Effect of Plasma Direct Irradiation on Oral Cancer Cells Using Torch Type Dielectric Barrier Discharge Plasma

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Abstract: Selective inactivation of the oral cancer cells is observed when the atmospheric oxygen plasma irradiates the oral cancer cells (HSC3) and normal cells (HaCaT) after removing culture medium. When the cells are irradiated with oxygen plasma, the intracellular active oxygen in cancer cells increases comparing with that in normal cells. Activity of caspase-3/7 in oral cancer cells, which is a kind of major biological reactions of the apoptosis, increases after oxygen plasma irradiation.

Keywords: atmospheric plasma, oral cancer cell, selective inactivation, caspase-3/7

1. Introduction

Recently, medical applications such as hemostasis, organ adhesion prevention, cell growth promotion and inactivation of cancer cells are spreading using low temperature atmospheric pressure plasmas close to room temperature. Among these treatments, cancer treatments using low temperature atmospheric pressure plasmas are attracting attention as a new cancer treatment method [1]. In plasma treatments, it has been reported that reactive oxygen species and reactive nitrogen species are involved in inactivation of cancer cells. There is a possibility that plasma irradiation induces apoptosis (programmed cell death) [2]. However, the detailed inactivation mechanism of cancer cells and the effect on normal cells, which are irradiated directly with oxygen plasma, have not yet been clarified.

Plasma activated media have cancer cell inactivating effects. Methods of administering PAM to cancer cells have been studied [3]. While, most of oral cancer cells and skin cancer cells those are model cells of this study appear in the epithelium, and it is possible to be directly irradiated with plasmas. In this study, the culture media is maximally removed before the plasma irradiation to cells. The purpose of this study is to investigate inactivation effects of the atmospheric oxygen plasma irradiation on oral cancer cells and normal cells.

2. Experimental procedure

Figure 1 shows the schematic diagram of experimental equipment. A cylindrical ceramic tube as a plasma torch with length 120 mm and inner diameter of 4 mm is covered with a rectangular grounded electrode. A cylindrical spiral metal electrode is set along the inner wall of the ceramic tube. The spiral electrode is connected to a high voltage and high frequency (10 kHz) power supply. The applied voltage is fixed at 4.2 kV. The dielectric barrier discharge (DBD) plasma generated in the ceramic tube is jet out by the gas flow in the tube. Material gas for the plasma production is pure oxygen gas, and the gas flow rate is 0.6 L/min. The distance from the cells to the torch edge is 12 mm. In order to prevent cell inactivation due to drying by the gas flow, the material gas is bubbled in pure water to give constant humidly. Period of the plasma irradiation to cells is varied from 5 to 30 secs.

Type of oral cancer cell line is HSC3. HaCaT is used as a normal cell line. HSC3 and HaCaT at a density of 10,000 cells/well in 96 well plate are cultured in a CO_2 incubator for 24 hours before the experiment. After the plasma irradiation, both cells are cultured in a CO_2 incubator for 24 hours. After the cultivation, the cell number is counted using a cell counting reagent. Intracellular active oxygen is observed using the total ROS activity assay kit. Activity of caspase-3/7 in the cancer cells is measured using a fluorescence light emission intensity from the Caspase-3/7 detection reagent.

3. Results and discussion

Figure 2 shows survival cancer cell (HSC3) and normal cell (HaCaT) numbers 24 hours after the plasma irradiation varying the plasma irradiation period. Number of the cells is normalized by that of control. The ozone concentration in the gas phase above the culture medium was 120 ppm. In both cells, the number of survival cells tended to decrease depending on the plasma irradiation period. Inactivation rate of the cancer cells is higher than

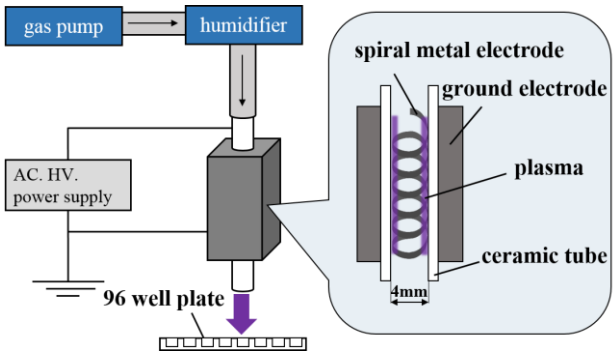

Fig. 1. Schematic diagram of experimental apparatus.

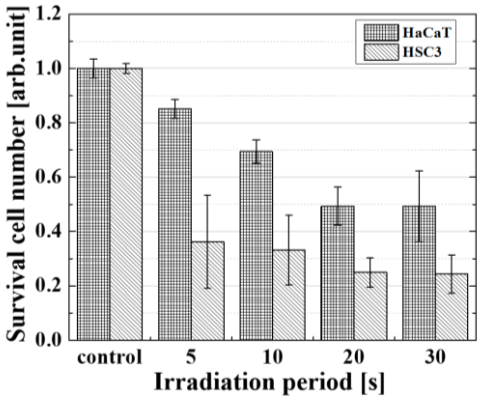

Fig. 2. Survival oral cancer cell and normal cell numbers irradiated by oxygen plasma varying irradiation period at applied voltage 4.2 kV.

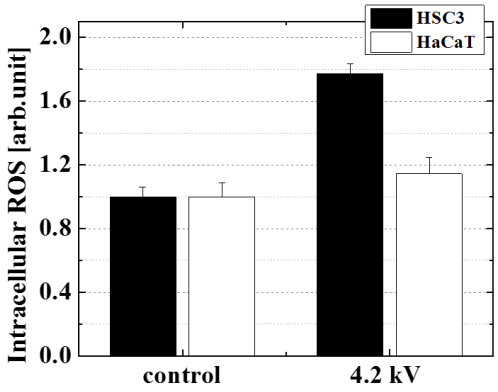

Fig. 3. Amount of intracellular ROS per cell after irradiation with oxygen plasma for 5 seconds.

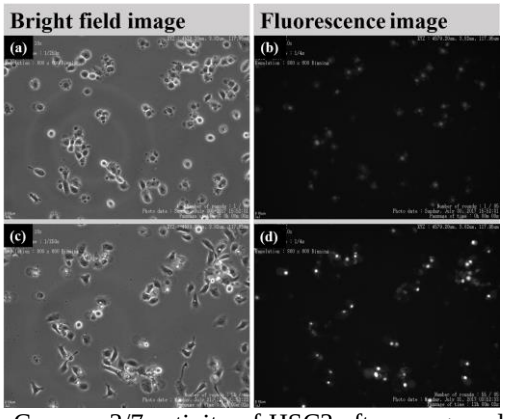

Fig. 4. Caspase-3/7 activity of HSC3 after oxygen plasma irradiation. (a), (b) Immediately after plasma irradiation. (c), (d) 12 hours after plasma irradiation.

that of normal cells at any irradiation period. In particular, at the irradiation time of 5 secs, the inactivation rate of normal cells is 15%. On the other hand, the survival rate of oral cancer cells is 64%. This result indicate that normal and cancer cells differ in resistance to active species in the plasma. One of the factors of cell inactivation by the low temperature atmospheric plasma is active oxygen species. It has been reported that cancer and normal cells are in the different consumption of active oxygen species and cancer cell is vulnerable to oxidative damage [4]. The appropriate increase of oxidative stress induces a gene mutation, acceleration of cell proliferation and apoptosis resistance. However, further oxidative stress lead cell dysfunction and cell death. Cancer cells lead to cell death earlier than normal cells when active oxygen species irradiate cells because antioxidative enzyme levels of cancer cells are lower than those of normal cells [5].

Figure 3 shows the amount of intracellular active oxygen species, which is normalized by control value. The amount of intracellular active oxygen species in HSC3 after the oxygen plasma irradiation increases approximately 1.8 times compared to control. On the other hand, that of HaCaT after plasma irradiation increased by approximately 1.1 times. This result supports the above hypothesis that antioxidative enzyme levels of cancer cells are lower. Accumulation of active oxygen species leads to the cell inactivation.

Caspases are a group of cysteine proteases that constitute signalling pathways that cause cells to undergo apoptosis. The effector caspase, caspase-3/7, has the function of degrading other proteins in the cell to cause apoptosis. Figure 4 shows the production of the caspase-3/7 of HSC3 after oxygen plasma irradiation at applied voltage 4.2 kV. Immediately after the HSC3 is irradiated with the oxygen plasma, the fluorescent light emission begins to be observed and the activity of caspase-3/7 increases. The light emission intensity increase with the elapsed time from the plasma irradiation and became maximum at 12 hours after the irradiation. This result implies that there are kinds of rate-controlling process in the gene expression in p53 or caspase pathway.

4. Conclusion

Selective inactivation of oral cancer cells is obtained after the plasma irradiation. When cells are irradiated directly with oxygen plasma, the increase in intracellular active oxygen species and the inactivation of oral cancer cells are observed. Immediately after the oxygen plasma irradiation to oral cancer cells, the activity of caspase-3/7 was observed. Oxygen plasma irradiation to HSC3 may induce caspase-dependent apoptosis in HSC3.

5. References

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