Chapter 4
Cancer Applications Overview

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Contents

Abstract In this chapter, we overview the progress of CAP application for cancer therapy. This includes summary of in vitro results, in vivo treatment, as well as first clinical trials.

In recent years, cold atmospheric plasma (CAP) has emerged as a possible new modality for cancer treatment [1]. The novelty of CAP is in various forms and it is typically associated with reactive species produced in plasma and electric field formed when plasma hits the tissue. Outside of cancer applications, CAP's efficacy has been tested in various applications such as disinfection, wound healing, dentistry, and cancer therapy [2–7]. High-level hypothesis of CAP interaction with cells and tissue is based on the notion that chemical elements of the CAP are

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M. Keidar (ed.), *Plasma Cancer Therapy*, Springer Series on Atomic, Optical, and Plasma Physics 115, https://doi.org/10.1007/978-3-030-49966-2_4

potentially toxic, such as reactive oxygen species (ROS), which might promote a "plasma killing effect," while others such as reactive nitrogen species (RNS) could produce a "plasma healing" effect. Forming various combinations of these species might provide great potential for activation of specific signaling pathways in cells. CAP treatment possesses powerful lethal capabilities against tumor cells both in vitro and in vivo and just as importantly, the normal counterpart cells have been shown to be less sensitive to the same CAP treatment [8]. All these aforementioned effects are believed to be related to the plasma chemistry. While different species are produced as a result of plasma treatment, the role of other plasma effects such as charged particles and the electric field is still not fully understood.

The general understanding of the CAP action on living tissue is associated with the ROS and RNS formation and transport from the gaseous to the liquid phase and through the biological barriers. Many RONS generated by CAP are also active components in cell biology [9]. It has been argued that analogy between cellular and plasma-generated RONS represents the major logic of plasma application in medicine including cancer therapy and that many species produced directly or indirectly by plasma will function in cell biology as endogenous species [8]. RONS play a central role in 'redox' or oxidation–reduction biology [10].

The timeline of plasma interaction with cells is shown in Fig. 4.1. Plasma interaction with living tissue is essentially a multi-scale process spanning from the initial burst at the timescale of nano- and microseconds (depending on specific plasma device) to seconds, followed by the timescale of minutes, which is related to RONS formation and transport across the cellular membrane and finally triggering various cellular pathways at the timescale of hours and days [11].

It should be pointed out that there are many important functions associated with reactive oxygen species. Watson [12] suggested that ROS is "a positive force for

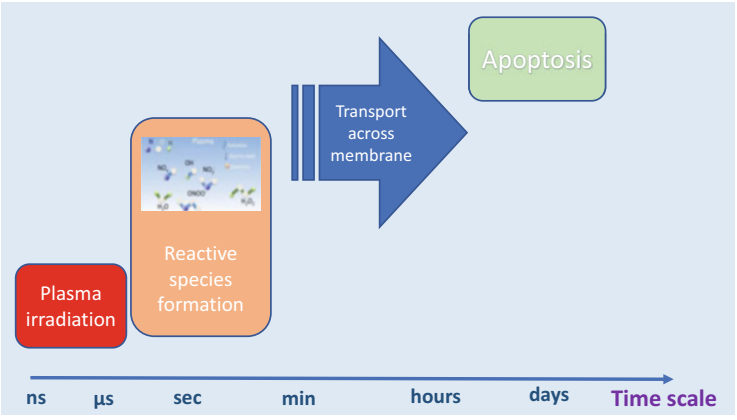

Fig. 4.1 Schematics of multi-scale nature of plasma interaction with cells. Initial plasma impact leads to the formation of long-life species, transport of these species across the gas–liquid interphase, cell membrane, activation of various cellular pathways, and eventually to cell apoptosis or programmable death

life" due to their role in apoptosis—an internal program leading to cell death. At the same time, ROS are also well recognized "for their ability to irreversibly damage key proteins and nucleic acid molecules [e.g., DNA and RNA]." In a balanced cell, a normal level of ROS is maintained by the antioxidant system. Watson noted that "The vast majority of all agents used to directly kill cancer cells (ionizing radiation, most chemotherapeutic agents and some targeted therapies) work through either directly or indirectly generating reactive oxygen species that block key steps in the cell cycle." The general understanding is that the effect of ROS on cell development depends on the level of ROS [13]. Low level of ROS supports cell proliferation and helps to maintain cell functionality while a high level of ROS causes oxidative stress leading to cell death. Healthy cell function is preserved by an antioxidant system that maintains the ROS level at a tolerable level. Cancer cell abnormal metabolism causes an aberrant high level of ROS [11]. To survive, a cancer cell mutates to regulate ROS. However, rise in the intracellular ROS level might cause irreparable DNA damage [14, 15]. At the same time, the level of ROS in cancer cells is near the limit at which cell death occurs. On the other hand, the ROS level in the corresponding normal cells is generally lower [13]. Thus, selectivity toward tumor cells by pro-oxidative therapy is achieved when anticancer therapy produces ROS near the "threshold" between levels of ROS in normal cells and cancer cells.

It has been hypothesized that such an approach seems to be relevant to the CAP-based anticancer therapy. ROS produced by CAP might lead to cancer cells' death by damaging the function of intracellular regulatory factors [16, 17]. To that end, multiple studies demonstrated that biologically active neutral short- and long-living ROS molecules are produced by CAP including OH, O, O (1D), O_2 ($1\Delta g$), O_3, HO_2, and H_2O_2 [18]. RNS species such as NO_2-, NO−, and NO+ are generated directly during the discharge in a gas phase and in the plasma-activated media [7, 19].

Turning attention to reactive nitrogen species, nitrite oxide (NO) plays a very significant role in cellular processes. In particular, NO is an important factor in the electron transport chain. It is also known that NO might affect the electron transport system by attacking cytochrome oxidase [20]. In turn, the breaking of the electron transport will increase the generation of superoxide reacting with NO to form peroxynitrite [21].

It has been well established that one of the important species formed in the course of plasma interaction with cell culture media is hydrogen peroxide (H_2O_2) [22, 23]. To that end, a model based on aquaporin (AQP), which is a well-known H_2O_2 channel on the cell membrane, has been proposed [24, 25]. Multiple studies inform that in general cancer tissues express more AQP channels than corresponding normal tissues. According to the proposed hypothesis [22, 23], after plasma treatment, H_2O_2 transport across the cellular membrane of the cancer cell is higher than that of the normal cell. Such differential H_2O_2 consumption rate might be the possible mechanism of the selective anticancer action of CAP. As such, the rise of intracellular ROS as a result of ROS diffusion across cell membrane correlates with the rise of extracellularly plasma-originated ROS. The reaction of cells to ROS excess is well documented. Catalase is the major enzyme that controls

concentrations of H_2O_2 in both cancer and normal cells [26]. For instance, a recent paper correlated the rate of H_2O_2 removal and activity of catalase for 15 cancer cell lines and 10 normal cell lines. This study showed that H_2O_2 produced from the oxidation of P-AscH- is a principle mediating factor in selective targeting of the cancer cells. More importantly, it was demonstrated that normal cells have a higher constant of H_2O_2 removal than that of cancer cells. This trend is illustrated schematically in Fig. 4.3. Granted, H_2O_2 is known to be a very strong oxidant but it has a slow reaction rate with the majority of biomolecules. As such, hydrogen peroxide accumulates in cells and the aforementioned process of H_2O_2 removal by catalase becomes a critical process for cell survival [24]. Detailed analysis of the biochemical process in cells caused by CAP is presented in Chap. 5.

Aforementioned processes suggest the possible role of plasmas in the biomedical application through effects on the cellular chemical balance by generating RONS. In that sense, what plasma creates is a reservoir for some critical species that can be utilized when the cell needs them. In addition, it should be pointed out that CAP is very different from chemotherapy and radiotherapy, though many chemotherapy and radiotherapy also increase intracellular RONS stress and further kill cancer cells. The primary difference is that CAP itself is the source of RONS including hydrogen peroxide, nitrite, nitrate, and nitric oxide.

The high-level model of plasma–cell interaction based on the aforementioned processes is shown schematically in Fig. 4.2. Current understanding of CAP action could be attributed to several cellular factors, i.e., higher expression of AQPs by the cancer cell membrane, the higher level of ROS in cancer cells, and lower expression of antioxidant enzyme (catalase) in cancer cells [22, 23].

4.1 Effect of CAP on Cell Cycle

According to conventional wisdom, a way to target cancer cells is to interfere with the cell cycle [27–29]. It stems from the fact that cancer cells proliferate at a faster rate than normal cells [30]. Several studies suggest that CAP interaction with cells, in particular, cancer cells, lead to modifications in the cell cycle. In fact, recent data confirmed that cancer cells are more susceptible to the effects of CAP because a greater percentage of cells are in the S phase.

Recall that one of the hallmarks of cancer is the approach to deregulate the mechanisms controlling cell cycle [31]. Note that the cell cycle or “life of cell” outlines the different stages taking place as a cell develops. In particular, the DNA synthesis occurs during the specific period of a cell cycle, which is called S-phase, the morphological changes in cell refer to the mitotic phase (M-phase), a phase of the cell division. The stages between M and S and between S and M phases are called G1 and G2 phases. It should be pointed out that non-proliferating cells are in the G1 phase of the cell cycle. Once cells proliferate, they move progressively from G1 to S (synthesis of DNA), G2, and then M (mitosis) phase where they divide. Cells in G1 can be either in quiescence (G0), terminally differentiate, or, in response to specific signals, be induced to proliferate. Most cells in normal adult tissues are in

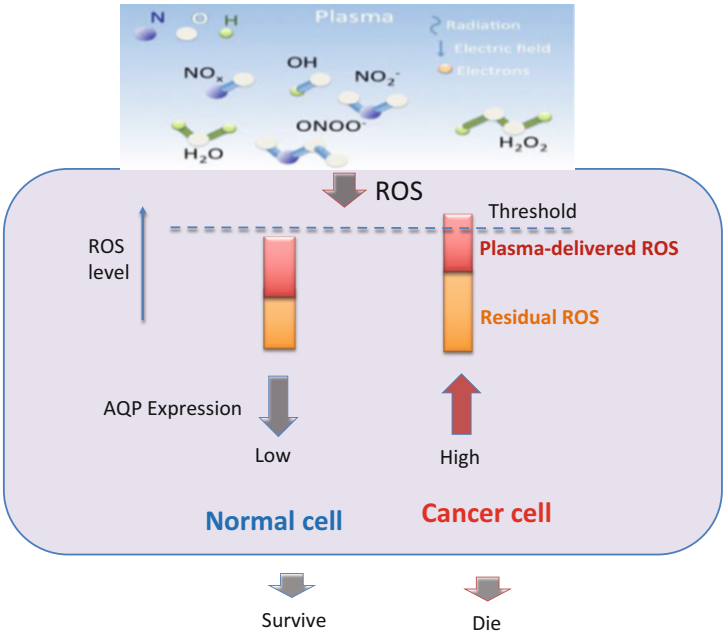

Fig. 4.2 Schematically represented model of the plasma interaction with cancer and normal cells explains plasma selectivity

the quiescent G0 phase. Between S and G2 and between G2 and M phases there are so-called "checkpoints." Cell progression through the cell cycle is monitored and controlled by these checkpoints. Checkpoints verify whether the processes at each phase of the cell cycle have been accurately completed before moving into the next phase.

Initial analysis of the CAP effect on glioblastoma [32] suggested that the CAP treatment causes significant arrest in the G2/Mphase in U87MG cells. Vandamme et al. [22] found that in U87MG cells, plasma treatment led to a significant decrease in the number of cells in the G0/G1 phase with a significant increase of cells in the S-phase. It was found that this effect depends on the plasma dose.

The effect of CAP treatment on the cell cycle was studied for 3 different cell lines. Bright-field images with 10X magnification of wild-type keratinocytes, 308, and PAM212 cells morphology, respectively, are shown in Fig. 4.3. Figures 10 d–l are DNA content measurement of control (untreated) cells in blue, and cells treated with CAP for 60 s in red. The cells were investigated after 4 and 24 h after CAP treatment (60 s). One can see that no significant shifts in the G1–G2 peak positions were observed for all cell types considered. It can be seen that CAP-induced robust G2/M–cell cycle increases in both carcinoma and papilloma cells (it is about two to threefold increase in about 24 h after CAP treatment), whereas normal keratinocytes showed almost no variation. It should be pointed out that this change diminished in about 48 h.

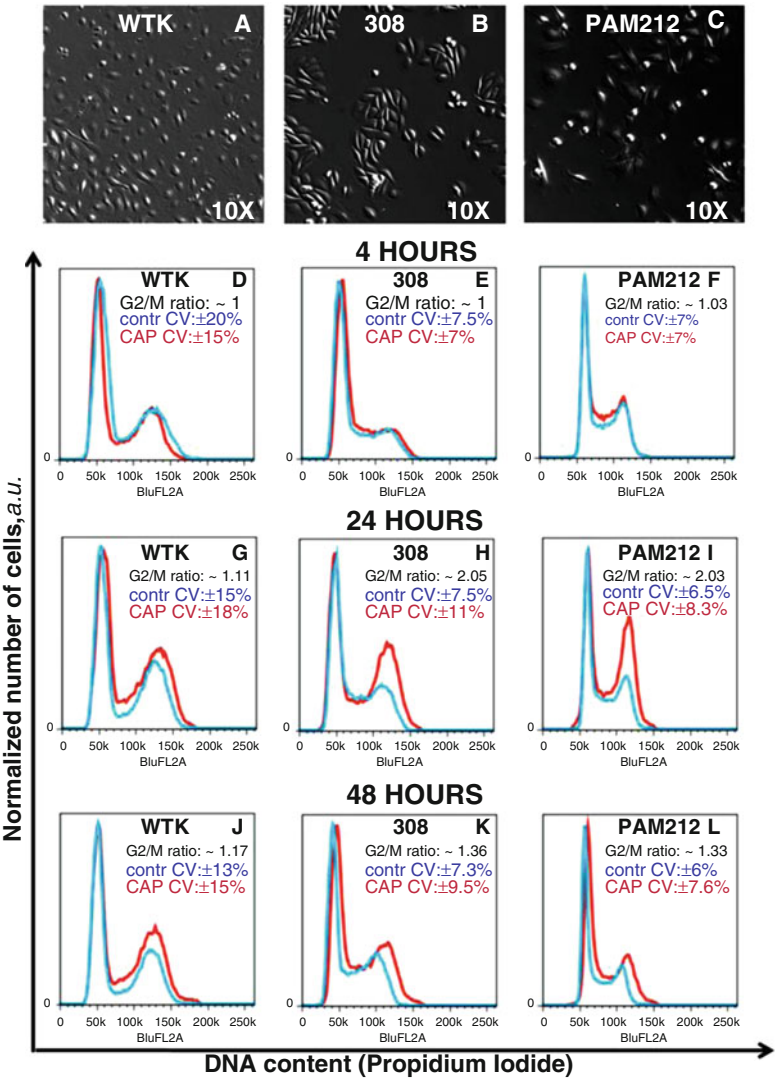

Fig. 4.3 Identification of the cell cycle change in the G2/M-phase. (**a**–**c**) Bright-field images of wild-type keratinocytes (WTK), epidermal papilloma (308 cells), and epidermal carcinoma (PAM212 cells) cells are shown with a magnification ×10. (**d**–**l**) show cell cycle studies: Propidium Iodide content (horizontal axis) and normalized number of cells (vertical axis) are shown. The controls are shown in blue and cells CAP treated for 60 s are in red. The ratio of the number of cells (treated to untreated) in the G2/M-phase with coefficients of variation (CV, in percent) is shown in the right top corner of each figure. (**d**–**f**) shows cell cycle measurements in about 4 h; (**g**–**i**) in about 24 h; and Figure (**j**–**l**) in about 48 h after the CAP treatment for WTK, 308, and PAM 212 cells, respectively. Reprinted with permission from [26]. Copyright (2012) by Macmillan Publishers Ltd

4.1.1 *In Vivo CAP Application. Subcutaneous Models*

To date, a number of studies have been performed in order to access the anticancer effect of CAP treatment in vivo. To that end, CAP treatments were mainly performed by treating the skin above the tumor sites. In addition, in several studies, micro-size μ-CAP devices were applied to guide the tiny CAP jet to affect the exposed tumorous tissues underneath the skin or the skull [6, 12]. All reported studies reported a strong effect of CAP on the growth of tumors.

The earliest in vivo experiments were performed by Vandamme et al. [33, 34]. They used the glioblastoma U87MG tumor mouse xenograft model to test the anticancer effect of CAP treatment. This pioneering work demonstrated a significant tumor volume decrease of 56% on the treated mice after the micro-duration pulsed floating electrode dielectric barrier discharge (FE-DBD), which delivered about 0.75 W at 200 Hz on the mouse skin.

Corresponding mouse length of survival increased by about 60% after the FE-DBD treatment. Both the tumor volume measurement and bioluminescence imaging (BLI) have been used to assess the anticancer effect of FE-DBD treatment. In this study, the authors also discovered an important trend that the five consecutive plasma treatments will cause a much better anticancer effect than a long single plasma treatment. Such a fractionated treatment strategy has been used in many subsequent studies (Fig. 4.4).

In the same year, Keidar et al. performed the study in vivo on a bladder tumor xenograft model on mouse through the direct CAP jet treatment (Fig. 4.5) [35].

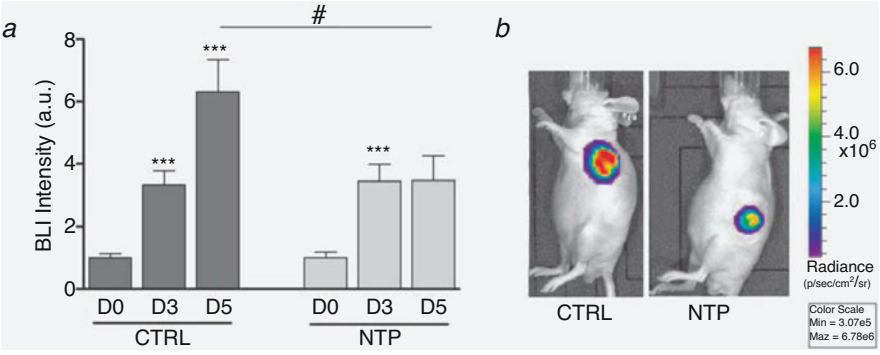

Fig. 4.4 The early in vivo demonstration of the antitumor effect of CAP. When the tumor reached 150 ± 50 mm^3, mice were randomly assigned into two groups: control (CTRL) and plasma, eight mice per group. Plasma treatment was delivered each day for five consecutive days (6 min, 200 Hz). Mice in both groups were sacrificed 24 h after the last treatment. (**a**) BLI imaging was performed before the first treatment (Day 0), during treatment course (Day 3), and 24 h after the end of treatment protocol (Day 5). Tumor BLI was normalized to signal at Day 0. (**b**) Representative BLI imaging of CTRL and NTP treated mice at Day 5. Reproduced with permission from Vandamme et al., International Journal of Cancer, 130, 2185 (2012). Copyright 2012 John Wiley & Sons on behalf of the Union for International Cancer Control

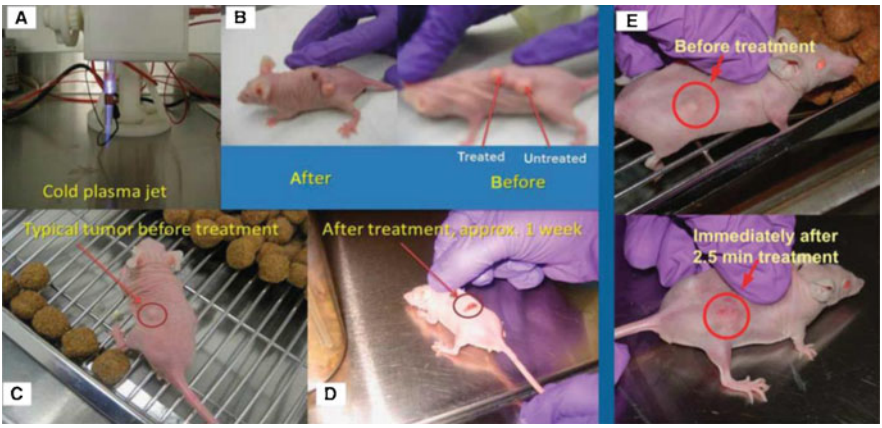

Fig. 4.5 The anticancer effect on the subcutaneous tumor model. (**a**) CAP jet; (**b**) typical images of mice with three tumors before and after the CAP treatment (shown after 24 h); (**c** and **d**) typical images of mice with a single tumor before and approximately 1 week after CAP treatment; (**e**) tumor before and immediately after the 2.5 min of CAP treatment [29]. Reproduced with permission from Keidar et al., British Journal of Cancer, 105.9, 1295 (2011). Copyright 2011 Nature Publishing Group (a division of Macmillan Publishers Ltd)

After just 2 min of CAP treatment, the tumor significantly decreased its size and could not be observed via gross inspection after 24 h [13]. In addition, the authors have performed the similar experiments on a murine xenograft melanoma model and achieved a promising result that the tumor growth has been completely inhibited over 3 weeks after the CAP treatment [29]. Similarly, the corresponding mouse survival rates were also strongly increased upon the CAP treatment. These two preliminary studies directly demonstrate the promising potential of the CAP treatment as an anticancer method with little or no toxic side effects. In addition, the similar anticancer effect has been observed on several other subcutaneously mouse xenograft models as described in Table 4.1.

According to many authors, the CAP-originated reactive species may play a dominant role in the anticancer mechanism of CAP treatment in vivo [11]. However, in general, a single reactive species such as H_2O_2 does not generate the same anticancer effect as the CAP treatment. For instance, in some work, it has been shown that using the gel/H_2O_2 mixture did not generate a tumor killing efficacy as significant as that of DBD did on the melanoma in the mouse [11]. The gel/H_2O_2 mixture led to delayed tumor growth, but the tumor eventually progressed through the treatment [11]. Based on this result, it is hypothesized that other reactive species rather than H_2O_2 may be involved in the interaction between CAP and the cancerous tissue. As an alternative notion, it can be argued that the biological effect of H_2O_2 on tumor tissues may be quite different from what is seen in vitro in a petri dish or multi-wells plate as compared to the in vivo setting.

Table 4.1 Summary of the anticancer CAP treatment in vivo

Year	Tumor types	Models	Anticancer effect	Authors/References
2011	Xenografted glioblastoma	Mouse	Medium volume decrease	[27]
2011	Xenografted bladder cancer	Mouse	Strong volume decrease	[13]
2011	Melanoma	Mouse	Strong volume decrease	[13]
2012	Xenografted pancreatic carcinoma	Mouse	Strong volume decrease	[3]
2012	Xenografted glioblastoma	Mouse	Medium volume decrease	[2]
2013	Xenografted neuroblastoma	Mouse	Medium volume decrease	[5]
2014	Xenografted head and neck cancer	Mouse	Medium volume decrease	[4]
2014	Melanoma	Mouse	Slight volume decrease	[10]
2015	Melanoma	Mouse	Strong volume decrease	[11]
2015	Xenografted adenocarcinoma	Mouse	Little volume change	[7]
2016	Xenografted breast cancer	Mouse	Strong volume decrease	[6]
2016	Xenografted glioblastoma	Mouse	Medium volume decrease	[8]
2017	Melanoma	Mouse	Medium volume decrease	[9]
2017	Glioblastoma	Mouse	Strong volume decrease	[12]
2017	Head and neck cancer	Human	Partial remission	[14]

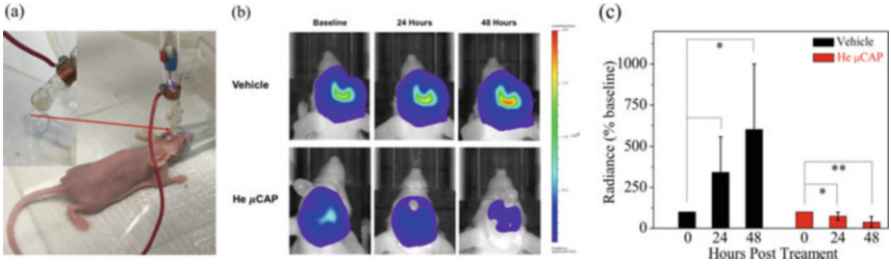

Fig. 4.6 Treatment of brain tumor with micro-cold atmospheric plasma (μCAP). (**a**) μCAP photo showing plasma delivery through an intracranial endoscopic tube; (**b**) bioluminescence images of tumor volume; (**c**) summary data of radiance intensity showing both helium (no discharge) treatment (marked as "vehicle") and CAP treatment [Ref. 27]. Reprinted with permission from Cancers 9.6 (2017): 61

4.1.2 In Vivo Treatment: Intracranial Model

Recently, the first intracranial mouse model was utilized in CAP treatment. To study the effect of CAP on a brain tumor, a micro-CAP device has been developed. This device was directly applied to the glioblastoma tumor by utilizing an implanted endoscopic delivery system as shown in Fig. 4.6 [36]. Tumor volume was assessed by the bioluminescence imaging in real time (as shown in Fig. 4.6b). These

experiments suggested that the tumor volume in the case of a control (i.e., helium only treatment) increased by about 600% after 2 days. At the same time, CAP treatment led to a decrease in the tumor volume by about 50% as shown in Fig. 4.6c.

Overall, by 2017, twenty-seven in vivo studies have been identified and concluded that there was a significant reduction in tumor size and an increase in survival rate [37]. To date, it should be pointed out that the in vivo applications were mostly performed on subcutaneous tumor xenografts in mice. Another study based on the use of a tumor chorioallantoic model was conducted [38]. Although the results corroborate the in vitro studies, the development of models closer to clinical situations is necessary. Only a single study was performed utilizing the intracranial model [27].

4.2 Clinical Studies

To date, several clinical studies have been performed. In Germany, two of such studies concerned with the application of CAP on patients with head and neck cancers as a palliative treatment or before tumor resection. The rationale of this therapeutic choice can be explained by the ability of CAP to decontaminate and to treat severely infected wounds or ulcerations [39] while inducing apoptosis in head and neck cancer cell lines. Summary of the first clinical studies was published by Metelmann et al. [33]. The trial enrolled 6 patients with locally advanced (pT4) squamous cell carcinoma of the oropharynx suffering from open infected ulcerations [33]. Patients were treated by a plasma jet in a cycle of 3 single applications within 1 week, each followed by an intermittence of 1 week. CAP treatment caused a reduction in odor and pain medication requirement, which improved the social function of patients and caused a positive emotional effect. The partial remission in two patients for at least 9 months has been observed. A moderate amount of apoptotic tumor cells and a desmoplastic reaction of the connective tissue was observed in the incisional biopsies [33].

In a most recent clinical follow-up, Metelmann and colleagues [40] investigated the effect of CAP on the surface of head and neck squamous cell carcinoma tumors. Overall, these results have shown an improvement in the quality of life of the patients, tumor reduction, and significant improvement in tumor decontamination.

In the USA, US Medical Innovation, LLC and GWU performed a clinical application by completing stage 4 colon cancer surgery to remove tumor and treat cancerous tissue remnants while sparing normal tissue at Baton Rouge General Medical Center in Baton Rouge, LA, in March 2015. Later in 2017, US Medical Innovation LLC used Canady Helios Cold Plasma and Hybrid Plasma Scalpels in the first clinical liver resection to remove and selectively kill liver cancer tumor cells [41]. More details of the clinical studies and perspectives of CAP application in health care are presented in Chap. 11.

4.3 Summary of Plasma Immunomodulation Studies

A recent new application of CAP in oncology involves plasma-based immunotherapy. There are two major reasons for recent significant interest in plasma immunomodulation studies in general and plasma immunotherapy of cancers in particular:

- First of all, most of the cancers are not superficial (like melanoma, or some types of carcinoma), but internal, that is located deep inside of the human body. Plasma species are in many cases simply unable to directly access the cancer tissue, even taking into account bystander and cell-to-cell communication effects. From this perspective, plasma stimulation of the immune system makes sense. Plasma species are able to stimulate major immune system factors either directly or through the intermediate contribution of bystander and cell-to-cell communication mechanisms; then the immune system suppresses malignancies wherever they are located in deep inside of the human body.
- The second aspect of interest in plasma immunomodulation studies is due to recent successes in cancer immunotherapy in general. Breakthroughs in cancer immunotherapies have demonstrated considerable success, though not without limitations mostly related to treatment side effects. Nonthermal plasma is highly multi-factor controllable substance, which provides an opportunity for extreme selectivity and suppression of side effects. It should be pointed out that CAP for cancer therapy has been emerging as a potential adjuvant treatment via induction of immunogenic cell death (ICD), which will be clarified below.

4.4 About Physical and Biochemical Mechanisms of Plasma Immunomodulation for Cancer Treatment

Cancer cells undergoing immunogenic cell death (ICD) stimulate a patient's immune system to mount an anticancer response. This is a major opportunity for plasma-based cancer immunotherapy. Underlying biochemical and biomedical mechanisms of NTP-induced ICD and relevant ICD consequences and their effect on cancer treatment are closely examined recently. Some novel experimental results in this direction (in particular, by Lin et al. [42]) are going to be shortly overviewed below.

The short-lived reactive oxygen and nitrogen species (e.g., hydroxyl radicals, atomic oxygen, nitric oxide) produced by plasma are the main effectors that elicit ICD, in particular, in melanoma. It has been demonstrated in vitro using dielectric barrier discharge DBD plasma systems, as well as validated in a vaccination assay in vivo. Plasma generation of reactive species appears to be dictated by many factors but especially by the total energy, which is a positive fact helping in defining effective dosimetry and standardization of plasma-medical treatment.

Recent advances in cancer immunotherapy have led to significant positive impacts on patient survival, especially in patients with cancers previously limited to first-line treatments. Cancer immunotherapy intends to assist a patient's natural cancer immunity cycle to fight cancer, and major success has been achieved with checkpoint inhibitors such as anti-PD-1/PDL-1 and anti-CTLA-4 therapies. However, the benefits of these treatments have been met with challenges including severe side effects and efficacy in only a subset of patients. Therefore, there is a considerable need to develop new treatment modalities that, in combination with current therapies, may help improve clinical outcomes by supporting different steps of the cancer immunity cycle. The goal of treatments should be to enable an effective, self-sustaining anticancer response in the patient.

4.5 Immunogenic Cell Death (ICD) as One of the Key Factors of Plasma-Stimulated Cancer Immunotherapy

There are several approaches to plasma immunomodulation for cancer treatment. Probably the most interesting approach to enhance the initial step of the cycle is to induce immunogenic cell death (ICD) in the tumor. ICD is a form of regulated cell death, characterized by the timely release of "danger signals" known as damage-associated molecular patterns (DAMPs).

Several damage-associated molecular patterns (DAMPs) have been linked to ICD (e.g., high-mobility group box 1 (HMGB1), adenosine triphosphate), the most critical and well-studied being surface-exposed calreticulin (CRT). CRT on the outer leaflet of the cell functions as an "eat-me" signal for uptake by dendritic cells. ICD inducers have been identified, and novel modalities continue to be explored. One such treatment is plasma generated at room temperature and atmospheric pressure, also known as nonthermal plasma (NTP). NTP treatment of mice has been shown to reduce tumor burden and extend survival in different cancer types.

The majority of these studies have used NTP for direct tumor cell killing or to induce cell senescence via reactive oxygen and nitrogen species (RONS)-mediated pathways. Small clinical studies with nonthermal plasma have only recently commenced for palliative and curative treatment of dermatological diseases, and to date, plasma has been effective with mild to no side effects [40, 43].

4.6 Immunogenic Cell Death: In Vitro, and In Vivo Studies, Understanding of Plasma-Chemical Mechanisms

Beginning in 2015, NTP has been investigated for its potential to induce ICD, and it has been reported to stimulate DAMP emission in multiple cancer cell lines in vitro [44, 45]. The first in vivo demonstration of NTP-induced ICD was performed

on Balb/c mice bearing subcutaneous, syngeneic CT26 colorectal tumors [46]. Tumors treated with a dielectric barrier discharge (DBD) plasma resulted in higher expression of DAMPs (CRT and HMGB1) and increased recruitment of CD11c+ and CD45+ immune cells into the tumor environment. In combination with a therapeutic vaccine, DBD plasma treatment also enhanced cancer-specific T-cell responses [46].

However, the underlying mechanisms by which NTP elicits ICD are still not fully understood. When plasma is generated, a complex environment of reactive species, charged particles, neutral molecules, ultraviolet radiation, and electric fields is present and can interact with the biological target. Reactive species produced by the DBD plasma in the presence of oxygen were reported as the major contributors for ICD induction, not the physical components [45].

The exact chemical species that are responsible remain unclear. These include RONS with lifetimes ranging from fractions of a second (e.g., atomic species, radicals) to days and weeks (e.g., hydrogen peroxide, nitrite anion). Knowing which short-lived and persistent species are required for ICD induction will be critical to the understanding and development of NTP technology for cancer immunotherapy.

To address the above challenges in oncology and the underlying questions in plasma chemistry and biological interactions, one should delineate the RONS generated by DBD plasma that are responsible for plasma-induced ICD [42]. Knowing the species in plasma that are critical to ICD induction leads to the development of an optimized clinical device, more details on the subject can be found in [42].

Summarizing, the number of open questions in plasma immunotherapy is still very high. On the other hand, the addition of plasma systems into the existing arsenal of cancer immunotherapies opens the great possibility for new combination strategies for safer and more robust control of cancer.

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