

Review

New Hopes for Plasma-Based Cancer Treatment

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Abstract: Non-thermal plasma represents a novel approach in cancer treatment. Both direct and indirect plasma treatments are available, with clinical trials of direct plasma treatment in progress. Indirect treatments involve chemotherapy (i.e., plasma-activated medium) and immunotherapy. Recent studies suggest that integrated plasma treatments could be an extremely effective approach to cancer therapy.

Keywords: plasma cancer treatment; plasma-activated medium (PAM); plasma-assisted immunotherapy

1. Introduction

Plasma-based cancer treatments represent a critical area in the field of plasma medicine [1–3]. Some pioneering *in vitro* [4] and *in vivo* [5] works have shown that non-thermal plasma exerts anti-tumor effects. Currently, two options for plasma cancer treatment are available: direct and indirect (Figure 1). Clinical trials of cancer treatments using non-thermal plasma (direct plasma treatments) are ongoing in Germany [6] and the USA [7]. Two different types of indirect plasma treatment have been proposed: plasma-assisted immunotherapy [8] and plasma-activated medium (PAM) [9,10]. Plasma is also considered as an adjuvant therapy, and the three main options are plasma in combination with chemotherapy [11,12], plasma to modulate tumor microenvironment [13,14], and plasma in association with electrotherapy [15]. These approaches have dramatically broadened the ways of using non-thermal plasma for treating cancers and other diseases.

Currently, two options for plasma cancer treatment are available: direct and indirect. Clinical trials of direct plasma treatments are ongoing. Indirect treatments include plasma-assisted cancer immunotherapy and plasma-activated medium (PAM) therapy.

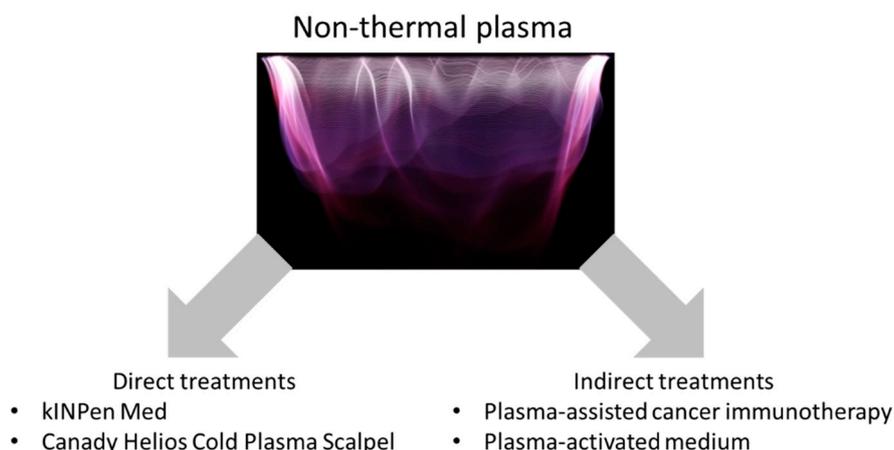


Figure 1. Two types of plasma-based cancer treatments.

2. Direct Treatments

Direct plasma treatments are the most straightforward. A variety of plasma sources have been developed for medical applications such as cancer treatment [16–20]. Metelmann et al. used a plasma source known as kINPen MED to treat advanced head and neck carcinoma ulcerations and patients in the final stages of disease [6,21]. Plasma treatment reduced both pain and odor. Only a few myeloid cells were present in tumor tissue of patients that received frequent plasma treatment, whereas numerous myeloid cells were found in tissue sections of patients that did not receive plasma treatment. Canady et al. treated liver cancer using a Canady Helios Cold Plasma Scalpel to remove cancerous tissue without damaging the blood supply to the remaining liver.

Since plasma needle was used for treatment of culture cells [22], various plasma sources have been developed for cancer treatment. The plasma jet and dielectric barrier discharge (DBD) have been developed and widely used in plasma cancer treatment. A pulsed DBD with microsecond pulses was used for treatment of xenograft model mouse of human glioblastoma cells [23]. Recently, nanosecond-pulsed plasma have been developed as potential tools in cancer treatment [24,25].

3. Indirect Treatment: Plasma-Assisted Cancer Immunotherapy

Several researchers have proposed the use of plasmas as immune modulators for treating cancer. The number of cells in the human body is estimated at about 40 trillion, and a small portion of these cells acquire mutations and become cancerous every day. However, the immune system typically removes mutated cells. It is only when cancerous cells avoid the immune system that cancerous disease develops. Recently, a variety of anti-cancer therapies designed to modulate the immune system have been developed [26–28]. These approaches include the use of cytokines, cell-based therapies, and immune checkpoint blockade. For example, the US Food and Drug Administration (FDA) approved the first cellular immunotherapy (sipuleucel-T) for prostate cancer in 2010 [29]. The FDA also approved the anti-PD1 monoclonal antibody, nivolumab, for adjuvant treatment of patients with melanoma involving the lymph nodes and patients with metastatic disease who have undergone complete resection [30].

A better understanding of the interactions between cancer cells and the immune system has increased interest in immunotherapies over the last decade [31,32]. Radiation and some chemotherapeutic drugs increase immunogenicity by triggering immunogenic cell death (ICD). Damaged or stressed cancer cells present “danger signals” known as damage-associated molecular pattern (DAMP) molecules. High-mobility group box 1, ATP, and calreticulin (CRT) are well-known DAMP molecules that are retained inside the cell in the healthy state and released only in response to stress or cell damage. Cancer cells usually induce immunosuppression; however, ICD-associated

DAMP molecules can reactivate anti-cancer immunity by triggering dendritic cell maturation and antigen presentation.

Several recent studies have suggested that non-thermal plasma treatment induces ICD and stimulates macrophages [25,33–35]. Non-thermal plasma treatment was shown to stimulate extracellular ATP secretion and enhance cell death via ICD-mediated macrophage stimulation. Plasma-generated reactive oxygen species (ROS) are major effectors of ICD. Non-thermal plasma elicits surface exposure of CRT, and N-acetyl cysteine, which is an ROS scavenger, reduces the externalization of CRT. These results suggest that intracellular ROS are responsible for plasma-induced CRT production. Tumor necrosis factor- α released from plasma-activated macrophages induces tumor cell death [36].

In the future, non-thermal plasma will be used to induce ICD in tumors to help dendritic cells find, eat, and present cancer cell antigen to elicit robust T cell immune responses [37]. It was shown that naïve T helper cells were less sensitive toward non-thermal plasma treatment, suggesting that plasma could be used as a tool to redox-control T cell phenotypes in cancer immunology [38]. Flow cytometric technique for microparticle characterization was established, and the number and size of microparticles released were shown to be modulated in THP-1 monocytes, polymorphonuclear leukocytes (PMN), and peripheral blood mononuclear cells (PBMC) after plasma exposure [39]. Interestingly, abscopal effects of non-thermal plasma treatment on tumor growth were observed, suggesting that plasma activated innate immune response [24].

4. Indirect Treatment: PAM

PAM has been proposed as a type of cancer chemotherapy. Various *in vitro* experiments have demonstrated that PAM exerts anti-tumor effects on many kinds of cancer cells [9,10,40,41]. In most cases, PAM induces intracellular ROS production and subsequent apoptosis of cancer cells. The mechanism through which PAM induces the apoptosis of cancer cells depends on the cell type [42,43]. In glioblastoma cells, down-regulation of survival and proliferation signaling networks plays a critical role in PAM-induced apoptosis [9,42,44]. Aquaporins, which transport hydrogen peroxide into the plasma membrane, are also key factors in apoptosis induction [45]. Many *in vivo* experiments have also demonstrated the anti-tumor effects of PAM against a variety of cancers. In a xenograft mouse model, PAM inhibited the growth of ovarian and pancreatic cancer tumor cells [10,41]. Intraperitoneal injection of PAM/plasma-activated Ringer's lactate (PAL) inhibited the metastasis of ovarian, gastric, and pancreatic cancer tumors in disease model mouse experiments examining peritoneal metastasis [46–48]. However, in apoptosis induced by PAL, less ROS are produced in comparison with PAM [49], suggesting that components generated in PAM control the redox balance.

5. Conclusions

Two major options are available for plasma-based cancer therapies: direct and indirect treatments. Direct plasma treatment methods have already been introduced clinically, whereas indirect plasma treatment methods such as plasma-assisted cancer immunotherapy and PAM therapy are new approaches currently under study. In the future, the overall survival of cancer patients could be significantly improved by combining direct and indirect plasma treatments.

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