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# Ubiquitin ligases in oncogenic transformation and cancer therapy

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## Abstract

The cellular response to external stress signals and DNA damage depends on the activity of ubiquitin ligases (E3s), which regulate numerous cellular processes, including homeostasis, metabolism and cell cycle progression. E3s recognize, interact with and ubiquitylate protein substrates in a temporally and spatially regulated manner. The topology of the ubiquitin chains dictates the fate of the substrates, marking them for recognition and degradation by the proteasome or altering their subcellular localization or assembly into functional complexes. Both genetic and epigenetic alterations account for the deregulation of E3s in cancer. Consequently, the stability and/or activity of E3 substrates are also altered, in some cases leading to downregulation of tumour-suppressor activities and upregulation of oncogenic activities. A better understanding of the mechanisms underlying E3 regulation and function in tumorigenesis is expected to identify novel prognostic markers and to enable the development of the next generation of anticancer therapies. This Review summarizes the oncogenic and tumour-suppressor roles of selected E3s and highlights novel opportunities for therapeutic intervention.

The proteome is the true driver or mediator of cellular functions and is therefore the preferred target of anticancer therapies. Most targeted therapies are directed against signalling circuits that are deregulated in cancer, exemplified by the targeting of protein kinases[1]. Altered activity of key regulatory proteins can be due to genetic or epigenetic modifications, which take place in the course of cell transformation. Signalling proteins are regulated by several site-specific post-translational modifications, of which `ubiquitylation` is second only to phosphorylation in abundance[2]. Ubiquitylation is

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SUPPLEMENTARY INFORMATION

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orchestrated by the sequential activity of ubiquitin-activating enzymes (E1s), ubiquitin-conjugating enzymes (E2s) and ubiquitin ligases (E3s) (BOX 1). Virtually every cellular protein is subject to ubiquitylation at least once in its lifetime, exemplifying the exquisite homeostatic control of this process. Ubiquitylation marks proteins for selective proteasomal or lysosomal degradation or marks entire organelles for autophagic clearance[3–5]. The two degradation pathways show extensive crosstalk and cooperation, as illustrated by the fact that proteasomes can be cleared by autophagy and that autophagy substrates can undergo proteasomal degradation in autophagy-deficient cellular compartments such as the nucleus[6,7]. Besides this fundamental role in maintaining a healthy proteome, ubiquitylation has multiple non-degradative functions, including the regulation of protein activity, localization and complex formation[8]. Consequently, ubiquitylation is associated with almost every cellular process, including the regulation of DNA integrity, gene expression and metabolism[8– 10]. Given the central role of ubiquitylation, it is not surprising that its deregulation is associated with a number of diseases, including cancer. The activity of many E3s is deregulated in cancer (Supplementary information S1 (table)) by epigenetic and genetic mechanisms and/or as a consequence of altered post-translational mechanisms, which are modified in response to extrinsic and intrinsic cues (reviewed in REF. 11) (FIG. 1).

Multiple mouse models have been developed to decipher the role of specific E3s in cancer (TABLE 1). E3s can elicit oncogenic (for example, MDM2, the E3 for p53) or tumour-suppressive (for example, von Hippel–Lindau disease tumour suppressor (VHL) and BRCA1) activity; however, shifting from one function to the other can be seen upon altered cellular signalling, as E3s regulate a diverse set of substrates, the regulation of which is influenced by the cellular context (for example, genetic background, cell lineage, differentiation state and stress level) and subcellular localization of the E3 (for example, speckle-type POZ protein (SPOP), the adaptor for the cullin 3–really interesting new gene (RING)–E3 ligase (CRL3) complex, functions as a tumour suppressor in the nucleus but as an oncogene in the cytoplasm[12,13]). Therefore, altered expression or post-translational modifications play key roles in the temporal and spatial function of E3s, diverting tumour suppressor E3s to oncogenes or vice versa. Given the substrate diversity of E3s, targeting one E3 potentially affects multiple processes required for the malignant phenotype (FIG. 2), pointing to E3s as desirable drug targets. However, a deeper, context-dependent understanding of each targeted E3 is required to appreciate possible tumour-promoting effects of putative inhibitors of E3s.

In this Review, we describe how deregulation of ubiquitylation affects malignant transformation, tumour progression and therapy resistance. We focus predominantly on the effects of deregulated ubiquitylation on DNA damage repair, cell cycle regulation, gene expression and signal transduction (FIG. 2), which dictate such pivotal cell fate decisions as senescence or quiescence, proliferation, differentiation or stemness and cell death. Lastly, given the role of selected ubiquitin-proteasome pathways in dictating the malignant phenotype, the opportunities for targeting deregulated ubiquitylation in cancer are also discussed.

## Ubiquitin ligases in genome maintenance

### Cell cycle control

Signals that control cell cycle entry, progression and arrest are commonly deregulated in cancer, and the subsequent disruption of DNA replication, DNA repair and chromosomal segregation often leads to genomic instability[14]. Deregulation of E3s that induce proteasomal degradation of cyclins and cyclin-dependent kinase (CDK) inhibitor proteins and/or that regulate the assembly of the DNA damage repair machinery thus contributes to the sustained proliferation and genomic instability observed in cancer cells (FIG. 3). Among the best-studied E3s that regulate cell cycle progression are the APC/C (anaphase-promoting complex; also known as the cyclosome) and its co-activators cell division cycle 20 (CDC20) or CDC20-like protein 1 (CDH1; also known as FZR1) and S-phase kinase-associated protein 1 (SKP1)–cullin 1–F-box protein (SCF) (and its component F-box proteins F-box/WD repeat-containing protein 7 (FBXW7), SKP2 and β-transducin repeat-containing protein (β-TRCP; also known as BTRC)) complexes.

Although APC/C itself is rarely mutated in cancer, increasing evidence supports a tumour-suppressor role for CDH1 and an oncogenic role for CDC20 (Supplementary information S1 (table)). *CDH1* functions as a haploinsufficient tumour suppressor (FIG. 3a). Aged $Cdh1^{+/-}$, but not wild-type (WT), mice show increased susceptibility to spontaneous epithelial tumours in various organs[15]. Accordingly, *Cdh1*-deficient mouse embryonic fibroblasts (MEFs) show reduced proliferation and accumulate chromosomal aberrations[15]. CDH1 knockdown in human bone osteosarcoma U2OS cells causes accumulation of cyclin A and cyclin B, premature entry into S-phase with reduced loading of pre-replication complexes onto DNA replication origins and accumulation of DNA double strand breaks (DSBs) during mitosis owing to the presence of replication intermediates[16].

Interest in the oncogenic potential of CDC20 arose when it was discovered that residual CDC20 activity upon activation of the spindle assembly checkpoint (SAC), (which sequesters CDC20 into the mitotic checkpoint complex, consisting of BUBR1 (also known as BUB1B), BUB3 and MAD2, to delay cyclin B1 degradation and induce mitotic arrest) promotes escape from antimitotic drug-induced apoptosis[17,18]. Consequently, blocking mitotic exit via CDC20 inhibition emerged as a more efficient means to induce apoptosis than spindle checkpoint-dependent mitotic poisons[18]. In a two-stage skin carcinogenesis mouse model, localized deletion of *Cdc20* results in massive metaphase arrest and apoptosis, and this phenotype is also observed *in vitro* in $Cdc20^{-/-}$ MEFs transformed with oncogenic $RAS^{G12V}$ and early region 1A (*E1A*) of human adenovirus type 5 (REF. 19). These findings prompted the development of two APC/C–CDC20 inhibitors: the small-molecule inhibitor, tosyl-L-arginine methyl ester (TAME), which binds to APC/C and prevents its activation by CDC20 or CDH1 (REF. 20), and apcin, which binds to CDC20 and blocks its interaction with the destruction box (D-box), which is present in almost all APC/C substrates[21]; apcin can act synergistically with TAME to block mitotic exit, exemplifying the difficulties in pharmacologically blocking the activity of the multisubunit APC/C efficiently[21]. Given that both inhibitors block CDH1 as well as CDC20 (REF. 20), caution is required to monitor for possible tumour-promoting consequences of CDH1 inhibition. The development of novel

CDC20-specific inhibitors could eliminate this concern. Recent advances in understanding how phosphorylation of the APC/C subunits stimulates CDC20 loading and activation of APC/C are an important step in this direction[22,23].

F-box proteins are often deregulated in cancer (Supplementary information S1 (table)), thereby affecting SCF complex activity (FIG. 3). FBXW7-containing SCF complexes mediate degradation of cyclin E (FIG. 3a), and impaired SCF–FBXW7 function leads to sustained proliferation and genomic instability[24,25]. *FBXW7* functions as a p53-dependent haploinsufficient tumour suppressor; thus, the effects of FBXW7 substrate accumulation upon heterozygous inactivation of *FBXW7* can be reversed by p53 expression[24–26]. In a mouse model, intestinal co-deletion of *Fbxw7* and *Trp53* results in advanced adenocarcinomas with increased cyclin E expression and a phenotype of chromosomal instability[24]. In addition, radiation-induced lymphomas in *Trp53*$^{+/-}$ mice but not *Trp53*$^{-/-}$ mice showed loss of heterozygosity and a 10% mutation rate of *Fbxw7* (REF. 26). However, rather than leading to cyclin E accumulation, deregulation of Aurora kinase A, another substrate of SCF–FBXW7, mediated genomic instability in this context[26].

The oncogenic SCF–SKP2 complex regulates a number of CDK inhibitors, of which the best studied is the tumour suppressor p27$^{KIP1}$ (REFS. 27–30) (FIG. 3a). Transgenic overexpression of *Skp2* in the mouse prostate induces hyperplasia, dysplasia and low-grade carcinoma[31], while co-expression of *Skp2* with *Nras*$^{G12V}$ or myristoylated *Akt1* in the mouse liver results in hepatocellular carcinoma (HCC)[32]. Conversely, *Skp2*-knockout efficiently inhibits tumour development in a conditional *Pten*-deficient and *Trp53*-deficient mouse prostate cancer model via activation of p27$^{KIP1}$-dependent, p21$^{CIP1}$-dependent and activating transcription factor 4 (ATF4)-dependent senescence[33]. Co-deletion of *Skp2, Rb1* and *Trp53* blocks tumorigenesis in the mouse pituitary and prostate in a p27$^{KIP1}$-dependent manner[34].

β-TRCP-containing SCF complexes play dual roles in cell cycle checkpoint control: they mediate cell cycle arrest via degradation of checkpoint kinase 1 (CHK1)-phosphorylated CDC25A[35] and relieve the arrest via degradation of WEE1, claspin, eukaryotic elongation factor 2 kinase (eEF2K) and Fanconi anaemia group M protein (FANCM) following phosphorylation by major M-phase kinases, such as polo-like kinase 1 (PLK1) and CDK1 (REFS 36–39) (FIG. 3a). In addition, SCF–β-TRCP promotes cell cycle arrest by targeting the degradation of casein kinase I (CKI)-phosphorylated MDM2, which leads to stabilization of p53 (REF. 40). Although these mechanisms might suggest a tumour-suppressor function for SCF–β-TRCP, the situation in human cancers is not clear. In part, this may be explained by its context-dependent opposing functions in cell cycle progression and arrest. However, many SCF–β-TRCP substrates are themselves tumour suppressors (for example, inhibitor of nuclear factor-κB (IκB), forkhead box protein O3 (FOXO3), p19$^{ARF}$ and repressor-element 1 (REI)-silencing transcription factor (REST)) and oncogenes (for example, TWIST1, MDM2 and β-catenin)[40–46], suggesting that the role of SCF–β-TRCP in cell cycle control may not entirely explain its effects in cancer.

In addition to the classical cell cycle regulators, studies in genetic mouse models and analysis of mutations in human cancers have pointed to the role of the E3 parkin in cell cycle

control[47–52] (FIG. 3a). Genetic alteration (including mutations and copy number loss) of *PARK2* (the gene encoding parkin; also known as *PRKN*) suggests a tumour-suppressive function for this E3[47,48,51]. In a mouse model of intestinal tumorigenesis, the rate of development of intestinal adenomas was found to be approximately fourfold higher in *Park2*$^{+/-}$ *Apc*$^{Min/+}$ mice than in *Apc*$^{Min/+}$ mice; increased tumour formation was associated with the loss of the inhibitory effect of parkin on cell proliferation[50]. In addition, knockdown of parkin in cancer cell lines is associated with multipolar spindles and the formation of micronuclei owing to cyclin E accumulation[51]. Notably, analysis of pan-cancer mutation data revealed that mutations in *PARK2* are mutually exclusive with alterations in G1 phase and S phase cell cycle regulators (cyclin D, cyclin E and CDK4)[48]. Furthermore, parkin can regulate cyclin D and cyclin E via formation of F-box only protein 4 (FBXO4)-containing or FBXW7-containing parkin–cullin–RING complexes, respectively[48]. Finally, parkin may interfere with cell cycle control by associating with CDC20 or CDH1, promoting degradation of several key mitotic regulators, including PLK1, NEK2, cyclin B1, securin, Aurora kinase A and Aurora kinase B, in an APC/C-independent manner[49]. In summary, the accumulation of mitotic regulators upon parkin loss of function may contribute to genomic instability, thereby promoting tumour formation.

In contrast to cancer, where parkin defects are associated with increased proliferation and increased tumorigenesis, in Parkinson disease, loss-of-function mutations of *PARK2* impair mitophagy, resulting in accumulation of damaged mitochondria and induction of apoptosis[53]. This difference might be explained in part by the post-mitotic nature of neurons compared with the proliferative phenotype of genetically unstable cancer cells, which may be able to circumvent apoptosis and tolerate mitochondrial damage; however, the roles of mitophagy and mitochondrial integrity in tumours, in which parkin function is lost through mutations, remain to be clarified (reviewed in REF. 54).

## DNA damage repair

Among the E3s, MDM2 and BRCA1 are known to link regulation of the DNA damage response and cell cycle checkpoints to cancer development (reviewed in REFS 55,56) (FIG. 3b). Briefly, MDM2 is overexpressed in a variety of cancers and promotes tumorigenesis primarily by targeting the degradation of p53, although the regulation of other substrates by MDM2 may contribute[55] (Supplementary information S1 (table)). Inhibitors that disrupt the interaction between p53 and MDM2 and/or the homologue MDMX (also known as MDM4) were developed[57], including the *cis*-imidazoline analogues (otherwise known as the nutlins) such as Nutlin-3 and RG7112, which are currently being assessed in clinical trials for haematological malignancies[58].

BRCA1 forms a heterodimer with BRCA1-associated RING domain protein 1 (BARD1) and mediates mono-ubiquitylation or non-degradative polyubiquitylation of its substrates[56]. The BRCA1–BARD1 complex is implicated in multiple cellular processes by virtue of its broad range of substrates, including histones, CtBPinteracting protein (CTIP; also known as RBBP8), oestrogen receptor-α (ERα), RNA polymerase II (RNAPII) and transcription initiation factor IIE (TFIIE) (Supplementary information S1 (table)). The aberrant role of BRCA1–BARD1 in homologous recombination (HR) and cell cycle control, and the

resulting genomic instability, is considered to be the key determinant in the aetiology of breast and ovarian cancer in women carrying *BRCA1* or *BRCA2* mutations[56]. Nonetheless, the importance of the E3 activity of BRCA1 in these processes is controversial. For example, a BRCA1–RING-domainmutant that lacks ligase activity cannot restore a cell cycle checkpoint or reverse γ-irradiation hypersensitivity in *BRCA1*-null human breast cancer cell lines[59]. Similarly, mice harbouring the clinically relevant missense $Brca1^{C61G}$ mutation, which confers aberrant E3 activity and reduces the interaction with BARD1, exhibit genomic instability and tumour development similar to that in $Brca1^{-/-}$ mice[60]. However, mice harbouring a $Brca1^{I26A}$ missense mutation (not found in human tumours), which allows heterodimer formation with BARD1 but disrupts the E3 activity of BRCA1, do show reduced tumour development compared with that in WT mice[61], suggesting that the E3 activity is not essential for tumour suppression. As BRCA1 functions as a scaffold protein for multiple protein complexes, protein–protein interactions and E3 activity are both likely to play important roles in BRCA1-mediated tumour suppression.

BRCA1 further links DNA DSB repair to the cell cycle[56,62]. DSB repair is regulated by ubiquitylation and deubiquitylation at multiple levels, starting with the recruitment of E3s RNF8 and RNF138 to DSBs, followed by a wave of ubiquitin signals that promote the recruitment of DNA repair factors to mediate chromatin remodelling at DSB sites[62]. Interestingly, the mode of DNA repair (that is, `non-homologous end joining` (NHEJ) or HR) is under cell cycle control (FIG. 3b). HR is restricted to the cell cycle phases when a sister chromatid is available for recombination and is thus suppressed during G1, partially via ubiquitin-dependent mechanisms[62–64] (FIG. 3). These mechanisms might be of therapeutic importance in cancer, because defective HR renders cells susceptible to inhibition of `base excision repair` (BER) mediated by poly (ADP-ribose) polymerase 1 (PARP1)[65–67]: the `deubiquitylating enzyme` (DUB) ubiquitin carboxyl-terminal hydrolase 11 (USP11) deubiquitylates partner and localizer of BRCA2 (PALB2) during S and G2 phases following DNA damage, allowing the formation of the BRCA1–PALB2–BRCA2 complex and HR repair to advance in these phases of the cell cycle[64]. USP11 is often overexpressed in cancer, confers resistance to PARP inhibitors[68] and may serve as a biomarker for PARP-inhibitor resistance. Conversely, its targeting may sensitize resistant tumours to PARP inhibition[68].

Furthermore, SCF–FBXW7 has been suggested to play a direct role in DNA DSB repair[69]. Activation of ataxia telangiectasia mutated (ATM), a crucial mediator of the DNA damage response, leads to phosphorylation of SCF–FBXW7 and its recruitment to the DSB sites, followed by K63-linked polyubiquitylation of X-ray repair cross-complementing protein 4 (XRCC4), a repair protein implicated in NHEJ. K63-ubiquitylated XRCC4 enhances its association with the KU70 (also known as XRCC6) and KU80 (also known as XRCC5) complex, thereby increasing NHEJ repair[69].

These examples establish the role of deregulated E3s in the uncontrolled proliferation and genomic instability that drive malignant transformation, tumour progression and therapy resistance. Individual E3s have a broad spectrum of substrates and thus can play multifaceted roles by serving as a nexus to coordinate cell growth, proliferation and survival under both favourable and hostile growth conditions (FIG. 2).

## Signal transduction regulation by ubiquitin ligases

E3s can regulate major growth-promoting pathways, including those targeted by current anticancer therapies such as the MAPK or PI3K–AKT–mTOR pathways[1,8] (FIG. 4a). Sustained activation of pathways that promote growth and survival constitutes a stressful environment; consequently, cancer cells must ensure that metabolic processes and stress signalling pathways are coordinately regulated in order to overcome these potentially deleterious conditions.

### Regulation of MAPK signalling

The MAPK and PI3K–AKT–mTOR pathways cooperate to drive cell growth, proliferation and survival[70] (FIG. 4a). They are the most hyperactivated pathways in cancer, and, not surprisingly, they are tightly regulated by the ubiquitin machinery. For example, the abundance of tyrosine kinase receptors at the cell surface is regulated by ubiquitin-dependent recycling (reviewed in REF. 4). Degradation of RAS by the E3 NEDD4 is part of a negative feedback loop in which RAS signalling induces transcriptional upregulation of NEDD4 that in turn limits the activity of WT RAS but not oncogenic RAS[71]. As NEDD4 is a known negative regulator of PTEN[72], NEDD4 upregulation increases PTEN degradation, further enhancing the malignant phenotype of RAS-driven tumours[71]. The RAS–NEDD4 relationship exemplifies how the genetic context or signalling state of a cell can affect the outcome of aberrant ubiquitylation: NEDD4 functions as a tumour suppressor in normal cells but as an oncogene in cells expressing hyperactivated and/or mutant RAS.

Biochemical and functional analysis suggested that the endosomal E3 RAB5 GDP/GTP exchange factor (RABEX5) promotes monoubiquitylation and diubiquitylation of HRAS and NRAS, which induces their localization to and retention in endosomes and thus suppresses their signalling output[73]. In non-small-cell lung cancer (NSCLC) cells expressing WT KRAS, the DUB OTU domain-containing ubiquitin aldehyde-binding protein 1 (OTUB1) suppresses RAS ubiquitylation in a proteolysis-independent manner, leading to increased RAS signalling[74]. However, *in vitro* studies suggest that monoubiquitylation of KRAS at K147 or HRAS at K117 can increase RAS signalling by inhibiting GTPase-activating protein (GAP)-mediated hydrolysis or increasing GTP–GDP exchange, respectively[75–77]. The expression of a KRAS-G12V;K147L-double mutant protein in mouse fibroblast NIH-3T3 cells reduces transformation monitored in subcutaneous mouse models, as compared to mice with KRAS-G12V alone, indicating that monoubiquitylation of KRAS-G12V may promote tumorigenesis[75]. However, as structural and mutational studies were limited to human embryonic kidney (HEK293) cells, further validation in cancer cells is required, and the cellular contexts and the E3s and DUBs that control the outcomes of monoubiquitylation remain to be defined (FIG. 4b).

BRAF, the downstream effector kinase of RAS, is also regulated by ubiquitylation. WT BRAF is targeted for degradation by the E3 RNF149 (REF. 78). Ubiquitin-dependent negative feedback control of BRAF activity has been observed in *Caenorhabditis elegans*[79], and a similar mechanism has been described in human cells[80]. ERK-mediated phosphorylation of either WT BRAF or BRAF-V600E, the most common mutant in melanoma, primes it for ubiquitin-dependent degradation[80]. In *C. elegans*, this was shown to

be mediated by an FBXW7 homologue[79], yet the human E3 remains to be defined[80]. BRAF-V600E, but not WT BRAF, requires the chaperone heat shock protein 90 (HSP90) for proper folding and stabilization[81]. When HSP90 activity is inhibited pharmacologically, its client proteins, including BRAF-V600E, are degraded by cullin-5-mediated ubiquitylation and proteasomal degradation[81,82]. CDH1 has also been suggested to limit BRAF signalling by multiple mechanisms[83]. In non-malignant cells, the APC/C–CDH1 complex induces BRAF proteolysis in a cell-cycle-dependent manner, but this process is suppressed in BRAF-V600E-expressing melanoma cells[83]. Interestingly, ERK and CDK4 both phosphorylate CDH1, which decreases its association with APC/C and leads to the accumulation of APC/C substrates, representing a positive feedback loop between hyperactivated ERK signalling and BRAF stability, as demonstrated in multiple melanoma cell lines and in BRAF-V600E-expressing immortalized mouse melanocytes. Consistent with this, treatment of cells with ERK inhibitors or CDK inhibitors decreases BRAF protein levels, suggesting that combined inhibition of these kinases is a useful therapy for cancers with hyperactivated BRAF[83]. Importantly, CDH1 can also limit signalling in cancer cells expressing WT BRAF (or dimerization-dependent BRAF mutants); in this case, direct binding of CDH1 prevents BRAF dimerization and full activation[83]. The dual-suppressor activity of CDH1 on BRAF illustrates the need to develop CDC20-specific inhibitors[21,83]. Although we are yet to fully define ubiquitin-mediated regulation of MAPK signalling, these examples provide the rationale for developing novel therapies that limit RAS signalling (for example, via OTUB1 inhibition) and suggest possible mechanisms of action of therapeutics already in clinical evaluation, including CDK4 and CDK6 inhibitors and HSP90 inhibitors.

## Regulation of PI3K–AKT–mTOR signalling

In contrast to the relative paucity of studies on the regulation of the MAPK pathway by ubiquitylation, multiple lines of evidence support a role for ubiquitin-mediated degradative and non-degradative pathways in regulating PI3K–AKT–mTOR signalling (FIG. 4c,d,e). The SCF–F-box and leucine-rich repeat protein 2 (FBXL2) complex has been demonstrated to regulate PI3K activation via degradation of the regulatory subunit p85β[84] (FIG. 4c). Mechanistically, SCF–FBXL2 marks p85β for degradation following dephosphorylation of p85β by protein tyrosine phosphatase PTPL1 (also known as PTPN13 and FAP1), thus preventing the competition between free p85β and active PI3K (made up of p110 (the catalytic subunit) and p85 heterodimers) for substrate binding[84]. Therefore, therapeutic targeting of FBXL2 may represent one route to limit PI3K signalling in cancer[84].

In addition to its effects on cyclins, parkin limits AKT activity and WNT–β-catenin signalling (by targeting β-catenin for proteasomal degradation), two major pathways regulating cell growth and survival[85]. Furthermore, *PARK2* copy number loss has been implicated in stimulating the PI3K–AKT pathway via mitochondrial dysfunction in PTEN-expressing but not PTEN-null cancers[86]: mechanistically, knockdown of *PARK2* in cancer cells impaired mitochondrial metabolism, reflected by decreased ATP levels, increased oxidative stress, activation of AMP-activated protein kinase (AMPK) and phosphorylation and activation of endothelial nitric oxide synthase (eNOS)[86]. The latter induces PTEN *S*-nitrosylation and concomitant ubiquitylation-dependent degradation[86] (FIG. 4c). In agreement with these *in vitro* findings[86], both the Cancer Cell Line Encyclopedia and The

Cancer Genome Atlas (TCGA) indicate that heterozygous deletion of *PARK2* frequently occurs in PTEN heterozygous cancer cells and tissues. Accordingly, *Pten*$^{+/-}$ mice crossed with mice carrying a targeted knockout of *Park2* exon 3 are significantly more tumour prone than *Pten*$^{+/-}$*Park2*$^{+/+}$ mice[86].

The SCF–SKP2-mediated K63-linked ubiquitylation of AKT1 and AKT2 promotes AKT recruitment to ERBB receptors and its concomitant activation[87]. ERBB activation in breast cancer cells elevates glucose uptake and glycolysis and increases cellular resistance to HER2-targeting therapies[87]. Interestingly, these AKT-mediated metabolic effects can be reversed by a SKP2 inhibitor, resulting in a p53-independent but p27$^{KIP1}$-dependent senescence[88]. This inhibitor reduces the viability of multiple cancer cell types (including p53-deficient cancer cells) *in vitro* and in xenograft tumour mouse models, pointing to a potential antitumour activity of this inhibitor in SKP2-overexpressing human cancers[88]. Of note, AKT1 can regulate SKP2 localization by direct phosphorylation of SKP2 at S72 (REFS 89,90) and via histone acetyltransferase p300 activation, which in turn mediates SKP2 acetylation, thereby inhibiting its interaction with CDH1 and enabling its retention in the cytoplasm; this is associated with more aggressive phenotypes in breast and prostate cancer[91]. Liverspecific loss of HIPPO signalling in a liver cancer mouse model also resulted in AKT–p300-mediated acetylation and cytoplasmic retention of SKP2 (REF. 92). In hepatocytes isolated from mouse strains with defective HIPPO signalling, cytoplasmic localization of SKP2 is associated with p27$^{KIP1}$ stabilization and, consequently, mitotic arrest and polyploidy[92]. The effect of AKT activity on SKP2 is also linked to the degradation of the transcriptional activators forkhead box protein O1 (FOXO1) and FOXO3, which positively regulate apoptosis; in turn, this enables increased proliferation of polyploid cells, genomic instability and increased formation of HCC in the HIPPO signalling-deficient liver[92].

The activity and stability of mTOR is regulated by a diverse set of E3s (FIG. 4e): among them is SCF–FBXW7, which binds, ubiquitylates and promotes mTOR degradation[93]. In a subset of breast cancer cell lines, loss of a single copy of *FBXW7* appears mutually exclusive with loss of a single copy of *PTEN* (a well-established, indirect, negative regulator of mTOR), substantiating the importance of FBXW7-mediated mTOR stabilization in tumorigenesis; thus, in addition to its effects on the cell cycle, SCF–FBXW7 loss may potently stimulate anabolic processes to promote tumour progression[93].

Activation of mTOR complex 1 (mTORC1) was previously shown to be regulated by K63-linked ubiquitylation mediated by the E3 tumour necrosis factor (TNF)-receptor-associated factor 6 (TRAF6) in complex with the scaffold protein p62 (also known as sequestosome 1)[94], and more recent work has demonstrated the role of ubiquitylation in the dynamic assembly of mTORC1 and mTORC2 (REF. 95). TRAF2 mediates K63-linked ubiquitylation of GβL (also known as mLST8; a component of both mTORCs) and disrupts its binding to the mTORC2-specific component mSIN1 (also known as MAPKAP1), thereby restricting formation of mTORC2 complexes[95]. Deubiquitylation of GβL by OTU domain-containing protein 7B (OTUD7B) re-establishes the GβL–mSIN1 interaction, mTORC2 formation and AKT phosphorylation (S473)[95]. The physiological relevance of this pathway can be illustrated in melanoma, where GβL mutants that lack the ubiquitylation site were identified,

and ectopic expression of these mutants in a melanoma cell line increased chemoresistance *in vitro* and tumour growth upon xenotransplantation in nude mice[95]. Second, OTUD7B amplifications were identified in a variety of cancers, including lung cancer, and homozygous deletion of *Otud7b* in the $Kras^{LA2}$ lung cancer mouse model inhibited KRAS-driven lung tumorigenesis[95].

## Adaptive stress responses

To illustrate the role of E3s in the adaptive stress response in cancer, we focus here on the E3s seven in absentia homologue 2 (SIAH2) and CRL3–kelch-like ECH-associated protein 1 (KEAP1), which exemplify ubiquitin-dependent crosstalk between cellular pathways and organelles to promote adaptive responses to stress while inhibiting cell death pathways. Members of the SIAH RING-finger family (SIAH1 and SIAH2) play important roles in fine-tuning the cellular response to `hypoxic tension`[96–98] and the `unfolded protein response` (UPR)[99]. Under hypoxic conditions, transcription and protein phosphorylation of SIAH1 and SIAH2 are upregulated, increasing their abundance and activity[96,97,100]. Activated SIAH1 and SIAH2 degrade prolyl hydroxylase 1 and prolyl hydroxylase 3, which limits prolyl hydroxylation of the transcription factor hypoxia-inducible factor 1α (HIF1α) and prevents its degradation by VHL[97]. In addition, SIAH1 increases the transcriptional activity of HIF1α by marking the serine/threonine homeodomain-interacting protein kinase 2 (HIPK2), which regulates HIF1α and other accessory components of the transcriptional machinery, for degradation[98]. SIAH2 negatively regulates the HIPPO signalling pathway by degrading large tumour suppressor kinase 2 (LATS2)[101]. In turn, activated transcriptional co-activator Yes associated protein 1 (YAP1) stabilizes HIF1α and cooperates in its target gene expression[101].

SIAH2 is overexpressed in a variety of cancers, and given its role in the regulation of hypoxia signalling, it is implicated to function as an oncogene in breast cancer, prostate cancer, HCC and melanoma[102–106]. SIAH1 and SIAH2 control of prolyl-hydroxylases has been shown to affect the regulation of ATF4 stability, contributing to the degree of ATF4 availability. Increased expression of ATF4 commits cells to death signalling programmes, in part by enhanced cooperation with C/EBP homologous protein (CHOP; also known as DDIT3), as shown under conditions of glucose and oxygen deprivation[107]. Notably, as ATF4 controls *SIAH2* transcription, the SIAH2–ATF4 axis constitutes a feedforward mechanism to determine the level of ATF4 availability and, consequently, the pathways it induces[107]. In addition to its role under hypoxic and endoplasmic reticulum stress conditions, SIAH2 has HIF-independent oncogenic roles that may rely on promoting the degradation of sprouty 2 (SPRY2), a negative regulator of RAS signalling[103]. Consistent with this, RAS-driven pancreatic tumours have been shown to be SIAH2-dependent[108].

KEAP1 is an adaptor protein for the CRL3 complex[109– 111]. Under normal conditions, CRL3–KEAP1 targets nuclear factor erythroid 2-related factor 2 (NRF2; also known as NFE2L2), the transcriptional master regulator of the antioxidant response, for proteasomal degradation[109–111]. Upon oxidative stress, CRL3–KEAP1 is directly inactivated through oxidant-induced or electrophile-induced conformational changes that mediate its dissociation from NRF2, leading to NRF2 stabilization[109–111]. Cancer cells rely on an

elevated anti-oxidant response to counter the deleterious effects of reactive oxygen species (ROS)[112,113]. Consistent with this, increased NRF2 activity, resulting from *KEAP1* mutations[114–120], *NRF2* promoter hypermethylation[118,121–123] or mutations in *NRF2* that disrupt KEAP1 association[124], is a negative prognostic marker in many cancers. Alterations in the KEAP1–NRF2 pathway have been implicated in the pathogenesis of lung cancer, where co-deletion of *Keap1* and *Trp53* in airway basal stem cells results in the development of lung tumours (upon transplantation of the cells into nude mice) that resemble human lung squamous cell carcinoma (SCC)[125].

The KEAP1–NRF2 axis is reportedly regulated by `succination`[126,127]. Hereditary leiomyomatosis and renal cell carcinoma (HLRCC) is an inherited cancer syndrome, in which patients are at risk of developing papillary renal cell carcinomas (pRCCs) owing to germline mutations in fumarate hydratase (*FH*), and pRCCs are associated with loss of heterozygosity at this locus[128]. FH-deficiency results in aberrant succination, and in pRCC, succination of KEAP1 inhibits its activity and leads to an increased antioxidant response[126,127]. Interestingly, the KEAP1–NRF2 axis is tightly connected to autophagy, which is activated downstream of many cellular stresses as a major survival pathway[99,129]. Accumulation of the autophagy receptor p62 induces binding to and inactivation of KEAP1 (REF. 130). The p62–KEAP1 interaction is increased by phosphorylation[131], allowing sustained NRF2 activation and increased proliferation of HCC cell lines *in vitro* and in xenograft mouse models[131]. This non-canonical pathway of NRF2 activation can be counteracted by tripartite motif 21 (TRIM21), which polyubiquitylates p62 at K7, thereby preventing its oligomerization and, consequently, NRF2 activation[132].

By modulating mitotic signalling as well as the cellular response to stress, E3s orchestrate a balance between cell growth and survival signals to promote tumour initiation and progression. In addition, E3s can regulate transcriptional programmes controlled by cellular stresses, exemplified by their impact on the HIF1α and NRF2 pathways.

## Regulation of gene expression by ubiquitin ligases

Ubiquitin can affect transcription by modulating the abundance and activity of transcriptional activators, modulating binding of transcriptional activators and formation of transcriptional complexes at genes and regulating chromatin structure[133]. We focus here on the deregulation of the oncogenic transcription factor MYC as an example of the mechanisms by which ubiquitylation affects transcription factors in cancer.

Turnover of MYC is regulated by multiple cancer-associated E3s and E3 complexes in a tissue-specific manner, including SCF–FBXW7 (REFS 134–136), CRL3–potassium channel tetramerization domain-containing protein 2 (KCTD2)[137], HUWE1 (REFS 138–140), CRL3–SPOP[141] and SCF–SKP2 (REF. 142). MYC is additionally stabilized by mutations in the phosphodegron motif, which disable MYC phosphorylation by glycogen synthase kinase 3 (GSK3) and thus prevent recognition by FBXW7 (REF. 143). Deregulation of the SCF–FBXW7–MYC axis has been shown to affect stemness features in T cell acute lymphoblastic leukaemia (T-ALL) and chronic myeloid leukaemia (CML) with different outcomes[134–136]. Ablation of *Fbxw7* in CML *in vivo* causes MYC accumulation, which

drives leukaemia-initiating cells out of the quiescent state and renders them sensitive to the tyrosine kinase inhibitor imatinib[134,135]. However, in T-ALL, mutant *Fbxw7*-knockin in mice cooperates with NOTCH1 to induce aggressive T-ALL. The accompanying increase in MYC levels expands the stem cell pool, and MYC inhibition results in disease remission[136]. Given that MYC functions as a transcriptional amplifier rather than as a reprogrammer[144], the distinct functions of MYC in maintaining the leukaemia-initiating cell phenotype may be explained by the differences in the genetic background of T-ALL and CML.

In a human glioma cell line and in CDKN2Adeficient mouse astrocytes, CRL3–KCTD2 was found to regulate MYC stability[137]. Interestingly, KCTD2 expression is lower in patient-derived glioma stem cells than in the non-stem-cell population[137]. Targeted inhibition of KCTD2 in human glioma cells or in CDKN2A-deficient mouse astrocytes leads to MYC-dependent regulation of stem cell markers and self-renewal capacity, increased glycolysis and increased *in vivo* intracranial tumour growth[137]. HUWE1, an X-linked member of the homologous to E6AP carboxy terminus (HECT) family of E3s, has been shown to control MYC regulation in skin and colon epithelia[138–140]. HUWE1 depletion accelerates tumour formation in the two-stage skin carcinogenesis model in mice, but this is reversed by concomitant MYC depletion[138]. A tumour-suppressor function for HUWE1 has been shown in colorectal cancer, where conditional knockout of *Huwe1* alone was sufficient to promote tumorigenesis in mice through a mechanism involving hyperactivated WNT signalling and MYC accumulation[139]. Ablation of *Myc* in the HUWE1-depleted intestine reduced intestinal hyperproliferation but maintained the mislocalization of Paneth cells mediated by WNT-dependent regulation of ephrin type-B receptor 3 (EPHB3)[139]. Likewise, co-deletion of *Huwe1* and *Apc* in the mouse intestine accelerated tumour development compared with deletion of only one copy of *Apc*[140]. This phenotype was associated with increased MYC levels and rapid accumulation of DNA damage, leading to loss of the second copy of *Apc*[140]. In contrast to the clear tumour-suppressor functions of *Huwe1* in knockout mouse models, other studies suggest a tumour-promoting function. In cultured cancer cell lines, HUWE1 mediates K63-linked polyubiquitylation of MYC, which is required for transcriptional activation of a subset of MYC target genes, and knockdown of HUWE1 diminishes cell growth[145]. Consistent with this, small-molecule inhibitors of HUWE1 repress the growth of tumour cell lines and orthotopic xenografts in mice by stabilizing MYC-interacting zinc-finger protein 1 (MIZ1; also known as ZBTB17), which accumulates at MYC-regulated promoters and represses MYC-activated target genes[146]. Given the evidence that the HUWE1–MYC axis can both support and suppress tumorigenesis, it is crucial that the exact mechanisms underlying their interaction are clarified. This should be facilitated by the availability of small-molecule inhibitors to HUWE1.

MYC can also be targeted for degradation by the SCF–SKP2 complex. Interestingly, MYC turnover in this context is associated with an increase in MYC transcriptional activity[142,147]. MYC has been shown to bind to the transcription polymerase-associated factor elongation complex (PAF1C) through an N-terminal region of MYC, which functions to target the MYC protein for degradation, known as MYC-box I[148]. Ubiquitin-dependent degradation of MYC by E3s that target another region, known as MYC-box II, such as SCF–SKP2 but not SCF–FBXW7, induces turnover of inhibitory MYC–PAF1C complexes and enables formation of MYC activator complexes that recruit transformation/transcription domain-

associated protein (TRRAP), histone acetyl-transferases and, consequently, the transcriptional regulators bromodomain-containing 4 (BRD4) and positive transcription elongation factor b (PTEFb) complex to activate transcription of MYC target genes[148]. Therefore, MYC turnover by SCF–SKP2 increases MYC transcriptional activity via the rapid replacement of inhibitory MYC complexes by activating ones.

Finally, MYC has been identified as a CRL3–SPOP substrate in prostate epithelial cells[141]. SPOP mutations found in prostate and endometrial cancers are thought to disrupt substrate binding, suggesting that SPOP plays a tumour-suppressor role in these tissues[12,141,149]. Accordingly, prostate-specific biallelic ablation of *Spop* in mice results in MYC accumulation and promotes the development of prostatic intraepithelial neoplasia[141]. CRL3–SPOP has also been implicated in the regulation of steroid receptor co-activator protein 3 (SRC3; also known as NCOA3), androgen receptor (AR) and ETS-related gene (ERG), key oncogenic signalling proteins in prostate cancer[12,150,151]. Notably, *SPOP* mutations and transmembrane protease serine 2 (*TMPRSS2*)–*ERG* gene fusions occur in a mutually exclusive manner in prostate cancer with both alterations inhibiting CRL3–SPOP-dependent ERG degradation[151,152]. Furthermore, the expression of mutant SPOP promotes prostate cancer cell proliferation and tumour growth via stabilization of AR[153]. Interestingly, a selected portion of AR transcriptional activity is regulated by SIAH2, which targets a transcriptionally inactive pool of nuclear receptor co-repressor 1 (NCOR1)-bound AR for degradation, promoting the expression of AR target genes implicated in lipid metabolism, cell motility and proliferation[154]. Such regulation was shown to promote the growth of prostate cancer cells under conditions of androgen deprivation *in vivo*, implicating SIAH2 in the development of castration-resistant prostate cancer. Coupled with the observation that SIAH2 controls (via regulation of the HIF-target genes *HES6, SOX9* and lysine demethylase 3A (*KDM3A*; also known as *JMJD1A*)) the development of a neuroendocrine type of prostate cancer often seen in castration-resistant tumours[102], the SIAH2 regulatory axis constitutes a promising therapeutic target for these aggressive, treatment-resistant tumours.

These studies illustrate how turnover of transcriptional activators or repressors modulates the output of specific oncogenic transcriptional networks. Although non-degradative ubiquitylation of transcription factors occurs, the relevance of these modifications to cancer is less well studied. In yeast, it was shown that fusion of a single ubiquitin moiety to a heterologous transcriptional activator LexA–VP16, composed of the bacterial LexA DNA binding domain and the activation domain from herpes simplex virus protein VP16, leads to its recognition by the AAA+ ATPase cell division control 48 (CDC48; a transitional endoplasmic reticulum ATPase known as p97 or VCP in mammals), which extracts monoubiquitylated LexA-VP16 from DNA without inducing its proteasomal degradation[155]. In mammalian cells, this mechanism limits transcriptional activity of the SMAD2 and SMAD3 complex, and depletion of p97 in breast cancer cells sufficiently induces transcription of SMAD target genes independently of transforming growth factor-β (TGFβ) stimulation[155]. By contrast, site-specific monoubiquitylation of p53 or interferon regulatory factor 1 (IRF1) within the DNA-binding domain promotes the interaction of these transcriptional activators with DNA; the cationic ubiquitin moiety directly interacts with anionic DNA, increasing the physical interaction between the transcription factor and DNA, thereby promoting transcriptional activity[156].

Finally, ubiquitylation is a common post-translational modification of histones, the best-studied examples of which are monoubiquitylation of histone 2A (H2A) and H2B. Ubiquitylation of histones affects transcription by altering chromatin structure and its access to the transcriptional machinery as well as by recruiting signalling molecules that mediate other modifications, which in turn regulate chromatin function[133]. Histones are ubiquitylated by E3s such as the RNF20 (also known as BRE1A)–RNF40 (also known as BRE1B) complex, the Polycomb repressive complex 1 (PRC1) and the BRCA1–BARD1 complex and deubiquitylated by DUBs such as USP22 or BRCA1-associated protein 1 (BAP1). An in-depth discussion of histone ubiquitylation can be found elsewhere[133,157,158].

## Cell death regulation by ubiquitin ligases

E3s are important regulators of mitochondrial (FIG. 5a) and receptor-mediated apoptotic and necroptotic pathways (FIG. 5b), and deregulation of these pathways confers a survival advantage on cancer cells. Several cancer-associated E3s target the anti-apoptotic protein myeloid cell leukaemia 1 (MCL1) for degradation[159–164], thereby sensitizing cells to apoptosis via a number of distinct pathways (FIG. 5a). For example, DNA damage induced by ultraviolet irradiation or the chemotherapeutic agents etoposide and cisplatin promotes HUWE1 binding to MCL1 via its BCL-2 homology domain 3 (BH3)-domain and marks MCL1 for proteasomal degradation[163]. Similarly, mitochondrial damage induces parkin to degrade MCL1, sensitizing the cells to apoptotic stimuli that induce `mitochondrial depolarization`[159]. Additionally, MCL1 targeting by the cell cycle regulators APC/C–CDC20 and SCF–FBXW7 links apoptosis to prolonged mitotic arrest[160,162]. Whereas CDK1–cyclin B-dependent phosphorylation of MCL1 induces its destruction via APC/C–CDC20 (REF. 160), CKII, JUN N-terminal kinase (JNK) and the p38 MAPK phosphorylate MCL1 to mark it for SCF–FBXW7-dependent degradation[162] during prolonged mitotic arrest. Finally, GSK3-mediated phosphorylation of `degrons` in MCL1 is linked to SCF–FBXW7-mediated degradation[164] and induces apoptosis following overexpression of the oncoproteins MYC, JUN or Notch. Therefore, stabilization of MCL1 explains the survival of cells upon loss of SCF–FBXW7 function and the corresponding upregulation of its targets MYC, JUN or Notch[164]. As the SCF–FBXW7–MCL1 axis reportedly confers resistance to the BH3-only mimetic ABT-737, anti-tubulin drugs and targeted protein kinase therapies such as regorafenib and sorafenib[162,164–166], targeting MCL1 may be a valuable approach to overcome the resistance of *FBXW7*-deficient cancer cells to these therapies. In this context, a study in human SCC cell lines demonstrated that vorinostat, a histone deacetylase (HDAC) inhibitor, upregulates the expression of pro-apoptotic BH3-only proteins, which in turn inhibit MCL1 and render *FBXW7*-deficient cancer cells susceptible to ABT-737 *in vitro* and in a xenograft mouse model[165].

Although SCF–FBXW7 is a *bona fide* tumour suppressor[167], it is noteworthy that *FBXW7* loss-of-function mutations are not detected in all cancers[167] (for example, multiple myeloma[168]), indicating that some cancers may rely on the activity of SCF–FBXW7. One possible explanation for such a dependency is that the accumulation of FBXW7 targets can be detrimental for cancer cells. Indeed, knockdown of FBXW7 in multiple myeloma cells dependent on the constitutive activation of non-canonical nuclear factor-κB (NF-κB) activity induces apoptosis and inhibits tumour growth *in vitro* owing to the accumulation of the

SCF–FBXW7 substrate p100 (also known as NF-κB2; a negative regulator of the non-canonical NF-κB pathway)[169]. Accordingly, expression of a p100 mutant that escapes recognition by SCF–FBXW7 inhibits tumour growth in mouse xenotransplantation experiments. Therefore, pharmacological inhibition of SCF–FBXW7 may be a valuable therapeutic approach in multiple myeloma[169] or, similarly (as discussed earlier), may increase the efficiency of imatinib in CML by driving leukaemia-initiating cells out of the quiescent state owing to accumulation of MYC[134,135].

Another example of the specificity of E3s in cell death regulation in different cancer types is the function of CRL3–SPOP in clear cell RCC (ccRCC). SPOP is over-expressed in almost 100% of ccRCC samples[170], where it is confined to the cytoplasm, in contrast to non-RCC cancers[13,171]. Cytoplasmic CRL3–SPOP drives the degradation of several negative regulators of proliferation (for example, PTEN, dual specificity protein phosphatase 7 (DUSP7) and the transcription factor GLI2) and apoptosis (for example, death domain-associated protein 6 (DAXX))[13]. Consequently, knockdown of SPOP inhibits proliferation and induces apoptosis of ccRCC cells but not of non-RCC cells, such as the cervical cancer cell line HeLa or the HEK293 cell line[13]. Small-molecule inhibitors of CRL3–SPOP–substrate interactions demonstrate selective killing of human ccRCC cells but not of cells lacking cytoplasmic accumulation of SPOP[171]. Thus, the cytoplasmic localization of SPOP seems to shift its function from a pro-apoptotic (in the nucleus) to an anti-apoptotic and pro-proliferative E3.

Mitochondrial apoptosis can be induced by mitochondrial calcium overload following increased calcium transfer from the endoplasmic reticulum to mitochondria[172]. The SCF–FBXL2 complex, which regulates PI3K signalling[84], has further been found to target the endoplasmic reticulum calcium transporter inositol 1,4,5-trisphosphate (IP3) receptor 3 (IP3R3) for degradation, thereby limiting mitochondrial calcium overload and thus apoptosis[173]. FBXL2 was found to compete with PTEN for IP3R3 binding, and consequently, IP3R3 degradation is increased in *PTEN*$^{-/-}$ cancer cells, thereby increasing apoptosis resistance in PTEN-deficient tumours[173]. Similarly, the tumour-suppressive DUB BAP1 has been demonstrated to deubiquitylate and stabilize IP3R3 (REF. 174). Consequently, heterozygous loss of BAP1 leads to decreased IP3R3 levels and resistance to apoptotic stimuli, as demonstrated in human fibroblasts or mesothelial cells derived from mutant *BAP1*$^{+/-}$ carriers and in mesothelioma cell lines[174].

Regulation of the extrinsic apoptosis pathway relies on the assembly of signalling complexes downstream of TNF superfamily receptors, a process controlled by K63-linked and M1-linked ubiquitylation (FIG. 5b). There are two groups of death receptors, which differ in the coordination and outcomes of their downstream signalling pathways. TNF receptor 1 (TNFR1; also known as TNFRSF1A)-like receptors preferentially induce inflammatory signalling by forming the receptor bound complex I. Upon stimulation with TNFR1-ligands, recruitment of the E3s cellular inhibitor of apoptosis 1 (cIAP1) and cIAP2 and `linear ubiquitin chain assembly complex` (LUBAC) mediates K63-linked and M1-linked polyubiquitylation of receptor-interacting serine/threonine-protein kinase 1 (RIPK1), which in turn triggers rapid and robust activation of NF-κB and MAPK signalling[175]. Formation of the secondary, receptor-free cytoplasmic complex II is largely dependent on the activity of

DUBs, specifically cylindromatosis (CYLD), A20 (also known as TNFAIP3) and ubiquitin thioesterase OTULIN, which destabilize complex I, abrogate NF-κB activation and release RIPK1 from complex I, which then forms the cytosolic complex II[175,176]. Thus, these DUBs regulate the switch from the pro-survival to the pro-death response[176]. Multiple components of this complex machinery are deregulated in cancer, and the aberrant activity of cIAPs or LUBAC as well as the loss of function of CYLD or A20 through gene inactivation contributes to tumorigenesis and resistance to therapy by inhibiting apoptosis and promoting inflammation via activation of oncogenic NF-κB signalling (reviewed in REFS 8,176).

By contrast, TNF-related apoptosis-inducing ligand (TRAIL) receptor (TRAILR; also known as TNFRSF10)-like signalling induces the formation of a membrane-associated death-inducing signalling complex (FIG. 5b), and inflammatory signalling and necroptosis are thought to be mediated by the formation of a secondary cytosolic receptor complex II (REF. 177). Recent studies indicate that RIPK1 (REFS 178,179) and LUBAC[179] can be directly recruited to membrane-bound TRAILR, suggesting that NF-κB activation can occur at the plasma membrane. Understanding the signalling circuits that are activated downstream of TRAILR is of special interest, as several TRAILR agonists have been evaluated as putative cancer therapeutics with limited clinical success (reviewed in REF. 177). The finding that TRAILR can induce migratory[180] and inflammatory responses that promote tumorigenesis[181] sheds light on the complex signalling circuits that respond to TRAIL. Therefore, fine tuning of LUBAC-mediated NF-κB activation may constitute one step to improve therapeutic effects of TRAILR agonists[179] and possible pathways that have been associated with deregulated LUBAC components.

## Ubiquitin ligases as therapeutic targets

The increasing recognition and understanding of the critical roles played by E3s in many fundamental cellular processes suggest that they could be targeted as a novel therapeutic modality. Possible routes to inhibit an oncogenic E3 by small molecules or peptides include repression of its expression, alteration of its subcellular localization, inhibition of its interaction with substrates, inhibition of its assembly into multisubunit complexes, inhibition of homodimerization or heterodimerization and inhibition of its catalytic domain[182,183]. Structure-based design, combined with advanced small-molecule screening technologies, is among the current approaches used for the development of E3 inhibitors. Pursuing these approaches has led to the development of E3 inhibitors that are currently being evaluated in the clinical and preclinical setting, including inhibitors of APC/C[20,21], MDM2 (REFS 57,58), SKP2 (REFS 88,184), SPOP[171] and cIAP[182,185]. However, it is not trivial to develop modulators of protein–protein interactions, and inhibition of E3s is currently one of the more challenging areas of drug development.

Targeting tumour suppressors in cancer therapy remains challenging generally. Approaches to target tumour suppressor E3s may involve re-expression of the repressed E3 (in cases where there are no inactivating mutations or deletions), exploration of genetic vulnerabilities by exploiting the concept of synthetic lethality (for example, PARP inhibitors in BRCA1-deficient or BRCA2-deficient cancers[65–67]) or inhibition of downstream oncogenic substrates. The latter may be achieved by the direct targeting of individual oncogenic

mediators (for example, targeting MCL1 in cancers with SCF–FBXW7 inactivation) or by a more general approach with proteolysis-targeting chimaera (PROTAC) technology, wherein bifunctional molecules are used to guide other E3s to degrade oncogenic substrates[183,186].

Yet, when considering E3s as therapeutic targets, a few important cautionary notes must be voiced. First, E3s can act as tumour suppressors or promoters in a sub-strate-dependent and context-dependent manner; thus, their targeting requires a deep understanding of their activity in a tissue-dependent and tumour-dependent manner. Second, specific post-translational modifications can convert an E3 from a tumour suppressor to a tumour promoter and vice versa, highlighting the complexity of their regulation and function. Third, because E3s have similar catalytic domains and target both tumour promoters and tumour suppressors, the ideal inhibitors would disrupt only the interactions of an E3 with substrates that are critical to cancer biology. Advances are being made, and the concept of targeting E3s as an anticancer therapy is gaining traction. Understanding the E3 structure as well as the post-translational modifications that regulate the spatial and temporal activity of E3s will undoubtedly guide the development of biologics and small-molecule inhibitors for this class of proteins.

## Conclusions

E3s are deregulated in cancer through diverse mechanisms, resulting in altered expression and/or activity of their target proteins. Here, we focus on the major regulatory hubs currently implicated in aberrant E3 function in cancer. As we better understand mechanisms that underlie deregulation of E3s, we recognize that the mechanisms that control their localization and activity — mainly post-translational modifications (that is, phosphorylation, which enables E3 interaction with substrates or affects its localization[96,187]) — merit further study. As deregulation of E3s alone is not sufficient to elicit the phenotypes seen in human cancers, their cooperation with additional genetic and epigenetic events should be appreciated and need to be defined. Given that tumour heterogeneity appears to be a key driver of tumour dormancy, metastasis and therapy resistance[113], it is expected that the temporal and spatial control of E3s would define tumour cell plasticity and/or heterogeneity.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Glossary

**Ubiquitylation**

An enzymatic reaction that leads to the attachment of ubiquitin moieties either to ubiquitin itself (creating polyubiquitin chains) or to other proteins via isopeptide linkages.

**Ubiquitin-activating enzymes (E1s)**
Enzymes that activate ubiquitin in an ATP-dependent manner.

**Ubiquitin-conjugating enzymes (E2s)**
Enzymes that first accept activated ubiquitin from a ubiquitin-activating enzyme (E1) and then transfer it to substrates.

**Ubiquitin ligases (E3s)**
Enzymes that facilitate substrate recognition and guide the transfer of activated ubiquitin from the ubiquitin-conjugating enzyme (E2) to specific substrates.

**Mitophagy**
The selective autophagic clearance of damaged mitochondria.

**Cyclins**
A family of regulatory proteins that show oscillating expression throughout the cell cycle and that are required for the activation of cyclin-dependent kinases.

**Cyclin-dependent kinase (CDK)**
A type of protein that belongs to a group of serine and threonine kinases that require cyclins for activation and regulate cell cycle progression.

**Haploinsufficient**
A state in which one copy of a gene is inactivated or deleted and the remaining functional copy is not sufficient to preserve normal function.

**Replication origins**
Sites in the DNA where the replication machinery is loaded at the onset of DNA synthesis.

**Spindle assembly checkpoint (SAC)**
A stage in the cell cycle that is activated during mitosis and meiosis to delay cell division until all chromosomes are correctly attached to the spindle.

**Phosphodegron**
One or multiple phosphorylated residues in a protein substrate that are necessary for recognition by some ubiquitin ligases.

**Destruction box (D-box)**
A conserved sequence of amino acids (RxxL) in proteins that is recognized by the APC/C (anaphase-promoting complex; also known as the cyclosome).

**Micronuclei**
Extranuclear bodies that form if chromosome fragments or entire chromosomes are not incorporated into the nucleus following cell division.

**Non-homologous end joining (NHEJ)**

An error-prone mechanism to repair DNA double strand breaks whereby the broken ends can be ligated, even with little or no sequence complementarity.

**Base excision repair (BER)**
A DNA repair mechanism that replaces bases that are damaged as a result of oxidation, deamination or alkylation.

**Deubiquitylating enzyme (DUB)**
A protease (cysteine protease or metalloproteinase) that cleaves the isopeptide linkage between the protein substrate (which can be ubiquitin itself) and the ubiquitin residue.

**Hypoxic tension**
The level of oxygen (usually measured as a percentage) in a given tissue or microenvironment. The lower the level of oxygen, the higher the tension.

**Unfolded protein response (UPR)**
A well-defined process that plays a critical role in restoring homeostasis following accumulation of potentially toxic misfolded proteins in the endoplasmic reticulum.

**Succination**
A process wherein fumarate reacts with cysteine residues in proteins by a Michael addition reaction to form *S*-(2-succinyl) cysteine.

**Mitochondrial depolarization**
The change in the resting potential (negative membrane potential) of mitochondria in the depolarizing direction (positive membrane potential), which is a critical step in the induction of mitochondrial apoptosis.

**Degrons**
Specific sequences of amino acids in a substrate that are necessary for recognition by the ubiquitin ligase.

**Linear ubiquitin chain assembly complex (LUBAC)**
An atypical ubiquitin ligase complex that consists of haeme-oxidized IRP2 ubiquitin ligase 1 (HOIL1; also known as RBCK1), HOIL1-interacting protein (HOIP; also known as RNF31) and the non-catalytic subunit shank-associated RH domain-interacting protein (SHARPIN). The LUBAC mediates M1-linked polyubiquitylation of its substrates and regulates, among other processes, nuclear factor-κB (NF-κB) and MAPK activation downstream of tumour necrosis factor receptor (TNFR) signalling.

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**Box 1**

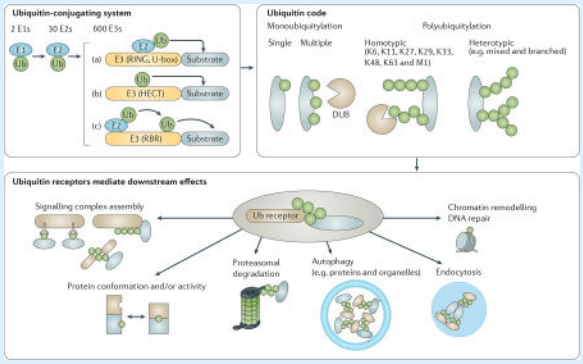
## The ubiquitin system

The human genome encodes two ubiquitin-activating enzymes (E1s), which activate ubiquitin (Ub) in an ATP-dependent manner and transfer it to one of ~30 ubiquitin-conjugating enzymes (E2s). The specificity and selectivity of the ubiquitin-conjugating system are conferred by more than 600 ubiquitin ligases (E3s), which are sub-classified into three groups according to their mode of ubiquitin ligation. (a) E3s containing really interesting new gene (RING) and UFD2 homology (U-box) domains belong to the first group; the most abundant (~600 members) is the family of RING E3s. The RING domain coordinates two $Zn^{2+}$ ions in a cross-brace arrangement to adopt the structure required for binding to E2s. RING E3s rely on the enzymatic activity of E2s to ubiquitylate substrates and can act either independently or as part of multisubunit E3 complexes. Examples of the latter are the cullin–RING–E3 ligase (CRL) family of complexes, including the S-phase kinase-associated protein 1 (SKP1)–cullin 1–F-box protein (SCF) complex and the APC/C (anaphase-promoting complex; also known as the cyclosome). U-box E3s (similar to RING E3s) function as scaffolds for E2s for ubiquitin transfer, but they do not require $Zn^{2+}$ coordination to adapt their structure. (b) The second group of E3s is the 28-member homologous to E6AP carboxy terminus (HECT) family, each of which contains an ~350 amino acid HECT domain that forms a thiol-ester bond with ubiquitin and then conjugates it to the substrate. According to the structure of the N-terminal domain, which serves as the substrate recognition domain, HECT E3s can be categorized into three subfamilies: NEDD4 and NEDD4-like E3s, which contain WW domains; HERC E3s, which harbour regulator of chromosome condensation 1 (RCC1)-like domains; and HECT E3s, which harbour neither WW nor RCC1-like domains. (c) The third group of E3s is the 14-member RING-between-RING (RBR) family. These enzymes have a RING1–in-between RING (IBR)–RING2 motif in which the RING1 domain binds to a ubiquitin-loaded E2 and transfers ubiquitin to the catalytic cysteine of the RING2 domain, which then conjugates ubiquitin to the substrate. Thus, RBR E3s function as hybrids of RING E3s and HECT E3s.

The fate of ubiquitylated proteins is largely determined by the ubiquitin chain topology. In a simplified view, K48-linked and K11-linked ubiquitin chains are usually associated with proteolysis; K63-linked or M1-linked ubiquitin chains mediate the assembly of signalling complexes, as illustrated by the nuclear factor-κB (NF-κB) pathway; and monoubiquitylation serves as a signal for chromatin regulation and protein sorting and trafficking. Ubiquitin is also phosphorylated, and this modification has been implicated in the removal of damaged mitochondria by autophagy[9,10,188–190]. The ubiquitin code is further modified by the proteolytic activity of ~100 deubiquitylating enzymes (DUBs) that, for example, remove K48-chains or K63-chains to prevent degradation or abrogate signalling events, respectively[10,191]. Our understanding of the role of the individual ubiquitin signals is constantly evolving. For example, monoubiquitylation was recently suggested to be a robust signal for proteasomal degradation[192].

Decoders of the ubiquitin code (ubiquitin receptors or readers) are proteins containing at least one of 20 structurally different ubiquitin-binding domains (UBDs) that serve to

recognize linkage-specific ubiquitin chains and mediate cellular processes (reviewed in REF. 9). As such, ubiquitin receptors mediate shuttling and binding of ubiquitylated cargo to the proteasome, initiate autophagy and/or mitophagy and orchestrate the assembly of signalling complexes[9]. Many linkage-specific ubiquitin binding proteins still await identification[193].



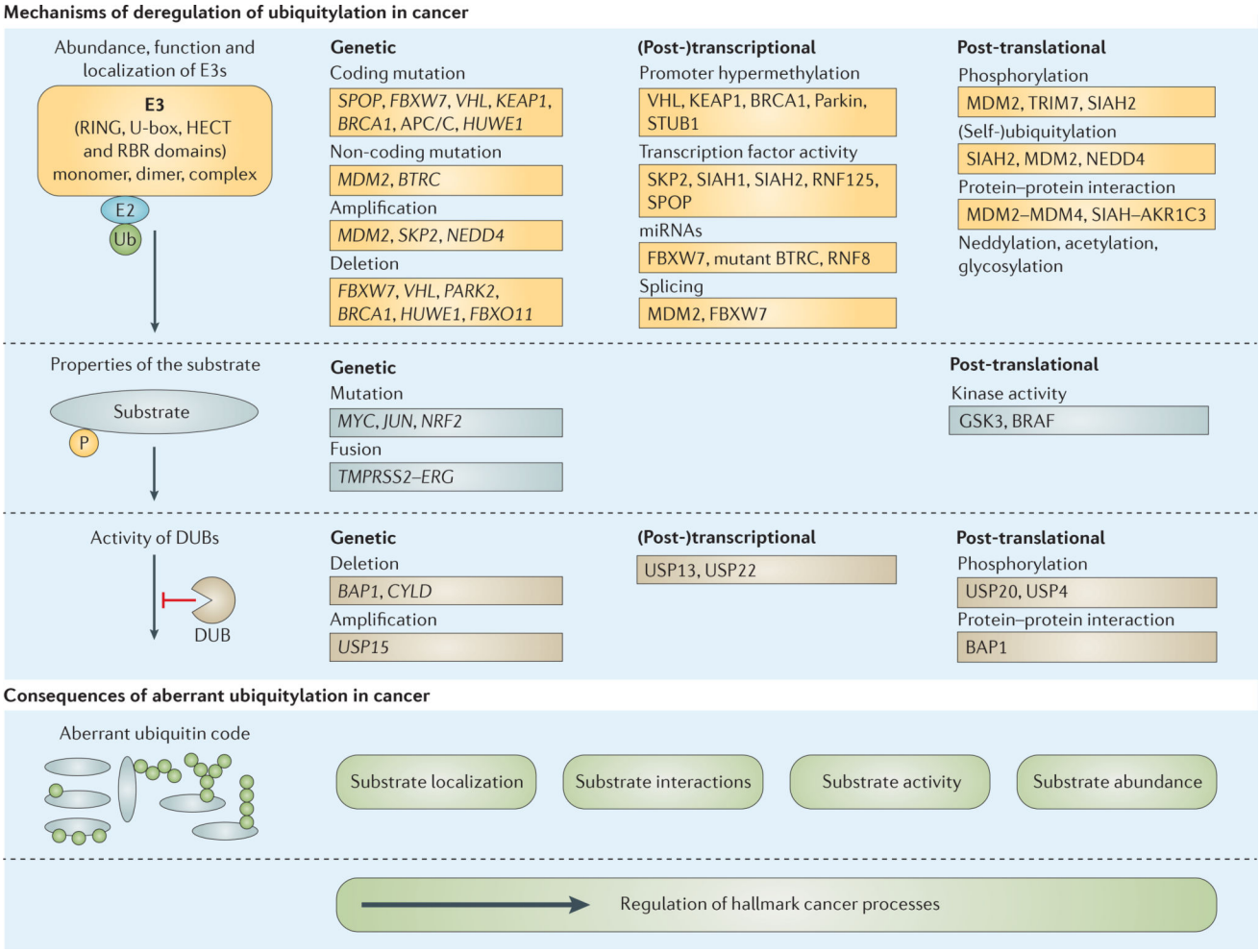



**Figure 1. Mechanisms underlying deregulated ubiquitylation in cancer**

Deregulated ubiquitylation in cancer can be attributed to epigenetic, genetic, transcriptional and post-translational mechanisms. Some ubiquitin ligases (E3s) are encoded by genes that are well recognized to confer susceptibility for familial cancers, such as the gene encoding von Hippel–Lindau disease tumour suppressor (VHL) in renal cell carcinoma (RCC)[194] or the gene encoding BRCA1 in breast cancer and ovarian cancer[56]. Large-scale analyses of cancer genomes have identified additional E3s that are altered by recurrent mutations or copy number changes in diverse cancers. The abundance and activity of E3s can also be regulated by post-translational mechanisms such as phosphorylation, ubiquitylation or protein–protein interactions, as demonstrated, for example, with the E3s MDM2 and SIAH2 (REFS 96,98,195,196). The activity and abundance of deubiquitylating enzymes (DUBs) are also regulated genetically and epigenetically, as recently reviewed[191]. In addition to deregulation of E3s and DUBs, the ubiquitin system is modulated by genetic alterations of the targeted substrates. For example, recognition of the ubiquitylation sites on MYC[143] and the fusion protein transmembrane protease serine 2 (TMPRSS2)–ETS-related gene (ERG)[152] is disrupted by mutations. Ubiquitylation is a dynamic and reversible process that responds to a variety of internal and external stresses, including DNA damage and hypoxic, oxidative and metabolic stresses, which are all encountered by cancer cells during malignant

transformation, during metastatic dissemination and in response to therapy. Each individual alteration in the ubiquitin system can have a profound effect on the regulation of cancer-associated pathways by modulating the localization, activity, signalling complex formation and abundance of major regulatory hubs. AKR1C3, aldo-keto reductase family 1 member C3; APC/C, anaphase-promoting complex; also known as the cyclosome; *BAP1*, BRCA1-associated protein 1; *BTRC*, encoding β-TRCP; *CYLD*, cylindromatosis; E2, ubiquitin-conjugating enzyme; *FBXO11*, F-box only protein 11; FBXW7, F-box/WD repeat-containing protein 7; GSK3, glycogen synthase kinase 3; HECT, homologous to E6AP carboxy terminus; KEAP1, kelch-like ECH-associated protein 1; *NRF2*, nuclear factor erythroid 2-related factor 2; *PARK2*, encoding parkin; RBR, RING-between-RING; RING, really interesting new gene; SIAH, seven in absentia homolgue; SKP2, S-phase kinase-associated protein 2; SPOP, speckle-type POZ protein; STUB1, STIP1 homology and U box-containing protein 1 (also known as CHIP); TRIM7, tripartite motif 7; U-box, UFD2 homology; Ub, ubiquitin; USP, ubiquitin carboxyl-terminal hydrolase.

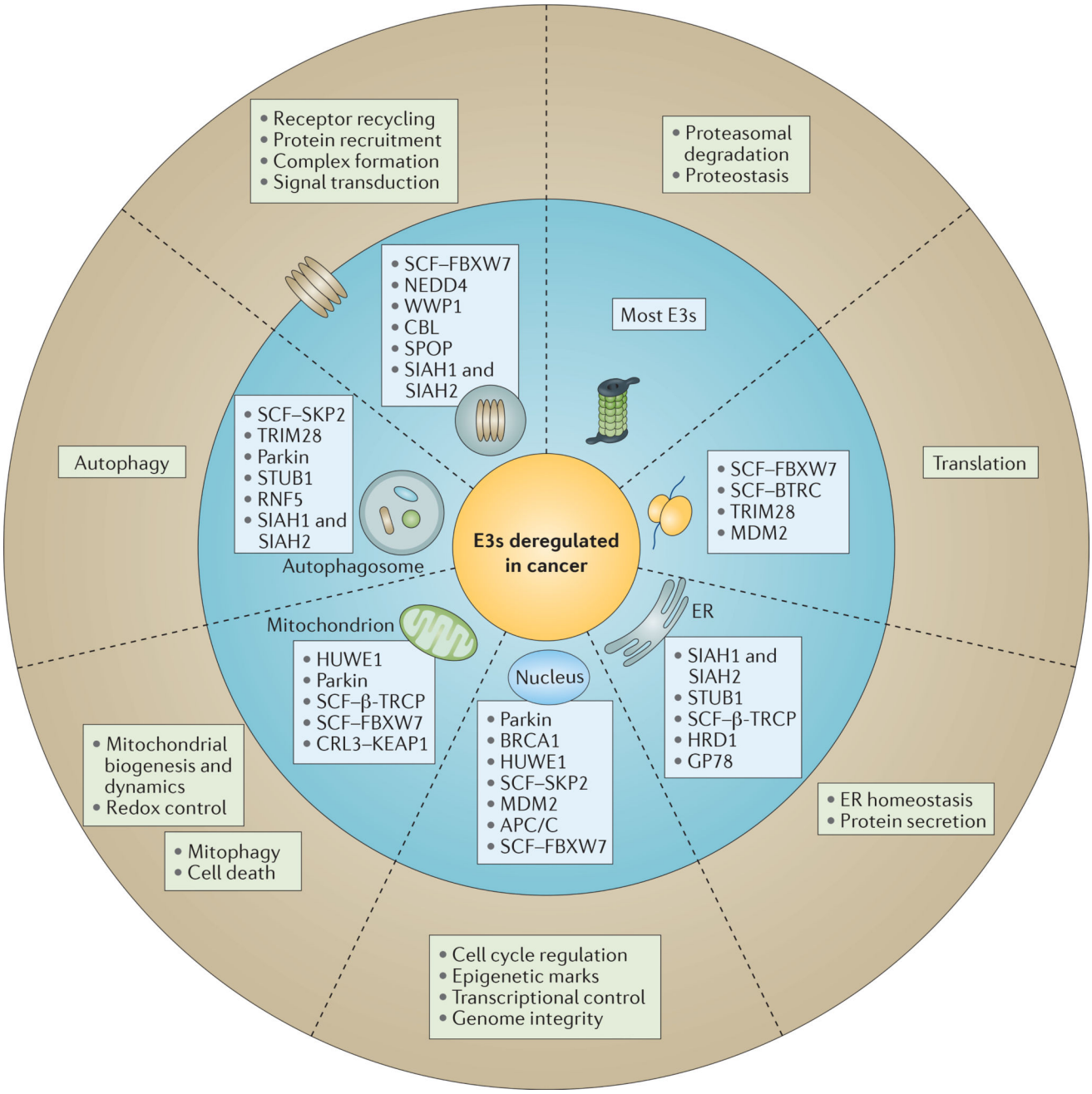



**Figure 2. Cellular processes affected by deregulated ubiquitylation in cancer**

Representative ubiquitin ligases (E3s) that are deregulated in cancer and the biological processes expected to be affected are depicted. As E3s ubiquitylate a diverse set of substrates, E3 loss or gain of function affects multiple cellular processes simultaneously. For example, S-phase kinase-associated protein 1 (SKP1)–cullin 1–F-box protein (SCF)–F-box/WD repeat-containing protein 7 (FBXW7) targets cell cycle regulators (for example, cyclin E), oncogenic transcription factors (for example, MYC), cell surface receptors (for example, NOTCH1), signalling molecules (for example, mTOR) and apoptosis regulators (for example, myeloid cell leukaemia 1 (MCL1)) for proteasomal degradation. Therefore,

SCF–FBXW7 loss of activity through mutations or deletions leads to genomic instability, increased proliferation and survival and the rewiring of transcriptional and signalling programmes that affect cancer cell migration, metabolism and stemness. Similarly, SCF–β-transducin repeat-containing protein (β-TRCP) can serve as a signalling hub to coordinate increased protein synthesis and pro-survival signals following pro-growth stimuli in normal cells and in cancer cells[197–199]. Thus, the β-TRCP signalling circuits provide a platform for therapeutic intervention, for example, in cancers characterized by activation of mTOR signalling. APC/C, anaphase-promoting complex; also known as the cyclosome; CRL3, cullin 3–really interesting new gene (RING)–E3 ligase; ER, endoplasmic reticulum; HRD1, also known as synoviolin; GP78, also known as AMFR; KEAP1, kelch-like ECH-associated protein 1; SIAH, seven in absentia homologue; SKP2, S-phase kinase-associated protein 2; SPOP, speckle-type POZ protein; STUB1, STIP1 homology and U box-containing protein 1; TRIM28, tripartite motif 28.

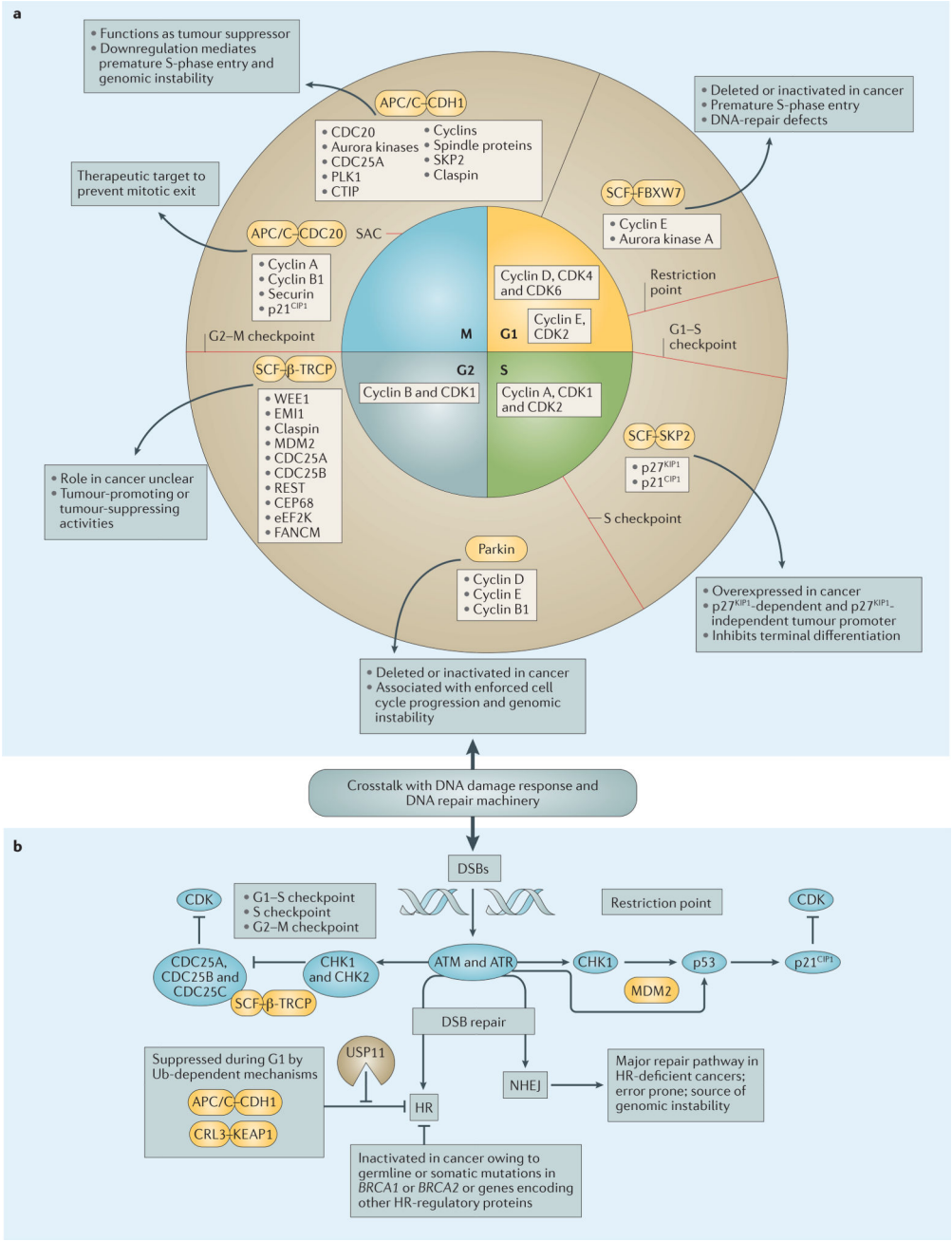



**Figure 3. Ubiquitin ligases coordinate the cell cycle and DNA damage repair to maintain genome integrity**

**a** | Phosphorylation and ubiquitylation coordinate the temporal activity of cyclin-dependent kinase (CDK)–cyclin complexes, thereby mediating cell cycle progression and checkpoint control. Displayed are some of the ubiquitin ligases (E3s), which are well-known cell cycle regulators, such as the APC/C (anaphase-promoting complex; also known as the cyclosome) and S-phase kinase-associated protein 1 (SKP1)–cullin 1–F-box protein (SCF)–F-box/WD repeat-containing protein 7 (FBXW7) complexes and parkin, whose function in the cell cycle is emerging. APC/C primarily mediates progression through mitosis by temporally coordinating the recruitment of co-activators (cell division cycle 20 (CDC20) or CDC20-like

protein 1 (CDH1)). Thus, APC/C–CDC20 mediates anaphase entry, while APC/C–CDH1 acts during mitotic exit and early G1. APC/C is also regulated by phosphorylation, binding of inhibitory molecules such as early mitotic inhibitor 1 (EMI1; also known as FBXO5) and the spindle assembly checkpoint (SAC)[200]. SCF is a four-protein complex consisting of the scaffold cullin 1, a really interesting new gene (RING) domain-containing component RBX1, the SKP1 adaptor protein and one of ~68 various F-box proteins (for example, FBXW7, SKP2 or β-transducin repeat-containing protein (β-TRCP)). Most F-box proteins mediate the recognition of substrates by binding to their `phosphodegron` motifs (although phosphorylation-independent mechanisms of substrate recognition exist), which contain residues that can be phosphorylated by multiple kinases. The SCF complex is active throughout the cell cycle; SCF substrates include a subset of cyclins and CDK inhibitors, through which the SCF complex regulates progression from G1 to the onset of mitosis. Some example substrates of each E3 are displayed in the cream boxes, and the effects of altered E3 function in cancer are indicated in the grey boxes. **b** | DNA double strand breaks (DSBs) mediate activation of DNA damage sensors ataxia telangiectasia mutated (ATM), ataxia telangiectasia and Rad3-related (ATR), checkpoint kinase 1 (CHK1) and CHK2. These in turn inactivate the E3 MDM2, leading to p53 stabilization and/or promoting SCF–β-TRCP-mediated degradation of CDK phosphatases CDC25A, CDC25B and CDC25C; both pathways converge on the attenuation of CDK activity. ATM and ATR also initiate the recruitment of the DNA repair machinery to the sites of DNA damage, which is in turn under the control of ubiquitylation. Suppression of homologous recombination (HR) during G1 involves competition between p53-binding protein 1 (53BP1) and BRCA1 at DSBs, APC/C–CDH1-mediated CtBP-interacting protein (CTIP) degradation and cullin 3–RING–E3 ligase (CRL3)–kelch-like ECH-associated protein 1 (KEAP1)-dependent inhibition of BRCA1–partner and localizer of BRCA2 (PALB2)–BRCA2 complex formation[62–64]. CEP68, centrosomal protein of 68 kDa; eEF2K, eukaryotic elongation factor 2 kinase; FANCM, Fanconi anaemia group M protein; NHEJ, non-homologous end joining; PLK1, polo-like kinase 1; REST, repressor-element 1 (REI)-silencing transcription factor; Ub, ubiquitin; USP11, ubiquitin carboxyl-terminal hydrolase 11.

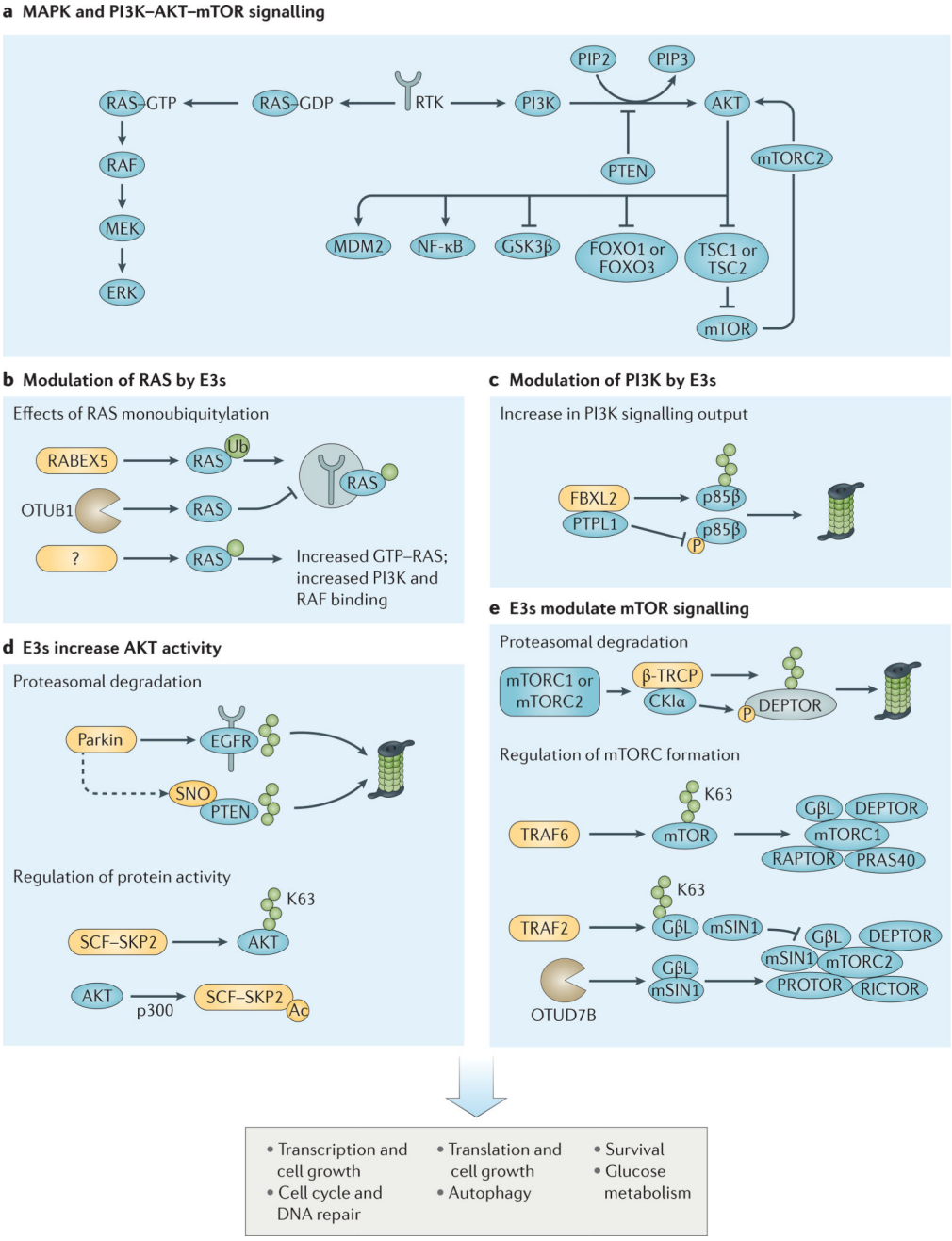



**Figure 4. Regulation of mitotic signalling by ubiquitin ligases**

**a** | Simplified schematics of the PI3K–AKT–mTOR and MAPK pathways are depicted. Ubiquitin ligases (E3s) and deubiquitylating enzymes (DUBs) regulate degradation, activity or complex assembly of receptor tyrosine kinases (RTKs), kinases and signalling molecules. **b** | Monoubiquitylation or diubiquitylation modulates RAS activity. Elevated expression of the DUB OTU domain-containing ubiquitin aldehyde-binding protein 1 (OTUB1) may contribute to sustained RAS activation, as demonstrated in lung cancer[74]. **c** | High levels of the PI3K regulatory subunit p85β compete with active PI3K (made up of p85–p110 heterodimers) for substrate binding, thereby limiting PI3K activity. Following

dephosphorylation of p85β by PTPL1, p85β is ubiquitylated by F-box and leucine-rich repeat protein 2 (FBXL2), leading to its proteasomal degradation and thereby increasing PI3K signalling output[84]. **d** | *PARK2* (which encodes E3 parkin) is frequently inactivated or downregulated in cancer by diverse mechanisms. Parkin loss indirectly increases AKT activity by affecting AKT upstream regulators. First, parkin loss leads to accumulation of parkin substrates, including the epidermal growth factor receptor (EGFR). Second, parkin loss impairs mitochondrial metabolism that results, from a cascade of events (indicated by the dotted line), in PTEN *S*-nitrosylation (by addition of the functional group *S*-nitrosothiol (SNO)), which primes PTEN for degradation. K63-linked polyubiquitylation of AKT by S-phase kinase-associated protein 1 (SKP1)–cullin 1–F-box protein (SCF)–SKP2 directly increases its activity. AKT can regulate stabilization and localization of SKP2, wherein AKT either directly phosphorylates SKP2 (REFS 89,90) (not shown) or mediates histone acetyltransferase p300 activation, which in turn leads to SKP2 acetylation (Ac) and thus stabilization and cytoplasmic retention of SKP2 (REF. 91). **e** | A casein kinase Iα (CKIα)–SCF–β-transducin repeat-containing protein (β-TRCP) auto-amplification loop mediates full activation of mTOR via DEP domain-containing mTOR-interacting protein (DEPTOR) degradation[199]. The dynamic assembly of mTOR complex 1 (mTORC1) and mTORC2 is under the control of ubiquitylation. Ubiquitin-dependent deregulation of these signalling hubs in turn alters cellular processes as diverse as cell growth, metabolism, DNA repair, transcription, translation and survival. FOXO, forkhead box protein O; GSK3β, glycogen synthase kinase 3β; NF-κB, nuclear factor-κB; OTUD7B, OTU domain-containing protein 7B; P, phosphorylation; PIP, phosphatidylinositol phosphate; PRAS40, proline-rich AKT1 substrate 1; PROTOR, proline-rich protein; RABEX5, RAB5 GDP/GTP exchange factor; RAPTOR, regulatory-associated protein of mTOR; RICTOR, rapamycin-insensitive companion of mTOR; TRAF, tumour necrosis factor (TNF)-receptor-associated factor; TSC1, tuberous sclerosis 1 (also known as harmartin); TSC2, also known as tuberin; Ub, ubiquitin.

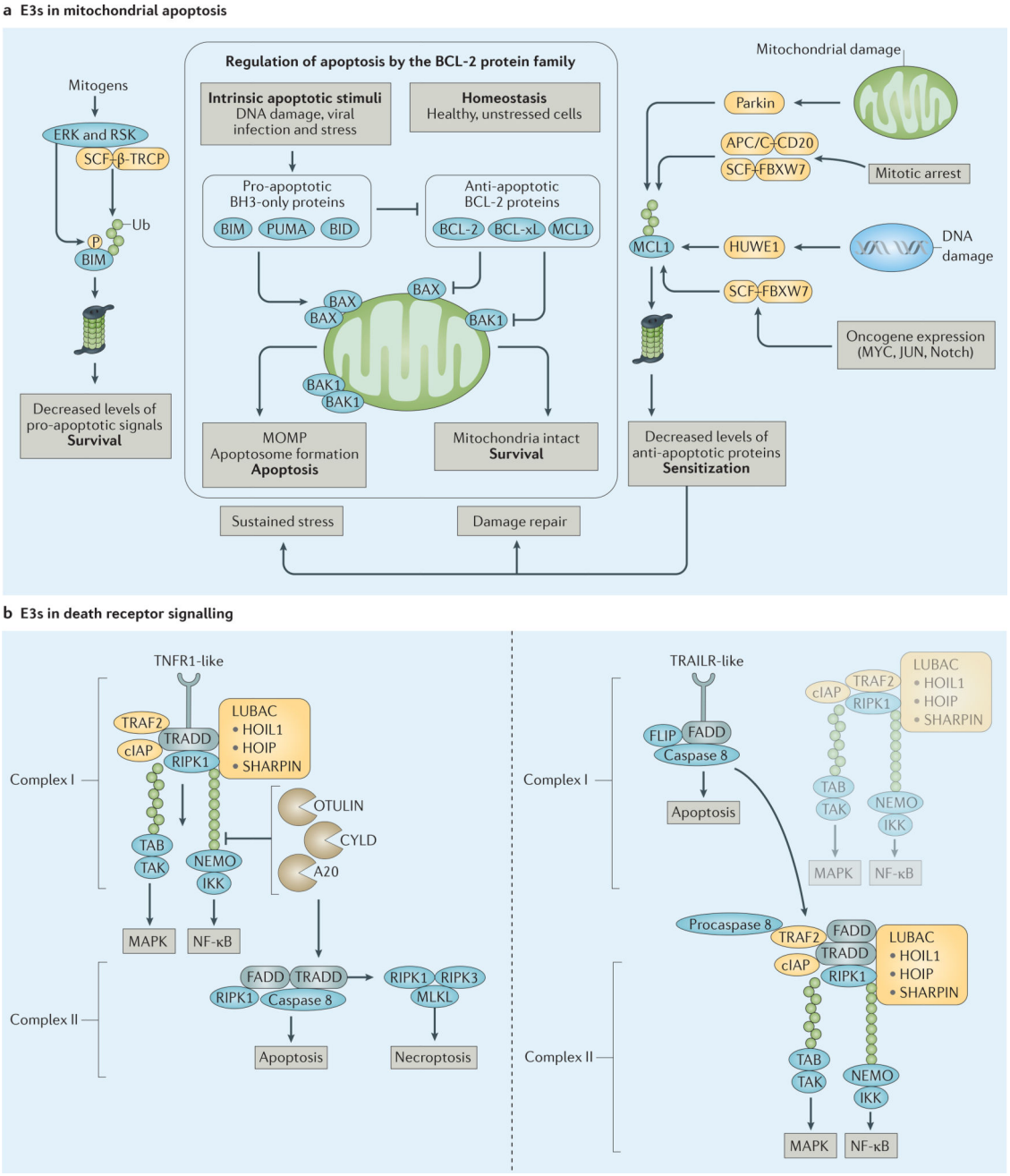



**Figure 5. Ubiquitin ligases regulate the intrinsic and extrinsic apoptotic pathways**

**a** | Cells maintain anti-apoptotic proteins at high levels, and pro-apoptotic BCL-2 homology domain 3 (BH3)-only proteins are transcriptionally upregulated mainly by stress-responsive transcription factors. This difference in protein abundance prevents oligomerization of the pro-apoptotic effector proteins BAX and BCL-2 antagonist/killer 1 (BAK1) and blocks mitochondrial outer membrane permeabilization (MOMP), thus inhibiting activation of caspase-dependent apoptosis. Increased mitogenic signalling can prevent apoptosis induction by targeting pro-apoptotic proteins for degradation, as exemplified by the S-phase kinase-associated protein 1 (SKP1)–cullin 1–F-box protein (SCF)–β-transducin repeat-

containing protein (β-TRCP)-dependent degradation of pro-apoptotic BH3-only protein BIM (also known as BCL2L11) following ERK-mediated and ribosomal S6 kinase (RSK)-mediated phosphorylation of BIM[197]. Consequently, knockdown of either β-TRCP or RSK induces apoptosis in non-small-cell lung cancer (NSCLC) cell lines[197]. Similarly, downregulation of anti-apoptotic proteins by ubiquitin-mediated mechanisms, as illustrated here for myeloid cell leukaemia 1 (MCL1), can lower the threshold for apoptosis induction and sensitize cells to apoptosis-inducing insults. Decreased activity of MCL1-targeting ubiquitin ligases (E3s) in cancer therefore increases apoptosis resistance. **b** | Receptors of the tumour necrosis factor (TNF) superfamily are made up of TNF receptor 1 (TNFR1)-like (including TNFR1, DR3 (also known as TNFRSF25) and DR6 (also known as TNFRSF21)) and TNF-related apoptosis-inducing ligand (TRAIL) receptor (TRAILR)-like (including CD95 (also known as FAS and TNFRSF6) and TRAILR). Activation of TNFR1-like receptors recruits TNFR type 1-associated DEATH domain protein (TRADD), receptor-interacting serine/threonine-protein kinase 1 (RIPK1), TNF receptor-associated factor 2 (TRAF2), cellular inhibitor of apoptosis 1 (cIAP1) or cIAP2 and the linear ubiquitin chain assembly complex (LUBAC). K63-linked and M1-linked polyubiquitylation of RIPK1 by cIAPs and LUBAC, respectively, recruits inhibitor of nuclear factor-κB kinase (IKK), TAK1-binding protein (TAB) and TAK (also known as MAP3K7) to stimulate robust and rapid nuclear factor-κB (NF-κB) and MAPK signalling. TNFR1-induced cell death depends on receptor internalization and formation of a secondary, receptor-free cytoplasmic complex (complex II) composed of either RIPK1–FAS-associated death domain protein (FADD)–caspase 8 or RIPK1–RIPK3–mixed lineage kinase domain-like protein (MLKL), which mediates either apoptosis or necroptosis (a programmed form of necrosis), respectively. Formation of complex II is largely dependent on the activity of deubiquitylating enzymes (DUBs), specifically cylindromatosis (CYLD), A20 and ubiquitin thioesterase OTULIN. Alterations in the activity of these DUBs or of the E3s cIAP and LUBAC are reported in diverse cancers, where they lead to apoptosis resistance while stimulating oncogenic NF-κB signalling[8,176]. By contrast, stimulation of TRAILR-like primarily forms the death-inducing signalling complex containing FADD, caspase 8, caspase 10 and FLICE-like inhibitory protein (FLIP; also known as CFLAR). Complex II contains FADD, procaspase 8 filaments, RIPK1 and TRAF2. As indicated by the lighter shaded complex, RIPK1 and LUBAC may be directly recruited to complex I, allowing MAPK and NF-κB activation independent of complex II formation. Understanding the ubiquitin-mediated mechanisms that result in pro-tumorigenic NF-κB activation upon TRAILR stimulation is one of the important steps towards improving the therapeutic effect of TRAILR agonists, which are currently being evaluated as anticancer drugs[179]. APC/C, anaphase-promoting complex; also known as the cyclosome; BCL-xL, also known as BCL2L1; BID, BH3, interacting domain death agonist; FBXW7, F-box/WD repeat-containing protein 7; HOIL1, haeme-oxidized IRP2 ubiquitin ligase 1 (also known as RBCK1); HOIP, HOIL1-interacting protein (also known as RNF31); NEMO, NF-κB essential modulator; P, phosphorylation; PUMA, p53 up-regulated modulator of apoptosis (also known as BBC3); SHARPIN, shank-associated RH domain-interacting protein; Ub, ubiquitin.

## Table 1

Examples of ubiquitin ligases in transgenic mouse models

| E3s | Phenotypes in transgenic mouse models | Refs |
|---|---|---|
| β-TRCP | *Btrc* overexpression in the mouse mammary gland increased cell proliferation and induced breast tumour development, which was associated with activation of the NF-κB pathway | 201 |
| | Overexpression of a dominant-negative mutant of *Btrc* (in which the F-box domain is deleted) in transgenic mice resulted in the development of intestinal, hepatic or urothelial tumours, which were associated with accumulation of β-catenin | 202 |
| BRCA1 | Conditional knockout of *Brca1* in mouse mammary epithelial cells resulted in the development of basal-like triple-negative breast carcinomas | 203 |
| | Co-deletion of *Brca1* and *Trp53* in mouse mammary epithelial cells accelerated the formation of breast carcinomas | 204,205 |
| CDC20 | *Cdc20*-knockout mice treated with a two-stage carcinogenesis protocol showed a massive arrest in metaphase and apoptosis of skin tumour cells | 19 |
| CDH1 | Aged *Cdh1*-heterozygous mice show increased susceptibility to spontaneous epithelial tumours in various organs (for example, mammary gland, lung, liver, kidney, testis and sebaceous gland) | 15 |
| FBXW7 | *Fbxw7*-knockout mice showed a higher incidence of γ-irradiation-induced tumour formation than wild-type mice. Loss of *Fbxw7* also changed the spectrum of tumours that developed in irradiated *Trp53*-deficient mice to include epithelial tumours from the lung, liver and ovary | 26 |
| | Conditional T cell lineage-specific knockout of *Fbxw7* in mice resulted in thymic lymphoma and acute leukaemia | 164 |
| | Knockout of *Fbxw7* in $Apc^{Min/+}$ mice accelerated intestinal tumour development | 206,207 |
| | Double knockout of *Fbxw7* and *Trp53* in the mouse intestine caused aggressive and metastatic adenocarcinomas that resembled human advanced colorectal cancer | 24 |
| HUWE1 | Knockout of *Huwe1* accelerated tumour formation in a two-stage skin carcinogenesis model, which was reversed by concomitant knockout of *Myc* | 138 |
| | Knockout of *Huwe1* in $Apc^{Min/+}$ mice accelerated intestinal tumour development, which was associated with increased MYC levels, rapid accumulation of DNA damage and loss of the second copy of *Apc* | 140 |
| MDM2 | Overexpression of *Mdm2* in mice induced spontaneous tumour formation. The frequencies of tumour formation in *Mdm2*-transgenic and *Trp53*-null mice were statistically indistinguishable from each other. *Mdm2*-transgenic mice (regardless of their *Trp53* status) showed a higher percentage of sarcomas than *Trp53*-null mice | 208 |
| Parkin | *Park2*-knockout mice showed increased hepatocyte proliferation and developed macroscopic hepatic tumours resembling hepatocellular carcinoma | 209 |
| | *Park2*-knockout mice accelerated lymphoma development upon γ-irradiation | 210 |
| | Knockout of *Park2* in $Apc^{Min/+}$ mice increased the development of intestinal tumours | 50 |
| SIAH2 | Knockout of *Siah2* inhibited the development of prostate neuroendocrine tumours and promoted the regression of atypical hyperplasia upon castration in the TRAMP model | 102,154 |
| | Knockout of *Siah2* in MMTV-PyMT mice delayed the progression of mammary tumours and was associated with vascular normalization in the tumour microenvironment | 104 |
| SKP2 | Transgenic overexpression of *Skp2* in the mouse prostate induces hyperplasia, dysplasia and low-grade carcinoma | 31 |
| | Co-expression of *Skp2* with $Nras^{G12V}$ or myristoylated *Akt1* in the mouse liver resulted in hepatocellular carcinoma | 32 |
| | *Skp2*-knockout inhibited tumour development in a $Pten^{-/-}Trp53^{-/-}$ mouse prostate cancer model via activation of $p27^{KIP1}$-dependent, $p21^{CIP1}$-dependent and ATF4-dependent senescence | 33 |
| | Co-deletion of *Skp2, Rb1* and *Trp53* blocked tumorigenesis in the mouse pituitary and prostate in a $p27^{KIP1}$-dependent manner | 34 |
| | *Skp2*-knockout inhibited DMBA-TPA-induced skin tumorigenesis in mice | 211 |
| SPOP | Prostate-specific knockout of *Spop* resulted in the upregulation of *Myc* and promoted development of prostatic intraepithelial neoplasia | 141 |

APC, adenomatous polyposis coli; ATF4, activating transcription factor 4; *Btrc*, encoding β-TRCP; CDC20, cell division cycle 20; CDH1, CDC20-like protein 1 (also known as FZR1); DMBA, dimethylbenzanthracene; E3s, ubiquitin ligases; FBXW7, F-box/WD repeat-containing protein 7; MMTV–PyMT, mouse mammary tumour virus–polyoma middle T antigen; NF-κB, nuclear factor-κB; Parkin, encoded by *Park2*; SIAH2, seven in

absentia homologue 2; SKP2, S-phase kinase-associated protein 2; SPOP, speckle-type POZ protein; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; TRAMP, transgenic adenocarcinoma of the mouse prostate.