석 사 학 위 논 문

ML364, USP2 Inhibitor, Sensitizes TRAIL-induced Apoptosis in Human Renal Carcinoma Caki Cells

계 명 대 학 교 대 학 원

의 학 과

이 탁 겸

지도교수 권 택 규

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이 논문을 석사학위 논문으로 제출함

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의학과 면역학 전공

이 탁 겸

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주 심 박 종 욱

부 심 권 택 규

부 심 김 신

계 명 대 학 교 대 학 원

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많이 부족하였던 저를 성장할 수 있도록 곁에서 가르침을 주신 우선민 박사님, 서승언 박사님 그리고 연구원 선생님과 동료학생들에게도 감사한 마음을 전합니다. 감사드립니다.

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1. Introduction

The ubiquitin-proteasome system (UPS) is a major pathway of protein quality control. UPS plays a role in multiple cellular processes including cell proliferation, DNA replication and apoptosis. UPS is a process wherein mono- or poly-ubiquitin is attached to the target protein that induces proteasomal degradation in 26S proteasome (1). Ubiquitination is a post-translational modification that is facilitated by an enzyme complex that contains E1 activating, E2 conjugating, and E3 ligase enzymes (2,3). Deubiquitinases (DUBs) remove ubiquitin from target proteins (4,5). Approximately 100 DUBs have been characterized and are categorized into two classes including cysteine proteases and metalloproteases (6). Throughout the process of cancer development, tumor suppressor proteins play a role in inhibiting tumor growth or inducing cell death in cancer cells. The ubiquitin-proteasome system can influence the stability and degradation of tumor suppressor proteins, which can impact the progression of cancer. Therefor several small molecule inhibitors of the UPS could be appropriately developed and designed into an efficacious anticancer drug.

USP2 is a cysteine protease which is well-characterized member of the USP family (7,8). It acts as a crucial regulator for diverse physiological and pathological phenomena encompassing tumorigenesis, inflammation, and circadian rhythm regulation (9). High level of USP2 is observed in prostate, hepatoma, bladder, and glioma cancers (10-14). ML364, a small molecule inhibitor of USP2, induces an increase in cellular cyclin D1 degradation and caused cell cycle arrest in colorectal cancer (15). These effects by ML364 support a

key role USP2 as a hopeful therapeutic target in cancer chemotherapy. Moreover, USP2 modulates the susceptibility to antineoplastic agents in prostate cells (16). Even though some researchers have reported that ML364 exerts the broad-spectrum anti-virulence effect (17). However, the underlying specific molecular mechanisms of ML364 on cancer cell death remain unclear.

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) binds to death receptors such as DR4 and DR5 in tumor cells, but not in normal cells, initiating signals for cell death (32-34). Therefore, TRAIL has attracted attention as a promising anticancer agent. However, most cancer cells exhibit resistance to TRAIL. Overexpression of anti-apoptotic proteins such as Bcl-2 family, c-FLIP, and IAP family, or downregulation of pro-apoptotic Bcl-2 family proteins, decrease TRAIL-induced cancer cell death (35). To overcome these limitations of TRAIL, many researchers have studied combination therapy with chemotherapeutic agents, which has shown to increase TRAIL sensitivity (36,37).

Survivin is a member of inhibitor of apoptosis proteins (IAP) and is highly expressed in various types of cancer. Additionally, Survivin is known to be involved in tumor cell resistance to chemotherapy and radiation (38).

In this study, I discovered the target substrate of USP2 and researched its molecular mechanisms. USP2 inhibition enhanced TRAIL-mediated apoptosis in cancer cells.

2. Metarials and Methods

2.1. Cell Lines and Cell Culture:

Human renal carcinoma cells (ACHN and Caki), human prostate cancer cells (DU145) and Human normal umbilical vein cells (EA.hy926) were acquired from American Type Culture Collection (Manassas, VA, USA), Human mesangial cells (MC) were bought from Lonza (Basel, Switzerland) and grown in Dulbecco's modified Eagle's medium (DMEM), including 10% fetal bovine serum, 5% penicillin/streptomycin (Welgene) and 100 μg/mL gentamicin (Thermo Fisher Scientific, Waltham, MA, USA). Human lung cancer cells (A549) were acquired from ATCC (Manassas, VA, USA), grown in Roswell Park Memorial Institute (RPMI) 1640 (Welgene, Gyeongsan, Korea), including 10% fetal bovine serum, 5% penicillin/streptomycin (Welgene) and 100 μg/mL gentamicin (Thermo Fisher Scientific, Waltham, MA, USA). All the cell lines were incubated at 37 ℃, 5% CO2 in humidified air. They were cultured by short tandem repeats profiling before use and were also routinely tested for bacterial infection during this study.

2.2. Antibodies and Reagents:

ML364 was bought from Cayman Chemical (Ann Arbor, MI, USA). z-VAD-fmk, human recombinant TRAIL and anti-survivin antibody were acquired from R&D System (Minneapolis, MN, USA). Cell Signaling Technology (Beverly, MA, USA) provided anti-USP2, anti-PARP, anti-cleaved caspase-3, anti-DR5 and anti-Bcl-xL antibodies. BD

Biosciences (San Jose, CA, USA) provided anti-Bim, anti-caspase-3, anti-cIAP1 and anti-XIAP antibodies. Santa Cruz Biotechnology (Santa Cruz, CA, USA) provided anti-cIAP2 antibody. Enzo Life Sciences (San Diego, CA, USA) provided anti-c-FLIP antibody. Abcam (Cambridge, MA, USA) provided the anti-DR4. MG132 was bought from Calbiochem (San Diego, CA, USA). Anti-actin antibody and cycloheximide were acquired from Sigma Chemical Co. (St. Louis, MO, USA).

2.3. Flow Cytometry Assay:

To analyze apoptosis, harvested cells were resuspended in 100 μL of phosphate-buffered saline (PBS) and fixed in 200 μL of 95% ethanol at 4 ℃. After, cells were incubated in 1.12% sodium citrate buffer including RNase at 37 ℃ for 30 min, then stained with propidium iodide. The level of apoptosis was analysed by Guava® easyCyte™ flow cytometer (Luminex Corporation, Austin, TX, USA).

2.4. DAPI Staining and DNA Fragmentation Assay:

Caki cells were treated with ML364 plus TRAIL. To detect the change of cellular nuclei, cells were fixed with 1% paraformaldehyde. The cells were washed with PBS and stained with 4′,6′-diamidino-2-phenylindole solution (Roche, Mannheim, Germany) for 5 min. The nucleus condensation was examined by fluorescence microscope (Carl Zeiss, Jena, Germany). For detection of apoptosis, DNA fragmentation was measured by Cell Death Detection ELISA PLUS kit (BoerhringerMannheim, USA). The samples were measured at 405 and 490 nm with spectrophotometry (BMG Labtech, Ortenberg, Germany).

2.5. Western Blotting Analysis:

Total cell were lysed using ERK lysis buffer including protease inhibitors (100 μM phenylmethylsulfonyl fluoride and 2 μM Aprotinin) and phosphatase inhibitor (1 mM Na3VO4). The protein sample were separated by 10% SDS-PAGE and transferred to PVDA membrane (GE Healthcare Life Sciences, Pittsburgh, PA, USA). The membranes were blocked with 5% nonfat dry milk and incubated overnight with the indicated primary antibody (1:700 dilution) at room temperature. Immunoreactive bands were detected with chemiluminescence kit (EMD Millipore, Darmstadt, Germany).

2.6. Reverse Transcription PCR (RT-PCR) and Quantitative PCR (qPCR):

Total RNA was extracted from cells using the TRIzol Reagent (Life Technologies, Gaithersburg, MD, USA). The quality and concentration of the RNA were determined by spectrophotometry (Thermo Scientific, Wilmington, Denmark). cDNA was acquired using M-MLV reverse transcriptase (Gibco-BRL, Gaithersburg, MD, USA). For RT-PCR, Blend Taq DNA polymerase (Toyobo, Osaka, Japan) with primers were used for targeting genes. For real time PCR, SYBR Fast qPCR Mix (Takara Bio Inc., Shiga, Japan) was used, and reactions were analyzed on Thermal Cycler Dice® Real Time System III (Takara Bio Inc., Shiga, Japan). The following primers were used for the amplification of the target genes survivin (forward) 5'-GCA CTT TCT TCG CAG TTT CC-3' and (reverse) 5'-GGA CCA CCG CAT CTC TAC AT-3', and β-actin (forward) 5'-CGA TTT CCC GCT CGG CCG TGG-3' and (reverse) 5'-GGC ATC GTC ACC

AAC TGG GAC-3'.

2.7. Transfection:

For siRNA transfection, human USP2 was knocked down using siRNA acquired from Bioneer (Daejeon, Korea) and used with Lipofectamine RNAiMAX Reagent (Invitrogen, Calshad, CA, USA). For plasmid transfection, pcDNA 3.1 vector was acquired from Invitrogen (Carlsbad, CA, USA). Flag-HA-USP2 plasmid was bought from Addgene and transfected using Lipidofect-P (Lipidomia, Seongnam, Korea).

2.8. Deubiquitination Assay and Immunoprecipitation Assay:

For in vitro deubiquitination assay, Caki cells were transfected with HA-ubiquitin plasmid, and treated with ML364 and proteasome inhibitor (MG132). Harvested cells were washed with PBS including 10 mM N-Ethylmaleimide (NEM), boiled for samples including 100 μL PBS/NEM plus 1% SDS for 10 min at 95 ℃. Total protein was extracted using RIPA lysis buffer including proteasome inhibitors and 5 mM NEM, lysed using syringe for 5-7 times and centrifuged. The supernatants were incubated with the indicated antibody overnight and reacted by attaching protein G agarose bead (Santa Cruz Biotechnology, St. Louis, MO, USA) for 2 h. Then, the supernatants washed with RIPA including 5 mM NEM and boiled using 2X sample buffer. Protein samples were separated on SDS-PAGE and then transferred onto PVDA membrane. The membrane was exposed denaturation condition overnight and blocked with 5% Bovine serum albumin (BSA) at least 3 h. Subsequently, the membrane was exposed using HRP-conjugated anti-Ub antibody. For

immunoprecipitation, the cells were lysed with RIPA lysis buffer including 10 mM NEM, 1 mMPMSF and sonicated all the samples. After then, centrifuged lysate's supernatants were incubated with the indicated antibody overnight at 4 ℃, and added Protein G agarose bead at 4 ℃ on rotator. Eluted immunoprecipitates were resolved on SDS-PAGE and inspected for association of proteins of interest using specific antibodies.

2.9. Statistical Analysis:

The data were analyzed using a one-way ANOVA and post-hoc comparisons (Student-Newman-Keuls) using the Statistical Package for Social Sciences 22.0 software (SPSS Inc.; Chicago, IL, USA).

3. Results

3.1. USP2 Inhibitor, ML364, Induces Downregulation of Survivin Expression:

To examine whether the differential expression levels of apoptosis-related proteins in USP2 inhibition condition, I explored the changes in apoptosis-associated proteins induced by USP2 specific inhibitor, ML364, in Caki cells. Expression level of survivin was decreased after 24 h after the USP2 inhibitor treatment (Figure 1A). However, the expression levels of other proteins were not changed by USP2 inhibitor (Figure 1A). Next, I investigated the effect of ML364 on diverse cancer cell lines (renal carcinoma, ACHN; prostate carcinoma, DU145; lung carcinoma, A549). All tested cells showed the downregulation of survivin expression after ML364 treatment (Figure 1B). To exclude the influence of ML364, which not only inhibits USP2 but also regulates targets such as USP8 and cyclin D1, I used specific siRNA for USP2. USP2 knockdown induced survivin downregulation (Figure 1C). Consequently, these data proposed that the pharmacological and genetic inhibition of USP2 induce downregulation of survivin protein expression.

3.2. USP2 Regulates Survivin Stability:

ML364 induced downregulation of survivin protein in a time-dependent manner. However, the mRNA expression level of survivin was not downregulated by ML364 treatment (Figure 2A). Next, I

investigated whether USP2 inhibition regulates survivin protein stability. Caki cells were treated with or without a pharmacological USP2 inhibitor (ML364) or knockdown of USP2 (siRNA) in the presence of cycloheximide (CHX) for various time points. Combined treatment with ML364 and CHX or USP2 siRNA markedly decreased survivin stability contrasted to CHX alone (Figure 2B,C). In addition, pretreatment with MG132, a proteasome inhibitor, inhibited the ML364-induced survivin downregulation (Figure 2D). Therefore, these results indicated that survivin protein stability is modulated by USP2.

3.3. USP2 Interacts with and Deubiqutinates Survivin:

To examine USP2 interaction with survivin, I performed immunoprecipitation (IP) assay using USP2 antibody. USP2 directly bound to survivin (Figure 3A). Further, I investigated ML364 -mediated survivin ubiquitination and found that ML364 markedly increased the polyubiquitination of survivin (Figure 3B). These data indicated that USP2 inhibition decreases survivin stabilization via the induction of survivin ubiquitination.

3.4. ML364 Sensitizes TRAIL-mediated Apoptosis:

Next, I investigated whether ML364 sensitizes TRAIL-mediated cell death. Combined treatment with a sub-lethal dosage of ML364 and TRAIL significantly increased the accumulation of the sub-G1 population and cleavage of poly (ADP-ribose) polymerase (PARP) in renal carcinoma (Caki and ACHN) and prostate carcinoma (DU145),

but not ML364 alone or TRAIL alone (Figure 4A). However, combined treatment did not induce the increase of sub-G1 population in normal human mesangial cells (MC) and normal human endothelial cells (EA.hy926) (Figure 4B). Taken together, these results proposed that USP2 inhibition effectively enhances cancer cells to TRAIL-mediated apoptosis.

3.5. Depletion of USP2 Enhances TRAIL-induced Apoptosis through Survivin Downregulation:

Next, I focused on the mechanism of ML364-mediated sensitization of TRAIL-induced apoptosis. Combined treatment induced nuclear condensation and fragmented DNA, but this was not observed in monotherapy (Figure 5A,B). As a result of examining the relevance of caspase activation in the combined treatment. combined treatment was an increase in caspase-3 activity (DEVDase) was confirmed, but no increase in activity was seen in the individual treatment (Figure 5C). Pan-caspase inhibitor, z-VAD-fmk, significantly inhibited the combined treatment-induced apoptosis and PARP cleavage (Figure 5D). The results showed that ML364 treatment downregulates survivin (Figure 1A). To verify the critical role of survivin in ML364-mediated TRAIL sensitization, I used overexpressed survivin. Ectopic expression of survivin significantly prevented the apoptosis and PARP cleavage by combined treatment in cancer cells (Figure 5E). These data proposed that survivin contribute to depletion of USP2-mediated TRAIL sensitization.

3.6. Overexpression of USP2 Inhibits TRAIL-induced Apoptosis:

To investigate whether the increase in TRAIL sensitivity upon USP2 inhibition is a common phenomenon in cancer cells, I conducted experiments by knockdown or overexpression USP2 in various cancer cell lines. As shown in Figure 6A, TRAIL alone considerably increased apoptosis in USP2 siRNA-treated cells. In addition, ectopic expression of USP2 attenuated the co-treatment with ML364 plus TRAIL-induced apoptosis (Figure 6B). Therefore, these results proposed that the inhibition of USP2-mediated survivin downregulation can modulate the TRAIL sensitization.

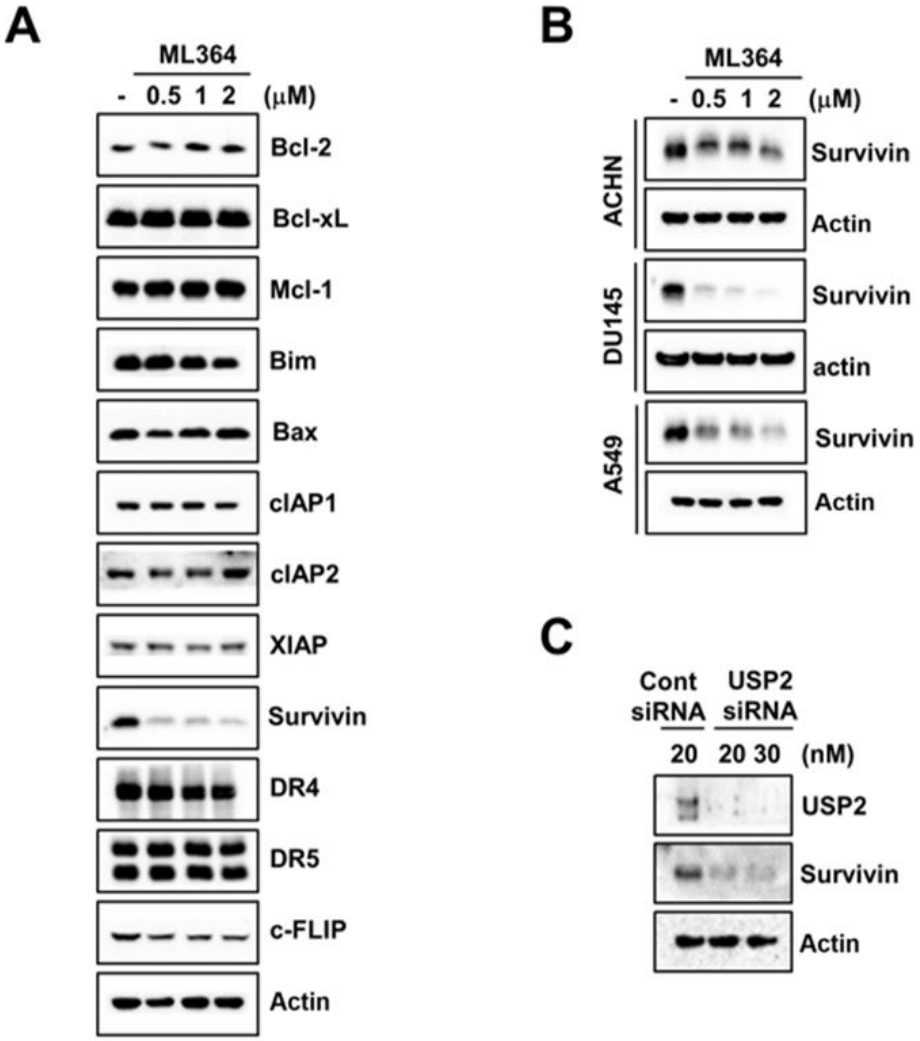

Figure 1. Knockdown of USP2 induces survivin downregulation in cancer cells. (A,B) Cancer cells were treated with various concentrations of ML364 for 24 h. (C) Caki cells were transfected with control (Cont, siRNA) or USP2 siRNA. The protein expression was determined by western blotting.

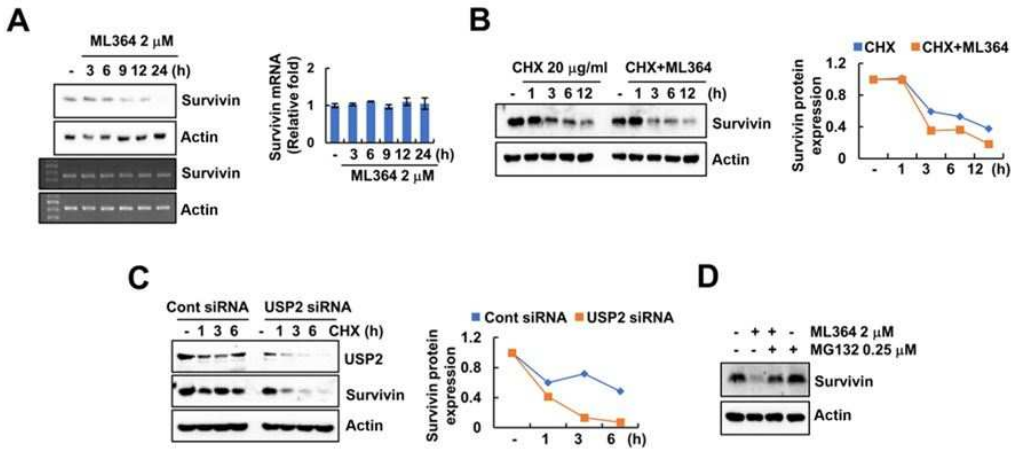

Figure 2. ML364 induces proteasome-mediated survivin degradation. (A) Caki cells were treated with 2 μM ML364 for the indicated times. The values in the graph represent the mean ± SD of three independent experiments. (B) Caki cells were pretreated with 20 μg/mL cycloheximide (CHX) in the presence or absence of 2 μM ML364 for the indicated times. (C) Caki cells were transfected with control or USP2 siRNA and then treated with 20 μg/mL CHX for the indicated times. (D) Caki cells were treated with 0.25 μM MG132 in the presence or absence of 2 μM ML364 for 24 h. The protein expression was determined using western blotting, RT-RCR and q-PCR.

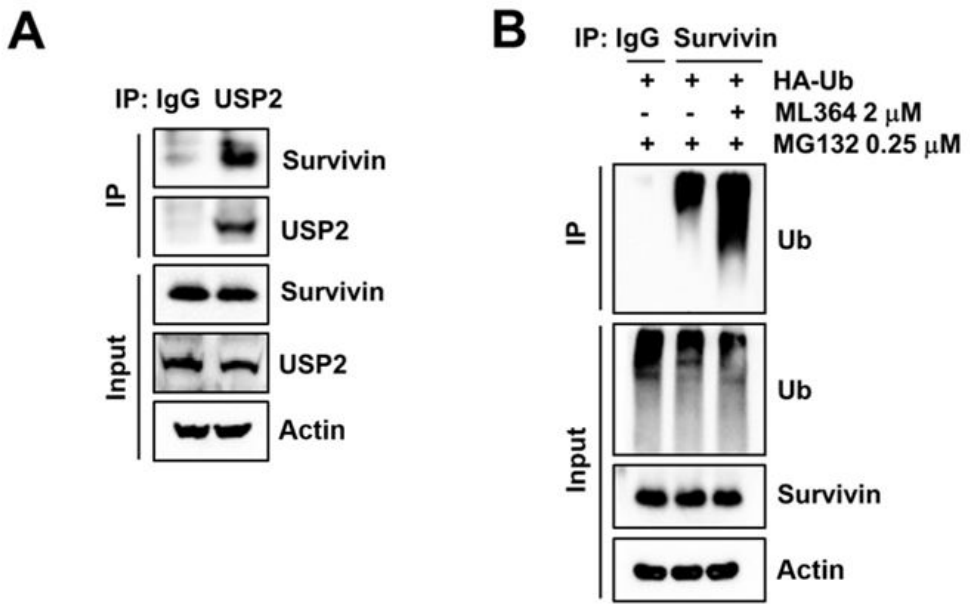

Figure 3. USP2 binds and deubiquitinates survivin. (A) Interaction between endogenous USP2 and survivin was examined by immunoprecipitation (IP) assay. (B) Caki cells were transfected with HA-Ub plasmid, and treated with 0.25 μM MG132 in the presence or absence of 2 μM ML364 for 24 h. IP assay and ubiquitination assay were detected by western blotting.

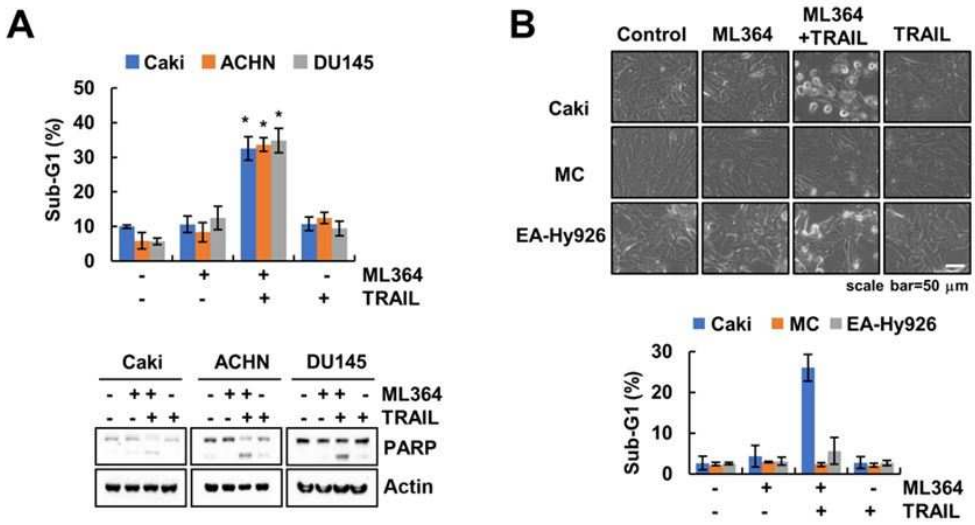

Figure 4. ML364 sensitizes TRAIL-mediated apoptosis in cancer cells. (A,B) Cancer (A,B) and normal (B) cells were treated with 2 μM ML364, 50 ng/mL TRAIL or combination for 24 h. The sub-G1 population (A) and protein expression (A,B) were determined using flow cytometry and western blotting, respectively. Cell morphology was assessed using a microscope. scale bar: 50 μm (B). Values in the graphs (A,B) represent the mean ± SD of three independent experiments. *P < 0.01 compared to the control.

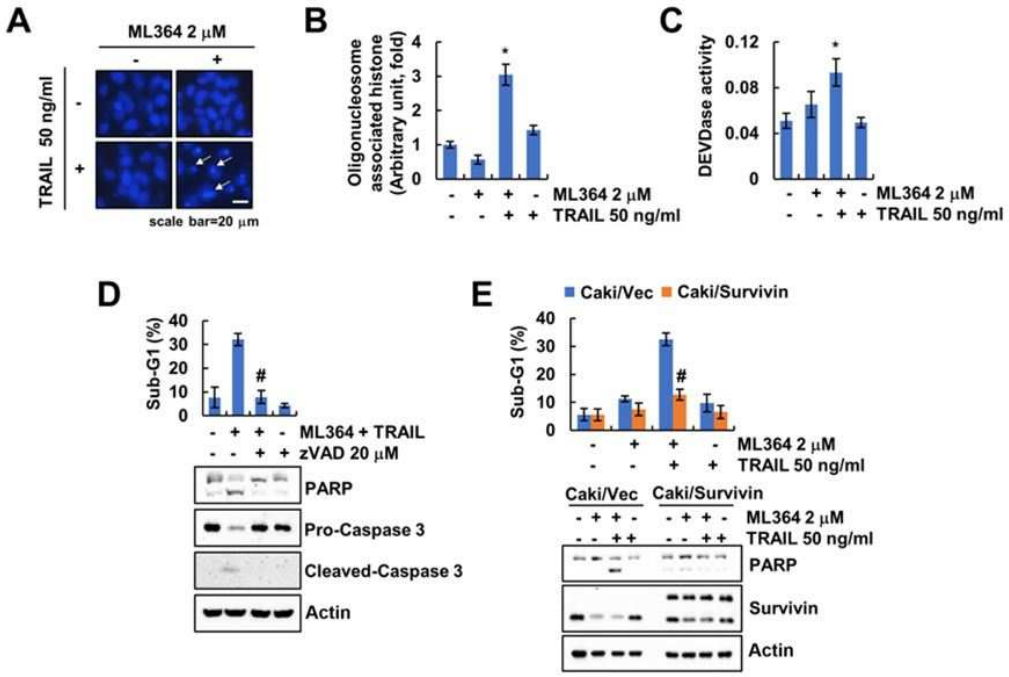

Figure 5. ML364-induced survivin downregulation contributes to sensitization of TRAIL induced apoptosis. (A-C) Caki cells were treated with 2 μM ML364, 50 ng/mL TRAIL or combination for 24 h. DNA fragmentation was analyzed using DAPI staining (A) and kit (B). DEVDase (caspase-3) colorimetric assay using DEVDase substrate (C). (D) Caki cells were pretreated with 20 μM zVAD and added with combination of 2 μM ML364 and 50 ng/mL TRAIL for 24 h. (E) Vector and survivin-overexpressed Caki cells were treated with 2 μM ML364, 50 ng/mL TRAIL or combination for 24 h. The sub-G1 population and protein expression were determined using flow cytometry and western blotting, respectively (D, E). Values in the graphs (B-E) represent the mean ± SD of three independent experiments. *P < 0.01 compared to the control. #P < 0.01 compared to ML364 and TRAIL-treated cells. scale bar: 20 μm.

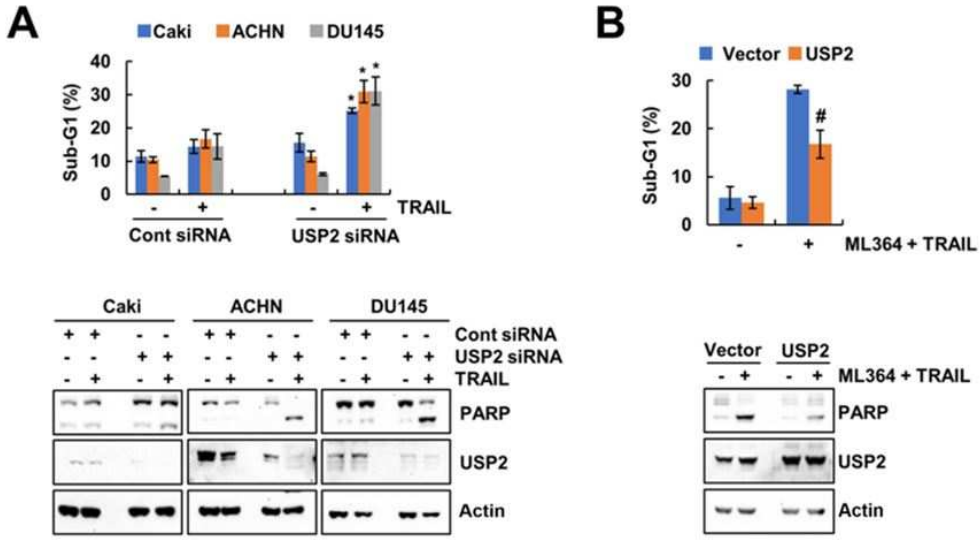

Figure 6. Knockdown of USP2 enhances TRAIL-induced apoptosis in cancer cells. (A) Cancer cells were transfected with control or USP2 siRNA, and treated with 50 ng/mL TRAIL for 24 h. (B) Caki cells were transfected with pcDNA3.1(+) (vector) and USP2 plasmid, and treated with combinations of 2 μM ML364 and 50 ng/mL TRAIL for 24 h. The sub-G1 population and protein expression were determined using flow cytometry and western blotting, respectively (A,B). Values in the graphs (A,B) represent the mean ± SD of three independent experiments. *P < 0.01 compared TRAIL treatment in control siRNA. #P < 0.01 compared to ML364 and TRAIL treatment in vector.

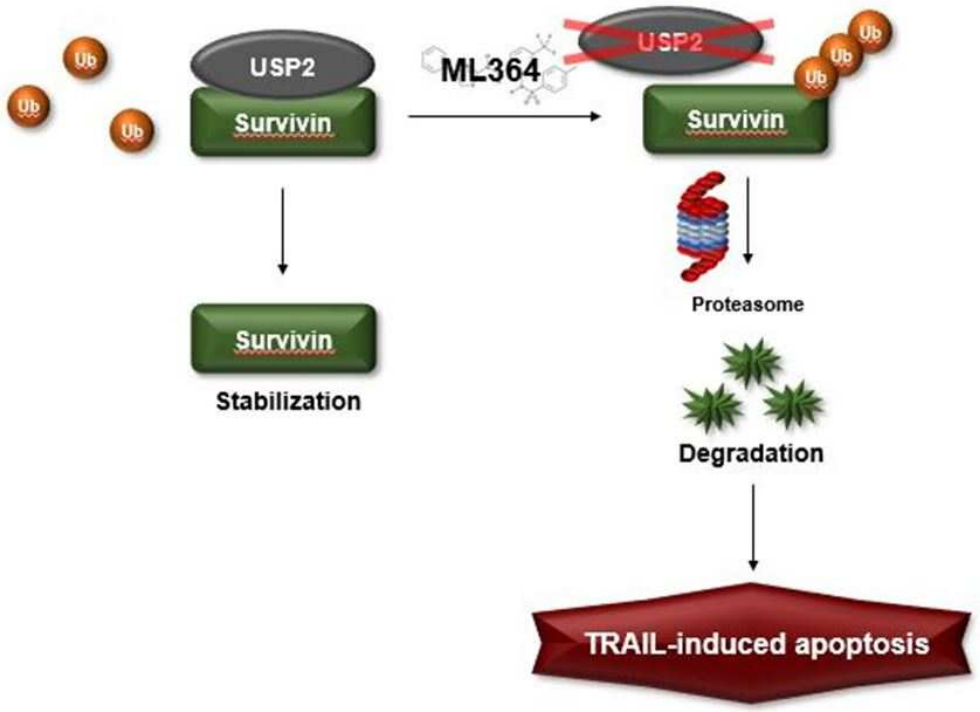

Figure 7. The scheme of the molecular mechanisms in ML364-mediated TRAIL sensitization. USP2 stabilizes survivin. ML364, a USP2 small-molecules, downregulates survivin expression through ubiquitination of survivin, thereby enhancing the sensitivity to TRAIL.

4. Discussion

The results demonstrated the novel function of USP2 as a DUB to regulate survivin stability. I found that inhibition of USP2 DUB activity by ML364 was associated with TRAIL sensitization via downregulation of survivin expression. Combined treatment with ML364 plus TRAIL induced apoptosis in cancer cells, but not in normal cells. These results suggest that ML364 could be an attractive TRAIL sensitizer.

ML364 is associated with regulating the stabilization of protein targets by inhibiting DUB activity, without affecting protein expression of USP2 (15). Therefore, I further confirmed that USP2 targets survivin by observing the downregulation of survivin when USP2 was knockdown using specific siRNA targeting USP2 (Figure 1C).

USP2 has been reported to function as an oncogene with high expression levels in various malignancies (10-14,18-22). Multiple studies have been reported the several molecular targets of USP2 in cancer cells. For example, USP2 interacts and stabilizes fatty acid synthase (10), MDM2 (18), MDMX (19), Cyclin D1 (20), Aurora-A (21), Smad4 (22), and β-catenin (23). USP2 knockdown via siRNA inhibited actinomycin plus TNF-α induced apoptosis via c-FLIP upregulation in hepatocytes (24). USP2 siRNA induced decrease of cellular levels of the ubiquitin-ligase Itch, a negative regulator of c-FLIP. In this study, c-FLIP did not upregulate by USP2 inhibitor, ML364 (Figure 1A). This contradiction may be attributed to differences between the cell lines that were used. However, the involvement of other c-FLIP regulatory pathways in ML364-treated cells cannot be completely ruled out. Furthermore, USP2 induced drug resistance in both immortalized

and transformed prostate cells. Overexpression of USP2 protected from cisplatin-induced oxidative stress by producing glutathione (16). Although USP2 is a regulator of oncogenic behavior in cancer through regulating protein stability of diverse substrates, USP2-targeted substrates and the underlying mechanisms of cancer cell death remain unclear.

In this study, to identify novel substrates of USP2 in cancer cell death, I investigated the levels of apoptotic regulatory proteins using genetic (siRNA) and pharmacological inhibitor. I found that USP2 depletion downregulated survivin protein levels (Figure 1). In addition, USP2 directly bound to survivin and induced survivin deubiquitination (Figure 3A,B), and the overexpression of survivin significantly inhibited apoptosis induced by the co-treatment with ML364 and TRAIL (Figure 5E). Therefore, I discovered that survivin is a specific substrate of USP2 and is associated with the inhibition of USP2-mediated sensitization of cancer cells to TRAIL.

Survivin expression is regulated at transcriptional and posttranslational levels. Survivin can be ubiquitinated and degraded by several E3 ubiquitin ligases, such as FBXL7, CUL9 and XIAP (25-27). DUB also participates in the regulation of survivin stability. Accumulating evidence indicates that survivin is deubiquitinated by several DUBs, such as USP1, USP35, USP9X and STAMBPL1 (28-31). Collectively, I showed that USP2 depletion sensitizes cancer cells to TRAIL-induced apoptosis through survivin downregulation. In addition, my findings provide the role of USP2 in ubiquitin-dependent survivin degradation.

5. Summary

USP2, a deubiquitinase, play an important role in many biological processes including cell proliferation, tumorigenesis and inflammation. Here, I explored the effect of TRAIL sensitiziation and its molecular mechanisms of ML364 in cancer cells. USP2 Inhibitor (ML364) and siRNA of USP2 only downregulated survivin expression at the post-translational level. In addition, USP2 bound to survivin, and ML364 increased ubiquitination of survivin. Furthermore, ML364 treatment and knockdown of USP2 enhances TRAIL-mediated apotposis in cancer cells, but not normal cells. Overexpression of survivin prevented combined treatment with ML364 plus TRAIL-indcued apoptosis. These data demonstrated that inhibition of USP2 sensitize TRAIL-mediated apoptosis through degradation of survivin.

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ML364, USP2 inhibitor, sensitizes TRAIL-induced apoptosis in human renal carcinoma Caki cells

Lee, Tak Gyeom

Department of Immunology
Graduate School

Keimyung University

(Supervised by Professor Kwon, Taeg Kyu)

(Abstract)

Ubiquitin-specific protease 2 (USP2) is one of deubiquitinase belong to ubiquitin-specific cysteine proteases subfamily. USP2 has been known to display various biological effects including tumorigenesis and inflammation. However, the function of USP2 and the mechanisms underlying chemotherapy remain unclear. Mechanistically, USP2 enhanced survivin stabilization by removing ubiquitin. Pharmacological inhibitors (ML364) and siRNA targeting USP2 induced downregulation of survivin expression. Combined treatment with ML364 plus TRAIL induced apoptotic cell death in cancer cells, but not in normal cells. Ectopic expression of survivin markedly inhibited the co-treatment with ML364 and TRAIL-induced apoptosis. Taken together, these results firstly

provide that ML364 enhances TRAIL-mediated apoptosis through UPS2-dependent survivin degradation in human renal carcinoma cells.

ML364에 의한 TRAIL 매개 세포사멸 향상 기전 연구

이 탁 겸
계명대학교 대학원
의학과 면역학 전공
(지도교수 권 택 규)

(초록)

유비퀴틴-프로테아좀 시스템은 유비퀴틴 리가아제와 탈유비퀴틴화 효소에 의해 타겟 단백질의 안정화를 조절한다. 탈유비퀴틴화 효소 중 하나인 ubiquitin-specific protease2는 종양 발생 및 증식, 염증과 같은 다양한 생물학적 과정을 조절다고 알려져 있다. 본 연구에서는 USP2의 새로운 타겟 단백질을 찾고, USP2 관련된 분자적 메커니즘 및 TRAIL에 대한 민감화 증진 효과에 대해 조사하였다. USP2는 항세포사멸 단백질인 survivin과 결합하고, 특정 USP2 저해제인 ML364는 survivin의 유비퀴틴화를 통해 단백질을 분해하였다. ML364에 의한 USP2 활성 억제 뿐만 아니라 siRNA를 이용한 USP2 결손은 TRAIL 매개 암세포사멸을 증진시켰다. 반면에 USP2 및 survivin 과발현된 세포에서는 ML364와 TRAIL 병합처리에 의한 세포사멸이 억제되었다. 이상의 연구 결과는 USP2의 새로운 타겟 기질로 survivin을 제시하며 USP2 활성 및 발현 저해가 항암제에 대한 저항성을 극복하는 새로운 전략이 될 것으로 사료된다.