

## Research Article

# LED Light-Activated Hypocrellin B Induces Mitochondrial Damage of Ovarian Cancer Cells

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**Objective.** Hypocrellin is a natural photosensitizer from a traditional Chinese herb. In the present study, our aim is to investigate the effect of LED light-activated hypocrellin B on mitochondria of ovarian cancer cells. **Material and Methods.** Ovarian cancer HO-8910 cells were incubated with hypocrellin B at the concentration of 2.5  $\mu\text{M}$  for 5 h and then irradiated by blue light from a novel LED source. Cell survival rate of HO-8910 cells was measured using MTT assay 24 h after photodynamic treatment of hypocrellin B. Mitochondrial morphology was observed using transmission electron microscopy (TEM). Mitochondrial membrane potential was measured using flow cytometry with JC-1 staining. **Results.** MTT assay showed that cell survival rate of HO-8910 cells in the photodynamic treatment group has significantly decreased down to  $27.22 \pm 1.26\%$  ( $P < 0.01$ ). Light irradiation alone or hypocrellin B alone showed no significant impact. In our TEM mitochondria of the cells after photodynamic treatment of hypocrellin B showed severe damage with swollen mitochondria that had nearly nonexistent cristae. Mitochondrial membrane potential remarkably decreased after photodynamic action of hypocrellin B. **Conclusion.** The findings demonstrated that photodynamic action of hypocrellin B significantly decreased cell proliferation of ovarian cancer HO-8910 cells, caused severe damage to mitochondrial structure, and induced mitochondrial membrane collapse.

## 1. Introduction

Ovarian cancer is one of the most common cancers threatening the health of women worldwide. Significant advances have been made in therapeutic modalities in the management of ovarian cancer. However, drawbacks exist in some of the commonly used modalities [1, 2]. Therefore, novel and more effective therapeutic strategies are needed for improving the clinical outcomes.

Photodynamic therapy (PDT) can eradicate the unwanted cells/tissues via the generation of reactive oxygen species (ROS) during photodynamic treatment, in which a photosensitizer is activated by light with an appropriate wavelength [3, 4]. The doubly selective advantages, including photosensitizer selectively retaining in tumor tissue and site-directed light irradiation, make PDT become a promising alternative to combat malignancies. Photosensitizer is a key component affecting the efficacy of photodynamic therapy. Hypocrellin B is a natural product isolated from a traditional Chinese Medicine *Hypocrella bambuase* [5, 6].

Growing evidence shows that hypocrellin B has significantly photodynamic activity with high quantum yields of singlet oxygen ( $^1\text{O}_2$ ) [7, 8]. Upon light activation hypocrellin B exhibits remarkably photodynamic eradication of cancer cells, virus, and bacteria. Our previous study showed that blue light from a novel LED source in our Lab could activate hypocrellin B, subsequently induced photocytotoxicity of ovarian cancer cells [9]. However, the biological mechanism is still unclear. On considering that mitochondria play an important role in cell growth and death, in the present study we focused on investigating the effect of photodynamic treatment of hypocrellin B on mitochondrial morphology and mitochondrial membrane potential of ovarian cancer cells.

## 2. Materials and Methods

**2.1. Sensitizer.** Hypocrellin B was used as a photosensitizer in our present study from Institute of Chemistry, Chinese Academy of Sciences. A stock solution was made in dimethyl

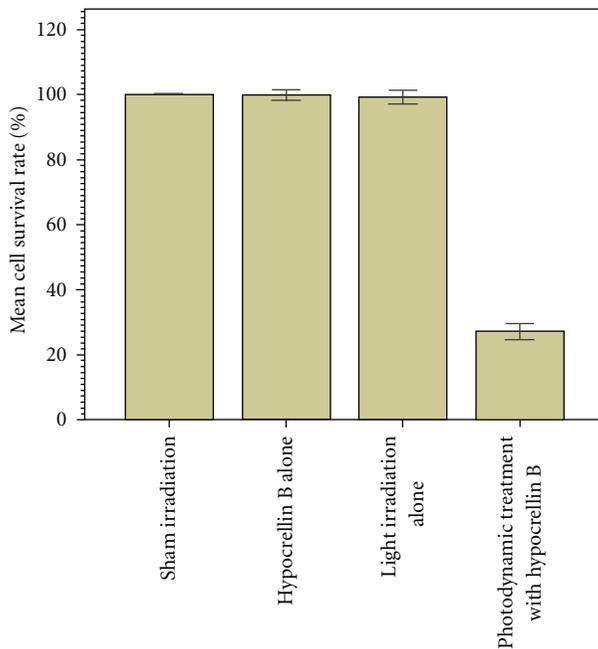


FIGURE 1: Cell survival rate of HO-8910 cells was measured using MTT 24 h after photodynamic action of hypocrellin B.

sulfoxide (DMSO) at a concentration of 100 mM and kept in the dark at  $-20^{\circ}\text{C}$ .

**2.2. Cell Culture.** Ovarian cancer HO-8910 cells were stored in the Institute of Ultrasound and Medicine Engineering, Chongqing under the approval of the ethics committee of Chongqing Medical University. The cells were grown in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS, Gibco), 50  $\mu\text{g}/\text{mL}$  penicillin, 50  $\mu\text{g}/\text{mL}$  streptomycin, and 10  $\mu\text{g}/\text{mL}$  neomycin.

**2.3. Photodynamic Treatment.** Photodynamic treatment of hypocrellin B in HO-8910 cells was the same as described by our previous study [9]. In brief, the cells were incubated with hypocrellin B (2.5  $\mu\text{M}$ ) at  $37^{\circ}\text{C}$  for 5 h in the dark. Unbound hypocrellin B was washed away and the cells were exposed for 17 s (except for the dark controls) to blue light emitted from LED source system with the wavelength of 470 nm and the power density of 60  $\text{mW}/\text{cm}^2$ . After light irradiation, the cells were then incubated for analysis. All experiments were randomly divided into 4 groups: photodynamic treatment of hypocrellin B, hypocrellin B treatment alone, light irradiation alone, and sham irradiation. The cells in the photodynamic treatment group were incubated with hypocrellin B before the exposure to light. The cells in light irradiation alone group were irradiated by light without incubation with hypocrellin B. Those in hypocrellin B treatment alone group were incubated with hypocrellin B without light irradiation. The cells in sham radiation group were treated by neither light irradiation nor hypocrellin B.

**2.4. Cell Survival Measurement.** To measure cell survival rates of ovarian cancer cells treated by photodynamic action

of hypocrellin B, HO-8910 cells ( $2 \times 10^4$  cells/well) were cultured overnight in 96-well microplates. Hypocrellin B (2.5  $\mu\text{M}$ ) was added into cells in each well and incubated for 5 h in the dark. Unbound hypocrellin B was removed and the cells were exposed (except for the dark controls) to blue light from LED system with the wavelength of 470 nm and the energy density of 1  $\text{J}/\text{cm}^2$ . After photodynamic action of hypocrellin B the cells were incubated for 20 h at  $37^{\circ}\text{C}$ . After that, 100  $\mu\text{L}$  MTT-containing medium was added into each well (0.5  $\text{mg}/\text{mL}$ , diluted with medium). After the incubation for 4 h at  $37^{\circ}\text{C}$ , MTT-containing medium was removed and 100  $\mu\text{L}$  DMSO was added into each well. The optical density (OD) was measured using an iEMS Analyzer (Lab-system, Type1401) at the wavelength of 570 nm. The cell survival rate is calculated using the following formula: cell survival rate (%) = OD of the treatment group/OD of the control group  $\times 100\%$ .

**2.5. Mitochondrial Morphological Observation.** Transmission electron microscopy (TEM) was performed to observe mitochondrial morphological changes of ovarian cancer HO-8910 cells. It was carried out 6 hours after hypocrellin B (2.5  $\mu\text{M}$ ) had been activated by blue light from LED system with the wavelength of 470 nm and the energy density of 1  $\text{J}/\text{cm}^2$ . Fixed cells were postfixed in 2%  $\text{OsO}_4$ , dehydrated in graded alcohol, and flat-embedded in Epon 812 (Electron Microscopy Sciences, Fort Washington, PA, USA). Ultrathin sections (100 nm) were prepared, stained with uranyl acetate and lead citrate, and examined under an electron microscopy (H-600; Hitachi, Japan).

**2.6. Mitochondrial Membrane Functional Measurement.** Mitochondrial membrane potential was measured using flow cytometry with JC-1 staining. Briefly, HO-8910 cells were incubated with hypocrellin B (2.5  $\mu\text{M}$ ) for 5 h. The cells were then irradiated by blue light from LED source with the energy density of 1  $\text{J}/\text{cm}^2$  and further incubated for 3 h. Finally, the treated cells were washed 3 times in PBS and stained with JC-1 for 20 min at  $37^{\circ}\text{C}$  in the dark. The washed cells were resuspended in PBS and then analyzed using flow cytometry (SE, Becton Dickinson) with the excitation setting at 488 nm, and signals were acquired at the FL-2 channel. At least 10,000 cells per sample were acquired in histograms and data analyzed by CellQuest software.

**2.7. Statistical Analysis.** The statistical analysis was performed using SPSS 13.0 for Windows. Differences between groups were analyzed by one-way ANOVA (analysis of variance). A  $P$  value  $< 0.05$  was considered significantly difference.

### 3. Results

**3.1. Inhibition of Cell Proliferation.** MTT assay showed that cell survival rate of the photodynamic treatment group was  $27.22 \pm 1.26\%$ , which was significantly lower than that of the sham group ( $P < 0.01$ ). Neither hypocrellin B treatment alone nor light irradiation alone had significant effect on cell survival (Figure 1).

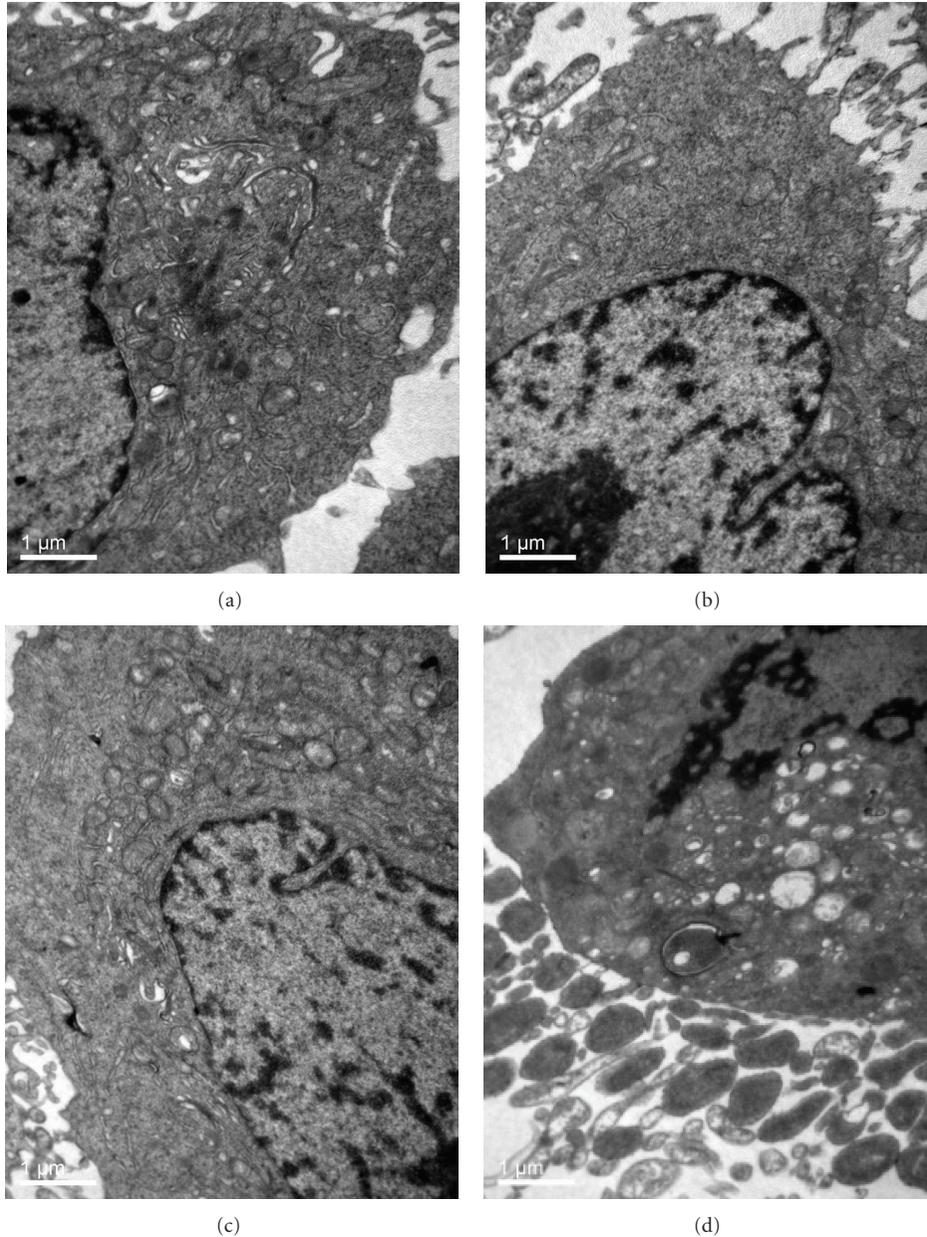


FIGURE 2: Mitochondrial morphological changes in HO-8910 cells were observed using TEM 6 h after photodynamic action of hypocrellin B ( $\times 15,000$ ). (a): Sham irradiation; (b): hypocrellin B treatment alone; (c): light irradiation alone; (d): photodynamic action of hypocrellin B.

**3.2. Mitochondrial Morphological Changes.** Mitochondria which maintained integrity of cell membrane and cristae were observed in ovarian cancer HO-8910 cells after sham irradiation, hypocrellin B treatment alone, and light irradiation treatment alone Figures 2(a), 2(b), and 2(c). After the combined treatment of light irradiation and hypocrellin B, swollen mitochondria were observed and some cells even showed an absence of cristae (Figure 2(d)).

**3.3. Changes in Mitochondrial Function.** Ovarian cancer HO-8910 cells were sensitized with  $2.5 \mu\text{M}$  hypocrellin B and then irradiated by LED light. After JC-1 staining, flow

cytometry was used to analyze the changes of mitochondrial membrane potential. Figure 3 showed that the spectral shift of the fluorescence curve to the left, indicating that mitochondrial membrane depolarization occurred in the treated HO-8910 cells.

#### 4. Discussion

Hypocrellin B from traditional Chinese herb *Hypocrella bambuase* has been confirmed as an efficient singlet oxygen generator upon light irradiation [5, 6, 10, 11]. Growing evidences show that light-activated hypocrellin B can markedly

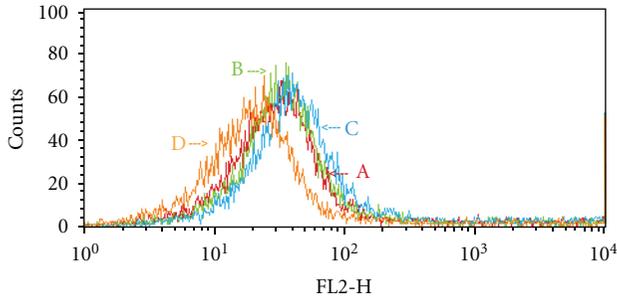


FIGURE 3: Mitochondrial membrane potential of HO-8910 cells was measured using flow cytometry with JC-1 staining 6 h after photodynamic action of hypocrellin B. The spectral shift of the fluorescence curve to the left indicates mitochondrial membrane depolarization. A: sham irradiation; B: hypocrellin B treatment alone; C: light irradiation alone; D: photodynamic action of hypocrellin B.

kill malignant cells and induce cell apoptosis [8, 9]. Our previous studies observed that hypocrellin B irradiated by blue light from LED source could cause significant damage to ovarian cancer HO-8910 cells [9]. In the present study, MTT assay showed that cell survival rate of HO-8910 cells in the photodynamic treatment group decreased significantly. Hypocrellin B treatment alone or light irradiation alone showed no significant impact. These data demonstrated that photodynamic action of hypocrellin B could decrease cell survival rate of ovarian cancer HO-8910 cells. However, the underlying mechanisms still need to be clarified.

Mitochondria play a central role in the regulation of cell growth and death [12, 13]. Mitochondria affect cellular breathing and energy metabolism directly or indirectly, subsequently decreasing cell proliferation and inducing cell death [14, 15]. In our TEM, we observed mitochondria with well-developed cristae in ovarian cancer HO-8910 cells after sham irradiation, hypocrellin B treatment alone, and light irradiation alone. After photodynamic treatment of hypocrellin B, some mitochondria displayed serious damage and disappearance of cristae. These alterations of mitochondrial structure would directly affect mitochondrial functions. In our present study, flow cytometry analysis with JC-1 staining also found that the spectral shift of the fluorescence curve was to the left, indicating that mitochondrial membrane depolarization occurred in HO-8910 cells treated by photodynamic action of hypocrellin B. The mitochondrial membrane depolarization can induce the release of mitochondrial cytochrome c into cytoplasm to initiate effector caspases such as caspases 3, 6, and 7. These effector Caspases can induce the generation of tBid to cause cell program death [16–18]. These findings suggest that mitochondrial morphological alterations and mitochondrial membrane depolarization are important events in photodynamic action of hypocrellin B on ovarian cancer HO-8910 cells. Hypocrellin B is a very good generator of ROS causing cell damage under light irradiation. It is well known that ROS has a very limited diffuse distance inside the cells; thus, ROS result in intracellular damage often close to the

sensitizer localization [19, 20]. Ali and Olivo have reported that hypocrellin B mainly localizes in lysosomes other than mitochondria [21], suggesting that lysosomes are the direct target of photodynamic action of hypocrellin B. Recent studies have observed that there is lysosomal/mitochondrial crosstalk inside the cell [22–25]. The damaged lysosomes can cause lysosomal membrane permeabilization (LMP) and release cathepsins into the cytosol, subsequently inducing the mitochondrial alterations via the bcl-2 family Bid or bax [23–25]. Therefore, hypocrellin B-mediated photodynamic action induced cell death of HO-8910 cells probably through lysosomal-mitochondrial axis.

## 5. Conclusion

Photodynamic action of hypocrellin B under LED light irradiation significantly decreased cell proliferation and caused damage to mitochondrial morphology and function of ovarian cancer cells, highlighting that mitochondrial damage might be an important event in photodynamic therapy of hypocrellin B on ovarian cancer.

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