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Interactions between a Plasma-Activated Medium and Cancer Cells

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ABSTRACT: A plasma-activated medium (PAM) is a medium that is irradiated with plasma; PAMs have garnered much attention from the plasma medicine community. PAMs induce apoptosis in many types of cancer cells and have a clinical benefit, especially in disseminated cancers such as peritoneal disseminated ovarian cancer and gastric cancer. Although the effectiveness of PAMs has been extensively reported, the mechanisms of the selective killing of cancer cells by PAMs still remain to be elucidated. Systems biology is a key to understanding the origins of the differences in cellular sensitivities to PAM.

I. INTRODUCTION

The application of nonthermal plasma to medicine was a big trend at the beginning of the 21st century; this field of medicine is often called plasma medicine.[1–6] Many *in vitro* and *in vivo* studies have demonstrated that such plasma is applicable to wound healing, blood coagulation, and cancer treatments.[7–13] It is important to understand the mechanisms of plasma treatment; however, the complexity of both plasma and biological systems makes it very difficult to understand these mechanisms. Some research groups in the plasma medicine community have started to consider applying the concept of systems biology to plasma medicine in order to better understand the mechanisms through which nonthermal plasma acts. In this article we introduce our perspective regarding how systems biology is useful for understanding plasma medicine.

A plasma-activated medium (PAM) is a medium that is irradiated with plasma. Components of PAMs are complex, and the mechanisms of physiological outputs by PAMs are not fully understood. It is believed that reactive species generated by plasma are key players that interact with cells and tissues. Understanding the mechanisms at work in this complex system will provide insights into improving effectiveness and safety when applying PAMs for various diseases.

II. PAM AS A TOOL FOR CANCER CHEMOTHERAPY

PAMs have broadened the ways in which nonthermal plasma can be applied, by providing a tool for cancer chemotherapy.[14,15] In traditional cancer chemotherapy antitumor drugs attack fast-dividing cancer cells; however, they also attack fast-dividing healthy cells. By contrast, a PAM selectively killed glioblastoma brain tumor cells rather than normal astrocytes.[16] The survival and proliferation signaling network is constitutively activated in glioblastoma, which leads to the inhibition of apoptosis and promotes cell

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growth. A PAM downregulated the survival and proliferation signaling network to induce apoptosis in glioblastoma brain tumor cells.[17] *In vivo* and *ex vivo* studies have also demonstrated that both plasma and the PAM generated by our previously reported plasma source are prominent tools for cancer therapy.[18–20] The effectiveness and safety of PAMs will be addressed in the future.

A PAM is considered a complex cocktail of products that are generated by interactions between plasma and air, plasma and water, and plasma and molecules in air and water. Plasma interacts with oxygen, nitrogen, and water in humid air to produce molecules such as oxygen radicals, nitrogen radicals, nitric oxide (NO), and hydroxyl radicals, and it moves into the liquid phase to generate molecules such as hydrogen peroxide (H_2O_2), nitrites, and nitrates.[21] NO is a well-known second messenger (a key player in signal transduction inside a cell), and H_2O_2 has also been recently recognized as a second messenger.[22,23] The combined data suggest that the mechanisms of selective killing of cancer cells by a PAM might be partly explained by the biology of free radicals.[24] However, recent studies in plasma medicine indicated that inputs from plasma or PAMs are complicated, and it will be necessary to combine diagnostics of plasma, studies of plasma and liquids, computational simulations, and molecular biology to completely understand the mechanisms of physiological outputs by plasma and PAMs.

III. THE USE OF SYSTEMS BIOLOGY FOR UNDERSTANDING INTERACTIONS BETWEEN PLASMA AND CANCER CELLS

There are several key players in the response of a cell to various extracellular stimuli (Fig. 1). On the plasma membrane, which is made of phospholipid bilayers, there are many types of receptors, such as ion channels, cell adhesion molecules, and glycoproteins. For example, the epidermal growth factor receptor binds extracellular epidermal growth factor to transduce signals into a cell.[25] Ion channels function as controllers that balance the concentrations of intracellular and extracellular ions.[26] Epithelial cells are connected by cell adhesion molecules.[27] Glycoproteins play important roles in cell recognition.[28] There may also be some membrane proteins whose functions are unknown. Underneath the plasma membrane are cortical actin cytoskeletons, which, together with actin-binding proteins, control cellular morphology and cellular dynamics.

Inside the plasma membrane there are several second messengers, such as cyclic adenosine monophosphate, cyclic guanosine monophosphate, calcium ions, phosphatidylinositols, and NO. NO is an important cellular signaling molecule that is involved in various physiological processes, including vasodilatation, neurotransmission, and protection against infection.[29] NO is synthesized from arginine by NO synthases in a cell, has a lifetime of only a few seconds, and freely penetrates cell membranes. NO is synthesized in the endothelium of blood vessels and diffuses into smooth muscles to react with guanylate cyclase and increase cyclic guanosine monophosphate, which results in the relaxation of smooth muscle and an increase in blood flow. NO has been reported to induce apoptosis in some cells and inhibit apoptosis in other cells.[30] Thus, high

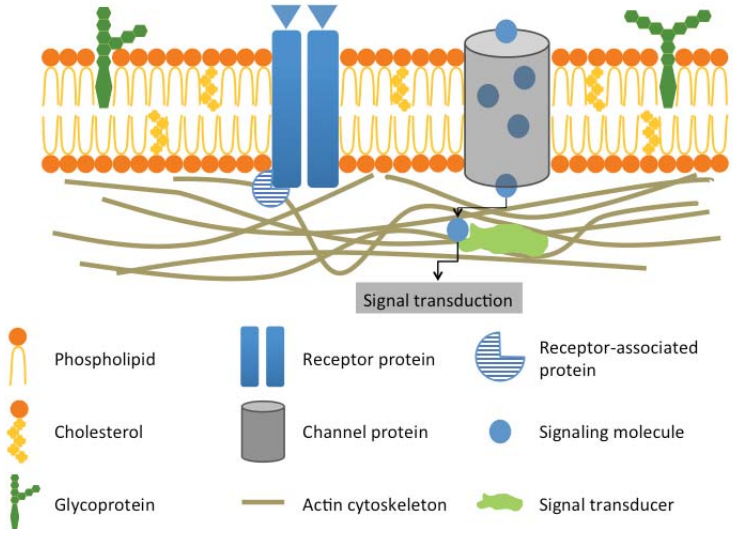

FIG. 1: Key players in cellular responses to extracellular stimuli. Various types of proteins on the plasma membrane receive and sense different extracellular environments. Actin cytoskeletons underlie the plasma membrane to form and change cell morphology. Inside the cell, signaling molecules such as nitric oxide and hydrogen peroxide are generated by extracellular signals for signal transduction.

concentrations of NO (more than ~1 μmol/L), or prolonged exposure to NO, induces apoptosis in some cells through various molecular mechanisms, whereas physiological concentrations of NO (less than ~100 nmol/L) protect cells from apoptosis.[31] H_2O_2 is a well-known reactive oxygen species and has recently been recognized as a second messenger.[32] Various growth factors and cytokines generate H_2O_2 in a cell through activation of nicotinamide adenine dinucleotide phosphate hydrogen oxidases. Low concentrations of H_2O_2 (less than ~10 μmol/L) induce antioxidants that protect a cell from oxidative stress, whereas high concentrations of H_2O_2 (more than ~10 μmol/L) induce apoptosis in a cell. In summary, both NO and H_2O_2 exert concentration-dependent and cell-type-specific effects; however, the mechanisms by which a cell senses and regulates the concentrations of these species are unclear.

The survival and proliferation signaling network is also modulated by NO and H_2O_2. For example, a key molecule in this signaling network, the phosphatase and tensin homolog (PTEN), is reversibly inactivated by H_2O_2 through oxidation of the essential cysteine residue of its active site.[33] PTEN is also inactivated by NO through S-nitrosylation, which is a covalent modification of cysteine residues that induces degradation of the PTEN protein via ubiquitination.[34]

Systems biology is a field of study in which biological phenomena are studied as a system, and it combines classical molecular biology, biochemistry, computational science, and systems engineering.[35] Biological systems receive various inputs from their environments and show several physiological outputs. The relationships between inputs

and outputs are determined by the structure of the signaling pathways. For example, the survival and proliferation signaling network has several negative feedback loops and amplifiers (Fig. 2), and mutations in this signaling network can cause changes in the strength of such regulatory functions.[36] In this way, differences in the sensitivities of cells with different mutations to various stimuli can be explained by differences in the structure of the signaling pathways.

Cellular sensitivities to PAMs are determined by the structure of the signaling pathways that regulate the survival and proliferation signaling network. When levels of NO and H_2O_2 that are higher than physiological levels are generated in a cell, the cell switches on apoptotic signals. The doses of NO and H_2O_2 that induce apoptosis depend on the cell type. Thus the different sensitivity of different cell types to a PAM might be explained by the structure of the signaling pathways of the survival and proliferation signaling network. Such system analyses are needed to understand the mechanisms of the selective killing of cancer cells by PAMs.

IV. CONCLUSION

We propose PAMs as a new type of cancer chemotherapy. A PAM potentially generates H_2O_2 and NO in a cell. Both NO and H_2O_2 are key signaling molecules in several sig-

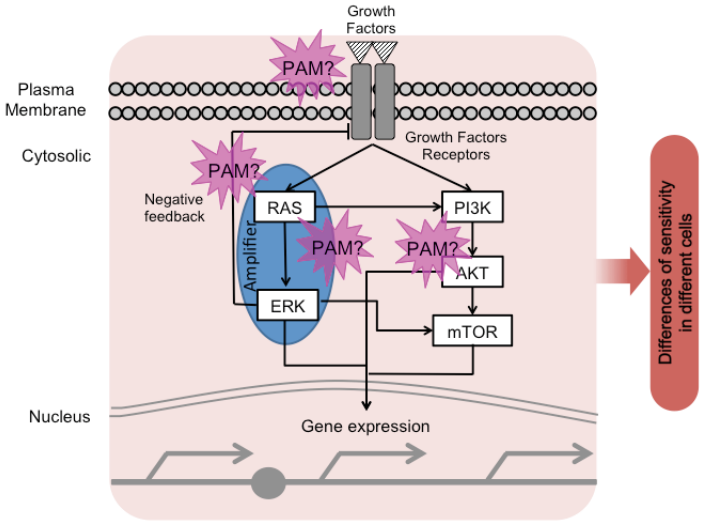

FIG. 2: Structure of signaling pathways in the survival and proliferation signaling network. There are several negative feedback loops and amplifiers in the signaling pathways of the survival and proliferation signaling networks. Differences in the sensitivities of different cell types to a plasma-activated medium (PAM) might be explained by changes in the strength of the structures of these signaling pathways. mTOR, mammalian target of rapamycin; PI3K, phosphoinositide 3-kinase.

naling networks and also interact with the survival and proliferation signaling network through S-nitrosylation and the oxidation of cysteine residues of the PTEN protein. Differences in the sensitivities of various cells to a PAM might be explained by differences in the structure of the signaling pathways in the survival and proliferation signaling network. In the future, interactions between plasma/PAMs and cells/tissues will be understood better using systems biology.

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