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Cellular Responses of Human Lung Cancer Cell Lines to Atmospheric Pressure Plasma-exposed Cell Culture Medium

ヒト肺がん由来培養細胞株の大気圧プラズマ照射培養液に対する細胞応答

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Recent progress of biomedical application of atmospheric pressure plasma shows that the biological effects are mainly due to reactive oxygen and nitrogen species. Here, we focused on reactive chemical species in plasma-exposed cell culture medium and cellular responses of mammalian cells after treatment of the cell culture medium. In this study, we examined two human lung cancer cell lines (A549 and NCI-H460). The contribution of hydrogen peroxide (H_2O_2) to the cellular responses was considered. Cellular responses in the both cell lines showed that the plasma-exposed medium and the H_2O_2 treatment gave similar reduction in viability and induction of apoptosis.

1. Introduction

Recently, non-thermal atmospheric pressure plasma has been studied in biological and medical applications. In particular, an atmospheric pressure plasma jet (APPJ) is widely used because it can treat subjects without thermal loading, and length of the plasma jet can be adjusted by flow condition of noble gas. It is generally considered that reactive oxygen and nitrogen species (RONS) in liquid produced by the plasma exposure play an important role. To elucidate the cellular responses induced by exposure to NTP, we focused on (1) identification and quantification of reactive chemical species in plasma-exposed cell culture medium, and (2) cellular responses of mammalian cells after treatment of the cell culture medium. In this study, we examined two human lung cancer cell lines (A549 and NCI-H460). The contribution of hydrogen peroxide (H_2O_2) to the cellular responses was considered.

2. Materials and methods

APPJ generator (Fig. 1) was constructed with a glass tube, a stainless steel wire as a high voltage electrode, and a stainless steel mesh as a ground electrode [1]. Dielectric barrier discharge was generated between the both electrodes, using a pulse power supply. A spin trap reagent (DMPO) and cell culture medium (high glucose DMEM supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin) were exposed to the APPJ.

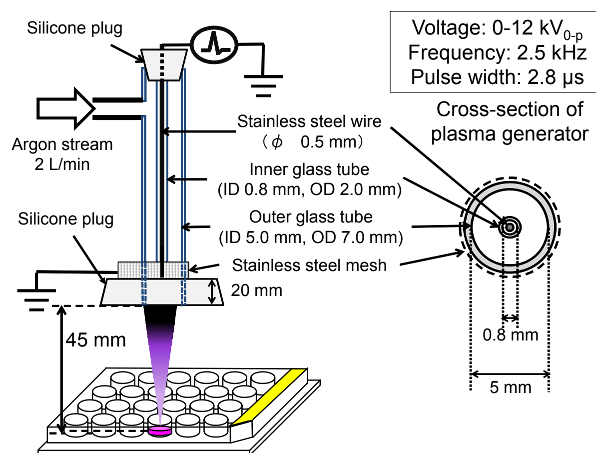

Fig.1. A schematic view of an argon plasma jet generator

In this study, we measured the production of OH radical using electron spin resonance (ESR) spectroscopy [2]. DMPO was used as a spin trap agent. 0.1 M DMPO was exposed for different time duration and discharge condition. After exposure, ESR signal was immediately measured. To determine the concentration of H_2O_2 in the plasma-exposed cell culture medium, Amplex Red Hydrogen Peroxide kit (Invitrogen) and a spectral scanning multimode reader (Varioskan Flash, Thermo scientific) were used. We also conducted

ROS detection with ROS-reactive fluorescent probes (APF and HPF, Invitrogen).

Cellular responses of human lung cancer cell lines (A549 and NCI-H460) to plasma-exposed cell culture medium were also studied. After treatment of the plasma-exposed cell culture medium, viability and cytotoxicity were assessed by fluorometric measurement of live-cell protease activity and dead-cell protease activity, respectively. Induction of apoptosis was also assessed by luminometric measurement of caspase3/7 activity. In addition, we also used cell culture medium containing H_2O_2 for comparison.

3. Results and discussion

First, OH radical production in aqueous solution was confirmed by ESR. As a result, a four-line ESR spectrum with an intensity ratio of 1:2:2:1 corresponding to the DMPO-OH spin adduct was observed [2]. The fluorometric measurement of H_2O_2 showed the H_2O_2 concentration in cell culture medium after 10 minutes of the APPJ exposure was 0.4 mM. Fluorescent intensity of the both ROS-reactive fluorescent probes was also increased with the exposure time.

Fig. 2 shows the cellular responses of human lung cancer cell lines to 16 hours treatment of the plasma-exposed cell culture medium and H_2O_2-containing cell culture medium. In the case of A549, treatment of both the plasma-exposed medium and H_2O_2-containing medium for 16 hours resulted in a dose-dependent decrease in apparent viability with cytotoxicity, but small increase in caspase-3/7 activity. However, in the case of NCI-H460, treatment of both the plasma-exposed medium and H_2O_2-containing medium for 16 hours resulted in a dose-dependent decrease in apparent viability with no cytotoxicity, but an increase in caspase-3/7 activity. Cellular responses in both cell lines showed that the plasma-exposed medium and the H_2O_2 treatment gave similar reduction in viability and induction of apoptosis. Thus, H_2O_2 should be the major cause of the cellular responses, as considered in previous reports [3, 4].

4. Conclusion

ROS detection in cell culture medium after APPJ exposure and cellular responses to the plasma-exposed medium to human lung cancer cell lines were conducted. It was shown that OH radical, $ONOO^-$, and H_2O_2 were produced in the cell culture medium. Cellular responses in both A549 and NCI-H460 cell lines showed that the plasma-exposed medium and the H_2O_2 treatment gave similar reduction in viability and induction of apoptosis.

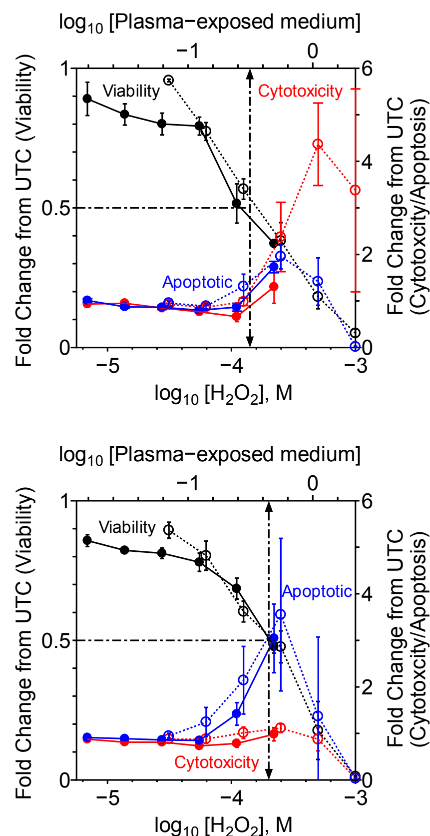

Fig.2. Cellular responses of human lung cancer cell lines to plasma-exposed cell culture medium (solid line with closed circle) and H_2O_2-containing cell culture medium (dotted line with open circle)
(upper) A549 (lower) NCI-H460

Acknowledgments

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