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ATM blocks tunicamycin-induced endoplasmic reticulum stress

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ABSTRACT

Endoplasmic reticulum stress (ER-stress) is associated with ataxia telangiectasia mutated (ATM) gene. We present here conclusive data showing that ATM blocks ER-stress induced by tunicamycin or ionizing radiation (IR). X-box protein-1 (XBP-1) splicing, GRP78 expression and caspase-12 activation were increased by tunicamycin or IR in Atm-deficient AT5BIVA fibroblasts. Activation of caspase-12 and caspase-3 by tunicamycin was significantly reduced in cells transfected with wild-type Atm (AT5BIVA/wtATM). Atm knockdown by siRNA, however, noticeably elevated ER-stress and chemosensitivity to tunicamycin. In summary, we present substantial data demonstrating that ATM blocks the ER stress signaling associated with cancer cell proliferation.

1. Introduction

Protein folding in the endoplasmic reticulum (ER) is carried out under the constant scrutiny of the ER quality control machinery [1]. The membrane of the ER in mammalian cells contains three sensors, PKR-like ER-resistant kinase (PERK), activating transcription factor 6 (ATF6) and inositol requiring enzyme 1 (IRE1) that can monitor the accumulation of unfolded proteins in the ER (ER stress) and activate elaborate defense mechanisms known collectively as the ER stress response to alleviate the burden of unfolded proteins [2–5], a signaling emanating from the ER that controls gene transcription, as well as protein translation. This ER to nucleus signaling cascade is referred to as the unfolded protein response (UPR), enhancing the clearance of misfolded proteins from the ER, and consequently alleviating ER stress [6]. ER stress has been associated with a variety of diseases including inflammation [7], neurodegenerative disorders such as Alzheimer's and Parkinson's diseases [8,9], and diabetes [10–12] and cancer [13–15].

Ataxia telangiectasia (A-T) is a human genetic disease whose hallmarks are genomic instability, neurodegeneration, immunodeficiency, premature aging, sterility, and a predisposition to cancer. ATM, the product of the A-T mutated gene (*Atm*) is a large, multifunctional kinase that regulates response to DNA double-strand breaks caused by ionizing radiation (IR), DNA damage agents, and DNA recombination. ATM is also involved in oxidative stress in the brain, testes, and thymus [16–20]. In this respect, Atm deficiency induced oxidative stress and ER stress in astrocytes [21]. Very recently, it was reported that ER stress and UPR are elevated in *Atm*-deficient thymocytes and thymic lymphoma cells [22]. However, functional role of ATM in ER stress induction has not yet been elucidated in detail.

In this study, using cells deficient in Atm expression, we show that ATM blocks ER stress induction and cell proliferation in response to tunicamycin. The role of ATM against ER stress was further confirmed in cells stably transfected with Atm plasmid either wild-type or kinase dead dominant negative mutant and with Atm SiRNA. We also present an unexpected result demonstrating that IR could clearly induce ER stress although IR has not been reported to trigger ER stress so far.

2. Materials and methods

2.1. Cell culture and transfection

The cell lines, human embryonic lung fibroblast MRC5CV1, Atm-deficient fibroblast AT5BIVA, AT5BIVA/mo, AT5BIVA/kdATM, and AT5BIVA/wtATM were maintained in RPMI 1640 medium supplemented with 2 mM L-glutamine, hydrocortisone (0.5 μg/ml) and 10% heat-inactivated fetal bovine serum, and were cultured in a humidified CO_2 incubator at 37 °C. Cells in the exponential phase

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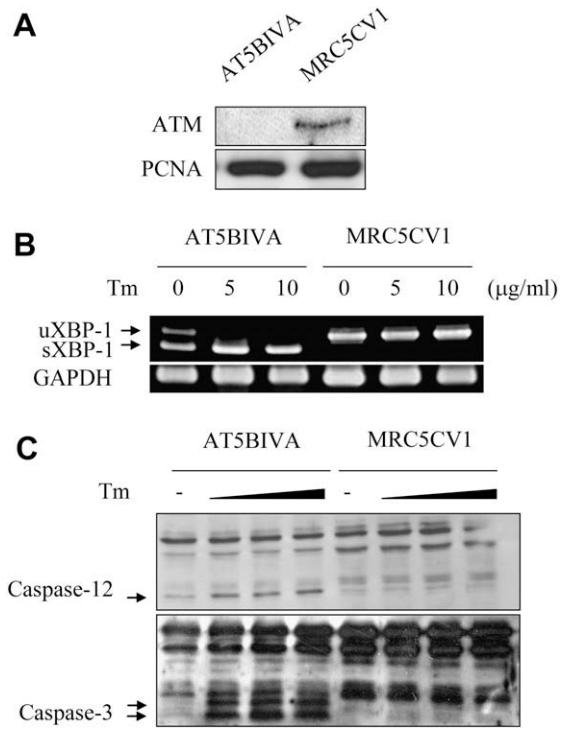

Fig. 1. ER stress-mediated activation of caspase-12 and caspase-3 in Atm deficient fibroblasts. Both AT5BIVA and MRC5CV1 cells were incubated in RPMI1640 medium with or without tunicamycin for 24 h. (A) Absence of ATM expression in AT5BIVA fibroblasts. Nuclear fraction was subjected to western blot analysis and immunoblotted with a specific antibody to ATM or PCNA. (B) Tunicamycin (Tm) increases ER-stress in Atm deficient cells. Total RNA was extracted for both the cells treated with tunicamycin for 24 h, and PR-PCR analysis was performed for the detection of XBP-1 splicing and GRP78 expression. To amplify XBP-1 mRNA, PCR was done for 35 cycles [94 °C, 30 s; 55 °C, 30 s; 72 °C, 1 min (7 min in the final cycle)] using primers 5′-AACTCCAGCTAGAAAATCAGC-3′ and 5′-CCATGGGAAGATGTTCTGGG-3′. Fragments, representing spliced (sXBP-1, 215 bp) and unspliced (uXBP-1, 241 bp), were detected by running on 2% agarose gel and staining with ethidium bromide. (C) Caspase activation by tunicamycin. Total cell lysate was subjected to western blot analysis and immuno-blotted with antibodies to caspase-12, caspase-3 and PARP.

of growth were transfected with appropriate plasmids with the use of lipofectamine (2 μg/ml; Invitrogene).

2.2. Antibodies

Antibodies to caspase-3 was from Calbiochem (San Diego, CA). Antibody to ATM was obtained from Oncogene (Cambridge, MA). Antibodies to caspase-12 and caspase-7 were purchased from Stratagene (Ann Arbor, Michigan). Antibodies to PARP, PCNA and GAPDH were from Santa Cruz Biotechnology (Santa Cruz, CA). And all the other antibodies were purchased from Sigma (St. Louis, MO, USA).

2.3. Materials

RPMI 1640 and fetal bovine serum were from Invitrogen (Carlsbad, CA). Sephadex G-25 columns and enhanced chemiluminescence reagents were purchased from Amersham Pharmacia Biotech (Amersham, NJ). Polyvinylidene difluoride (PVDF, 0.22 μm) membrane was obtained from Bio-Rad (Hercules, CA). Luciferase activity was measured with a detection kit from Promega (Madison, WI). Atm siRNA was obtained from Santa Cruz Biotechnology. CCK-8 solution was purchased from Dojindo Molecular Technologies Inc. (Rockville, MD). DEVD-fmk was from Calbiochem. Tunicamycin and all the other reagents were purchased from Sigma.

2.4. Measurement of XBP-1 splicing

To amplify XBP-1 mRNA, PCR was done for 35 cycles [94 °C, 30 s; 55 °C, 30 s; 72 °C, 1 min (7 min in the final cycle)] using primers 5′-AACTCCAGCTAGAAAATCAGC-3′ and 5′-CCATGGGAAGATGTTCTGGG-3′. Fragments, representing spliced (sXBP-1, 215 bp) and unspliced (uXBP-1, 241 bp), were detected by running on 2% agarose gel and staining with ethidium bromide.

2.5. Real-time PCR

After cell stimulation with an appropriate agent and lysis, total RNA was extracted and the expression of GRP78 was determined by real-time PCR using the primers 5′-CATCACGCCGTCCTATG TCG-3′ and 5′-CGTCAAAGACCGTGTTCTCG-3′.

2.6. Luciferase reporter gene assay

Cells were subjected to transient transfection by incubation for 16 h with the luciferase reporter plasmid pGRP78-Luc containing the promoter region of GRP78 chaperone or with a mock vector using lipofectamine reagent. At appropriate times after exposure to tunicamycin, cells were lysed in reporter lysis buffer, the lysate was centrifuged for 2 min at 14000×*g*, and the supernatant was used for the measurement of luciferase activity with a kit.

2.7. Immunoblotting analysis

After growth to subconfluency in 100 or 150 mm dishes, cells were harvested by centrifugation and washed with ice-cold phosphate-buffered saline (PBS). Cell pellet was suspended in a hypotonic solution, a solution containing 10 mM HEPES–KOH (pH 7.9), 10 mM KCl, 2 mM $MgCl_2$, 0.1 mM EDTA, 0.2 mM NaF, 0.4 mM phenylmethylsufonyl fluoride (PMSF), leupeptin (10 μg/ml), aprotinin (10 μg/ml), 0.1 mM Na_3VO_4 and 1 mM DTT and incubated on ice for 12 min. After the addition of NP-40 to a final concentration of 0.15% the lysate was vigorously mixed for 15 s and then centrifuged at 15000 rpm for 1 min at 4 °C. The resulting supernatant was stored at −70 °C as the cytoplasmic extract, and the nuclear pellet was resuspended in a high salt buffer, a solution containing 50 mM HEPES–KOH (pH 7.9), 50 mM KCl, 300 mM NaCl, 0.1 mM EDTA, 0.2 mM NaF, leupeptin (10 μg/ml), aprotinin (10 μg/ml), 0.4 mM PMSF, 0.1 mM Na_3VO_4, 1 mM DTT and 10% glycerol. The resulting suspension was incubated for 30 min on ice with occasional vortex and then centrifuged at 15000 rpm for 30 min at 4 °C. The protein concentration was determined with Bio-Rad protein assay reagent. Equal amounts of cytoplasmic or nuclear extract were subsequently fractionated by 10–15% SDS–PAGE. The fractionated proteins were transferred to a polyvinylidene difluoride (PVDF) membrane. Membranes were blocked in 5% skim milk (or non-fat dry milk) diluted in PBS (or TBS) containing 0.1% Tween-20 for 1 h and incubated with the primary antibodies, at 4 °C, overnight. The secondary antibodies were used at a 1:1000 dilution. Immune complexes were detected with enhanced chemiluminescence reagents.

2.8. Cell Counting Kit-8 (CCK-8) assay

For cell proliferation assay, semi-confluent cells on a 24 well plate were transfected with SiRNA for 4 h and incubated in the presence of tunicamycin for 24 h. After adding 50 μl of the CCK-8 solution to each well of the culture plate for 2 h, an aliquot (100 μl) from each well was transferred to 96 well microtiter plate and the absorbance was measured using a microplate reader (Dynatech MR700) at 450 nm with a reference wavelength at 650 nm.

3. Results

3.1. ATM inhibits tunicamycin-induced ER stress cascade in ATM deficient cells

We previously reported enhanced caspase-3 degradation and apoptosis in Atm deficient AT5BIVA fibroblasts but not in MRC5CV1 cells normally expressing ATM [23] (Fig. 1A). In order to determine whether ER stress is involved in increased apoptotic death of *Atm*-deficient cells, both AT5BIVA and MRC5CV1 cells were treated with tunicamycin at 5–10 μg/ml for 6 h. We found that tunicamicin significantly induced XBP-1 splicing only in Atm-deficient cells (Fig. 1B). Spliced XBP-1 (sXBP-1, 215 bp) from its unspliced form (uXBP-1, 241 bp), in turn, can bind to the ER-stress response element (ERSE) of genes involved in translational attenuation and apoptosis, and of chaperons including GRP78/Bip. When both the cells were treated with tunicamycin at varying concentrations for 24 h, a hallmark of ER stress-mediated apoptosis, caspase-12, was found to be activated in AT5BIVA but not in MRC5CV1 cells, and the downstream caspase-3 activation and subsequent PARP cleavage also occurred only in *Atm*-deficient cells (Fig. 1C). These results suggested that increased ER stress induction in *Atm*-deficient cells could be associated enhanced chemosensitization of the cells to tunicamycina and other chemical chaperones.

3.2. ER stress induction by tunicamycin and ionizing radiation could be reduced in cells stably transfected with wild-type ATM

Since ER stress was increased in *Atm*-deficient thymic lymphoma cells [22] and in AT5BIVA (Fig. 1), it could be expected that ATM might inhibit the tunicamycin-induced ER stress. When AT5-BIVA cells expressing wild-type ATM (wt) or mock vector (mo) were treated with tunicamycin, it was revealed that XBP-1 mRNA splicing was enhanced in *Atm*-deficient AT5BIVA/mo cells in response to tunicamycin while it was very weak in AT5BIVA/wtATM cells (Fig. 2A). Unexpectedly, ionizing radiation (IR) could also

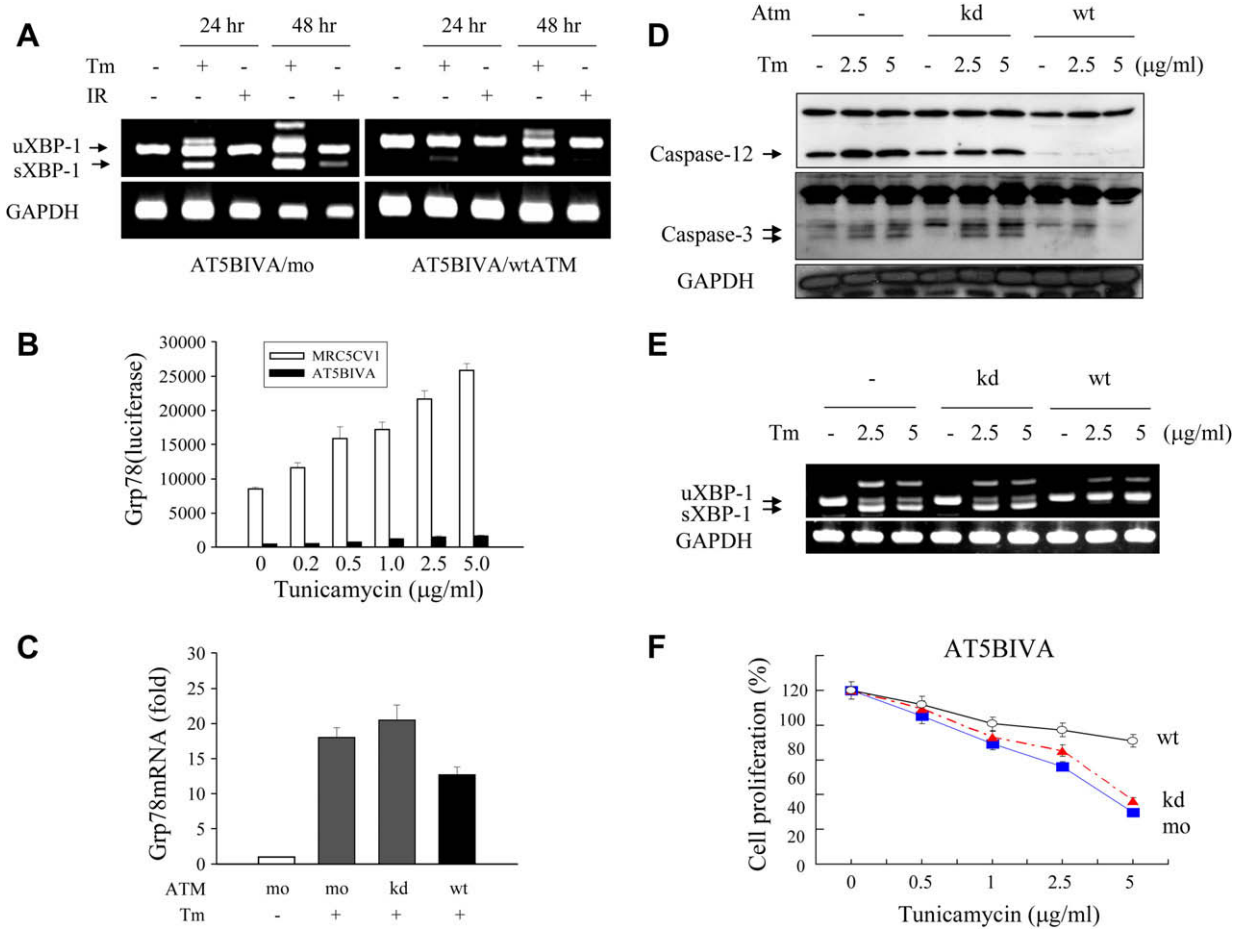

Fig. 2. ATM inhibits tunicamycin-induced XBP-1 splicing and GRP78 expression but enhances chemoresistance. (A) Inhibition of XBP-1 splicing by ATM. Both AT5BIVA and AT5BIVA/wtATM cells transfected with mock vector and wild-type Atm, respectively, were challenged with tunicamycin at 2 μg/ml for different times. Total RNA was extracted and subjected to RT-PCR analysis for measurement of XBP-1 mRNA splicing. The arrow indicates spliced form of XBP-1. (B) Reporter gene assay showing inhibition of tunicamycin-induced GRP78 expression in ATM expressing cells. Both AT5BIVA and MRC5CV1 cells were transfected with pGRP78-luc plasmid in lipofectamine for 16 h and then treated with tunicamycin for another 6 h at various concentrations. Luciferase activity was measured with a detection kit according to the methods provided by the manufacturer. (C) Real-time PCR analysis for the determination of the inhibitory effect of ATM on GRP78 expression by tunicamycin. AT5BIVA cells transfected with mock vector (mo), kinase dead mutant (kd) or wild-type (wt) Atm were incubated in RPMI1640 medium in the presence of tunicamycin (2 μg/ml) for 24 h. After cell lysis, total RNA was extracted and the expression of GRP78 was determined by real-time PCR using the primers 5′-CATCACGCCGTCCTATGTCG-3′ and 5′-CGTCAAAGACCGTGTTCTCG-3′. All the bars are means ± S.E. from a representative triplicate experiment. (D) Western blot analysis for the evaluation of proteins involved in apoptotic cell death. All three cell lines, AT5BIVA, AT5BIVA/kdATM and AT5BIVA/wtATM were treated with tunicamycin (Tm) for 24 h, lysed, and the total cell lysates were subjected to western blot analysis and immuno-blotted with specific antibodies to caspase-12 and caspase-3. (E) Reduction of ER stress by ATM. Cells were treated with tunicamycin for 24 h, lysed, and the total RNA extracted was subjected to RT-PCR analysis for measurement of XBP-1 mRNA splicing. Unspliced (uXBP-1) and spliced (sXBP-1) forms were marked by arrows. (F) ATM increases chemoresistance to tunicamycin. All three cell lines, AT5BIVA, AT5BIVA/kdATM and AT5BIVA/wtATM were treated with tunicamycin for 48 h in 96 multititer plate, and the cell proliferation was measured with a cell counting kit (CCK-8) solution as described in Section 2.

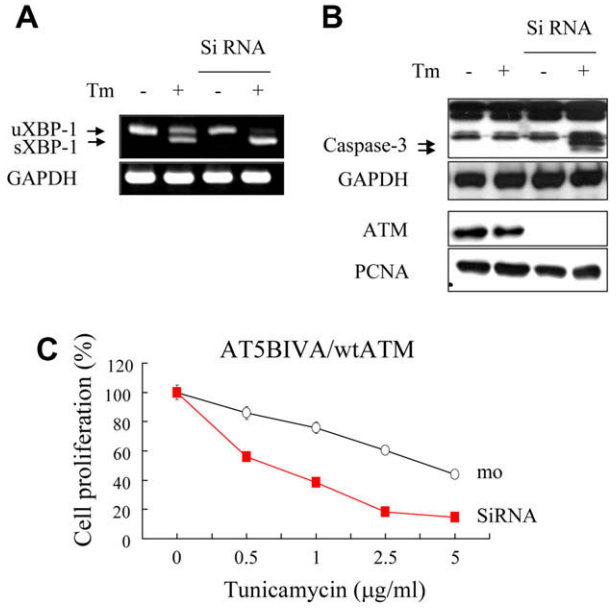

Fig. 3. Atm SiRNA enhances tunicamycin-induced ER stress, caspase-3 activation and chemosensitivity by Atm SiRNA. (A) Inhibition of ATM expression enhances tunicamycin-induced XBP-1 splicing. AT5BIVA/wtATM cells were transfected with Atm siRNA, treated with tunicamycin (2.5 μg/ml) for 48 h, and the total RNA was prepared for RT-PCR analysis with appropriate primers to XBP-1 gene. (B) Inhibition of ATM expression enhances tunicamycin-induced caspase-3 activation. AT5BIVA/wtATM cells transfected with Atm siRNA and treated with tunicamycin (2.5 μg/ml) for 48 h were lysed and the total cell lysate was subjected to western blot analysis and immuno-blotted with a specific antibody to caspase-3. The nuclear fraction was used for the detection of ATM level. (C) Inhibition of ATM expression enhances chemosensitivity to tunicamycin. AT5BIVA/wtATM cells transfected with Atm siRNA or mock vector were treated with tunicamycin for 48 h in 24 well plate and the cell proliferation was evaluated as above. All the points show means ± S.E. from a representative triplicate experiment.

induce XBP-1 splicing in AT5BIVA/mo cells although not strong. In some cases depending on the culture conditions, however, we could also see upper-most XBP-1 band demonstrating the heterodimer form of uXBP-1/sXBP-1. Binding of spliced XBP-1 to the promoter of GRP78/BiP chaperone is required for its efficient expression [24,25]. When both AT5BIVA and MRC5CV1 cells were transfected with pGRP78-luc reporter gene and treated with tunicamycin, GRP78 expression was significantly increased in AT5BIVA while there were little differences in MRC5CV1 cells (Fig. 2B). Real-time PCR analysis also showed that the extent of GRP78 expression by tunicamycin was significantly reduced in AT5BIVA cells transfected with wild-type Atm compared to the cells expressing mock vector or kinase dead mutant Atm (kd) (Fig. 2C). All these results indicate that ATM reduces tunicamycin-induced ER stress induction by interfering with XBP-1 splicing and subsequent GRP78 chaperone expression.

3.3. ATM blocks tunicamycin-induced caspase-12 activation and cell apoptosis

Caspase-12 activation has been implicated in ER stress-mediated apoptosis [26,27]. To examine whether ATM inhibition of XBP-1 splicing is closely associated with caspase-12 activation, AT5BIVA cells harboring mock, kinase dead or wild-type Atm were treated with various concentrations of tunicamycin for 24 h. Western blot analysis showed that activation of caspase-12 and caspase-3 in response to tunicamycin was only inhibited in AT5BIVA/wtATM cells (Fig. 3D). RT-PCR analysis also revealed the reduced XBP-1 splicing in AT5BIVA/wtATM cells (Fig. 3E), suggesting the inhibitory effect of ATM against IRE-1 mediated XBP-1 splicing and caspase-12 activation.

In an attempt to determine whether ATM is a key regulatory factor for ER stress-mediated cell death, cell proliferation of AT5BIVA cells harboring mock, kinase dead or wild-type Atm was measured at 48 h after tunicamycin treatment. It was revealed that AT5BIVA/wt cells showed resistance to tunicamycin when compared to the other two cells (Fig. 3F).

3.4. ATM knockout enhances the tunicamycin-induced ER stress, caspase-3 activation and cell apoptosis

ATM inhibition of ER stress was further confirmed when AT5BIVA/wtATM cells were transfected with ATM siRNA for 48 h. XBP-1 splicing in AT5BIVA/wtATM cells was rarely detectable at 24 h after tunicamycin treatment, however, it could weakly be seen at 48 h (Fig. 2A). When tunicamycin was treated to AT5BIVA/wtATM cells for 48 h with Atm siRNA, it was found that XBP-1 splicing was enhanced by Atm siRNA (Fig. 3A). Caspase-3 activation was also increased in cells transfected with Atm siRNA (Fig. 3B). Accordingly, Atm siRNA transfection of AT5BIVA/wt cells enhanced chemosensitivity to tunicamycin (Fig. 3C). These results clearly show that ATM protects cells from ER stress-induced cell death.

3.5. Requirement of caspase-3 activation for ER-stress-induced cell death by tunicamycin

To confirm that caspase-3 activation by tunicamycin was responsible for the increased death of Atm-deficient cells, AT5BIVA cells were pretreated with a caspase-3 specific inhibitor DEVD-fmk for 30 min before tunicamycin treatment. PARP cleavage, a marker of caspase-3 activation, was inhibited by DEVD-fmk (Fig. 4A). Moreover, AT5BIVA cells showed resistance to tunicamycin-induced cell death when pretreated with DEVD-fmk (Fig. 4B). These results indicate that increased caspase-3 activation in the

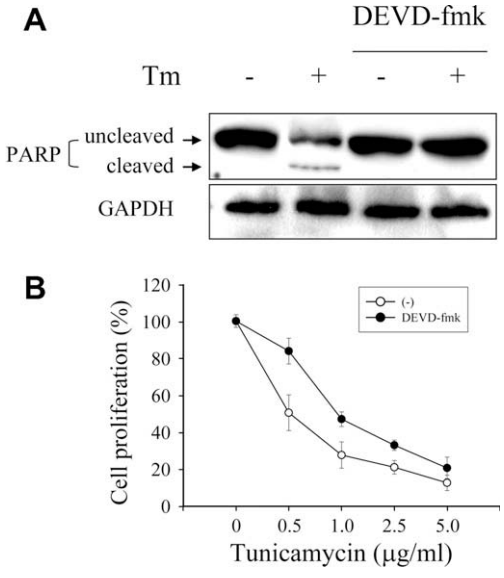

Fig. 4. Caspase-3 is associated with tunicamycin-induced cell death. (A) Caspase-3 inhibitor DEVD-fmk reduces PARP cleavage. AT5BIVA cells were treated with DEVD-fmk at 30 μM for 30 prior to tunicamucin treatment (2.5 μg/ml) for another 48 h. After cell lysis, the lysate was subjected to western blot analysis for the detection of PARP and GAPDH. (B) DEVD-fmk increases survival of AT5BIVA cells in response to tunicamycin. AT5BIVA cells were treated with tunicamycin for 48 h in the presence or absence of caspase-3 inhibitor DEVD-fmk, and the cell proliferation was measured with a cell counting kit (CCK-8) solution as described in Section 2. All the points show means ± S.E. from a representative triplicate experiment.

absence of ATM plays a key role in cell death in response to tunicamyin.

4. Discussion

Although ATM deficiency has been associated with ER stress induction [21,22], until now, no reports have demonstrated that ionizing radiation (IR) elicits ER stress induction. Unexpectedly, our study showed that IR can induce ER stress (Fig. 2A). The reason for the failure of detection of ER stress induction in response to IR by other groups could be that different level of ATM is expressed depending on cell types. In our study, ATM is expressed in AT5BIVA cells but too quickly degraded to be detectable (Fig. 1A). XBP-1 splicing could only be detectable after two days of IR exposure to AT5BIVA cells (Fig. 2A). Thus, although more detailed examination of IR-induced ER-stress is required, this result is the first implicating a new biological function of IR for cell stress regulation and survival.

On the other hand, the observation that ER stress induction by either IR or tunicamycin could be blocked by ATM expression (Fig. 2A) suggests a possibility that not only tunicamycin but also other chemical chaperones could be negatively affected by ATM in ER stress induction. In this respect, ATM seems to play a pivotal role for stress-related human diseases. Atm SiRNA was used in our study to determine the association of ATM with ER stress and apoptosis (Fig. 3). Although wortmannin is known to be effective against ATM kinase activity, the high concentration required for ATM inhibition [28] affects other cellular proteins. So, a compound specifically inhibit ATM could help unravel the function of ATM in ER stress signaling.

Atm knockdown by SiRNA augmented the tunicamycin-induced XBP-1 splicing as well as caspase-3 activation in AT5BIVA/wtATM cells (Fig. 3A and B). In the absence of SiRNA, however, an unexpected result was that caspase-3 activation could not be seen although XBP-1 splicing was apparent when AT5BIVA/wtATM cells were treated with tunicamycin for 48 h (Fig. 3A and B, second lanes). Hence, two possibilities could be raised from these results; one is that XBP-1 splicing was not sufficient for caspase-3 activation in AT5BIVA/wtATM cells treated with tunicamycin. Another factor affected by ATM could be crucial in this respect. Since ATM controls proteins of diverse biological functions including cell cycle and apoptosis [29,30], cooperative or synergistic activity of XBP-1 with other factors might enhance caspase-3 activation. Another possibility is that XBP-1 splicing by tunicamycin did not reach the threshold level for capase-3 activation. However, based on the finding that even increasing the concentration of tunicamycin could not induce caspase-3 activation (data not shown), it could be strongly suggested that another factor affected by ATM could synergistically regulate the XBP-1-mediated caspase-3 activation.

There has been accumulating evidences demonstrating that ER stress is closely associated with cell apoptosis. It was previously reported that bortezomib (PS-341, Velcade), a potent anticancer drug candidate, sensitized pancreatic cancer cells to ER stress-mediated apoptosis [31]. In addition, ER stress-dependent upregulation of DR5 induced pancreatic cancer cell apoptosis [32]. In our study, ATM knockdown by SiRNA enhanced the apoptotic death of AT5BIVA/wtATM cells (Fig. 3C). Conversely, Atm transfection into AT5BIVA cells increased the chemoresistance of the cells (Fig. 2F). Since ATM is a sensor of IR, and tunicamycin increased the radiosensitization of PC3 prostate cancer cells (data not shown), our results suggest that a specific ATM inhibitor could be a useful enhancer of chemosensitization of cancer cells.

Although Atm deficiency has been reported to induce ER stress through oxidative stress [18,19], there has been no conclusive evidence that ATM prevents the ER stress. Our data clearly show that IR- or tunicamyin-induced ER stress could be inhibited by ATM. Hence, it is suggested that regulation of ATM could be an efficient way for enhanced chemosensitization of tumor cells through ER stress-mediation.

Acknowledgements

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References

[1] Ellgaard, L. and Helenius, A. (2001) ER quality control: towards an understanding at the molecular level. Curr. Opin. Cell Biol. 13 (4), 431–437.
[2] Kaufman, R.J. (1999) Stress signaling from the lumen of the endoplasmic reticulum: coordination of gene transcriptional and translational controls. Genes Dev. 13 (10), 1211–1233.
[3] Mori, K. (2000) Tripartite management of unfolded proteins in the endoplasmic reticulum. Cell 101 (5), 451–454.
[4] Patil, C. and Walter, P. (2001) Intracellular signaling from the endoplasmic reticulum to the nucleus: the unfolded protein response in yeast and mammals. Curr. Opin. Cell Biol. 13 (3), 349–355.
[5] Urano, F., Bertolotti, A. and Ron, D. (2000) IRE1 and efferent signaling from the endoplasmic reticulum. J. Cell Sci. 113 (Pt 21), 3697–3702.
[6] Travers, K.J., Patil, C.K., Wodicka, L., Lockhart, D.J., Weissman, J.S. and Walter, P. (2000) Functional and genomic analyses reveal an essential coordination between the unfolded protein response and ER-associated degradation. Cell 101 (3), 249–258.
[7] Wellen, K.E. and Hotamisligil, G.S. (2005) Inflammation, stress, and diabetes. J. Clin. Invest. 115 (5), 1111–1119.
[8] Imai, Y., Soda, M., Inoue, H., Hattori, N., Mizuno, Y. and Takahashi, R. (2001) An unfolded putative transmembrane polypeptide, which can lead to endoplasmic reticulum stress, is a substrate of Parkin. Cell 105 (7), 891–902.
[9] Katayama, T., Imaizumi, K., Sato, N., Miyoshi, K., Kudo, T., Hitomi, J., Morihara, T., Yoneda, T., Gomi, F., Mori, Y., Nakano, Y., Takeda, J., Tsuda, T., Itoyama, Y., Murayama, O., Takashima, A., St George-Hyslop, P., Takeda, M. and Tohyama, M. (1999) Presenilin-1 mutations downregulate the signalling pathway of the unfolded-protein response. Nat. Cell Biol. 1 (8), 479–485.
[10] Gu, F., Nguyen, D.T., Stuible, M., Dube, N., Tremblay, M.L. and Chevet, E. (2004) Protein–tyrosine phosphatase 1B potentiates IRE1 signaling during endoplasmic reticulum stress. J. Biol. Chem. 279 (48), 49689–49693.
[11] Nakatani, Y., Kaneto, H., Kawamori, D., Yoshiuchi, K., Hatazaki, M., Matsuoka, T.A., Ozawa, K., Ogawa, S., Hori, M., Yamasaki, Y. and Matsuhisa, M. (2005) Involvement of endoplasmic reticulum stress in insulin resistance and diabetes. J. Biol. Chem. 280 (1), 847–851.
[12] Ozcan, U., Cao, Q., Yilmaz, E., Lee, A.H., Iwakoshi, N.N., Ozdelen, E., Tuncman, G., Gorgun, C., Glimcher, L.H. and Hotamisligil, G.S. (2004) Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. Science 306 (5695), 457–461.
[13] Leclerc, D. and Rozen, R. (2008) Endoplasmic reticulum stress increases the expression of methylenetetrahydrofolate reductase through the IRE1 transducer. J. Biol. Chem. 283 (6), 3151–3160.
[14] Lovat, P.E., Corazzari, M., Armstrong, J.L., Martin, S., Pagliarini, V., Hill, D., Brown, A.M., Piacentini, M., Birch-Machin, M.A. and Redfern, C.P. (2008) Increasing melanoma cell death using inhibitors of protein disulfide isomerases to abrogate survival responses to endoplasmic reticulum stress. Cancer Res. 68 (13), 5363–5369.
[15] Shuda, M., Kondoh, N., Imazeki, N., Tanaka, K., Okada, T., Mori, K., Hada, A., Arai, M., Wakatsuki, T., Matsubara, O., Yamamoto, N. and Yamamoto, M. (2003) Activation of the ATF6, XBP1 and grp78 genes in human hepatocellular carcinoma: a possible involvement of the ER stress pathway in hepatocarcinogenesis. J. Hepatol. 38 (5), 605–614.
[16] Barlow, C., Dennery, P.A., Shigenaga, M.K., Smith, M.A., Morrow, J.D., Roberts 2nd, L.J., Wynshaw-Boris, A. and Levine, R.L. (1999) Loss of the ataxia-telangiectasia gene product causes oxidative damage in target organs. Proc. Natl. Acad. Sci. USA 96 (17), 9915–9919.
[17] Browne, S.E., Roberts 2nd, L.J., Dennery, P.A., Doctrow, S.R., Beal, M.F., Barlow, C. and Levine, R.L. (2004) Treatment with a catalytic antioxidant corrects the

neurobehavioral defect in ataxia-telangiectasia mice. Free Radic. Biol. Med. 36 (7), 938–942.
[18] Chen, P., Peng, C., Luff, J., Spring, K., Watters, D., Bottle, S., Furuya, S. and Lavin, M.F. (2003) Oxidative stress is responsible for deficient survival and dendritogenesis in purkinje neurons from ataxia-telangiectasia mutated mutant mice. J. Neurosci. 23 (36), 11453–11460.
[19] Ito, K., Hirao, A., Arai, F., Matsuoka, S., Takubo, K., Hamaguchi, I., Nomiyama, K., Hosokawa, K., Sakurada, K., Nakagata, N., Ikeda, Y., Mak, T.W. and Suda, T. (2004) Regulation of oxidative stress by ATM is required for self-renewal of haematopoietic stem cells. Nature 431 (7011), 997–1002.
[20] Kamsler, A., Daily, D., Hochman, A., Stern, N., Shiloh, Y., Rotman, G. and Barzilai, A. (2001) Increased oxidative stress in ataxia telangiectasia evidenced by alterations in redox state of brains from Atm-deficient mice. Cancer Res. 61 (5), 1849–1854.
[21] Liu, N., Stoica, G., Yan, M., Scofield, V.L., Qiang, W., Lynn, W.S. and Wong, P.K. (2005) ATM deficiency induces oxidative stress and endoplasmic reticulum stress in astrocytes. Lab. Invest. 85 (12), 1471–1480.
[22] Yan, M., Shen, J., Person, M.D., Kuang, X., Lynn, W.S., Atlas, D. and Wong, P.K. (2008) Endoplasmic reticulum stress and unfolded protein response in Atm-deficient thymocytes and thymic lymphoma cells are attributable to oxidative stress. Neoplasia 10 (2), 160–167.
[23] Kwon, O., Kim, K.A., He, L., Kim, S.O., Kim, M.S., Cha, E.Y., Yoon, B.D., Sok, D.E., Jung, M., Ahn, J.S. and Kim, B.Y. (2008) Ionizing radiation can induce GSK-3beta phosphorylation and NF-kappaB transcriptional transactivation in ATM-deficient fibroblasts. Cell Signal. 20 (4), 602–612.
[24] Rao, R.V. and Bredesen, D.E. (2004) Misfolded proteins, endoplasmic reticulum stress and neurodegeneration. Curr. Opin. Cell Biol. 16 (6), 653–662.
[25] Yoshida, H., Matsui, T., Yamamoto, A., Okada, T. and Mori, K. (2001) XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. Cell 107 (7), 881–891.
[26] Szegezdi, E., Fitzgerald, U. and Samali, A. (2003) Caspase-12 and ER-stress-mediated apoptosis: the story so far. Ann. N.Y. Acad. Sci. 1010, 186–194.
[27] Yoneda, T., Imaizumi, K., Oono, K., Yui, D., Gomi, F., Katayama, T. and Tohyama, M. (2001) Activation of caspase-12, an endoplastic reticulum (ER) resident caspase, through tumor necrosis factor receptor-associated factor 2-dependent mechanism in response to the ER stress. J. Biol. Chem. 276 (17), 13935–13940.
[28] Li, N., Banin, S., Ouyang, H., Li, G.C., Courtois, G., Shiloh, Y., Karin, M. and Rotman, G. (2001) ATM is required for IkappaB kinase (IKKk) activation in response to DNA double strand breaks. J. Biol. Chem. 276 (12), 8898–8903.
[29] Barlow, C., Brown, K.D., Deng, C.X., Tagle, D.A. and Wynshaw-Boris, A. (1997) Atm selectively regulates distinct p53-dependent cell-cycle checkpoint and apoptotic pathways. Nat. Genet. 17 (4), 453–456.
[30] Morgan, S.E. and Kastan, M.B. (1997) P53 and ATM: cell cycle, cell death, and cancer. Adv. Cancer Res. 71, 1–25.
[31] Nawrocki, S.T., Carew, J.S., Pino, M.S., Highshaw, R.A., Dunner Jr., K., Huang, P., Abbruzzese, J.L. and McConkey, D.J. (2005) Bortezomib sensitizes pancreatic cancer cells to endoplasmic reticulum stress-mediated apoptosis. Cancer Res. 65 (24), 11658–11666.
[32] Abdelrahim, M., Newman, K., Vanderlaag, K., Samudio, I. and Safe, S. (2006) 3, 3'-diindolylmethane (DIM) and its derivatives induce apoptosis in pancreatic cancer cells through endoplasmic reticulum stress-dependent upregulation of DR5. Carcinogenesis 27 (4), 717–728.