

[Figure 1]

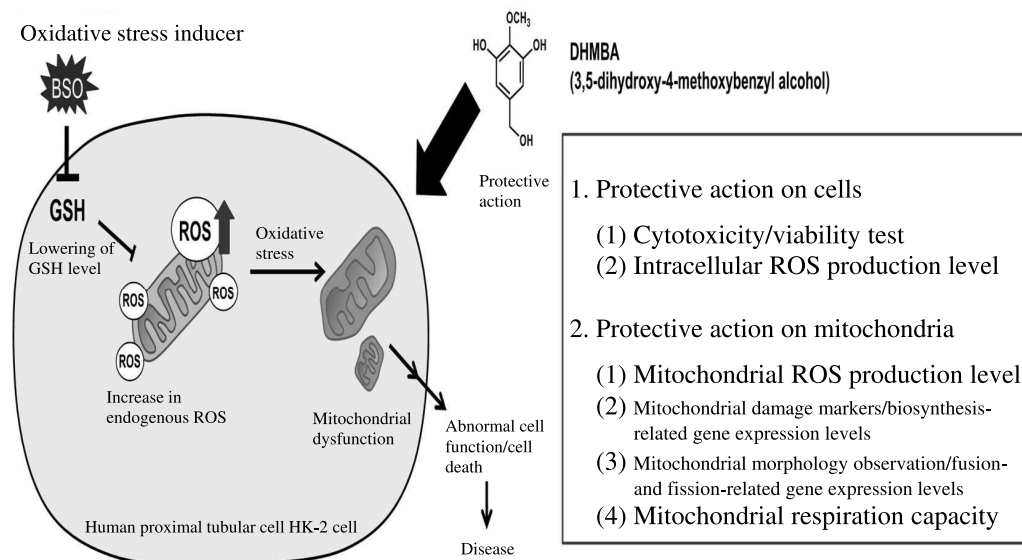


Fig. 1. Strategy for searching for effects of improving mitochondrial functions

[Figure 2]

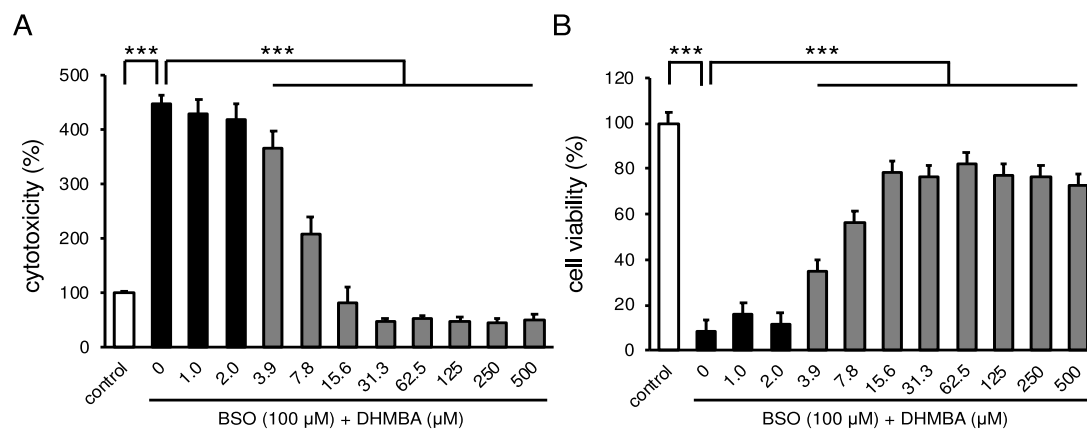


Fig. 2. Cytoprotective effect of DHMBA under oxidative stress stimulation

After HK-2 cells were cultured with 100 μ M BSO and 1 μ M to 500 μ M DHMBA, cytoprotective testing by cytotoxicity (A) and cell viability (B) was performed. Mean $\pm$ SD (n = 5 to 6).

[Figure 3]

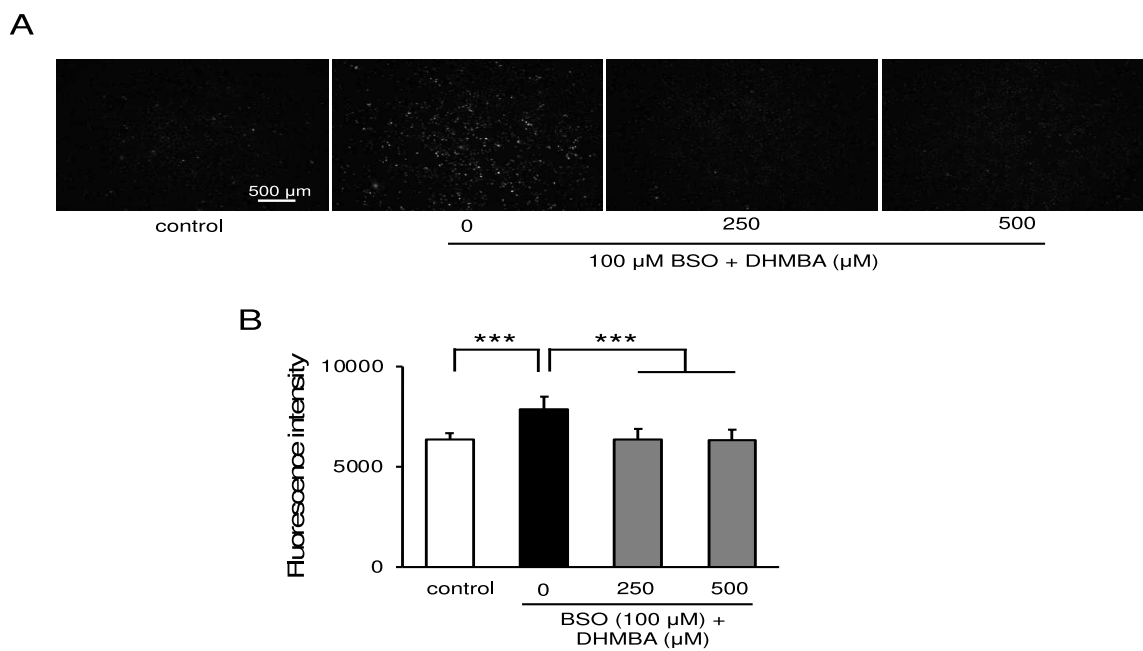


Fig. 3. Effect of DHMBA reducing intracellular ROS under oxidative stress stimulation

After HK-2 cells were cultured with 100 μ M BSO and 250 μ M and 500 μ M DHMBA, intracellular ROS fluorescence images (A) and fluorescence intensity (B) were measured by DCFDA staining. Mean $\pm$ SD (n = 6).

[Figure 4]

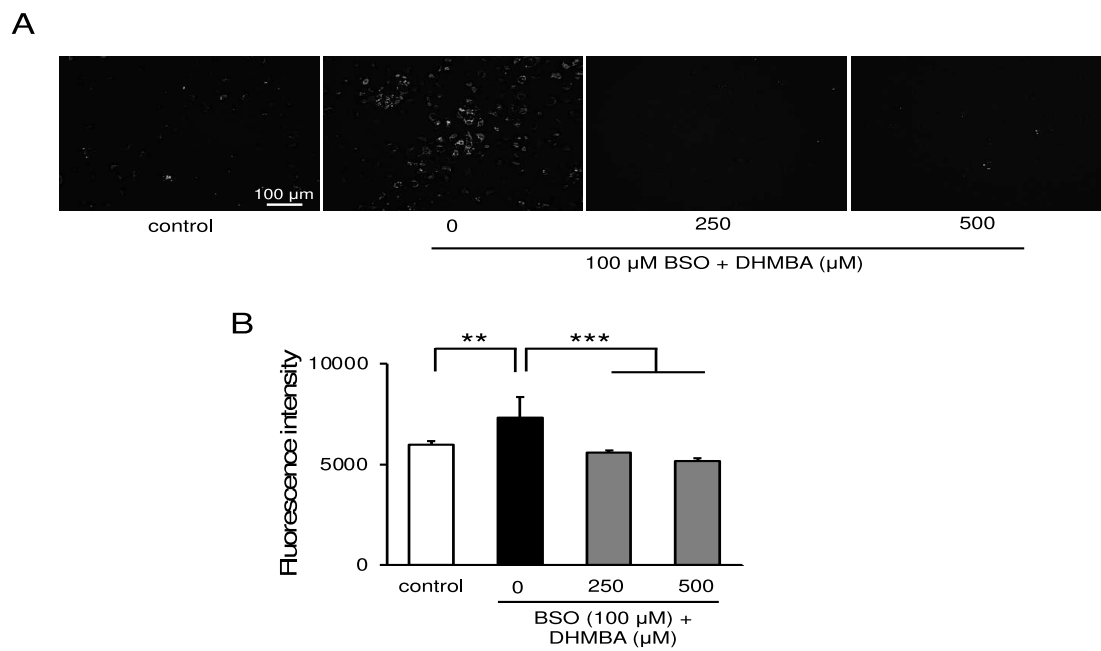


Fig. 4. Effect of DHMBA reducing intramitochondrial ROS under oxidative stress stimulation

After HK-2 cells were cultured with 100 μM BSO and 250 μM and 500 μM DHMBA, intramitochondrial ROS fluorescence images (A) and fluorescence intensity (B) were measured by DHR123 staining. Mean $\pm$ SD ($n = 6$).

[Figure 5]

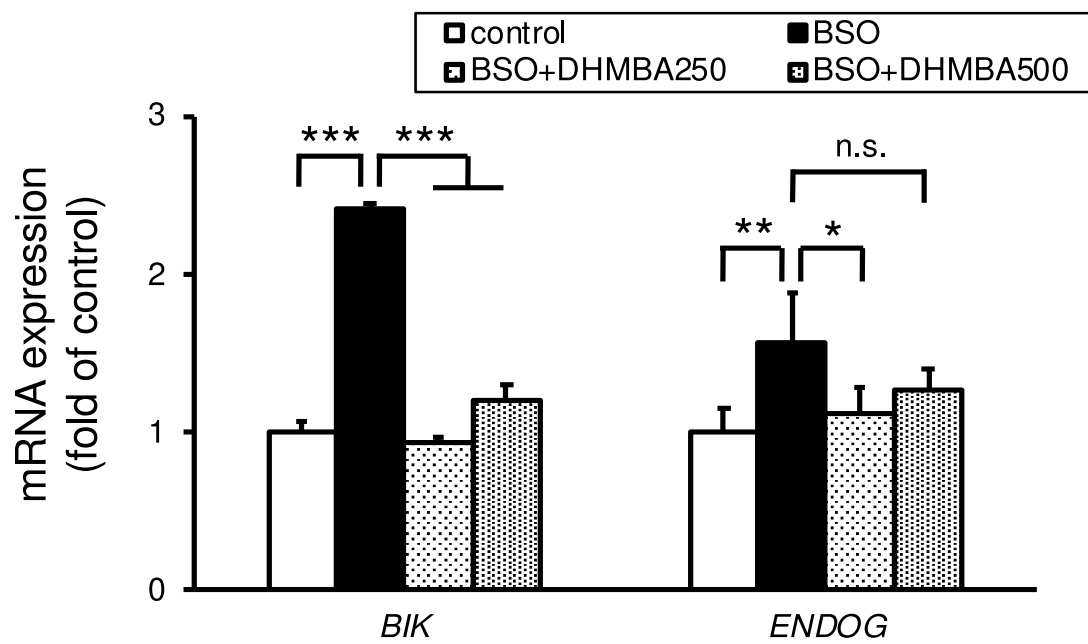


Fig. 5. Changes in expression levels of cells and mitochondrial damage-related genes by DHMBA under oxidative stress stimulation. After HK-2 cells were cultured with 100 μ M BSO and 250 μ M and 500 μ M DHMBA, the gene expression levels were measured by means of qPCR. Mean $\pm$ SD (n = 4).

[Figure 6]

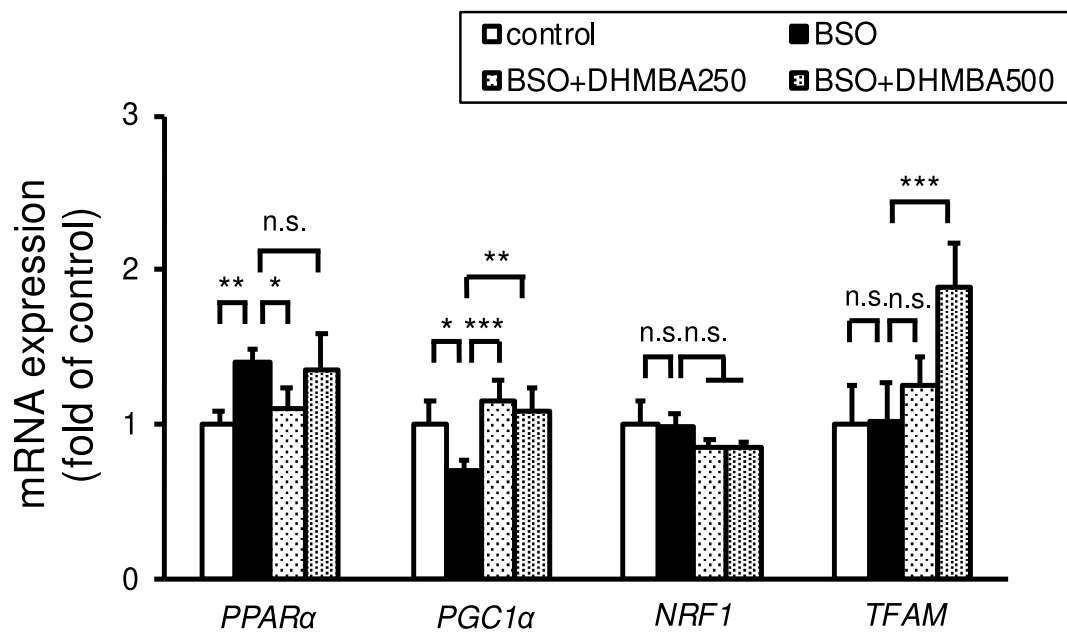


Fig. 6. Changes in expression levels of mitochondrial biosynthesis-related genes by DHMBA under oxidative stress stimulation. After HK-2 cells were cultured with 100 μ M BSO and 250 μ M and 500 μ M DHMBA, the expression levels of mitochondrial biosynthesis-related genes were measured by means of qPCR. Mean $\pm$ SD (n = 4).

[Figure 7]

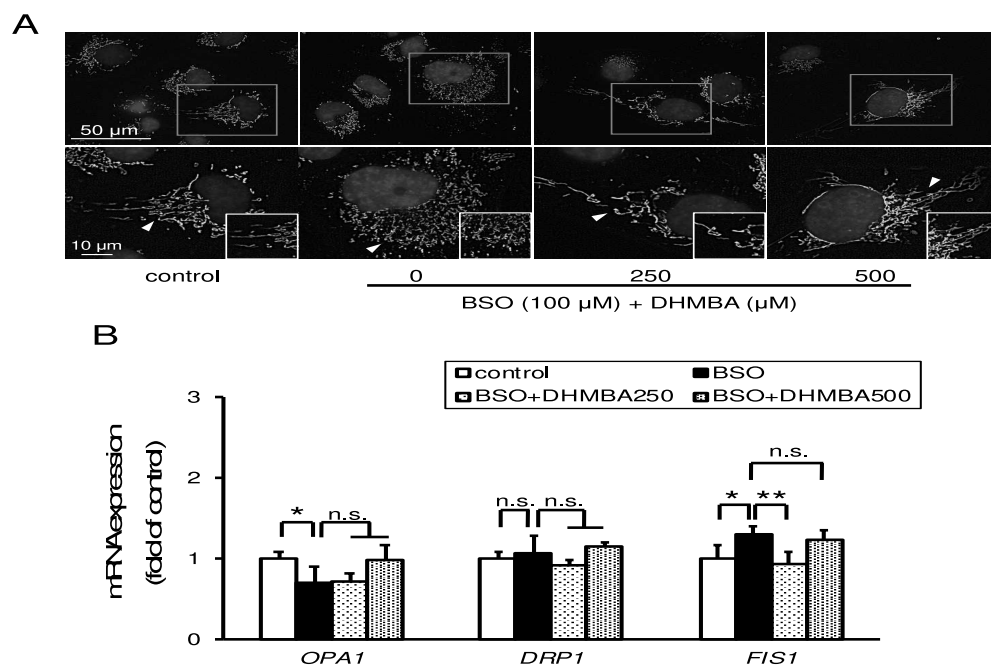


Fig. 7. Effect of DHMBA on mitochondrial morphology under oxidative stress stimulation and changes in expression levels of fusion- and fission-related genes

After HK-2 cells were cultured with 100 μ M BSO and 250 μ M and 500 μ M DHMBA, observation of mitochondrial morphology with MitoTracker[®] Green FM fluorescent stain (A) and measurement of expression levels of mitochondrial fusion- and fission-related genes by means of qPCR (B) were performed. Mean $\pm$ SD (n = 4).

[Figure 8]

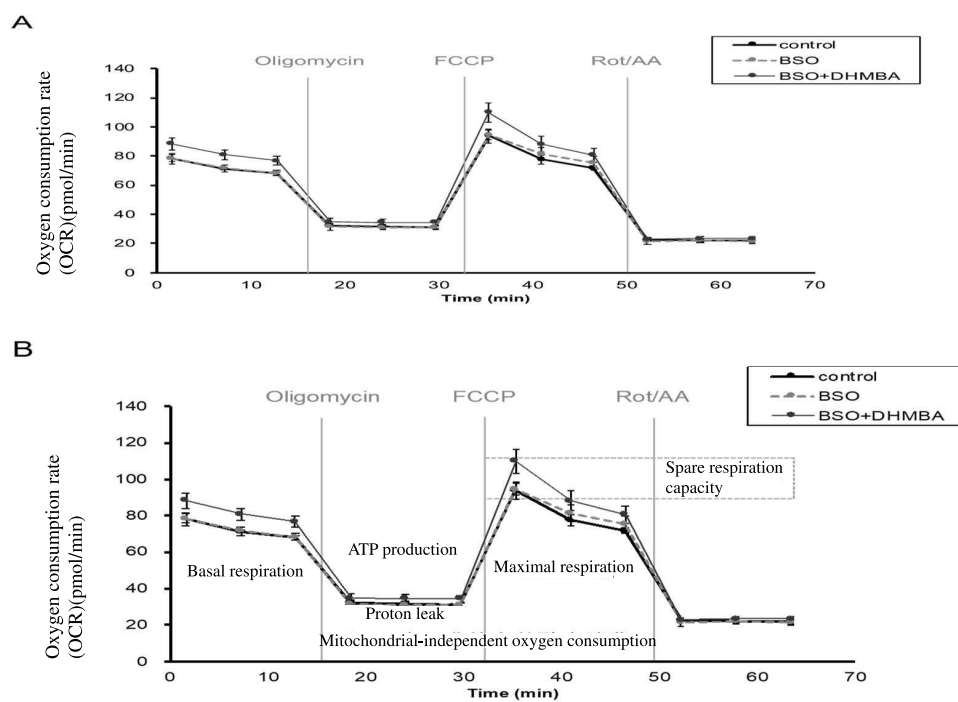


Fig. 8. Effect of DHMBA on mitochondrial oxygen consumption rate
 After HK-2 cells were cultured with 100 μ M BSO and 500 μ M DHMBA for 24 hours, the oxygen consumption rate was measured using an extracellular flux analyzer (A). Fluctuations in the oxygen consumption rate can be used to calculate key indicators in the assessment of mitochondrial functions (B). Mean $\pm$ SD (n = 3 to 4).

[Figure 9]

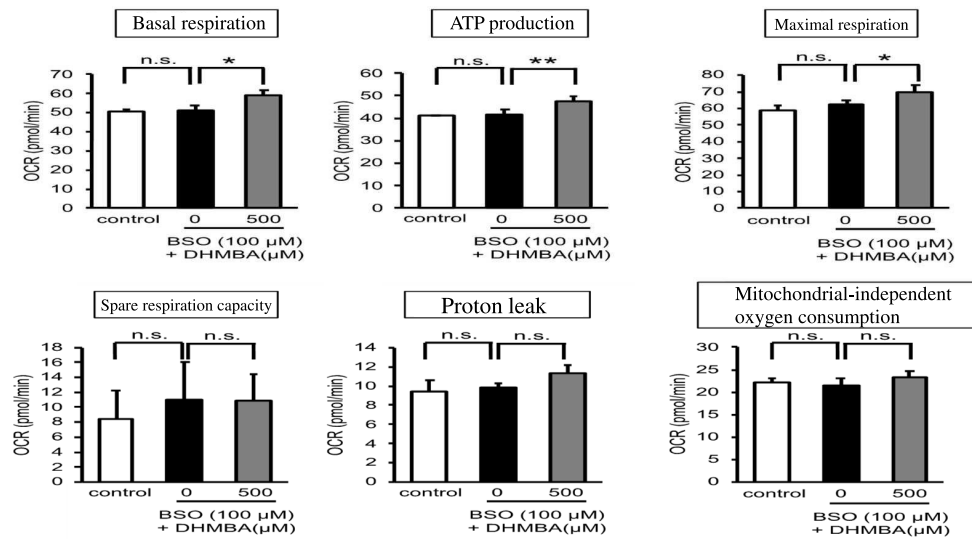


Fig. 9. Effect of DHMBA on mitochondrial functions

After HK-2 cells were cultured with 100 μM BSO and 500 μM DHMBA for 24 hours, the oxygen consumption rate was measured using an extracellular flux analyzer, and key indicators in the assessment of mitochondrial functions (basal respiration, ATP production, maximal respiration, spare respiration capacity, proton leak, mitochondrial-independent oxygen consumption) were calculated. Mean $\pm$ SD (n = 3 to 4).