



Cullin 3 Ubiquitin Ligases in Cancer Biology: Functions and Therapeutic Implications

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Cullin-RING ubiquitin ligases are the largest E3 ligase family in eukaryotes and are multiprotein complexes. In these complexes, the Cullin protein serves as a scaffold to connect two functional modules of the ligases, the catalytic subunit and substrate-binding subunit. To date, eight members of the Cullin family proteins have been identified. In the Cul3 ubiquitin ligases, Bric-a-brac/Tramtrack/Broad complex (BTB) domain-containing proteins function as a bridge to connect Cul3 and substrates. While the BTB domain is responsible for Cul3 binding, these proteins usually contain an additional domain for substrate interaction, such as MATH, kelch, Zn finger, and PAM, Highwire, and RPM-1 (PHR domain). With the existence of a large number of BTB proteins in human, the Cul3 ubiquitin ligases ubiquitinate a wide range of substrates involving in diverse cellular functions. In this review, we will discuss recent advances on the functions of Cul3 ubiquitin ligases in cancer development, progression, and therapeutic response and the dysregulation of Cul3-mediated ubiquitination events in human malignancies. In particular, we will focus on three Cul3 substrate adaptors, kelch-like ECH-associated protein (Keap1), kelch-like family member 20 (KLHL20), and speckle type BTB/POZ protein (SPOP), with the intent to highlight novel targets in cancer therapy.

Keywords: Cul3 ubiquitin ligases, cancer, Keap1, KLHL20, SPOP

INTRODUCTION

The ubiquitin-proteasome system controls a wide range of physiological processes and disease conditions, including cancer. In this system, the addition of ubiquitin moiety to the lysine residue of protein is mediated by a cascade of enzymatic reactions involving E1 activating enzyme, E2 conjugation enzyme, and E3 ubiquitin ligase, in which substrate specificity is conferred by E3 ubiquitin ligase (1, 2). Cullin-RING multiprotein complexes comprise the largest family of ubiquitin ligases, in which one particular Cullin serves as a scaffold for linking two functional modules: the catalytic RING finger protein Roc1 or Roc2 and the substrate-binding module for bringing substrate within the proximity to the catalytic module (3). The human Cullin family consists of eight members: Cul1, Cul2, Cul3, Cul4A, Cul4B, Cul5, Cul7, and Cul9. In the Cul3 family of ubiquitin ligases, the Bric-a-brac/Tramtrack/Broad complex (BTB) domain-containing protein functions as the substrate adaptor to bridge Cul3 and substrate and, therefore, is in analogous to the Skp1-F-box heterodimer in the Cul1 complex (4, 5). Structural analysis indicates that the BTB domain adopts a five α -helical fold resembling other Cullin-binding proteins in the Cullin-RING ligase complexes, such as Skp1

and ElonginC (6). However, two features are unique for the BTB-domain proteins among the substrate adaptors of Cullin family. First, BTB proteins are capable of dimerization and, therefore, can organize two Cul3 molecules in one E3 ligase complex. Second, many BTB proteins contain additional domains and can be classified into subfamilies based on these domains, such as MATH, kelch, Zn finger, and PAM, Highwire, and RPM-1 (PHR). These additional domains are responsible for the interaction of BTB proteins with the substrate of Cul3 complex. Although human genome encodes ~200 BTB proteins, not all of them serve as substrate adaptors of Cul3 ubiquitin ligases. For instance, BTBD12, which lacks a 3-box region critical for binding Cul3, does not copurify with Cul3 from cells (7). In addition, KLHL39, which contains certain non-conserved residues in the BTB domain, fails to bind Cul3 (8).

With the existence of a large number of substrate adaptors, Cul3 ubiquitin ligases have recently been shown to participate in diverse cellular processes, such as cell cycle regulation, protein trafficking, development, and stress responses. In human, functional alterations of this family of ubiquitin ligases are associated with several disease states, such as muscle diseases, metabolic disorders, and cancers (4). This review will provide insights into the functions of Cul3 ligases in tumorigenesis and progression, their dysregulation in human cancers, and therapeutic implications. In particular, we will focus on Cul3 complexes containing the following three substrate adaptors, kelch-like ECH-associated protein (Keap1), kelch-like family member 20 (KLHL20), and speckle type BTB/POZ protein (SPOP). All three proteins bind Cul3 through their BTB domain and elicit profound effects on tumorigenesis and progression.

THE DUAL ROLES OF Keap1–Nrf2 PATHWAY IN CANCER

Kelch-like ECH-associated protein is a Cul3 substrate adaptor containing BACK and kelch-repeat domains in addition to the BTB domain (9–11). Keap1 was first discovered as a key inhibitor of the transcription factor Nf-E2-related factor 2 (Nrf2) (12, 13), which binds to the antioxidant response element (ARE) present in the promoters of downstream genes encoding proteins participating in the cellular antioxidant responses and detoxification of xenobiotics and drugs (14). Under basal conditions, Keap1-based Cul3 complex targets Nrf2 for ubiquitin-dependent degradation (9–11). In the presence of oxidative or electrophilic stress, a number of reactive cysteine residues in Keap1 are covalently modified, leading to its conformational change to prevent Nrf2 ubiquitination. Consequently, Nrf2 is stabilized and undergoes nuclear translocation. Through this mechanism, the Keap1–Nrf2 pathway plays a major role in anti-oxidation and cell defense responses.

Since oxidative stress plays an important role in carcinogenesis, the chemopreventive function of Nrf2 is expected to suppress the initiation of carcinogenesis. In support of this notion, Nrf2^{-/-} mice are more prone to chemical carcinogen-induced tumor formation in the stomach, bladder, and skin (15–19). In addition, Nrf2 deficiency accelerates tumor growth in a mouse

lung cancer model induced by B-Raf^{V600E} (20). These findings suggest that activation of Keap1–Nrf2 pathway could be used as a chemopreventive strategy.

Although the chemopreventive function of Keap1–Nrf2 pathway protects normal cells from carcinogenesis, once tumor is formed, cancer cells hijack this pathway for acquiring survival and growth advantage to cope with stressed conditions. For instance, increased Nrf2 expression in cancer cells decreases their sensitivity to a variety of chemotherapeutic agents as well as ionizing radiation, whereas Nrf2 knockdown sensitizes them to cancer therapy (21–24). A similar chemoresistant phenotype is found in cancer cells with elevated Nrf2 activity due to reduced Keap1 expression (21). Although Nrf2-induced activation of antioxidant enzymes accounts for one mechanism of its chemoresistance/radioresistance effect, Nrf2 can also cross talk with other pathways to affect tumor-cell survival. For instance, increased Nrf2 expression is shown to interfere with p53-induced apoptosis (25). Apart from conferring the resistance of tumor cells to therapy, Keap1–Nrf2 pathway also promotes proliferation. It has been found that Nrf2 expression is elevated in response to several oncogenes, such as K-Ras, B-Raf, and Myc. As a consequence, Nrf2-mediated antioxidant responses suppress ROS production in response to the activation of oncogenes, and Nrf2 deficiency suppresses oncogene-induced proliferation and tumorigenesis (26). Evidence has emerged that the proliferative effect of Keap1–Nrf2 pathway is associated with metabolic reprogramming (27). Several genes in the pentose phosphate pathway, such as glucose-6-phosphate dehydrogenase (*G6PD*), phosphogluconate dehydrogenase (*PGD*), transaldolase 1 (*TALDO1*), and transketolase (*TKT*), are Nrf2 targets. Nrf2 also activates other metabolic genes, such as malic enzyme 1 (*ME1*), phosphoribosyl pyrophosphate amidotransferase (*PPAT*), methylenetetrahydrofolate dehydrogenase 2 (*MTHFD2*), and isocitrate dehydrogenase 1 (*IDH1*). These enzymes support NADPH generation, purine production, and glucose flux, thereby providing cancer cells with energy and building blocks of DNA and RNA for accelerating proliferation.

The dual functions of Nrf2 in cancer are nicely demonstrated by a mouse model of carcinogenesis. It has been found that Nrf2^{-/-} mice show increased tumor formation at 8th week, after the administration of carcinogen urethane, but become less advanced in malignant progression at 16th week (28). Such finding also suggests that transient activation of Nrf2 in normal cells is protective but prolonged Nrf2 activity enhances tumor survival and progression.

DYSREGULATION OF Keap1–Nrf2 PATHWAY IN CANCER

Accumulating evidence indicates that Nrf2 expression is aberrantly elevated in many types of cancer. Dysregulation of Nrf2 in tumors can be mediated by multiple mechanisms. Among them, somatic mutations in the components of Keap1–Nrf2 pathway have been found in many cancer types. Loss-of-function mutations in *KEAP1* were initially identified in human lung adenocarcinoma cell lines, which cause reduced affinity of

Keap1 to Nrf2 (29). Since then, Keap1 mutations have also been found in several other cancer types, such as NSCLC, gallbladder, ovarian, and liver cancers (21, 30–33). In some cases, Keap1 mutations have a dominant-negative effect on wild-type Keap1, and thus, a heterozygous mutation is sufficient to cause Nrf2 activation (34). As to Nrf2, gain-of-function mutations have been found in several cancers, including lung, head and neck, and esophageal carcinoma (35, 36). The mutations are found exclusively within the DLG and ETGE motifs of Nrf2, which are both required for binding to Keap1 (37). Genetic alterations of *KEAP1* or *NFE2L2* (Nrf2 gene) in cancers, especially in lung cancers, are also uncovered by large-scale omic project (38). In addition to Keap1 and Nrf2, somatic mutations leading to Nrf2 accumulation in cancers have also been discovered in other key components of the Keap1–Nrf2 pathway, such as Cul3 and Rbx1 (39–41).

Kelch-like ECH-associated protein downregulation in cancers can also be mediated by epigenetic mechanisms. For instance, hypermethylation of the *KEAP1* promoter has been found in lung, prostate, malignant glioma, and colorectal cancers, leading to Nrf2 accumulation (42–44). In some cases, *KEAP1* hypermethylation is associated with poor prognosis of patients (45). Besides DNA methylation, miRNA-induced silencing is another mechanism for modulating Keap1–Nrf2 pathway in cancer. For instance, miR-200a, which is frequently repressed in cancer, is found to target Keap1, thereby indirectly regulating Nrf2 (46). In addition, downregulation of several Nrf2 targeting miRNAs in esophageal squamous cell carcinoma is found to associate with poor prognosis (47).

Additional mechanism for regulating Keap1–Nrf2 pathway involves proteins that disrupt the Keap1–Nrf2 interaction. One such protein is p62 (also known as sequestosome 1), which contains an STGE motif that is similar to the ETGE motif in Nrf2. Through this motif, p62 functions as a pseudosubstrate of Keap1 by competing with Nrf2 for Keap1 binding (48–51). Interestingly, p62 is a transcriptional target of Nrf2, indicating the existence of feedback regulation between the two proteins (49). By acting as an autophagic cargo, p62 level is elevated in response to the blockage autophagic flux. In this context, the elevated p62 sequesters Keap1 in the autophagosome, thereby stabilizing Nrf2 (52). Moreover, p62 phosphorylation through an mTOR-dependent mechanism increases its affinity to Keap1, leading to persistent Nrf2 activation to enhance tumor growth (53). While p62 interrupts Keap1–Nrf2 pathway by binding to Keap1, the p53 downstream target p21 binds to the ETGE and DLG motifs of Nrf2 to prevent its recruitment to Keap1 (54). A similar effect was observed from the tumor suppressor BRCA1, which interacts with the ETGE motif to prevent the binding of Nrf2 to Keap1 (55).

Dysregulation of Keap1–Nrf2 pathway in cancer can also be mediated by metabolites. In the hereditary type 2 papillary renal cell carcinoma, homozygous loss-of-function mutation in the fumarate hydratase leads to the accumulation of fumarate, a metabolite of the Krebs cycle. The excessive fumarate forms adduct on the cysteine residues of Keap1, thereby preventing Nrf2 ubiquitination to promote tumorigenesis (56, 57).

TARGETING Keap1–Nrf2 PATHWAY FOR CANCER PREVENTION AND CANCER TREATMENT

Given the dual roles of Keap1–Nrf2 pathway in cancer, manipulation of this pathway could in principle offer therapeutic benefits. For instance, compounds that activate Nrf2 may be used for cancer prevention, whereas Nrf2 inhibitors could be used as adjuvants in chemotherapy to overcome chemoresistance. Among the Nrf2 activators, many are naturally existing phytochemicals. The prototype and most studied agent is SFN found in cruciferous vegetables. SFN has been shown to exert chemopreventive effect against several cancer types, such as colon, skin, lung, and stomach cancers (58). To date, certain Keap1–Nrf2 activating agents have been tested in clinical trials for their chemopreventive effects against various types of cancer (59, 60). As to Nrf2 inhibitors, a number of small molecules have been identified to inhibit Nrf2 expression or activity, such as IM3829 and brusatol (61, 62). In addition to directly manipulating Nrf2, autophagy pathway that intersects with Keap1–Nrf2 pathway *via* p62-dependent degradation may also be used as a strategy to modulate the activity of Nrf2.

KLHL20–DAPK PATHWAY IN INTERFERON RESPONSES

Kelch-like family member 20 possesses the same domain architecture as Keap1, that is, an N-terminal BTB domain, followed by a BACK domain and six kelch repeats. Similar to Keap1 and many other KLHL proteins, KLHL20 binds to Cul3 through its BTB domain to function as a substrate adaptor of Cul3 ubiquitin ligase (63). This protein was uncovered in our laboratory as an interacting partner of death-associated protein kinase (DAPK), a tumor-suppressor protein involved in several cell death paradigms, including apoptosis, autophagic death, and programmed necrosis (64–66). In addition to promoting cell death, DAPK elicits other anticancer functions, such as suppressing cell migration and adhesion and promoting cytoskeleton remodeling (67, 68). Consistent with these pleiotropic tumor-suppressive functions, DAPK expression or activity is often suppressed in tumors by epigenetic, posttranscriptional, or posttranslational mechanisms (64, 69–71). The finding that DAPK binds to the kelch-repeat domain of KLHL20 suggests its function as a substrate of the Cul3–KLHL20 ubiquitin ligase. Subsequent biochemical analyses have validated this notion. Moreover, KLHL20-dependent ubiquitination results in the degradation of DAPK by proteasomes. Through this mechanism, KLHL20 antagonizes the cell death-promoting effect of DAPK (63).

Death-associated protein kinase was originally discovered based on its involvement in interferon (IFN)-induced cell death (72). Interestingly, we found that the KLHL20-mediated DAPK ubiquitination and degradation can also be modulated by IFN, in particular, IFN- α and IFN- γ (63). In response to IFN- α/γ treatment, KLHL20 is relocated to a subnuclear domain called PML–nuclear body (PML–NB). This is due to IFN- α/γ -induced transcriptional upregulation of promyelocytic leukemia (PML)

(73, 74), the major component of PML–NBs, along with the competition between PML and DAPK for KLHL20 binding (63). As a consequence, DAPK can no longer gain access to KLHL20 and is, therefore, stabilized under IFN- α/γ treated conditions. The stabilization of pro-death DAPK explains its contribution to IFN-induced apoptosis and autophagic death. In certain multiple myeloma cells where IFN- α/γ cannot induce PML and PML–NBs, DAPK is persistently ubiquitinated and degraded by KLHL20. Importantly, this mechanism contributes to the resistance of these multiple myeloma cells to IFN-based therapy. Thus, the KLHL20–DAPK pathway plays a determining role in the efficacy of IFN-based anticancer therapy.

KLHL20 PROMOTES THE DEGRADATION OF TUMOR-SUPPRESSOR PML

Death-associated protein kinase is not the only tumor-suppressor protein targeted by KLHL20. The finding that PML competes with DAPK for KLHL20 binding suggests its function as a KLHL20 substrate. The *PML* gene was identified at the break point of the t(15:17) chromosome translocation of acute promyelocytic leukemia, which results in the generation of oncogenic PML–RAR α fusion protein (75). The PML protein is crucial for the assembly of PML–NBs and elicits pleiotropic antitumor effects, such as suppression of proliferation, angiogenesis, cell migration, and metastasis, and promotion of apoptosis and senescence (76–79). Additionally, PML regulates cancer cell metabolism and suppresses cancer stem cell maintenance (80, 81). Consistent with these tumor-suppressive functions, the expression of PML protein, but not its mRNA, is frequently lost or reduced in a wide range of human malignancies, such as colon, lung, prostate, breast, brain tumors, germ cell tumors, and non-Hodgkin's lymphoma (82). Evidence has emerged that ubiquitin-mediated proteasomal degradation is a key mechanism for PML degradation in tumors (83–85). The Cul3–KLHL20 complex is one of the ubiquitin ligases that target PML for ubiquitination and proteasomal degradation. However, two consecutive posttranslational modifications are required for PML binding to KLHL20, that is, phosphorylation at S518 by CDK1/2 followed by prolyl isomerization of the pS518–P519 peptide bond by Pin1 (86). This mechanism allows a cell cycle-dependent regulation of PML. Accordingly, PML abundance is gradually declined with the progression of cell cycle (87), correlating with the gradual increase of CDK1/2 activity. Furthermore, since CDK1/2 activity and Pin1 expression are frequently upregulated in tumors, KLHL20-dependent PML ubiquitination and degradation is expected to be enhanced in tumors. Through degradation of PML, KLHL20 is expected to elicit oncogenic roles by blocking PML tumor-suppressive effects. Indeed, KLHL20 confers tumor-promoting functions, such as transformation, migration, and survival, which are dependent on PML downregulation (86).

KLHL20 IN TUMOR HYPOXIA RESPONSES

The finding that KLHL20 is a transcriptional target of hypoxia-inducible factor-1 (HIF-1) unravels an additional layer of the

regulation of KLHL20-mediated PML ubiquitination (86). HIF-1 and its paralog HIF-2 are key molecules to mediate the adaptation of hypoxia by transcriptional activation of a large panel of genes containing “hypoxia responsive element” (HRE) on their promoters (88). This transcriptional program plays crucial roles in many aspects of cancer biology, including immortalization, autocrine growth, metabolic reprogramming, invasion, metastasis, angiogenesis, cancer stem cell maintenance, and resistance to chemotherapy and radiotherapy (89). The promoter of *KLHL20* contains two HREs, which are both involved in hypoxia-induced transactivation (86). Due to the induction of KLHL20 by HIF-1, PML ubiquitination and degradation is potentiated under hypoxia conditions. Interestingly, PML is itself a negative regulator of HIF-1 protein translation through a mechanism involving mTOR repression (76). Thus, the HIF-1-induced, KLHL20-mediated PML degradation together with the PML-induced, mTOR-mediated HIF-1 α downregulation should constitute a double-negative feedback loop to maximize HIF-1 α accumulation in hypoxia. Indeed, evidence has supported the participation of KLHL20/PML pathway in this feedback regulation to lead to a robust induction of both HIF-1 α and HIF-2 α in response to hypoxia (86). Thus, KLHL20-mediated PML ubiquitination results in not only the inhibition of PML tumor-suppressive functions but also a robust induction of various tumor hypoxia responses to contribute to the aggressiveness of diseases.

DYSREGULATION OF KLHL20 IN CANCER

Since HIF-1 α is frequently upregulated in tumors through hypoxia-dependent or -independent mechanism (90), KLHL20 expression is expected to be upregulated in certain cancers. In line with this notion, KLHL20 expression is elevated in prostate cancers compared to its expression in benign prostatic hyperplasia. Furthermore, this upregulation correlates with dysregulation of several other key molecules in the KLHL20–PML pathway, including HIF-1 α upregulation, Pin1 upregulation, and PML downregulation (86). More importantly, patients displaying the signature of high HIF-1 α , high KLHL20, high Pin1, and low PML expression pattern are found to be progressively increased with disease progression. These clinical findings support the significance of KLHL20–PML pathway in the progression of prostate cancer and suggest a promise for targeting this pathway in the treatment of aggressive prostate cancers.

In addition to the regulation of its expression level, the activity of Cul3–KLHL20 E3 ligase can be regulated in tumors by an inhibitor (8). Interestingly, this inhibitor, called KLHL39, shares a similar domain structure with KLHL20. However, due to the presence of certain atypical residues in its BTB domain, KLHL39 fails to bind Cul3. Rather, it interacts with KLHL20 through the kelch domain of two proteins. We found that KLHL39 cannot serve as a substrate of the Cul3–KLHL20 ubiquitin ligase but disrupts the interaction of KLHL20 with its substrate such as PML and DAPK. Surprisingly, KLHL39 also blocks the binding of KLHL20 to Cul3 through an unknown mechanism. Through these dual inhibitory roles, i.e., inhibition of KLHL20 binding to Cul3 and substrates, KLHL39 blocks KLHL20-dependent ubiquitination and degradation of DAPK and PML, leading to an

increase of their steady-state levels. Clinically, low expression of KLHL39 in human colon cancer correlates with low expression of PML and DAPK, higher tumor grade, lymph node metastasis, and distant metastasis. Furthermore, by comparing the primary tumors with lymph node metastases of the same patient, low expression of each KLHL39, DAPK, and PML is more frequently observed in the metastatic lesions. Consistent with the clinical observations, KLHL39 suppresses colon cancer migration, invasion, and metastasis, and these tumor-suppressive effects are all mediated through a PML- and DAPK-dependent manner. These findings indicate a tumor-suppressive function of KLHL39 by blocking KLHL20-dependent ubiquitination of PML and DAPK.

THE TUMOR-SUPPRESSIVE FUNCTIONS OF SPOP

Speckle type BTB/POZ protein comprises an N-terminal MATH domain, a BTB domain, a 3-box domain, and a C-terminal nuclear localization sequence. Similar to other BTB proteins, SPOP serves as a substrate adaptor of Cul3 ubiquitin ligase, and substrate binding is mediated by its MATH domain, which binds to a SPOP-binding consensus (SBC) motif φ - π -S-S/T-S/T (φ = non-polar; π = polar) on the substrate (7). The linkage of SPOP to cancer was first revealed by cancer genomic analyses, which uncovers SPOP as a significantly mutated gene in human prostate cancers (91). Subsequent analyses using larger prostate cancer patient cohorts confirmed this finding (92–95). Most of these SPOP mutations occur in the MATH domain, suggesting that mutations impair substrate binding. To date, a number of SPOP substrates have been identified in the context of prostate cancer, including androgen receptor (AR), steroid receptor coactivator (SRC)-3, DEK, ERG, and SENP7 (96–101).

Androgen receptor signaling is crucial for prostate cancer initiation, progression, and development of resistance to antiandrogen therapy (102). AR is found as a *bona fide* substrate of SPOP-based Cul3 ubiquitin ligase and an SBC motif in the hinge region of AR mediates its interaction with SPOP (97). SPOP-mediated AR ubiquitination leads to its proteolysis in the proteasome. Importantly, prostate cancer-associated SPOP mutants fail to target AR for ubiquitination, whereas AR splicing mutants lacking hinge domain are refractory to SPOP-mediated degradation. This study also revealed that SPOP-mediated AR degradation is antagonized by androgens and promoted by antiandrogens, suggesting that ligand binding-induced conformational change of AR could affect its recruitment to SPOP. In addition to AR, SPOP binds to SRC-3, a preferred coactivator of hormone-activated AR (103, 104), and targets it for Cul3-mediated ubiquitination and degradation (99). Again, prostate cancer-associated SPOP mutants cannot target SRC-3 for degradation (105). Thus, these SPOP mutants could enhance AR functions in prostate cancers by inhibiting the turnover of both AR and its coactivator SRC-3.

The role of AR in prostate cancer initiation is mediated in part by the translocation of oncogenic ETS family transcription factors, such as ERG and ETV1, to the loci of androgen regulated genes including TMPRSS2 (106, 107). Among them, the most common fusion is TMPRSS2-ERG, which occurs in >50% of

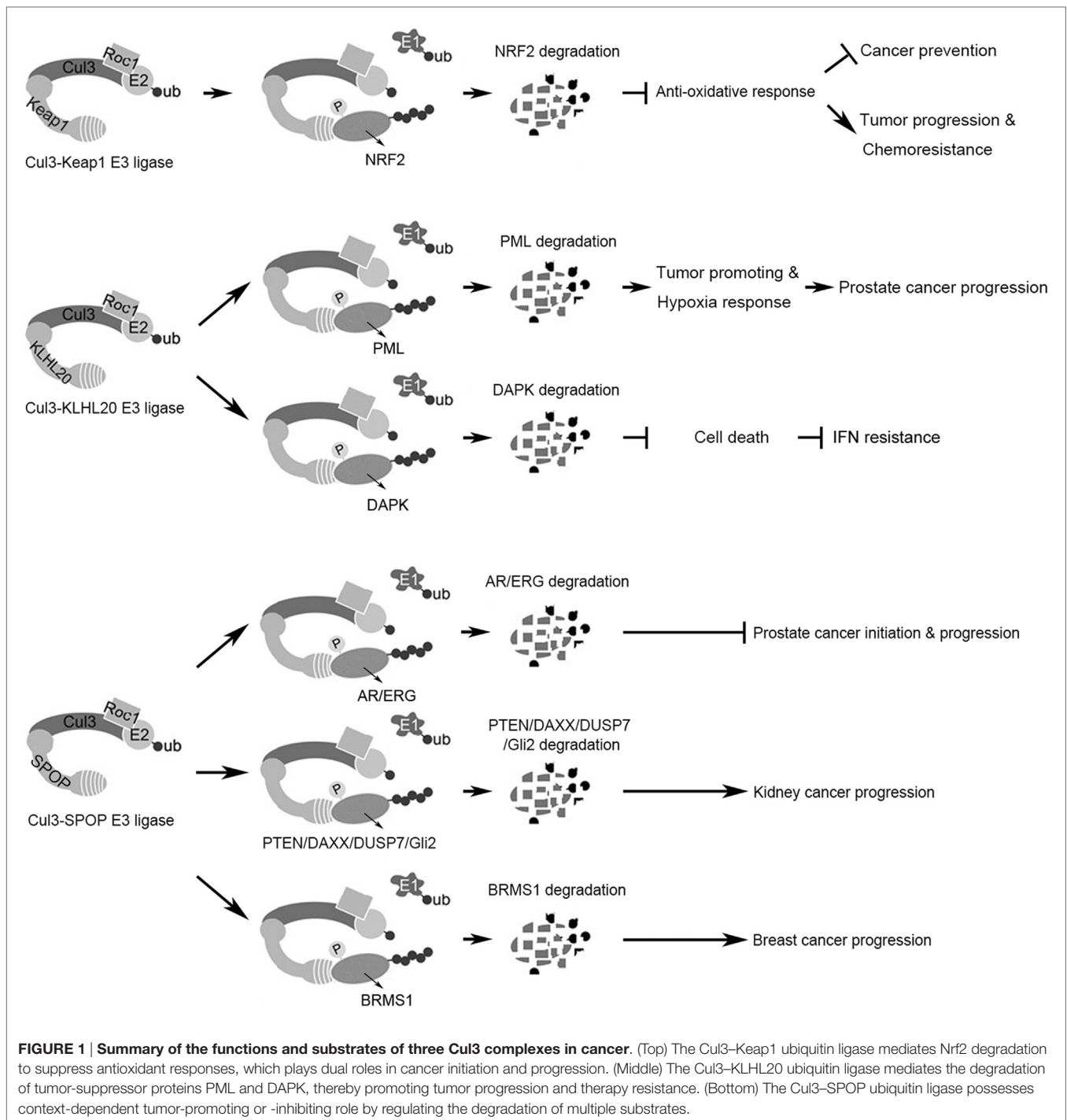
prostate cancers. This fusion allows AR-induced ERG overexpression, which elicits oncogenic functions such as proliferation, migration, and invasion (108). Recent studies indicate that ERG is targeted to SPOP-based Cul3 ubiquitin ligase for ubiquitination and degradation, and an SBC motif in the N-terminus of ERG is responsible for SPOP recognition (96, 98). Importantly, prostate cancer-associated SPOP mutants fail to induce ERG degradation, whereas the majority of TMPRSS2-ERG fusions encoding N-terminal truncated ERG proteins are resistant to SPOP-mediated degradation. Since these two types of genetic alterations, i.e., SPOP mutations and TMPRSS2-ERG fusions, similarly lead to ERG stabilization, it is conceivable that their incidences are mutually exclusive in prostate cancers (93).

Using mass spectrometry-based ubiquitylome analysis, several SPOP substrates have been discovered from prostate cancer cells, such as DEK, TRIM24, and NCOA3 (100). Among them, DEK stabilization contributes to prostate cancer invasion and stem cell-like property and DEK upregulation correlates with SPOP mutations, in prostate cancer. Besides these substrates, SENP7 desumoylase is also identified as a SPOP substrate (101). The SPOP-SENP7 axis promotes prostate cancer senescence, which is impaired by the presence of prostate cancer-associated SPOP mutants. Collectively, SPOP targets the degradation of multiple tumor-promoting proteins in prostate cancer to contribute to the carcinogenesis process.

Of note, SPOP mutations in the MATH domain are also found in endometrial cancers (109). In this cancer type, wild-type SPOP, but not cancer-associated SPOP mutants, targets estrogen receptor- α for ubiquitination and degradation (110). In breast cancer, SPOP represents one of the highest loci for loss of heterozygosity (99). Progesterone receptor, which contributes to the development of breast cancer, is found to function as a substrate of SPOP in this cancer type (111). These findings suggest that SPOP governs the turnover of distinct hormone receptors to participate in the carcinogenesis of several cancer types.

THE TUMOR-PROMOTING FUNCTIONS OF SPOP

In contrast to the aforementioned cancer types, SPOP plays a tumor-promoting role in kidney cancer. SPOP high expression occurs in 99% of clear cell renal cell carcinoma (ccRCC) (112), the most prevalent type of kidney cancer. The pathology of ccRCC is tightly associated with HIF-1 accumulation resulted from deficiency of VHL, which acts as a substrate adaptor of Cul2 ubiquitin ligase (113). Importantly, SPOP is a transcriptional target of HIF-1 and hypoxia potentiates the cytoplasmic accumulation of SPOP (114). This cytoplasmic retention of SPOP confers tumor-promoting activities, which is opposite to the function of SPOP in the nucleus. Mechanistically, SPOP controls the ubiquitination and degradation of several tumor suppressors residing in the cytoplasm, such as PTEN, ERK phosphatases, Daxx, and Gli2. In addition to kidney cancer, SPOP is reported to mediate ubiquitination and destabilization of breast cancer metastasis suppressor 1 (BRMS1) in breast cancer, thereby derepressing metastasis-associated genes (115). Thus, SPOP elicits context-dependent



functions in cancer development, which is influenced in part by its different subcellular distributions.

CONCLUDING REMARKS

A significant number of recent studies shed light on the biological functions of Cul3 E3 ligases that regulate tumor development, progression, and therapeutic response. In particular,

Keap1, KLHL20, and SPOP are the most reported Cul3 substrate adaptors for their impacts on various cancer types. These three proteins mediate Cul3-dependent ubiquitination on multiple substrates to influence on tumor initiation, progression, and therapeutic response (Figure 1). While KLHL20 mainly plays a tumor-promoting role, SPOP elicits both tumor-promoting and suppressive effects depending on its subcellular localization and cell context. As to Keap1, its role in cancer varies with the stages

in the multistep carcinogenesis process. Further insights into the functional and mechanistic basis of KLHL20 and SPOP in cancer development can be obtained from studies with suitable animal models, especially genetically engineered mouse models. In this regard, KLHL20 conditional knockout mice are recently generated (116), which could offer a powerful tool for studying its function in multistep carcinogenesis in a tissue-specific manner. In addition, systematic identification and characterization of substrates of these three adaptors and other Cul3 substrate adaptors can potentially facilitate a more comprehensive understanding on the functions of Cul3 ubiquitin ligases in cancer biology and their clinical implications. Such information will

be helpful for designing new therapeutic strategies for cancer intervention.

AUTHOR CONTRIBUTIONS

Both the authors collected and reviewed literatures and wrote the manuscript.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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