AT-406, an IAP Inhibitor, Activates Apoptosis and Induces Radiosensitization of Normoxic and Hypoxic Cervical Cancer Cells

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Abstract. IAP antagonists increased the antitumor efficacy of X-irradiation in some types of cancers, but their effects on hypoxic cancer cells remain unclarified. We aims to investigate the radiosensitizing effect of an IAP inhibitor AT-406 on cervical cancer cell lines under both normoxia and hypoxia conditions. Hela and Siha cells were treated to investigate the effects of drug administration on cell proliferation, apoptosis, and radiosensitivity. Western blot analysis was used to determine the role of AT-406 in inhibition of IAPs. The pathway of apoptosis was characterized by caspases activity assay. AT-406 potently sensitized Hela cells but not Siha cells to radiation under normoxia. Notably, the radiosensitizing effect of AT-406 on hypoxic cells was more evident than on normoxic cells in both cell lines. Further mechanism studies by western blot showed that under normoxia AT-406 decreased the level of cIAP1 in Hela cells in a dose-dependent manner; while additional downregulation of XIAP expression was induced by AT-406 treatment under hypoxia in both cell lines. Finally, AT-406 works on both extrinsic death receptor and intrinsic mitochondrial apoptosis pathways to activate apoptosis. Totally, AT-406 acts as a strong radiosensitizer in human cervical cancer cells, especially in hypoxic condition.

[Supplementary Figures: available only at http://dx.doi.org/10.1254/jphs.14079FP]

Keywords: AT-406, inhibitor of apoptosis protein (IAP), radiosensitization, cervical cancer, hypoxia

Introduction

Cervical cancer is the third most common malignancy in women (1, 2), with 85% of the total number of cases occurring in developing countries; in such countries, cervical cancer is the second most common cause of malignancy-related death in women (3). Radiation therapy is used either as a definitive therapy for cervical cancer patients with locally advanced disease or as an adjuvant therapy following radical hysterectomy for patients with pathological risk factors. However, intrinsic tumor radioresistance promotes high recurrence and decreases five-year survival. Apoptosis evasion, a characteristic of human cancers, is a critical cause of treatment resistance that frequently occurs in various human cancers, including cervical cancer. Cells in the hypoxic region of tumors also resist radiation by reducing oxygen radical generation (4).

Cell death by apoptosis theoretically proceeds via two major pathways, namely, the extrinsic (death receptor) and the intrinsic (mitochondrial) pathways (5). The extrinsic and intrinsic pathways are initiated by specific ligands or various intracellular stimuli that activate the effector caspases, ultimately fragmenting DNA and inducing cell death (6 – 8). Both pathways are involved...
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in radiation-induced apoptosis (9). Inhibitors of apoptosis proteins (IAPs), including X-linked inhibitor of apoptosis protein (XIAP), cellular IAPs (cIAP1, cIAP2), NAIP, Survivin, Livin/ML-IAP, and Bruce, are a family of endogenous anti-apoptotic proteins characterized by one or several baculoviral IAP repeat (BIR) domains (10). Some IAPs contain a RING finger domain at their C-terminus showing E3 ubiquitin ligase activity, which is required for the ubiquitination and proteasomal degradation of different substrates (11). XIAP exerts the most pronounced anti-apoptotic function by effectively binding to and thereby inhibiting active caspase-3, -8, and -9. The E3 ligase activity of XIAP contributes to proapoptotic factor degradation (12). CIAP1 and CIAP2 tend to be involved in NF-κB activity regulation and caspase-8 activation suppression (13). Aberrant IAP expression occurs in combination with the cancer, particularly in hypoxic microenvironments (14). Previous studies on IAP amplification and overexpression demonstrated that IAPs, such as cIAP1 and XIAP, are possibly novel predictive markers for radiotherapy resistance in individual cervical squamous cell carcinoma (CSCC) patients (15, 16). IAPs serve a critical function in interfering with the efficacy of radiotherapy and chemotherapy.

Smac/DIABLO, a second mitochondria-derived activator of caspases/direct IAP-binding protein with low isoelectric point, is a natural antagonist of IAP-mediated caspase inhibition and is secreted from the intermitochondrial membrane upon apoptosis induction (17). Smac/DIABLO binds to IAPs via the N-terminal AVPI tetrapeptide motif, thereby blocking the ability of IAPs to inhibit apoptosis (18). However, a recent study reported the overexpression of cIAP1 by 11q22 amplification in CSCC-derived cell lines (15). In several cancers, the decreased expression of Smac/DIABLO, which is associated with worse prognosis, has been reported (19). Consequently, small-molecule IAP antagonists were designed to act as IAP inhibitors to improve the efficacy of radiotherapy.

AT-406 is an IAP antagonist that shows higher sensitivity to these IAPs than Smac AVPI peptide with 50-fold to 100-fold binding affinities (20). AT-406 was designed to mimic the interaction between the Smac AVPI peptide and the XIAP BIR3 protein, thereby restoring caspase-9 activity. AT-406 effectively and rapidly induces cIAP1 degradation at concentrations as low as nanomolar affinities; and such rapid induction is consistent with the high binding affinity of AT-406 to cIAP1 (20). Smac protein expression selectively induces cIAP1/2 degradation, but not XIAP degradation, in some cancer cell lines, including Hela cells (21). The function of AT-406 in human cervical cancer is unknown as of this writing.

Thus, we aimed to investigate the potential radiosensitizing effect of AT-406 and the molecular mechanisms underlying its efficacy in cancer cell lines Hela and Siha. We also determined whether AT-406 is a potent and active IAP antagonist and can function in hypoxic microenvironments.

Materials and Methods

Cell culture, irradiation, and reagents

Hela and Siha human cervical cancer cell lines were obtained from Shanghai Institute of Cell Biology (Shanghai, China). Both cell lines were cultured in Dulbecco’s modified Eagle’s medium (DMEM) (Gibco, NY, USA) supplemented with 10% fetal bovine serum (Hyclone, Utah, USA) and 1% penicillin/streptomycin (Invitrogen, Carlsbad, CA, USA). The hypoxic group of cells was cultured in 0.8% – 1.0% pO2. The irradiation group of cells was subjected to a 2, 4, 6, and 8 Gy X-ray irradiation using a medical linear accelerator (Elekta Precise, Stockholm, Sweden) at room temperature. The IAP antagonist compound AT-406 was purchased from Selleck Chemicals (Houston, TX, USA) and dissolved in dimethyl sulfoxide (DMSO) to create a 10 mM stock solution. Rabbit monoclonal antibodies against cIAP-2, XIAP, STAT3, p-STAT3, and HIF-1α were purchased from Cell Signaling Technology (Beverly, MA, USA). Goat polyclonal antibody against cIAP-1 was purchased from R&D Systems (Minneapolis, MN, USA). Mouse antibody against β-actin was purchased from Millipore (Billerica, MA, USA). Bicinchoninic Acid (BCA) Protein Quantification Kit was purchased from Vazyme Biotech (Piscataway, NJ, USA). Caspase-3, -8, and -9 colorimetric assay kits were purchased from Biovision (Milpitas, CA, USA). Caspase family inhibitor Z-VAD-FMK was purchased from Biovision.

Measurement of cervical cell proliferation

Cell proliferation rate was estimated using Cell Counting Kit-8 (CCK-8; Dojindo Laboratories, Kumamoto). The 96-well plates were seeded with 5,000 cells/well in sextuplicate. The cells were allowed to adhere and were treated with increasing AT-406 concentrations. A 10-μl portion of CCK-8 reagent was added to each well in the dark after 24 and 48 h of AT-406 treatment. The cells were incubated for 2 h at 37°C, and the absorbance of the plates at 450 nm was read using a microplate reader. The relative cell viability of each sample was calculated by normalizing the absorbance to that of the corresponding control. IC50 values were calculated using the SPSS 19.0 software.
Clonogenic survival assay
Hela and Siha cells were trypsinized as single-cell suspensions and seeded in triplicate at serial densities into 60-mm dishes. The cells were allowed to adhere and were treated with DMSO (control) or AT-406 (5 or 10 μM) for 24 h under normoxic or hypoxic conditions. Subsequently, cells were exposed to different radiation doses (0, 2, 4, 6, or 8 Gy). The cells were incubated for 10–14 d, and colonies containing more than 50 cells were counted under a microscope. The cells were fixed for 30 min with 70% ethanol and stained for 1 h with Giemsa. The cell survival curves were fitted to a single-hit multi-target model, and the survival enhancement ratio (SER) of each sample was calculated as the ratio of the mean inactivation dose in the control cells treated with X-rays alone divided by the mean inactivation dose in cells treated with AT-406 and X-rays. Each experiment was performed thrice.

Apoptosis assay
Annexin-V/FITC and propidium iodide (PI) dual staining (Annexin-V/PI) was performed to determine the percentage of apoptotic cells. The cells were seeded into six-well plates and treated with or without AT-406 under normoxic or hypoxic conditions for 24 h. Subsequently, cells were subjected to X-ray irradiation (8 Gy). The cells treated with AT-406 (10 μM) and radiotherapy in the caspase inhibitor assay were subjected to Z-VAD-FMK pretreatment. The cells were collected 24 h after irradiation and analyzed with an Annexin-V/FITC Apoptosis Detection kit (BD Bioscience, Oxford, UK) by flow cytometry.

DNA double-stranded break (DSB) repair assay
Phospho-γH2AX foci immunofluorescence was detected to monitor DNA DSBs and DSB repair capacity of the cells. The cells were seeded on coverslips and treated with either DMSO (control) or AT-406 (10 μM) under normoxic or hypoxic conditions for 24 h prior to 4 Gy irradiation. The cells were fixed with 4% paraformaldehyde for 20 min at room temperature at 0.5, 2, 8, and 24 h after irradiation and were maintained in a humidified chamber overnight at 4°C for phosphorH2AX S139 antibody (γH2AX, Millipore) staining. Cells were subsequently stained with Alexa 488 Fluor secondary antibody (Invitrogen) for 1.5 h at room temperature. The nuclei were finally counterstained with 2 μg/ml 4, 6-diamidino-2-phenylindole, and the samples were mounted in 3 μl of Vecta Shield mounting medium for confocal microscopy.

Western blot analysis
Total proteins were extracted from the cells using SDS Lysis Buffer for 24 h (to detect cIAP-1, cIAP-1, and XIAP) or 12 h (to detect STAT3 and p-STAT3) after the last AT-406 treatment under normoxic or hypoxic condition. The protein concentrations of the supernatants were determined by BCA assay. Equal amounts of protein were loaded into each well. The proteins were separated by 10% SDS–PAGE and blotted onto polyvinylidene fluoride membranes (Millipore). The membranes were blocked, probed overnight with primary antibodies against cIAP-1, cIAP-1, XIAP, STAT3, p-STAT3, HIF-1α, and β-actin, and incubated with conjugated secondary antibodies for 1 h at 37°C. The immunoblotted proteins were visualized by ECL reagents, and the signals were detected using the Chemidoc XRS imaging system (Quantity One Quantitation software; BioRad Laboratories, Hercules, CA, USA). Relative concentrations were measured using the public domain NIH Image J Program.

Caspase activity assay
Caspase-3, -8, and -9 activities were measured by colorimetric assay using a caspase colorimetric protease kit. Hela and Siha cells were treated with AT-406 (10 μM), radiation (8 Gy), or both under normoxic and hypoxic conditions. The cells were incubated for 24 h, and the cell lysate containing 200 μg of protein was incubated with 5 μL of 4 mM pNA-conjugated substrates at 37°C for 1.5 h. DEVD-pNA, IETD-pNA, and LEHD-pNA were used as substrates for caspase-3, -8, and -9, respectively. The amount of released pNA was measured at 405 nm using an ELISA microplate reader.

Statistical analysis
PRISM statistical analysis software (GraphPad Software Inc., San Diego, CA, USA) was used for statistical analysis. The mean ± standard error (S.E.) of the triplicate assays was calculated, and the differences between treatment groups were determined using two-tailed Student’s t-test. P < 0.05 was considered statistically significant. Difference in cell survival was assessed by analysis of variance.

Results
AT-406 inhibited human cervical cancer cells proliferation
CCK-8 assay was performed at 24 and 48 h after AT-406 administration at various concentrations up to 500 μM to determine the sensitivity of different human cervical cancer cell lines to AT-406 as a single agent. IC50 values for Hela cells and Siha cells at 24 h were 83.32 and 64.26 μM, respectively. Hela and Siha cells showed high resistance to AT-406 (IC50 > 50 μM) at all time points (Fig. 1). Thus, AT-406 itself is not a cyto-
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AT-406 significantly enhanced cervical cancer cell radiosensitivity

The clonogenic survival assay was performed to assess potential AT-406 radiosensitization activity. Normoxic or hypoxic cervical cancer cells were treated with low AT-406 concentrations (5 and 10 μM). The results of CCK-8 analysis showed that AT-406 cell inhibition was less than 20% at these concentrations. Under normoxic and hypoxic conditions, Hela cells treated with AT-406 at 10 μM prior to irradiation showed a significant survival curve shift with SERs of 1.81 and 2.07, respectively, compared with untreated Hela cells (Fig. 2A).

However, AT-406 only showed a slight radiosensitizing effect on normoxic Siha cells, with an SER of 1.43, even at a high dose of 10 μM. AT-406 at 5 and 10 μM significantly reversed the radioresistance of hypoxic Siha cells, with SERs of 1.28 and 1.72, respectively. Such cells showed the ability to form colonies after irradiation (Fig. 2B). The surviving fraction (SF) was calculated as the number of counted colonies divided by the number of plated colonies, with correction for plating efficiency (PF, the number of observed colonies divided by the number of plated cells). SF data were fitted into the single-hit multi-target model formula, $SF = 1 - (1 - e^{-D/D_0})^n$.

SF2 values of Siha cells treated with AT-406 at 5 μM were 79.24% and 76.36% under normoxic and hypoxic conditions, respectively. The SF2 value at 10 μM AT-406 was 74.17% for normoxic Siha cells, whereas the value decreased to 67.33% for hypoxic Siha cells. These data demonstrate that AT-406 suppressed Hela cell colony formation under both normoxic and hypoxic conditions, and preferentially enhanced Siha cell radiosensitivity under hypoxic conditions.

AT-406 enhanced radiation-induced apoptosis in both normoxic and hypoxic Hela cells and preferentially enhanced apoptosis in hypoxic Siha cell

Annexin-V/PI staining was performed to quantify cervical cell line apoptosis after AT-406 treatment for
24 h. As shown in Fig. 3, A and B and Supplementary Fig. 1, a and b (available in the online version only), 8-Gy radiation caused moderate apoptosis levels in Hela cells under both normoxic and hypoxic conditions, and the pro-apoptosis effect of single-agent AT-406 treatment (5 or 10 μM) was low. Apoptotic events were remarkably induced when irradiation was combined with AT-406 treatment. Apoptosis induction rate under hypoxic condition was more significant than that under normoxic condition. The difference between combination and radiation-only treatment groups was statistically significant (P < 0.05). In normoxic Siha cells, AT-406 showed low radiation-enhancing activity for apoptosis induction (Fig. 3C and Supplementary Fig. 1c). The combination of irradiation and AT-406 induced significant apoptosis levels and exhibited a synergistic effect in hypoxic Siha cells (Fig. 3D and Supplementary Fig. 1d).

AT-406 impaired DSB repair after cervical cancer cell irradiation

DSBs, as major determinants of cytotoxicity of radiation therapy, are highly deleterious. The formation of nuclear DSBs triggers phosphorylation of histone H2AX on Ser-139 (defined as gammaH2AX), which helps repair DNA damage. Hundreds of γH2AX copies form foci at DSB sites (22). The phospho-γH2AX foci levels at 0.5, 2, 8, and 24 h after 4-Gy irradiation were determined by immunofluorescence to investigate the effect of AT-406 on X-ray-induced DSB repair kinetics (Fig. 4A). The peak foci value appeared in Hela cells 2 h after irradiation. The treatment combination of AT-406 and 4 Gy X-ray increased phospho-γH2AX foci formation at 2 h by 1.6- and 1.7-fold compared with X-radiation alone under normoxic and hypoxic conditions, respectively. DSB repair gradually led to the decrease in the number of foci in Siha cells from 0.5 to 24 h, but the numbers of foci among hypoxic cells were significantly different in the combination treatment and in the simple X-ray treatment at peak time. AT-406 treatment resulted in slow γH2AX foci decay after irradiation in both Hela and Siha cells (Fig. 4B). Under normoxic conditions, AT-406 markedly increased the induction and persistence of irradiation-induced γH2AX foci in Hela cells.

AT-406 reduced cIAP1 expression under normoxic condition and preferentially inhibited XIAP under hypoxic condition

The levels of the three most important members of the IAP family, namely, cIAP1, cIAP2, and XIAP, were measured in Hela and Siha cells to determine whether IAP family members are involved in the radiosensitizing effects of AT-406 in cervical cancer cell lines. Hela and Siha cells were treated with increasing AT-406 concentrations from 0 to 10 μM for 24 h under normoxic condition. Western blot analysis showed that all three members of the IAP family were strongly expressed in the tested cervical cancer cell line. AT-406 treatment significantly decreased cIAP1 expression in Hela cells, but had insignificant effect on cIAP2 and XIAP levels. However, none of the three IAP family members were affected by AT-406 treatment in Siha cells (Fig. 5A). The effect of AT-406 on hypoxic cells was also determined. The expression levels of HIF-1α, a cell hypoxia marker, in both cell lines significantly increased after exposure to 1% O2 for 24 h (Fig. 5B). AT-406 treatment failed to affect HIF-1α expression. The IAP-inhibiting activity of AT-406 in hypoxic cells was different from that in normoxic cells. The XIAP expression in both cell lines was downregulated by AT-406 treatment under hypoxic condition, but moderate cIAP1 decrease was still observed in the Hela cells after AT-406 treatment. The STAT3 and p-STAT3 protein expression levels in the control and AT-406-treated (1 or 10 μM) groups were compared. We found an association between inhibition of XIAP and decreasing phosphorylation of STAT3 in both Hela and Siha hypoxia cells (Fig. 5C).

AT-406 radiosensitization was associated with caspase activation in cervical cancer cells

The direct involvement of caspase activation in AT-
406 radiosensitization was determined. Western blot analysis demonstrated that AT-406 promoted cIAP-1 degradation and disrupted XIAP binding to active caspases in cervical cancer cells. Cells were treated with AT-406, radiation alone, or both AT-406 and irradiation under normoxic and hypoxic conditions, and caspase-3, -8, and -9 activities were measured (Fig. 6A). Table 1A shows that the combination treatment on Hela cells caused a 7-, 18.6-, and 13.8-fold activation of caspase-3, -8, and -9, respectively, under normoxic condition. The

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effect of the combination treatment was significantly superior to that of the single treatment, and such findings were supported by the same results under hypoxic conditions. AT-406 single treatment caused 3.1-, 9.8-, and 3.1-fold activation of caspase-3, -8, and -9, respectively. Neither single nor combination treatment affected the caspase activation of Siha cells, whereas AT-406 used as a single treatment caused 4.1-, 6.4-, and 4.6-fold activation of caspase-3, -8, and -9, respectively, under hypoxic conditions. AT-406 radiosensitization prior to irradiation caused 7.1-, 15.0-, and 13.2-fold activation of caspase-3, -8, and -9, respectively (Table 1B). The AT-406-induced radiosensitization of Hela cells was mediated by both extrinsic death receptors and intrinsic mitochondrial apoptosis pathways under normoxic conditions, whereas AT-406 merely activated relevant apoptosis pathways in Siha cells. However, AT-406-induced radiosensitization was mediated by the two apoptosis pathways in both Hela and Siha cells under hypoxic conditions. Pretreatment with the pan-caspase inhibitor Z-VAD-FMK completely negated the increase in radiation-induced apoptosis by AT-406, and Z-VAD-FMK treatment alone failed to affect cell apoptosis (Fig. 6B and Supplementary Fig. 2, available in the online version only).
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Discussion

Radiotherapy is a crucial cervical cancer treatment, but its outcomes are still far from satisfactory. Radiotherapy leads to poor five-year survival and locoregional control rate. The mechanism underlying the failure of radiotherapy involves molecular defects in the apoptosis pathway, and such defects are partly caused by the genetic mutation of cancer cells and the emergence of a hypoxic microenvironment. Overexpression of cIAP1, cIAP2, or XIAP is observed in many malignant tumors and is associated with radioresistance (23). Therefore, novel IAP antagonists that inhibit members of the IAP family were identified to enhance the effects of both chemotherapy and radiation therapy (24–29).

Cai et al. reported that AT-406 showed single-agent activity in multiple cancer types, such as breast and ovarian cancers (21). However, information is lacking on the efficacy and mechanism of action of AT-406 in cervical cancer as a single agent or in combination with front-line radiotherapy. AT-406 functioned as a potential radiosensitizer by enhancing apoptosis pathway activation in human cervical cancer cells. AT-406 itself was not cytotoxic, with an IC_{50} of > 50 µM after 24 h of application to Hela and Siha cells. AT-406 at 10 µM significantly enhanced the radiosensitivity of cancer cells. Irradiation inhibits tumor function primarily by inducing DSB formation. The γH2AX foci analysis demonstrated that AT-406 radiosensitized cervical cancer cells by inhibiting DSB repair. Flow cytometry was performed to further explore the mechanism underlying AT-406 radiosensitization. Radiation and AT-406 treatment showed synergistic effects on apoptosis induction in cervical cancer cell lines. The results of western blot analysis and caspase activation assay showed that AT-406, as an IAP antagonist, served an important function in both extrinsic and intrinsic apoptosis pathways by downregulating cIAP1 and XIAP in cervical cancer cells. AT-406 sensitized Hela cells to X-ray irradiation under normoxic conditions by downregulating cIAP1, but failed to affect Siha cells. For the first time, we suggest that AT-406 significantly inhibits IAP expression and promotes radiosensitivity of hypoxic cancer cells.

In a previous study, cIAP1 and XIAP were shown to directly bind specific caspases via their BIR domains. AT-406, a potent IAP antagonist, acted as an antagonist of IAPs. AT-406 binds to cIAP1 and XIAP proteins with Ki values of 66.4 and 1.9 nM, respectively (21). The cIAP1 expression, rather than XIAP expression, evidently increased in Hela cells after AT-406 treatment. Other studies also found that IAP antagonists induced rapid cIAP1 degradation, which is a key early event in IAP antagonist–induced apoptosis in sensitive cancer cells (28, 29). This phenomenon may be associated with the function of the RING-finger of cIAP1 as an active E3 center. The IAP antagonist AT-406 also triggers auto-ubiquitination and subsequent rapid proteasomal cIAP1 degradation (30). However, AT-406 treatment failed to downregulate the expression of any member of the IAP family in normoxic Siha cells. This failure to downregulate expression might explain the poor response of Siha cells to AT-406-mediated radiosensitization under normoxic conditions.

A different AT-406 function in IAP inhibition was observed in hypoxic cells. AT-406 treatment nearly completely eliminated XIAP expression in both Hela and

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**Table 1.** AT-406 radiosensitization is associated with caspase activation in cervical cancer cells

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<td></td>
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A) Effect of AT-406 on Hela cell caspase activation. B) Effect of AT-406 on Siha cell caspase activation.
Siha cells under hypoxic conditions and downregulated the cIAP1 level in Hela cells (Fig. 5B). Brunckhorst et al. demonstrated that AT-406-induced apoptosis is mainly correlated with XIAP downregulation (31). AT-406 exhibited high IAP inhibition under hypoxia, and the downregulation of these IAPs by AT-406 contributed to the enhancement of radiation-induced apoptosis in hypoxic cells. Thus, IAP antagonists have potential as sensitizing agents for radiotherapy in hypoxic cancer cell treatment. XIAP and cIAP1 degradation activated caspase activity and initiated both intrinsic and extrinsic apoptosis pathways. The abovementioned findings supported our conclusion that the radiosensitization of AT-406 was cancelled by the caspase inhibitor Z-VAD-FMK.

Many studies showed IAPs inhibitors could induce apoptosis through the inhibition of STAT3, NF-kB, and PPARγ in various types of human cancer cells (32 – 34). However, reports on the combination of IAP family members and hypoxic microenvironment are few. Kwon et al. suggested that hypoxia/reoxygenation induced the transient activation of PI3K/Akt/XIAP survival signaling pathways through the epidermal growth factor/Ras/Raf cascade in post-ischemic renal epithelial cells (35). Wang et al. found that the anti-tumor activity of XIAP inhibitor might be associated with cell growth and apoptosis, e.g., PI3K/Akt and JAK/STAT, in gastric cancer cells (36). Therefore, the PI3K/AKT and JAK/STAT pathway experiment showed that AT-406-induced XIAP inhibition was associated with reduced STAT3 phosphorylation. The mechanisms underlying AT-406 activity under hypoxia and the way by which AT-406 inhibits XIAP expression, which is not affected by AT-406 under normoxic condition, should be studied in the future.

We presented strong evidence that AT-406 potently sensitizes cervical cancer cells to X-ray irradiation under both normoxic and hypoxic conditions by decreasing cIAP1 or XIAP levels and activating caspase-related apoptosis pathways. These results support the use of IAP inhibitors as a part of a novel adjuvant approach to enhance the effects of radiotherapy in cervical cancer patients. More investigations should be performed to determine the in vivo radiosensitization effect of AT-406.

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