Rubratoxin B induces apoptosis in p53-null cells

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ルブラトキシン B は p53 欠損細胞にアポトーシスを誘導する

Summary

Exposure to rubratoxin B caused homozygous null *p53* cells to shrink, condense their chromosomes and fragment their nuclei, showing that rubratoxin B induces apoptosis in p53-null cells

Key words: rubratoxin B, ルブラトキシン B; apoptosis, アポトーシス; p53, p53

Introduction

Rubratoxin B is a potent hepatotoxic^{1,2)} and teratogenic^{3,4)} mycotoxin produced by the fungi *Penicillium rubrum* and *Penicillium purpurogenum*^{1,5)}. We previously reported that rubratoxin B induced internucleosomal fragmentation (DNA ladder)⁶⁾ and brought about nuclear fragmentation⁶⁾, suggesting that the toxicity of rubratoxin B is the consequence of apoptosis^{6,7)}. To date, p53-dependent^{8,9)} and independent^{10,11)} apoptosis have been reported. The p53 tumor suppressor protein preserves genetic stability by mediating either a G₁ arrest^{12,13)} or apoptosis^{8,10)} in response to DNA damage. In this report, to elucidate the mechanism of apoptosis induced by rubratoxin B, we investigated p53-dependency using homozygous null *p53* cells.

Materials and Methods

Chemicals and cells Rubratoxin B was purchased from Makor Chemicals (Israel). Cultured homozygous null *p53* mouse embryo fibroblast cell (p53-null cell)¹⁰⁾ was generously given by Dr. A. Fanidi.

The p53-null cell was cultured in Dulbecco's Modified Eagles medium containing 10% fetal calf serum.

Morphological study Four times 10⁵ cells in 2 ml of medium with rubratoxin B were cultured in each well of a 6-well plate for 24 hr, followed by microscopic examination. For examination of nuclei, 4 × 10⁵ cells in 2 ml of medium with 10 µg/ml rubratoxin B were cultured in a glass bottom culture dish for 24 hr, then stained with 5 µg/ml Hoechst 33258 at 37°C for 30 min¹⁴⁾. The nuclei were examined by fluorescence microscopy.

Results and Discussion

As shown in Fig. 1, exposure to rubratoxin B caused p53-null cells to shrink, similar to the results of other cultured cells^{7,15)}. Seeing that more than half of the cells floated after the 24 hr-treatment of 40 µg/ml of rubratoxin B (Fig. 1C), p53-null cells are more sensitive than HeLa and HepG2 cells which did not float under the same experimental condition⁷⁾. Since shrinkage of the cells is one of the characteristics of apoptosis, our results suggested the possibility that rubratoxin B induces apoptosis in p53-null cells.

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Fig. 1 Morphological change of p53-null cells by rubratoxin B. (A), Control; (B), treated with 20 $\mu\text{g}/\text{ml}$; (C), treated with 40 $\mu\text{g}/\text{ml}$ of rubratoxin B for 24 hr. (Magnification $\times 100$)

Fig. 2 Morphological change of p53-null cell nuclei by rubratoxin B. (A), Control; (B-E), treated with 10 $\mu\text{g}/\text{ml}$ of rubratoxin B for 24 hr. The cells were stained with Hoechst 33258, then the nuclei were examined by fluorescence microscopy (Magnification $\times 400$)

To confirm that the shrinkage of the cells was the consequence of apoptosis, we investigated the morphological change of nuclei induced by rubratoxin B, since the condensation of chromatin and nuclear

fragmentation are the typical morphological changes of apoptosis¹⁶. Compared to the control (Fig. 2A), rubratoxin B-treated chromatin was condensed (Fig. 2B-2E). Furthermore, we observed crescents

in apposition to the nuclear membrane (Fig. 2B, 2C), and several dense spheres (Fig. 2D, 2E) as a result of nuclear fragmentation. Therefore, our results showed that rubratoxin B induced apoptosis in p53-null cells. Although these changes are often accompanied by DNA ladder, we could not detect under these experimental conditions (data not shown), as in the case of HeLa cells⁶⁾.

Rubratoxin B induced apoptosis in HL-60 cells⁶⁾. The p53 protein and mRNA were not detected in HL-60 cells, probably because of major deletions in the coding region of *p53* gene¹⁷⁾. Inasmuch as rubratoxin B induces apoptosis in two independent p53-deficient cell lines, our results strongly suggest that rubratoxin B can induce p53-independent apoptosis. There is a possibility, however, that the activity of p53 in HL-60 cells is not completely lost. HL-60 cells retain the 5' part of *p53* gene¹⁷⁾, and only the first 214 amino-terminal residues of murine p53 was sufficient to induce p53-dependent apoptosis¹⁸⁾. Further studies are needed to clarify whether rubratoxin B induces apoptosis exclusively mediating p53-independent pathway or not.

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