

Ubiquitin and ubiquitin-like proteins in cancer pathogenesis

Daniela Hoeller*, Christina-Maria Hecker* and Ivan Dikic**

Abstract | Ubiquitin and ubiquitin-like proteins (Ubls) are signalling messengers that control many cellular functions, such as cell proliferation, apoptosis, the cell cycle and DNA repair. It is becoming apparent that the deregulation of ubiquitin pathways results in the development of human diseases, including many types of tumours. Here we summarize the common principles and specific features of ubiquitin and Ubls in the regulation of cancer-relevant pathways, and discuss new strategies to target ubiquitin signalling in drug discovery.

Ubl

Ubiquitin-like proteins are small proteins that have significant sequence and structural similarity to ubiquitin and are conjugated in a similar way.

Small ubiquitin-related modifier

SUMO belongs to the family of ubiquitin-like proteins. Three ubiquitously expressed paralogues are known. SUMO2 and SUMO3 are highly similar to each other (95%), whereas SUMO1 shows about 50% sequence similarity with SUMO2.

Tagging proteins by post-translational modifications is an important cellular strategy that enables the cell to react dynamically to intracellular or environmental changes caused by exposure to stress factors, growth stimuli or differentiation signals. Among many possible modifications, ubiquitylation is one of the most abundant inducible and reversible protein modifications involved in cellular homeostasis and signalling¹⁻³. Ubiquitylation is a three-step process that results in the attachment of the small protein ubiquitin to lysine residues on a substrate protein (BOX 1). Originally described as a destruction tag for misfolded or disused proteins, ubiquitin has recently entered the centre stage in many fundamental processes such as the cell cycle, DNA repair, endocytosis, antigen processing and apoptosis⁴. It is evident that the role of ubiquitin in these processes is not restricted to targeting key proteins for degradation, thereby permanently switching off their specific functions. Instead, ubiquitin has been discovered to be signalling competent and able to trigger molecular events in the cell⁵.

Ubiquitin has a 3-dimensional structure with a complex surface architecture and is able to form various ubiquitin chains, creating a protein modifier with a greater versatility than phosphate. Moreover, it seems that many pathways and/or single proteins are regulated not only by ubiquitin, but also by ubiquitin-like protein (Ubl) modifications including sumoylation (small ubiquitin-related modifier; SUMO), neddylation (NEDD8), ISGylation (interferon-stimulated gene 15; ISG15) and fatylation (FAT10), in a functionally distinct manner (BOX 1, 2). As a way to recognize the messages that are encoded by these different tags, the cell developed a series of small modular domains that non-covalently bind to ubiquitin and Ubls. To date, around 15 families

of ubiquitin-binding domains (UBDs) have been shown to bind specifically to ubiquitin, whereas SUMO is recognized by the SUMO-interacting motif, which binds to SUMO in a manner distinguishable from that of UBDs binding to ubiquitin⁵⁻⁷. These domains are able to read and interpret specific ubiquitin or Ubl signals into appropriate cellular phenotypes.

Quite expectedly, alterations to the ubiquitin-conjugation machinery and the ubiquitin-signalling networks have been found in many pathologies, including inflammatory diseases and cancer^{3,8}. In this Review we summarize recent advances in our understanding of ubiquitin-dependent mechanisms in cancer pathogenesis, and describe examples of the cross-talk between ubiquitin and Ubls in several oncogenic pathways. We also evaluate the potential of these findings for new therapeutic interventions.

Ubiquitin and cancer

As ubiquitylation affects almost all cellular processes, including apoptosis and the cell cycle, it is not surprising that alterations in the ubiquitin and Ubl systems have direct or indirect roles in the genesis of different types of tumours (TABLE 1). A large body of experimental and clinical data indicate that defects in the ubiquitin-dependent proteolysis of important house-keeping genes or cell-cycle components are intimately linked with cancer pathogenesis (reviewed in REFS 8-11). In these processes, the deregulation of ubiquitin ligases seems to be particularly relevant, as important ligases such as *CBL* (involved in growth-factor-receptor downregulation) or *SKP2* (of the SKP1-CUL1-F-box (SCF) ligase complexes involved in cell-cycle control) are oncogenes^{11,12}. It was proposed that

*Institute of Biochemistry II, Goethe University School of Medicine, University Hospital, Building 75, Theodor-Stern-Kai 7, D-60590 Frankfurt, Germany.

**Mediterranean Institute for Life Sciences, Mestrovicovo Setaliste bb, 21 000 Split, Croatia.

Correspondence to I. D. e-mail: ivan.dikic@biochem2.de
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At a glance

- Ubiquitin is an 8 kDa polypeptide that can be covalently attached to other proteins through a process called ubiquitylation (also known as ubiquitination). Similar to phosphorylation, ubiquitylation is an inducible and reversible process that changes the properties of the modified substrate; for example, its subcellular localization, stability or enzymatic activity.
- The attachment of ubiquitin to substrates requires the sequential action of three enzymes: ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2) and ubiquitin ligase (E3). This process can be reversed by de-ubiquitylating enzymes (DUBs) that remove ubiquitin from substrates.
- Several ubiquitin-like modifiers (Ubls) have been identified, such as SUMO, NEDD8, ISG15 and FAT10. They possess a distinct primary sequence but share characteristic features with ubiquitin, including the 3-dimensional fold and the mode of their conjugation to substrates.
- Protein domains that bind to ubiquitin (UBDs) or Ubls have crucial roles in the recognition of modified proteins and the translation of the encoded information into the proper cellular response. Most UBD-containing proteins undergo monoubiquitylation themselves, which causes their auto-inhibition by intramolecular UBD–ubiquitin interactions.
- Alterations to the ubiquitin and Ubl systems are associated with various pathologies, such as inflammatory diseases and cancer. Moreover, many disease-related pathways are not only regulated by a single ubiquitin or Ubl, but by a sophisticated cross-talk of different types of modifications.
- Examples of such ubiquitin or Ubl interplays in oncogenic pathways include p53, nuclear factor κ B and DNA repair pathways, which are regulated by monoubiquitylation, polyubiquitylation, sumoylation and neddylation.
- Bortezomib is the first compound that targets the ubiquitin-system to be approved for clinical use in human cancers. It inhibits the active site of one of the proteasome subunits and shuts down the proteasomal protein-degradation system.
- New, more specific anticancer drugs that target the ubiquitin-system are being developed, including selective inhibitors of E3 enzymes like MDM2. Moreover, DUBs and the binding interface of ubiquitylated proteins and UBDs have emerged as promising drug targets.

the oncogenic potential of these ligases is mediated by the proteasomal or lysosomal degradation of several cell-specific proto-oncogenes or tumour-suppressor genes^{8,10}. However, the situation is more complicated, as genetic and biochemical evidence shows that **FBW7**, which is a component of the ubiquitin ligase SCF complex, is a *bona fide* tumour suppressor and targets several oncogenes for degradation⁸. On the other hand, ubiquitin-specific proteases, known as de-ubiquitylating enzymes (DUBs), keep the balance in ubiquitin-signalling networks by removing ubiquitin conjugation from specific cellular targets, and potentially functioning as tumour suppressors.

Knowledge about the temporal and spatial dynamics of the ubiquitylation and de-ubiquitylation of target proteins is of fundamental importance for understanding the physiological and pathological consequences of ubiquitin signalling. In analogy to protein-tyrosine phosphatases, which counteract phosphorylation by protein-tyrosine kinases, DUBs that remove the ubiquitin or Ubl tag from substrates have emerged as players in tumour pathogenesis and are potential drug targets. The human genome contains about 90 putative DUBs¹³. The specificity of these enzymes seems to be limited towards certain target proteins *in vivo*, and is probably defined by a combination of the ubiquitin moiety (monoubiquitin, Lys48-

linked or Lys63-linked polyubiquitin), the modified protein and subcellular localization. Interestingly, DUBs and E3 ligases often interact with each other¹³, and E3 and DUB activity can even reside in the same protein¹⁴.

Ubl modifiers, such as SUMO and ISG15, are over-expressed and conjugated to many proteins in tumours (TABLE 1), and are presumably implicated in tumorigenesis through the modification of several oncogenes and tumour suppressors^{15,16}. However, direct evidence to support this paradigm is still sparse.

The molecular understanding of the role of ubiquitin and Ubl modifications in cancer-related signalling pathways is further complicated by the finding that the key molecules in these pathways are often modified by different types of ubiquitin modifications (mono-, multi- and polyubiquitylation) and sometimes other Ubl molecules. These post-translational modifications involve a specialized set of molecular interactions that are coupled to distinct cellular phenotypes³ (BOX 2). Recent reports imply that cross-talk within the ubiquitin and/or Ubl family might be a general concept that is applicable to several signalling pathways. One intriguing example is p53, the functions of which are kept in check by a sophisticated interplay between mono- and polyubiquitylation, sumoylation and neddylation¹⁷. In the following sections we describe the functional significance of such ubiquitin–Ubl interplays in the regulation of **p53**, nuclear factor κ B (NF κ B) and DNA-repair pathways, and discuss evidence of their deregulation in tumorigenesis.

Cell-cycle control, DNA-damage tolerance and repair.

Dealing with DNA damage represents a fundamental cellular process that promotes the survival of intact cells and the death of irreparably damaged cells. One of the first protective events after DNA damage is to stop the cell cycle, which prevents the proliferation of aberrant, potentially malignant cells. A series of sensor and repair systems check whether the damage can be corrected and, if needed, initiate apoptosis. In tumour cells this protective strategy is often hindered, therefore enabling uncontrolled proliferation. The crucial involvement of ubiquitin ligases in cell-cycle control and cancer has been covered in a recent review⁸, and shows the importance of the degradation-dependent ubiquitin function in these processes. However, several studies in yeast and mammalian cells point out another role of ubiquitin and Ubls in DNA repair, apoptosis and cell-cycle control. As described below, this role depends on their signalling properties rather than on the destructive potential of ubiquitylation.

The p53 pathway. The transcription factor p53 is a tumour-suppressor protein that functions by stopping cell-cycle progression or promoting apoptosis when a cell is exposed to genotoxic stress. In unstressed cells, nuclear p53 is kept at low levels mainly by a negative-feedback loop that involves the action of the p53 target **MDM2**, which is a RING-type E3 ligase that mediates p53 polyubiquitylation and degradation in nuclear proteasomes^{18,19}. Besides MDM2 there are at least three additional RING-containing E3 ligases that function as negative regulators of p53: PriH2, **COP1**

NEDD8

This protein belongs to the family of ubiquitin-like proteins and modifies specific ubiquitin E3 ligases to influence their catalytic ubiquitylation activity.

Interferon-stimulated gene 15

This protein consists of two ubiquitin-like folds, and is conjugated to substrates after interferon 1 stimulation. ISG15 is important in the regulation of the immune response.

FAT10

A ubiquitin-like protein that consists of two ubiquitin-like folds and is conjugated to substrates after interferon- γ and tumour-necrosis factor- α induction. Its expression has been shown to cause apoptosis.

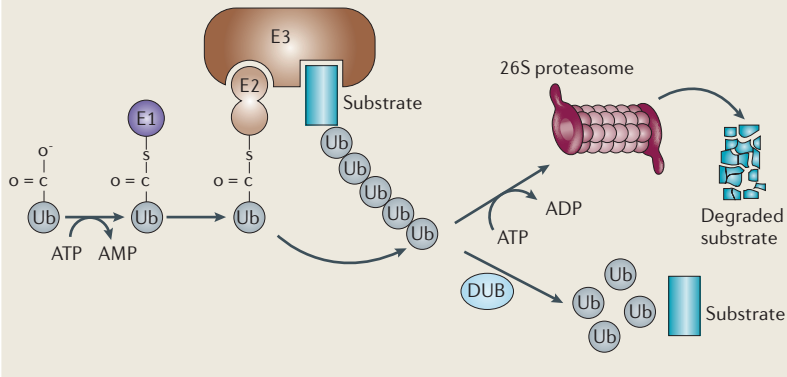
Box1 | Ubiquitin conjugation

Ubiquitin (Ub) is a highly conserved 8 kDa protein that becomes covalently attached to lysine residues of target proteins in an inducible and reversible manner. This occurs through a three-step process involving three different types of enzymes.

Ubiquitin is activated in an ATP-dependent manner by a ubiquitin-activating enzyme (E1), and is then transferred to a ubiquitin-conjugating enzyme (E2) through a thioester bond. A ubiquitin-protein ligase (E3) specifically attaches ubiquitin to the ε-amino group of a lysine residue in the target protein¹. Although only a few E1 enzymes are known, humans have more than 20 different E2s. E3 ligases are primarily responsible for substrate recognition. To provide specificity about 500–1,000 different E3 ligases exist in humans⁹. After the attachment of a Lys48-linked polyubiquitin chain to a substrate it is degraded in the 26S proteasome; the attached ubiquitin moieties can be recycled. Ubiquitylation reactions are reversible by de-ubiquitylating enzymes (DUBs), of which several types are known at present.

This conjugation process is similar for ubiquitin-like (Ubl) proteins, such as small ubiquitin-related modifier (SUMO). However, only one E1 (UBA1) and one E2 (UBC9) are known to catalyse sumoylation. There are several E3 ligases, such as PIAS family proteins, RANPB2, PC2 or TOPORS. Interestingly, SUMO E3 ligases do not seem to be as crucial for mediating sumoylation as ubiquitin E3 ligases are for ubiquitylation, but rather enhance the conjugation event¹¹⁰. The sentrin-specific protease protein family catalyses de-sumoylation⁸⁷.

Fewer components of the NEDD8 and ISG15 conjugation machinery have been identified. For NEDD8, one E1 (APPBP1), one E2 (UBC12) and one E3 (RBX1) are known¹¹¹. Interestingly, ubiquitin E3 ligases were recently shown to also function as NEDD8 E3 ligases¹¹². For ISG15 only one E1 (UBE1L), one E2 (UBCH8) and one de-ISGylation enzyme (UBP43) are known¹¹¹.



and **CHIP**⁹. After DNA damage the N-terminal region of p53 becomes phosphorylated by several kinases, including ataxia telangiectasia mutated (**ATM**), ataxia telangiectasia and Rad3 related (**ATR**), **CHK1**, **CHK2** and **DNAPK**, which leads to dissociation from MDM2 and the accumulation of transcriptionally active p53 in the nucleus (FIG. 1). This basic mode of MDM2-mediated p53 regulation by proteasomal degradation is fine-tuned by a set of additional modifications that include the monoubiquitylation, sumoylation and neddylation of both p53 and MDM2. If MDM2 is expressed at a low level it will primarily monoubiquitylate p53, which leads to the nuclear export but not to the proteasomal degradation of p53 (REF. 20) (FIG. 1). The nuclear export of monoubiquitylated p53 might serve to temporarily segregate it from its main site of action but keep it in reserve. Alternatively or additionally, p53 activity can be redirected to cytoplasmic sites such as the mitochondria, where it might trigger apoptosis in an MDM2-dependent manner^{21–24}. Even though the role of MDM2 is becoming fairly well understood, recent findings

confirm that the complexity of p53 ubiquitylation is ever increasing, as new modes of MDM2 binding are discovered^{25,26} and the number of molecules involved in p53 ubiquitylation continues to grow^{27,28}.

p53 also undergoes sumoylation *in vitro* and *in vivo*^{29–33}. However, reports about the functional effect of p53 sumoylation on its activity are inconsistent, and range from transcriptional activation to repression^{17,30–34} (FIG. 1). This might be due to different cell systems and/or assay conditions, and reflects the great functional flexibility of the SUMO modification. A recent example of transcriptional activation by sumoylation involves the SUMO E3 ligase **PIASy**. In human fibroblasts, PIASy stimulated p53 sumoylation and consequent senescence or apoptosis pathways depending on the cell state³². Interestingly, efficient p53 sumoylation *in vivo* requires its direct interaction with MDM2 (REF. 35), which indicates that ubiquitylation and sumoylation might be functionally interconnected. However, the modifications target distinct acceptor lysines located in the C terminus of p53: Lys386 for SUMO and multiple additional lysines for ubiquitin, whereas in most other transcription factors that are differentially regulated by both modifications, one single lysine is targeted by either ubiquitin or SUMO. A simple competitive function of the two modifications on p53 is therefore unlikely to have a role *in vivo*.

To add another layer of complexity to p53 regulation, three of the lysines that are ubiquitylated can alternatively be neddylated³⁶. And again, MDM2 has its RING finger in the 'pie' by mediating the neddylation of p53 *in vitro* and *in vivo*, and also neddyating itself. The physiological role of these modifications and how they are regulated remains to be determined. However, there is some evidence that the neddylation of p53 negatively regulates its transcriptional activity³⁶.

Furthermore, herpes-virus-associated ubiquitin-specific protease (**HAUSP**) was shown to mediate the de-ubiquitylation and stabilization of p53, and the overexpression of HAUSP is sufficient to induce p53-dependent effects such as cell-growth arrest or apoptosis^{37,38}. Surprisingly, disruption of the **HAUSP** gene in human cells by targeted homologous recombination results in significant stabilization and functional activation of p53 (REFS 39,40). The finding that MDM2 is another physiological target of HAUSP suggests that the de-ubiquitylation of different targets of HAUSP might determine the steady-state level of p53 (REFS 39,40). Not unexpectedly, mutations in the **HAUSP** and **MDM2** genes are frequently associated with the risk of different types of cancer, including **non-small-cell lung cancer**, soft-tissue carcinoma and **colorectal cancer**^{41–43}.

NFκB signalling. The transcription factor NFκB is a key regulator of several cellular processes, such as apoptosis, innate and adaptive immune responses, cell migration and proliferation. Its activation of anti-apoptotic signalling has been connected to tumour progression and resistance to chemotherapeutic agents that function by inducing the apoptosis of malignant cells^{44,45}. The NFκB family consists of five members called **RelA**, **RelB**, **c-Rel**, **NFκB1** and **NFκB2**. RelB, c-Rel and RelA

26S proteasome
A large 2.5 MDa multisubunit protein that is the main proteolytic system in eukaryotic cells. It degrades polyubiquitylated proteins.

UBDs
Ubiquitin binding domains bind to ubiquitin and have crucial roles in the recognition of modified proteins and the translation of encoded information into the proper cellular response.

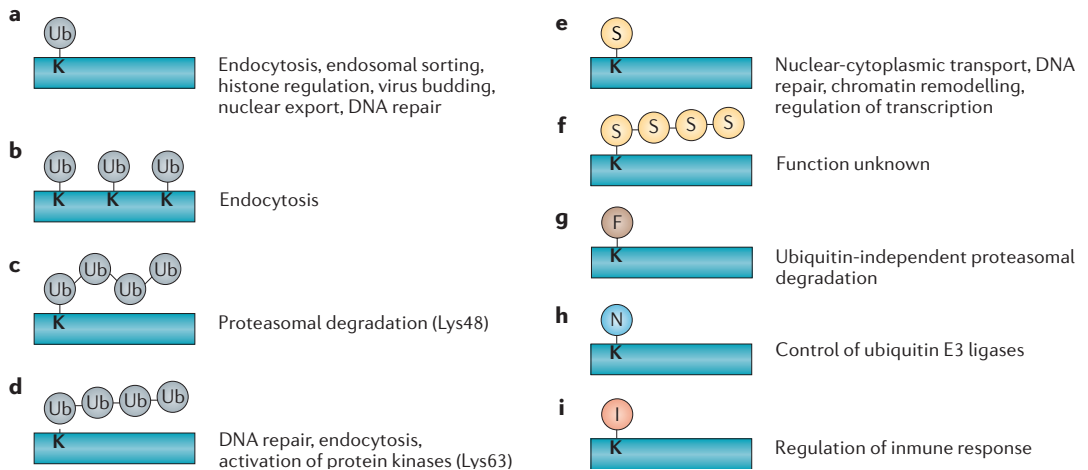
Box2 | Different types of modifications

The types of ubiquitin (Ub) modifications are diverse, as are their functions. In the simplest form, a single ubiquitin molecule is attached (part **a**), which is defined as monoubiquitylation. It is involved in several processes including endocytosis, endosomal sorting, histone regulation and DNA repair. Alternatively, a substrate that contains several lysine (K) residues can be tagged with several single ubiquitin molecules (part **b**), which has a role in endocytosis.

Furthermore, ubiquitin itself has seven Lys residues that can be involved in chain formation *in vivo*. Ubiquitin chains linked through Lys48 and 63 are the best characterized. Linkage through Lys48 (part **c**) has been shown to represent a signal for the proteasomal degradation of modified substrates. Lys63-linked chains (part **d**) seem to have completely different, degradation-independent functions that are involved in processes such as DNA repair and protein sorting³. Besides these well-characterized types of ubiquitylation, new, less well-understood modes have been reported that involve Cys instead of Lys as the ubiquitin acceptor¹¹³, or mixed ubiquitin chains that consist of Lys48 and Lys63 linkages¹¹⁴.

Although the 3-dimensional structures of ubiquitin-like (Ubl) modifiers are similar to that of ubiquitin, their functions are quite different. Monosumoylation (S) was shown to have roles in the regulation of subcellular localization, DNA repair, chromatin remodelling and the regulation of transcription (part **e**), whereas the function of SUMO chains that were observed for SUMO paralogues 2 and 3 is still unknown (part **f**)⁸⁷.

Fatylation (F) has recently been shown to mediate rapid degradation by the proteasome (part **g**)¹¹⁵. Neddylation (N) has been observed for only a few proteins (part **h**). Interestingly, several ubiquitin-E3 ligases were reported to be neddylated, which provides a close link between neddylation and ubiquitylation events^{36,112}. In addition to neddylation, conjugation with the Ubl ISG15 (I) was shown to interfere in the ubiquitin modification system by modifying an E2. Furthermore, ISGylation regulates the immune response and is inducible by interferons (part **i**).



SKP1–CUL1–F-box ligase complexes

An E3 ligase protein complex that consists of three invariable components: RBX1 (RING-finger protein), CUL1 (scaffold protein) and SKP1 (adaptor protein), and one variable component, the F-box protein.

Nuclear factor κB

A transcription factor that consists of either a homo- or a heterodimer of proteins such as NFκB1, RelA, NFκB2 and RelB.

Ataxia telangiectasia mutated

ATM is a crucial signal-transducing kinase for mediating certain forms of DNA damage such as double-strand breaks. The activation of many proteins has been shown to be ATM-dependent. These include CHK1 and CHK2 kinases, BRCA1, NBS1, and p53, among others.

contain a DNA-binding domain (called the Rel homology domain) through which they interact with the NFκB-binding sites of target genes and a transactivation domain responsible for transcriptional activation. By contrast, NFκB1 and NFκB2 have a DNA-binding domain but lack a transactivation domain. Therefore, to promote efficient gene transcription *in vivo* they either form a heterodimer with RelA, RelB or c-Rel, or recruit the co-activator **BCL3** (B-cell CLL/lymphoma 3), which provides the transactivation domain. A central step in the regulation of NFκB activity is its translocation from the cytoplasm to the nucleus. This process is regulated by several different ubiquitin modifications to various members of the NFκB pathway⁴⁶.

NFκB can be triggered by distinct signalling pathways in response to several extracellular cues such as radiation, inflammation, tumour-necrosis factor-α (TNFα), bacterial lipopolysaccharides (LPS), interleukin 1β and chemotherapeutics, and intracellular cues such as metabolic products causing DNA lesions. Irrespective of the activating agent, or whether the signalling cascade is initiated in the cytoplasm or the nucleus, all but one of the pathways described so far converge on the cytoplasmic

IκB kinase (IKK) complex, which is responsible for the phosphorylation of IκB leading to NFκB liberation and activation. IKK is a multiprotein complex consisting of catalytic subunits (**IKKα** and **IKKβ**) and a regulatory subunit (**IKKγ**) (FIG. 2a). Although IKKα and IKKβ are the catalytically active components of IKK, it is IKKγ that underlies an unorthodox system of post-translational regulation that involves phosphorylation, ubiquitylation and sumoylation^{47–52}. Notably, the type of IKKγ modification is dependent on the mode of IKK activation. For example, the stimulation of cell-surface receptors such as T-cell receptors (TCRs) or TNFα receptors results in the Lys63-linked polyubiquitylation of IKKγ^{47,48}, whereas IKKγ undergoes transient sumoylation in response to DNA-damaging agents or stress conditions such as heat shock and hydrogen peroxide^{51,52} (FIG. 2a). To achieve NFκB activation, the SUMO tag on IKKγ has to be replaced by monoubiquitin. This requires the action of another protein that is induced by DNA double-strand breaks: the protein kinase ATM, which is a well-known regulator of the cell cycle, DNA repair and cell death. ATM binds to nuclear IKKγ and phosphorylates it at Ser85, which is followed by the addition of monoubiquitin⁵³. This

Table 1 | Summary of misregulated proteins involved in ubiquitin or ubiquitin-like regulation

Modifier	Pathway	Deregulated protein	Type of deregulation	Substrate	Modification	Tumour type/disease	Refs
Ubiquitin	Cell cycle	MDM2	SNP in the promotor region	p53	Polyubiquitylation	Non-small-cell lung cancer, soft-tissue carcinoma, colorectal cancer	41,43
		HAUSP	Downregulation	p53, MDM2	De-ubiquitylation	Non-small-cell lung cancer	42
		SCF (SKP2)	Upregulation of SCF	p27 (KIP)	Polyubiquitylation	Malignant melanoma, lymphoma	116,117
		APC		Cyclin B, securin	Polyubiquitylation	Colorectal cancer	118
	DNA repair	FANCL	Defect of PHF9	FANCD2	Monoubiquitylation	Fanconi anaemia-related cancers	119
	NFκB signalling	CYLD	Mutation	IKKγ	De-ubiquitylation	Cylindromatosis	59
		IAP2	Mutation	BCL10	Polyubiquitylation	MALT lymphomas	120
	RTK signalling	CBL	Deregulation	RTKs	Multiple monoubiquitylation	Lymphoma, AML and gastric carcinoma	121
SUMO		SEN1	Chromosomal translocation		De-sumoylation	Infantile teratoma, thyroid oncocyctic adenoma	122,123
	Cell cycle	RB	Mutation	RB	Sumoylation	Retinoblastoma tumour	124
				p14 ^{ARF}	Sumoylation	Melanoma	125
		PIAS3	Upregulation		Sumoylation	Lung, breast, prostate, colon, rectum and brain tumour	126
		PIASy	Downregulation		Sumoylation	Myelodysplastic syndrome	127
		SUMO1	Deregulation		Sumoylation	Anaplastic large-cell lymphoma	128
		SUMO2, UBA2	Deregulation		Sumoylation	Hepatocellular carcinoma	129
Apoptosis	UBC9	Upregulation	BCL2	Sumoylation	Ovarian tumour	106	
NEDD8	NEDD8	Downregulation		Neddylation	Prostate malignancy	130	
FAT10	FAT10	Upregulation		Fatylation	Hepatocellular carcinoma, stomach, intestinal, colorectal, uterine, cervical and ovarian cancer	131	
ISG15	RAR-induced differentiation	UBE1L	Downregulation		ISGylation	Acute promyelocytic leukaemia, lung tumour	93,133
		ISG15	Deregulation		ISGylation	Melanoma	134
		ISG15	Upregulation		ISGylation	Adult T-cell leukaemia, bladder, breast, colorectal, ovarian and prostate cancer	132,135, 136
		UBP43	Upregulation		De-ISGylation	AML	137,138

AML, acute myeloid leukaemia; APC, adenomatosis polyposis coli; CYLD, cylindromatosis; FANCL, Fanconi anaemia, complementation group L; HAUSP, herpes-virus-associated ubiquitin-specific protease; IAP, inhibitor of apoptosis; ISG15, interferon-stimulated gene 15; MALT, mucosa associated lymphoid tissue; NFκB, nuclear factor κB; PIAS3, protein inhibitor of activated STAT3; RAR, retinoic acid receptor; RB, retinoblastoma; RTK, receptor tyrosine kinase; SCF, SKP1-CUL1-F-box; SENP1, sentrin specific peptidase 1; SNP, single nucleotide polymorphism; SUMO, small ubiquitin-related modifier; UBC, ubiquitin-conjugating enzyme; UBE1L, ubiquitin-activating enzyme E1-like; USP, ubiquitin-specific peptidase.

happens at the expense of SUMO because both moieties use the same acceptor lysines, Lys277 and Lys309. Monoubiquitylated IKKγ seems to function as an export vehicle for ATM. Together they exit the nucleus and encounter the cytoplasmic catalytic IKK subunits IKKα and IKKβ that are activated by ATM. Modification of either Lys277 or Lys309 seems to be sufficient to activate this pathway, because single mutation of the residues only slightly weakens sumoylation and ultimately the activation of NFκB, whereas the double mutant is fully inactive⁴⁹. Providing two acceptor lysines might serve the quantitative fine-tuning or the integration of as-yet-unknown

signals that converge on IKKγ. In any case, blocking the sumoylation or ubiquitylation of IKKγ should prevent IKKγ-dependent ATM export and NFκB activation in response to genotoxic stress.

Besides IKKγ, IκB is a direct target of both SUMO and ubiquitin. However, the modifiers of IκB do not function sequentially in a cooperative way but in an antagonistic manner. The activation of IKK leads to the phosphorylation and subsequent polyubiquitylation of IκB lysine residues Lys21 and Lys22, which results in the proteasomal degradation of IκB (FIG. 2b). This event can be counteracted by the sumoylation of Lys21, which leads to the stabilization

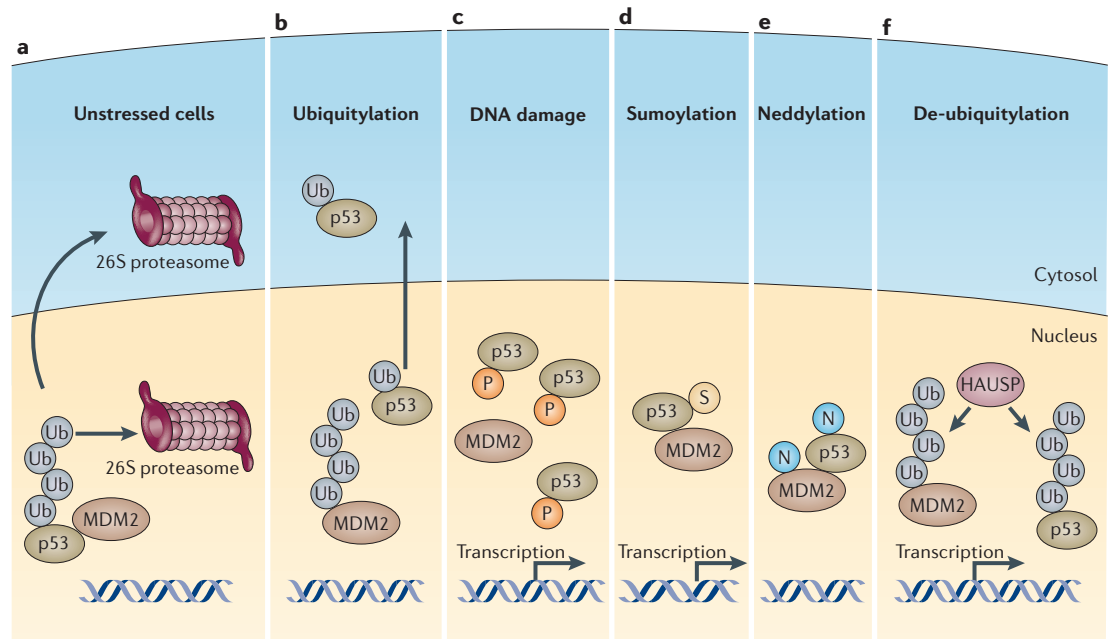


Figure 1 | Different modification states of p53 and MDM2. **a** | In unstressed cells a small amount of the transcription factor p53 is present in the nucleus, so that the level of transcriptional activity is low. This is enabled by two different modification strategies: at high expression levels of MDM2, p53 is polyubiquitylated by MDM2 and degraded. Both proteasomes in the nucleus and in the cytosol can be involved in this degradation process. **b** | On the other hand, MDM2 at low expression levels can monoubiquitylate p53, which subsequently translocates from the nucleus to the cytosol. In the cytosol, p53 monoubiquitylation can be extended to polyubiquitin, which leads to its degradation. It can also be deubiquitylated and shuttled back into the nucleus. **c** | DNA damage activates several kinases, which phosphorylate (P) p53 at its interaction surface with MDM2. As a result, ubiquitylation by MDM2 is blocked and transcriptionally active p53 is stabilized in the nucleus. **d** | Both MDM2 and p53 can be modified by other Ubiquitin-like proteins, although the function of these modifications is not yet clear. Sumoylation (S) seems to be induced by DNA damage, and either increases or decreases the transcriptional activity of p53. **e** | MDM2 was shown to mediate the neddylation (N) of p53, and seems to reduce its transactivation activity. Surprisingly, the induction of DNA damage and subsequent phosphorylation of p53 does not prevent its neddylation but actually increases this modification. **f** | The de-ubiquitylating enzyme herpes-virus-associated ubiquitin-specific protease (HAUSP) is able to catalyse the de-ubiquitylation of both MDM2 and p53, presenting another mechanism to alter the balance between MDM2 and p53, which in turn determines the transcriptional activity of p53. Ub, ubiquitin.

of IκB (FIG. 2c)⁵⁴. However, it remains to be clarified whether ubiquitin and SUMO directly compete for the respective acceptor lysines. Rather than direct competition, another explanation of the stabilizing effect of sumoylation could be that the sumoylated protein is sequestered and therefore made unavailable for ubiquitylation.

Two DUBs that have pivotal roles in NFκB signalling have been identified so far: **A20** and cylindromatosis (**CYLD**). A20 functions in the cytokine-induced (classical) NFκB-activation pathway upstream of the IKK complex^{14,55}, and has both de-ubiquitylating and ubiquitin ligase activity¹⁴. Its ability to edit ubiquitin modifications on signal mediators and the functional relevance of this activity have been reviewed in great detail^{46,56}. A genetic link between A20 and risk of cancer has not been established yet, although elevated levels of A20 are associated with the resistance of cancer cell lines and human breast carcinoma to TNFα-induced apoptosis^{57,58}.

CYLD was identified as a tumour-suppressor gene that was mutated in familial cylindromatosis (characterized by many tumours of the skin appendages)⁵⁹, and is also proposed to de-ubiquitylate components of the

NFκB-signalling pathway⁶⁰ (FIG. 3). Reduced or absent expression of **CYLD** was also observed in human skin tumours such as **basal-cell carcinomas** and **squamous-cell carcinomas**⁶⁰, as well as in several other tumours such as **kidney, liver** and **uterine cervix**^{61–63}, which suggests that **CYLD** has a more general role as a tumour suppressor. In a small-interfering-RNA (siRNA) screen that targeted DUBs that were implicated in cancer-related pathways, **CYLD** was identified as a regulator of NFκB⁶⁴. The ubiquitously expressed **CYLD** contains a C-terminal ubiquitin hydrolase domain that removes Lys63-linked polyubiquitin from several mediators of the classical cytokine-induced NFκB pathway, including TNF receptor associated factor 2 (**TRAF2**)^{65–67}, TNF receptor associated factor 6 (**TRAF6**)⁶⁶ and **IKKγ**⁶⁵. However, the analysis of **CYLD**^{-/-} mice led to the identification of **BCL3** as a crucial *in vivo* target of **CYLD** in the development of cylindromatosis-related skin tumours in mice⁶⁰. **BCL3** is a transcriptional co-activator, and is also referred to as non-inhibitory or atypical IκB. As such it associates with NFκB1 or NFκB2 homodimers (both of which lack transactivation domains), but not

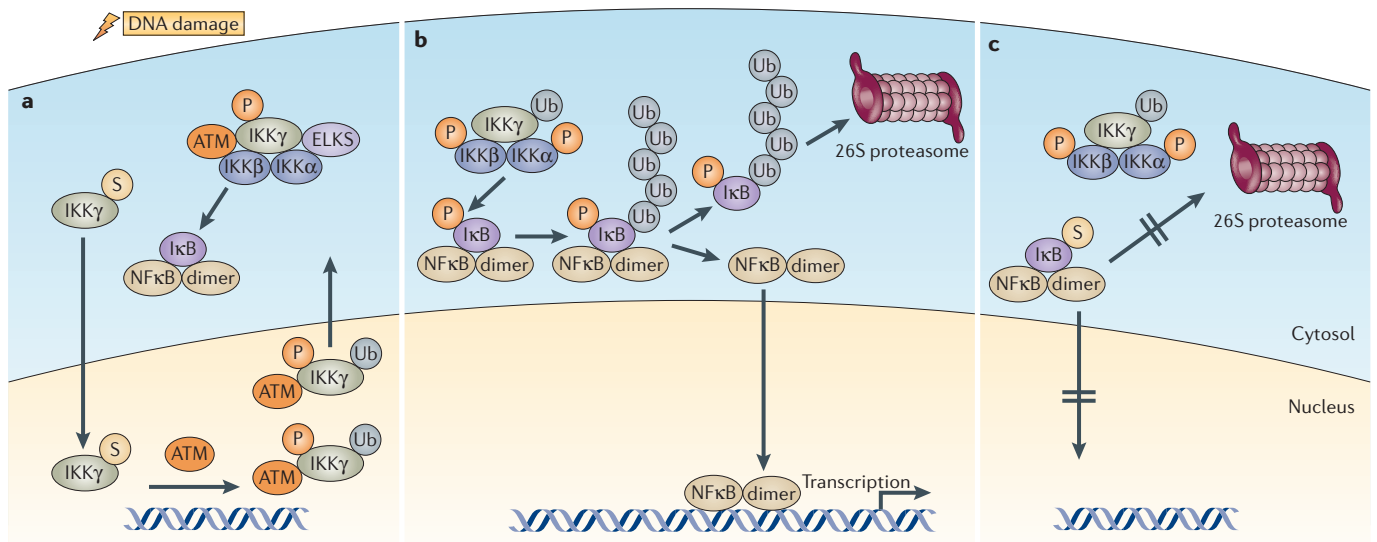


Figure 2 | Ubiquitin and Ubl modifications in the NFκB pathway. a | After DNA damage IKKγ is sumoylated (S), which promotes its nuclear localization. The kinase ATM (ataxia telangiectasia mutated) is activated independently of the sumoylation of IKKγ. ATM catalyses the phosphorylation (P) of nuclear IKKγ, which is followed by the attachment of ubiquitin to IKKγ. Ubiquitylated IKKγ translocates to the cytoplasm with ATM, where it associates and activates the IκB kinase (IKK) complex, which consists of IKKα, IKKβ and IKKγ. Recent reports have shown that another IKK regulator, a protein rich in glutamate, leucine, lysine and serine (ELKS) is recruited to and activates the IKK complex⁵³. **b** | Various stimuli, such as tumour-necrosis factor-α (TNFα), interleukin 1, lipopolysaccharide or DNA damage, can induce the nuclear factor κB (NFκB) pathway. All different stimuli result in the activation of the IKK complex. IKK phosphorylates IκB, which is subsequently polyubiquitylated with Lys48-linked chains and degraded by the 26S proteasome. NFκB is then able to translocate to the nucleus and activate the transcription of several genes that are crucial for inflammatory reactions, immune response or apoptosis. **c** | Alternatively, IκB might be sumoylated at the same lysine that is used for the attachment of the polyubiquitin chain. This impairs the degradation of IκB and subsequent translocation of NFκB to the nucleus.

RelA–NFκB1 heterodimers, which are downstream of the classical inflammation-induced NFκB pathway. Cytoplasmic BCL3 is inactive and requires Lys63–polyubiquitin to enter the nucleus⁶⁰. Nuclear association with BCL3 switches NFκB1 or NFκB2 from a repressive to a transcriptionally active state and promotes the expression of target genes including **cyclin D1**, which promotes proliferation. Treatment with 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and UVB light promote the de-ubiquitylation of BCL3 by CYLD, thereby limiting the extent of nuclear translocation (FIG. 3). In accordance with this mechanism, CYLD-deficient animals show an increased susceptibility to large skin tumours, which are the result of increased tumour cell proliferation.

Translesion synthesis. Despite the existence of elaborate repair systems that are able to iron out many types of DNA damage, the cell is often forced to replicate across lesions to avoid the stalling of replication. This is accomplished through a process known as translesion DNA synthesis (TLS), during which sites of base damage are bypassed by specialized TLS polymerases that are capable of replicating damaged DNA at the expense of the induction of mutations. It is thought that TLS is probably responsible for the carcinogenic properties of DNA damage. This capability is due in part to a loose and flexible active site, which makes the polymerases highly mutagenic. Remarkably, although this system is

inherently mutagenic, its mutation rate is sufficiently tolerable to protect us against cancer.

During DNA replication, unmodified PCNA (proliferating cell nuclear antigen) functions as a processivity factor for replicative polymerases. PCNA is monoubiquitylated at the highly conserved Lys164 in response to certain types of DNA damage^{68–71} (BOX 3). This modification is recognized by the recently identified UBDs ubiquitin-binding motif and ubiquitin-binding zinc finger (UBM and UBZ) that are present in TLS polymerases^{72,73}, and leads to the accumulation of the low-fidelity polymerases POLI or POLη at stalled replication forks, where they displace the high-fidelity polymerase POLδ. POLI and POLη contain an additional PCNA interaction site (PIP motif), but only the presence of an intact UBD and ubiquitylated Lys164 enables efficient interaction with PCNA and therefore TLS⁷². After the lesion has been bypassed it is necessary for the cell to replace the error-prone TLS polymerases again with POLδ to ensure accurate DNA replication. The details of this switch-back mechanism are not yet fully understood, but it is probable that the de-ubiquitylation of PCNA contributes to the process⁷⁴.

POLI and POLη are also monoubiquitylated *in vivo* in a process that requires their intact UBDs⁷². This process is known as ‘coupled monoubiquitylation’ owing to the fact that UBDs are required for both binding to ubiquitin and the monoubiquitylation of the host protein, a common

UBM and UBZ
Ubiquitin-binding motif (UBM) and ubiquitin-binding zinc finger (UBZ) were recently discovered to be involved in the TLS response. UBMs do not bind to the hydrophobic Ile44 patch of ubiquitin.

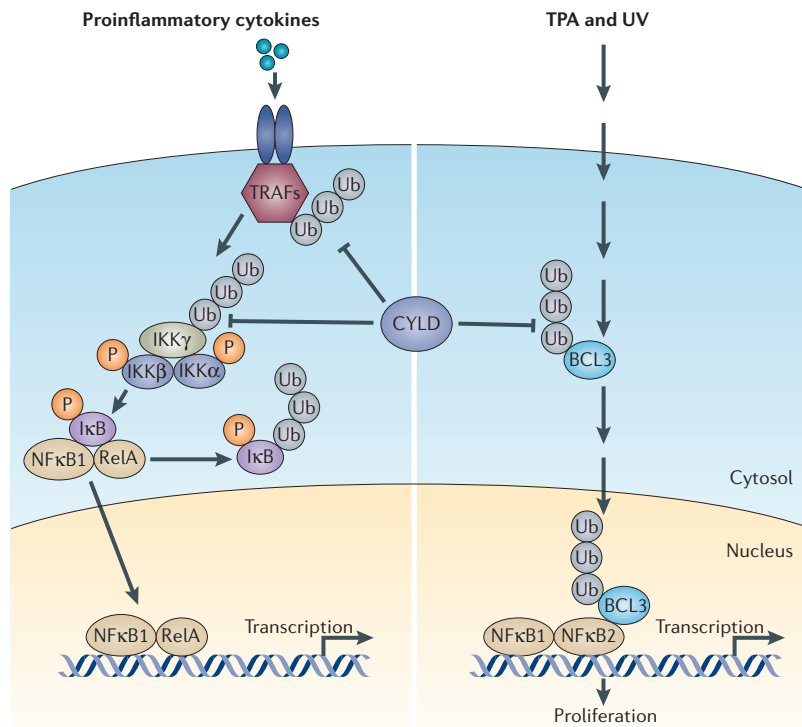


Figure 3 | CYLD interferes at different steps of the NFκB pathway depending on the stimulus received by the cell and the cell type. In the classical pathway (left side) nuclear factor κB (NFκB) is activated by various inflammatory signals such as tumour-necrosis factor-α (TNFα), interleukin 1 or lipopolysaccharides. The activation of this pathway leads to the Lys63-linked polyubiquitylation of TRAFs (TNF receptor associated factors) and IKKγ, and consequently the activation of the IKK complex. IKK phosphorylates IκB, which is then polyubiquitylated and degraded by the 26S proteasome. The unbound NFκB dimer shuttles to the nucleus and activates transcription. Cyldromatosis (CYLD) deubiquitylates TRAFs and IKKγ, which is part of the IKK complex. Therefore, CYLD inhibits the action of the IKK complex and the transcriptional activation of NFκB. The treatment of CYLD-deficient mice with UV light or 12-O-tetradecanoylphorbol-13 acetate (TPA) induces the development of tumours of hair follicle keratinocytes, which mimics the pathogenesis of familial cylindromatosis. In keratinocytes (right side) it is not the classical pathway that is affected by CYLD, but the BCL3-linked pathway. Stimulation with TPA or UV light induces the polyubiquitylation of BCL3, which is required for its nuclear translocation, and BCL3 and NFκB1- and NFκB2-dependent proliferation. Under normal conditions CYLD deubiquitylates BCL3, and therefore inhibits its translocation to the nucleus and transcriptional activation of proliferative genes. However, in CYLD-deficient mice this negative-regulatory mechanism is missing and cells are hyperproliferative.

phenomenon for many ubiquitin-binding proteins^{75–78}. Although its function has long been obscure, recent findings indicate that monoubiquitylation provokes the intramolecular binding of the UBD to the attached ubiquitin, thereby efficiently inactivating the ability of these proteins to interact with ubiquitylated targets *in trans*⁷⁸. The exact timing and the physiological significance of Pol-ubiquitylation remains to be studied in detail, but it is conceivable that it might have a role in discharging TLS polymerases from ubiquitylated PCNA after having bypassed the lesion and/or preventing their recruitment under certain physiological conditions, thereby creating an inactive but ready-to-use pool of TLS polymerases.

The UV-induced monoubiquitylation of PCNA provides the signal for switching from high-fidelity

replicative polymerases to TLS polymerases^{69,70}. But how does the cell control PCNA ubiquitylation to avoid the excessive deployment of POLη or even more error-prone TLS polymerases like POLι? Insights into this process were gained from the discovery that ubiquitin specific peptidase 1 (USP1) functions as a DUB for PCNA⁷⁴. In fact, cells in which *USP1* expression has been reduced by siRNA show increased PCNA monoubiquitylation in the absence and presence of DNA damage and a higher frequency of both spontaneous and damage-induced mutagenesis. Strikingly, the stability of USP1 is regulated by UV light that initiates USP1 self cleavage. In this way, constitutive PCNA de-ubiquitylation by USP1 is halted and the monoubiquitylated form can accumulate to recruit TLS polymerases. It remains to be determined exactly how UV treatment causes USP1 to cease from functioning on PCNA and target itself instead.

The dark side of the low-fidelity TLS polymerases is shown in patients who suffer from a variant of Xeroderma pigmentosum (XPV), a UV-induced skin tumour syndrome. XPV is caused by mutations in the TLS polymerase *POLη* gene, which result in a truncated inactive protein^{79,80}. In the absence of *POLη*, TLS is believed to be catalysed by different (but as yet undetermined) polymerase(s) that incorporate(s) incorrect nucleotides, generating the mutations that promote skin cancer. In culture conditions, fibroblasts isolated from XPV patients show increased sensitivity to toxic effects, particularly UV light irradiation⁸⁰. The ubiquitin-binding ability of overexpressed *POLη* in these cells is essential to overcome sensitivity and promote the TLS response⁷².

Besides the direct removal of DNA lesions by error-prone TLS polymerases, the eukaryotic cell has developed an error-free damage-avoidance pathway that bypasses the lesion instead of repairing it^{81,82} (BOX 3). The mechanism that underlies this pathway is not fully understood, but it has been recently shown that the assembly of Lys63-linked polyubiquitin chains on Lys164 of PCNA guards against TLS-induced mutations⁸³. Specifically, it has been shown that cells that are unable to form Lys63-linked polyubiquitin chains are more sensitive to UV-induced mutations. This effect was associated with an increased recruitment of TLS polymerases to foci that contained PCNA in irradiated cells⁸³.

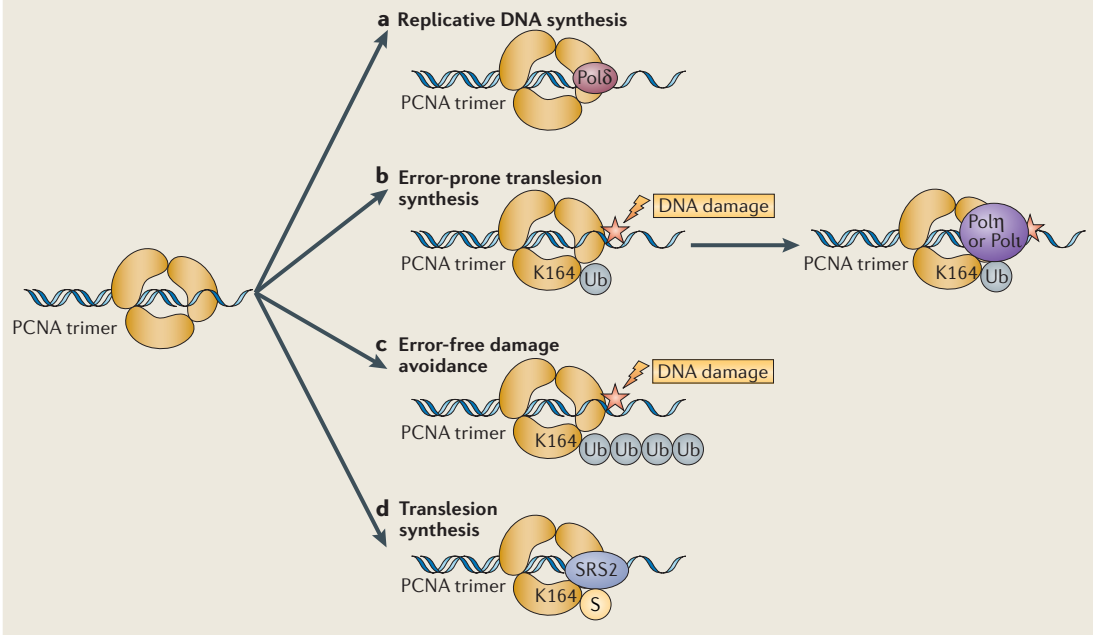
The role of Lys164 of PCNA is further expanded in yeast, as it is not only targeted by mono- and polyubiquitylation, but can be alternatively modified with SUMO⁸⁴. Although controversial, a competitive mechanism seemed to be self-evident to account for the negative effect on ubiquitin-dependent TLS, as both modifications target the same acceptor lysine. It has now been shown that during S-phase SUMO-modified PCNA recruits the anti-recombinogenic DNA helicase SRS2, which prevents the assembly of the homologous recombination machinery^{84–86}. In turn, this effect seems to pave the way for ubiquitin-dependent damage bypass that would otherwise be less favourable. Therefore, although SUMO and ubiquitin modify the same lysine of PCNA in yeast, they do not act antagonistically but rather cooperatively to coordinate the appropriate response to DNA damage (BOX 3).

Box 3 | Translesion synthesis polymerases

During DNA replication, induced or spontaneous lesions will result in stalled replication forks. Because the genome needs to be fully replicated before mitosis, it is essential that cells have mechanisms for dealing with these stalled replication forks. This can occur by either an error-prone or error-free damage-tolerance pathway. The error-prone pathway involves low-fidelity translesion synthesis (TLS) polymerases, which incorporate any base opposite the lesion⁶⁸ so that replication can continue. The recruitment of the determined polymerase is dependent on the modification status of PCNA (proliferating cell nuclear antigen). During DNA replication, unmodified PCNA functions as a processivity factor for replicative polymerases (POL δ) (part a). When DNA damage occurs replication is stalled, and PCNA is monoubiquitylated. Monoubiquitylation activates TLS by recruiting Y-family polymerases (POL η or POL ι) to the ubiquitin (Ub) moiety (part b). By contrast, the less-well understood error-free pathway (part c) requires the assembly of Lys63-linked ubiquitin chains on Lys164 of PCNA, which decreases the use of TLS polymerases.

Y-family polymerases have similar structures and include catalytic sites, and either a ubiquitin-binding motif (UBM) or a ubiquitin-binding zinc-finger (UBZ) at the C terminus, by which they are able to bind to monoubiquitylated PCNA at stalled replication forks⁷². Furthermore, members of the Y-family polymerases have been shown to be monoubiquitylated themselves. Their UBM or UBZ can therefore bind to the ubiquitin moiety intramolecularly.

In yeast, Lys164 of PCNA has also been shown to undergo sumoylation (part d). PCNA is sumoylated in S-phase independently of DNA damage, and results in the recruitment of the helicase SRS2. This mechanism seems to set the course for TLS as it blocks homologous recombination.



Y-family polymerases

These are translesion synthesis (TLS) polymerases of low fidelity, such as POL ι , POL η , POL κ and REV1, which are involved in the error-prone DNA-repair pathway.

Wnt

The family of secreted cysteine-rich glycoproteins that act as short-range ligands to locally activate receptor-mediated pathways.

KAI1

A member of the tetraspanin family that is capable of inhibiting the progression of tumour metastasis without affecting primary tumorigenicity.

Cancer-relevant pathways affected by Ubls

Different ubiquitin-like modifiers are also implicated in cancer pathogenesis (TABLE 1). The sumoylation of transcription factors, cofactors or proteins that are involved in chromatin remodelling have been shown to modulate transcriptional activity⁸⁷ and to regulate signalling pathways, such as the Wnt pathway, which is linked to various human cancers such as colon, hepatocellular carcinoma, leukaemia and melanoma^{88,89}. After stimulation with the Wnt ligand, β -catenin enters the nucleus and recruits a chromatin-remodelling complex to activate transcription⁹⁰. Among other proteins, reptin was identified to be a part of the β -catenin chromatin-remodelling complex⁹¹. Reptin was shown to be modified by SUMO, which represses the expression of the metastasis-suppressor gene KAI1 and results in the promotion of tumour metastasis⁹². The abrogation of SUMO modification of reptin by the downregulation of SUMO E2 enzyme ubiquitin conjugating enzyme (UBC9), or the overexpression of

the desumoylating enzyme sentrin-specific protease 1 (SENPI), promotes the expression of KAI1 and therefore inhibits the invasive activity of cancer cells⁹².

In addition, enzymes that are involved in ISG15 conjugation, such as the ubiquitin-activating enzyme E1-like protein (UBE1L), or de-ISGylation, such as UBP43, are affected in lung cancers⁹³ and acute promyelocytic leukaemia (APL)⁹⁴. APL is a distinct subtype of myeloid leukaemia that is characterized by the accumulation of tumour cells that are blocked at the promyelocytic stage of myeloid maturation. It is associated with a reciprocal translocation between chromosomes 15 and 17, which results in the expression of fusion proteins like promyelocytic leukaemia (PML)–retinoic acid receptor alpha (RAR α)⁹⁵. This fusion protein functions as a dominant-negative inhibitor of several retinoid-dependent signalling pathways, and results in the blockage of cells in an immature stage of differentiation. Pharmacological doses of all trans retinoic acid (RA) can overcome this

block through the recruitment of a co-activator complex. This, in turn, leads to the activation of RA target genes that trigger terminal myeloid differentiation⁹⁶ and other biological effects that lead to clinical remissions in APL. The expression of *UBE1L* mRNA is reduced in leukaemic cells and increased after treatment with RA. Upregulation of *UBE1L* after RA treatment results in the degradation and, consequently, the downregulation of the PML–RAR α fusion protein⁹⁴. As ISG15 or enzymes involved in conjugation with ISG15 are deregulated in various types of cancers (TABLE I), their detailed functions and potential tumour-suppressor effects need to be further elucidated.

Targeting ubiquitin in drug discovery

Although most attempts to identify new drugs for cancer treatment have concentrated on protein phosphorylation and the development of kinase inhibitors, there is an increasing interest in therapeutically exploiting modulators of the ubiquitin system. The biggest success so far in targeting ubiquitin-dependent processes has been the development of bortezomib, the first proteasome inhibitor to be approved for clinical use in human cancers⁹⁷. Bortezomib is a targeted small molecule that inhibits the active site of one of the proteasome subunits. Rather than inhibiting a selected signalling molecule, it shuts down the entire proteasomal protein degradation system⁹⁷. Bortezomib is clinically used for the treatment of relapsed multiple myeloma, and it shows anti-tumour activity against **non-Hodgkin lymphoma**⁹⁷. Surprisingly, bortezomib shows selective cytotoxicity to cancer cells compared with normal cells. Why it has the observed beneficial and selective anticancer effects is only vaguely understood. Nevertheless, the successful story of bortezomib has been driving investment in the next generation of ubiquitin-pathway anticancer therapeutics, which are expected to be more specific.

Increasing knowledge of the selective roles of ubiquitin ligases and their links to malignancies has spurred considerable interest in modulating their activity as a more specific means of developing anticancer therapeutics. There are currently two main strategies for interfering with their functions. On one hand, small-molecule inhibitors are designed to selectively block the catalytic activity of enzymes, whereas others are designed to prevent the interaction between ligases and specific substrates. Among targets with the most promise are E3 ligases that are involved in the regulation of apoptosis or the cell cycle. In this respect, progress has been made in the discovery of MDM2 ligase inhibitors, which target the p53 pathway. The small molecule HLI98 has been designed to inhibit the E3 ligase activity of MDM2. It selectively blocks p53 ubiquitylation by targeting the RING-finger domain of MDM2 but not the N-terminal part, which mediates binding to p53 (REF. 98). Despite significant concerns about the similarity among RING fingers and the level of similarity in E2-interaction sites between different E3 ubiquitin ligases, the example of HLI98 serves as a proof-of-principle of selectively targeting ubiquitin ligases. It is also a promising start for the development of related inhibitors with improved clinical parameters.

In addition, small-molecule inhibitors such as the Nutlins and RITA (reactivation of p53 and induction of tumour cell apoptosis) have been identified to target protein–protein interactions between p53 and MDM2 (REFS 99,100) rather than influence their enzymatic function. The Nutlins are cis-imidazoline derivatives that bind MDM2 in the p53-binding pocket, and show strong anti-tumour effects in mice with surprisingly few side effects after oral administration¹⁰¹. RITA originally scored as a hit in a functional chemical library screen designed to identify compounds that specifically arrest the growth of a p53-positive cancer cell line¹⁰². RITA does not seem to bind MDM2, but binds the N terminus of p53. This seems to either prevent the recognition of p53 by MDM2 or to stabilize the N-terminal α -helix domain of p53 in the MDM2 pocket or both¹⁰⁰. However, in nuclear magnetic resonance chemical-shift-perturbation experiments RITA did not block the formation of a p53–MDM2 complex *in vitro*, but Nutlins did¹⁰³. Both inhibitors induce p53-dependent apoptosis in human cancer cells, which suggests that MDM2 antagonists might have clinical use in the treatment of tumours with wild-type p53. Current efforts in the field are focusing on the discovery of specific inhibitors that target other E3 ligases, deubiquitylating enzymes, the sites of interaction of E3 ligases with their cognate E2s and inhibitors of E1¹⁰⁴. These approaches are not exclusive for ubiquitin, but are rather being pioneered as a platform for the development of drugs that also target Ubl modifiers. For example, there are a number of approaches that target UBC9, the E2 enzyme for sumoylation that is upregulated in a large number of human tumours^{105,106}.

The most promising and most challenging approach is to therapeutically interfere with the ubiquitin- and Ubl-driven protein–protein interactions in cells. It is believed that targeting protein–protein interactions will increase the specificity of drug action, as there are many more modification-driven interactions than existing enzymes. It is, however, well known that targeting protein–protein interfaces is a complex and difficult task, and targeting the ubiquitin and Ubl interactomes is not an exception to this rule. The main difficulties include the typical flatness and hydrophobic nature of the binding surface between ubiquitin and UBDs, and the low affinity that is achieved through many interactions. Significant progress has been made recently in identifying small-molecule inhibitors of several protein–protein interaction systems (REFS 107,108 and D. Vucic, personal communication). The major breakthrough was based on findings that the binding energy of protein–protein interactions is localized around a small number of amino-acid residues, so-called ‘hot spots’¹⁰⁹. Good targets for small-molecule inhibition are those that have small hot spots that can be covered by a drug-sized molecule, and perhaps those hot spots that have been shown to bind to small peptides¹⁰⁹. In the case of ubiquitin–UBD interactions, a small molecule could be an effective and potent inhibitor if it mimicked the binding of UBDs to the hydrophobic surface centred around Ile44 on ubiquitin, which is the required surface for binding most UBDs. Although targeting

All trans retinoic acid
A form of vitamin A used as a drug for the treatment of acute promyelocytic leukaemia.

ubiquitin and Ubl interactomes is still in its infancy, the progress that has been made in the field and the importance of this modification will ensure that this topic receives increased attention in the near future.

Conclusions and future perspectives

Ubiquitin and Ubl modifiers are crucial signals that control many biological processes — from cell proliferation to programmed cell death. They function in pathologies such as inflammation and cancer, and therefore represent an important class of targets for human therapeutics. Current efforts are focused on understanding how the ubiquitin-linked pathways participate in the aetiology of different tumour types. By targeting ubiquitin pathways in malignancies one

could, as has been shown with bortezomib, develop promising tools for anticancer therapies. However, it is becoming obvious that targeting several steps in oncogenic pathways is required to overcome the robustness of malignant cells. One of the challenges in the development of successful anticancer strategies will be to account for the distinct roles of ubiquitin and Ubl pathways and their cross-talk with other post-translational modifications, notably protein phosphorylation, during malignant transformation and metastasis. A combinatorial use of inhibitors of protein kinases with newly developed anti-ubiquitin drugs holds the promise of targeting cancer cells through several means, which should result in more effective therapy.

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Competing interests statement

The authors declare no competing financial interests.

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