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Nonequilibrium atmospheric pressure plasma with ultrahigh electron density and high performance for glass surface cleaning
Cancer therapy using non-thermal atmospheric pressure plasma with ultra-high electron density

Hiromasa Tanaka,1,2 Masaaki Mizuno,2 Shinya Toyokuni,3 Shoichi Maruyama,4 Yasuhiro Kodera,5 Hiroko Terasaki,6 Tetsuo Adachi,7 Masashi Kato,8 Fumitaka Kikkawa,9 and Masaru Hori1

1Institute of Innovation for Future Society, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8603, Japan
2Center for Advanced Medicine and Clinical Research, Nagoya University Graduate School of Medicine, Tsurumai-cho 65, Showa-ku, Nagoya 466-8550, Japan
3Department of Pathology and Biological Responses, Nagoya University Graduate School of Medicine, Tsurumai-cho 65, Showa-ku, Nagoya 466-8550, Japan
4Department of Nephrology, Nagoya University Graduate School of Medicine, Tsurumai-cho 65, Showa-ku, Nagoya 466-8550, Japan
5Department of Gastroenterological Surgery (Surgery II), Nagoya University Graduate School of Medicine, Tsurumai-cho 65, Showa-ku, Nagoya 466-8550, Japan
6Department of Ophthalmology, Nagoya University Graduate School of Medicine, Tsurumai-cho 65, Showa-ku, Nagoya 466-8550, Japan
7Laboratory of Clinical Pharmaceutics, Gifu Pharmaceutical University, 501-1196 Gifu, Japan
8Department of Occupational and Environmental Health, Nagoya University Graduate School of Medicine, Tsurumai-cho 65, Showa-ku, Nagoya 466-8550, Japan
9Department of Obstetrics and Gynecology, Nagoya University Graduate School of Medicine, Tsurumai-cho 65, Showa-ku, Nagoya 466-8550, Japan

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Cancer therapy using non-thermal atmospheric pressure plasma is a big challenge in plasma medicine. Reactive species generated from plasma are key factors for treating cancer cells, and thus, non-thermal atmospheric pressure plasma with high electron density has been developed and applied for cancer treatment. Various cancer cell lines have been treated with plasma, and non-thermal atmospheric plasma clearly has anti-tumor effects. Recent innovative studies suggest that plasma can both directly and indirectly affect cells and tissues, and this observation has widened the range of applications. Thus, cancer therapy using non-thermal atmospheric pressure plasma is promising. Animal experiments and understanding the mode of action are essential for clinical application in the future. A new academic field that combines plasma science, the biology of free radicals, and systems biology will be established. © 2015 AIP Publishing LLC.

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I. INTRODUCTION

Non-thermal atmospheric pressure plasma is one of the most attractive tools that human kind has ever produced. This technology has been used for micro-fabrication to produce compact electronic circuit cards. Reactions induced by plasma have been widely used to produce various materials. Recently, these reactions have been directed at biological targets that include bacteria, mammalian cells, plants, and animals.1-7

Electrons, ions, radicals, and light (ultra violet and vacuum ultra violet) are present in atmospheric pressure plasma. These components react with nitrogen, oxygen, and water (humidity) in air to produce radicals with a relatively short half-life such as oxygen radicals, nitrogen radicals, hydroxyl radicals, and nitrogen oxide, in the gas phase.8 These radicals react with other molecules in the gas phase and liquid phase to produce hydrogen peroxide, nitrite, and nitrate.9 Reactive molecules that are produced by plasma in the gas phase and liquid phase interact with the cellular surface or move inside cells.10 As a result, reactive oxygen species (ROS) and reactive nitrogen species are detected in plasma-treated cells.

The biology of free radicals is useful for understanding the mechanisms of plasma-cell/tissue interactions.11 In addition, systems biology is involved in understanding the complex system of plasma-cell/tissue interactions.12 In this review, we discuss new possible roles for non-thermal atmospheric pressure plasma with ultra-high electron density and the possibility of developing a new science, “plasma medicine,” which combines plasma science, the biology of free radicals, and systems biology.

In addition to reactive species, electric fields produced by plasma are also important factor which affect cells and tissues. The recent computational studies revealed that intracellular electric fields produced by dielectric barrier discharge (DBD) can penetrate surface cells of skin, which may exceed the threshold for electroporation.13

II. NON-HERMAL ATMOSPHERIC PRESSURE PLASMA WITH ULTRA-HIGH ELECTRON DENSITY FOR CANCER THERAPY

The radicals in plasma are thought to be especially important for physiological responses because cells/tissues
consist of biological materials that are affected by radicals. In biology, free radicals mediate various biological reactions that lead to physiological responses. Free radicals produce not only biochemical damage to proteins and lipids but also redox signaling that mediates oxidative stress.

Thus, we hope to be able to use non-thermal atmospheric pressure plasma that produces a high flux of radicals for medical and biological applications. Recently, non-thermal atmospheric pressure plasma with ultra-high electron density has been developed.\(^{14}\) The electron density was in the order of \(10^{16}\) cm\(^{-3}\), while the electron density of the conventional DBD plasmas is considered in the order of \(10^{14}\) cm\(^{-3}\). It was applied for sterilization of \textit{Penicillium digitatum}.\(^{15-17}\) This technology has been also used for treatment of cancers such as ovarian cancer, gastric cancer, glioblastoma, and melanoma.\(^{18-30}\)

Safety studies are important aspects of clinical application. When non-thermal atmospheric pressure plasma with ultra-high electron density is used to directly treat rat liver, lipid peroxidation and DNA modification are detected.\(^{31}\) Oxidative damage by plasma is dose dependent.

III. PLASMA-ACTIVATED MEDIUM (PAM) FOR CANCER THERAPY

Plasma not only directly affects cells but also indirectly induces physiological mediators that affect cells by producing reactive species around cells. Cells are usually surrounded by liquid, and cells are grown in medium. In one experiment, medium was treated with non-thermal atmospheric pressure plasma with ultra-high electron density (Figure 1) and used to replace the medium in which glioblastoma brain tumor cells were cultured.\(^{20}\) This plasma-treated medium induces apoptosis of the glioblastoma cells. This medium with anti-tumor effects was denoted “plasma-activated medium” or PAM.

PAM has also been used to treat ovarian and gastric cancer cells.\(^{19,32}\) Surgery is the primary method of treatment if cancers are isolated and solid. Paclitaxel or cisplatin is the first-line chemotherapy for several cancers including ovarian and gastric cancers. However, cancer cells often gain resistance to these chemotherapeutic agents, and many patients with ovarian or gastric cancers experience a recurrence.

Paclitaxel- and cisplatin-resistant ovarian cancer cell lines have been created and treated with PAM.\(^{32}\) Interestingly, PAM was effective at killing both anti-cancer drug-resistant ovarian cancer cell lines and their parent cell lines. A typical feature of advanced or recurrent ovarian and gastric cancers is that tumors spread within the peritoneum. These peritoneal metastases are the most common feature of ovarian and gastric cancers, and such advanced cancers are traditionally considered incurable and terminal. We expect that PAM may be useful for treating intraperitoneal malignant ascites and may improve the poor prognosis. Combination therapy of plasma/PAM and anti-cancer drugs will also be promising for treating cancers.\(^{23,33}\)

A recent study showed that PAM may also be useful for treating age-related macular degeneration (AMD).\(^{34}\) AMD is an eye disease that leads to the deterioration of the center of the retina and is the most common cause of vision loss in aged people. Wet AMD is caused by choroidal neovascularization (CNV). PAM suppresses laser-induced CNV and does not show retinal toxicity. Thus, PAM is expected to be a novel therapeutic agent for suppressing CNV.

IV. INTRACELLULAR SIGNALING NETWORKS AND SYSTEMS BIOLOGY

Cells respond to various inputs such as nutrients, growth factors, and stress from the surrounding environment. Membrane-bound receptors on cells receive such inputs, and then the signal is transduced via dimerization or polymerization of receptors, conformational change of the receptors, phosphorylation of tyrosine, serine, and threonine residues. When membrane-bound receptors are activated, signaling molecules such as kinases are recruited to the plasma membrane, are activated by the membrane-bound receptors, and phosphorylate downstream signaling molecules. Some activated signaling molecules move into the nucleus where they bind and activate transcription factors that induce gene expression. Other activated signaling molecules regulate cytoskeleton-binding proteins and control cellular morphology. Thus, various inputs induce physiological outputs such as cell death, cell growth, and morphological changes by regulating the cytoskeleton, gene expression, and other signaling events.

Many signaling pathways have been characterized. Cross-talk and feedback loops among signaling pathways have been identified. Thus, signaling pathways form a network that induces many physiological outputs. Systems biology addresses how these signaling networks function as a system using classical molecular biological and biochemical approaches, mathematics, informatics, and genome-wide experiments such as microarray, next-generation sequencing, and high-content imaging techniques.

V. INTRACELLULAR MOLECULAR MECHANISMS OF CELL DEATH MEDITATED BY PLASMA OR PAM

The survival and proliferation signaling network is one of the most essential signaling networks and includes the Phosphoinositide 3-kinase (PI3K)-AKT signaling pathway and Raus-associated sarcoma (RAS)-Mitogen-activated...
Protein Kinase (MAPK) signaling pathway. Activation of this signaling network leads to induction of cell growth and cell division and inhibition of apoptosis (Figure 2). Cancer cells often harbor mutations in the survival and proliferation network, resulting in constitutive activation of the signaling network. Thus, signaling molecules in the survival and proliferation network are attractive targets for modern molecular targeted drugs.

The glioblastoma brain tumor cell line U251SP harbors mutations in Epidermal-growth-factor receptor (EGFR) and Phosphatase and tensin homolog (PTEN), which lead to constitutive activation of both the PI3K-AKT pathway and RAS-MAPK pathway. PAM treatment of this glioblastoma cell line down-regulates both pathways. Based on these results, an intracellular molecular model in which PAM induces apoptosis of glioblastoma cells by down-regulating the survival and proliferation signaling network was constructed.

Several signaling pathways trigger apoptosis, and many extrinsic and intrinsic ligands induce apoptosis (Figure 3).

Death receptors such as the FasL receptor and tumor necrosis factor-alpha receptor mediate the extrinsic pathway. When Fas ligand and tumor necrosis factor-alpha bind to their receptors, Caspase 8 is cleaved through an adaptor molecule called Fas-associated death domain (FADD), which activates downstream caspases to induce apoptosis. DNA damage alters the mitochondrial membrane potential by activating p53 and down-regulating Bcl-2. Then, cytochrome c is released from mitochondria and binds to Apaf-1 and Caspase-9 to activate caspase cascade reactions that lead to apoptosis. Endoplasmic reticulum stress such as accumulation of unfolded proteins and disorders of calcium and glucose homeostasis also induces apoptosis.

Many signal transduction pathways that induce apoptosis involve cleavage of caspases, which are cysteine proteinases. Initiator caspases such as Caspase-8, 9, and 12 and effector caspases such as Caspase-3, 6, and 7 are involved, and apoptotic signals are mediated by caspase cleavage cascades. Caspase-independent pathways also play a role in apoptosis. Apoptosis-inducing factor is a major important pro-apoptotic caspase-independent factor, and poly (ADP-ribose) polymerase-1 activation is necessary for apoptosis-inducing factor release from mitochondria. PAM induces caspase-independent apoptosis in the non-small lung carcinoma cell line, A549.

Non-thermal atmospheric pressure plasma induces lipid peroxidation and DNA damage. PAM contains hydrogen peroxide. ROS that plasma and PAM produce that enter cells may be derived from hydrogen peroxide in PAM or intrinsic ROS production due to signal transduction that plasma and PAM trigger (Figure 4). ROS can activate both caspase-dependent and -independent apoptosis. ROS is generated in mitochondria and induces DNA damage and oxidation of protein cysteine (-SH). The mechanisms by which plasma and PAM induce ROS and interactions of the ROS redox signaling network with other signaling networks remain to be elucidated.

VI. SELECTIVE KILLING OF CANCER CELLS BY PLASMA OR PAM

Conventional chemotherapies usually have side effects such as hair loss and decreased blood cell counts because...
these anti-cancer drugs target rapidly dividing, normal cells. If cancer cells are selectively killed, the anti-cancer therapy will have a greater benefit without unacceptable toxicity to the patient. Non-thermal atmospheric pressure plasma with high electron density selectively kills ovarian cancer cells compared to normal fibroblasts.18 Glioblastoma brain tumor cells are selectively killed by PAM compared to normal astrocytes.20 Other groups have also succeeded in selective killing of cancer cells.26,41

Regardless of whether the effects are direct or indirect, the appropriate irradiation time of the plasma should be determined. When the treatment time is too long, both cancer cells and normal cells are killed by plasma, whereas when the treatment time is too short, neither cancer cells nor normal cells are killed. Thus, investigation of the therapeutic range of plasma and PAM is necessary. Sensitivities to plasma or PAM are different for different cells. The intracellular molecular mechanisms that mediate selective killing of cancer cells remain to be elucidated. The structure of intracellular signaling networks may be key to understanding the different sensitivities to plasma or PAM in different cells. Mutations in cancer cells are responsible for the different structure of signaling networks. Thus, plasma or PAM treatments should be designed based on the particular intracellular signaling structure that the cancer cells harbor.

VII. CONCLUDING REMARKS

Non-thermal atmospheric pressure plasma with high electron density is a promising tool for not only cancer therapy but also other diseases such as AMD. PAM has potentially broad applications to diseases such as cancerous peritonsillitis. Plasma and PAM induce apoptosis of cancer cells, and the mechanisms depend on the cell type and plasma conditions. The effectiveness and safety should be tested using disease model animal experiments so that plasma or PAM will be clinically applicable in the future.

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