Zanthoxylum fruit extract from Japanese pepper promotes autophagic cell death in cancer cells

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Citation
Oncotarget, 7(43), 70437-70446
https://doi.org/10.18632/oncotarget.11926

Issue Date
2016-10-26

Doc URL
http://hdl.handle.net/2115/64034

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SUPPLEMENTARY FIGURES

Supplementary Figure S1: Effects of ZFE on morphology and proliferation of human colon cancer cells. a. Effect of ZFE on the morphology of the indicated cells. Cells were incubated with 200 μg/ml of ZFE or 0.2% v/v DMSO (control) for 24 h. Scale bars, 50 μm. b. Cells were incubated with 200 μg/ml of ZFE or 0.2% v/v DMSO (control) for the indicated time and cell viability was measured using a cell proliferation assay kit. Data are the mean ± SD of three independent experiments. *p < 0.05 vs. control at 48 h. ††p < 0.01, vs. control at 72 h (Student’s t-test). Ctrl; control.
Supplementary Figure S2: Knockdown of ATG5 protein inhibits the anticancer effect of ZFE in DLD-1 cells. 

**a.** DLD-1 cells were transfected with scrambled siRNA or ATG5 siRNA #2 (10 nM final concentration) or subjected to transfection in the absence of siRNA (Mock). Twenty-four hours after transfection, RNAs were extracted, and quantitative RT-PCR was performed to measure knockdown efficiency. Fold-changes in ATG5 mRNA levels were calculated by the ΔΔCt method using GAPDH as a reference gene. Error bars represent S.D. of mean values (n=3). **p < 0.01 vs. control (Dunnett’s test).**

**b.** DLD-1 cells were transfected with scrambled siRNA or ATG5 siRNA #2 (10 nM final concentration) or subjected to transfection in the absence of siRNA (Mock). Twenty-four hours after transfection, cell lysates were subjected to Western blotting with the indicated antibodies. Similar results were obtained in three independent experiments.

**c.** Effect of ZFE on the morphology of ATG5 knockdown DLD-1 cells. Transfected DLD-1 cells were incubated with 200 μg/ml of ZFE for 24 h. Scale bars, 50 μm.

**d.** DLD-1 cells were transfected with scrambled siRNA or ATG5 siRNA #2 (10 nM final concentration) or subjected to transfection in the absence of siRNA (Mock). Twenty-four hours after transfection, the cells were harvested and treated with 200 μg/ml of ZFE or 0.2% v/v DMSO (control) for the indicated time. Cell viability was measured using a cell proliferation assay kit. Error bars represent S.D. of mean values (n=3). **p < 0.01 at 72 h (Student’s t-test). Ctrl; control.
Supplementary Figure S3: Effects of ZFE on phosphorylation of JNK in intestinal cells. a. Cells were treated with 200 μg/ml of ZFE for the indicated time. The cell lysates were prepared and subjected to Western blotting with the indicated antibodies. Similar results were obtained in three independent experiments. b. IEC-6 cells were treated with 200 μg/ml of ZFE for the indicated time. The cell lysates were prepared and subjected to Western blotting with the indicated antibodies. Similar results were obtained in three independent experiments.
Supplementary Figure S4: Sanshools have no anticancer activity in DLD-1 cells. a. Effect of sanshools (mixture of HAS and HBS at the same concentration) on the morphology of DLD-1 cells. Cells were incubated with 38 μM of sanshools or 0.2% v/v DMSO (control) for 24 h. Scale bars, 50 μm. b. DLD-1 cells were treated with 38 μM of sanshools or 0.2% v/v DMSO (control) for the indicated time. The cell lysates were prepared and subjected to Western blotting with the indicated antibodies. Similar results were obtained in three independent experiments. c. DLD-1 cells were incubated with 38 μM of sanshools or 0.2% v/v DMSO (control) for the indicated time and cell viability was measured using a cell proliferation assay kit. Data are the mean ± SD of three independent experiments. Ctrl; control.