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Induction of autophagy does not alter the anti-tumor effects of HDAC inhibitors

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Dear Editor,

HDAC inhibitors (HDACis) can suppress the growth and/or survival of tumor cells and thus far two HDACis, vorinostat and romidepsin, have been approved by the FDA for the treatment of hematological malignancies.[1] Vorinostat is a potent HDACi that mediates tumor cell-selective apoptosis and we and others have previously shown that the induction of apoptosis correlates with therapeutic efficacy in mouse models of hematological cancer.[2–5] Although apoptosis may be the preferred mode of HDACi-induced cell death, it is clear that in tumor cells with nonfunctional apoptotic cascades, HDACi are capable of inducing a caspase-independent form of cell death.[5–7] We recently demonstrated that Eμ-*myc* lymphomas devoid of *Apaf-1* displayed a delayed cell death in response to HDACi treatment, and loss of *Apaf-1* failed to affect therapeutic efficacy.[5] The delayed HDACi-induced cell death was concomitant with biochemical and morphological changes characteristic of autophagy.[5] Herein we utilized the genetically tractable Eμ-*myc* mouse model to address conflicting reports in the field regarding the importance of autophagy in regulating the anti-tumor responses of HDACi.[6–8]

To determine whether autophagy had any role in regulating the response of Eμ-*myc/Apaf-1*$^{-/-}$ cells to HDACi, we knocked down the expression of two key autophagy proteins (Atg5 and Atg7) using constitutive short-hairpin RNAs (shRNAs). We developed two distinct shRNAs against *Atg5* and one shRNA against *Atg7* that efficiently silenced the expression of Atg5 and Atg7, respectively (Figure 1a). To determine if knockdown of Atg5 or Atg7 impaired HDACi-mediated autophagy, we treated *Atg5*- and *Atg7*-shRNA-expressing Eμ-*myc/Apaf-1*$^{-/-}$ lymphomas with vorinostat and determined the ratio of LC3-I/LC3-II by western blot. In Eμ-*myc/Apaf-1*$^{-/-}$ cells with decreased expression of Atg5 or Atg7, vorinostat-induced processing of LC3-I to LC3-II was greatly reduced (Figure 1a).

We next evaluated the response of *Atg5*- and *Atg7*-shRNA-expressing Eμ-*myc/Apaf-1*$^{-/-}$ lymphomas to increasing concentrations of vorinostat over time. Consistent with our previous results,[5] a delay in the kinetics of cell death was observed in vorinostat-treated Eμ-*myc/Apaf-1*$^{-/-}$ compared with Eμ-*myc* lymphomas (Figure 1b). However, depletion of *Atg5* or *Atg7* did not affect vorinostat-mediated tumor cell death, indicating that inhibition of autophagy neither protected Eμ-*myc/Apaf-1*$^{-/-}$ cells from HDACi-mediated cell death nor potentiated its anticancer effect. To evaluate the response of *Atg5*- and *Atg7*-shRNA-expressing Eμ-*myc/Apaf-1*$^{-/-}$ lymphomas to vorinostat *in vivo*, we transplanted lymphomas into recipient mice and treated tumor-bearing mice with vorinostat. Consistent with our *in vitro* data, *Atg5*- and *Atg7*-shRNA-expressing lymphomas were equally responsive to vorinostat and cleared with similar kinetics as control-shRNA-expressing lymphomas (Figure 1c). Finally, we determined whether inhibition of autophagy had any effect on the therapeutic efficacy of vorinostat in Eμ-*myc/Apaf-1*$^{-/-}$ lymphomas. We observed an enhanced survival in mice bearing *Atg5*-shRNA Eμ-*myc/Apaf-1*$^{-/-}$ lymphomas after treatment with vorinostat (median survival vorinostat: 43.5 days, median survival vehicle: 25 days, $P<0.0084$), which was similar to the therapeutic response seen with control-shRNA-expressing lymphomas (median survival vorinostat: 48 days compared with vehicle: 23.5 days, $P<0.0001$). In conclusion, our data demonstrate that there is no evidence supporting an essential role for autophagy in regulating the response of apoptosis-deficient tumors to HDACi. Recent data suggest that in tumor cells with apoptotic defects, inhibition of autophagy may potentiate the therapeutic response mediated by HDACi.[7] In contrast, we provide evidence that combining autophagy inhibitors with HDACi may not be clinically beneficial in lymphomas with apoptotic defects.

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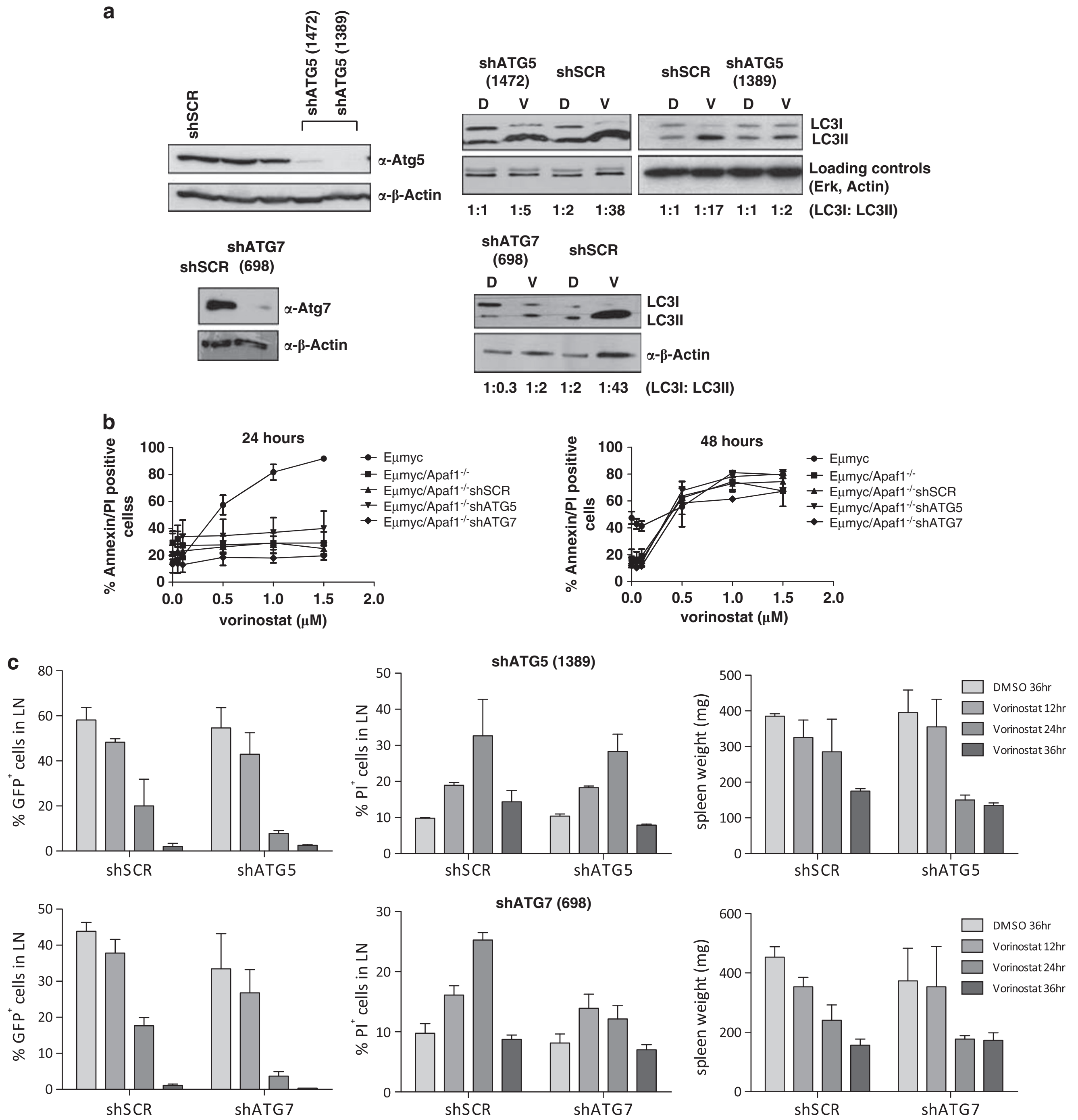

Figure 1 Vorinostat does not require a functional autophagic pathway to induce tumor cell death in E*μ-myc/Apaf-1*$^{-/-}$ lymphomas. **(a)** E*μ-myc/Apaf-1*$^{-/-}$ lymphomas were retrovirally transduced with constructs expressing shRNAs targeting *Atg5* (clone 1472 and 1389, respectively), *Atg7* (clone 698) or a scrambled control. Knockdown efficiency was tested via western blot using antibodies specific for Atg5 and Atg7 (Cell Signaling Technologies, Inc., Danvers, MA, USA, no. 2630 and 2631). Anti-*β*-actin was used as a loading control. Results are a representative of at least three separate westerns. *Atg5*-shRNA-expressing E*μ-myc/Apaf-1*$^{-/-}$ (1389 and 1472), *Atg7*-shRNA-expressing E*μ-myc/Apaf-1*$^{-/-}$ (698) and control-shRNA-expressing E*μ-myc/Apaf-1*$^{-/-}$ lymphomas were treated with 1 *μ*M vorinostat (V) or DMSO (D) for 24 h and LC3 processing was determined by western blot using antibody specific for LC3 (NanoTools Antikoerpertechnik GmbH & Co. KG., Teningen, Germany, no. 0260-100/LC3-2G6). Anti-p44/42 ERK (Cell Signaling Technology, Inc., Danvers, MA, USA, no. 9107) or anti-*β*-actin were used as loading controls. Quantitative western analysis was performed using ImageJ software (public domain software by Wayne Rasband), National Institute of Health, USA. Results are representative of at least three separate westerns. **(b)** E*μ-myc*, E*μ-myc/Apaf-1*$^{-/-}$ and E*μ-myc/Apaf-1*$^{-/-}$ lymphomas expressing the various shRNA constructs were treated with increasing concentrations of vorinostat for 24 and 48 h. Cell viability was assessed by annexin V/propidium iodide staining and error bars indicate ± S.E.M. of at least three independent experiments. **(c)** C57Bl/6 mice bearing *Atg5*-shRNA-expressing E*μ-myc/Apaf-1*$^{-/-}$ (1389), *Atg7*-shRNA-expressing E*μ-myc/Apaf-1*$^{-/-}$ (698) or control-shRNA-expressing E*μ-myc/Apaf-1*$^{-/-}$ lymphomas were treated with one dose of vorinostat (200 mg/kg). Spleen and lymph nodes were harvested at the indicated time points (h) following HDACi treatment and the percentage of lymphoma cells in lymph nodes was determined using flow cytometry in the presence of propidium iodide. Each time point is representative of 2–3 mice

Conflict of Interest

The authors declare no conflict of interest.

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