RINGs of good and evil: RING finger ubiquitin ligases at the crossroads of tumour suppression and oncogenesis

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Abstract | The ubiquitin-proteasome system has numerous crucial roles in physiology and pathophysiology. Fundamental to the specificity of this system are ubiquitin-protein ligases (E3s). Of these, the majority are RING finger and RING finger-related E3s. Many RING finger E3s have roles in processes that are central to the maintenance of genomic integrity and cellular homeostasis, such as the anaphase promoting complex/cyclosome (APC/C), the SKP1–cullin 1–F-box protein (SCF) E3s, MDM2, BRCA1, Fanconi anaemia proteins, CBL proteins, von Hippel–Lindau tumour suppressor (VHL) and SIAH proteins. As a result, many RING finger E3s are implicated in either the suppression or the progression of cancer. This Review summarizes current knowledge in this area.

Oncogenes
Genes with protein products that can promote cancer development. Oncogenes frequently undergo amplification or activating mutations and act in a genetically dominant manner.

Tumour suppressor genes
(TSGs). Genes whose protein products, when lost or mutated, are permissive for the development of cancer. TSGs frequently undergo deletion or inactivating mutations of both alleles and act in a genetically recessive manner.

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Consequent to the determination that many RING finger-containing proteins are ubiquitin-protein ligases (E3s)[1], and therefore the primary determinants of substrate specificity in ubiquitylation (also known as ubiquitination) (BOXES 1,2,3), it has become evident that many RING finger E3s are implicated in malignancy. Oncogenic transformation is characterized by dysregulated cell growth signals that lead to limitless autonomous proliferation, insensitivity to anti-growth or pro-apoptotic signals, dysregulation of the cell cycle and genomic instability. Solid tumours also acquire the ability to induce angiogenesis, which allows for the expansion of the primary tumour and facilitates metastasis[2]. RING finger E3s are implicated in all of these steps. Some are bona fide oncogenes, whereas others are products of tumour suppressor genes (TSGs). However, a single E3 can also have opposing functions in malignancy owing to multiple substrates or multiple roles of a single substrate (TABLE 1; and below).

We focus below on specific RING finger E3s that are of particular therapeutic interest because of their roles in signalling, maintaining genomic stability and responses to hypoxia; a more comprehensive listing of the RING finger E3s that are associated with cancer is provided in TABLE 1.

RING fingers and the genome

A hallmark of cancer is altered genomic stability. This can manifest in ways that include a failure to repair mutations or other DNA damage, as well as gross chromosome abnormalities, including aneuploidy.

Cell cycle. The cell cycle must proceed in an orderly manner to ensure genomic integrity and to prevent dysregulated proliferation. Fundamental to this is the precisely timed degradation of key regulators, including cyclins, cyclin-dependent kinase inhibitors, securin and transcription factors such as MYC and JUN. Two multisubunit RING finger ubiquitin ligase families are crucial to cell cycle progression and regulation[3–6]. The anaphase-promoting complex/cyclosome (APC/C) consists of at least 13 subunits. Although the APC/C is substantially more complex, it shares properties with the cullin RING ligase (CRL) (BOX 3) superfamily, including a cullin-like subunit at its core, a small RING finger protein and interchangeable substrate recognition subunits. Two substrate recognition elements have been identified for the APC/C: cell division cycle 20 (CDC20) and CDH1 (also known as FZR1). APC/C^{CDC20} and APC/C^{CDH1} are sequentially activated, with APC/C^{CDC20} active from prometaphase to telophase and APC/C^{CDH1} primarily active in G1 phase of the cell cycle. From late G1 until prometaphase the APC/C is inactive. The regulation of the APC/C is complex and includes temporal ubiquitin-mediated degradation of CDC20, CDH1 and an E2 UBCH10 (also known as UBE2C, UBCX and UBCC), which is one of several used by the APC/C. Also contributing to the regulation of the

At a glance

- RING finger ubiquitin-protein ligases (E3s) are the most abundant class of E3 that mediate protein ubiquitylation (also known as ubiquitination). They regulate crucial cellular functions, such as the cell cycle, DNA repair, cell signalling and responses to hypoxia. Genetic alterations, including activating and inactivating mutations, gene amplifications, translocations and deletions, have been described for many RING finger E3s. RING finger E3s are validated oncogenes (such as *MDM2*) or tumour suppressor genes (such as *BRCA1* and von Hippel–Lindau tumour suppressor (*VHL*)) because of their role in regulating crucial cell functions.
- The cell cycle is regulated by the S phase kinase-associated protein 1 (SKP1)–cullin 1 (CUL1)–F-box protein (SCF) and anaphase-promoting complex/cyclosome (APC/C) multisubunit RING finger E3s. These complexes are targeted to specific substrates via interchangeable substrate recognition subunits, including F-box proteins for SCF and cell division cycle 20 (CDC20) and CDH1 for APC/C. These multisubunit E3s have a large number of substrates with oncogenic and tumour suppressive effects. Genetic alterations to components of these E3 complexes that result in loss of function (such as FBW7, CDH1 and CDC20) or gain of function (such as SKP2 and β-transducin repeat-containing protein (β-TrCP)) are implicated in the development of cancer.
- RING finger E3s have central roles in DNA damage responses and DNA repair. For example, MDM2 targets p53 for degradation. *MDM2* is amplified, overexpressed or activated in other ways in cancers and is a means of inactivating the tumour suppressor p53. The BRCA1 and the Fanconi anaemia (FANC) E3s have essential roles in the repair of DNA damage; both E3s function as tumour suppressors.
- RING finger E3s have important roles in both positively and negatively regulating signal transduction. A prominent example of negative regulation is the CBL family of RING finger E3s that target activated receptor tyrosine kinases (RTKs) for degradation. Mutations that inactivate CBL E3 function have been described in myeloid neoplasms and result in the hyperactivation of RTKs and intracellular signalling pathways.
- The response to hypoxia is regulated by the multisubunit $CRL2^{VHL}$ RING finger E3 and the single subunit RING finger E3 SIAH. The VHL complex targets the hypoxia-inducible factor-α (HIFα) transcription factors for proteasomal degradation, which prevents the expression of angiogenic and growth-promoting genes under normoxic conditions. Inactivating mutations of VHL are found in familial and sporadic clear cell cancer of the kidney, resulting in the stabilization of the HIFα transcription factor subunits and consequently abnormally high expression of angiogenic and growth genes. By contrast, the SIAH RING finger E3s stabilize HIFα under hypoxic conditions.
- Targeting RING finger E3s for the treatment of cancer is being actively explored. For example, small-molecule inhibitors have been developed that interfere with the MDM2–p53 interaction or that inhibit MDM2 E3 activity, thus stabilizing p53. These approaches have demonstrated antitumour activity in preclinical studies, but the clinical efficacy of interfering with MDM2 function remains to be determined. Targeting the loss of activity of RING finger E3s that are tumour suppressors will require novel approaches such as the synthetic lethality that is induced by poly(ADP-ribose) polymerase (PARP) inhibition in cells that are deficient in BRCA1 or BRCA2.

Aneuploidy
Abnormal number of chromosomes resulting in more or less than the normal diploid number of chromosomes. Cancer cells are frequently aneuploid.

Cyclins
Proteins that control the progression of the cell cycle by activating cyclin-dependent kinases.

Securin
A protein that forms a complex with separase and thereby inhibits separase activity and prevents chromosome separation at anaphase. Securin is dephosphorylated and degraded by APC/C^{CDC20} at the onset of anaphase.

Haploinsufficient TSG
A gene for which the loss of one allele is sufficient to promote cancer development.

Amplification
Increased copy number of a gene within the genome; this is a common mechanism to increase the activity of oncogenes.

APC/C is the phosphorylation-dependent degradation of the APC/C pseudosubstrate, early mitotic inhibitor 1 (EMI1; also known as FBX05) (discussed below)[6].

Hemizygous frameshift and point mutations in several subunits of APC/C have been found in colon cancer cell lines and tumours[7]. Overexpression studies of one mutant subunit suggested that APC/C mutations can act in a dominant-negative manner to inhibit function and to cause the inappropriate progression of the cell cycle[7]. Also, altered APC/C function can lead to genomic instability owing to decreased degradation of securin or cyclin B1 and cyclin B2 by APC/C^{CDC20} and can consequently lead to aberrant chromosome segregation[8]. In addition, CDH1-deficient mice ($Fzr1^{-/-}$ mice) display genomic instability, and $Fzr1^{+/-}$ mice develop epithelial tumours, supporting a role for *FZR1* as a haploinsufficient TSG[9].

A second ligase family that has been strongly implicated in cell cycle regulation is the S phase kinase-associated protein 1 (SKP1)–cullin 1 (CUL1)–F-box protein (SCF) family of the CRL superfamily, in which each of up to 69 F-box proteins (in humans) can potentially serve as substrate recognition elements (BOX 3), although less than 20% of these currently have defined substrates[5]. The activity of SCF E3s towards substrates during the cell cycle can be regulated by the level of the F-box protein, as is the case for SKP2. SCF^{SKP2} targets the G1/S cyclin-dependent kinase inhibitor p27 for proteasomal degradation. Therefore, increased SKP2 levels would be predicted to result in a failure to appropriately control the G1/S checkpoint and would also be predicted to be oncogenic[3]. Indeed, amplification of *SKP2* is found in multiple carcinomas[5]. For some F-box proteins, including both F-box/WD repeat-containing protein 7 (FBW7) and β-transducin repeat containing protein (β-TrCP; also known as FBW1A), the regulation of activity occurs by substrate phosphorylation. SCF^{FBW7} targets substrates that promote cell cycle progression, and therefore proliferation, including cyclin E, MYC, JUN and NOTCH. Consistent with this, *FBW7* is a TSG and loss of FBW7 function is seen in a number of carcinomas[3,10]. $SCF^{β\text{-}TrCP}$ has a substantial number of phosphorylation-dependent substrates and has a more complex role in the cell cycle and in tumorigenesis. $SCF^{β\text{-}TrCP}$ targets the APC/C inhibitor EMI1 during late G2, thereby relieving one level of APC/C inhibition. Among its other substrates are proteins that either oppose (for example, IκB and WEE1) or promote (for example, CDC25 and β-catenin (see below)) cell cycle progression and proliferation. In addition, if Fanconi anaemia group M protein (FANCM) is not degraded before mitosis by $SCF^{β–TrCP}$, then this can lead to inappropriate activity of the FANC ubiquitin ligase (see below) and can result in chromosome abnormalities. $SCF^{β–TrCP}$ also targets the transcriptional repressor RE1 silencing transcription factor (REST; also known as NRSF), which can potentially

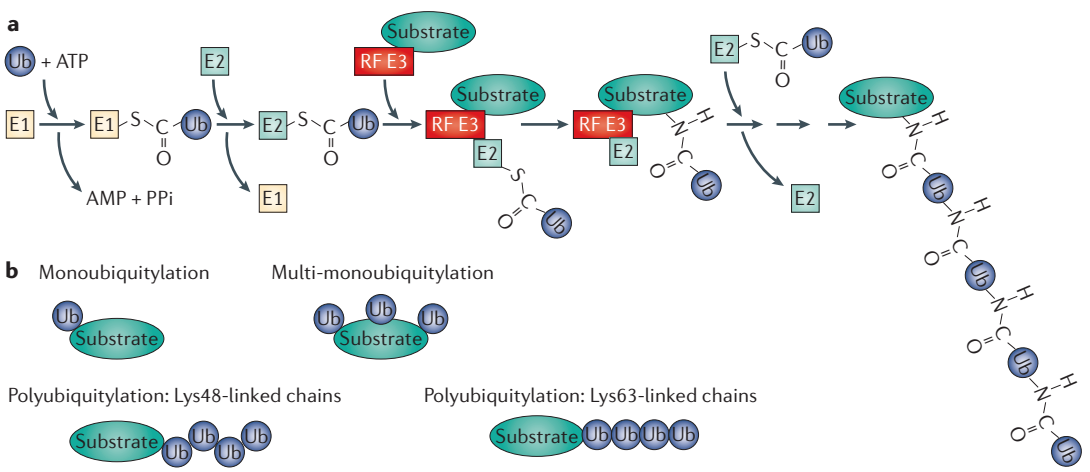
function as either a tumour suppressor or an oncogenic protein[4,11,12]. Additionally, $SCF^{\beta\text{–}TrCP}$ targets multiple pro-apoptotic proteins for degradation (such as BCL2L11 (also known as BIMEL), NF-κB inhibitor-α (IκBα), IκBβ and programmed cell death protein 4 (PDCD4))[5,13]. Given the diverse roles of its targets, $SCF^{\beta\text{–}TrCP}$ can primarily function as an oncogene as a consequence of its pro-survival roles, and it could also potentially function as a tumour suppressor in a context-dependent manner. The E3 activity of many SCF E3s, including $SCF^{\beta\text{–}TrCP}$, towards substrates is regulated by serine and threonine phosphorylation of the substrate. For this reason, whether a particular SCF E3 functions as an oncogene or a tumour suppressor may be determined by what substrates are phosphorylated in a particular cell.

MDM2: keeping the 'guardian of the genome' in check. The cellular response to genomic damage is largely mediated by the tumour suppressor p53, which blocks cell proliferation by cell cycle arrest or which can induce apoptosis, particularly in transformed cells. p53 is best characterized as a transcription factor, although there is increasing evidence for cytoplasmic roles for this protein[14]. Its importance is underscored by the discovery of mutations that inactivate p53 in up to 50% of human cancers and the finding that cellular alterations that suppress p53 activity are present in many other cancers[15].

The RING finger E3 and oncoprotein MDM2 (also known as HDM2 in humans) is a major regulator of p53 that is under the direct transcriptional control of p53 (REFS 16,17). MDM2 binds directly to p53 and targets itself and p53 for ubiquitylation and proteasomal degradation[18–22] (FIG. 1). Amplification or overexpression of *MDM2* and mutations in p53 represent an alternative means of escaping growth control in cancer[16]. The relationship between MDM2 and p53 was established by early embryonic lethality in *Mdm2*$^{-/-}$ mice and their rescue by crossing with *Trp53*$^{-/-}$ mice[23,24]. The sensitive nature of the p53–MDM2 relationship is underscored by the decrease in tumour formation in mice with

Box 1 | **Ubiquitylation by RING finger E3s**

Ubiquitylation is a multienzyme process (see part **a** of the figure) in which ubiquitin (Ub) is first activated in an ATP-dependent reaction that leads to a high-energy thiolester linkage between the carboxy-terminal glycine residue of ubiquitin and the active site cysteine residue on the E1 (ubiquitin-activating enzyme) protein. Ubiquitin is transferred to the active site cysteine of one of approximately 40 E2s (ubiquitin-conjugating enzymes or ubiquitin carrier proteins), where a second high-energy thiolester linkage is formed. RING finger (RF) E3s interact with the substrate and a ubiquitin-charged E2 and mediate the direct transfer of ubiquitin from E2 to the substrate, most frequently either forming an isopeptide bond between the C terminus of ubiquitin with an internal lysine or forming a peptide bond with the amino terminus of the substrate. Less frequently observed are linkages with internal serine, threonine or cysteine residues of the substrates. Ubiquitin can also be added to the growing end of a nascent ubiquitin chain on a substrate (see right side of part **a** of the figure), usually through linkages between internal lysines of the most distal of the substrate-bound ubiquitins and the C terminus of the newly added ubiquitin. Linkages through the N terminus of a bound ubiquitin chain can also occur. After multiple rounds, polyubiquitin (also known as multiubiquitin) chains are formed. RING finger E3s can mediate the addition of a single ubiquitin to the substrate (monoubiquitylation; see part **b** of the figure), the addition of single ubiquitin molecules to multiple different sites on the substrate (multi-monoubiquitylation) or the addition of polyubiquitin chains, potentially on any of the lysines of ubiquitin. Lys48-linked chains, which are best known for their role in targeting proteins for degradation by the 26S proteasome, are often depicted (based on structure) as having a kinked appearance. By contrast, Lys63-linked chains, which are implicated in a number of non-proteasomal functions, are often depicted as having a linear arrangement. Ubiquitylation is central to diverse processes that regulate protein fate and function, most prominently proteasomal degradation, but also processes such as endocytosis, activation of signal transduction cascades and DNA repair. The outcome of ubiquitylation is specified by several factors, including the type of ubiquitylation of the substrate, cellular location and context, interaction with specific ubiquitin receptors, and by the opposing activity of specific deubiquitylating enzymes.



reduced MDM2 and dramatic apoptosis in multiple tissues seen with an inducible p53 in mice lacking *Mdm2* (REFS 25,26).

There are other crucial players in this relationship. The importance of MDMX (also known as MDM4 and HDMX) was established through genetic studies that are analogous to those for MDM2 (REFS 15,17). MDMX shares the domain structure of MDM2 and can bind p53; however, it lacks E3 activity and does not include the subcellular localization signals that are found in MDM2 (FIG. 1). MDM2 can either form homodimers or heterodimerize with MDMX through their RING fingers[27–29], and both types of dimers are active as E3s; MDMX has little tendency to form homodimers[15,17,30]. MDMX has crucial roles in MDM2 stability, presumably by decreasing MDM2 homodimers and thereby limiting MDM2 homodimer ubiquitylation in *trans*. Importantly, the relative levels of these proteins influence p53 activity in ways that remain to be fully established but that are thought to include enhanced ubiquitylation of p53 when MDMX is expressed[31,32]. Additionally, the capacity of MDM2 to export p53 to the cytoplasm for degradation is expected to be negatively affected by competition with MDMX[33]. The p53–MDM2–MDMX relationship is further complicated by all three being substrates for the deubiquitylating enzyme (DUB; also known as deubiquitinating enzyme) herpes virus-associated ubiquitin-specific protease (HAUSP; also known as USP7). Genotoxic stress-induced phosphorylation of MDM2 and MDMX decreases their binding to HAUSP, leading to rapid MDM2 and MDMX degradation, thereby contributing to p53 stabilization[15].

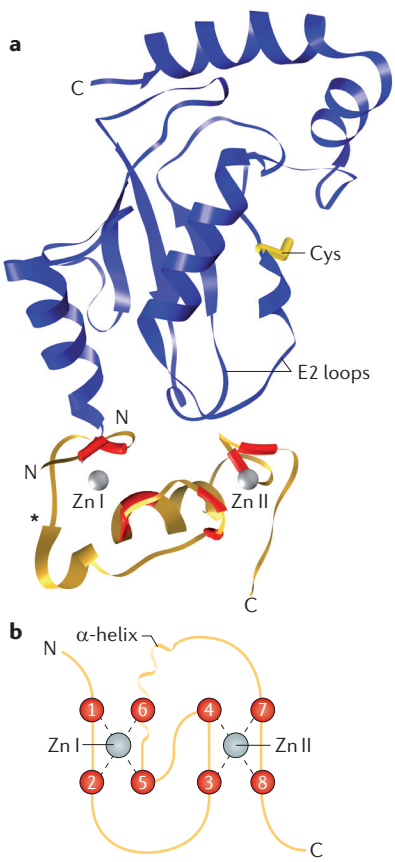
The levels or availability of other proteins that increase with genotoxic stress bind to MDM2 and decrease its activity towards p53. The tumour suppressor ARF, which is the product of the *CDKN2A* locus, binds to the central acidic domain of MDM2 and inhibits its activity towards p53 (REFS 34,35); ARF is inactive in a high proportion of tumours that retain wild-type p53 (REF. 36). Several ribosomal proteins, L5 (also known as RPL5), L11 (also known as RPL11) and L23 (also known as RPL23)[37], bind MDM2 — in a region that is similar to that described for ARF — and function cooperatively[37]. Free levels of these proteins increase with the inhibition of ribosomal biogenesis during genomic stress[38,39].

Both ARF and these ribosomal proteins can localize to the nucleolus, where MDM2 is similarly re-localized by ARF. This has led to the hypothesis that increases in ARF expression sequester MDM2 in the nucleolus, which thus prevents its interaction with p53. A cryptic nucleolar localization signal that is located in the MDM2 RING finger is revealed by ARF binding[40], which suggests that ARF has the potential to induce substantial conformational changes in MDM2. However, the story is more complex as L11 does not seem to affect E3 activity. Moreover, both L5 and an ARF peptide inhibit p53 ubiquitylation by MDM2. Furthermore, ARF inhibition of p53 degradation does not require nucleolar localization[36,37]. A unifying hypothesis is that ARF and these ribosomal proteins bind MDM2 in a region that is distinct from the RING finger, which leads to a conformational change in the MDM2 RING finger that both inhibits E3 activity and reveals the MDM2 nucleolar localization signal.

In addition to the direct effects of interacting proteins, the E3 activity of MDM2 towards p53 is affected by other protein modifiers. Phosphorylation of p53, MDM2 and MDMX, which are often induced in response to DNA damage or other genomic stress, can affect interactions and ubiquitylation[15]. The acetylation of carboxy-terminal lysines on p53 competes for MDM2-mediated ubiquitylation and thereby activates p53; recently, acetylation of other lysine residues of p53 has been shown to activate p53 by blocking MDM2 binding[14]. A provocative observation is that MDM2 can mediate p53 neddylation on lysines, overlapping those modified with ubiquitin, and that this inhibits p53-mediated transcription[16]. In addition to MDM2 and MDMX, more than ten other E3s (most of which are RING fingers) have been

Box 2 | **RING finger E3 structure**

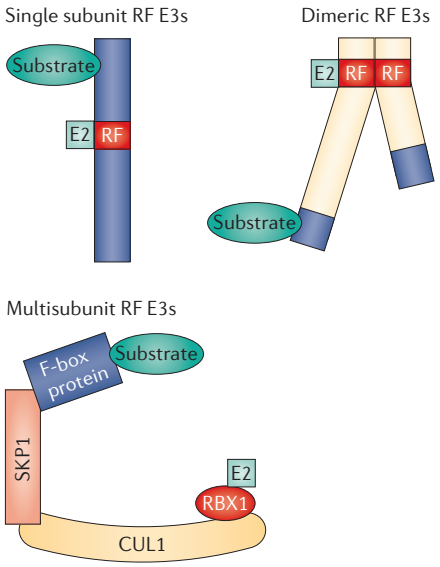
The vast majority of known E3s contain a RING finger, which is a small (~40–60 amino acid) domain that coordinates two zinc (Zn) ions. Active RING finger domains interact with a subset of the ~40 E2s that are expressed in humans and mediate the transfer of ubiquitin from the active site of the E2 to acceptor residues on target proteins or on the growing end of ubiquitin chains. A ribbon diagram, based on a model of the RING finger of CNOT4 bound to the E2 UBCH5B (blue; catalytic cysteine of E2 in yellow) is shown in part **a** of the figure (the amino and carboxyl termini are indicated; protein databank identification number: 1UR6)[155], with a schematic of the RING finger shown in part **b** of the figure. The RING finger includes eight residues (shown in red in all parts of the figure) that coordinate the two Zn ions (shown in grey) in a cross-braced pattern. Residues 1, 2, 5 and 6 interact with one Zn ion and residues 3, 4, 7 and 8 interact with the second Zn ion. Most coordinating amino acids are cysteines, there are often one to two histidines, which are generally not found in the first or last pair of coordinating residues, and in rare cases (such as RBX1) an acidic residue can function as a coordinating residue. The Zn I region interacts with the N terminus and other regions (E2 loops) of the E2. The α-helix, which generally includes the final coordinating residue for Zn I (that is, residue 6) and the Zn II region interact with the E2 loops. RING fingers have conserved spacing between most of the coordinating residues but there is considerable variability in the loop between the second and third coordinating residues (indicated by an asterisk in part **a** of the figure) and in the length of the region containing the α-helix between the sixth and seventh residues (see part **c** of the figure: coordinating residues are numbered in red, X indicates intervening amino acids followed by spacing in numbers). The leukocyte-association protein (LAP) domain or plant homeodomain (PHD) is a variation on the RING finger. The U-box resembles the RING finger but its structure is determined by interactions between U-box amino acids rather than by the coordination of Zn ions. Many proteins containing these domains are established E3s.



c

N–(1)–X(2)–(2)–X(9–27)–(3)–X(1–3)–(4)–X(2)–(5)–X(2)–(6)–X(4–48)–(7)–X(2)–(8)–C

Box 3 | **RING finger E3 types**



RING finger E3s can be single subunit E3s in which the RING finger (RF; shown in red in the figure) is surrounded by protein-interacting motifs (shown in dark blue). A number of RING finger E3s exist as homodimers or heterodimers where the RING finger and/or surrounding regions serve as the site of dimerization and enhance or direct E3 activity (for example, BRCA1–BRCA1-associated RING domain 1 (BARD1) and MDM2–MDMX). For a number of heterodimeric E3s, only one RING finger functionally interacts with E2 (for example, BRCA1 and MDM2). The most complex RING finger E3s are multisubunit E3s, which include the cullin RING ligase (CRL) superfamily (including the S phase kinase-associated protein 1 (SKP1)–cullin 1 (CUL1)–F-box protein (SCF) and CRL2 E3 families), the anaphase promoting complex/cyclosome (APC/C) and the Fanconi anaemia (FANC) E3 complex. In CRLs, a cullin protein serves as a scaffold to assemble multiple proteins, including a small RING finger protein (RBX1), adaptor proteins (such as SKP1 in the SCF and elongin B–elongin C in CRL2) and substrate targeting proteins (such as F-box proteins for the SCF complex, von Hippel–Lindau tumour suppressor protein (VHL) and SOCS box proteins for the CRL2 family). RING finger E3s recognize substrates through diverse protein–protein interactions. This regulated recognition is modulated by other post-translational modifications that include phosphorylation, glycosylation and sumoylation, and the activity of CRLs is generally activated by neddylation. There are not general consensus sites for ubiquitylation, although the binding sites for E3s on target proteins can be highly specific and often dictate sites of ubiquitylation. Proteins may be targeted by multiple E3s and, conversely, E3s can have multiple substrates, including themselves. Moreover, different E3s might recognize the same or different sites on proteins and a single E3 can have multiple ways to recognize target proteins.

implicated in ubiquitylating p53, although for a number of these this role has not been confirmed (TABLE 1; see Supplementary information S1 (table)). One consideration is that some of these function either cooperatively with MDM2–MDMX or further ubiquitylate p53 in the nucleus or perhaps after export to the cytoplasm[17].

Up to 50% of malignancies retain wild-type p53 and in most of these there is increased MDM2 activity towards p53 as a consequence of amplification of *MDM2*, increased MDM2 expression owing to polymorphisms, alterations in ARF activity or other factors[16,36]. Thus, inhibiting the physical and functional interactions between these proteins is of great therapeutic interest. The small molecule RITA binds to p53 and blocks interactions with MDM2. This results in p53-dependent apoptosis in cell lines and the inhibition of growth in xenografts[41]. The function of RITA has been extended to human papillomavirus (HPV) E6-dependent degradation of p53 by the HECT domain E3 E6-associated protein (E6AP; also known as UBE3A), which is consistent with RITA inducing a conformational alteration in p53 (REF. 42). Nutlins are small molecules with half-maximal inhibitory concentrations (IC_{50}s) of ~100 nM that bind MDM2 and competitively inhibit its binding to p53 (FIG. 1); they have been shown to have activity in preclinical models both alone[43] and in combination with other treatments in xenografts of prostate cancer and neuroblastoma[44,45]. With nutlins validating the inhibition of protein–protein interactions as a way of activating p53, and the crystal structure of the MDM2–p53 interface well defined, there is the potential for structure-based refinement of this approach to targeting p53–MDM2 binding[46]. Another approach to reactivate p53 is through inhibiting the E3 activity of MDM2, ideally through small molecule ARF mimetics. There are several small molecules that inhibit MDM2 activity either in general or specifically towards p53 (REFS 47–49). Although these establish proof-of-principle for the inhibition of E3 activity, neither the molecular basis for their action nor their *in vivo* efficacy is known. With knowledge of the MDM2–MDMX RING finger dimer structure[29] there is the potential to design inhibitors that disrupt the active dimeric E3. However, as the animal studies described above demonstrate, there is a fine line between achieving a therapeutic level of p53 activation and causing undesirable widespread apoptosis[25,26].

DNA repair-associated RING fingers and cancer. Together with DNA damage-activated kinases and other protein-modifying enzymes, RING finger and RING finger-like E3s have crucial roles in sensing and repairing DNA damage, regulating cell cycle progression and minimizing the propagation of damaged DNA and chromosome abnormalities (Supplementary information S2 (table)). RING finger E3s carry out essential roles in the five best-characterized forms of DNA repair (Supplementary information S3 (table)). Particularly illustrative of the importance of RING finger E3s in repair processes are BRCA1 and the FANC E3 complex.

BRCA1 is a tumour suppressor that is frequently mutated in familial breast and ovarian cancer[50]. The role of BRCA1 in DNA repair was established as a consequence of its colocalization with RAD51 in nuclear foci during S phase and colocalization with RAD51, and proliferating cell nuclear antigen (PCNA) upon DNA damage[51,52]. This led to the discovery that cells that are deficient in BRCA1 have a defect in the repair of double-stranded breaks by homologous recombination (HR)[53,54]. The central role of BRCA1 in DNA repair is underscored by its recruitment to DNA as a dimer with BRCA1-associated RING domain 1 (BARD1) (discussed below) in several different protein complexes that have functions in sensing DNA damage, controlling both G2/M and DNA replication checkpoints, and recruiting DNA repair enzymes[55]. A key BRCA1–BARD1 complex that accumulates at DNA damage foci and is involved in the G2/M checkpoint is comprised of abraxas (also known as FAM175A), RAP80, MERIT40 (also known as BABAM1), BRE (also known as BRCC45) and the DUB BRCC36. A notable feature of all five of these complex members is that they each include ubiquitin-binding domains and that they bind ubiquitylated proteins[55]. Such associations are a recurrent theme in DNA damage complexes, as is the presence of DUBs (discussed below). Thus, an important

Table 1 | **RING finger E3s as oncogenes and tumour suppressor genes**

E3	Function	Role in cancer*	Refs
Cell cycle			
APC/C ligases	Multisubunit E3s that regulate the cell cycle	Tumour suppressor complexes. Evidence of mutations in several subunits that disrupt function in colon cancer cells, resulting in increased accumulation of cyclins and progression of the cell cycle. Some experimental data suggest that compromised function of APC/C by loss of substrate recognition subunits, CDC20 or CDH1 can result in genomic instability consistent with a role for APC/C as a tumour suppressor	7–9
β-TrCP (FBW1A)	F-box protein, functions as a substrate recognition component of the SCF E3 complex, which is involved in the degradation of phosphorylated cell cycle and signalling molecules. β-TrCP targets the SCF complex to multiple proteins that have both pro- and anti-proliferative effects, such as BCL2L11 (also known as BIMEL), CDC25A, β-catenin, IκB, PDCD4, SMAD3, SMAD4 and WEE1	A potential oncogene. Transgenic mice overexpressing β-TrCP have an increased incidence of epithelial tumours. Consistent with its function as an oncogene, β-TrCP is overexpressed and associated with poor prognosis in many human epithelial cancers. However, β-TrCP mutations described in gastrointestinal malignancies are associated with stabilization and the accumulation of nuclear β-catenin, consistent with a role as a TSG. This suggests that the oncogenic or tumour suppressive effects of β-TrCP are context-dependent	5,13
COP1 (RFWD2)	RING finger component of a multisubunit E3 that targets multiple transcription factors, including members of the JUN and ETS family for proteasomal degradation. Also reported to target p53 for proteasomal degradation (see Supplementary information S1 (table))	A TSG. *Rfwd2*-hypomorphic mice develop thymic lymphomas, teratomas and uterine tumours. Rare deletions of *RFWD2* have been described in lymphoblastic lymphoma, melanoma and prostate cancer. There is an inverse correlation between low COP1 expression and high expression of JUN and ETS family members in prostate cancer. Translocations of ETS family genes that delete COP1-binding sites stabilize ETS proteins	158,159
EMI1 (FBXO5)	Inhibits activity of APC/C^{CDH1} and allows progression from G1 to S phase. In some cells it also functions to inactivate APC/C^{CDC20}	A potential oncogene. EMI1 is overexpressed in breast, colon, ovarian, uterine and lung cancer. Predicted to allow cell cycle progression when APC/C^{CDH1} is inhibited	160–162
FBXW7	F-box and WD repeat-containing protein that functions as a recognition component of an SCF E3 complex. Substrates include cyclin E, MYC, NOTCH and JUN	A haploinsufficient TSG. Loss-of-function mutations identified in cholangiocarcinoma, T-ALL, breast, bladder, ovarian, liver, lung, bone and endometrial cancers. There is evidence that mutant alleles dimerize with and inhibit wild-type alleles	10,163
SKP2	F-box protein that functions as a recognition component of the SCF E3 complex that targets p27 and other cell cycle proteins	An oncogene. *SKP2* is amplified in human epithelial cancers, including small cell and non-small-cell lung cancer, glioblastoma, squamous cell oesophageal cancer, cervical cancer and thyroid cancer, and is overexpressed in many human tumours. Cooperates with activated RAS in transformation assays. Transgenic mice expressing SKP2 and activated NRAS develop lymphomas with increased frequency and decreased latency compared with activated NRAS alone. High expression of SKP2 correlates with high-grade lymphoma	3, 164–166
Genomic integrity			
BARD1	RING finger protein without E3 activity. Heterodimerizes with BRCA1 and enhances BRCA1 E3 activity. Functions in HR DNA repair pathway	A TSG. Patients with familial breast cancer have been described with homozygous deletion or inactivating mutations	56, 167–169
BRCA1	RING finger E3. Functions in transcription and HR DNA repair pathway	A TSG. Deleted or inactivated in patients with familial breast and ovarian cancer	56,170
CUL7	Cullin protein. Forms an SCF-like multisubunit E3 complex. Heterodimerizes with PARC (also known as CUL9) and inactivates p53	A potential oncogene. Prevents MYC-induced apoptosis and cooperates with MYC in transformation assays. Seems to function by inactivating p53	171–173
FANC core complex	Multisubunit E3 that monoubiquitylates FANCD2 and FANCI and regulates DNA repair. FANCL contains a RING finger-like PHD domain with E3 activity	A tumour suppressor. Mutations in individual FANC E3 subunits lead to Fanconi anaemia, which is associated with an increased risk of cancer	73
MDM2	RING finger E3. Inactivates p53 by ubiquitylation and proteasomal degradation. Other substrates identified (see Supplementary information S1 (table))	An oncogene. Transforming protein when overexpressed, it is amplified in human cancers	15,174
MDMX (MDM4)	RING finger protein without E3 activity. Dimerizes with MDM2 and enhances p53 ubiquitylation	An oncogene. Enhances degradation of p53 by MDM2. Transforming protein when overexpressed. Amplified in human cancers	31, 175–177
PARC (CUL9)	Cullin-like protein that forms a multisubunit E3 complex with the RING finger protein RBX1	A potential oncogene. Sequesters p53 in the cytoplasm and prevents p53 activation	172,178
PIRH2 (RCHY)	RING finger E3 that ubiquitylates and targets p53 for proteasomal degradation	A potential oncogene. Overexpressed in lung cancer resulting in p53 degradation. For additional information see Supplementary information S1 (table)	179,180

Table 1 (cont.) | **RING finger E3s as oncogenes and tumour suppressor genes**

E3	Function	Role in cancer*	Refs
Signal transduction			
CBLs	RING finger E3s that target activated kinases for ubiquitylation and degradation	Oncogenes. Dominant-negative forms of *Cbl* function as oncogenes in mice and NIH-3T3 cells. Mutations that create dominant-negative forms of CBL have been found in human myeloid neoplasms. Mutations in CBL-binding sites on kinases or in negative regulators of CBL have been described in various cancers	113
FBXW5	F-box and WD repeat-containing protein. Substrate binding component of CRL4 E3 ($CRL4^{FBW5}$)	A potential oncogene. Targets the tumour suppressor protein TSC2 for proteasomal degradation	181
Hakai (CBLL1)	Single subunit RING finger E3 that targets E-cadherin for degradation	A potential oncogene or prometastatic gene. Promotes cell migration, proliferation and anchorage-independent growth	88,182
IAPs	RING finger E3s that autoubiquitylate and ubiquitylate caspases and TRAFs. They regulate NF-κB signalling and also negatively regulate caspase activation	Oncogenes and/or TSGs that inhibit apoptosis and promote cell proliferation. Overexpressed in many malignancies. Translocations creating MALT1–cIAP2 fusion protein are seen in 25% of MALT lymphomas. These fusions delete the RING finger of cIAP2 and overexpression activates the NF-κB pathway. Homozygous deletions of the chromosome region containing *BIRC2* and *BIRC3* (which encode cIAP1 and cIAP2, respectively) described in multiple myeloma are associated with increased NF-κB activity	95, 183–187
TRAFs	A family of RING finger E3s that positively and negatively regulate NF-κB activation	Oncogenes and/or TSGs. Missense mutations identified in *TRAF2* and *TRAF5* in 2–5% of B cell lymphomas. Overexpression experiments of one such mutation in *TRAF2* demonstrated increased NF-κB activity, although the mechanism is not defined. By contrast, inactivating mutations or homozygous deletions of *TRAF2* and *TRAF3* have been described in multiple myeloma associated with increased NF-κB activity and are consistent with a TSG function for these TRAFs	183, 186,188
TRC8 (RNF139)	RING finger E3 with sterol-sensing domain involved in protein biosynthesis	A TSG. Disruption of *RNF139* by translocations is found in patients with familial clear cell renal cancer, patients with thyroid cancer and a patient with dysgerminoma. Overexpression of TRC8 suppresses tumour cell growth	189–191
Hypoxia			
VHL	Recognition component of the cullin-based $CRL2^{VHL}$ E3 complex. Targets HIF transcription factor for degradation under conditions of normoxia	A TSG lost in von Hippel–Lindau syndrome, which is associated with CNS tumours, haemangioblastomas, pheochromocytomas and clear cell kidney cancer. VHL is inactivated in 40–80% of sporadic clear cell kidney cancer	131–133
SIAHs	RING finger E3s	Potential oncogenes. Ubiquitylates and targets proline hydroxylases for proteasomal degradation, which results in stabilization of HIFα transcription factors	152,153
Metastasis			
gp78 (AMFR)	RING finger E3 implicated in endoplasmic reticulum-associated degradation (ERAD)	A potential oncogene or prometastatic gene. Promotes sarcoma metastasis by degrading metastasis suppressor KAI1 (also known as CD82)	192

β-TrCP, β-transducin repeat containing protein; AMFR, autocrine motility factor receptor; APC/C, anaphase-promoting complex/cyclosome; BARD1, BRCA1-associated RING domain 1; BIRC, baculoviral IAP repeat containing; CDC, cell division cycle; CNS, central nervous system; COP1, constitutive photomorphogenesis protein 1 homologue; CRL, cullin RING ligase; CUL, cullin; EMI1, early mitotic inhibitor 1; FANC, Fanconi anaemia; FBXW, F-box/WD-repeat containing protein; HIF, hypoxia-inducible factor; HR, homologous recombination; KAI1, kangai 1; MALT, mucosa-associated lymphoid tissue lymphoma translocation; NF-κB, nuclear factor-κB; PDCD4, programmed cell death protein 4; PHD, plant homeodomain; RFWD2, RING finger and WD repeat domain protein 2; RNF139, RING finger protein 139; SCF, SKP1–cullin 1–F-box protein; SKP2, S-phase kinase-associated protein 2; T-ALL, T cell acute lymphoblastic leukaemia; TRAF, TNF receptor-associated factor; TSC2, tuberin; TSG, tumour suppressor gene; VHL, von Hippel–Lindau tumour suppressor.
*Genes are designated as oncogenes or TSGs when there is functional evidence for their role in cancer and genetic mutations consistent with that role have been found in human cancers. For oncogenes this generally consists of activating mutations and/or gene amplification. For TSGs, this generally consists of inactivating mutations and/or deletions. Genes are designated as potential oncogenes or TSGs when functional studies are consistent with that role but no mutations have been reported in human tumours to date. In some instances a protein may act as an oncoprotein or a tumour suppressor depending on the cellular context.

concept is that protein ubiquitylation at sites of DNA damage, primarily by RING finger and RING finger-related E3s, provides a means of recruiting other specific proteins with ubiquitin-binding domains, and this accumulation can be partly regulated by DUBs.

BRCA1 includes an amino-terminal RING finger, a nuclear localization sequence, a coiled-coil domain and two C-terminal BRCA1 C-terminal (BRCT) domains[55]. The BRCA1 RING finger has intrinsic E3 activity[1]. However, in cells it exists as a stable heterodimer with the related RING finger protein BARD1, which greatly increases the activity of the BRCA1 RING finger *in vitro*, but which lacks E3 activity by itself[56,57]. BRCA1 and BARD1 dimerize through their RING fingers, and in cells the loss of expression of either protein results in the degradation of the other[58–60]. How the BARD1

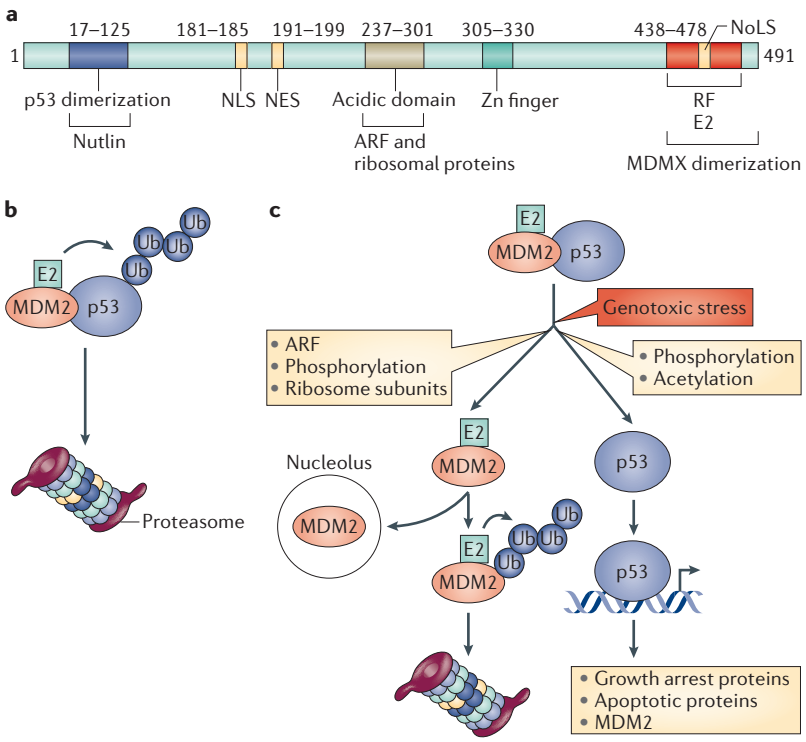


Figure 1 | **MDM2. a** | A linear representation of MDM2 with the positions of defined domains (boundaries are approximate) is shown. MDMX has a similar domain structure but lacks the nuclear localization signal (NLS), the nucleolar localization signal (NoLS) and the nuclear export signal (NES) that are found in MDM2. Interacting proteins are listed below the domains of MDM2 with which they interact, and the site of nutlin binding is also shown. **b** | MDM2, predominantly as a dimer with MDMX (not shown), binds *in vivo* to, and ubiquitylates (Ub), p53, which exists largely as a tetramer. This results in the proteasomal degradation of p53. **c** | In response to genotoxic and other stresses, kinases and acetylases promote the modification of p53, as well as MDM2, so that their binding is reduced and sites of ubiquitylation are unavailable. Additionally, the expression of ARF is increased and small ribosome subunits become available. These inhibit the activity of MDM2 towards p53 and result in targeting MDM2 to the nucleolus and potentially increase auto-ubiquitylation and degradation of MDM2 in the context of the homodimer. This degradation may be further facilitated by stress-induced phosphorylation of MDM2 leading to decreased binding of herpes virus-associated ubiquitin-specific protease (HAUSP (not shown)). As a consequence of its stabilization and dissociation from MDM2, p53 activity increases, resulting in the induction of growth arrest proteins (such as p21) or pro-apoptotic proteins (such as the BH3-only proteins p53-upregulated modulator of apoptosis (PUMA) and NOXA), as well as increased *MDM2* expression.

RING finger enhances the activity of BRCA1 *in vitro* and stabilizes it in cells is unknown. Interestingly, it was recently shown that BRCA1 sumoylation by protein inhibitor of activated STAT (PIAS) proteins is crucial to the cellular and *in vitro* activity of the BRCA1–BARD1 complex[61].

The nature of BRCA1, including its large size and involvement as a common player in multiple forms of DNA damage repair, can partly account for its prominent association with cancer. However, the plethora of germline cancer-associated missense and nonsense mutations throughout the coding region do not provide a ready explanation for its differential association with familial breast and ovarian cancer. Similarly, although the BRCA1–BARD1 dimer associates with a large number of proteins, the most important substrates for ubiquitylation may remain to be determined.

Among established BRCA1 substrates is CTBP-interacting protein (CTIP; also known as RBBP8), which is the binding partner of the transcriptional repressor carboxy-terminal binding protein (CTBP)[62]. *CTIP* is considered to be a TSG on the basis of it being mutated in a number of cancers and its association with other tumour suppressors, including BRCA1 (REF. 63). CTIP binds the BRCT domains of BRCA1 in a phosphorylation-dependent manner. In response to ionizing radiation, ubiquitylated CTIP is found in an insoluble chromatin-containing fraction of cell lysates, suggesting a role for BRCA1-mediated ubiquitylation of CTIP in checkpoint arrest in response to DNA damage[62]. On the basis of negative data from cycloheximide chase experiments it was concluded that BRCA1-mediated CTIP ubiquitylation does not result in proteasomal degradation. This would be consistent with the capacity of BRCA1 to form non-canonical Lys6 polyubiquitin chains.

BRCA1 binds both progesterone receptor (PR) and oestrogen receptor-α (ERα; also known as ESR1) through interactions that primarily involve its N-terminal regions[64–66]. BRCA1 decreases the transcriptional activity of both PR and ERα, but the roles that ubiquitylation and proteasomal degradation have remain to be fully determined. Convincing evidence exists that PR is a target for polyubiquitylation and degradation in breast cancer cells[67]; however, other studies suggest that the role of BRCA1 is to decrease PR activity without degradation[68].

BRCA1 has been shown to target ERα for monoubiquitylation[66]. The specific lysine in ERα that is ubiquitylated by BRCA1 *in vitro* overlaps with lysines in a region of ERα that is acetylated by the histone acetyltransferase p300. Activated BRCA1, but not RING-mutant BRCA1, induces decreased ERα acetylation, and there is evidence that acetylation is directly correlated with ERα transcriptional activity. Thus, an emerging model is that BRCA1-mediated monoubiquitylation competes with acetylation to inhibit ERα transcriptional activity[69]. However, a role for BRCA1 in the proteasome-mediated degradation of ERα cannot be excluded.

An apparent paradox is that, most commonly, BRCA1-mutant breast cancers do not express ERα or PR, but both of these receptors interact with BRCA1. Currently, a satisfying explanation is lacking. However, several lines of evidence suggest that despite the ERα$^-$ PR$^-$ phenotype, ovary-derived hormones and ERα have crucial roles in the genesis of BRCA1-mutant tumours. For example, the incidence of mammary carcinomas in mice with conditional *Brca1* deletion targeted to the mammary gland is increased by exogenous oestrogen. Consistent with the suppression of ERα-mediated transcription by BRCA1, the loss of BRCA1 results in the increased sensitivity of cells to oestrogen-induced proliferation[70,71]. In adult patients with germline *BRCA1* mutations, oophorectomy results in a 56% decrease in the incidence of breast cancer[72]. Thus, it may be that early in tumorigenesis *BRCA1* loss or mutation alters the regulation of these receptors and thereafter the manifestation of *BRCA1* loss is primarily on genomic stability.

In addition to the well-established involvement of BRCA1 in breast and ovarian cancer, there is an association of an E3 that contains a RING finger-like domain (PHD domain E3) (BOX 2) with Fanconi anaemia. This recessive genetic disease of childhood is caused by defective DNA repair proteins. Children who survive its early multi-system effects are susceptible to a number of malignancies[73]. This rare genetic disease occurs as a consequence of mutations in any one of at least 13 different FANC genes, the loss of which results in a failure to repair DNA crosslinks during DNA synthesis. Failure to repair crosslinks has catastrophic consequences for the surrounding DNA. Crosslink repair requires the sequential action of several different DNA damage response pathways, including translesion synthesis (TLS), which is intrinsically mutagenic in itself, nucleotide excision repair (NER) and HR. Accordingly, DNA damage response foci show reactivity for other proteins that are involved in DNA repair, including RAD51, PCNA and BRCA1 (REF. 73). Beyond the 13 FANC genes, biallelic mutations in either of two other genes have recently been identified as being associated with Fanconi anaemia[74,75].

The 13 FANC proteins can be subdivided accordingly: eight components of the core FANC E3 ubiquitin ligase, two substrates for transient monoubiquitylation and three other effector proteins (including BRCA2 (also known as FANCD1)). These effector proteins are recruited to sites of DNA damage together with the ubiquitylation substrates. FANCM has a central role as it is recruited to sites of DNA damage and recruits the other FANC proteins. Crucial to the activity of the FANC E3 ligase is FANCL, which has a RING finger-like PHD domain that is responsible for its activity. FANCL functions together with the E2 UBE2T to add a single ubiquitin to specific lysine residues on FANCD2 and FANCI. This transient monoubiquitylation leads to their binding to chromatin, with FANCD2 ubiquitylation being essential for DNA repair, and FANCI ubiquitylation possibly enhancing repair[73]. An interesting feature of this E2–E3 pair is that a fairly limited region of FANCL, which includes its PHD domain, is sufficient for a high-affinity interaction with UBE2T[76]. Such an interaction is unusual for E2s, which must dissociate from the RING finger to reload with ubiquitin[77]. However, this finding might make intuitive sense, as the FANCL substrates only undergo monoubiquitylation.

Monoubiquitylated FANCD2 is required to 'unhook' the crosslinked nucleotide from one of the parental strands, as well as for nucleotide insertion on the opposite strand[78]. Deubiquitylation of FANCD2 and FANCI is mediated by the ubiquitin-specific peptidase 1 (USP1) and USP1-associated factor 1 (UAF1; also known as WDR48) complex[73]. This reversibility is essential for crosslink repair. Recently, a crucial link between FANC-mediated ubiquitylation and repair was made with the discovery that the endonuclease FANCD2/FANCI-associated nuclease 1 (FAN1) binds specifically to ubiquitylated FANCD2 (REFS 79–81). The FANC repair pathway must be inactivated during mitosis to prevent the formation of radial chromosomes and other abnormalities. This is accomplished by ubiquitylation and degradation of FANCM. FANCM is phosphorylated by kinases that include polo-like kinase 1 (PLK1), which leads to ubiquitylation by $SCF^{\beta\text{-}TrCP}$ and proteasomal degradation[82].

The defects in DNA repair in tumours with BRCA1 or BRCA2 loss has led to a synthetic lethal approach to targeting these tumours. The inhibition of poly(ADP-ribose) polymerase (PARP), which is involved in the repair of single-stranded DNA breaks, leads to the formation of double-stranded DNA breaks, which are usually repaired by HR. Cells deficient in BRCA1 or BRCA2 (and thus HR) are therefore selectively sensitive to PARP inhibition[83,84]. Clinical trials using PARP inhibitors in patients with BRCA1- or BRCA2-deficient breast and ovarian cancers have shown promising single agent activity[85–87].

RING fingers and transmembrane signalling

Regulated transmembrane signalling is essential for normal homeostasis and development. Many RING finger E3s are implicated in the regulation of signal transduction. For example, the E3 CBL (discussed below) mediates the ubiquitylation and thus the downregulation of many activated receptor tyrosine kinases (RTKs), such as epidermal growth factor receptor (EGFR); Hakai (also known as CBLL1) downregulates E-cadherin; neuregulin receptor degradation protein 1 (NRDP1) downregulates ERBB3; and the RING finger-related U-box protein CHIP mediates chaperone-dependent ubiquitylation and downregulation of ERBB2 (REFS 88–91). Similarly, the $SCF^{\beta\text{-}TrCP}$ RING finger E3 ubiquitylates and negatively regulates activated prolactin receptor, regulates the endocytosis of the growth hormone receptor and negatively regulates WNT signalling by targeting β-catenin for proteasomal degradation[92–94]. Multiple RING finger E3s both positively and negatively regulate nuclear factor-κB (NF-κB) signalling, and some are implicated in the development of lymphoid malignancies[95] (TABLE 1).

CBL. Dysregulated signalling in cancer cells is frequently caused by activating mutations and/or amplification of RTKs[96]. This results in autonomous activation of growth and survival signalling pathways. Activated RTKs are negatively regulated by their internalization and intracellular trafficking to lysosomes[97]. This is driven by RTK ubiquitylation, which serves as a docking site for ubiquitin-binding proteins that mediate the trafficking of RTKs from the early endosome to the multivesicular body, ultimately leading to lysosomal degradation[97]. The CBL proteins mediate ubiquitylation and lysosomal degradation of many RTKs and thereby downregulate their function[98–103] (FIG. 2).

The CBL proteins (in mammals these are CBL, CBLB and CBLC) are a family of single subunit RING finger E3s that contain multiple protein interaction motifs surrounding the RING finger[104]. All CBL proteins have a highly conserved N-terminal tyrosine kinase-binding (TKB) domain that is composed of a four-helix bundle, a calcium-binding EF hand and an atypical SH2 domain[104]. The TKB domain mediates interactions between CBL proteins and phosphorylated tyrosine residues on CBL substrates. The RING finger and TKB domain are separated by an α-helical

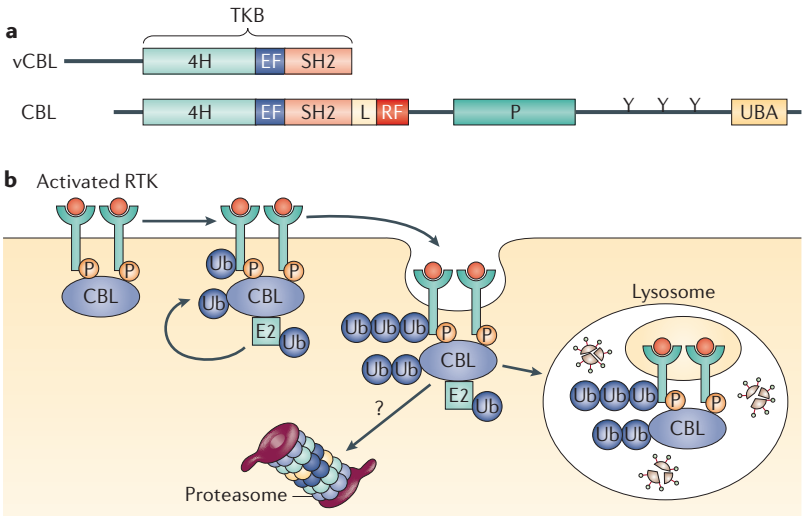


Figure 2 | **CBL. a** | A schematic structure of the vCBL and CBL proteins is shown. The tyrosine kinase-binding (TKB) domain is comprised of a four-helix bundle (4H), an EF hand (EF) and a variant SH2 domain (SH2). The linker (L), RING finger (RF), proline-rich (P) and ubiquitin-associated (UBA) domains are indicated. The tyrosines (Y) at sites of phosphorylation in the carboxyl terminus of CBL are also indicated. **b** | CBL proteins mediate ubiquitylation (Ub) and the downregulation of receptor tyrosine kinases (RTKs). Whether CBL proteins associated with activated complexes are degraded in lysosomes or proteasomes is unresolved. P, phosphorylation.

linker region, which is crucial for the regulation of CBL E3 function and is implicated in the transforming mutations discussed below. Based on the crystal structure, the linker region contacts the TKB, the RING finger and the E2 (REF. 105). Phosphorylation of a conserved tyrosine residue in the linker region activates E3 activity by causing a conformational change in CBL that modulates affinity for the E2 (REFS 102,106,107). C-terminal to the RING finger, CBL proteins have proline-rich domains, which mediate interactions with SH3-containing proteins, and tyrosines that become phosphorylated and mediate interactions with SH2 proteins (such as the p85 subunit of PI3K).

The CBL proteins catalyse the formation of mono-ubiquitin or Lys63-linked polyubiquitin chains on EGFR — these are modes of ubiquitylation that are associated with endocytic trafficking and not with proteasomal degradation[108,109]. Also, the CBL proteins function as adaptor proteins through a diverse array of interactions[110]. This adaptor function contributes to the E3-dependent activities of CBL proteins by targeting specific substrates for ubiquitylation and degradation. However, there are also non-E3-dependent activities served by the adaptor function of the CBL proteins, such as the recruitment of proteins that are involved in internalization of the target RTK, localization of the CBL proteins to specific compartments within the cell and the activation of signalling pathways[110].

vCBL was identified as the product of the transforming gene of the Cas NS-1 murine retrovirus, which causes leukaemia and lymphomas in mice[111]. The oncogenic nature of vCBL can be attributed to a dominant-negative function of this truncated CBL protein, which lacks an intact RING finger[112]. Mutations that abrogate the E3 activity of CBL are found in ~5% of human myeloid neoplasms[113]. This loss of activity is largely due to homozygous missense mutations, frameshifts or deletions in and around the linker and RING finger domains. These associations underscore the importance of the RING finger in the essential function of CBL proteins. As other CBL isoforms are expressed and active in these cells, these mutants are also likely to function in a dominant-negative manner. Further evidence supporting a tumour suppressor role for CBL proteins derives from studies in which mice that are null for both *Cbl* and *Cblb* in haematopoietic stem cells develop early onset myeloid neoplasms[114]. However, several studies suggest that oncogenic CBL proteins may also exhibit a gain-of-function activity that results in the aberrant activation of the JAK–STAT and PI3K–AKT pathways, in particular[115–117]. Whether the apparent gain-of-function is predominantly due to a dominant-negative effect on other CBL proteins or due to the adaptor function of CBL is not clear.

The RTK fms-like tyrosine kinase 3 (FLT3) has been implicated in the pathogenesis of myeloid leukaemias and is frequently the target of activating mutations[118]. Interestingly, cells expressing mutant CBL proteins show increased activity of FLT3, which is consistent with FLT3 being the relevant target of the CBL proteins, which thus contributes to the oncogenic activity of mutant CBL proteins[115,116,119]. This is supported by the finding that loss of FLT3 suppresses the development of myeloid neoplasms in mice harbouring a RING finger mutation in CBL[120]. However, many RTKs interact with and are ubiquitylated by CBL, and thus there are other substrates that may contribute to the oncogenic effects of CBL in myeloid neoplasms[110].

The role of CBL in the development of other malignancies requires further study. In non-small-cell lung cancer, mutations in CBL that do not disrupt E3 activity have been found, but the importance of these has not yet been determined[121]. CBL function could also be disrupted by mutations in the substrates of the CBL proteins or by aberrant activity or overexpression of negative regulators of CBL[122,123]. Because oncogenic mutations in CBL proteins predominantly lead to loss of function, novel strategies will be needed to develop therapies for tumours with CBL mutations. One approach could be to target FLT3 or downstream signalling pathways such as PI3K and AKT that are activated in myeloid neoplasms containing CBL mutations[115–117,119,120,124].

A causative oncogenic role for loss-of-function mutations of CBLB has not been demonstrated. However, the function of CBLB in negatively regulating the immune responses provides a potential therapeutic opportunity for CBLB inhibition. *In vitro* studies of T lymphocytes isolated from *Cblb*-deficient mice have demonstrated that CBLB negatively regulates the CD28 co-stimulation pathway. Thus, CBLB positively controls the induction of anergy[125–127]. Interestingly, $CD8^+$ cytotoxic T lymphocytes in $Cblb^{-/-}$ mice mediate enhanced regression of tumours[128,129]. Similarly, knock-in mice expressing a RING finger-mutant form of CBLB show enhanced antitumour immunity, demonstrating the essential role of the RING finger E3 activity in regulating antitumour immunity[130]. Together, these

Anergy
When lymphocytes fail to mount an immune response to an antigen.

data suggest that inhibition of CBLB, alone or in combination with adoptive immunotherapy, may enhance immunotherapy of tumours.

Responses to hypoxia

VHL. VHL was first discovered as the TSG that is inactivated in the familial kidney cancer syndrome von Hippel–Lindau disease[131]. This syndrome is characterized by the development of benign and malignant tumours of multiple organs, most notably clear cell kidney cancers. Also, approximately 57% of sporadic clear cell cancers of the kidney contain inactivating mutations of VHL, and 98% of these have loss of heterozygosity (LOH) at the *VHL* locus[132]. Additional studies suggest that *VHL* is epigenetically silenced in some clear cell kidney cancers[133]. Xenograft studies using a renal carcinoma cell line that expresses a VHL mutant have demonstrated that reconstitution of the wild-type VHL protein into the cell line suppresses tumorigenicity, which is consistent with the proposed function of *VHL* as a TSG[134].

VHL forms a complex with elongin B and elongin C, cullin 2 and the small RING finger protein RBX1 (REFS 135–137). This architectural similarity to the multisubunit SCF RING finger E3s (FIG. 3) led to the demonstration that the VHL complex is an E3 (REFS 138,139). VHL is a substrate-targeting subunit of the CRL2 or cullin 2–elongin B–elongin C (CBC) family of RING finger E3s[140,141]. The identification of the crucial $CRL2^{VHL}$ targets, the three hypoxia inducible factor-α (HIFα) proteins, stemmed from the observations that the mRNA for hypoxia-associated genes such as vascular endothelial growth factor A (*VEGFA*), solute carrier family 2 member 1 (*SLC2A1*; which encodes GLUT1) and platelet-derived growth factor-β (*PDGFB*) were expressed at similar levels in VHL-deficient cells grown in either normoxic or hypoxic conditions and that the levels of the mRNA for these proteins did not change with hypoxia[140,142,143]. By contrast, in cells expressing wild-type VHL, the mRNAs for these genes were upregulated by hypoxia. The reintroduction of VHL into VHL-deficient cells reduced the expression of these genes under normoxic conditions and restored their hypoxia-induced upregulation[140,142,143]. Many of these genes were previously shown to be regulated by the HIFα family of transcription factors (HIF1α–3α), and subsequent work demonstrated that VHL interacted with HIFα proteins and induced rapid oxygen-dependent proteasomal degradation of HIFα proteins[140,141]. In normal cells, HIFα proteins are stable under hypoxic conditions. This allows for their dimerization with the constitutively expressed HIF1β (also known as ARNT) and their translocation to the nucleus. The complex binds to hypoxia-responsive elements that are associated with target genes and enhances their transcription. In the absence of VHL, these genes are constitutively expressed and can lead to tumour development.

Under normoxic conditions, HIFα proteins are hydroxylated on a specific proline residue, creating a binding site for the hydroxyproline-binding pocket of the VHL protein, which leads to $CRL2^{VHL}$-mediated ubiquitylation and proteasomal degradation[144–146]. Three oxygen-dependent prolyl hydroxylases (PHD1–3; also known as EGLN1–3) have been identified in mammalian cells that catalyse proline hydroxylation of HIFα proteins and thus enhance VHL binding under normoxic conditions[147] (FIG. 3).

Although $CRL2^{VHL}$ can target all three HIFα proteins for degradation, the degradation of HIF2α seems to be the most crucial target for $CRL2^{VHL}$ (REFS 148–151).

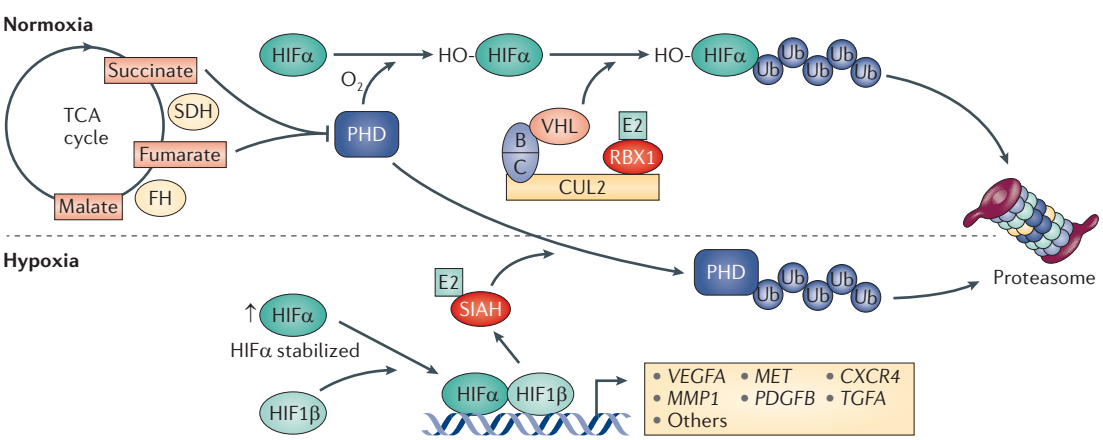


Figure 3 | **Hypoxia.** Hypoxia-inducible factor-α (HIFα) proteins are subunits of the HIF transcription factors, which regulate the expression of many genes that are associated with the response to hypoxia and proliferation. Under normoxic conditions the prolyl hydroxylase (PHD) proteins hydoxylate HIFα proteins, which creates a binding site that is recognized by the cullin RING ligase 2 (CRL2)–von Hippel–Lindau tumour suppressor protein (VHL) E3 complex ($CRL2^{VHL}$). This leads to proteasomal degradation of HIFα proteins. The PHD proteins are ubiquitylated (Ub) and degraded by the SIAH RING finger E3s that are themselves transcriptionally upregulated by HIF transcription factors in response to hypoxia[152]. The stability of HIFα proteins is also regulated by fumarate hydratase (FH) and succinate dehyrogenase (SDH), which are tumour suppressors that are associated with kidney cancer[156]. The loss of FH and SDH results in the accumulation of fumarate and succinate, respectively, which competitively inhibit PHD activity and prevent hydroxylation and VHL-mediated degradation of HIFα proteins, leading to the inappropriate upregulation of hypoxia-inducible genes[157]. MMP1, matrix metalloproteinase 1; *PDGFB*, platelet-derived growth factor-β; TCA, tricarboxylic acid; *TGFA*, transforming growth factor-α; *VEGFA*, vascular endothelial growth factor A.

Whether there are other substrates of the $CRL2^{VHL}$ RING finger E3 that contribute to its role as a TSG has not been demonstrated.

SIAHs. A second class of RING finger E3s, the SIAH proteins[1], regulates HIFα protein stability by functioning upstream of VHL (FIG. 3). SIAH1 and SIAH2 bind PHD1 and PHD3 and target them for proteasomal degradation[152]. *SIAH2* expression is transcriptionally upregulated under hypoxic conditions. Accordingly, *Siah2*$^{-/-}$ cells have an impaired response to hypoxia owing to the stabilization of PHD proteins, the ongoing hydroxylation of HIFα proteins and the constitutive recognition and degradation of HIFα proteins by $CRL2^{VHL}$ (REF. 152). Consistent with a role for SIAH2 in mediating the response to hypoxia, *Siah2*-null mice have an impaired increase in red blood cells in response to chronic hypoxia[152]. SIAH proteins have other roles that are potentially both oncogenic and tumour suppressive[153].

Conclusions and perspectives

Since the discovery more than 12 years ago that RING finger proteins are, in general, ubiquitin-protein ligases, it has become apparent that the ubiquitin-conjugating system has a far greater role in both malignancy and tumour suppression than was previously appreciated. We now understand that many processes that influence the course of cancer development and progression are regulated by RING finger proteins. The trajectory of publications in this area suggests that we are just seeing the 'tip of the iceberg'. However, even among some of the best-studied, cancer-relevant RING finger E3s and substrates, there are many questions that are yet to be answered. We understand fairly little about how MDM2–MDMX is regulated *in vivo* and we know even less about the functional interactions between this E3 and the numerous other RING finger E3s that have been identified for p53. Although we are beginning to 'drill down' on the ligases and ubiquitin chain linkages that are involved in DNA repair, there is still much to be learned about how ubiquitylation by RING finger E3s leads to the recruitment of the DNA repair complexes and the importance of the dynamics of ubiquitylation and deubiquitylation and what the overall role of proteasomal degradation is in these processes. In cell signalling we understand that ubiquitylation can induce protein trafficking to the lysosome, but in the context of RTK signalling complexes it is unclear to what extent proteasomal degradation and lysosomal degradation intersect in the degradation of complexes that include transmembrane proteins and associated signalling complexes and the extent to which types of ubiquitin linkages generated by RING finger E3s specify fate.

The therapeutic success of the proteasome inhibitor bortezomib demonstrates the potential efficacy of targeting the ubiquitin–proteasome system[154]. A question of great importance is the extent to which all of the information we are now garnering about RING finger function can be applied towards the development of targeted therapeutics of greater specificity than proteasome inhibitors. Preclinical data using nutlins provide encouragement that targeting RING finger E3–substrate interactions might be beneficial in the treatment of cancer, and one might envisage similar approaches to limit cell proliferation by interfering, for example, with SKP2–p27 interactions. Related to this, as a number of RING finger–E3 interactions are phosphorylation dependent, an alternative means of disrupting interactions is the inhibition of specific kinases. However, when it comes to inhibiting RING finger E3 activity itself, the jury is still out. Proof-of-principle has been established for MDM2, but 12 years on the number of reports of inhibitors that are specific for RING finger E3s is limited. Part of this challenge probably derives from the fact that RING finger domains activate ubiquitin transfer from the E2 directly to the substrate, but they are not bona fide catalysts with active sites. Thus, approaches would probably have to be focused on disrupting the RING structure or the RING–E2 interface, which seems to be fairly highly conserved among E2–E3 pairs. In many instances in which the loss of activity of RING finger or RING finger-like E3s is associated with the development of cancer (such as VHL, BRCA1, FANCL and CBL) novel approaches may be necessary. The promising results with PARP inhibition in BRCA1- and BRCA2-deficient tumours suggests that such a synthetic lethal approach may be applicable in some instances in which tumours arise because of loss of E3 activity. Additionally, ubiquitylation is a reversible process involving, in many cases, specific deubiquitylating enzymes. Therefore, in situations in which compromised ubiquitylation has a role in cancer, the inhibition of specific deubiquitylating enzymes may represent a viable therapeutic modality. With the accumulation of structural and functional data and the elucidation of the pathways that are controlled by RING finger E3s, there is great potential for seeing this knowledge used in the development of new, targeted therapeutics.

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Competing interests statement

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