SIRT1 Inhibitor Enhances Hsp90 Inhibitor-mediated Abrogation of Hsp90 Chaperone Function and Potentiates the Cytotoxicity of Hsp90 Inhibitor in Chemo-resistant Human Cancer Cells

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The present investigation was undertaken to examine the effectiveness of the combination treatment of an Hsp90 inhibitor and a SIRT1 inhibitor on suppressing the growth of chemo-resistant human cancer cells. We showed that inhibition of SIRT1 effectively potentiated the cytotoxicity of 17-allylamino-17-demethoxygeldanamycin (17-AAG) and reversed Hsp90 inhibitor resistance in multidrug-resistant (MDR) human ovarian HeyA8-MDR cells. Amurensin G, a potent natural SIRT1 inhibitor, enhanced Hsp90 inhibitor-mediated abrogation of the Hsp90 chaperone function and accelerated degradation of mutated p53 (mut p53), an Hsp90 client protein, by up-regulation of ubiquitin ligase CHIP. Knockdown of CHIP significantly attenuated amurensin G-induced mut p53 degradation. Down-regulation of mut p53 reduced the expression of heat shock factor1 (HSF1)/heat shock proteins (Hsps), a major cause of Hsp90 inhibitor resistance, which led to sensitization of the MDR cells to the Hsp90 inhibitor by the SIRT1 inhibitor. Amurensin G potentiated cytotoxicity of the Hsp90 inhibitor in HeyA8-MDR cells through suppression of 17-AAG-induced Hsp70 and Hsp27 induction via down-regulation of mut p53/HSF1, and it caused activation of PARP and inhibition of Bcl-2. Our data suggests that SIRT1 inhibitors could be used to sensitize MDR cells to Hsp90 inhibitors, possibly through suppression of the mut p53/HSF1-dependent pathway, and a novel mut p53-directed action of SIRT1 inhibition could effectively prevent mut p53 accumulation in MDR cells.

Key words : Cancer, HSF1, Hsp70, Hsp90 inhibitor, SIRT1 inhibitor

Introduction

Heat shock protein 90 (Hsp90) is being considered as an important molecular target relevant to cancer, since Hsp90 is key to the stability and function of a host of proteins, which contribute to key characteristics of cancer cells: (1) self-sufficiency in growth signals (EGFR, Bcr-Abl, ErbB-2, Raf and SRC), (2) invasion of apoptosis (AKT, mut p53, Bcl-2, and survivin), (3) resistance to antigrowth signals (CdK4 and cyclin D), (4) tissue invasion and metastasis (c-MET and MMP-2), (5) sustained angiogenesis (VEGFR, FAK, and HIF-1), and (6) limitless replicative potential (h-TERT) [4]. Inhibition of Hsp90 leads to degradation of these oncogenic clients via the ubiquitin-proteosome pathway. Despite the fact that Hsp90 is an abundant protein, Hsp90 inhibitors selectively accumulate in tumors rather than in normal tissues, thus destroying tumor cells over normal cells. This therapeutic selectivity of Hsp90 inhibitors results from the presence of a predominantly high-affinity, activated form of Hsp90 in tumors whereas Hsp90 in normal tissues is in a low-affinity, inactive form [17, 27].

Therefore, several small-molecule inhibitors of Hsp90 of diverse chemotypes have shown potent antitumor activity in a wide-range of malignancies, and are currently in clinical or late-stage preclinical investigation as a therapeutic approach for the treatment of cancer. The early clinical trials involved 17-allylamino-17-demethoxygeldanamycin (17-AAG, an ansamycin derivative), an inhibitor of Hsp90. Although some promising results of 17-AAG were reported in HER2+ breast cancer and multiple myeloma, where encouraging activity was observed in combination with the proteasome inhibitor bortezomib and trastuzumab, respectively [17, 26, 27, 33, 34] and a prolonged disease stabilization was achieved in phase II studies in various additional tumor types expressing particular Hsp90 client proteins [26, 30, 33, 36].

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p53 is reported to participate in the regulation of multidrug resistance (MDR). Wild-type p53 can inhibit *MDR1* gene expression, while mutated p53 (mut p53) can activate the promoter of the *MDR1* gene that encodes P-glycoprotein (P-gp) [3, 8, 12, 30, 36]. Indeed, the high-level of MDR observed in cancer cells is attributable to p53 mutations and/or a loss of p53 function acquired during chemotherapy, and the chemo-resistant phenotype of mut p53 tumors may be a result of up-regulation of *MDR1* by mut p53 protein [6, 17, 18, 35]. Mut p53 cancers are surprisingly dependent on their hyperstable mut p53 protein for survival since mut p53 and Hsp90 were shown to physically interact, which is due to massive up-regulation of Hsp90 chaperone machinery during malignant transformation [21]. Naturally unfolded mut p53 may form stable complex with Hsp90 multichaperone machinery and Hsp90 activity is crucial to prevent degradation of mut p53 through functional inactivation of E3 ligase such as carboxyl terminus of Hsp70-interacting protein (CHIP) and causes an accumulation of dysfunctional p53 in human cancer cells [22, 28]. Therefore, the effect of Hsp90 inhibitor on MDR cells would enhance if the level of mut p53 can be reduced by an increased degradation of mut p53.

Heat shock proteins (HSPs) induction in cancer cells is triggered by heat shock factor 1 (HSF1) that binds to heat shock elements (HSEs) in the promoters of HSPs. *MDR1* genes also contain HSE in the promoter region and its production is also stimulated by heat shock [38, 40]. Binding of Hsp90 inhibitor to the Hsp90 ATP/ADP-binding domain induces a stress response through the release and activation of HSF1, which can bind HSE within promoters of *Hsp* genes, resulting in increased transcription and translation of these genes [32] and play an important role in resistance to 17-AAG [14, 24, 25]. Knockdown of HSF1 in mut p53 cancer cells induces rapid destabilization of mut p53 and reduces its half-life, along with reduction of Hsps levels [22].

It has been known that pharmacological inhibition of SIRT1, a NAD(+)-dependent class III histone deacetylase (HDAC) [5, 13, 42], or RNAi-mediated depletion of SIRT1 activity leads to a marked reduction in HSF1 occupancy on the *Hsp70* promoter and hampers the stress-inducible expression of *Hsp* genes [1, 41]. Therefore, we hypothesized that destabilizing mut p53 by SIRT1 inhibitor may provide a novel therapeutic efficacy of the Hsp90 inhibitors. Since pharmacologically effective mut p53-targeting small molecule approaches have not been properly explored, we therefore investigated whether amurensin G, a potent natural product inhibitor of SIRT1 [29], could enhance the cytotoxic effect of 17-AAG in MDR cells harboring mut p53 by degradation of mut p53 and prevention of Hsps induced by Hsp90-targeted therapy.

Materials and Methods

Cell culture and reagents

For this study, we used HeyA8 human ovarian cancer cell line and its MDR subline HeyA8-MDR, MDR MCF-7 human breast cancer cells (MCF7-MDR) [19] and MDR human yelogenous leukemia K562 (K562-MDR, originally named CD44^{high} K562) [20]. These cells were maintained in DMEM medium supplemented with 10% fetal bovine serum (FBS, GIBCO BRL, Life Technologies, Inc.) and incubated at 5% CO_2 at 37°C and 95% humidity. 17-AAG, AUY922 and BIIB021 were purchased from Enzo Life Sciences Inc. (Farmingdale, New York, USA) and Selleck Chemicals (Houston, TX, USA), respectively. EX527 was purchased from BioVision Inc. (Milpitas, CA, USA). Amurensin G, a natural SIRT1 inhibitor, was supplied Prof. Oh (Seoul National University, Seoul, Korea) as described previously [29].

Cell proliferation assay

Cell proliferation was measured by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Exponentially growing cells (2×10^4 cells/well) were plated in plated in a 96-well plate and incubated in growth medium containing the indicated concentrations of 17-AAG and/or amurensin G at 37°C for 96 hr. Optical densities of samples at 570 nm were measured using an ELISA reader. Measured optical densities were proportional to viable cell numbers. Inhibition of cell proliferation was expressed as percentages of untreated control cell growth. At least two separate experiments were performed in triplicate. Interaction between 17-AAG and amurensin G was assessed using Compu-Syn Software (ComboSyn, Paramus, NJ, USA). A combination index (CI) <0.9 represents drug synergism, 0.9 < CI <1.1 implies nearly additive interactions, and CI >1.1 indicates antagonism. All experiments were carried out in triplicate.

Western blot analysis

Isolated cell protein lysates were separated by SDS-PAGE and blotted onto nitrocellulose membranes (Hybond-ECL,

GE Healthcare, USA), which were then incubated with specific antibodies and then with secondary antibody conjugated with horseradish peroxidase. Specific antigen-antibody complexes were detected by enhanced chemiluminescence (PerkinElmer Life science, MD, USA). Western blot analysis was performed with specific primary antibodies against SIRT1 and CHIP (Cell Signaling Technology, MA, USA), Hsp27 (Epitomics, CA, USA), p53, PARP, Bcl-2, HSF1, P-gp (Santa Cruz Biotechnology, CA, USA), Hsp70 and Hsp90 (Enzo Life Sciences, Inc., NY, USA), and β-actin (actin) or β-tubulin (tubulin) (Sigma‐Aldrich, St. Louis, MO, USA) was used as the loading control.

Preparation of siRNA transfection

The siRNAs used for the targeted silencing of CHIP and scrambled were purchased from Bioneer (Daejeon, Korea). In brief, MCF7-MDR cells (2×10^5 cells/ml) were seeded on 6-well plates and transfected with 0.2 μM of CHIP siRNA or scrambled control siRNA (Bioneer, Daejeon, Korea) using oligofectamine reagent, according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). Cells were then incubated at 37°C for 4 hr in serum free media containing 10% FBS. After 48 hr, cells were treated with 17-AAG for an additional 24 hr and collected for Western blotting to determine the levels of indicated proteins.

RT-PCR analysis

Endogenous mRNAs of MCF7-MDR cells were isolated using RNeasy Mini Kits (Qiagen, Hilden, Germany) and assessed by RT-PCR using a PC-818 program Temp Control System (ASTEC, Japan). For cDNA synthesis, 1 μg of RNA was retrotranscribed by semiquantitative PCR using the following oligonucleotides: SIRT1 (forward), 5'-AAGCCAGG AGGCTTTGATGT-3'(reverse), 5'-GAGAGGGCCTCAGGTA GTGC-3', p53 (forward), 5'-AAAGAGGCTGTGAACGGGAT-3', (reverse), 5'-CAGCAGATGGTTGATGGAGG-3'. β-actin (forward) 5'-ACCAACTGGGACGACATGGA-3' and (reverse) 5'-GTGAGGATCTTCATGAGGTA-3'. The resulting total cDNA was used in PCR, which was performed in total volume of 20 μl using Taq polymerase (Solgent, Korea). Amplification conducted over 30 cycles (denaturation 94°C for 60 sec, annealing 56°C for 60 sec, and amplification 72°C for 90 sec, followed by a final extension at 72°C for 10 min. The PCR products were analyzed by ethidium bromide-stained 2% agarose electrophoresis and quantified using image analyzing software (Quantity-one; Bio-Rad Laboratories,

CA, USA).

Statistical analysis

The results obtained were expressed as the mean ± S.E. of at least three independent experiments. The statistical significance of differences assessed using the Student's t-test. *$p<0.05$, **$p<0.01$ and ***$p<0.001$ was considered statistically significant in all experiments.

Results

Degradation of mut p53 in MDR cells is accelerated by SIRT1 inhibition

Since mut p53 and SIRT1 could activate the *MDR1* promoter and expression of P-gp [11, 30], there is a possibility that down-regulation/degradation of mut p53 could suppress *MDR1*/P-gp by SIRT1 inhibition. It has been reported that the chaperone-associated ubiquitin ligase CHIP rather than MDM2 could induce the degradation of mut p53 [9, 28] and the functional inactivation of CHIP is the cause of aberrant stabilization of mut p53. We therefore investigated whether amurensin G, a SIRT1 inhibitor, could affect the expression of CHIP and mut p53 (Fig. 1A). When MCF7-MDR cells were treated with various concentrations of amurensin G for 24-, 48- or 72 hr, the levels of mut p53 as well as SIRT1 were decreased in the MDR cells with a dose-dependent manner after treatment of amurensin G. The mut p53 level was dramatically decreased at 48- and 72 hr after treatment with amurensin G. Following exposure of MCF7-MDR cells to amurensin G, protein level of CHIP was significantly increased, which led to the reduction of mut p53 level, indicating the acceleration of CHIP-mediated degradation of mut p53 by amurensin G. Since no significant change of mut p53 mRNA level was detected in the amurensin G-treated MDR cells (Fig. 1B), amurensin G might down-regulate mut p53 at post-transcription level. To confirm the effect of amurensin G on mut p53 destabilization, MCF7-MDR cells were treated with amurensin G in the presence or absence of MG132 proteasome inhibitor. We showed that proteasome inhibition by MG132 rescued amurensin G-mediated down-regulation of mut p53 (Fig. 1C). These results indicate that amurensin G could accelerate CHIP-mediated mut p53 degradation in MDR cells. To confirm the role of SIRT1 on CHIP-mediated mut p53 degradation, MCF7-MDR cells were treated with indicated doses of amurensin G after CHIP knock-down. The amurensin G-mediated mut p53
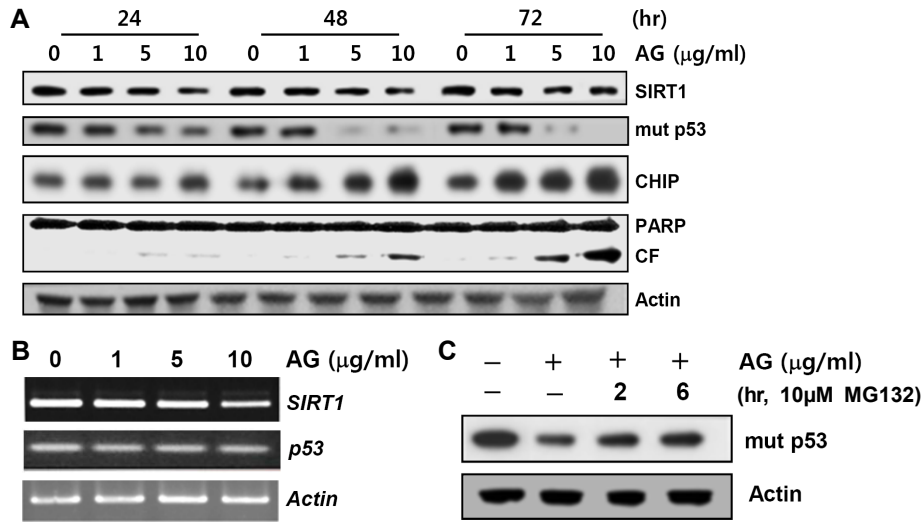
Fig. 1. Amurensin G down-regulates mut p53 in at post-transcriptional level via up-regulation of CHIP. (A) MCF7-MDR cells were treated with graded single doses of amurensin G (AG) for 24-, 48- or 72 hr. Western blot analysis was performed to determine the levels of SIRT1, mut p53, CHIP and cleavage of PARP. (B) MCF7-MDR cells were treated with the indicated doses of AG for 24 hr followed by extraction of total RNA. cDNA fragments of *SIRT1, p53* and *actin* were amplified by RT-PCR. (C) MCF7-MDR cells were treated with AG (10 μg/ml) for 24 hr. Then, the cells were treated with proteasome inhibitor MG-132 (10 μM) for 2- or 6 hr. The level of mut p53 protein was detected by Western blot analysis. The level of actin was used as a loading control.

degradation was not occurred by CHIP knock-down whereas amurensin G treatment of the cells transfected with control siRNA showed a significant decrease of mut p53 in a dose-dependent manner, indicating acceleration of CHIP-mediated mut p53 degradation by SIRT1 inhibition.

CHIP-mediated mut p53 degradation leads to down-regulation of HSF1

It has been reported that mut p53 positively regulates HSF1 levels and activity [23]. We also confirmed that the level of HSF1 was decreased in amurensin G-treated MCF7-MDR cells transfected with control siRNA but not CHIP knock-downed cells. Therefore, our results suggest that SIRT1 inhibition could accelerate CHIP-mediated mut p53 degradation in MDR cells. Next, we determined whether the relationship between HSF1/mut p53 and CHIP in another MDR cells (Fig. 2B). K562-MDR cells also showed that CHIP-mediated mut p53 degradation was accelerated by SIRT1 inhibition, and mut p53 positively regulated HSF1 expression and negatively regulated CHIP expression in the cells co-treated with SIRT1 inhibitor (amurensin G or EX 527) and Hsp90 inhibitor (17-AAG or AUY922). These results suggest that mut p53 positively associated with HSF1 expression and was negatively regulated by CHIP expression.

MDR cells harboring mut p53 exhibit resistance to Hsp90 inhibitors

When we assessed the expression of mut p53 and CHIP in HeyA8-MDR and parental HeyA8 cells by western blot analysis, a large increase in mut p53 was detected in HeyA8-MDR cells whereas no detectable mut p53 in HeyA8 cells harboring wild-type p53 since wild-type p53 is a ubiquitous protein and not detectable under normal condition. Conversely, the expression of CHIP in HeyA8 cells was increased compared to HeyA8-MDR cells (Fig. 3A, *upper*). We next compared the cell viability between HeyA8 and HeyA8-MDR cells by the MTT assay after exposure to serial concentrations of Hsp90 inhibitor 17-AAG or BIIB021. HeyA8-MDR cells showed significant resistance to both 17-AAG and BIIB021 compared to HeyA8 cells (Fig. 3A, *lower*), indicating resistance of MDR cells to Hsp90 inhibitors. We therefore determined the changed protein levels of 17-AAG-treated both cells. The cleavage/activation of PARP by treatment of 17-AAG were occurred in HeyA8 cells but not in HeyA8-MDR cells overexpressing P-gp (Fig. 3B). Hsp90 inhibitor induces the release and activation of HSF1, which increases transcription and translation of *Hsps* genes, and it leads to resistance of cancer cells to Hsp90 inhibitors [14, 24]. Our results showed that expression of Hsp70/Hsp90 in HeyA8-MDR cells was more enhanced than in HeyA8 cells
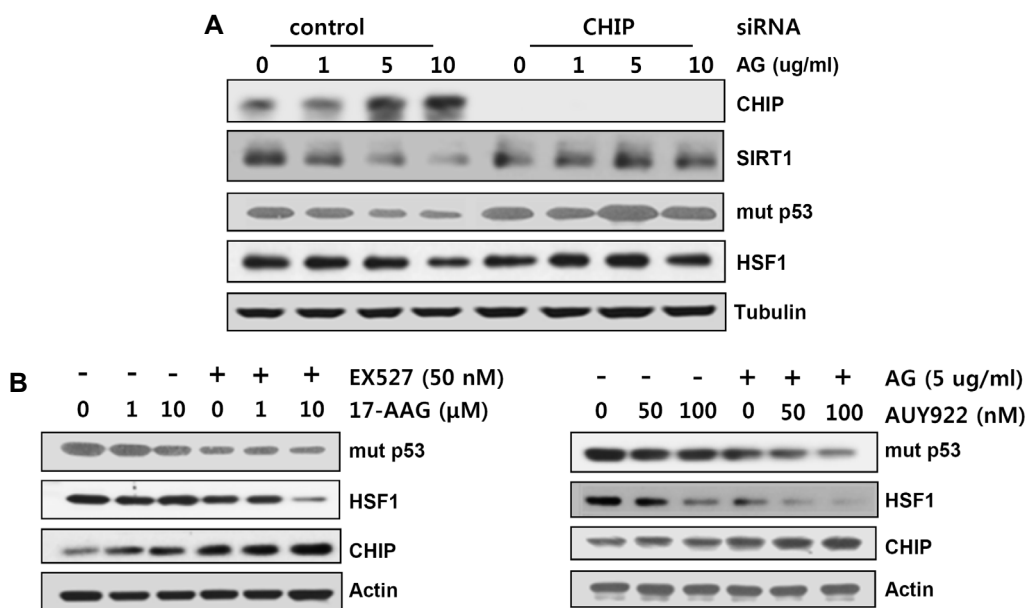
Fig. 2. CHIP is required for in the acceleration of 17-AAG-mediated- mut p53 degradation by SIRT1 inhibition (A) MCF7-MDR cells transfected with 20 nM SIRT1 siRNA or scrambled control siRNA as control for 48 hr were treated with AG (1-, 5- or 10 μg/ml) for 24 hr, and the changed levels of CHIP, SIRT1, mut p53 and HSF1 were determined by western blot analysis. The level of tubulin was used as a loading control. (B) K562-MDR cells were pretreated with SIRT1 inhibitor (5 μg/ml AG or 50 nM EX527) for 6 hr followed by Hsp90 inhibitor (1- or 10 μM 17-AAG or 50- or 100 nM AUY922) for additional 24 hr, western blot analysis was performed to determine changed levels of mut p53, HSF1 and CHIP. The level of tubulin or actin was used as a loading control.

after exposure of 17-AAG due to activation of HSF1 since the activation of HSF1 evidenced by an electrophoretic mobility shift in the MDR cells. Since expression of SIRT1 as well as HSF1 also could induce *MDR1*/P-gp gene [29, 40], we confirmed up-regulation of SIRT1 in HeyA8-MDR cells, which might be associated with the expression of P-gp. These results suggest that resistance of MDR cells to Hsp90 inhibitors might be resulted from activation of HSF1 and subsequent up-regulation of Hsps.

SIRT1 inhibitor potentiates cytotoxicity of Hsp90 inhibitor in MDR cells via down-regulation of HSF1/ mutp53 through suppression of 17-AAG-induced Hsps

Therefore, we next determined whether SIRT1 inhibition suppressed 17-AAG-induced expression of HSPs such as Hsp70 and Hsp27 through down-regulation of HSF1, which resulted in enhanced 17-AAG susceptibility of HeyA8-MDR cells. When HeyA8-MDR cells were treated with serial concentrations of 17-AAG in the presence or absence of amurensin G, 17-AAG-mediated cytotoxicity against HeyA8-MDR cells was significantly enhanced by amurensin G. The CI values were lower than 0.5 at all concentrations, indicating a synergistic effect of combination of both agents. It means

that amurensin G could increase the susceptibility of HeyA8-MDR cells to 17-AAG. We also showed that amurensin G could suppress 17-AAG-mediated Hsp70 and Hsp27 induction through down-regulation of HSF1/mut p53, which led to cleavage/activation of PARP and down-regulation of Bcl-2 and, it sensitized HeyA8-MDR cells to 17-AAG (Fig. 4B). Taken together, our results indicate that sensitization of HeyA8-MDR cells to 17-AAG by amurensin G is associated with SIRT1 inhibition-mediated down-regulation of HSF1/mut p53 and subsequent suppression of 17-AAG-mediated induction of Hsps.

Discussion

In this study, we showed that SIRT1 inhibition by amurensin G treatment effectively enhanced Hsp90 inhibitor 17-AAG cytotoxicity, and it could suppress the 17-AAG resistance of HeyA8-MDR cells. This result may occur because SIRT1 inhibition facilitates disruption of Hsp90 multichaperone complex, and up-regulation/activation of CHIP accelerates degradation/down-regulation of mut p53 and HSF1, which leads to the suppression of 17-AAG-induced Hsps and sensitization of MDR cells to 17-AAG.
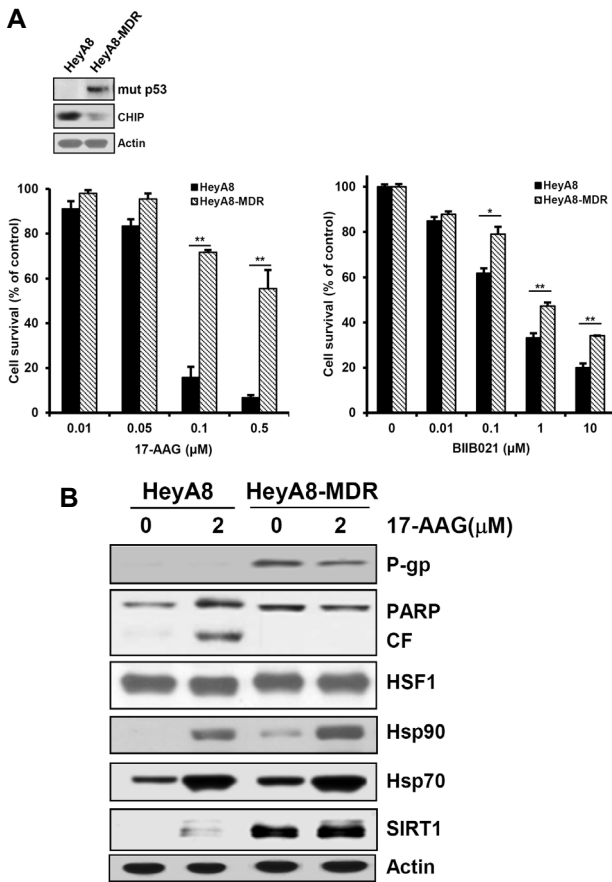
Fig. 3. MDR cells expressing mut p53 and P-gp exhibit resistance to Hsp90 inhibitors. (A) HeyA8 and its MDR subline HeyA8-MDR cells were treated with serial concentrations of 17-AAG or BIIB021. Percentage of cell survival was determined after 96 hr of incubation using MTT assay. Results are the means ± SEs of three experiments. *$p<$0.05, **$p<0.01$ and ***$p<0.001$. Western blot analysis was performed to monitor the protein levels of mut p53 and CHIP. (B) Both cells were treated with 2 μM 17-AAG for 24 hr, and Western blot analysis was performed to determine the changed levels of P-gp, HSF1, Hsp90 and Hsp70 proteins and the activation of PARP (cleaved form of PARP). The level of actin was used as a loading control.

In cancer, an uncontrolled cell proliferation may be facilitated by the increased levels of a variety of Hsp90 client proteins [4]. While successfully targeting Hsp90 in cancer patients to achieve a significant therapeutic benefit is still a work in progress, there has been considerable progress in understanding the molecular, cellular and organismal contributions of Hsp90. Currently, several small-molecule inhibitors of Hsp90 of diverse chemotypes have shown potent antitumor activity in a wide-range of malignancies, and are currently in clinical or late-stage preclinical investigation
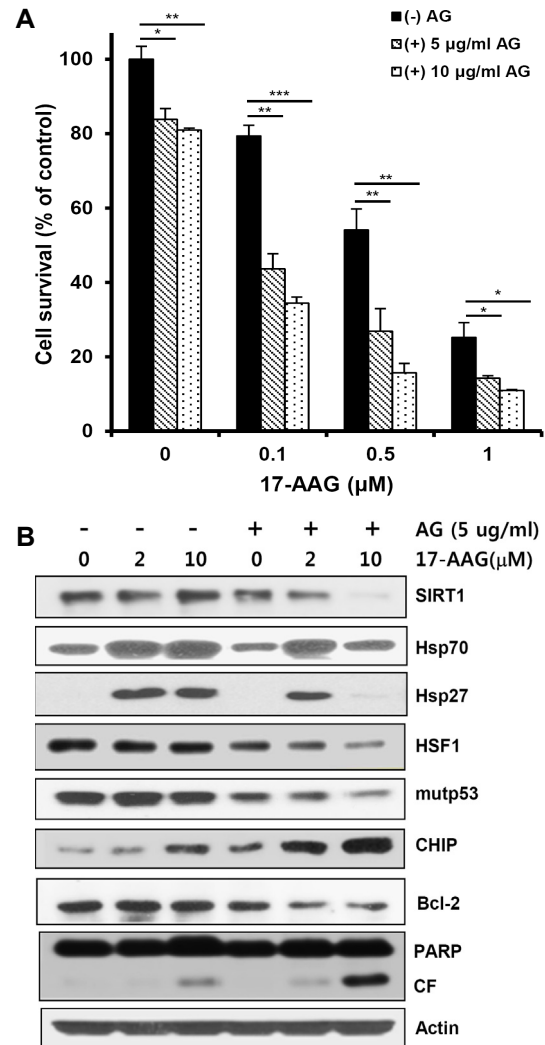
Fig. 4. Suppression of 17-AAG-induced Hsps via down-regulation of HSF1/mutp53 and subsequent PARP activation by amurensin G caused a potentiation of 17-AAG cytotoxicity in MDR cells. (A) HeyA8-MDR cells were pretreated with amurensin G (AG; 5- or 10 μg/ml) for 6 hr followed by various concentrations of 17-AAG for 96 hr. Cell survival was determined by MTT assay. Results are the means ± SEs of three experiments. Each bar represents the mean ± S.D. of triplicate experiments. **$p<$0.01 and ***$p<0.001$. (B) HeyA8-MDR cells were pretreated with amurensin G (AG; 5 μg/ml) for 6 hr followed by 17-AAG (2- or 10 μM) for additional 24 hr, Western blot analysis was performed to determine the changed levels of SIRT1, Hsp70, Hsp27, HSF1, CHIP, mut p53 and Bcl-2 proteins and the activation of PARP. The level of actin was used as a loading control.

as a therapeutic approach for the treatment of cancer [2, 15, 21]. In the long term, reaching the full therapeutic potential of Hsp90 inhibitors may require concomitant inhibition of Hsp70 isoforms or blockade of HSF1. Activation of HSF1

is considered to limit the activity of Hsp90 inhibitors and occurs uniformly in response to the Hsp90 inhibitors because HSF1-dependent transcriptional induction of Hsp70 and Hsp27, and to some degree Hsp90 itself, protects cancer cells from apoptosis. Indeed, HSF1 knock-out cells are much more sensitive to Hsp90 inhibitors than are their wild type counterparts [2]. Efforts are underway to identify and validate inhibitors of HSF1, Hsp70, and Hsp27, and to explore their combination with Hsp90 inhibitors [7, 10, 21]. Subsequently, it is becoming increasingly evident that the combinatorial targeting of HSP90 and the heat shock response could be a more effective therapeutic approach [31].

It has been known that HDAC6, a cytoplasmic non-histone HDAC that deacetylates Hsp90 and functions as an obligate positive regulator of the Hsp90 chaperone activity, is critically important in enabling aberrant stability of mut p53, and suberoylanilide hydroxamic acid, a pan-HDAC inhibitor, shows preferential cytotoxicity in mut p53 cancer cells by destabilizing mut p53 through inhibition of the HDAC6-Hsp90 chaperone axis [21]. In our study, we suggest that SIRT1 also might involve Hsp90 multichaperone complexes. It has been reported that CHIP, which is a co-chaperone of Hsp90/Hsp70 complex, is involved in 17-AAG-induced mut p53 degradation as an ubiquitin ligase [11]. Most importantly, CHIP is required for amurensin G-induced mut p53 degradation in MDR cells. SIRT1 inhibition induced up-regulation of CHIP, which leads to destabilizing/degradation of mut p53 through disruption of Hsp90 multichaperone complexes, and knock-down of CHIP significantly attenuated amurensin G-induced mut p53 degradation, suggesting that CHIP participates in amurensin G-induced mut p53 degradation.

Since SIRT1 is required to maintain the stability of HSF1 after activation and to prevent its rapid degradation [41], we also showed that treatment of MDR cells with SIRT1 inhibitor resulted in down-regulation of HSF1 as well as mut p53. HSF1 actions were mediated via a mut p53-dependent mechanism. Mut p53 via direct interaction with activated HSF1 facilitates binding of HSF1 to its DNA-binding sites and stimulates transcription of HSPs and HSF1 activation by mut p53 renders cells resistant to proteotoxic stress [23]. Importantly, our result showed SIRT1 inhibition accelerated down-regulation of mut p53 through both HSF1 and CHIP-mediated pathway. The accelerate degradation of mut p53 by amurensin G further mediates inactivation of survival signaling pathways and enhances 17-AAG induced cytotoxicity. Taken together, the present study suggests that SIRT1 inhibitors would be used to sensitize MDR cells to Hsp90 inhibitors, possibly through suppression of mut p53/HSF1-dependent pathway.

Acknowledgement

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초록 : SIRT1 inhibitor에 의한 Hsp90 inhibitor의 Hsp90 샤페론 기능 억제 및 항암제 내성세포의 Hsp90 inhibitor에 대한 세포독성 증강

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　본 연구는 Hsp90 inhibitor 및 SIRT1 inhibitor의 병용처리가 항암제 다제내성(MDR) 인간 암세포의 증식 억제에 효과적임을 밝혔다. SIRT1 활성 억제가 Hsp90 inhibitor인 17-AAG의 세포 독성의 효과를 증강시켰으며, 이로 인해 Hsp90 inhibitors에 대한 내성을 극복시킬 수 있음을 인간 자궁암세포인 HeyA8의 MDR 변이주인 HeyA8-MDR 세포에서 확인하였다. SIRT1 inhibitor는 Hsp90 inhibitor에 의한 Hsp90 샤페론 기능 억제를 증강시키며, ubiquitin ligase CHIP의 발현 증강을 유발하여, Hsp90 client protein 인 mutant p53 (mut p53)의 분해를 촉진시킨다. Mut p53 의 발현 감소는 암세포의 Hsp90 inhibitor 내성 획득의 가장 중요한 원인으로 지적되는 heat shock factor 1 (HSF1)/heat shock proteins (Hsps)의 발현 억제와 관련됨을 알 수 있었으며, 이는 항암제 다제내성 세포에서 SIRT1 inhibitor에 의하여 Hsp90 inhibitor에 대한 감수성이 증강되는 분자적 기전임을 밝혔다. 그러므로, SIRT1 억제에 의한 mut p53/HSF1 발현 감소가 MDR 암세포의 Hsp90 inhibitors 내성 극복에 매우 유효함을 시사하는 결과를 얻었다.