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プラズマが誘起する細胞内の生体応答と生化学反応
Intracellular responses and biochemical reactions induced by plasma

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Introduction

The reactions that occur in liquids irradiated with non-thermal plasmas have been studied. In indirect irradiation, plasma acts as a source of a rich variety of reactive species, such as OH and NO. Light can also play roles in plasma-induced effects. Since the interaction is mostly limited in liquid reactions, long-lived species are regarded as participating in interaction occurring at biological surfaces. This variety of plasma-activated aqueous species induced biological responses of cells and biochemical reactions inside the cells.

Recently, the nonthermal plasma has come into attraction as a potential antitumor therapy. In cancer therapy, for example, modifications of liquids in contact with cells or tissues play an important role for biological effects on cell membranes resulting from plasma-induced changes in electric fields, responding to the reactive species. We have studied radical kinetics and electrical effects to fully elucidate these phenomena [1-15]. (Fig. 1)

In our previous publications, non-equilibrium atmospheric pressure plasma (NEAPP) irradiated to a culture medium, a so-called plasma-activated medium (PAM), was first reported in 2011 to induce apoptotic cell-death together with selective cytotoxicity of cancer cells against normal cells. This antitumor effect of PAM was extensively reported in vivo with a xenografting mouse model with subcutaneous injection to implanted chemoresistant ovarian cancer cells.[16] Thereafter, a variety of cancerous cells was killed in the PAM cultivation.[17-27] Reactive oxygen and nitrogen species (RONS) such as hydrogen peroxide (H_2O_2) and nitrite (NO_2^-) are contained in PAM, which are generated by irradiation of NEAPP with entrained air and excited molecules dissolved in the original culture media.[1] The killing of cancer cells was caused in part by the synergistic effect of H_2O_2 and NO_2^-.[2] In terms of oxidative stress, the antitumor effect was inhibited by pretreatment with a reactive oxygen species (ROS) scavenger, N-actyl cysteine (NAC).[16] Genetic mutations in cancer-driver genes, tumor suppressors, and amplified oncogenes are linked to specific alterations in metabolic activity in cancer cells.[28,29] Therefore, PAM-specific apoptotic cell death is discussed with respect to metabolomic profiles of intracellular metabolites.

Plasma-induced intracellular responses

Human glioblastoma cell lines (U251SP) were cultured in the PAM. Cell survivals were gradually decreased as elapsed the PAM incubation period, and the cell survival was almost zero after 24 h of incubation. Cells death by the PAM began to appear after 2 h of incubation and then all cells were dying by 24 h of incubation. At 0.5 h of incubation, the cell fate was determined and all cells were dying together with changes to the intracellular metabolites. Thus, the metabolism of cell death by PAM incubation was then analyzed by quantitative more than 100 metabolites of cells for 0.5 h of incubation. (i) metabolites that involved both a coenzyme of glycolysis, e.g., nicotinamide adenine dinucleotide (NAD+) and an energy source of cell

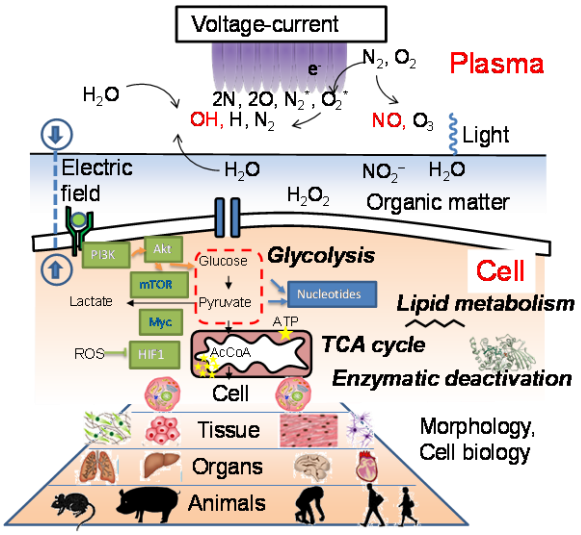

Fig. 1 Dynamic behavior of plasmas should be characterized in situ at all hierarchical levels (i.e., liquid, cell, tissue, organ, body) for understanding of plasma induced biological responses.

proliferation, adenosine triphosphate (ATP), decreased in only PAM cultivated cells. (ii) TCA cycle's metabolites decreased. The TCA cycle was not significantly affected by the PAM; therefore, downstream of the metabolic pathway of the TCA cycle, such as the branched chain amino acids (BCAA) pathway, also decreased in terms of total intracellular amino acids of cancer cells incubated with the control medium or PAM. (iii) the metabolites involving glyceraldehyde 3-phosphate (G3P) etc. of the pentose phosphate pathway (PPP) were increased only in the PAM. The metabolites related to the pathways for glycolysis and PPP were in particular modified by PAM incubation. Moreover, a frequent mutation of isocitrate dehydrogenase 1 (IDH1) in glioblastoma occurs in most cases. The IDH1 enzyme catalyzes the oxidative decarboxylation of isocitrate to α-ketoglutarate (2-OG) and simultaneously converts NADP+ to NADPH.[30]

Plasma-activated biochemical reactions

The NEAPP treatments generated the plasma activated organic substances in aqueous phases. Very recently, we found acetyl- and pyruvic acid–like groups in solution by nuclear magnetic resonance (NMR) analyses.[3] These long-lived species are regarded as participating in interaction occurring at biological surfaces, since the interaction is mostly limited in liquid reactions. The instantaneous stress intensity induces severe effects to targets and the effects are cumulative. Thus, either dose or dose-rate of plasma provides thermal and non-thermal effects.[31] The overall physicochemical antitumor effect was resulted by the dose rate of plasma intensity showing the non-thermal effects on the biological responses in regard with biochemical reactions.

Concluding remarks

Controlling the intracellular responses and biological reactions is difficult due to the nonequilibrium states that are thermodynamically unfavorable. Holistic views of cell metabolism may overcome this challenge. Metabolomic profiles of cells were shown differently the intracellular states induced by the plasma-activated organics. As a consequence, we should point out that the dynamic behavior of plasmas should be characterized *in situ* at all hierarchical levels (i.e., liquid, cell, tissue, organ, body) for understanding of plasma induced biological responses.

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