *USP17* transcription through NCOR2 upregulation. Prostate cancer samples were found to express low levels of NCOR2, which inversely correlated with USP17 expression, and USP17 expression correlated positively with BRD4 in these tissues (Jin et al., 2018). There was also a positive correlation between USP17 expression and expression of both BRD4 and EZH2 in OSCC tissues (F. Luo et al., 2020).

Cancer invasiveness and metastasis: Through its involvement in cell migration pathways, more and more evidence is emerging that USP17 has key roles in cancer metastasis. Matrix metalloproteinases (MMPs) are important for matrix remodelling and promoting cancer invasion (Shay et al., 2015). A panel of prostate cancer cell lines were found to express USP17 to high levels, and its depletion resulted in a reduction of *MMP2*, *MMP9* and *MMP14* mRNA levels along with decreased cellular migration and invasion (Baohai et al., 2019). Similar results were found in in NSCLC cell lines, where USP17 knockdown caused decreases in MMP3 and MMP9 expression while also displaying reduced

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invasiveness (S. Zhang et al., 2016). USP17 levels significantly correlated with NSCLC adenocarcinoma disease recurrence at distant sites, implying a role in promoting metastasis (McFarlane et al., 2013).

Through SNAIL1 de-ubiquitination and EMT regulation, USP17 can influence breast cancer migration. MDA-MB-231 cells injected into mouse tail veins showed reduced metastatic nodule formation in the lung when USP17 was knocked down, which could be rescued by USP17 expression (T. Liu et al., 2017). In line with this, USP17 and SNAIL1 protein levels both correlated positively with metastatic breast carcinoma, and additionally USP17 levels correlated with those of SNAIL1 in triple-negative breast cancer (T. Liu et al., 2017; Y. Wu et al., 2017). Moreover, higher USP17 expression significantly increased the risk of distant metastasis in patients (Y. Wu et al., 2017). Other EMT-related substrates of USP17 can also promote the invasiveness of breast cancer. IL-6 treatment of SUM159 cells induced USP17 expression and increased SLUG and TWIST protein levels with an associated increase in cell invasiveness, which could be reversed by USP17 depletion (in addition to destabilisation of SLUG and TWIST) (Yiwei Lin et al., 2017). Accordingly, protein levels of USP17 were also correlated with TWIST in primary breast tumours (Yiwei Lin et al., 2017).

2.4. USP17 as a tumour suppressor

Despite an abundance of evidence of its oncogenic potential, there are some reports suggesting that USP17 has tumour suppressor characteristics. Ectopic USP17 expression significantly attenuated anchorage-independent clonal formation in HeLa cells (Ramakrishna et al., 2011). Deletion of USP17 HABMs diminished this effect, suggesting that HABMs are important for USP17 anti-tumorigenic properties (Ramakrishna et al., 2012). Low USP17 expression has also been linked to increased glioma tumorigenesis, where overexpression of USP17 led to reduced protein levels of RAS and MYC and attenuated cell proliferation, whereas low USP17 expression was more likely to present in more advanced tumours (Hu et al., 2016). How USP17 modulates RAS and MYC protein levels in glioma cells is unclear.

There is also some evidence suggesting USP17 has tumour suppressor properties in breast cancer. Analysis of *USP17* mRNA found that levels were significantly lower in cancerous versus normal breast tissue, and through the downregulation of AEP levels and inhibition of ERK signalling, ectopic USP17 expression reduced tumorigenesis and

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proliferation in MDA-MB-231 cells (X. Chen et al., 2019). Combination of HDAC inhibitor and BET inhibitor (JQ1) treatment inhibited proliferation and promoted apoptosis in various breast cancer cell lines through the induction of USP17, which ablated RAS signalling (Borbely et al., 2015). By de-ubiquitinating and stabilising Hippo pathway regulators, there is also the potential for USP17 to act as a tumour suppressor through downstream inhibition of YAP/TAZ transcriptional activity (H. T. Nguyen et al., 2017). The tumour suppressor gene effects of USP17 likely reflects its roles in proliferation and apoptosis, which can be positively or negatively regulated. For example, ERK cascade-driven proliferation of cells can be promoted through USP17 de-ubiquitination of substrates such as ELK-1, whereas by controlling the stability of Hippo substrates such as ITCH, USP17 can mediate apoptosis, with the outcome dependent on which pathway predominates.

2.5. USP17 as a therapeutic target

In recent years, the targeting of DUBs through small molecule inhibitors has gained traction as an anticancer therapeutic strategy (Yuan et al., 2018). These range from broad spectrum pan-DUB inhibitors to more selective and specific DUB inhibitors (Colland et al., 2009; Tian et al., 2011; Schauer et al., 2020). Numerous publications have shown that USP17 depletion inhibits cell proliferation in many different cellular contexts (McFarlane et al., 2010; Pereg et al., 2010; C. Song et al., 2017; Ducker et al., 2019; B. Hu et al., 2019). NRF2-dependent chemoresistance in colon cancer cell lines HCT116 and RKO could be re-sensitised to paclitaxel and camptothecin treatment following USP17 depletion (Q. Zhang et al., 2019). The abundance of data identifying USP17 as an oncogene in a variety of carcinomas suggest it as a promising target for development of therapeutics. However, to our knowledge, to date no small molecule screens looking for USP17-specific inhibitors have been reported. The partly specific DUB inhibitor WP1130 (Degrasyn) is able to inhibit activity of several DUBs including USP5, USP9x, USP14 and USP24 (Kapuria et al., 2010; H. Luo et al., 2019). It has also been found to directly bind to the USP domain of USP17, with predictive modelling suggesting the inhibitor docks at the ubiquitin cleavage site (Y. Wu et al., 2017). WP1130 treatment reduced MDA-MB-157 and MDA-MB-231 breast cancer cell migration and invasion to a similar degree to USP17 depletion, and treatment of mice harbouring

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MDA-MB-231-derived cancers with WP1130 significantly attenuated tumour growth (Y. Wu et al., 2017).

Aside from targeting USP17 through use of DUB-binding inhibitors, disruption of protein-protein interactions and signalling pathways important for DUB activity have also been explored. The regulation of USP17 activity through phosphorylation by CDK4/6 is a possible signalling target. The specific CDK4/6 inhibitor PD0332991 (Palbociclib) inhibited breast cancer metastasis in MDA-MB-231-derived mouse tumour models, and blocked USP17 de-ubiquitination and stabilisation of SNAIL1 protein (T. Liu et al., 2017). In another study, the use of PD0332991 was also shown to rescue sensitivity of DU145 cell-derived prostate tumours in mice to JQ1 (Jin et al., 2018). This was largely through the destabilisation of USP17 target BRD4, as the combinatorial effect of JQ1 and PD0332991 was nullified by concomitant USP17 depletion (Jin et al., 2018).

A screen of several small molecules identified the DNA methyltransferase inhibitor PaTrin-2 as able to effectively suppress USP17 expression through MGMT inhibition, which in combination with HDAC inhibitors (Entinostat, Mocetinostat or Abexinostat) destabilised MCL1 and promoted apoptosis in ovarian cancer cell lines (Xiaowei Wu et al., 2019). Combination of Mocetinostat (or other HDAC inhibitors) and JQ1 can synergistically stimulate USP17 expression, likely through MGMT expression (elevated by HDAC inhibitors), HDAC10 inhibition (inactivated by HDAC inhibitors) and/or BRD4 inhibition (inactivated by BET inhibitors) (Borbely et al., 2015; Xiaowei Wu et al., 2019; Jin et al., 2018). Therefore, to overcome chemoresistance of cancer cell lines to BET and HDAC inhibitors, PD0332991 or PaTrin-2 were used in the above examples to downregulate (and hence rebalance) USP17 activity/expression (Xiaowei Wu et al., 2019; Jin et al., 2018). Collectively, these examples illustrate the potential for use of combination therapy to reduce treatment resistance in cancers exhibiting aberrant USP17 expression or activity.

3.1. Concluding remarks

USP17 comprises a family of highly similar proteins, derived from a variable copy number gene that has undergone multiple duplications. Expression of *USP17* genes are tightly controlled in a cell-cycle dependent manner, reflected by the multitude of USP17 substrates that have key functions in cell-cycle control, proliferation and apoptosis. Through DUB

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activity, USP17 can hydrolyse a range of ubiquitin linkage topologies, often (but not exclusively) protecting its substrates from proteasomal degradation. USP17 exerts control over a variety of cellular processes, with clients from a multitude of inter-linked signalling pathways. Particularly, we have outlined that through its contradictory roles in ERK and Hippo signalling, USP17 can promote cell proliferation or apoptosis. Dysregulation of USP17 expression is frequently reported in cancer, due largely to having targets involved in cell-cycle progression, tumorigenesis and metastasis. As such, USP17 displays oncogenic potential in many cellular contexts, and presents itself as a potential therapeutic target. However, in certain examples it can also act as a tumour suppressor, highlighting the importance of understanding the specific molecular mechanisms at play in a given tumour microenvironment. Moving forward, further elucidation of how USP17 achieves specificity through substrate interaction is required, in addition to how expression patterns of USP17-like proteins vary in different tissues. This will in turn allow greater insight into how USP17 modulates its target proteins and the signalling pathways they impact upon.

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The authors report no declarations of interest.

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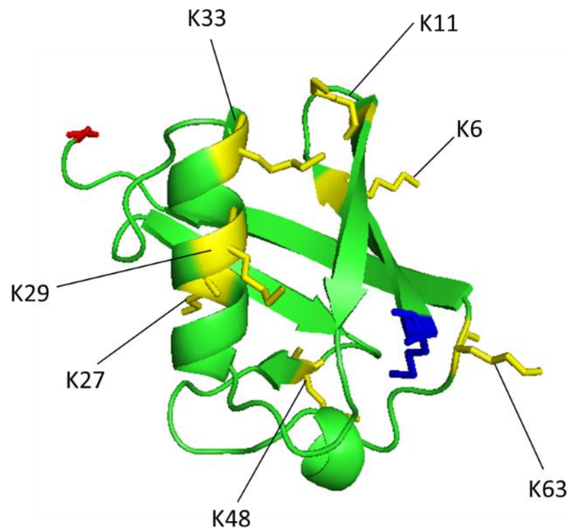

Figure 1: Structure of ubiquitin (human), with all lysine residues and termini labelled. Lysine = yellow, amino-terminus (M) = blue, carboxyl-terminus (G) = red. Structure taken from (Vijaykumar et al., 1987) - PDB 1UBQ – re-rendered with PyMOL.

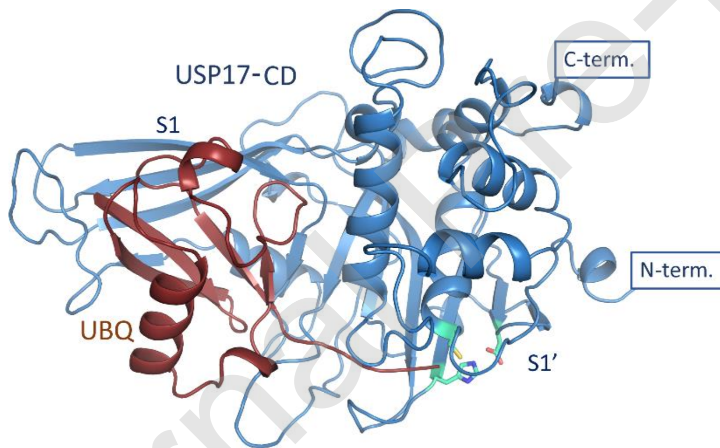

Figure 2: Homology model of the USP17 USP catalytic domain (CD = blue- residues 72-390), with ubiquitin (UBQ = red) placed into the S1' binding pocket. Catalytic triad residues (C89, H334 and D350) are highlighted in green.

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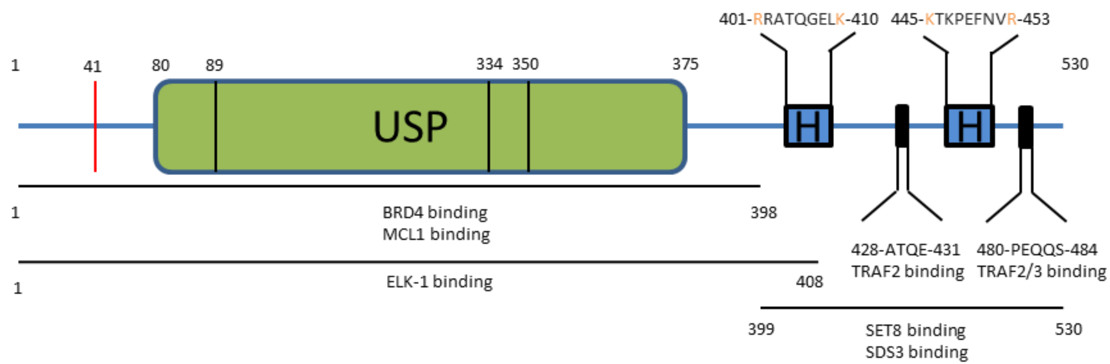

Figure 3: Schematic of USP17 domain structure (USP17L2 - human). USP domain contains catalytic triad C89, H334 and D350, and amino-terminal phosphorylation site S41 is labelled with red bar. Hyaluronan binding sites are highlighted (H), with arginine and lysine residues key to interaction in orange. TRAF2/3 binding motifs and identified binding regions for various USP17 substrates are also highlighted.

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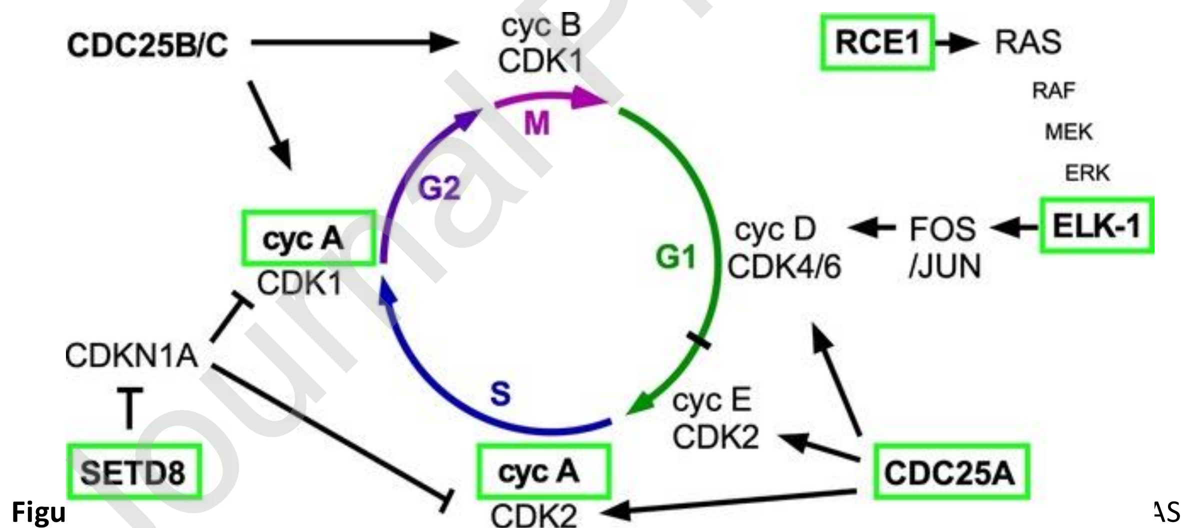

Figu AS activation through the removal of K63-linked polyubiquitin chains from RCE1; 2) de-repression of ELK-1 transcription through removal of mono-ubiquitin to drive IEG expression; 3) stabilisation of CDC25A, which proceeds to de-phosphorylate CDKs and drive forward G1; 4) stabilisation of Cyclin A, which interacts with CDK1/2 to drive forward the cell cycle; 5) SETD8 stabilisation to repress *CDKN1A* (p21) transcription.

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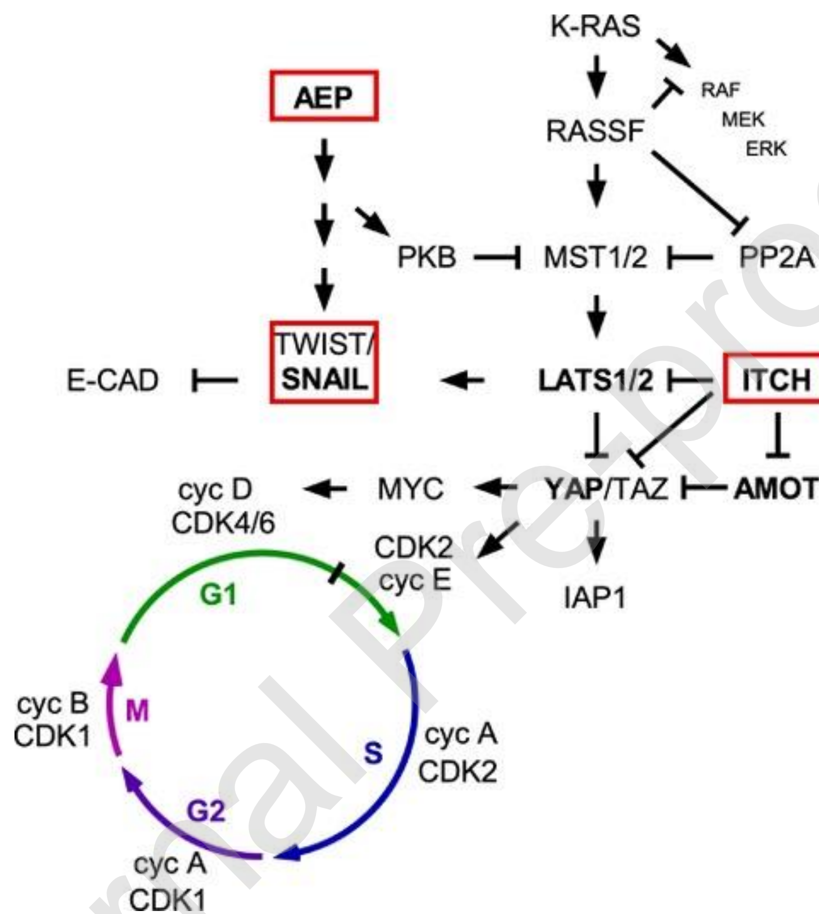

Figure 5: Anti-proliferative substrates of USP17 (in red boxes) include 1) stabilisation of ITCH to promote ubiquitination and degradation of YAP, AMOT, LATS and inhibit signals that promote G1 progression; 2) stabilisation of SNAIL/ TWIST to inhibit *CHD1* (E-Cadherin) expression and promote EMT; 3) down-regulation of AEP (removal of K63-linked ubiquitin chains) leading to lower PKB (and ERK) activity and increased Hippo pathway activity, along with loss of TWIST expression and elevated E-Cadherin levels.

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Target	Involvement	Ubiquitin type/ linkage	Ref
RCE1	CC+P	Polyub/ K63	(Burrows et al., 2009)
SDS3	CC+P, A	Polyub/ K63	(Ramakrishna et al., 2011)
CDC25A	CC+P/D, S	Polyub	(Pereg et al., 2010; Van der Laan et al., 2013)
ELK-1	CC+P	Monoub	(Ducker et al., 2019)
SETD8	CC+P	Polyub	(Fukuura et al., 2019)
Cyclin A	CC+P	Polyub	(B. Hu et al., 2019)
BRD4	CC+P, C	Polyub	(Jin et al., 2018; F. Luo et al., 2020)
ITCH	CC+P, A	Polyub, K48	(H. T. Nguyen et al., 2017)
AMOT	CC+P, A	Polyub, K48 + K63	(H. T. Nguyen et al., 2017)
LATS1/2	CC+P, A	Polyub	(H. T. Nguyen et al., 2017)
AEP	CC+P, CM	Polyub/ K63	(Yingying Lin et al., 2014; X. Chen et al., 2019)
SMAD4	CC+P, CM	Polyub	(C. Song et al., 2017)
HAS2	CM	Polyub/ K48 + K63	(Mehić et al., 2017)
SNAIL1	CM	Polyub/ K48	(T. Liu et al., 2017; Y. Wu et al., 2017)
SLUG	CM	Polyub	(Yiwei Lin et al., 2017)
TWIST	CM	Polyub	(Yiwei Lin et al., 2017)
TRAF2/TRAF3	II+I, S	Polyub/ K63	(Lu et al., 2018)
HDAC2	II+I	Polyub/ K48 + K63	(H. Song et al., 2015)
IL-33	II+I	Polyub/ K48 + K63	(Ni et al., 2015)

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RIG-1	II+I	Polyub/ K48 + K63	(R. Chen et al., 2010)
MDA-5	II+I	Polyub	(R. Chen et al., 2010)
H2AX	CC+D	Monoub	(Delgado-Diaz et al., 2014)
DEC1	CC+D	Polyub	(Kim et al., 2014)
MCL1	A, C	Polyub, K48	(Xiaowei Wu et al., 2019)
NRF2	C	Polyub/ K48	(Q. Zhang et al., 2019)

Table 1: List of *bona fide* USP17 substrates, the cellular processes they are implicated in regulating in the publications identifying them as USP17 clients and the identified ubiquitin modifications/linkages hydrolysed by USP17.

Key - CC+P = Cell cycle and proliferation, CC+D = Cell cycle and DNA damage, S = Stemness, CM = Cell migration, A = Apoptosis, C = Chemoresistance, II+I = Innate immunity and inflammation

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Effect of altering USP17/DUB3 expression on cell proliferation			
Cell line	**USP17 OE**	**USP17 K/D**	**Ref**
HeLa	Inhibit	Inhibit	(McFarlane et al., 2010; Ramakrishna et al., 2011; Ducker et al., 2019)
HEK293T	-	Inhibit	(Ducker et al., 2019)
Ba/F3	Inhibit	-	(Burrows et al., 2004)
U2OS	Increase	Inhibit	(C. Song et al., 2017; Pereg et al., 2010)
MG-63	Increase	Inhibit	(C. Song et al., 2017)
MDA-MB-231	Inhibit	Not significant	(X. Chen et al., 2019; T. Liu et al., 2017)
MCF-7	-	Inhibit/Increase	(Fukuura et al., 2019; X. Chen et al., 2019)
U87-MG	Inhibit	-	(M. Hu et al., 2016)
U251-MG	Inhibit	-	(M. Hu et al., 2016)
HA1800	-	Increase	(M. Hu et al., 2016)
A549	-	Inhibit	(McCann et al., 2018; B. Hu et al., 2019)
HCC827	-	Inhibit	(McCann et al., 2018)
H1975	-	Inhibit	(McCann et al., 2018)
DU145	-	Inhibit	(Jin et al., 2018; Baohai et al., 2019)
PC3	-	Inhibit	(Baohai et al., 2019)
BJ	Inhibit	-	(H. T. Nguyen et al., 2017)
H1299	Increase	-	(Lu et al., 2018)
D121	Increase	-	(Lu et al., 2018)

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H157	-	Inhibit	(F. Luo et al., 2020)
HSC-2	-	Inhibit	(F. Luo et al., 2020)
OVCAR3	-	Inhibit	(B. Zhou et al., 2015; Xiaowei Wu et al., 2019)
OVCA433	-	Inhibit	(Xiaowei Wu et al., 2019)

Table 2: Effects of USP17 overexpression (OE) and knockdown (K/D) on cell proliferation, where proliferation was assessed by a standard assay (e.g. MTT, CCK-8, trypan blue).

References

Abdul Rehman, S. A., Kristariyanto, Y. A., Choi, S. Y., Nkosi, P. J., Weidlich, S., Labib, K., Hofmann, K., & Kulathu, Y. (2016). MINDY-1 Is a Member of an Evolutionarily Conserved and Structurally Distinct New Family of Deubiquitinating Enzymes. *Molecular Cell*, *63*(1), 146–155.

Adler, J. J., Heller, B. L., Bringman, L. R., Ranahan, W. P., Cocklin, R. R., Goebl, M. G., Oh, M., Lim, H. S., Ingham, R. J., & Wells, C. D. (2013). Amot130 adapts atrophin-1 interacting protein 4 to inhibit yes-associated protein signaling and cell growth. *Journal of Biological Chemistry*, *288*(21), 15181–15193.

Alkan, C., Kidd, J. M., Marques-Bonet, T., Aksay, G., Antonacci, F., Hormozdiari, F., Kitzman, J. O., Baker, C., Malig, M., Mutlu, O., Sahinalp, S. C., Gibbs, R. A., & Eichler, E. E. (2009). Personalized copy number and segmental duplication maps using next-generation sequencing. *Nature Genetics*, *41*(10), 1061–1067.

Baek, K. H., Kim, M. S., Kim, Y. S., Shin, J. M., & Choi, H. K. (2004). DUB-1A, a Novel Deubiquitinating Enzyme Subfamily Member, Is Polyubiquitinated and Cytokine-inducible in B-lymphocytes. *Journal of Biological Chemistry*, *279*(4), 2368–2376.

Baek, K. H., Mondoux, M. A., Jaster, R., Fire-Levin, E., & D'Andrea, A. D. (2001). DUB-2A, a new member of the DUB subfamily of hematopoietic deubiquitinating enzymes. *Blood*, *98*(3), 636–642.

Baohai, X., Shi, F., & Yongqi, F. (2019). Inhibition of ubiquitin specific protease 17 restrains prostate cancer proliferation by regulation of epithelial-to-mesenchymal transition

Journal Pre-proof

Journal Pre-proof

(EMT) via ROS production. *Biomedicine and Pharmacotherapy*, *118,* 108546.

Batlle, E., Sancho, E., Francí, C., Domínguez, D., Monfar, M., Baulida, J., & De Herreros, A. G. (2000). The transcription factor Snail is a repressor of E-cadherin gene expression in epithelial tumour cells. *Nature Cell Biology*, *2*(2), 84–89.

Borbely, G., Haldosen, L., & Dahlman-wright, K. (2015). Induction of USP17 by combining BET and HDAC inhibitors in breast cancer cells. *Oncotarget*, *6*(32), 33623–33635.

Boyartchuk, V. L. (1997). Modulation of Ras and a-Factor Function by Carboxyl-Terminal Proteolysis. *Science*, *275*(5307), 1796–1800.

Bracken, A. P., Pasini, D., Capra, M., Prosperini, E., Colli, E., & Helin, K. (2003). EZH2 is downstream of the pRB-E2F pathway, essential for proliferation and amplified in cancer. *EMBO Journal*, *22*(20), 5323–5335.

Brandl, M., Seidler, B., Haller, F., Adamski, J., Schmid, R. M., Saur, D., & Schneider, G. (2010). IKKα controls canonical TGFβ-SMAD signaling to regulate genes expressing SNAIL and SLUG during EMT in Panc1 cells. *Journal of Cell Science*, *123*(24), 4231–4239.

Brunet, S., Sardon, T., Zimmerman, T., Wittmann, T., Pepperkok, R., Karsenti, E., & Vernos, I. (2004). Characterization of the TPX2 Domains Involved in Microtubule Nucleation and Spindle Assembly in *Xenopus* egg extracts. *Molecular Biology of the Cell*, *15*(12), 5318–5328.

Burrows, J. F., Kelvin, A. A., McFarlane, C., Burden, R. E., McGrattan, M. J., De La Vega, M., Govender, U., Quinn, D. J., Dib, K., Gadina, M., Scott, C. J., & Johnston, J. A. (2009). USP17 regulates Ras activation and cell proliferation by blocking RCE1 activity. *Journal of Biological Chemistry*, *284*(14), 9587–9595.

Burrows, J. F., McGrattan, M. J., & Johnston, J. A. (2005). The DUB/USP17 deubiquitinating enzymes, a multigene family within a tandemly repeated sequence. *Genomics*, *85*(4), 524–529.

Burrows, J. F., McGrattan, M. J., Rascle, A., Humbert, M., Baek, K. H., & Johnston, J. A. (2004). DUB-3, A Cytokine-inducible Deubiquitinating Enzyme That Blocks Proliferation. *Journal of Biological Chemistry*, *279*(14), 13993–14000.

Burrows, J. F., Scott, C. J., & Johnston, J. a. (2010). The DUB/USP17 deubiquitinating enzymes: a gene family within a tandemly repeated sequence, is also embedded within the copy number variable beta-defensin cluster. *BMC Genomics*, *11*, 250.

Casey, P. J., Solski, P. A., Der, C. J., & Buss, J. E. (1989). P21Ras Is Modified By a Farnesyl

Journal Pre-proof

Isoprenoid. *Proceedings of the National Academy of Sciences of the United States of America*, *86*(21), 8323–8327.

Centore, R. C., Havens, C. G., Manning, A. L., Li, J. M., Flynn, R. L., Tse, A., Jin, J., Dyson, N. J., Walter, J. C., & Zou, L. (2010). CRL4Cdt2-mediated destruction of the histone methyltransferase Set8 prevents premature chromatin compaction in S phase. *Molecular Cell*, *40*(1), 22–33.

Chan, S. W., Lim, C. J., Chong, Y. F., Pobbati, A. V., Huang, C., & Hong, W. (2011). Hippo pathway-independent restriction of TAZ and YAP by angiomotin. *Journal of Biological Chemistry*, *286*(9), 7018–7026.

Chanmee, T., Ontong, P., Mochizuki, N., Kongtawelert, P., Konno, K., & Itano, N. (2014). Excessive hyaluronan production promotes acquisition of cancer stem cell signatures through the coordinated regulation of twist and the transforming growth factor β (TGF-β)-snail signaling axis. *Journal of Biological Chemistry*, *289*(38), 26038–26056.

Chen, R., Zhang, L., Zhong, B., Tan, B., Liu, Y., & Shu, H. B. (2010). The ubiquitin-specific protease 17 is involved in virus-triggered type i IFN signaling. *Cell Research*, *20*(7), 802–811.

Chen, X., Wang, C., Liao, K., Zhou, S., Cao, L., Chen, J., Xu, C., & Lin, Y. (2019). USP17 suppresses tumorigenesis and tumor growth through deubiquitinating AEP. *International Journal of Biological Sciences*, *15*(4), 738–748.

Choy, E., Chiu, V. K., Silletti, J., Feoktistov, M., Morimoto, T., Michaelson, D., Ivanov, I. E., & Philips, M. R. (1999). Endomembrane trafficking of ras: The CAAX motif targets proteins to the ER and Golgi. *Cell*, *98*(1), 69–80.

Colland, F., Formstecher, E., Jacq, X., Reverdy, C., Planquette, C., Conrath, S., Trouplin, V., Bianchi, J., Aushev, V. N., Camonis, J., Calabrese, A., Borg-Capra, C., Sippl, W., Collura, V., Boissy, G., Rain, J. C., Guedat, P., Delansorne, R., & Daviet, L. (2009). Small-molecule inhibitor of USP7/HAUSP ubiquitin protease stabilizes and activates p53 in cells. *Molecular Cancer Therapeutics*, *8*(8), 2286–2295.

Cui, Y., Li, Q., Li, H., Wang, Y., Wang, H., Chen, W., Zhang, S., Cao, J., & Liu, T. (2017). Asparaginyl endopeptidase improves the resistance of microtubule-targeting drugs in gastric cancer through IQGAP1 modulating the EGFR/JNK/ERK signaling pathway. *OncoTargets and Therapy*, *10*, 627–643.

Cui, Y., Wang, Y., Li, H., Li, Q., Yu, Y., Xu, X., Xu, B., & Liu, T. (2016). Asparaginyl

Journal Pre-proof

endopeptidase promotes the invasion and metastasis of gastric cancer through modulating epithelial-to-mesenchymal transition. *Oncotarget*, *7*(23), 34356–34370.

Dai, X., Gan, W., Li, X., Wang, S., Zhang, W., Huang, L., Liu, S., Zhong, Q., Guo, J., Zhang, J., Chen, T., Shimizu, K., Beca, F., Blattner, M., Vasudevan, D., Buckley, D. L., Qi, J., Buser, L., Liu, P., … Wei, W. (2017). Prostate cancer-Associated SPOP mutations confer resistance to BET inhibitors through stabilization of BRD4. *Nature Medicine*, *23*(9), 1063–1071.

De La Vega, M., Burrows, J. F., McFarlane, C., Govender, U., Scott, C. J., & Johnston, J. A. (2010). The deubiquitinating enzyme USP17 blocks N-Ras membrane trafficking and activation but leaves K-Ras unaffected. *Journal of Biological Chemistry*, *285*(16), 12028–12036.

de la Vega, M., Kelvin, A. A., Dunican, D. J., McFarlane, C., Burrows, J. F., Jaworski, J., Stevenson, N. J., Dib, K., Rappoport, J. Z., Scott, C. J., Long, A., & Johnston, J. A. (2011). The deubiquitinating enzyme USP17 is essential for GTPase subcellular localization and cell motility. *Nature Communications*, *2*, 259.

Delgado-Diaz, M. R., Martin, Y., Berg, A., Freire, R., & Smits, V. A. J. (2014). Dub3 controls DNA damage signalling by direct deubiquitination of H2AX. *Molecular Oncology*, *8*(5), 884–893.

Dhillon, A. S., Meikle, S., Yazici, Z., Eulitz, M., & Kolch, W. (2002). Regulation of Raf-1 activation and signalling by dephosphorylation. *EMBO Journal*, *21*(1–2), 64–71.

Doil, C., Mailand, N., Bekker-Jensen, S., Menard, P., Larsen, D. H., Pepperkok, R., Ellenberg, J., Panier, S., Durocher, D., Bartek, J., Lukas, J., & Lukas, C. (2009). RNF168 Binds and Amplifies Ubiquitin Conjugates on Damaged Chromosomes to Allow Accumulation of Repair Proteins. *Cell*, *136*(3), 435–446.

Donzelli, M., & Draetta, G. F. (2003). Regulating mammalian checkpoints through Cdc25 inactivation. *EMBO Reports*, *4*(7), 671–677.

Ducker, C., Chow, L. K. Y., Saxton, J., Handwerger, J., McGregor, A., Strahl, T., Layfield, R., & Shaw, P. E. (2019). De-ubiquitination of ELK-1 by USP17 potentiates mitogenic gene expression and cell proliferation. *Nucleic Acids Research*, *47*(9), 4495–4508.

Evanko, S. P., & Wight, T. N. (1999). Intracellular Localization of Hyaluronan in Proliferating Cells. *Journal of Histochemistry & Cytochemistry*, *47*(10), 1331–1341.

Fueller, F., Bergo, M. O., Young, S. G., Aktories, K., & Schmidt, G. (2006). Endoproteolytic

Journal Pre-proof

processing of RhoA by Rce1 is required for the cleavage of RhoA by Yersinia enterocolitica outer protein T. *Infection and Immunity*, *74*(3), 1712–1717.

Fukuura, K., Inoue, Y., Miyajima, C., Watanabe, S., Tokugawa, M., Morishita, D., Ohoka, N., Komada, M., & Hayashi, H. (2019). The ubiquitin-specific protease USP17 prevents cellular senescence by stabilizing the methyltransferase SET8 and transcriptionally repressing p21. *Journal of Biological Chemistry*, *294*(44), 16429–16439.

Gallinari, P., Di Marco, S., Jones, P., Pallaoro, M., & Steinkuhler, C. (2007). HDACs, histone deacetylation and gene transcription: from molecular biology to cancer therapeutics. *Cell Research*, *17*, 195–211.

Geley, S., Kramer, E., Gieffers, C., Gannon, J., Peters, J. M., & Hunt, T. (2001). Anaphase-promoting complex/cyclosome-dependent proteolysis of human cyclin A starts at the beginning of mitosis and is not subject to the spindle assembly checkpoint. *Journal of Cell Biology*, *153*(1), 137–147.

Gille, H., Sharrocks, A. D., & Shaw, P. E. (1992). Phosphorylation of transcription factor P62TCF by MAP Kinase stimulates ternary complex-formation at C-FOS promoter. *Nature*, *358*(6385), 414–417.

Gondo, Y., Okada, T., Matsuyama, N., Saitoh, Y., Yanagisawa, Y., & Ikeda, J. E. (1998). Human megasatellite DNA RS447: copy-number polymorphisms and interspecies conservation. *Genomics*, *54*(1), 39–49.

Gualdrini, F., Esnault, C., Horswell, S., Stewart, A., Matthews, N., & Treisman, R. (2016). SRF Co-factors Control the Balance between Cell Proliferation and Contractility. *Molecular Cell*, *64*(6), 1048–1061.

Guo, C., Zhang, X., & Pfeifer, G. P. (2011). The tumor suppressor RASSF1A prevents dephosphorylation of the mammalian STE20-like kinases MST1 and MST2. *Journal of Biological Chemistry*, *286*(8), 6253–6261.

Haahr, P., Borgermann, N., Guo, X., Typas, D., Achuthankutty, D., Hoffmann, S., Shearer, R., Sixma, T. K., & Mailand, N. (2018). ZUFSP Deubiquitylates K63-Linked Polyubiquitin Chains to Promote Genome Stability. *Molecular Cell*, *70*(1), 165-174.e6.

Haglund, K., & Dikic, I. (2005). Ubiquitylation and cell signaling. *EMBO Journal*, *24*(19), 3353–3359.

Harper, J. W., & Elledge, S. J. (2007). The DNA Damage Response: Ten Years After. *Molecular Cell*, *28*(5), 739–745.

Journal Pre-proof

Journal Pre-proof

Hermanns, T., Pichlo, C., Woiwode, I., Klopffleisch, K., Witting, K. F., Ovaa, H., Baumann, U., & Hofmann, K. (2018). A family of unconventional deubiquitinases with modular chain specificity determinants. *Nature Communications*, *9*(1).

Hewings, D. S., Heideker, J., Ma, T. P., Ahyoung, A. P., El Oualid, F., Amore, A., Costakes, G. T., Kirchhofer, D., Brasher, B., Pillow, T., Popovych, N., Maurer, T., Schwerdtfeger, C., Forrest, W. F., Yu, K., Flygare, J., Bogyo, M., & Wertz, I. E. (2018). Reactive-site-centric chemoproteomics identifies a distinct class of deubiquitinase enzymes. *Nature Communications*, *9*(1).

Hjortland, N. M., & Mesecar, A. D. (2016). Steady-state kinetic studies reveal that the anti-cancer target Ubiquitin-Specific Protease 17 (USP17) is a highly efficient deubiquitinating enzyme. *Archives of Biochemistry and Biophysics*, *612*, 35–45.

Hu, B., Deng, T., Ma, H., Liu, Y., Feng, P., Wei, D., Ling, N., Li, L., Qiu, S., Zhang, L., Peng, B., Liu, J., & Ye, M. (2019). Deubiquitinase DUB3 Regulates Cell Cycle Progression via Stabilizing Cyclin A for Proliferation of Non-Small Cell Lung Cancer Cells. *Cells*, *8*(4), 297.

Hu, M., Chen, H., Han, C., Lan, J., Xu, Y., Li, C., Xue, Y., & Lou, M. (2016). Expression and functional implications of USP17 in glioma. *Neuroscience Letters*, *616*, 125–131.

Hu, Min, Li, P., Li, M., Li, W., Yao, T., Wu, J. W., Gu, W., Cohen, R. E., & Shi, Y. (2002). Crystal structure of a UBP-family deubiquitinating enzyme in isolation and in complex with ubiquitin aldehyde. *Cell*, *111*(7), 1041–1054.

Huen, M. S. Y., Grant, R., Manke, I., Minn, K., Yu, X., Yaffe, M. B., & Chen, J. (2007). RNF8 Transduces the DNA-Damage Signal via Histone Ubiquitylation and Checkpoint Protein Assembly. *Cell*, *131*(5), 901–914.

Hussain, S., Zhang, Y., & Galardy, P. J. (2009). DUBs and cancer: The role of deubiquitinating enzymes as oncogenes, non-oncogenes and tumor suppressors. *Cell Cycle*, *8*(11), 1688–1697.

Jaworski, J., de la Vega, M., Fletcher, S. J., McFarlane, C., Greene, M. K., Smyth, A. W., Van Schaeybroeck, S. V., Johnston, J. A., Scott, C. J., Rappoport, J. Z., & Burrows, J. F. (2014). USP17 is required for clathrin mediated endocytosis of epidermal growth factor receptor. *Oncotarget*, *5*(16), 6964–6975.

Jin, X., Yan, Y., Wang, D., Ding, D., Ma, T., Ye, Z., Jimenez, R., Wang, L., Wu, H., & Huang, H. (2018). DUB3 Promotes BET Inhibitor Resistance and Cancer Progression by Deubiquitinating BRD4. *Molecular Cell*, *71*(4), 592-605.

Journal Pre-proof

Kalluri, R., & Weinberg, R. (2009). The basics of epithelial-mesenchymal transition. *The Journal of Clinical Investigation*, *119(6)*, 1420–1428.

Kapuria, V., Peterson, L. F., Fang, D., Bornmann, W. G., Talpaz, M., & Donato, N. J. (2010). Deubiquitinase inhibition by small-molecule WP1130 triggers aggresome formation and tumor cell apoptosis. *Cancer Research*, *70*(22), 9265–9276.

Kim, J., D'Annibale, S., Magliozzi, R., Low, T. Y., Jansen, P., Shaltiel, I. A., Mohammed, S., Heck, A. J. R., Medema, R. H., & Guardavaccaro, D. (2014). USP17- and SCF TrCP-Regulated Degradation of DEC1 Controls the DNA Damage Response. *Molecular and Cellular Biology*, *34*(22), 4177–4185.

Kolattukudy, P. E., & Niu, J. (2012). Inflammation, endoplasmic reticulum stress, autophagy, and the monocyte chemoattractant protein-1/CCR2 pathway. *Circulation Research*, *110*(1), 174–189.

Komander, D., Clague, M. J., & Urbé, S. (2009). Breaking the chains: structure and function of the deubiquitinases. *Nature Reviews. Molecular Cell Biology*, *10*(8), 550–563.

Komander, D., & Rape, M. (2012). The ubiquitin code. *Annual Review of Biochemistry*, *81*, 203–229.

Lin, Yingying, Qiu, Y., Xu, C., Liu, Q., Peng, B., Kaufmann, G. F., Chen, X., Lan, B., Wei, C., Lu, D., Zhang, Y., Guo, Y., Lu, Z., Jiang, B., Edgington, T. S., & Guo, F. (2014). Functional role of asparaginyl endopeptidase ubiquitination by TRAF6 in tumor invasion and metastasis. *Journal of the National Cancer Institute*, *106*(4).

Lin, Yiwei, Wang, Y., Shi, Q., Yu, Q., Liu, C., Feng, J., Deng, J., Mark Evers, B., Zhou, B. P., & Wu, Y. (2017). Stabilization of the transcription factors slug and twist by the deubiquitinase dub3 is a key requirement for tumor metastasis. *Oncotarget*, *8*(43), 75127–75140.

Liu, C. Y., Zha, Z. Y., Zhou, X., Zhang, H., Huang, W., Zhao, D., Li, T., Chan, S. W., Lim, C. J., Hong, W., Zhao, S., Xiong, Y., Lei, Q. Y., & Guan, K. L. (2010). The hippo tumor pathway promotes TAZ degradation by phosphorylating a phosphodegron and recruiting the SCFβ-TrCP E3 ligase. *Journal of Biological Chemistry*, *285*(48), 37159–37169.

Liu, T., Yu, J., Deng, M., Yin, Y., Zhang, H., Luo, K., Qin, B., Li, Y., Wu, C., Ren, T., Han, Y., Yin, P., Kim, J. J., Lee, S. B., Lin, J., Zhang, L., Zhang, J., Nowsheen, S., Wang, L., … Lou, Z. (2017). CDK4/6-dependent activation of DUB3 regulates cancer metastasis through SNAIL1. *Nature Communications*, *8*, 1–12.

Journal Pre-proof

Lu, C. H., Yeh, D. W., Lai, C. Y., Liu, Y. L., Huang, L. R., Lee, A. Y. L., Jin, S. L. C., & Chuang, T. H. (2018). USP17 mediates macrophage-promoted inflammation and stemness in lung cancer cells by regulating TRAF2/TRAF3 complex formation. *Oncogene*, *37*(49), 6327–6340.

Luo, F., Zhou, Z., Cai, J., & Du, W. (2020). Dub3 facilitates growth and inhibits apoptosis through enhancing expression of ezh2 in oral squamous cell carcinoma. *OncoTargets and Therapy*, *13*, 1447–1460.

Luo, H., Jing, B., Xia, Y., Zhang, Y., Hu, M., Cai, H., Tong, Y., Zhou, L., Yang, L., Yang, J., Lei, H., Xu, H., Liu, C., & Wu, Y. (2019). WP1130 reveals USP24 as a novel target in T-cell acute lymphoblastic leukemia. *Cancer Cell International*, *19*(1), 1–14.

Mailand, N., Falck, J., Lukas, C., Syljuåsen, R. G., Welcker, M., Bartek, J., & Lukas, J. (2000). Rapid destruction of human Cdc25A in response to DNA damage. *Science*, *288*(5470), 1425–1429.

Matallanas, D., Romano, D., Al-Mulla, F., O'Neill, E., Al-Ali, W., Crespo, P., Doyle, B., Nixon, C., Sansom, O., Drosten, M., Barbacid, M., & Kolch, W. (2011). Mutant K-Ras Activation of the Proapoptotic MST2 Pathway Is Antagonized by Wild-Type K-Ras. *Molecular Cell*, *44*(6), 893–906.

Matallanas, D., Romano, D., Yee, K., Meissl, K., Kucerova, L., Piazzolla, D., Baccarini, M., Vass, J. K., Kolch, W., & O'Neill, E. (2007). RASSF1A Elicits Apoptosis through an MST2 Pathway Directing Proapoptotic Transcription by the p73 Tumor Suppressor Protein. *Molecular Cell*, *27*(6), 962–975.

McCann, A. P., Smyth, P., Cogo, F., McDaid, W. J., Jiang, L., Lin, J., Evergren, E., Burden, R. E., Van Schaeybroeck, S., Scott, C. J., & Burrows, J. F. (2018). USP17 is required for trafficking and oncogenic signaling of mutant EGFR in NSCLC cells 06 Biological Sciences 0601 Biochemistry and Cell Biology. *Cell Communication and Signaling*, *16*(1), 1–12.

McClellan, A. J., Laugesen, S. H., & Ellgaard, L. (2019). Cellular functions and molecular mechanisms of non-lysine ubiquitination. *Open Biology*, *9*(9).

McFarlane, C., Kelvin, A. A., De La Vega, M., Govender, U., Scott, C. J., Burrows, J. F., & Johnston, J. A. (2010). The deubiquitinating enzyme USP17 is highly expressed in tumor biopsies, is cell cycle regulated, and is required for G1-S progression. *Cancer Research*, *70*(8), 3329–3339.

McFarlane, C., McFarlane, S., Paul, I., Arthur, K., Scheaff, M., Kerr, K., Stevenson, M., Fennell,

Journal Pre-proof

D. A., & Johnston, J. A. (2013). The deubiquitinating enzyme USP17 is associated with non-small cell lung cancer (NSCLC) recurrence and metastasis. *Oncotarget*, *4*(10), 1836–1843.

Mehić, M., de Sa, V. K., Hebestreit, S., Heldin, C.-H., & Heldin, P. (2017). The deubiquitinating enzymes USP4 and USP17 target hyaluronan synthase 2 and differentially affect its function. *Oncogenesis*, *6*(6), e348.

Mevissen, T. E. T., & Komander, D. (2017). Mechanisms of deubiquitinase specificity and regulation. *Annual Review of Biochemistry*, *86*, 159–192.

Moon, K. J., Mochizuki, K., Zhou, M., Jeong, H. S., Brady, J. N., & Ozato, K. (2005). The bromodomain protein Brd4 is a positive regulatory component of P-TEFb and stimulates RNA polymerase II-dependent transcription. *Molecular Cell*, *19*(4), 523–534.

Nguyen, H. T., Andrejeva, D., Gupta, R., Choudhary, C., Hong, X., Eichhorn, P. J. A., Loya, A. C., & Cohen, S. M. (2016). Deubiquitylating enzyme USP9x regulates hippo pathway activity by controlling angiomotin protein turnover. *Cell Discovery*, *2*, 1–13.

Nguyen, H. T., Kugler, J. M., & Cohen, S. M. (2017). DUB3 deubiquitylating enzymes regulate Hippo pathway activity by regulating the stability of ITCH, LATS and AMOT proteins. *PLoS ONE*, *12*(1), 1–15.

Nguyen, L. K., Matallanas, D. G., Romano, D., Kholodenko, B. N., & Kolch, W. (2015). Competing to coordinate cell fate decisions the MST2-Raf-1 signaling device. *Cell Cycle*, *14*(2), 189–199.

Ni, Y., Tao, L., Chen, C., Song, H., Li, Z., Gao, Y., Nie, J., Piccioni, M., Shi, G., & Li, B. (2015). The deubiquitinase USP17 regulates the stability and nuclear function of IL-33. *International Journal of Molecular Sciences*, *16*(11), 27956–27966.

Nishioka, K., Rice, J. C., Sarma, K., Erdjument-Bromage, H., Werner, J., Wang, Y., Chuikov, S., Valenzuela, P., Tempst, P., Steward, R., Lis, J. T., Allis, C. D., & Reinberg, D. (2002). PR-Set7 is a nucleosome-specific methyltransferase that modifies lysine 20 of histone H4 and is associated with silent Chromatin. *Molecular Cell*, *9*(6), 1201–1213.

Okada, T., Gondo, Y., Goto, J., Kanazawa, I., Hadano, S., & Ikeda, J. E. (2002). Unstable transmission of the RS447 human megasatellite tandem repetitive sequence that contains the USP17 deubiquitinating enzyme gene. *Human Genetics*, *110*(4), 302–313.

Otto, J. C., Kim, E., Young, S. G., & Casey, P. J. (1999). Cloning and Characterization of a Mammalian Prenyl protein-specific protease. *The Journal of Biological Chemistry*,

8379–8382.

Pagano, M., Pepperkok, R., Verde, F., Ansorge, W., & Draetta, G. (1992). Cyclin A is required at two points in the human cell cycle. *The EMBO Journal*, *11*(3), 961–971.

Peinado, H., Ballestar, E., Esteller, M., & Cano, A. (2004). Snail Mediates E-Cadherin Repression by the Recruitment of the Sin3A/Histone Deacetylase 1 (HDAC1)/HDAC2 Complex. *Molecular and Cellular Biology*, *24*(1), 306–319.

Pereg, Y., Liu, B. Y., O'Rourke, K. M., Sagolla, M., Dey, A., Komuves, L., French, D. M., & Dixit, V. M. (2010). Ubiquitin hydrolase Dub3 promotes oncogenic transformation by stabilizing Cdc25A. *Nature Cell Biology*, *12*(4), 400–406.

Praskova, M., Khoklatchev, A., Ortiz-Vega, S., & Avruch, J. (2004). Regulation of the MST1 kinase by autophosphorylation, by the growth inhibitory proteins, RASSF1 and NORE1, and by Ras. *Biochemical Journal*, *381*(2), 453–462.

Ramakrishna, S., Suresh, B., Bae, S. M., Ahn, W. S., Lim, K. H., & Baek, K. H. (2012). Hyaluronan binding motifs of USP17 and SDS3 exhibit anti-tumor activity. *PLoS ONE*, *7*(5), 1–9. 2

Ramakrishna, S., Suresh, B., Lee, E. J., Lee, H. J., Ahn, W. S., & Baek, K. H. (2011). Lys-63-specific deubiquitination of SDS3 by USP17 regulates HDAC activity. *Journal of Biological Chemistry*, *286*(12), 10505–10514.

Robinson, D., Van Allen, E. M., Wu, Y. M., Schultz, N., Lonigro, R. J., Mosquera, J. M., Montgomery, B., Taplin, M. E., Pritchard, C. C., Attard, G., Beltran, H., Abida, W., Bradley, R. K., Vinson, J., Cao, X., Vats, P., Kunju, L. P., Hussain, M., Feng, F. Y., … Chinnaiyan, A. M. (2015). Integrative Clinical Genomics of Advanced Prostate Cancer. *Cell*, *162*(2), 454.

Romano, D., Matallanas, D., Weitsman, G., & Preisinger, C. (2010). Proapoptotic Kinase MST2 Coordinates Signaling Crosstalk between RASSF1A , Raf-1 , and Akt. *Cancer Research*, *2*, 1195–1204.

Romano, D., Nguyen, L. K., Matallanas, D., Halasz, M., Doherty, C., Kholodenko, B. N., & Kolch, W. (2014). Protein interaction switches coordinate Raf-1 and MST2/Hippo signalling. *Nature Cell Biology*, *16*(7), 673–684.

Roy, M., Li, Z., & Sacks, D. B. (2005). IQGAP1 Is a Scaffold for Mitogen-Activated Protein Kinase Signaling. *Molecular and Cellular Biology*, *25*(18), 7940–7952.

Saitoh, Y., Miyamoto, N., Okada, T., Gondo, Y., Showguchi-Miyata, J., Hadano, S., & Ikeda, J.

Journal Pre-proof

Journal Pre-proof

E. (2000). The RS447 human megasatellite tandem repetitive sequence encodes a novel deubiquitinating enzyme with a functional promoter. *Genomics*, *67*(3), 291–300.

Salah, Z., Melino, G., & Aqeilan, R. I. (2011). Negative regulation of the Hippo pathway by E3 ubiquitin ligase ITCH is sufficient to promote tumorigenicity. *Cancer Research*, *71*(5), 2010–2020.

Schauer, N. J., Magin, R. S., Liu, X., Doherty, L. M., & Buhrlage, S. J. (2020). Advances in Discovering Deubiquitinating Enzyme (DUB) Inhibitors. *Journal of Medicinal Chemistry*, *63*(6), 2731–2750.

Shaw, J. A., Page, K., Blighe, K., Hava, N., Guttery, D., Ward, B., Brown, J., Ruangpratheep, C., Stebbing, J., Payne, R., Palmieri, C., Cleator, S., Walker, R. A., & Coombes, R. C. (2012). Genomic analysis of circulating cell-free DNA infers breast cancer dormancy. *Genome Research*, *22*(2), 220–231.

Shay, G., Lynch, C. C., & Fingleton, B. (2015). Moving targets: Emerging roles for MMPs in cancer progression and metastasis. *Matrix Biology*, *44–46*, 200–206.

Shen, T., & Huang, S. (2012). The Role of Cdc25A in the Regulation of Cell Proliferation and Apoptosis. *Anti-Cancer Agents in Medicinal Chemistry*, *12*(6), 631–639.

Shih, C. T., Chang, Y. F., Chen, Y. T., Ma, C. P., Chen, H. W., Yang, C. C., Lu, J. C., Tsai, Y. S., Chen, H. C., & Tan, B. C. M. (2017). The PPARγ-SETD8 axis constitutes an epigenetic, p53-independent checkpoint on p21-mediated cellular senescence. *Aging Cell*, *16*(4), 797–813.

Shin, J.-M., Yoo, K.-J., Kim, M.-S., Kim, D., & Baek, K.-H. (2006). Hyaluronan- and RNA-binding deubiquitinating enzymes of USP17 family members associated with cell viability. *BMC Genomics*, *7*, 292.

Song, C., Liu, W., & Li, J. (2017). USP17 is upregulated in osteosarcoma and promotes cell proliferation , metastasis , and epithelial – mesenchymal transition through stabilizing SMAD4. *Tumour Biology, 39(7):*1010428317717138

Song, H., Tao, L., Chen, C., Pan, L., Hao, J., Ni, Y., Li, D., Li, B., & Shi, G. (2015). USP17-mediated deubiquitination and stabilization of HDAC2 in cigarette smoke extract-induced inflammation. *International Journal of Clinical and Experimental Pathology*, *8*(9), 10707–10715.

Thiery, J. P. (2002). Epithelial-mesenchymal transitions in tumor progression. *Nature Reviews Cancer*, *2*(6), 442–454.

Journal Pre-proof

Thin, T. H., Li, L., Chung, T. K., Sun, H., & Taneja, R. (2007). Stra13 is induced by genotoxic stress and regulates ionizing-radiation-induced apoptosis. *EMBO Reports*, *8*(4), 401–407.

Tian, X., Isamiddinova, N. S., Peroutka, R. J., Goldenberg, S. J., Mattern, M. R., Nicholson, B., & Leach, C. (2011). Characterization of selective ubiquitin and ubiquitin-like protease inhibitors using a fluorescence-based multiplex assay format. *Assay and Drug Development Technologies*, *9*(2), 165–173.

Torres-Adorno, A. M., Lee, J., Kogawa, T., Ordentlich, P., Tripathy, D., Lim, B., & Ueno, N. T. (2017). Histone deacetylase inhibitor enhances the efficacy of MEK inhibitor through NOXA-mediated MCL1 degradation in triple-negative and inflammatory breast cancer. *Clinical Cancer Research*, *23*(16), 4780–4792.

Uhlén, M., Fagerberg, L., Hallström, B. M., Lindskog, C., Oksvold, P., Mardinoglu, A., Sivertsson, Å., Kampf, C., Sjöstedt, E., Asplund, A., Olsson, I. M., Edlund, K., Lundberg, E., Navani, S., Szigyarto, C. A. K., Odeberg, J., Djureinovic, D., Takanen, J. O., Hober, S., … Pontén, F. (2015). Tissue-based map of the human proteome. *Science*, *347*(6220).

Van Der Laan, S., Golfetto, E., Vanacker, J. M., & Maiorano, D. (2014). Cell cycle-dependent expression of Dub3, Nanog and the p160 family of nuclear receptor coactivators (NCoAs) in mouse embryonic stem cells. *PLoS ONE*, *9*(4), 1–12.

Van der Laan, S., Tsanov, N., Crozet, C., & Maiorano, D. (2013). High Dub3 Expression in Mouse ESCs Couples the G1/S Checkpoint to Pluripotency. *Molecular Cell*, *52*(3), 366–379.

Vijaykumar, S., Bugg, C. E., & Cook, W. J. (1987). Structure of Ubiquitin refined at 1.8 A resolution. *Journal of Molecular Biology*, *194*(3), 531–544.

Viñas-Castells, R., Beltran, M., Valls, G., Gómez, I., García, J. M., Montserrat-Sentís, B., Baulida, J., Bonilla, F., De Herreros, A. G., & Díaz, V. M. (2010). The hypoxia-controlled FBXL14 ubiquitin ligase targets SNAIL1 for proteasome degradation. *Journal of Biological Chemistry*, *285*(6), 3794–3805.

Vincent, T., Neve, E. P. A., Johnson, J. R., Kukalev, A., Rojo, F., Albanell, J., Pietras, K., Virtanen, I., Philipson, L., Leopold, P. L., Crystal, R. G., de Herreros, A. G., Moustakas, A., Pettersson, R. F., & Fuxe, J. (2009). A SNAIL1-SMAD3/4 transcriptional repressor complex promotes TGF-β mediated epithelial-mesenchymal transition. *Nature Cell Biology*, *11*(8), 943–950.

Wang, C., An, J., Zhang, P., Xu, C., Gao, K., Wu, D., Wang, D., Yu, H., Liu, J. O., & Yu, L. (2012). The Nedd4-like ubiquitin E3 ligases target angiomotin/p130 to ubiquitin-dependent degradation. *Biochemical Journal*, *444*(2), 279–289.

Wang, Y., Liu, J., Ying, X., Lin, P. C., & Zhou, B. P. (2016). Twist-mediated Epithelial-mesenchymal Transition Promotes Breast Tumor Cell Invasion via Inhibition of Hippo Pathway. *Scientific Reports*, *6*, 24606.

Wu, S., Wang, W., Kong, X., Congdon, L. M., Yokomori, K., Kirschner, M. W., & Rice, J. C. (2010). Dynamic regulation of the PR-Set7 histone methyltransferase is required for normal cell cycle progression. *Genes and Development*, *24*(22), 2531–2542.

Wu, Xiaowei, Luo, Q., Zhao, P., Chang, W., Wang, Y., Shu, T., Ding, F., Li, B., & Liu, Z. (2019). MGMT-activated DUB3 stabilizes MCL1 and drives chemoresistance in ovarian cancer. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(8), 2961–2966.

Wu, Xinchao, Liu, D., Tao, D., Xiang, W., Xiao, X., Wang, M., Wang, L., Luo, G., Li, Y., Zeng, F., & Jiang, G. (2016). BRD4 regulates EZH2 transcription through upregulation of C-MYC and represents a novel therapeutic target in bladder cancer. *Molecular Cancer Therapeutics*, *15*(5), 1029–1042.

Wu, Y., Wang, Y., Lin, Y., Liu, Y., Wang, Y., Jia, J., Singh, P., Chi, Y.-I., Wang, C., Dong, C., Li, W., Tao, M., Napier, D., Shi, Q., Deng, J., Mark Evers, B., & Zhou, B. P. (2017). Dub3 inhibition suppresses breast cancer invasion and metastasis by promoting Snail1 degradation. *Nature Communications*, *8*, 14228.

Yang, H., Wang, L., Zhao, J., Chen, Y., Lei, Z., Liu, X., Xia, W., Guo, L., & Zhang, H. T. (2015). TGF-β-activated SMAD3/4 complex transcriptionally upregulates N-cadherin expression in non-small cell lung cancer. *Lung Cancer*, *87*(3), 249–257.

Yang, T., Buchan, H. L., Townsend, K. J., & Craig, R. W. (1996). MCL-1, a member of the BCL-2 family, is induced rapidly in response to signals for cell differentiation or death, but not to signals for cell proliferation. *Journal of Cellular Physiology*, *166*(3), 523–536.

Yin, L., Yu, V. C., Zhu, G., & Chang, D. C. (2008). SET8 plays a role in controlling G1/S transition by blocking lysine acetylation in histone through binding to H4 N-terminal tail. *Cell Cycle*, *7*(10), 1423–1432.

Yuan, T., Yan, F., Ying, M., Cao, J., He, Q., Zhu, H., & Yang, B. (2018). Inhibition of Ubiquitin-Specific Proteases as a Novel Anticancer Therapeutic Strategy. *Frontiers in*

Journal Pre-proof

Pharmacology, *9*, 1080.

Zeng, Y., Zhu, J., Shen, D., Qin, H., Lei, Z., Li, W., Huang, J. A., & Liu, Z. (2016). Repression of Smad4 by miR205 moderates TGF-induced epithelial-mesenchymal transition in A549 cell lines. *International Journal of Oncology*, *49*(2), 700–708.

Zhang, Q., Zhang, Z., Du, H., Li, S., Tu, R., Jia, Y., Zheng, Z., Song, X., Du, R., & Zhang, X. (2019). DUB3 deubiquitinates and stabilizes NRF2 in chemotherapy resistance of colorectal cancer. *Cell Death and Differentiation*, *26*(11), 2300–2313.

Zhang, S., Yuan, J., & Zheng, R. (2016). Suppression of Ubiquitin-Specific Peptidase 17 (USP17) Inhibits Tumorigenesis and Invasion in Non-Small Cell Lung Cancer Cells. *Oncology Research*, *24*(4), 263–269.

Zhao, B., Li, L., Tumaneng, K., Wang, C. Y., & Guan, K. L. (2010). A coordinated phosphorylation by Lats and CK1 regulates YAP stability through SCFβ-TRCP. *Genes and Development*, *24*(1), 72–85.

Zhao, B., Ye, X., Yu, J., Li, L., Li, W., Li, S., Yu, J., Lin, J. D., Wang, C. Y., Chinnaiyan, A. M., Lai, Z. C., & Guan, K. L. (2008). TEAD mediates YAP-dependent gene induction and growth control. *Genes and Development*, *22*(14), 1962–1971.

Zheng, H., Shen, M., Zha, Y. L., Li, W., Wei, Y., Blanco, M. A., Ren, G., Zhou, T., Storz, P., Wang, H. Y., & Kang, Y. (2014). PKD1 Phosphorylation-Dependent Degradation of SNAIL by SCF-FBXO11 Regulates Epithelial-Mesenchymal Transition and Metastasis. *Cancer Cell*, *26*(3), 358–373.

Zhou, B. P., Deng, J., Xia, W., Xu, J., Li, Y. M., Gunduz, M., & Hung, M. C. (2004). Dual regulation of Snail by GSK-3β-mediated phosphorylation in control of epithelial-mesenchymal transition. *Nature Cell Biology*, *6*(10), 931–940.

Zhou, B., Shu, B., Xi, T., Su, N., & Liu, J. (2015). Dub3 expression correlates with tumor progression and poor prognosis in human epithelial ovarian cancer. *Biomedicine and Pharmacotherapy*, *70*(C), 84–89.

Zhu, W., Shao, Y., Yang, M., Jia, M., & Peng, Y. (2016). Asparaginyl endopeptidase promotes proliferation and invasiveness of prostate cancer cells via PI3K/AKT signaling pathway. *Gene*, *594*(2), 176–182.

Zhu, Y, Pless, M., Inhorn, R., Mathey-Prevot, B., & D'Andrea, a D. (1996). The murine DUB-1 gene is specifically induced by the betac subunit of interleukin-3 receptor. *Molecular and Cellular Biology*, *16*(9), 4808–4817.

Journal Pre-proof

Journal Pre-proof

Zhu, Yuan, Lambert, K., Corless, C., Copeland, N. G., Gilbert, D. J., Jenkins, N. A., & D'Andrea, A. D. (1997). DUB-2 is a member of a novel family of cytokine-inducible deubiquitinating enzymes. *Journal of Biological Chemistry*, *272*(1), 51–57.

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