Investigation of compositions in plasma-irradiated buffer evoking TRP-channel mediated calcium response

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Although plasma medicine is a rapidly emerging field and medical applications using non-equilibrium atmospheric pressure plasma are promising, the interaction mechanism between the plasmas and living cells remains unclear. In order to enhance understandings of the interaction, we focused on transient receptor potential (TRP) channel(s) on cell membrane as biological targets of APP-produced reactive species and investigated the concentrations of hydrogen peroxide (H_2O_2) and the potency of the reactive species for evoking calcium responses through TRP channel. The results suggest that the precursor(s) of H_2O_2 may be responsible for the APP-induced TRP-channel-mediated calcium responses.

1. Introduction

Plasma medicine is a rapidly emerging field, and a number of researchers have reported innovative applications of non-equilibrium atmospheric pressure plasma (APP) [1, 2]. While the fact that reactive species are key components of APP in the plasma medical treatment is now widely accepted, the interaction mechanism between the reactive species and living cells remains unclear. Because the first contact of the species with cells is considered to be just the membrane lipids or the membrane proteins, we have intensively investigated the APP-induced changes in cell membrane transports.

Thus, we experimentally showed that unclassified reactive species in APP-irradiated solution can trigger physiologically relevant Ca^{2+} influx through transient receptor potential (TRP) channel(s) on cell membrane [3]. However, it is still challenging to specify the key species and the key member of TRP family. Therefore, we tried to measure the APP-produced reactive species and the induced calcium response through TRP channels.

2. Experimental Apparatus

APP was generated using low frequency (LF) (frequency: 8 - 10 kHz, voltage: 5 - 12 kV) with Helium gas flow, which was exposed to the biological buffer. The plasma-irradiated solution was put on a hot plate (37°C) for a retention time $\boldsymbol{t_r}$, and added to mouse fibroblast cells 3T3-L1 (indirect plasma irradiation) or added to hydrogen peroxide monitoring probe. Real-time changes in the amount of the intracellular calcium ion concentration ($[Ca^{2+}]_i$) were obtained using a calcium indicator fluo 4 and a confocal microscope.

3. Experimental Results and Discussion

Figure 1 shows that (a) the concentration of hydrogen peroxide (H_2O_2) in plasma-irradiated solution at varying $\boldsymbol{t_r}$ and (b)time course of changes in the average $[Ca^{2+}]_i$ of 3T3-L1 cells stimulated with plasma-irradiated solution at $\boldsymbol{t_r}$ = 30 s, 300 s, and 600 s. The concentration of H_2O_2 increased but the production rate decreased with time after APP irradiation, which indicating that APP-irradiated solution gradually lost the capacity to produce the precursor of H_2O_2 with time. As $\boldsymbol{t_r}$ increased, the APP-induced increase in $[Ca^{2+}]_i$ was lower. These results suggest that the precursor(s) of H_2O_2 is responsible for the APP-induced calcium response. In the presentation, I will show the result on the mechanism of not only calcium responses but also other membrane transports evoked by APP irradiation.

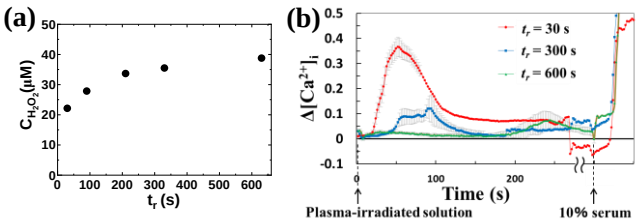

Fig. 1. (a) The concentration of hydrogen peroxide (C_{H2O2}) in plasma-irradiated solution at varying $\boldsymbol{t_r}$ and (b)time course of changes in the average $[Ca^{2+}]_i$ of 3T3-L1 cells stimulated with plasma-irradiated solution at $\boldsymbol{t_r}$ = 30 s, 300 s, and 600 s.

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