CYLD Inhibits Tumorigenesis and Metastasis by Blocking JNK/AP1 Signaling at Multiple Levels

Paula Miliani de Marval, Shazia Lutfeali, Jane Y. Jin, Benjamin Leshin, M. Angelica Selim, and Jennifer Y. Zhang

Abstract

CYLD has been recognized as a tumor suppressor due to its dominant genetic linkage to multiple types of epidermal tumors and a range of other cancers. The molecular mechanisms governing CYLD control of skin cancer are still unclear. Here, we showed that K14-driven epidermal expression of a patient-relevant and catalytically deficient CYLD truncated mutant (CYLDm) sensitized mice to skin tumor development in response to 7,12-dimethylbenz(a)anthracene (DMBA)/(12-O-tetradecanoylphorbol-13-acetate) TPA challenge. Tumors developed on transgenic mice were prone to malignant progression and lymph node metastasis and displayed increased activation of c-Jun-NH2-kinase (JNK) and the downstream c-Jun and c-Fos proteins. Most importantly, topical application of a pharmacologic JNK inhibitor significantly reduced tumor development and abolished metastasis in the transgenic mice. Further in line with these animal data, exogenous expression of CYLDm in A431, a human squamous cell carcinoma (SCC) cell line, markedly enhanced cell growth, migration, and subcutaneous tumor growth in an AP1-dependent manner. In contrast, expression of the wild-type CYLD inhibited SCC tumorigenesis and AP1 function. Most importantly, CYLDm not only increased JNK activation but also induced an upregulation of K63 ubiquitination on both c-Jun and c-Fos, leading to sustained AP1 activation. Our findings uncovered c-Jun and c-Fos as novel CYLD targets and underscore that CYLD controls epidermal tumorigenesis through blocking the JNK/AP1 signaling pathway at multiple levels. Cancer Prev Res 4(6): 851–9. ©2011 AACR.

Introduction

Human cyld (chromosome 16q12-13) encodes a deubiquitinase that primarily removes lysine-63 (K-63) linked polyubiquitin chains from an array of target proteins including TRAF2/6, IKKγ, Bcl3, p1k1, Tak1, and Ick (1–8). K63-ubiquitin is distinct from K48-ubiquitin with the former leading to protein activation and the latter targeting protein for degradation. Thus, CYLD generally acts a negative regulator to target protein function (9). CYLD was initially discovered as a tumor suppressor due to its autosomal-dominant genetic linkage to multiple types of cutaneous adnexal tumors including Brooke–Spiegler syndrome (BSS), familial cylindromas, multiple familial trichoepithelioma (MFT), and spiradenoma (9, 10). Up to 51 different truncation and missense mutations have been characterized thus far in skin tumors; all of these mutations result in the production of catalytically deficient CYLD mutants (CYLDm; ref. 10). In addition, LOH of the normal allele has been detected in about 70% of tumors carrying a CYLDm (11–14). These data underscore that the catalytic function of CYLD is important for tumor suppression. Moreover, CYLD loss of function has been associated with many other cancers including melanoma and myeloma (15, 16), as well as breast, colon, liver, kidney, and cervical cancers (17–21). Despite the broad relevance of CYLD to cancer, the molecular mechanisms governing CYLD effects on tumorigenesis are poorly understood.

The NF-κB pathway is a major downstream target of CYLD and is predicted to be the central regulator in driving the pathogenesis of skin cancers associated with CYLD deficiency (2–4). In particular, Bcl3, a noncanonical NF-κB subunit and a direct downstream target of CYLD, is identified as an essential regulator for the TPA-induced hyperproliferation of cyld−/− keratinocytes (22). However, clinical trials targeting NF-κB for the cure of cylindromas have not been satisfactory (23), which could be attributed to the common issues associated with bench-to-bedside applications. On the other hand, recent studies using either mouse or human tissue models have shown that NF-κB inhibits epidermal malignancy (24–26), suggesting that there could be other key regulators acting downstream of CYLD to promote malignancy.

In this study, we used transgenic mouse and human squamous cell carcinoma (SCC) models to investigate how CYLD loss of function leads to abnormal signal transduction and promotes tumorigenesis. We showed that expression of a catalytically deficient and patient-relevant CYLD
mutant (CYLD<sup>m</sup>) sensitizes the epidermis to malignancy and metastasis in a JNK/AP1-dependent manner. We also showed that CYLD<sup>m</sup> enhanced, whereas wild-type CYLD inhibited human SCC tumorigenesis both in vitro and in vivo. Moreover, we found that CYLD<sup>m</sup> not only increased JNK activity but also increased K63 ubiquitination on both c-Jun and c-Fos and ultimately potentiated AP1 transcriptional activity. Our findings indicate that the abnormal induction of the JNK/AP1 signaling pathway underlies epidermal tumorigenesis associated with CYLD loss of function.

**Material and Methods**

**Plasmids**

*K14-CYLD<sup>m</sup>* (HA-CYLD.932) expression construct was generated with the PCR product with pcDNA.HA-CYLD as a template (2). The purified PCR product was cloned into the pENTR1A vector (Invitrogen) and then gateway cloned into pBskII.K14 plasmid (27), which was then linearized with *KpnI* and *Smal* for the generation of transgenic mice. LZRS. CYLD<sup>WT</sup> and LZRS.CYLD<sup>m</sup> were generated by using the *Pmel* fragment from pcDNA.HA.CYLD and the PCR fragment encoding HA-CYLD.932. All plasmids were sequence verified at Duke DNA sequencing core facility. Retroviruses were produced in phoenix cells as described (28).

**Cell culture and gene transfer**

A431 and 293T cells were obtained from American Type Culture Collection and cultured in 5% FBS in Dulbecco’s modified Eagle’s media (DMEM). A431 cells were confirmed to express cytokeratin 14 by immunostaining, but no additional cell line authentication was conducted by the authors. DNA transfection was carried out with GenJet transfection reagent (SignaGen Laboratories) followed by selection with puromycin (1 μg/mL) for 3 to 4 days for stable expression of LacZ, CYLD<sup>WT</sup>, or CYLD<sup>m</sup>. For protein analysis, cells were serum starved for 24 hours and then incubated with fresh media containing 5% FBS and 25 ng/mL epidermal growth factor (EGF) for 1 hour. Protein extracts were collected in RIPA (radioimmunoprecipitation assay) or NP-40 lysis buffer supplemented with the cocktails of inhibitors for protease (Roche) and ubiquitin hydrolyase (20 mM/L N-ethylmaleimide and 5 μM/L ubiquitin aldehyde. For ubiquitin analysis, 293T cells stably expressing LacZ, CYLD<sup>WT</sup>, or CYLD<sup>m</sup> were further transfected with pcDNA plasmids encoding AU1–ubiquitin<sup>WT</sup> or mutants that contained 6 lysine to arginine mutations (29) and then used for protein analysis. Dual luciferase gene reporter analysis, cell growth, and soft agar colony formation were carried out as previously described (25). For scratch-wound assay, transfected A431 cells were grown to near confluence and wounded with a 10-μL pipette tip. Images were taken at 0 and 18 hours postwounding.

**Animal studies**

Animal studies were conducted in accordance with protocols approved by Duke Animal Care and Use Committee. K14-CYLD<sup>m</sup> transgenic mice were generated in Duke transgenic facility using FVB mice. Two independent lines of transgenic mice were identified by PCR genotyping and validated by immunoblotting with epidermal protein lysates. For the 2-stage carcinogenesis, transgenic and wild-type littermates (n = 20, 1–3 days old) were treated with 1 dose of 50 μg 7,12-dimethylbenz[a]anthracene (DMBA; Sigma) in 50 μL acetone as previously described (30). Three weeks later, mice were shaved on the dorsal skin and treated with 2.5 μg 12-O-tetradecanoylphorbol-13-acetate (TPA; Sigma) in 200 μL acetone biweekly for a total of 20 weeks. Tumors on each mouse were counted weekly following TPA treatment. Those scored as SCC on the basis of the invaginated growth pattern were confirmed by histologic analysis at the endpoint of growth. JNK inhibition was achieved by topical treatment with 250 μg SP600125 (LC Laboratories) in 200 μL DMSO (dimethyl sulfoxide) 30 minutes before each biweekly TPA application for a total of 20 weeks. Of note, no apparent health problems other than tumor growth were observed on WT and transgenic mice following the topical applications of DMBA, TPA, SP600125, or control vehicle. Statistical values were obtained through Student’s t-test analysis using GraphPad InStat 3.05.

For subcutaneous tumor growth, A431 cells were transduced with retrovirus for expression of LacZ, CYLD<sup>WT</sup>, or CYLD<sup>m</sup>. Cells were trypsinized 3 days postinfection and suspended in DMEM at 5 × 10<sup>6</sup>/mL and then mixed with Matrigel (BD Bioscience) at 1:1 ratio. A cell suspension of 200 μL was injected subcutaneously into CB17.SCID mice as previously described (31). The tumors were measured on days 28 and 35.

**Protein analysis**

For immunoprecipitation (IP), protein lysates (250 μg per sample) isolated from A431 or 293T cells were incubated with polyclonal antibodies against c-Fos, c-Jun, or ubiquitin (Santa Cruz Biotechnology) for 2 hours at 4°C followed by 2-hour incubation with Protein A Agarose beads. The beads were washed 3 times with NP-40 lysis buffer and then eluted for immunoblotting with antibodies against CYLD, c-Fos, or c-Jun, or phosphorylated c-Jun (p-c-Jun; GenScript). Immunohistochemistry and immunofluorescent staining were carried out with paraffin and frozen tissue sections, respectively, as described (31). Mouse keratinocytes were isolated from newborns and cultured to about 80% confluence as described (32). Cells were then treated with 0 or 100 nmol/L TPA along with or without 10 μmol/L SP600125 for 24 hours prior to whole-cell protein analysis and nuclei isolation. Nuclear proteins were extracted in 25 μmol/L HEPES, pH 7.9, 400 μmol/L NaCl, 1 μmol/L DTT, 20% glycerol, and 0.2 μmol/L EDTA in the presence of protease and phosphatase inhibitor cocktails (Roche), and 1.5 μg protein of each sample were used for AP1-gel mobility shift assay using odyssey dye-conjugated AP1 oligonucleotides and assay kit (Li-cor Biotechnology).
Results

Expression of CYLD<sup>m</sup> in mice leads to epidermal hyperplasia

To examine how CYLD affects epidermal homeostasis and tumorigenesis, we generated transgenic mice with keratin 14 promoter-driven expression of a human CYLD mutant 932 (CYLD<sup>m</sup>) which lacks the 21 amino acid residues at the C-terminal end (Supplementary Fig. S1A). CYLD<sup>m</sup> was catalytically deficient when tested with TRAF2/6 as substrates (4); and consistently, it increased IκBα phosphorylation and NF-κB gene reporter function (Supplementary Fig. S1B and C). Epidermal expression of CYLD<sup>m</sup> was verified by both immunoblotting and immunostaining with an antibody against CYLD or the HA-epitope in 2 independent lines of transgenic mice (Fig. 1A and Supplementary Fig. S1D). The transgenic mice had no obvious developmental abnormalities other than mild epidermal hyperproliferation as indicated by the increased numbers of Ki-67–positive and nucleated cells (Supplementary Fig. S1D and E).

CYLD<sup>m</sup> promotes epidermal tumor development and malignant progression

To determine the role of CYLD<sup>m</sup> in tumor development, we subjected both WT and transgenic mice to a 2-stage skin carcinogenesis protocol. Newborn mice were initiated with 1 topical dose of DMBA followed by TPA twice weekly for 20 weeks. Tumor incidence and multiplicity were scored in each group for 40 weeks. Transgenic mice reached a 100% tumor incidence by week 13 following TPA promotion and developed an average of 11.4 tumors per mouse by week 21. In contrast, WT mice did not reach the 100% tumor incidence until week 21 and developed an average of 6.4 tumors per mouse (Fig. 1B). In addition, 66% of the transgenic lesions, as compared with 25% of WT lesions, developed clinical features of SCC by week 32 (Fig. 1C), as judged by the invaginated growth pattern accompanied either with or without a cauliflower-like or ulcerated appearance. In corroboration with the clinical features, transgenic tumors showed histologic features of malignancy, including the epidermal tissue invasion down to the dermis and the increased numbers of atypical and Ki-67–positive cells (Fig. 1D). In addition, these tumors displayed signs of epithelial-mesenchymal transformation (EMT) as indicated by the absence of E-cadherin and the presence of mesenchymal cell markers including N-cadherin and vimentin. In contrast, the tumors developed on WT mice retained the expression of E-cadherin and were negative of N-cadherin and vimentin (Fig. 1D). The WT tumors generally maintained an epithelial cell morphology; although, some of them were hyperproliferative and locally invasive. These data indicate that CYLD<sup>m</sup> not only sensitizes mouse skin to tumor development but also promotes malignant conversion.

Skin tumors developed on transgenic mice metastasize to lymph nodes

The aggressive nature of the transgenic tumors prompted us to conduct whole body necropsy following the collection of primary tumors. Surprisingly, over 50% of the
Figure 2. Tumors developed on K14-CYLDM transgenic mice metastasize to lymph node in a JNK/AP1-dependent manner. A, inguinal lymph nodes of WT (WT/LN) and Tg (Tg/LN) mice were collected at 32 weeks following DMBA/TPA challenge. Clinical images of representative lymph nodes were shown along with histologic analysis by H&E staining or immunostaining for cytokeratin 5 (K5) and Ki-67 (brown). Nuclei (blue). B, immunostaining of the primary skin tumors and the lymph node tumors with antibodies against p-JNK, c-Fos, and RelA (brown). Nuclei (blue). C, mouse keratinocytes isolated from WT and Tg mice were treated with or without 100 nmol/L TPA and 10 μmol/L SP600125 and then collected for immunoblotting for p-c-Jun and Actin and for AP1-gel mobility shift assay. D, WT and Tg siblings (n = 15–20) were subject to DMBA/TPA carcinogenesis either with or without topical SP600125 prior to each TPA application. The percentage of mice which developed at least 1 tumor and the average papilloma numbers per mouse were shown in the graph in a weekly time-course manner. E, percentage of mice developed primary, sarcomatoid, and lymph node tumors. The denominators and numerators above each column represent the total number of mice initiated for the study and the number of mice that developed indicated tumors, respectively.
transgenic mice, as compared with none of the WT siblings, had tumors in the lymph nodes located primarily at the axillary and inguinal regions (Fig. 2A). The lymph node tumors displayed a mixture of keratinized and spindle cell morphologies, expressed cytokeratin 5 (K5), an epidermal cell marker, and were highly proliferative as indicated by the high number of Ki-67–positive cells (Fig. 2A). These results indicate that CYLDm promotes epidermal tumors to metastasize to lymph node.

**JNK/AP1 activity underlies tumorigenesis and metastasis**

Among the known downstream targets, NF-κB has been presumed as the major culprit in the tumorigenesis associated with CYLD mutation (3, 4, 22). However, NF-κB inhibitors showed a limited efficacy in a recent clinical trial (23), suggesting that other CYLD downstream targets might be involved in the tumorigenesis. Specifically, we examined the JNK signaling pathway whose receptor-mediated induction was subject to CYLD inhibition (5). As predicted, primary and lymph node tumors from the transgenic mice displayed strong nuclei localization of phosphorylated JNK (p-JNK) and c-Fos, an AP1 subunit involved epidermal malignant conversion (ref. 9; Fig. 2B). In contrast, RelA, a major NF-κB subunit that translocates from cytoplasm to nuclei on activation (33), was primarily located in the cytoplasm of tumor cells (Fig. 2B). In addition, AP1 activity was increased in keratinocytes isolated from transgenic mice as shown by immunoblotting for p-c-Jun and AP1-gel mobility shift assay (Fig. 2C). Treatment with the JNK-specific inhibitor SP600125 reduced p-c-Jun levels, indicating that c-Jun activation is dependent on JNK function. In contrast to cyld−/− keratinocytes (22), the transgenic tumors did not show a significant increase of nuclear Bcl3 (Supplementary Fig. S2). These data indicate that JNK/AP1 but not NF-κB activation is increased in tumors expressing CYLDm and are consistent with our previous findings showing a clinical relevance of NF-κB loss of function and JNK gain of function in human SCC (24, 25, 31, 34). To determine whether JNK/AP1 is essential for the tumorigenesis enhanced by CYLDm, we challenged mice with the DMBA/TPA protocol and incorporated the topical treatment of SP600125 before each TPA application. SP600125 significantly reduced tumor multiplicity and incidence in both WT and transgenic mice (Fig. 2D). In addition, SP600125 prevented the transgenic skin tumors from progressing into sarcomatoid SCC or metastasis to lymph node or other internal organs (Fig. 2E). Of interest, the transgenic mice have normal profiles of T lymphocytes as analyzed by flow cytometry (Supplementary Fig. S3), which is in concordance with the notion that CYLD regulates T-cell development in a cell autonomous manner (7). Thus, the tumor-prone phenotype of the transgenic mice is unlikely caused by potential immune defects. Taken together, these findings underscore that JNK/AP1 signaling pathway underlies tumor development and metastasis caused by CYLD deficiency.

**CYLD loss of function promotes human SCC in an AP1-dependent manner**

Next, we tested CYLDm-effects on A431, a spontaneous human SCC cell line. We found that CYLDm significantly

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**Figure 3.** CYLD controls human SCC through suppressing JNK/AP1 function. A, cell growth. B, soft agar colony formation. A431 cells were transduced with retroviruses for expression of LacZ, CYLDWT, or CYLDm along with or without DNc-Jun. Graphs represent average numbers of (A) cells or (B) colonies ± SD. C, cell migration. Cells transduced as above were grown to near confluence and then scratch-wounded. Images were taken at 0 and 18 hours postwounding. D, subcutaneous tumor growth. Cells transduced as above were injected subcutaneously into CB.17SCID mice (5 × 10^5 cells per mouse; 5–10 mice per group). Tumors were measured on day 28 and 35, and average tumor volumes were shown ± SD.
increased the rate of monolayer cell growth and 3-dimensional soft agar colony formation, whereas CYLDWT decreased them (Fig. 3A and B). AP1 inhibition by expression of DNc-Jun, a dominant-negative c-Jun mutant (33), significantly reduced the number of soft agar colonies. In addition, CYLDm induced an increased rate of cell migration as assessed by a scratch-wounding assay, whereas CYLDWT markedly slowed cell migration (Fig. 3C). Again, AP1 inhibition by siRNA-mediated gene silencing of c-Jun and c-Fos abolished the CYLDm effect on cell migration (Supplementary Fig. S4). Moreover, CYLDm noticeably enhanced subcutaneous tumor growth of A431 cells in immunodeficient mice. In contrast, CYLDWT abolished tumor growth in mice (Fig. 3D). These findings indicate that CYLDWT inhibits, whereas CYLDm promotes tumorigenesis of human SCC in an AP1-dependent manner.

**CYLD suppresses AP1 function by regulating c-Jun/c-Fos ubiquitination**

To further confirm that AP1 is subject to CYLD regulation at a functional level, we conducted luciferase gene reporter analysis. We found that CYLDm increased AP1-driven expression both in the presence and absence of EGF, whereas CYLDWT reduced AP1 activity in both conditions (Fig. 4A). These data are in line with the recent finding showing that CYLD controls JNK activity at a level upstream of MKK7 (5). In addition, CYLDWT markedly reduced, whereas CYLDm significantly potentiated AP1 activity driven by exogenous c-Fos expression; conversely, gene silencing of c-Jun or c-Fos abolished AP1 induction by CYLDm (Fig. 4A). These data suggest that there could be a direct link between CYLD and AP1 subunits. To test for this link, we first examined the protein levels of c-Jun and c-Fos in response to altered CYLD function in 293T cells. In response to EGF, c-Fos and c-Jun, and p-c-Jun, a product of JNK activation, were increased in cells expressing LacZ or CYLDm but not in those expressing CYLDWT (Fig. 4B). Moreover, co-IP analysis revealed that EGF increased c-Jun/c-Fos dimerization, a process necessary for c-Fos function (36); this induction was enhanced by CYLDm and reduced by CYLDWT (Fig. 4C). Surprisingly, both CYLDm and CYLDWT were also precipitated by the c-Fos antibody (Fig. 4C). The interactions between c-Jun, c-Fos, and CYLD were also observed in A431 cells as shown by the bidirectional co-IP analyses with 3 different antibodies (Supplementary Fig. S5A). These findings suggest that CYLD have an enzyme–substrate relationship with c-Jun and c-Fos. We tested this idea by IP for c-Jun and c-Fos and immunoblotting for ubiquitin and vice versa and found that the levels of ubiquitinated c-Fos and c-Jun were increased by EGF and further augmented by CYLDm but diminished by CYLDWT; the same result was obtained by IP for ubiquitin and immunoblotting for c-Fos (Fig. 5A). Because the IP ubiquitination analyses were carried out under stringent buffer conditions that disrupted protein–protein interactions, the observed change of ubiquitination is unlikely a result of protein complex formation but rather is directly linked to c-Jun and c-Fos.

**Figure 4.** CYLDm increases AP1 activity (A) AP1-luciferase gene reporter analysis. 293T cells were cotransfected with AP1-firefly luciferase along with LacZ, CYLDWT, or CYLDm expression constructs and then treated with EGF for 4 hours or cotransfected with the c-Fos expression construct or siRNA oligonucleotides targeting c-Jun (sic-Jun), c-Fos (sic-Fos), or nonsilencing control (siCon). Graphs represent the means of relative luciferase reading units (RLU) from triplicate experiments ± SD. B, Immunoblotting for CYLD, c-Fos, c-Jun, p-c-Jun, and actin. Protein lysates were isolated from 293T cells stably expressing LacZ, CYLDWT, or CYLDm and treated with or without EGF. C, IP of protein lysates collected as in B for c-Fos followed by immunoblotting for CYLD, c-Fos, or c-Jun. IgH, IgG heavy chain.
CYLD loss of function is not only relevant to cutaneous adnexal tumors but also to many other cancers including SCC (22, 42). It is worth noting that *cyld*<sup>−/−</sup> mice are sensitive to chemically induced carcinogenesis, but the tumors developed on these mice are not more malignant than those of WT mice (22). We predict that the differential tumor growth phenotypes observed in *cyld*<sup>−/−</sup> and CYLD<sup>m</sup> transgenic mice could be explained by multiple possibilities. First, CYLD<sup>m</sup> might have dominant negative effects such that the N-terminus of CYLD possesses oncogenic functions that are independent of the C-terminal catalytic function. Such a scenario is in line with the fact that every patient-relevant *cyld* mutation characterized so far produces a catalytically deficient CYLD mutant (10). Second, CYLD is required for endothelia cell migration (43); thus, its absence in endothelial cells of CYLD<sup>m</sup> transgenic mice might result in an impairment of angiogenesis and consequently affect tumor progression. In contrast, expression of CYLD<sup>m</sup> is limited to epidermal cells in the transgenic mice. Third, the differences in mice genetic backgrounds might also contribute to the differential sensitivity to carcinogenesis, which can be addressed by cross-breeding of the transgenic and knockout mice in future studies. *K14-CYLD<sup>m</sup>* transgenic mice did not develop spontaneous skin tumors, indicating that other genetic or environmental challenges are required to promote tumorigenesis. Because cutaneous adnexal tumors are frequently located on the exposed areas, UV irradiation has been considered as the major cause of tumor initiation. However, recent studies have shown that the pubic area is also susceptible to cylindromatosis, a phenomenon that has been previously underreported. This datum suggests that hormonal factors might be involved in tumor induction in patients (37). Future efforts are necessary to determine how...
UV, hormonal factors, and LOH of the WT cyld allele contribute to CYLD<sup>−/−</sup>-driven epidermal malignancy.

Bcl3 is a direct substrate of CYLD, and on activation via K63 ubiquitination, it forms heterodimers with p50/p52 to induce expression of cyclin D1. Thus, Bcl3 is recognized as an important regulator in skin carcinogenesis of cyld<sup>−/−</sup> mice (22). Interestingly, despite the inhibitory role of CYLD on NF-κB, neither Bcl3 nor RelA displayed increased induction in the CYLD<sup>−/−</sup> transgenic tumors. It is possible that this is due to the negative cross-talk from JNK/AP1 as described in our previous studies (34). These findings implicate that NF-κB is unlikely the sole key regulator in the malignant tumor phenotype developed on transgenic mice. To this end, we found that JNK and its downstream c-Jun and c-Fos proteins were highly activated in both primary and metastatic tumors from the transgenic mice. In addition, CYLD<sup>−/−</sup> increased the basal levels of c-Jun and c-Fos and sustained their activation status in response to EGF treatment. Moreover, both CYLD<sup>Wt</sup> and CYLD<sup>−/−</sup> interacted with c-Jun and c-Fos but with opposite effects; the latter increased c-Jun/c-Fos K63-ubiquitination and potentiated their transcriptional activity. Presumably, K63 ubiquitination excludes the K63 ubiquitination (3, 22). In contrast to the canonical NF-κB pathway which suppresses epidermal growth and neoplasia (24, 26, 44), Bcl3 and the JNK/AP1 signaling cascades support epidermal growth and tumorigenesis (22,25,31). Taken together, our data established an important and broad role for CYLD in malignant and metastatic tumor development and identified c-Jun and c-Fos as novel CYLD downstream regulators. These findings provide mechanistic insights to therapeutic targeting of the JNK/AP1 pathway for cancers associated with CYLD loss of function.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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Paula Miliani de Marval, Shazia Lutfeali, Jane Y. Jin, et al.

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