Oxidative modification of DNA bases in human lung cancer cells treated with non-thermal atmospheric-pressure plasma

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Abstract: We investigated oxidative damage to nuclear and mitochondrial DNA in A549 human lung cancer cells exposed to a helium atmospheric-pressure plasma jet (APPJ). APPJ irradiation increased intracellular RONS levels. The formation of 8-oxoguanine (8-oxoG), a representative oxidized form of a DNA base, was observed in both nuclear and mitochondrial DNA. We also demonstrated that the 8-oxoG level gradually decreased during cell culture, suggesting that 8-oxoG was removed from nuclear DNA after APPJ irradiation.

Keywords: Plasma medicine, oxidative stress, DNA damage, base modification.

1. Introduction

Non-thermal atmospheric-pressure plasma (NTAPP) has emerged as a useful tool in medicine and life science research. For example, NTAPP irradiation has been studied in cancer therapy, wound healing, and the prevention of infectious diseases. In addition, the use of NTAPP to introduce cell-impermeable molecules has become a promising approach in life sciences and medical research. The biological effects are mainly due to oxidative reactions induced by reactive oxygen and nitrogen species (RONS) supplied by NTAPP irradiation. For example, plasma-delivered RONS in liquids lead to elevated intracellular RONS levels and stimulate various cellular responses.

Intracellular RONS can induce damage to biological molecules. For example, some RONS induce oxidative damage to nucleic acids, including strand breaks and base modifications. Plasma-induced DNA damage is a possible trigger of apoptotic cancer cell death as well as a biological indicator useful in evaluations of genotoxicity and carcinogenicity. Therefore, a number of studies have examined how NTAPP treatment of cultured cells affects intracellular DNA.

In this study, we investigated oxidative damage to nuclear and mitochondrial DNA (mtDNA) in A549 human lung cancer cells exposed to a helium atmospheric-pressure plasma jet (APPJ). APPJ irradiation elevated intracellular RONS level. The formation of 8-oxoguanine (8-oxoG), a representative oxidized form of a DNA base, was observed after APPJ irradiation. In addition, we observed that the 8-oxoG level decreased after additional incubation.

2. Materials and methods

Fig. 1 shows a schematic illustration of the experimental setup. A helium atmospheric-pressure plasma jet (He-APPJ) was used in this study. The APPJ generator consisted of a quartz glass tube with two electrodes (10 mm width) spaced 5 mm apart. The upper electrode was powered (18 kV_{p-p} sinusoidal voltage at 18 kHz), and the other was grounded. The distance between the grounded electrode and the nozzle was 10 mm. We used A549 human lung carcinoma cells. One milliliter of a 4.0×10^5 cells/ml suspension prepared with D-PBS (-) in one well of a 24-well tissue culture test plate was irradiated with the He-APPJ for 1–5 min.

The changes in intracellular RONS levels were determined using a general oxidative stress fluorescence probe CM-H_2DCFDA. Before He-APPJ irradiation, A549 cells were loaded with the fluorescent probe. After He-APPJ irradiation, the fluorescence intensity of the cells was measured using flow cytometry.

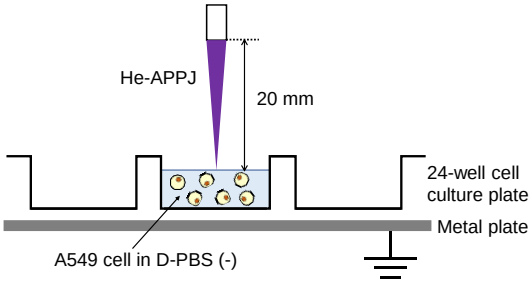

Fig. 1. Schematic illustration of He-APPJ irradiation to a cell suspension.

We investigated the oxidation of a guanine base (8-oxoG formation). Immunofluorescence staining was used to detect 8-oxoG. Following He-APPJ irradiation, the cells were fixed and denaturated. To investigate 8-oxoG formation in nuclear DNA, the cells were treated with HCl. NaOH was used to detect 8-oxoG in mtDNA. After the denaturation, cells were permeabilized and incubated with an anti-8-oxoG antibody labeled with FITC. The cells were analyzed using a flow cytometer.

3. Results and discussion

Fig. 2 shows the results of flow cytometric analysis of intracellular RONS. Fig. 2 shows representative flow cytometry histograms. APPJ irradiation of A549 cells resulted in increased 2'-7'dichlorofluorescein (DCF) fluorescence intensity compared with the control. Longer irradiation time resulted in a greater increase in fluorescence intensity. As DCF fluorescence intensity is correlated with intracellular RONS level, this result suggests that APPJ irradiation stimulates the production of intracellular RONS.

Fig. 3 shows the results of 8-oxoG immunostaining in APPJ-irradiated cells treated with HCl or NaOH. The APPJ irradiation time was 5 min. Fig. 3 (a) and 3 (b) show representative flow cytometry histograms. Compared with the control, APPJ irradiation led to increased FITC fluorescence intensity after treatment with both HCl and NaOH. Fig. 3 (c) shows the relative median FITC

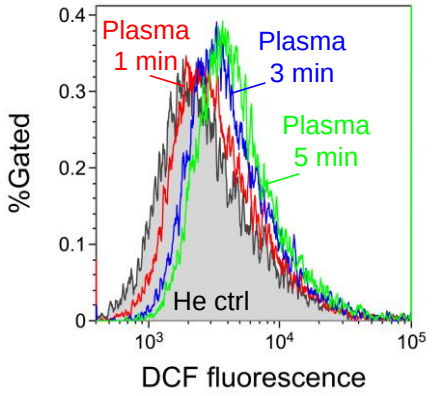

Fig. 2. Intracellular RONS production in APPJ-irradiated A549 cells. Representative flow cytometry histograms are shown.

fluorescence intensity compared with the control. A statistically significant increase in FITC fluorescence intensity was observed under the conditions of treatment with both HCl and NaOH. Therefore, these results suggest that APPJ irradiation stimulates 8-oxoG formation in nuclear DNA and mtDNA. This result suggests that APPJ irradiation enhances RONS accumulation in mitochondria.

Fig. 4 shows 8-oxoG formation and removal in nuclear DNA associated with 5 min of APPJ irradiation. A549 cells were irradiated with APPJ in D-PBS (−), centrifuged, and divided into three sample tubes. The cells in one tube were immediately subjected to 8-oxoG immunostaining, whereas the cells in the other tubes were resuspended in a cell culture medium and inoculated into a cell culture plate. After 2 or 4 h of culture, the cells were harvested and immunostained. FITC fluorescence intensity was significantly greater than the control after 2 and 4 h of culture. However, the relative fluorescence intensity gradually decreased with increasing culture incubation time. These results demonstrate that the 8-oxoG level in nuclear DNA increases after APPJ irradiation. The decrease in 8-oxoG level during culture post-APPJ irradiation suggests 8-oxoG is later removed from nuclear DNA.

4. Conclusion

This study demonstrated the formation and removal of 8-oxoG in A549 cells exposed to He-APPJ. APPJ irradiation elevated the levels of intracellular RONS and 8-oxoG in nuclear DNA. The 8-oxoG level decreased after 4 h of additional incubation, suggesting the removal of 8-oxoG in APPJ-irradiated cells. Furthermore, the present study also revealed 8-oxoG formation in mtDNA in APPJ-irradiated cells. These results will further understanding of the cellular responses to CAP treatment.

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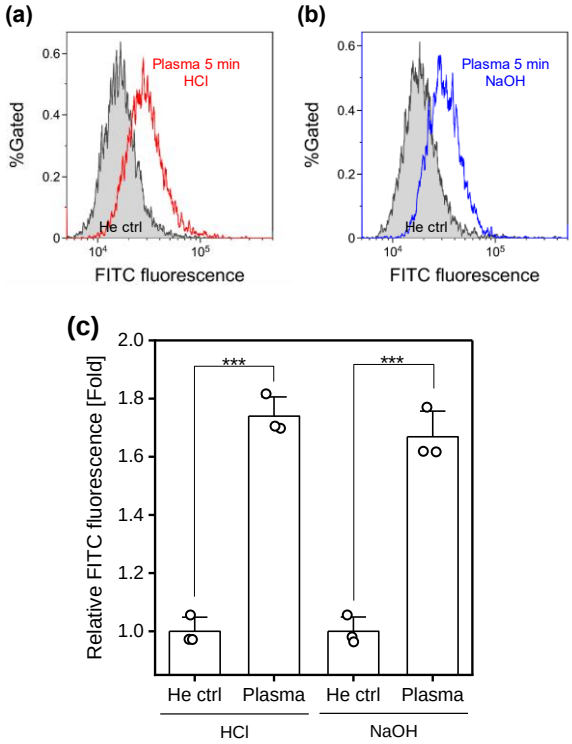

Fig. 3. Formation of 8-oxoguanine (8-oxoG) in plasma-irradiated A549 cells. Cells were treated with APPJ, and 8-oxoG levels were measured using flow cytometry and immunostaining.

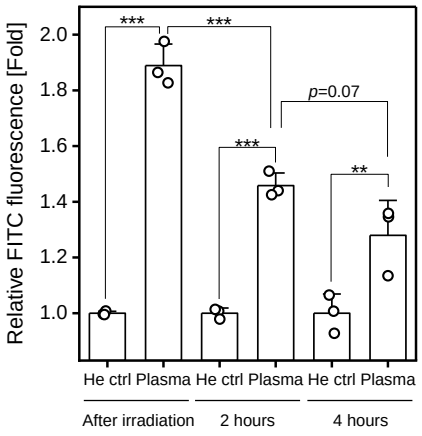

Fig. 4. Formation and removal of nuclear 8-oxoG in plasma-irradiated A549 cells. APPJ-irradiated cells were subjected to immunofluorescence staining immediately after APPJ irradiation or after 2 and 4 h of culture.

References

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