Minireview

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Molecular mechanisms of non-thermal plasma-induced effects in cancer cells

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Abstract: Plasma is the fourth state of matter with higher energy than gas; non-thermal plasma (NTP) is currently available. As NTP is useful in sterilization, promoting wound healing and cancer treatments, the molecular mechanisms of plasma-induced effects in living cells and microorganisms are of significant interest in plasma medicine with medical-engineering collaboration. Molecular mechanisms of plasma-induced effects in cancer cells will be described in this minireview. Both direct and indirect methods to treat cancer cells with NTP have been developed. NTP interacts directly with not only cancer cells but also the liquids surrounding cancer cells and the immune cells that target them. Reactive oxygen and nitrogen species play key roles in NTP-induced effects; however, other mechanisms have been suggested. The complex interactions between NTP, cells and liquids have been extensively studied. In the future, details regarding NTP-induced effects on gene regulatory networks, signaling networks, and metabolic networks will be elucidated.

Keywords: biochemical networks; plasma cancer therapy; plasma medicine; reactive oxygen species (ROS).

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Introduction

Plasma is the fourth state of matter, in addition to solid, liquid and gas. Innovative technologies for generating non-thermal plasmas (NTP) at atmospheric pressure have recently been developed and applied in several industries, as well as in medicine and biology. NTP has been clinically used in wound healing (Awakowicz et al., 2009; Heinlin et al., 2010; Lademann et al., 2010; Isbary et al., 2012; Schmidt et al., 2013; von Woedtke et al., 2013; Akimoto et al., 2016). NTP has also been studied in blood coagulation applications (Kalghatgi et al., 2007; Ikehara et al., 2015; Ueda et al., 2015; Miyamoto et al., 2016), and cancer treatments (Kieft et al., 2004; Fridman et al., 2007; Vandamme et al., 2010; Keidar et al., 2011; Schlegel et al., 2013; Tanaka et al., 2014a,b). The molecular mechanisms of plasma-induced effects have been extensively studied. The novel academic field that combines plasma science and medical science is often referred to as 'plasma medicine' (Fridman et al., 2008; Kong et al., 2009; Morfill et al., 2009; von Woedtke et al., 2013, 2014; Tanaka et al., 2017).

NTP and plasma-activated medium (PAM) induce reactive oxygen and nitrogen species (RONS) in cells

Numerous *in vitro* and *in vivo* experiments have demonstrated that NTP selectively kills cancer cells (Kieft et al., 2004; Keidar et al., 2013; Schlegel et al., 2013; Tanaka et al., 2015a,b). The most readily observed effect associated with NTP is oxidative stress, which is typically induced by the formation of reactive oxygen and nitrogen species (RONS) in cells. Various hypotheses to explain the selective killing of cancer cells by NTP have been proposed. Reactive oxygen species (ROS) are generally produced in cancer cells at much higher levels than in normal cells (Toyokuni et al., 1995). Thus, cancer cells are more vulnerable than normal cells to ROS generated by NTP (Toyokuni, 2016).

Bauer and Graves hypothesized that NTP-derived singlet oxygen induces the selective killing of cancer cells (Bauer and Graves, 2016). Cancer cells express membrane-associated NADPH oxidase-1, NOX1, which results in generating extracellular superoxide anions, however, they also express membrane-bound catalase and superoxide dismutase to cope with intracellular RONS-dependent apoptosis induction. NTP generates the variety of RONS. These are superoxide anions, hydrogen peroxide (H_2O_2), hydroxyl radicals (•OH), nitric oxide (•NO), nitrogen dioxide, peroxynitrite, nitrite, nitrate and singlet oxygen. Singlet oxygen can inactivate membrane-associated catalase, which leads to the generation of secondary singlet oxygen and apoptosis, selectively in cancer cells.

Plasma-activated medium (PAM) is culture medium irradiated with NTP. PAM also selectively kills cancer cells and induces apoptosis (Tanaka et al., 2013, 2014a,b, 2015a,b, 2016; Yan et al., 2014, 2015, 2016; Mohades et al., 2015; Judee et al., 2016; Kumar et al., 2016). The reactions between plasma and liquids have been extensively studied and reviewed by Bruggeman et al. (2016). Plasma which consists of electrons, ions, radicals and light interacts with oxygen, nitrogen and water in humid air and evaporation from the interface between plasma and bulk liquid to produce molecules such as •NO and •OH. Molecules such as H_2O_2, nitrites and nitrates were generated in the interface between plasma and bulk liquid and transported into bulk liquid (Figure 1; Tanaka et al., 2015a,b; Bruggeman et al., 2016). These bioactive molecules trigger signaling cascades that mediate plasma-induced effects in cells (Adachi et al., 2016). In the presence of H_2O_2, iron promotes the generation of •OH via the Fenton reaction. •OH are highly reactive and short-lived, and they interact with DNA, protein and carbohydrate molecules. Recently, the role of aquaporins in the toxicity of PAM has been reported (Yan et al., 2017). In A549 cells, exposure to PAM leads to elevated intracellular levels of ferrous ion and the generation of •OH (Adachi et al., 2016). Injury of fibroblast cells induced by treatment with H_2O_2 is significantly suppressed by pretreatment with 'mild PAM' which was prepared under relatively mild conditions (Horiba et al., 2017).

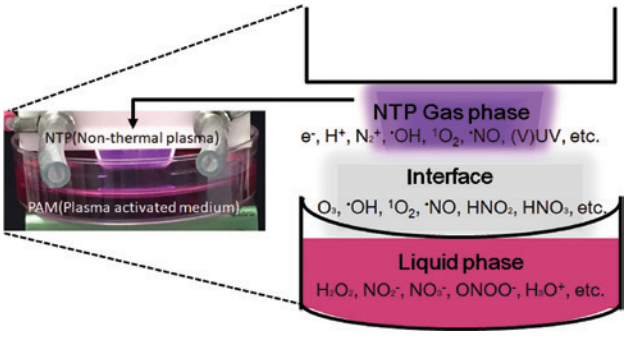

Figure 1: Interactions between NTP and liquid in PAM.
Plasma interacts with oxygen, nitrogen and water in humid air and evaporation from the interface between plasma and bulk liquid to produce molecules such as nitric oxide (•NO) and hydroxyl radicals (•OH). Molecules such as hydrogen peroxide (H_2O_2), nitrites and nitrates were generated in the interface between plasma and bulk liquid and transported into bulk liquid (Bruggeman et al., 2016).

There are some important hypotheses. Singlet oxygen can inactivate membrane-associated catalase, which leads to the generation of secondary singlet oxygen (Bauer and Graves, 2016). Aquaporins might be key transmembrane channels of H_2O_2 generated by NTP and PAM (Yan et al., 2017). Fenton reactions might be key reactions in cell-based H_2O_2 generation (Adachi et al., 2016). Thus, oxidative stress plays an important role in NTP- and PAM-treated cells, and controlling the redox balance using NTP or PAM is a key factor for successful clinical applications.

Signal transduction pathways are affected by NTP and/or PAM

Hallmarks of cancer cells include resistance to death, sustained proliferative signaling, evasion of growth suppressors, invasiveness and metastasis, replicative immortality and enhanced angiogenesis (Hanahan and Weinberg, 2011), regulated by multiple intracellular signaling networks. Survival and proliferation signaling networks play a pivotal role in both cancerous and normal cells. Two signaling pathways that have been extensively studied are the Ras/MAPK signaling pathway and the PI3K/AKT signaling pathway. In cancer, constitutive and aberrant activation of components of these pathways results in increased cell proliferation and survival. It was demonstrated that exposure to PAM downregulates these pathways in glioblastoma cells, inducing apoptosis (Tanaka et al., 2013, 2014a,b). Treatments using NTP and PAM are thus expected to become prominent therapeutic options for exploiting vulnerabilities in cancer cells.

NTP and PAM directly and indirectly influence cell signaling networks, in turn affecting gene regulatory and metabolic networks. Obtaining a comprehensive understanding of these networks will be important to accurately determine the effects of NTP and PAM on specific cellular processes.

Many signal transduction pathways in various cells including the PI3K/AKT pathway and the Ras/MAPK pathway in glioblastoma cells are affected by NTP and PAM (Table 1). For example, Chang et al. have reported that NTP

Table 1: Signal transduction pathways affected by NTP and PAM.

Cell line	Input/output	Affected pathways	References
U251SP (glioblastoma)	PAM/apoptosis	PI3K/AKT pathway, RAS/MAPK pathway	Tanaka et al., 2013, 2014a,b
SCC25 (oral cancer)	NTP/apoptosis	ATM/p53 pathway	Chang et al., 2014
Mel007 (melanoma)	NTP/apoptosis	TNF/ASK1 pathway	Ishaq et al., 2014a
A549 (lung cancer)	PAM/apoptosis	ER stress pathway	Adachi et al., 2014
SNUC5 (colon cancer)	NTP/apoptosis	ER stress pathway	Ruwan Kumara et al., 2016
SM2 (mesothelioma)	NTP/autophagy/ ferr optosis	Autophagic pathway/ferroptosis	Shi et al., 2017; Toyokuni et al., 2017; Furuta et al., 2018

NTP and PAM induce apoptosis and other types of cell death by affecting various signal transduction pathways on various cell lines.

induced apoptosis in p53 wild-type oral cavity squamous cell carcinoma through DNA-damage-triggering sub-G_1 arrest via the ATM/p53 pathway (Chang et al., 2014). Ishaq et al. reported that NTP induced the TNF/ASK1 pathway-mediated apoptosis through ROS production in melanoma (Ishaq et al., 2014a,b). Endoplasmic reticulum stress pathways are also affected by NTP and PAM to induce apoptosis on colon cancer cells (Ruwan Kumara et al., 2016) and lung cancer cells (Adachi et al., 2014).

Recently, cell death forms other than apoptosis caused by NTP have been identified. For example, Shi and co-workers reported that NTP induced autophagy in mesothelioma, possibly leading to ferroptosis (Shi et al., 2017; Toyokuni et al., 2017). Furuta et al. have recently hypothesized that NTP may induce ferroptosis in cancer cells by increasing catalytic Fe(II) via simultaneous destruction ferritin core and reduction of Fe(III) (Furuta et al., 2018). Ferroptosis is a recently defined type of non-apoptotic programmed cell death that is driven by catalytic Fe(II)-dependent lipid peroxidation (Dixon et al., 2012; Stockwell et al., 2017). Many researchers have reported that NTP can also stimulate macrophages and induce immunogenic cell death (Miller et al., 2014, 2016; Lin et al., 2015).

The plasma membrane and membrane-bound receptors serve as the interface between extracellular stimuli and intracellular biochemical reactions. NTP and PAM are thought to directly affect these components and indirectly affect intracellular components via the production of RONS (Bruggeman and Leys, 2009; Tanaka et al., 2017). Stress-sensing proteins are activated by NTP and/or PAM treatment, ultimately inducing transcription factors to bind target sequences on the genome and stimulate expression of genes to respond to the NTP and/or PAM (Ishaq et al., 2014a,b; Kang et al., 2014; Horiba et al., 2017; Nakamura et al., 2017). A central objective of plasma science research is to comprehensively understand the changes in the signaling, gene regulatory and metabolic networks affected by NTP and/or PAM and control the associated biochemical networks by fine-tuning NTP and/or PAM treatments.

Concluding remarks

The current studies strongly indicated some important roles of RONS in plasma cancer treatments. Understanding the molecular mechanisms of NTP-induced effects in cells is key for the successful clinical application of NTP irradiation. To control redox balance using plasma and/or PAM, molecular mechanisms from the point of NTP application to the resulting physiologic responses must be elucidated in detail. Molecular mechanisms of selective killing of cancer cells must be also elucidated in detail. Although considerable data have been generated to date regarding the molecular mechanisms underlying NTP-induced effects, a more comprehensive understanding of how NTP affects gene regulatory networks, signaling networks and metabolic networks is still needed.

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