Altered Cross-linking of HSP27 by Zerumbone as a Novel Strategy for Overcoming HSP27-mediated Resistance

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1. Introduction

HSPs have diverse roles in the regulation of signal transduction and in numerous aspects of cell growth and death. Indeed, HSP90, HSP70, and HSP27 have each been implicated in promoting cancer. Most HSP27 exists as large oligomeric complexes ranging from 100-800 kDa, which are probably stabilized by complex interactions between dimeric building blocks. The functional properties of HSP27 are dependent on the quaternary structure of the protein. For example, HSP27 acts as a chaperone and binds to cytochrome c or Daxx as a dimer. Therefore, the oligomerization pattern of HPS27 is believed to have HSP27-mediated protective functions.

In this study, zerumbone (ZER), the cytotoxic component isolated from *Zingiber zerumbet* Smith, induced cross-linking of HSP27 protein by its insertion between the disulfide bond of HSP27, and ZER-mediated altered cross-linking of HSP27 modified normal HSP27 dimerization, which resulted in a sensitizing effect to tumors after treatment with radiation. Therefore, altered cross-linking by ZER may be a novel strategy for inhibition of HSP27-mediated resistance.

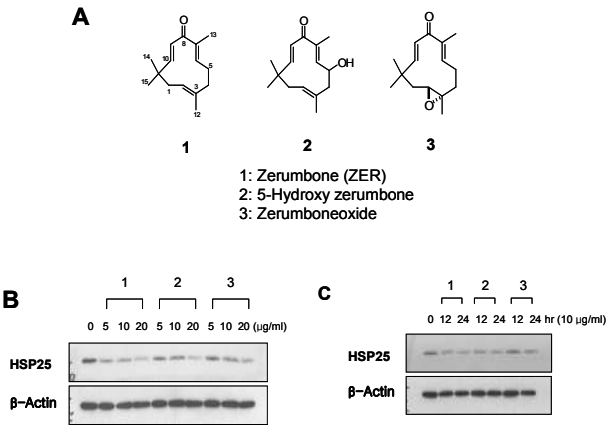
2. Materials and Methods

Zerumbone were isolated from the rhizomes *of Zingiber zerumbet* smith. We performed Immunoprecipitation and Western blotting to study interaction between HSP27 and Cytochrome c, PKC δ. In addition, colony forming assay and Annexin V-FITC/PI staining were performed to measure radiosensitivity. LC-MS was used for identification of HSP27.

3. Results

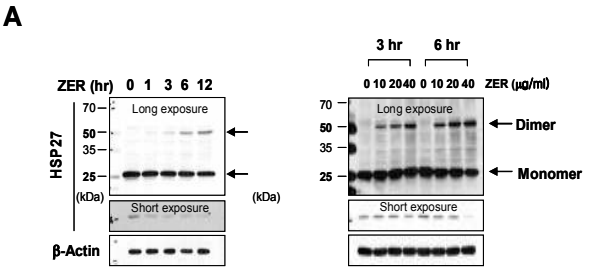
ZER inhibits monomeric HSP27 protein expression.
We selected three compounds (ZER, 5-hydroxy-ZER, and zerumboneoxide; Fig. 1A) by Western blotting as inhibitors of HSP27 protein expression from natural compounds. These three compounds showed inhibition of HSP27 protein in NCI-H1299, which is a lung adenocarcinoma cell with high expression of HSP27 (Fig. 1B). We selected ZER (compound #1) because it had greater inhibition of HSP27 protein expression in a time- and dose-dependent manner (Fig. 1C).

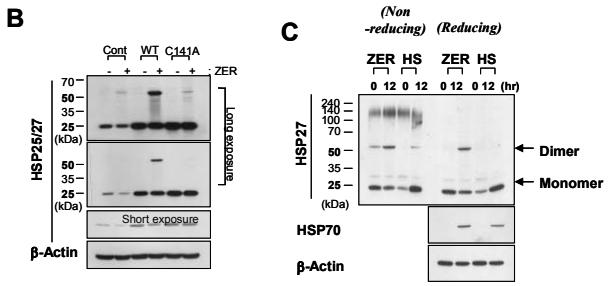
Fig. 1. Zerumbone inhibited monomeric HSP27 expression



Inhibition of the monomeric form of HSP27 by ZER was correlated with production of cross-linking of HSP27.
Mass spectrophotometry revealed that approximately 50kDa of protein in SDS-gel was the cross-linking form of two HSP27 proteins (data not shown). Cross-linking of HSP27 occurred in a time- and dose-dependent manner with inhibition of monomeric HSP27 protein (Fig. 2A). ZER induced cross-linking of HSP25-WT however, in the case of HSP25-C141A (dimerization-deficient mutant), cross-linking by ZER was blocked and inhibition of monomeric HSP25 was also attenuated (Fig. 2B). Normal dimerization of HSP27 by heat shock (HS), which is induced by a disulfide bond, was only detected in non-reducing gel. But, ZER induced cross-linking of HSP27 detected in reducing and non-reducing gel (Fig. 2C).

Fig. 2. Inhibition of monomeric HSP27 expression by ZER was dependent on the production of cross-linking of HSP27.

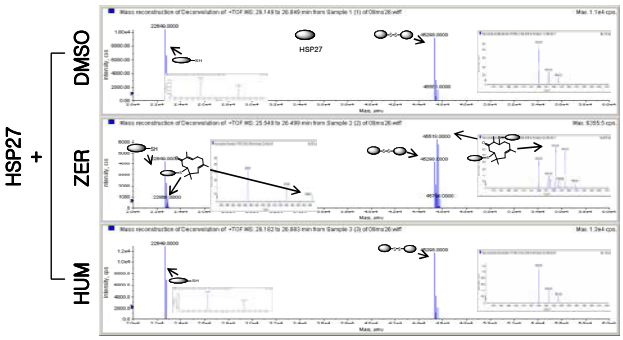




The α, β-unsaturated carbonyl group in ZER was essential for altered cross-linking of HSP27.

Structure analysis using mass spectrometry revealed a modified residue 0f HSP27 in ZER treated condition. However, in the case of HUM treatment, which lacks an α, β-unsaturated carbonyl group these phenomena were not observed (Fig. 3). From the results, the α, β-unsaturated carbonyl group in ZER is a key moiety for altered cross-linking of HSP27.

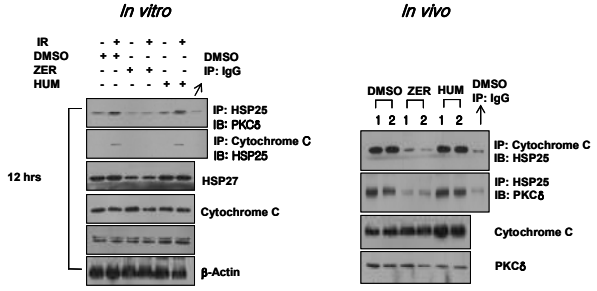
Fig. 3. The α, β-unsaturated carbonyl group in ZER was essential for covalent complex formation between ZER and HSP27.



ZER-mediated cross-linking of HSP27 inhibited its binding activity with apoptotic molecules.

ZER was treated to NCI-H1299 cells (*in vitro*) or at the tumor site of NCI-H1299 cell-xenografted mice (*in vivo*). The binding activity of HSP27 with cytochrome c or PKCδ after radiation was inhibited by ZER treatment, while in the case of HUM, this inhibition was not shown (Fig. 4). From the data, ZER-mediated cross-linking of HSP27, which is specific for HSP27 protein, may be a functional defective form.

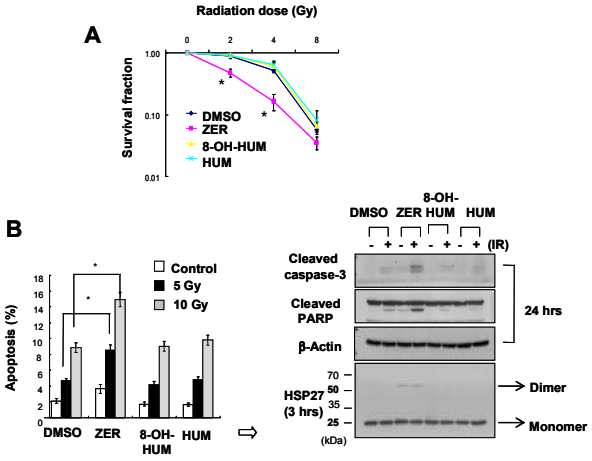
Fig. 4. ZER-mediated cross linking of HSP27 altered the oligomerization patterns of HSP27 and its binding activity with apoptotic molecules.



ZER exhibited sensitization to the radiation.

Pre-treatment of ZER for 3 hrs before radiation decreased clonogenic survival by radiation (Fig. 5A) and increased cell death (Fig. 5B). Thus, we suggest that ZER has a greater radio-sensitization effect in HSP27-overexpressed cells, such as NCI-H1299.

Fig. 5. Zerumbone-induced radiosensitization.



4. Conclusion

In summary, ZER was involved in altered cross-linking of HSP27, which correlated to its sensitization to radiation. Hence, as human HSP27 belongs to the survival protein family that participates in the maintenance of aggressively-growing and therapy-resistant tumors, the kind of small molecules, such as ZER described herein, may have some future potential therapeutic use in overcoming HSP27-mediated radio- or chemo-resistance.

References

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