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Broad-spectrum ubiquitin-specific protease inhibition as a mechanism for the cytotoxicity of YM155 in cancers

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Protein ubiquitination is a dynamic and reversible process involved in gene transcription, protein metabolism, and cellular apoptosis. Ubiquitin specific proteases (USPs), as the largest family of deubiquitinating enzymes, are able to remove the ubiquitin from target proteins, rescuing them from degradation. Here, we characterized the small molecule antitumor agent YM155 as a broad-spectrum USP inhibitor. By inhibiting the deubiquitinase activity of multiple USPs, YM155 causes the degradation of oncogenic substrate proteins, such as c-Myc and intracellular domain of Notch1. In cancers driven by these proteins, YM155 induces profound cell apoptosis and markedly inhibits tumor growth in xenograft models. Together, these findings demonstrate that YM155 is a broad-spectrum USP inhibitor, and a potential drug candidate for cancers which depend on hyper-active oncogenic proteins that are regulated by the ubiquitin-proteasome pathway.

Keywords YM155, A broad-spectrum USP inhibitor, c-Myc, Notch1, Ubiquitin-proteasome pathway

Deubiquitinases (DUBs) are key components of ubiquitin-proteasome system with their ability to specifically deconjugate ubiquitin from targeted proteins[1]. DUBs play a crucial role in reversal of ubiquitin signaling and recycling ubiquitin[2]. The ubiquitin-specific proteases represent the largest subset of DUB family, with more than 50 members[3]. As cysteine proteases, the enzymatic capability of USP primarily depends on the catalytic site[4]. After binding of a substrate, the catalytic cysteine is deprived of a proton by histidine, and undergoes a nucleophilic attack, releasing the ubiquitin from the substrates[5]. A large number of substrates for USPs are oncoproteins and tumor suppressor proteins, therefore, USPs might be attractive targets for novel cancer therapies[6]. Increasing number of USP inhibitors have been reported with potential in cancers therapy[7–9]. Compound VLX1570 was the first USP inhibitor to enter clinical trials in 2015 (NCT02372240). However, this trial had to be prematurely terminated due to severe toxicity[10]. Several other USP inhibitors are currently undergoing clinical testing (NCT02321293, NCT03272503) in cancer or other disease associated with USPs enzyme[11,12], with more potent USP inhibitors in preclinical stage.

YM155 (also referred to as Sepantronium bromide), a small compound (1-(2-methoxyethyl)-2-methyl-4,9-dioxo-3-(pyrazine-2-ylmethyl)-4,9-dihydro-1*H*-naphtho[2,3-*d*] imidazolium bromide), was originally identified as a survivin inhibitor[13]. It is known to exert anti-tumor effects in various types of cancer[14,15]. In human multiple myeloma cells, YM155 exhibits robust cytotoxic activity through downregulation of survivin and Mcl-1 protein[16]. In pancreatic cancer cells, YM155 reduces survival through modulation of EGFR and survivin expression[17]. Additional studies revealed that YM155 is an oxidative stress inducer which can induce autophagy-dependent reactive oxygen species (ROS)-mediated DNA damage in human cancers. The compound was quite safe in human, however, results from multiple phase II clinical studies have proven disappointing[19,20], possibly due to an inaccurate understanding of the molecular target of YM155.

As cysteine proteases, the activities of USPs are inhibited by the oxidation of ROS[21]. Naphthoquinones (with a naphthalene ring), the prominent type of quinones, bear the ability to accept electrons to form semiquinones and hydroxyquinones. These species can be oxidized again by molecular oxygen generating ROS[22]. Naphthoquinone derivatives have been reported to target USP and inhibit its activity[23]. YM155 is a small molecule agent with a typical naphthoquinone core[24]. In our previous study, it is demonstrated that YM155 decreases MYCN protein level through inhibiting USP7 deubiquitinase activity[25]. However, inhibition of USP7 alone is not sufficient to

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explain the relative broad anti-cancer activity of YM155 in the literature. In this report, we set out to further explore the molecular basis of YM155 activity, especially within the broader USP target space based on its naphthoquinone core structure.

An *in vitro* assay for high throughput screening demonstrated that YM155 as a broad-spectrum USPs inhibitor. The activity of YM155 to inhibit multiple deubiquitinase was evaluated by enzymatic assays in living cells. YM155 was shown to accelerate degradation of oncogenic c-Myc and ICN1 proteins which depend on USPs for protein stabilization. Furthermore, our experiments demonstrated that YM155 induces cell apoptosis *in vitro* and inhibits xenograft tumor growth *in vivo*. These results establish a novel mechanism for the cytotoxicity of YM155 in cancers, and will support its translation for future clinical use.

Materials and methods

Cell culture and reagents

Human B lymphoma cells, SU-DHL-2, SU-DHL-10 (purchased from Cell Bank, Nanjing Cobioer Biosciences), SU-DHL-4 (purchased from Cell Bank, Shanghai Institutes for Biological Sciences), were cultured in RPMI 1640 (Hyclone, SH30809.01) supplemented with 10% fetal bovine serum (Gibco, 10099–141 C). OCI-LY7 (purchased from Cell Bank, Nanjing Cobioer Biosciences), was cultured in IMDM (Gibco, 31980-030) supplemented with 20% fetal bovine serum (Gibco, 10099–141 C). Human Burkitt's lymphoma cell, Ramos (purchased from Cell Bank, Nanjing Cobioer Biosciences), was cultured in RPMI 1640 (Hyclone, SH30809.01) supplemented with 10% fetal bovine serum (Gibco, 10099–141 C). Human T cell acute lymphoblastic leukemia, CCRF-CEM (purchased from Cell Bank, Shanghai Institutes for Biological Sciences), was cultured in RPMI 1640 (Hyclone, SH30809.01) supplemented with 10% fetal bovine serum (Gibco, 10099–141 C). Human Leukemia cells, Jurkat and MOLT-4 (purchased from Cell Bank, Cell Resource Center, Peking Union Medical College (PCRC)), Loucy (purchased from Cell Bank, ATCC), were cultured in RPMI 1640 (Hyclone, SH30809.01) supplemented with 10% fetal bovine serum (Gibco, 10099–141 C). Human prostate cancer cells, 22RV1 and DU145 (purchased from Cell Bank, Shanghai Institutes for Biological Sciences), were cultured in RPMI 1640 (Hyclone, SH30809.01) supplemented with 10% fetal bovine serum (Gibco, 10099–141 C). Human lung cancer cells, HCC827 (purchased from Cell Bank, Shanghai Institutes for Biological Sciences), HCC4006 (purchased from Cell Bank, Nanjing Cobioer Biosciences), were cultured in RPMI 1640 (Hyclone, SH30809.01) supplemented with 10% fetal bovine serum (Gemini, 900 – 108). Cultures were incubated at 37 °C, in 5% CO_2. Human lymphoma cell, P493 (purchased from Wuhan University School of Medicine), was cultured in RPMI 1640 (Hyclone, SH30809.01) supplemented with 10% fetal bovine serum (Gibco, 10099–141 C). The cells were induced with tetracycline HCl (INALCO, 1758–9302) at 0.2μM concentration for 72 h. Cultures were incubated at 37 °C, in 5% CO_2.

Cell apoptosis analysis by flow cytometry

Cells were grown in 6-well plates. The suspensions were centrifuged to collect the pellets after the indication treatment. Then the cells were stained with propidium iodide (PI) and Annexin V (Thermo Sicentific, V13245). Cells were analyzed by flow cytometer (BD, USA). Data were analyzed using FlowJo.

Western blot

Cells were lysed in lysis buffer with protease inhibitors and phosphatases inhibitors (Beyotime Biotechnology). Briefly, protein concentrations were determined with a Micro BCA™ Protein Assay Kit (Thermo). 20–80 μg protein was loaded in each well of SDS-PAGE, and transferred to polyvinylidene fluoride membrane. The following antibodies were used to detect: USP28 (Abcam, ab126604), USP16 (Proteintech, 14055-1-AP), Ub (Abcam, ab7780), c-Myc (Abcam, ab32072), K48-linkage specific poly ubiquitin antibody (CST, 8081 S), mono and poly-ubiquitylated conjugates, mAb (FK2) (UBIQUIGENT, 67-0006-001), K63-linkage specific ubiquitin antibody (Abcam, ab179434), Notch1 (CST, 4380 S), rabbit monoclonal Cleaved Notch1 (CST, 4147 S), mouse GAPDH (ZSGB-Bio, TA-08). Secondary antibody: anti-mouse IgG, HRP-linked antibody (CST, 7076 S), anti-rabbit IgG, HRP-linked antibody (CST, 7074 S).

Deubiquitinating (DUB) enzymes activity assay

The samples were added to the half volume 96-well plates then incubated for 30 min at room temperature. Initiated reactions by quickly adding 5 μl of Ubiquitin-AMC (Cayman, 701490) to all samples. The fluorescence was detected at an excitation wavelength of 355–365 nm and an emission wavelength of 455–465 nm.

Deubiquitinating enzyme activity assay using auto-ubiquitylation products as the substrate

Auto-ubiquitylation products was produced following manufacturer's instruction of DUBscan™ Kit (Ubiquigent, UK). UBE1, UBE2W, UBE2N, E3 chip, and UBE2V1 (Ubiquigent), Ubiquitin (Genemay Inc, USA), DTT (Sigma, USA) and full-length USP28 (R&D, USA) were used in this assay. DUB test-well was used for the auto-ubiquitylation reaction, consisting of ATP, ubiquitin, E1, E2, and E3. Then incubated for 1 h at 37 °C. Used EDTA to stop the reaction. Incubated the USP28 with or without YM155 with this auto-ubiquitylation substrate for 0.5–1 h at 37°C, respectively. Analyse by SDS-PAGE and Western blotting. The Mono- and Poly-ubiquitylated substrates (with or without USP28 and/or YM155 treatment) were detected using monoclonal antibody (FK2).

Cell thermal shift assay (CETSA)

DU145, 22RV-1 or SU-DHL-4 cell lysates were collected and centrifuged at 20,000 g for 20 min at 4 °C. The supernatant was added into DMSO or YM155 at indicated concentration, respectively. The mixture was aliquoted into different tubes after incubated at room temperature for 30 min. Then heated it 3 min at indicated temperature. The precipitated and soluble proteins were separated by centrifugation at 20,000 g for 20 min at 4 °C. The soluble proteins were detected with Western blot.

Xenograft model

Four-to-six weeks old female (BALB/c-nu) mice were purchased from SPF Biotechnology Co.,Ltd (China). SU-DHL-4 and OCI-LY7 cells (2×10^6) were subcutaneously (s.c.) injected into the flanks of mice. Following tumor development, mice were randomized into 2 groups: vehicle (saline) or YM155. YM155 was administered at 2 mg/kg/day by continuous s.c. infusion for 7 days using a micro-osmotic pump (Alzet model 1007D). HCC827 and HCC4006 cells were also used to produce xenografted model. YM155 was administered at 5 mg/kg/day for 9 days by i.p. Tumor diameter was measured twice a week, and the body weight was measured every day. Tumor volume was calculated by the formula: volume = length× width× width×0.5.

Results

YM155 is a small molecule agent with a typical naphthoquinone core (Fig. 1A). We identified YM155 as a broad-spectrum USPs inhibitor using a high throughput ubiquitin-rhodamine assay with 29 DUBs from diverse families (Fig. 1B). YM155 inhibited enzyme activity of 23 USPs to different extent in this panel. However, YM155 has limited activity against other families of DUBs, such as Machado-Josephin domain (MJD) family and Jab1/MPN domain-associated metalloisopeptidase (JAMM) family. To further verify the activity of YM155 against USPs, we incubated YM155 with recombinant USP2, USP16 or USP28, followed by incubation with ubiquitin-amino-methylcoumarin (Ub-AMC) as the substrate. It was confirmed that the deubiquitinating enzyme activities of USP2, USP16 and USP28 were inhibited by YM155 in a dose-dependent and time-dependent manner (Fig. 1C). In previous study, we have demonstrated that the activity of USP7 was inhibited by YM155[25]. In summary, YM155 is a broad-spectrum USP inhibitor.

To further verify that YM155 inhibits the activity of USPs via direct binding, we employed the cellular thermal shift assay (CETSA), a method that allows rapid and simple assessment of direct target engagement in a cellular context. In Fig. 2A, USP28 protein was stabilized by YM155 treatment at 52 °C and 55 °C, compared to control group. Consistent with the results, accumulation of USP28 was also increased by YM155 in a concentration manner (Supplementary Fig. 1A). These results confirmed that YM155 can bind to USPs in the context of cellular environment. We further evaluated the function of YM155 as an inhibitor of USPs. *In vitro* auto-ubiquitylation reactions were prepared with E1 activating enzyme, E2 conjugating enzyme, E3 ligase, ubiquitin and ATP. As shown in Fig. 2B, the ubiquitin-linked substrates were markedly reduced after incubation with USP28 for 1 h (lane 2 compared with lane 1). The de-ubiquitination activity of USP28 was blocked by increasing amount of YM155 in dose-dependent manner (lane 3, lane 4 and lane 5 compared with lane 2) and time-dependent manner (Supplementary Fig. 1B). To further evaluate the function of YM155 in inhibiting the enzyme activity of USP2 in a native cellular context, we repeated the experiment using lymphoma cell lysate (Fig. 2C). Addition of recombinant USP28 protein significantly decreased the visualized multiple-ubiquitin chain bands (lane 2 compared with lane 1). However, the deubiquitination activity was inhibited by YM155 (lane 3 compared with lane 2). Our previous research demonstrated that YM155 can also inhibit the enzyme activity of USP7[25]. In summary, YM155 acts as a broad-spectrum-USP inhibitor.

USPs are involved in protein stabilization, therefore inhibition of USPs can lead to accelerated turn-over of the substrates. We examined the fate of several well-known targets of USP7, USP28 and USP16 after YM155 treatment, including activating Notch1 (intracellular domain of Notch1, ICN1), AR-V7 and c-Myc. ICN1 has been reported to be a direct substrate for USP7 and USP28[26,27]. As shown in Fig. 3A, YM155 inhibits ICN1 level

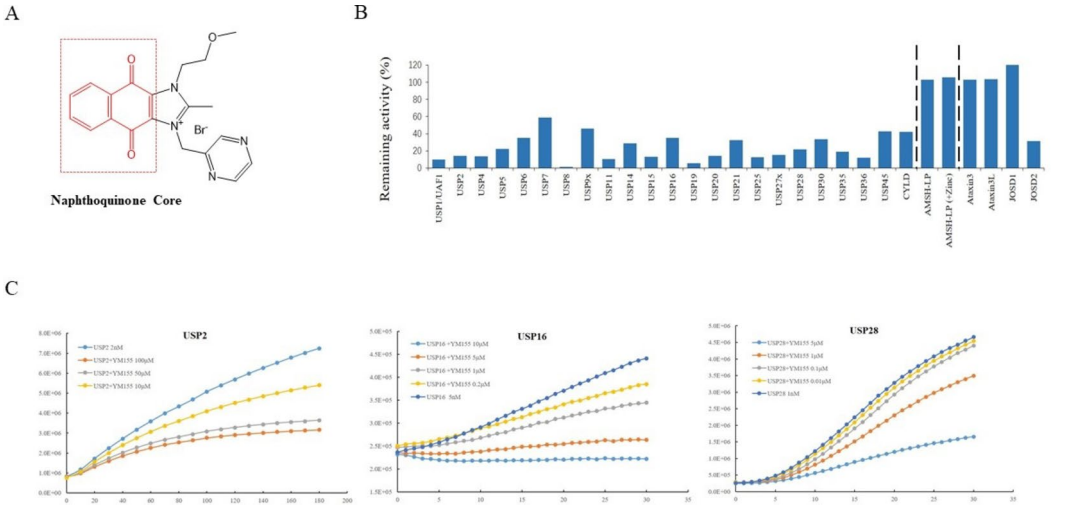

Fig. 1. YM155 is a small-molecule USP inhibitor. (**A**) A chemical structure of YM155. Red: Naphthoquinone core. (**B**) Commercial DUB screen (one replicate) with 10 μM YM155. Data is reported as a percentage of enzyme activity in the presence of YM155 relative to 100% activity DUB enzyme controls. (**C**) Increasing concentrations of YM155 (10 μm, 50 μm and 100 μm YM155 with USP2; 0.2 μm,1 μm, 5 μm and 10 μm YM155 with USP16; 0.01 μm, 0.1 μm, 1 μm and 5 μm YM155 with USP28) were incubated with USPs. After 20 min, substrate (Ub-AMC) was added. Then fluorescence intensity was measured.

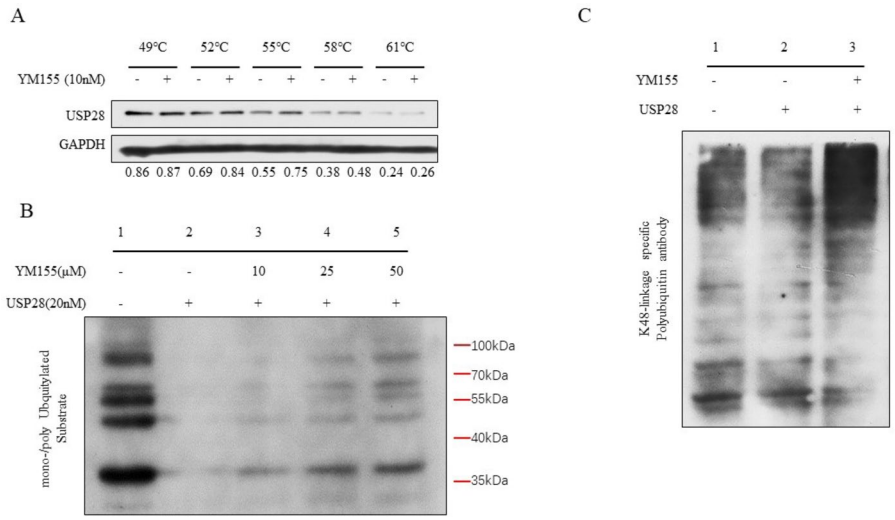

Fig. 2. YM155 blocks the activity of USP28. (**A**) CETSA was performed to measure the binding ability of YM155 to USP28 in DLBCL cell line (SU-DHL-4). Cell lysates were incubated with YM155 for 30 min, then the lysates were heated at the indicated temperature. Western blot of USP28 were shown. GAPDH was used as a loading control. Each band intensity of USP28 was normalized with respective GAPDH. The numbers below the images indicate the relative intensity of USP28 protein. (**B**) The effect of YM155 on the deubiquitinating activity of USP28 was detected *in vitro*. Ubiquitin-linked E3 ligase was used as the substrate (lane 1). The substrate was incubated with full length USP28 for 30 min (lane 2). The substrate was incubated with USP28 and 10μM, 25μM or 50μM YM155, respectively (lane 3, lane 4 and lane 5). USP28: 20nM. (**C**) The effect of YM155 on deubiquitinating activity of USP28 was detected in DLBCL cell line lysate (OCI-LY7). The cell lysate was used as substrate (lane 1). The lysate was incubated with USP28 (lane 2) and USP28 with YM155 (lane 3). USP28:25 nM; YM155 200 μM; antibody: anti-Ubiquitin (linkage-specific K48).

in a dose-dependent manner. ICN1 level decreased after 72 h of YM155 treatment in T-ALL cell lines, consistent with the blockage of USP7 and USP28 activity. Androgen receptor (AR) signaling axis plays a critical role in the development, function and homeostasis of the prostate cancer[28]. AR-v7 is the most clinically relevant variant, and USP7 has been implicated to be associated with AR-v7 isoform in prostate cancer (PC) cells[29]. Consistent with this notion, YM155 significantly reduced the half-life of AR-v7 protein (Fig. 3B). Myc is a short-lived protein and its stability is precisely regulated by the ubiquitin-proteasome system. Several USPs are known to regulate Myc stabilization such as USP7, USP16 and USP28[30–32]. YM155 significantly shortened the half-life of endogenous c-Myc in prostate cancer cell lines and B-cell lymphoma cell lines (Fig. 3C and D and Supplementary Fig. 2). These data indicate thatYM155 accelerates the degradation multiple well-known oncogenic driver proteins via broad-spectrum inhibition of multiple USPs. This might lead to new clinical research strategy targeting cancers which depend on the dysregulation of these oncogenic proteins.

We further evaluated the roles of YM155 in a cellular context. Several T-ALL cell lines are known to have ligand-independent activation of ICN, including CCRF-CEM, Jurkat, and MOLT-4 (Supplementary Fig. 3A). YM155 induces dramatic apoptosis in a dose-dependent manner (Fig. 4A). In contrast, YM155 treatment leads to little additional apoptosis in LOUCY cell, which does not possess activated Notch oncoprotein, even at 50nM dose. Similarly, YM155 treatment leads to much more pronounced apoptosis in SU-DHL-4, OCI-LY7 and SU-DHL-10 (with c-Myc translocation) lymphoma cells than that in SU-DHL-2 (without c-Myc translocation) (Fig. 4B and Supplementary Fig. 3B). These data indicate that even though YM155 has a broad activity against multiple USPs, its ability to kill cancer cells largely depends on the existence of substrate of USPs which is the oncogenic driver proteins, whereas normal cells or cells without such substrate are spared.

Since inactivation of oncogenic c-Myc (chromosomal translocation or gene amplification) can lead to tumor regression in c-Myc-driven cancer[33], we proposed that YM155might inhibits tumor growth by downregulating c-Myc via USPs inhibition. To verify the hypothesis, DLBCL cell lines were treated with low concentration of YM155, and its effect on c-Myc protein was analyzed. As expected, c-Myc protein level was significantly reduced in cells with c-Myc translocation. In contrast, c-Myc protein level was extremely low in cells without c-Myc translocation, and was not changed after YM155 treatment (Supplementary Fig. 3C). To further establish the relationship between c-Myc expression level and cellular sensitivity to YM155, we used a tet-off system, which allows the silencing c-Myc transgene expression by the administration of tetracycline. As shown in Fig. 5A, c-Myc expression was decreased in P493 cells (Myc-off) in response to tetracycline treatment compared with that in control cells (Myc-on), and Myc-off cells were less sensitive to YM155 compared with Myc-on cells (Fig. 5B). Taken together, cells with oncoprotein, such as ICN and c-Myc, which are substrates of USPs are more sensitive to YM155.

These *in vitro* results prompted us to test the *in vivo* effects of YM155. SU-DHL-4, OCI-LY7 and SU-DHL-2 cell lines were used to develop tumor xenograft models. YM155 inhibited tumor growth by 100% and 88.4% in SU-DHL-4 and OCI-LY7 xenografts, respectively, compared with control group (Fig. 6A and B). On day 7,

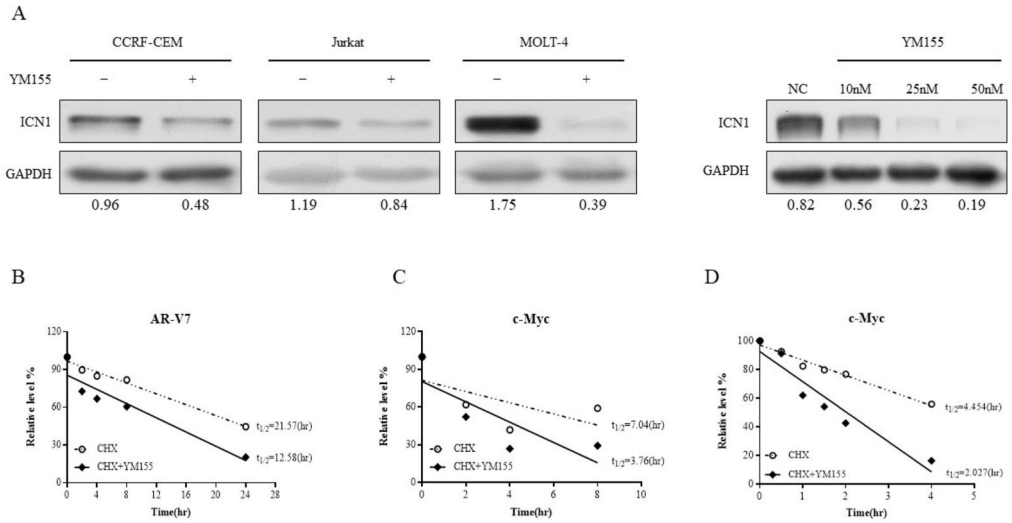

Fig. 3. YM155 affects the targets of USPs in living cells. (**A**) Left panel: Western blot analysis of ICN1 protein in T-ALL cell lines (CCRF-CEM, Jurkat and MOLT-4) treated with 10 nM YM155 for 72 h. Right panel: Western blot analysis of ICN1 protein in T-ALL cell line CCRF-CEM after YM155 treatment for 48 h at indicated concentration. GAPDH was used as a loading control. Each band intensity of ICN1 was normalized with respective GAPDH. The numbers below the images indicate the relative intensity of ICN1 protein. (**B**) Quantification of AR-V7 protein stability assays. Human prostate cancer cells 22RV-1 were treated with CHX or CHX + YM155. Lysates were prepared at the indicated times following treatment. AR-V7 was normalized to GAPDH. CHX: cycloheximide (a protein synthesis inhibitor). C and D: Quantification of c-Myc protein stability assays. Human prostate cancer cells DU145 (**C**) and human diffuse large B cell lymphoma cells SU-DHL-10 (**D**) were treated with CHX or CHX + YM155. Lysates were prepared at the indicated times following treatment.

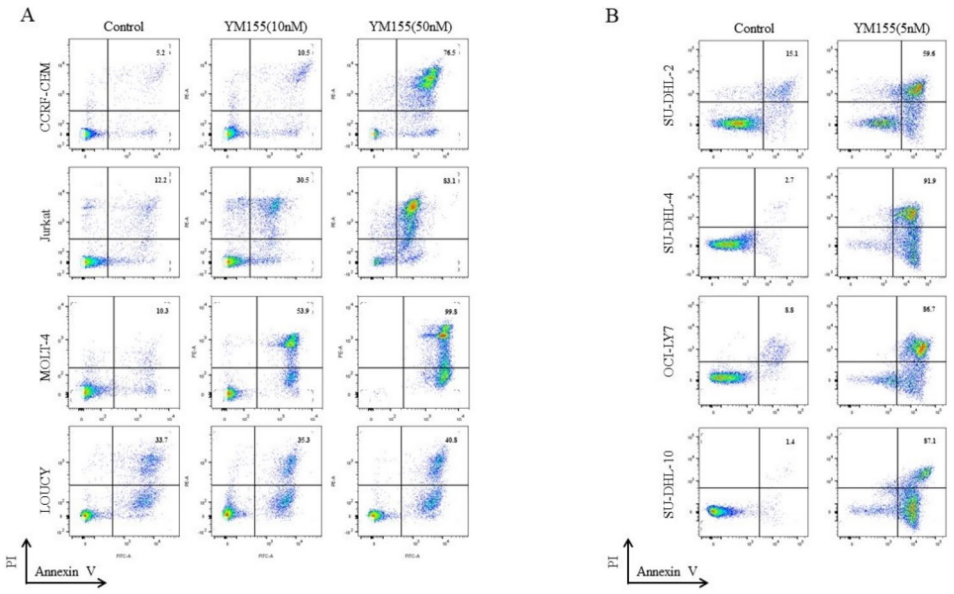

Fig. 4. YM155 induces apoptosis in T-ALL and DLBCL cells. (**A**) Cells were treated with YM155 as indicated concentration for 72 h. The percentage of apoptosis (early and late), as quantified by Annexin V/PI staining and analyzed by flow cytometry. CCRF-CEM, Jurkat and MOLT-4 cells: with ICN; LOUCY: without ICN. (**B**) Cells were treated with 5nM YM155 for 48 h. The percentage of apoptosis (early and late), as quantified by Annexin V/PI staining and analyzed by flow cytometry. SU-DHL-4, OCI-LY7 and SU-DHL-10: with c-Myc translocation; SU-DHL-2: without c-Myc translocation.

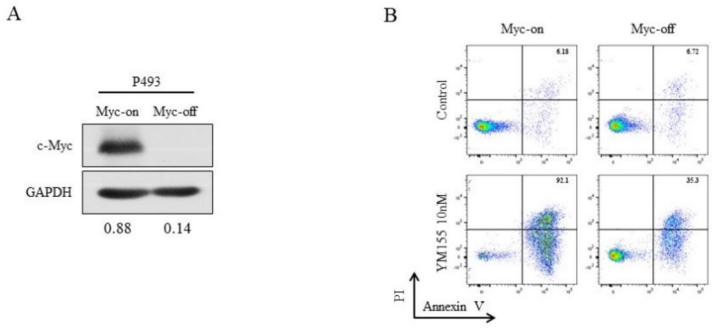

Fig. 5. The efficacy of YM155 inducing cell apoptosis dependent on c-Myc status. (**A**) Western blot analysis of c-Myc expression in P493 cells treated with 200nM tetracycline for 24 h. GAPDH was used as a loading control. Each band intensity of c-Myc was normalized with respective GAPDH. The numbers below the images indicate the relative intensity of c-Myc protein. (**B**) Myc-on and Myc-off cells were treated with 10nM YM155 for 72 h. The percentage of apoptosis (early and late), as quantified by Annexin V/PI staining and analyzed by flow cytometry.

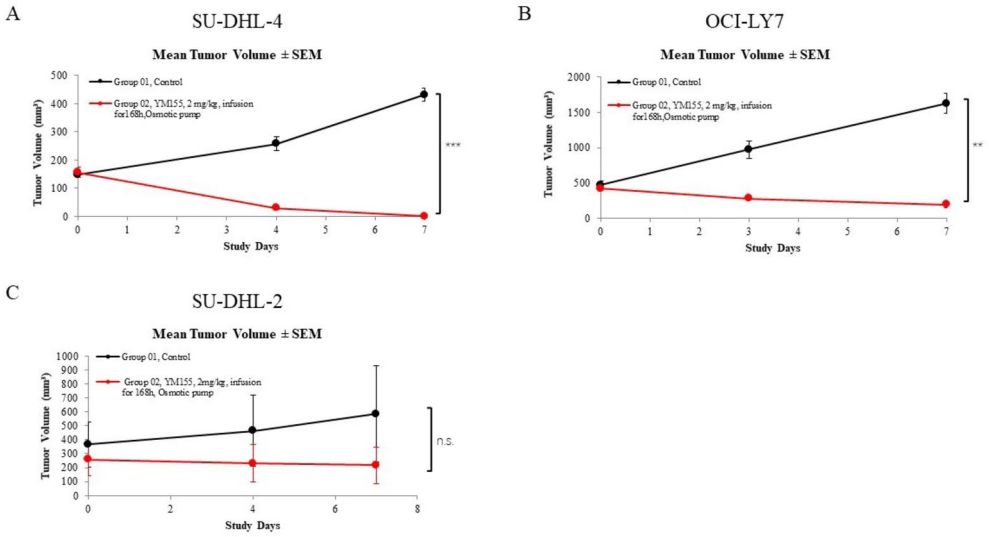

Fig. 6. YM155 inhibited the xenografted tumor growth in DLBCL. (**A–C**) Inhibition of xenografted SU-DHL-4 (**A**, $n=5$), OCI-LY7 (**B**, $n=4$) and SU-DHL-2 (**C**, $n=3$) tumor growth in mice receiving YM155 treatment. YM155 was administered at 2 mg/kg/day by continuous subcutaneously (s.c.) infusion for 168 h using a micro-osmotic pump. Tumor volume on day 7 was compared with the control group. The data presented are means ± SEM. $^{**}P<0.01$; $^{***}P<0.001$; n.s. no significant difference.

tumor regressions induced by YM155 were 100% and 54.9% ($P<0.001$ and $P<0.01$), respectively. However, SU-DHL-2 tumor which does not have c-Myc translocation, only showed a modest tumor reduction after YM155 treatment, and the tumor regression rate was 15.1% ($P>0.05$) (Fig. 6C). Similar observation was also made in solid tumor models. YM155 was more effective in HCC827 (cells with c-Myc amplification) lung cancer xenograft model than in HCC4006 (cells without c-Myc amplification) model (Supplementary Fig. 4).

Discussion

YM155, a small molecule inhibitor of survivin, is known to exert anti-tumor effects in various cancers. However, the underlying mechanism of action remains controversial. Some studies have reported the cytotoxic activities of YM155 were independent of survivin inhibition. Recent research of the YM155 structure revealed a quinone-based core, which has been reported to be involved in inhibition of USP2 via the oxidation mechanism[23]. Other reports showed that YM155 can lead to the degradation of critical proteins involved in tumor growth and survival, such as EGFR and Mcl-1[34,35]. Therefore, we hypothesize that YM155 might exert the anti-cancer activity by inhibiting USPs via quinone/semiquinone cycling mechanism.

Ubiquitylation and deubiquitylation have key roles in maintaining protein homeostasis. Dysregulation and/or imbalance of these processes have been implicated in a wide range of human diseases, such as cancer,

inflammation, and many neurological diseases. Exploration of protein turnover mechanism has led to the development of anti-cancer drugs such as thalidomide and lenalidomide, which works by accelerating protein degradation via the addition of ubiquitin to target proteins. Theoretically, this can be also achieved by inhibiting deubiquitylation of target proteins. USPs, as the most widely studied enzymes in the DUB family have been considered as promising cancer targets. Several USP inhibitors have been developed during the past decade, which are categorized into broad-spectrum and sub-type-specific USP inhibitors. PR-619 exhibits a broader inhibitory profile and more efficient USPs inhibition. Another molecule P22077 has a more selective profile toward USP7. Complete inhibition of USP7 was observed at higher concentrations of P22077 than PR-619. Current development efforts are mainly focused on improving compound selectivity against specific USP target, while sparing other USPs to reduce potential toxicity. A potential drawback for this approach is the loss of pharmacological potency against fast evolving cancer cells. As a broad-spectrum USP inhibitor, YM155 has the advantage of simultaneously degrading multiple proteins that are important for cancer cell proliferation and survival. Oncoproteins with short half-life are more prone to perturbed by USP inhibition. Myc family proteins are master regulators of cell proliferation and have very short intracellular half-life. Consistent with this hypothesis, both n-Myc and c-Myc proteins can be effectively degraded by YM155 treatment, which lead to rapid apoptosis in cancer cells with high level Myc protein (such as with gene translocation, amplification and high level transcription). Inactivation of Myc cause a cell cycle arrest and apoptosis[36]. Therefore, YM155 is a novel drug to induce tumor regression in Myc-driven tumor via Myc protein degradation. A phase II clinical trial of YM155 is on-going to prove this concept in c-Myc driven high grade B cell lymphoma (HGBCL) and Burkitt's lymphoma (BL).

Data availability

All data generated or analyzed during this study are included in this published article and its supplementary information files.

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Author contributions

Xiang Li: design of the work, the acquisition, analysis, or interpretation of data，drafted the work or revised it critically for important intellectual content.Na He: the acquisition of data. Yan Lv: the acquisition of data. Haiyue Wang: the acquisition of data. Ming Zhang: the acquisition of data. Heiyan Zhai: interpretation of data. Zhen Yang: interpretation of data. Yi Yang: the acquisition of data. Dagang Guo: drafted the work or revised it critically for important intellectual content. Zhixiang Cao: drafted the work or revised it critically for important intellectual content. Yiyou CHEN: design of the work, drafted the work or revised it critically for important intellectual content，agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Declarations

Competing interests

The authors declare no competing interests.

Ethical approval

All studies in animals were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) and Animal Welfare Committee of Beijing Percans Oncology (Cothera Bioscience, Inc., China R&D center) under the supervision of the Regulations of Beijing Laboratory Animal Management (approval no. IACUC201807). We confirm that all methods were performed in accordance with the ARRIVE guidelines and regulations.

Additional information

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