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Inhibition of Autophagy Increases 2-Methoxyestradiol-Induced Cytotoxicity in SW1353 Chondrosarcoma Cells

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ABSTRACT
Chondrosarcoma is a cartilage tumor and is the second most common malignant bone cancer. Unlike many tumors, chondrosarcomas are resistant to conventional chemotherapy and radiotherapy. Autophagy is a homeostatic mechanism through which cellular proteins and organelles are subjected to lysosomal degradation and recycling. Autophagy could play a dual role in cancer by facilitating either cell death or cell survival. To determine whether autophagy plays a role in cell death in chondrosarcoma, we have studied the effect of the anti-tumor compound 2-methoxyestradiol (2-ME) in chondrosarcoma cells in culture. Transmission electron microscopy imaging indicates that 2-ME treatment leads to the accumulation of autophagosomes in human chondrosarcoma (SW1353 and Hs819T) cells. Also, 2-ME induces the conversion of microtubule-associated protein LC3-I to LC3-II, a protein marker that is correlated with the formation of autophagosomes. Our results show that siRNAs directed against ATG3 blocks 2-ME-induced autophagosome formation in chondrosarcoma cells. In addition, treatment with Bafilomycin A1 (Baf) and 3-methyladenine (3-MA), the inhibitors of autophagy, further increased the cell death in 2-ME-treated chondrosarcoma cells. Taken together, our studies demonstrate that autophagy causes resistance to cytotoxicity in chondrosarcoma cells, and the efficacy and anti-tumor effects of drugs in chondrosarcoma could be enhanced by modulating autophagy. J. Cell. Biochem. 117: 751–759, 2016. © 2015 Wiley Periodicals, Inc.

KEY WORDS: AUTOPHAGY; 2-METHOXYESTRADIOL; CHONDROSARCOMA; SW1353

Chondrosarcoma is a rare tumor with an estimated incidence of 1 per 200,000 per year in the US but it accounts for 16–20% of bone sarcomas [Unni, 1996]. The majority of chondrosarcoma patients are older than 40 years [Giuffrida et al., 2009]. Although chondrosarcoma grows slowly and rarely metastasizes, the current treatment options are limited to surgical procedures due to the radio- and chemo-resistance of this tumor [Gelderblom et al., 2008]. The mechanism of chemoresistancy has been previously investigated in chondrosarcoma, but has not been fully understood [Bovee et al., 2005]. Since there is a lack of systemic treatment the survival rate in chondrosarcoma patients has stayed the same for decades, and hence there is a demand for effective chemotherapeutics [Damron et al., 2007].

2-ME is a metabolite of mammalian estrogen, 17β-estradiol (E_2) which has been shown to induce apoptosis in chondrosarcoma and other tumor cells [Zhu and Conney, 1998]. 2-ME and its analogs have been reported to induce apoptosis and autophagy in many tumors but not in chondrosarcoma [Chen et al., 2008]. Autophagy is an evolutionarily conserved process that maintains a balance between synthesis and degradation of cell contents, for example, organelles and proteins especially during energy deprivation or metabolic stress [Klionsky and Emr, 2000; Green and Levine, 2014]. In cancer, autophagy seems to have a dual role as it could increase stress tolerance and survival of tumor cells or it could lead to tumor suppression and cell death [Yang et al., 2011]. Various pathways by which 2-ME mediates its anti-tumor effects have been studied in many cell types [D'Amato et al., 1994; Mukhopadhyay and Roth, 1997; Maran et al., 2002; LaVallee et al., 2003; Shogren et al., 2007; Ganapathy et al., 2009; Benedikt et al., 2010; Koganti et al., 2014; Ma et al., 2014; Si et al., 2014;

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Chen et al., 2015; Lee and Nevo, 2015]. Although 2-ME has been shown to inhibit chondrosarcoma cell growth [Fong et al., 2007], the molecular mechanism of action of 2-ME in chondrosarcoma cells has not been completely understood. In this study, we have investigated whether autophagy plays a role in the anti-growth actions of 2-ME in chondrosarcoma cells.

MATERIALS AND METHODS

CELL CULTURE

SW1353 and Hs819T cells purchased from the American Type Culture Collection (ATCC) were cultured in 10% FBS DMEM/F12 media at 37°C and treated with vehicle control, 2-ME, 17β-estradiol (E_2), Bafilomycin A1 (Baf), and 3-Methyladenine (3-MA) as indicated. Cell growth was monitored by plating chondrosarcoma cells at 5×10^4 cells per well into 24-well plates containing 1 ml/well medium. After allowing the cells to attach overnight, the media in the wells were replaced with fresh 1 ml medium. The cells were treated with the test compounds or vehicle and maintained for 72 h. At the end of treatment, the cell viability was measured by MTS-based cell viability assay systems as per the manufacturer's protocol using CellTiter 96® Aqueous One Solution Cell Proliferation Assay Kit, purchased from Promega (Madison, WI).

TRANSMISSION ELECTRON MICROSCOPY (TEM)

SW1353 and Hs819T cells were grown on aclar in 12-well plates and were treated with Vehicle (Veh), 2-ME (20 μM), and 17β-estradiol (E_2; 20 μM) for 48 h. After treatment, the cells were fixed using Trump's fixative and processed for TEM at Mayo Clinic's electron microscopy core facility.

WESTERN ANALYSIS

Following treatment, chondrosarcoma cells were harvested, and the cytoplasmatic extracts prepared were analyzed by western blot hybridization using anti-LC3-I/II (Cell Signaling technology, Beverly, MA), anti-ATG3 (Cell Signaling Technology), and anti-actin antibodies (Sigma, St. Louis, MO). Quantitation of the corresponding bands was carried out using densitometry and analyzed using Quantity One software (Bio-Rad, Hercules, CA).

siRNA TRANSFECTION

SW1353 cells were plated in 24-well or 6-well plastic plates, each well containing 5×10^4 and 1×10^6 cells respectively, and maintained in the incubator at 37°C for 24 h. The next day the cells were transfected with a pool of ATG3 siRNAs [i) 5′ GAGAGUGGAUUGUUGGAAA 3′; ii) 5′GCGGAUGGGUAGAUACAUA 3′; iii) 5′GAGCAACGGCAGCCUU UAA3′; iv) 5′ACAAGACACUUCACAAUGU 3′] and non-template control siRNAs [i) 5′UGGUUUACAUGUCGACUAA3′; ii) 5′ UGGUUU ACAUGUUGUGUG A3′; iii) 5′UGGUUUACAUGUUUUCUG A3′; iv) 5′ UGGUUU ACAUGUUUUCCU A3′] as per the manufacturer's protocol (Dharmacon, Lafayette, CO). Twenty-four hours after transfection, cells were treated with vehicle control and 2-ME (20 μM). At the end of 48 h of treatment, cells were harvested and processed as described above for western blot hybridization.

STATISTICAL ANALYSIS

StatView version 5.0.1 (SAS Institute Inc.) was used for statistical analysis of the data and all of the values are expressed as mean +/− standard error of mean. Statistical analysis was accomplished by using oneway analysis of variance (ANOVA). When the overall ANOVA *F*-test was found to be significant, pair-wise comparisons were performed using Tukey's studentized range test in order to maintain the overall experiment-wise error rate. $P < 0.01$ was considered statistically significant.

RESULTS

EFFECT OF 2-ME ON CHONDROSARCOMA CELL GROWTH

We have studied the dose effects of 2-ME on SW1353 and Hs819T cells. Our results show that SW1353 cells were resistant at lower doses and sensitive at higher doses (5–40 μM) to 2-ME-mediated cytotoxic effects. Compared to the vehicle control 100% [± 0.6], the cell survival decreased to 84% [± 2.8], 70.7% [± 1.4], 57% [± 2.1], 44% [± 2.7], 37.8% [± 3.9], and 23.3% [± 3.8] at 1, 2, 5, 10, 20, and 40 μM concentrations of 2-ME, respectively (Fig. 1A). The results show that Hs819T cells exposed to 2-ME showed no decreases in cell survival at 1 and 2 μM concentrations compared to vehicle control 100% ± 0.3% (Fig. 1B). Whereas 2-ME at 5, 10, 20, and 40 μM concentrations decreased the Hs819T cell survival to 78%[± 1.3], 73% [± 2.1], 76% [± 3.2], and 76% [± 1.4], respectively (Fig. 1B).

2-ME INDUCES AUTOPHAGOSOMES IN CHONDROSARCOMA CELLS

To investigate whether autophagy is involved in 2-ME-mediated actions in chondrosarcoma cells, we have utilized TEM to detect autophagic vacuoles in chondrosarcoma cell. The results show that multilamellar bodies are formed after 48 h in 20 μM 2-ME treated SW1353 (Fig. 2Aii and iii) and Hs819T cells (Fig. 2Bii and iii). Our results did not reveal any multilamellar bodies in vehicle-treated SW1353 or Hs819T cells (Fig. 2Ai and Bi).

2-ME INDUCES MARKERS OF AUTOPHAGY

The autophagosome-associated protein, microtubule-associated protein 1 light chain 3 (LC3) is a marker of autophagy. In the course of autophagy LC3-I (cytosolic) is converted to LC3-II (membrane-bound) [Klionsky and Emr, 2000; Green and Levine, 2014]. We found that 2-ME at 20 μM enhances accumulation of LC3 II in SW1353 and Hs819T cells (Fig. 3). Our results show that the LC3-II/LC3-I ratio in SW1353 cells increases after 2-ME treatment by 5.4-fold and 4.2-fold at 24 and 48 h, respectively, compared to Veh-treated SW1353 cells (Fig. 3iA and B). Our results show that the LC3-II/LC3-I ratio in Hs819Tcells increases after 2-ME treatment by 2.0-, 2.4, 2.0-, 3.0-, and 2.8-fold at 4, 8, 16, 24, and 48 h respectively compared to Veh treated cells (Fig. 3iiA and B). In parallel, we have measured the effect of 2-ME at various concentrations at 24 h in SW1353 chondrosarcoma cells (Fig. 4). The results show that the LC3 II/LC3 I ratio does change with 1 μM concentrations but increases by 1.3-, 2.8-, 2.8-, 5.5-, and 2.9-fold at 2, 5, 10, 20, and 40 μM concentrations of 2-ME (Fig. 4).

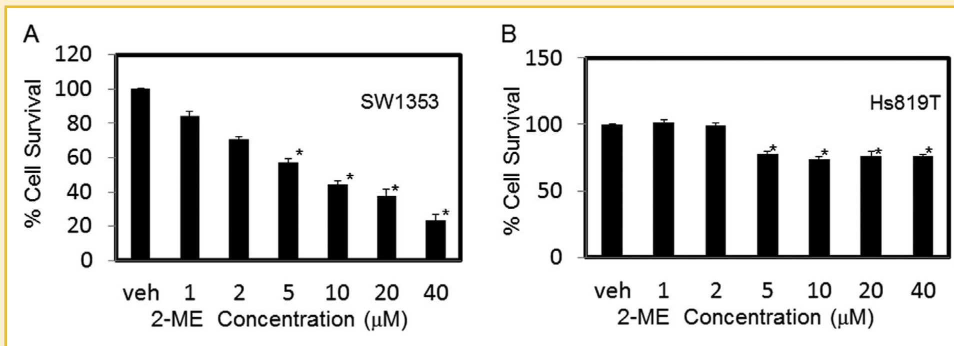

Fig. 1. 2-ME treatment decreases cell survival in SW1353 (A) and Hs819T (B) cells. Cells were treated with Vehicle (Veh) and 2-ME at indicated concentrations for 3 days. The cell survival was measured by MTS assay. Values are the mean $\pm$ SE (N $=$ 6 replicate cultures). $^{*}P < 0.01$ versus veh.

The effect of 2-ME on autophagic flux in chondrosarcoma cells was further assessed by measuring the p62 protein expression following 2-ME treatment. Our results show that 2-ME decreases p62 levels by 5-fold in SW1353 (Fig. 5A and B) and showed a minimum decrease in Hs819T (Fig. 5C and D) chondrosarcoma cells, at 24 h of treatment compared to the control vehicle-treated cells.

E_2 DOES NOT INDUCE AUTOPHAGOSOME FORMATION AND THE MARKERS OF AUTOPHAGY IN CHONDROSARCOMA CELLS

In order to determine whether the parent estrogn has a similar effect as 2-ME, we have studied the effect of 20 μM 17-β estradiol (E_2) on autophagosome formation and LC3 protein levels at 4, 8, 16, 24, and 48 h in SW1353 (Fig. 6) and Hs819T (Fig. 7) cells. Our results show

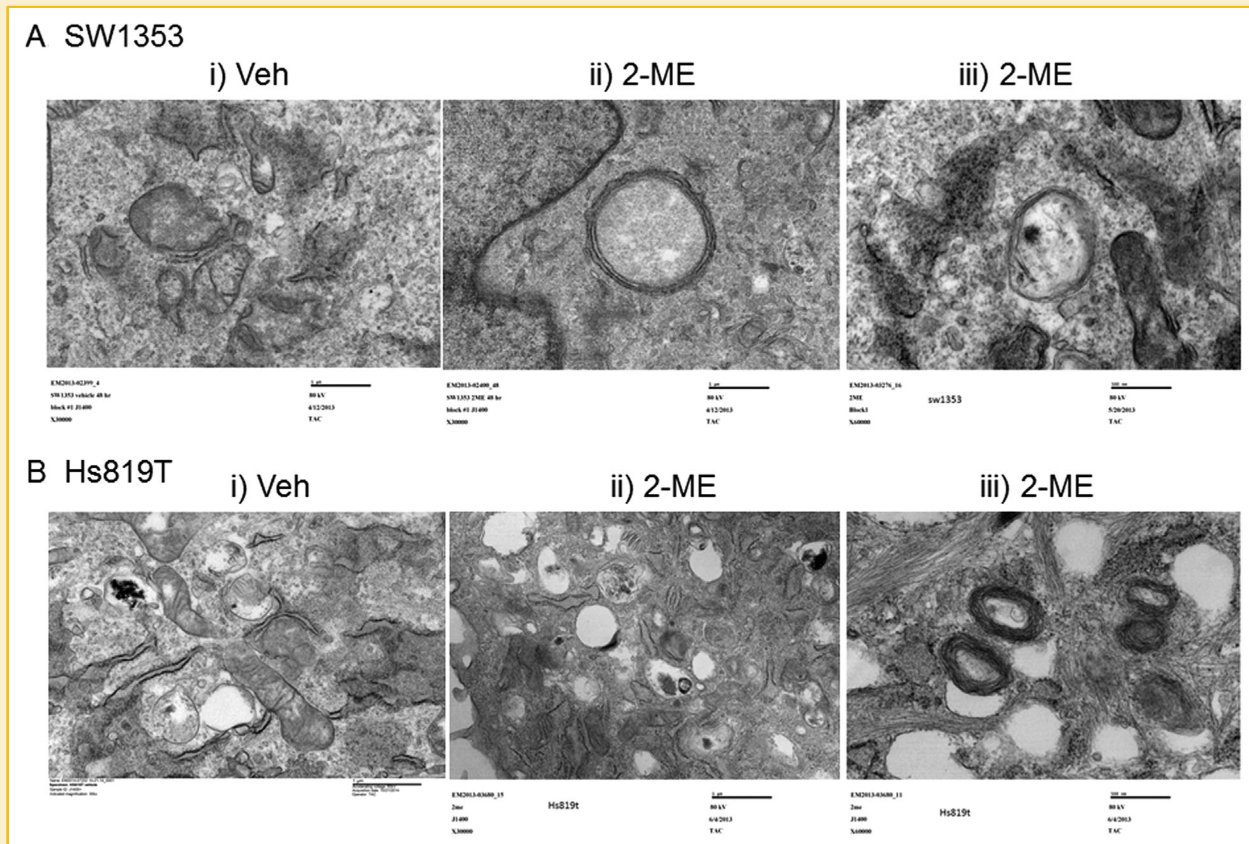

Fig. 2. 2-ME induces autophagosome formation in chondrosarcoma cells. The cells were treated with Veh and 2-ME (20 μM) for 48 h and analyzed using TEM. (A) TEM images of SW1353 cells. (B) TEM images of Hs819T cells. (i) Veh 30000X; (ii) 2-ME 30000X; (iii) 2-ME 60000X.

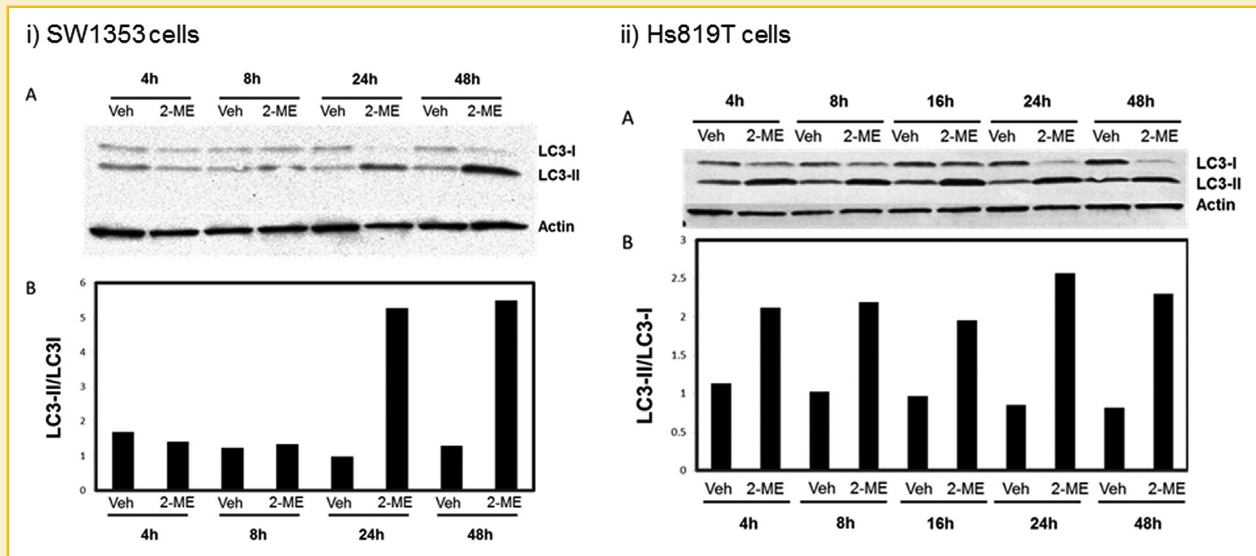

Fig. 3. 2-ME increases LC3-II/LC3-I ratio in chondrosarcoma cells. Protein extracts were prepared at various times from Veh and 2-ME (20 μM)-treated SW1353 (i) and Hs819T (ii) cells and analyzed by western blot hybridization using anti-LC3 and anti-actin antibodies. (A) Western blot; (B) Quantitation of the blot.

that E_2 does not induce autophagosome formation. Also, the results show that E_2 has minimal or no effect on LC3 protein levels and in the conversion of LC3-I to LC3-II in SW 1353 (Fig. 6B and C) and Hs819T cells (Fig. 7B and C).

2-ME-MEDIATED EFFECT ON AUTOPHAGIC FLUX IS REVERSED BY INHIBITORS OF AUTOPHAGY

We have investigated the effect of 2-ME on autophagic flux in the presence of the autophagy inhibitor, bafilomycin (Baf). Our results show Baf treatment blocks 2-ME-mediated conversion of LC3-I to LC3-II in chondrosarcoma cells (Fig. 8).

We have studied the effect of 2-ME on the conversion of LC3-I to LC3-II in chondrosarcoma cells that have been depleted with ATG3 expression following transfection with siRNAs directed against the ATG3 gene. Our results show that 2-ME-mediated conversion of LC3-I to LC3-II is blocked in chondrosarcoma cells transfected with siRNAs directed against the ATG3 gene but not in untransfected cells or cells transfected with non-template siRNAs (Fig. 9A and B). The western analysis shown in Fig. 9C confirms that siRNA transfections lead to the down regulation of ATG3 protein levels without affecting the control actin protein levels.

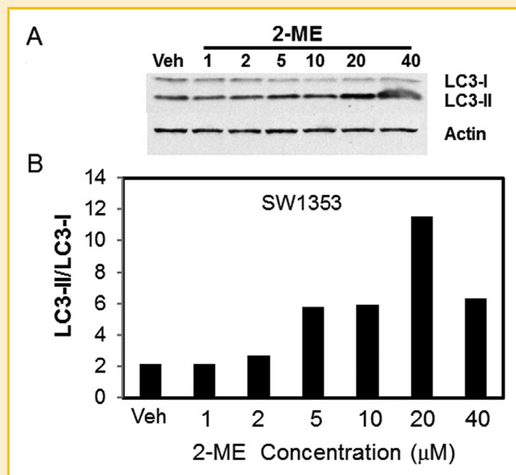

Fig. 4. Dose-dependent effects of 2-ME on LC3-II/LC3-I ratio. Protein extracts prepared from SW1353 cells treated with Veh and 2-ME at 1, 2, 5, 10, 20, and 40 μM were analyzed by western blot hybridization using anti-LC3 and anti-actin antibodies. (A) Western blot; (B) Quantitation of the blot.

INHIBITION OF AUTOPHAGY INCREASES 2-ME-MEDIATED CELL DEATH

To determine whether autophagy plays a role in 2-ME-mediated cell death, we investigated the effect of two autophagy-inhibitors, Bafilomycin (Baf), and 3-methyladenine (3-MA) on 2-ME-mediated effects in chondrosarcoma cells (Fig. 10). Our results show that compared to vehicle control 100% ± 3.1, the cell viability decreased in the presence of 2-ME, Baf, and Baf plus 2-ME to 42% [± 2.4], 54% [± 3.1] and 13% [± 0.6], respectively (Fig. 10A). The cell viability of SW1353 cells treated with Baf plus 2-ME was significantly decreased compared to treatment with 2-ME or Baf alone. The cell viability of SW1353 cells treated with Baf plus 2-ME was decreased by 4.75- and 3.74-fold, compared to cells treated with 2-ME or Baf alone, respectively. Similar results were found with 2-ME treatment in the presence of the autophagy inhibitor, 3-MA (Fig. 10B). Compared to the control 100 [± 0.6], the cell viability decreased to 51% [± 4.7], 61% [± 5.1], and 34% [± 3.7] in the presence of 2-ME, 3-MA, and 3-MA plus 2-ME, respectively. Our results show that the cell viability in presence of the 3-MA and 2-ME combination decreased by 1.7- and 2.6-fold compared to either 3-MA or 2-ME treatment, respectively (Fig. 10B).

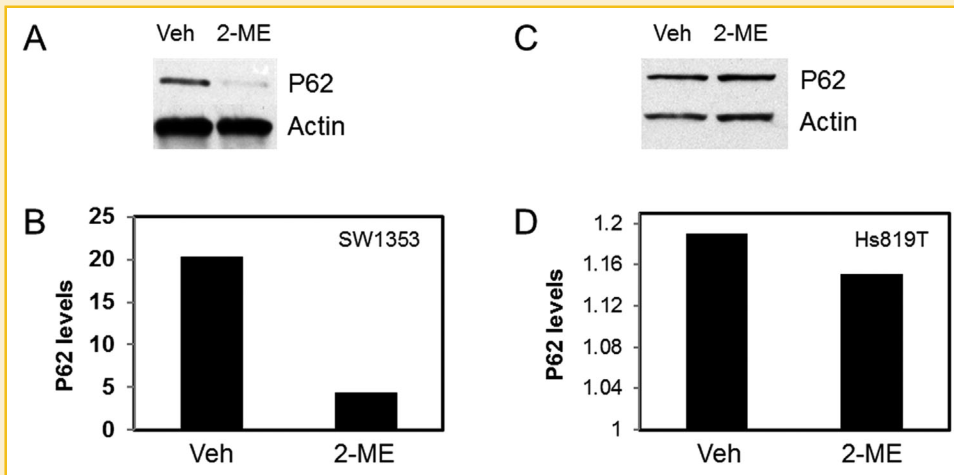

Fig. 5. 2-ME downregulates p62 expression in chondrosarcoma cells. Protein extracts prepared from SW1353 (A and B) and Hs819T (C and D) cells following 24 h of treatment with Veh and 2-ME (20 μM) were analyzed by western blot hybridization using anti-p62 and anti-actin antibodies. (A and C) Western blot; (B and D) Quantitation of the blot.

DISCUSSION

We have shown in this report that 2-ME treatment induces autophagy in chondrosarcoma cells. The results demonstrate that induction of autophagy causes resistance to cell killing and the inhibition of autophagy increases the cytotoxicity in 2-ME-treated chondrosarcoma cells. Chondrosarcoma is a malignant tumor and is resistant to conventional chemotherapy. The cellular mechanisms that contribute to chemoresistance in chondrosarcoma are yet to be fully investigated. Hence, surgical resection is widely recommended as a treatment in chondrosarcoma [Bjornsson et al., 1998; Schwab et al., 2007; Herget et al., 2011; Gunay et al., 2013; Angelini et al., 2014; Van Gompel and Janus, 2015]. Our work suggests that autophagy could be further explored in enhancing the efficacy of existing systemic treatment in chondrosarcoma.

Our results show that chondrosarcoma cells are resistant at lower concentration of 2-ME, and at very high concentration the cell survival is decreased considerably in SW1353 but not much in

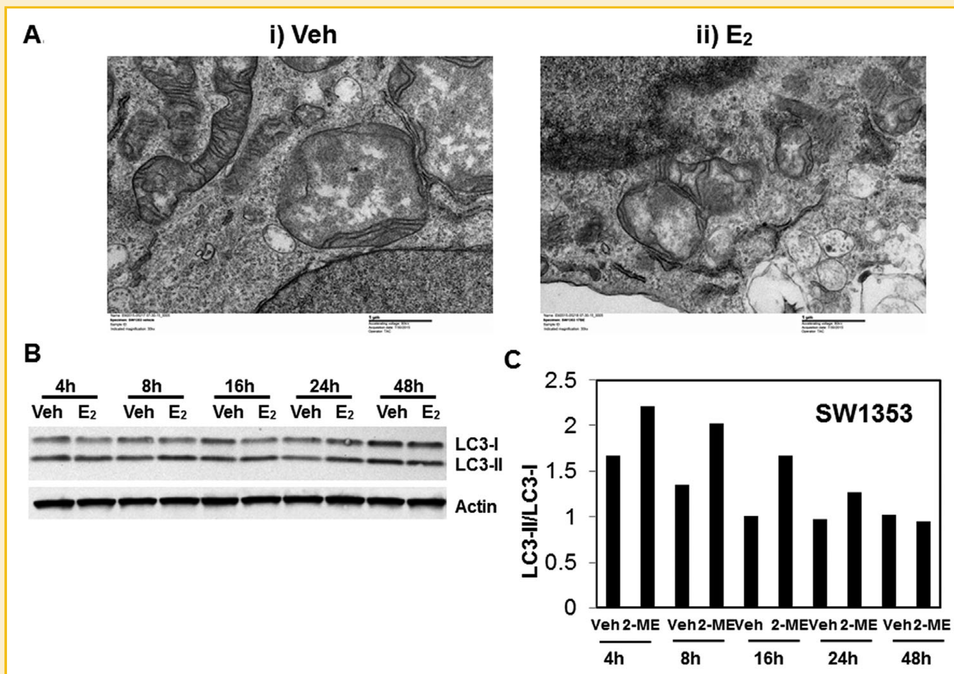

Fig. 6. Effect of E_2 on SW1353 chondrosarcoma cells. The cells were treated with Veh and E_2 (20 μM) for 48 h and analyzed by TEM and western blot hybridization using anti-LC3 and anti-actin antibodies. (A) TEM images: (i) Veh 30000X; (ii) E_2 30000X; (B) Western blot; (C) Quantitation of the blot.

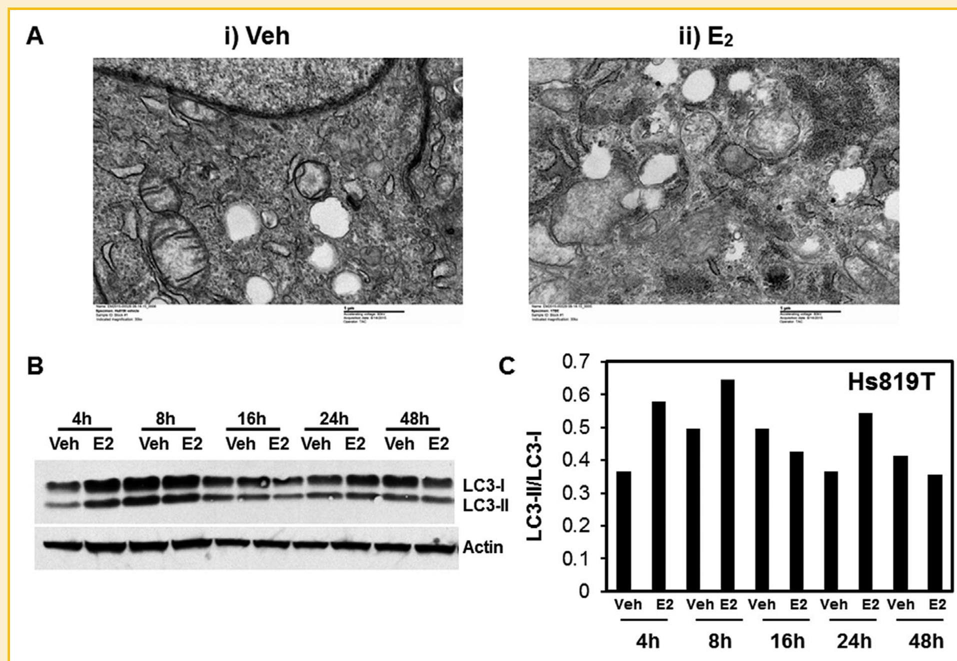

Fig. 7. Effect of E_2 on Hs819T chondrosarcoma cells. The cells were treated with Veh and E_2 (20 μM) for 48 h and analyzed by TEM and western blot hybridization using anti-LC3 and anti-actin antibodies. (A) TEM images: (i) Veh 30000X; (ii) E_2 30000X; (B) Western blot; (C) Quantitation of the blot.

Hs819T chondrosarcoma cells. 2-ME treatment is accompanied by autophagosome formation and conversion of LC3-I protein into LC3-II protein in two different chondrosarcoma (SW1353 and Hs819T) cell lines. The effect is specific to the anti-tumor metabolite, 2-ME and the parent estrogen, 17β-estradiol does not induce autophagosome formation and the conversion of LC3-I to LC3-II in chondrosarcoma cells. It was found that the increase in LC3-II to LC3-I ratio is observed at a much earlier time point in Hs819T cells, which shows more resistance to 2-ME-mediated anti-growth and cell killing effects. This shows that the high resistance may be associated with an earlier induction of autophagic flux in Hs819T cells. Further work is necessary to understand the differential responses to 2-ME treatment and induction of the autophagy markers in chondrosarcoma cell lines. In addition, the results show that p62 protein is downregulated following exposure to 2-ME in chondrosarcoma cells. The decrease in p62 levels was significant in SW1353 cells but was only very minimal in Hs819T cells. The literature points out that the involvement of p62 in autophagy induction is complex and cell type-dependent. The p62 protein which serves as a molecular adaptor in the autophagic apparatus has been shown to be degraded following an autophagic flux [Eskelinen et al., 2007; Belaid et al., 2014; Green and Levine, 2014]. However, it has also been shown that p62 is not required for autophagy in Drosophila fat cells [Pankiv et al., 2010]. The actural role of p62 in the regulation of autophagy is also controversial. It has been found that p62 could participate in autophagy through the regulation MTORC1 [Duran et al., 2011] or through disrupting the association of Bcl2 and Beclin1 [Zhou et al., 2013]. Thus, the differential response and the downregulation of p62 in SW1353 and Hs819T cells further reveals that the physiological significance and role of p62 in 2-ME-induced autophagy needs to be investigated further by following its regulation and interactions with other proteins.

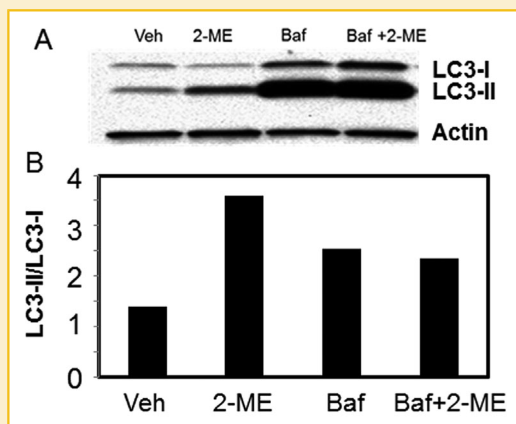

Fig. 8. Autophagy inhibitor blocks 2-ME-mediated conversion of LC3-I to LC3-II. Protein extracts prepared at 48 h from SW1353 cells following Veh and 2-ME (20 μM) treatment were analyzed by Western blot hybridization using anti-LC3 and anti-actin antibodies. (A) Western blot; (B) Quantitation of the blot.

Autophagy-related protein 3 (ATG3) functions as an ubiquitin conjugating enzyme analog that catalyzes the transfer of ATG 8 to phosphatidylethanolamine, a critical step for autophagy [Green and Levine, 2014]. Our data show that 2-ME treatment in chondrosarcoma cells transfected with ATG3 siRNAs does not lead to an increase in LC3-II/LC3-I ratio compared to cells treated with 2-ME

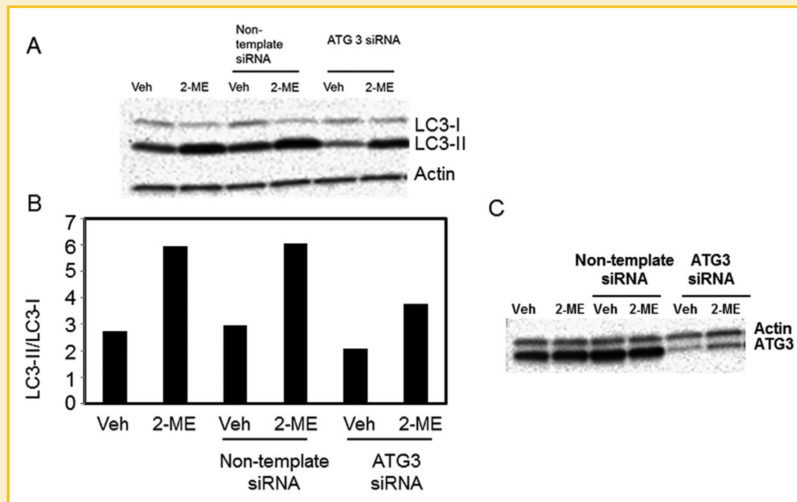

Fig. 9. ATG3 siRNA decreases LC3-II/LC3-I ratio in 2-ME-treatred chondrosarcoma cells. SW1353 cells were treated with Veh and 2-ME (20 μM) following siRNA transfection. Protein extracts prepared at 48 h were analyzed by western blot hybridization using anti-LC3, anti-actin and anti-ATG3 antibodies. (A) Western blot for measuring LC3 protein levels; (B) Quantitation of the blot; and (C) Western blot for measuring ATG3 protein levels.

and transfected with non-template siRNAs. This confirms the induction of autophagy, and further suggests that ATG3 is required for 2-ME-mediated regulation of autophagy in chondrosarcoma cells.

2-ME and its analogs have been shown to induce autophagy [Chen et al., 2008; Lorin et al., 2010; Yang et al., 2011]. In contrary to the current observations, 2-ME-mediated induction of autophagy leads to cell death in some cancer cells. 2-ME treatment induces oxidative stress which leads to autophagy-mediated cell death in transformed and cancer cells [Chen et al., 2008]. Also, 2-ME-mediated induction of autophagy leads to cell death in another type of musculoskeletal tumor, Ewing sarcoma. 2-ME induces autophagy through Damage-regulated autophagy modulator (DRAM) protein in Ewing sarcoma cells [Lorin et al., 2010]. 2-ME induces RNA-dependent protein kinase (PKR) dependent autophagy in osteosarcoma cells [Yang et al., 2013]. Also, 2-ME-induces autophagosome formation in chondrocytes in culture (S. Reumann, K. Shogren, M. Yaszemski and A. Maran, unpublished observations) which have been shown to be resistant to the anti-growth effects of 2-ME [Fong et al., 2007]. Other investigations have found autophagy as a protective mechanism in chondrocytes against steroid compounds and mitochondrial dysfunctions. [Akasaki et al., 2014; Liu et al., 2014; Lopez de Figueroa et al., 2015]. Although further experimental verification is required, this preliminary finding suggests that autophagy induction could be a resistance mechanism to anti-growth effects in chondrosarcoma (tumor) but not in chondrocytes (normal cells).

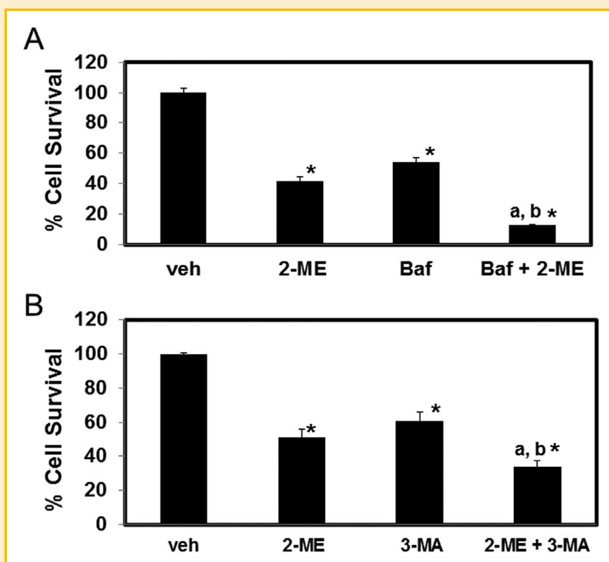

Fig. 10. Autophagy inhibitors enhance 2-ME-mediated cell death in chondrosarcoma cells. SW1353 cells were treated with Veh, 2-ME (20 μM), 100 nM Bafilomycin (Baf) or 50 μM 3-methyladenine (3-MA) for 3 days. The cell survival was measured by MTS assay. Values are the mean ± SE (N = 6 replicate cultures). $^{*}P < 0.01$ versus veh; $^{a}P < 0.01$ versus 2-ME; $^{b}P < 0.01$ versus 3-MA or Baf.

A previous report by Fong et al. shows that 2-ME induces cell cycle arrest and cell death in JJ012 chondrosarcoma cells [Fong et al., 2007]. Current results show that different concentrations are required for achieving cell death in the two chondrosarcoma cell lines, SW1353 and Hs819T. The differences in sensitivity to 2-ME could be partly due to the fact that these cells were established from different patients. It is further possible that the different rate of autophagic flux could be contributing to the differential resistances in the presence of 2-ME. This possibility is further supported by increases in 2-ME-mediated cell death in the presence of the autophagy inhibitors in chondrosarcoma cells.

Autophagy and its role in chemoresistance has been observed before in other tumors [Liu et al., 2011], although autophagy has been understudied in chondrosarcoma. Pharmacological inhibitors have been shown to sensitize several tumor cells for anti-growth

effects induced by various agents [Wu et al., 2012]. Our results show that inhibition of autophagy sensitizes chondrosarcoma cells and enhances 2-ME-mediated cytotoxicity in chondrosarcoma cells. Baf, which is known for inhibiting vacuolar ATPase (V-ATPase) and promoting autophagic vacuoles in cells [Klionsky and Emr, 2000], blocks 2-ME-mediated increases in the LC3-II levels in chondrosarcoma cells. In the presence of Baf treatment, the levels of both LC3-I and LC3-II are increased. This could be due to various possibilities. It is plausible that the increased flux could lead to increased levels of LC3-I and make the conversion of LC3-I to LC3-II a rate-limiting one. Baf enhances 2-ME-mediated anti-tumor effects and the chondrosarcoma cell survival decreases in the combined presence of Baf and 2-ME compared to 2-ME treatment alone. This indicates that 2-ME-mediated autophagy could involve V-ATPase-dependent effects. Previous reports show that Baf inhibits autophagy through blocking the fusion of autophagosomes and lysosomes. Similarly, a moderate upsurge in cell death, when cells were exposed to the 2-ME and autophagy inhibitor 3-MA combination suggests that the induction of autophagy via 2-ME may not be fully dependent on the phosphoinositide 3-kinase (PI3K)-pathway which is blocked by 3-MA treatment [Klionsky and Emr, 2000; Wu et al., 2012]. Thus, our results show that both 3-MA and Baf which work at early and late stages of autophagic flux through different mechanisms could enhance 2-ME-mediated cell death. Further studies are required to fully understand the molecular mechanisms associated with 2-ME-mediated induction of autophagy. Overall, our investigation points out that autophagy could be induced by chondrosarcoma cells as a pro-survival response against anti-tumor therapies. Autophagy may enable chondrosarcoma cells to adapt to metabolic stress and advocate cell survival during apoptosis. As reviewed by White, autophagy in response to metabolic stress can prevent cell death by apoptosis and in apoptosis-defective cells inhibition of autophagy can lead to necrotic cell death [White, 2008]. Considering this possibility, the cell death mechanism following the administration of 2-ME and Baf might be due to induction of necrosis which needs further investigation. Thus, our work reveals that the regulation of autophagy might be an important event in chondrosarcoma and could be explored further clinically. In conclusion, inhibition of autophagy may be a promising strategy to increase the efficacy of 2-ME and other anticancer drugs in the treatment of chondrosarcoma.

ACKNOWLEDGMENT

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