Plasma Medicine, 6(1): 47–57 (2016)

Genotoxic and Cytotoxic Effects of Plasma-Activated Media on Multicellular Tumor Spheroids

Judée Florian, Nofel Merbahi,* & Mohammed Yousfi

LAPLACE, Université Paul Sabatier de Toulouse, Centre National de la Recherche Scientifique, Institut National Polytechnique de Toulouse, France

*Address all correspondence to: Nofel Merbahi, Laboratoire Laplace, Université de Toulouse, Toulouse 31062, France; Tel.: 33-5-6155-7590; Fax: 33-5-6155-6362, E-mail: merbahi@laplace.univ-tlse.fr

ABSTRACT: The purpose of this study was to evaluate the effect of plasma-activated media (PAM) on a multicellular tumor spheroid (MCTS) obtained using HCT116 colon carcinoma cells. PAM was processed by a helium plasma jet during 60–240 seconds of exposure. Spheroids were immersed in PAM at different transfer times (from 15 minutes to 48 hours). The inhibitory effect of PAM on cell growth was first evaluated. This growth inhibition is associated with the regionalized accumulation of DNA damage detected by histone H2AX phosphorylation, leading to massive cell death and loss of the MCTS proliferative region. The use of a hydrogen peroxide (H_2O_2) scavenger (catalase) in PAM induced the reduction of DNA-damaged cells in MCTS, thus pointing out the role of H_2O_2 in DNA damage, but growth inhibition was still observed. Finally, when HCT116 colorectal cancer cells and GM637 human fibroblast cells were immersed in PAM, the expression of γH2AX showed that only HCT116 cells were damaged, emphasizing an interesting selectivity of PAM to inactivate only the cancerous cells in the framework of in vitro cases; this selectivity has already been noted in the literature.

KEY WORDS: colon carcinoma cancer cell, low-temperature plasma, plasma-activated media, genotoxic and cytotoxic effects

I. INTRODUCTION

Colorectal cancer is the third most prevalent cause of cancer death. According to the statistics, in 2014 about 136,830 new cases were screened and 50,310 patients died of colorectal cancer in United States[1]; it was estimated that, in France, 17,700 patients died in 2012.[2] Surgery is the usual strategy against colorectal cancers,[3] whereas additional chemotherapy and radiotherapy can be used if tumors have grown through several layers of the colon and/or extend into the nearby tissues.[3] The recurrence of colorectal cancer is estimated between 10% and 30%.[4] The drawback of surgery as a therapeutic approach is the need to remove healthy tissue surrounding the region affected by the cancer. Therefore, new therapeutic strategies are needed to more efficiently and more selectively treat colorectal cancer.

The effects of low-temperature plasmas generated at atmospheric pressure on various cancer cell lines have been described for *in vitro* cases.[5–15] These studies showed that low-temperature plasmas induce cancer cell inactivation, arrest growth, cause apoptotic cell death and DNA damage, and selectively ablate some cancer cells. *In vivo* research also demonstrated that cold plasma treatment of subcutaneous tumors (grown from cell lines) inhibits tumor growth and causes cell death and apoptosis.[16–19] This work was done

2151-805X/16/$35.00

in the context of low-temperature plasmas generated at atmospheric pressure to study their effects on cancer cell inactivation. It is a continuation of a previous work[20] devoted to the role of the plasma jet in the direct treatment of multicellular tumor spheroids (MCTSs), a model that mimics the 3-dimensional organization and regionalization of a microtumor region. A preliminary experiment studying plasma-activated media (PAM) and their effects on MCTSs was presented in Ref. 20.

Therefore, this work investigates the genotoxic and cytotoxic effects of PAM on MCTSs. Moreover, a specific plasma-activated species (hydrogen peroxide [H_2O_2]) and its effect on DNA damage are evaluated. The selective inactivation of tumor cells by the PAM is also analyzed, specifically comparing the effects of PAM on HCT116 human cancer colorectal cells and GM637 human fibroblast cells.

II. MATERIALS AND METHODS

The materials and methods used in this study have been detailed in previous publications.[20,21] Only the main steps and the biological procedure are described herein.

A. Helium Plasma Jet

A dielectric barrier discharge configuration was used to produce a helium plasma jet.[21] It comprises two aluminum electrodes (each 20 mm wide, separated by a 10-mm gap) wrapped around a quartz tube (inner diameter, 4 mm; outer diameter, 6 mm). Helium is injected through the tube at 3 Std. L/min using a mass flow controller. The electrical supply generates positive high-voltage square pulses with the following characteristics: 9-kV voltage, 9.69-kHz frequency, and 1-µs pulse duration. Detailed information about the present low-temperature plasma jet can be found elsewhere.[21]

B. Multicellular Tumor Spheroids

Spheroids were created using HCT116 colorectal cancer cells from ATCC or GM637 human fibroblast cells. Cells were cultured in a culture medium (Dulbecco's modified Eagle medium d5796; Invitrogen) supplemented with 10% fetal calf serum (FCS) and 2 mmol/L glutamine and penicillin/streptomycin in a controlled atmosphere (37°C and 5% CO_2) before centrifugation in low-attachment well plates [20]. A centrifugation method using 500 cells/well generates spheroids through cell aggregation.[22] Five days' growth of spheroids in an incubator are necessary to obtain a 400-µm diameter. In this configuration no necrotic core is observed in the spheroids in any of the measurements.

C. PAM Production and Spheroid Treatment

Use of PAM for cancer cell inactivation have been described in the literature (see, e.g., Refs. 6, 15, and 20). In this work, PAM was generated as follows: 100 µL of culture medium (d5796) in 96-well plates was exposed to the plasma jet at a distance of 2 cm

between the quartz tube output and the upper surface of the culture medium. After exposure, the culture medium is called PAM because of the presence of many active aqueous species. Duration of exposure to the plasma jet (t_{exp}) ranged from 60 to 240 seconds. HCT116 spheroids are treated by transferring 80 µL of PAM after a selected delayed transfer time (t_{trans}) ranging from 15 minutes to 48 hours. During t_{trans}—that is, between exposure and PAM transfer—the PAM were stored in an incubator at 37°C.

D. Growth Inhibition

A calibrated eyepiece reticle was used to measure the spheroids' diameter 9 days after transfer in PAM. Between the 2 measurements, spheroids were stored in a controlled atmosphere (37°C and 5% CO_2).

E. Detection of DNA Damage

According to our previous work,[20] DNA-damaged cells detach 5 hours after treatment; therefore, the spheroids were fixed 4 hours after treatment with PAM (just before the cells detach). Fixation was achieved by a 2-hour incubation in formalin (Sigma), followed by 2 hours of incubation in 15% sucrose and finally 24 hours in 30% sucrose. Sections (5 µm thick) were taken from along the entire thickness of the sample and deposited on glass slides before being placed in a vacuum at room temperature overnight. After a blocking step in phosphate-buffered saline (PBS), 10% FCS, and 0.5% Triton X-100, sections were incubated with primary antibodies. DNA damage was detected using primary antibodies against phosphorylated mouse monoclonal histone H2AX (Merck Millipore, Billerica, MA) conjugated with secondary Alexa 488 anti-mouse antibodies (Molecular Probes, Eugene, OR). Nuclei were stained using DAPI (4′,6-diamidino-2-phenylindole). The fluorescence of the cryosections was evaluated using an Epifluorescence DM5000 microscope (Leica) equipped with a charge-coupled device camera (Roper COOLsnap ES). Metavue and ImageJ software were used to process the fluorescence images.

F. Data Statistics

Data are shown as means ± standard deviations or the most relevant images from at least 4 independent experiments for each condition. The data were statistically evaluated using the Student *t*-test.

III. RESULTS

A. Exposure Time and Transfer Time are the Key Parameters in DNA Damage

To investigate the effect of exposure time on growth inhibition and DNA damage, spheroids were immersed in the culture media treated by the helium plasma jet for different exposure times (60–240 seconds). Figure 1 displays the evolution of spheroid volume as

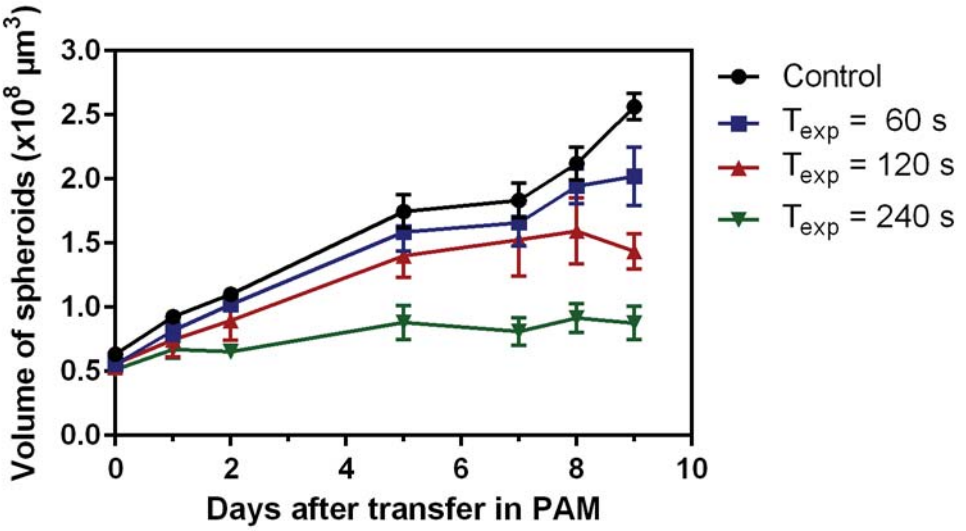

FIG. 1: Evolution of the volume of HCT116 spheroids after plasma-activated media (PAM) treatment for different exposures times (t_{exp} = 60, 120, and 240 seconds) and a 1-hour transfer time. Data are shown as the means ± standard deviations from 4 independent experiments.

a function of exposure time. Control spheroids, which were not exposed to PAM, grew as expected during the period being analyzed. The volume inhibition was dependent on the duration of exposure to the plasma jet and was detected 1 day after the transfer of the MCTSs in PAM. Weak growth inhibition was detected in the case of PAM obtained during the shortest exposure times (t_{exp} = 60 and 120 seconds) compared with the results obtained at the longest exposure time (t_{exp} = 240 seconds), after which an important growth inhibition effect was observed.

This growth inhibition can be correlated to the detachment of cells with DNA damage.[20] Figure 2 displays an example of spheroids cryosections for a 4-hour t_{trans} in PAM obtained with different exposure times (t_{exp} = 60, 120, and 240 seconds). DNA damage detected in the outermost region of HCT116 spheroids depended on how long PAM was exposed to the plasma jet. In the case of a t_{exp} of 60 seconds, only the first cell layer was damaged; deeper cell layers of the spheroid show DNA damage when PAM was exposed for 240 seconds. These results confirm that the concentration of aqueous active species produced by the plasma jet in the culture medium depends on the exposure time.

To quantify the effects of the exposure time and the transfer time on the MCTSs, DNA damage in the cryosections was analyzed using ImageJ software. The percentage of the spheroid presenting with DNA damage was estimated by the ratio of the volume of the spheroid without DNA damage (the portion stained using DAPI; center of images in Fig. 2) to the total volume of the spheroid (center of images and outer rings in Fig. 2).

Whatever the transfer time, the proportion of cells with DNA damage increases with exposure time (Fig. 3). For a t_{trans} less than 2 hours, the prevalence of DNA damage is

relatively constant and corresponds to about 19% for PAM obtained during 60 seconds of exposure and 32% at 120 seconds of exposure; it finally reached 50% of the volume of a spheroid at 240 seconds of exposure time. This rate of DNA damage is not directly proportional to the exposure time; the proportionality factor calculated by dividing the DNA damage by the exposure time decreases with t_{exp}. This ratio is equal to 0.32 for 60 seconds of exposure, 0.27 for 120 seconds, and 0.21 for 240 seconds. This factor indicates that it is more difficult to induce genotoxicity in the deeper layers of the spheroid, which is consistent with the measurement of oxygen penetration.[23] The natural structure of the spheroid makes it difficult for reactive species to penetrate inside the spheroid. To increase the rate of DNA damage, it is necessary to produce further genotoxic species in the culture medium, which therefore requires longer exposure.

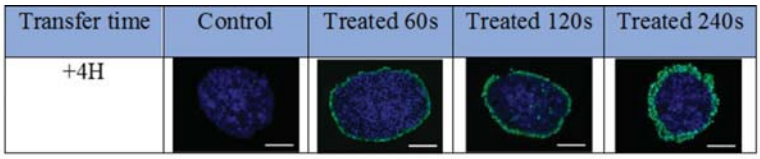

FIG. 2: DNA damage induced by plasma-activated media (PAM) obtained during 3 exposure times (t_{exp} = 60, 120, and 240 seconds) and 4 hours (+4H) after the transfer of multicellular tumor spheroid in PAM (t_{trans} = 4 hours). Nuclei are stained using DAPI (center), and DNA damage is stained by phosphorylation of the histone variant H2AX (outer ring). Displayed images are representative from 4 independent experiments. Scale bar, 100 μm.

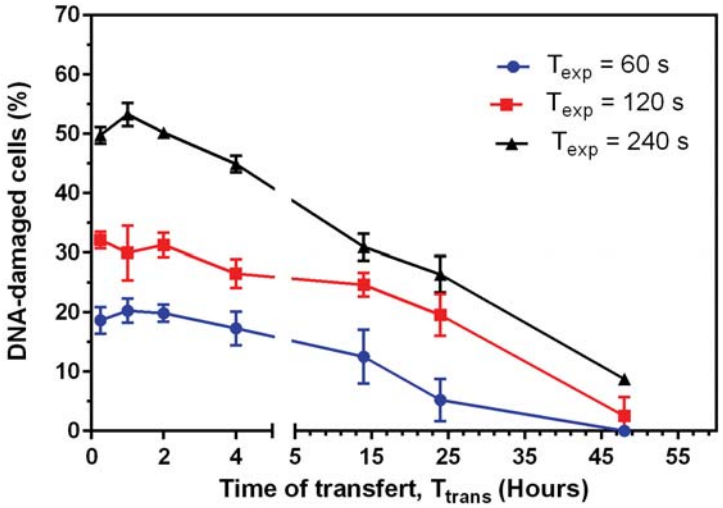

FIG. 3: Influence of transfer times (between 15 minutes and 48 hours) and exposures times (t_{exp} = 60, 120, and 240 seconds) on the percentage of cells in the multicellular tumor spheroid with DNA damage. Data are shown as the means ± standard deviations from 4 independent experiments.

The proportion of cells in an MCTS with DNA damage is also dependent on the t_{trans}. There is a decrease of the same order of magnitude for the curves corresponding to each t_{trans} (Fig. 3). This means more genotoxic species are produced during longer exposure times, which leads to a longer duration of the genotoxic and cytotoxic effects of PAM. In the case of a 48-h t_{trans}, no DNA damage occurs at t_{exp} = 60 seconds, and only 9% of the MCTS had DNA damaged with PAM exposed for 240 seconds.

Interactions between active species produced by the plasma jet and the culture medium generate various aqueous chemically active species including hydroxyl radicals (OH), H_2O_2, ozone (O_3), atomic oxygen (O), superoxide anions (O_2^-), nitric oxide (NO), and peroxynitrite anions ($ONOO^-$).[24] In this study we focused on H_2O_2 because it is one of the chemical species generally highlighted in the literature[25,26] and it has a long lifetime in culture media.[15]

The role of H_2O_2 in the DNA damage of MCTSs was studied by adding catalase (Sigma), which quenches H_2O_2.[27] For this analysis, 30 $\mu g \cdot mL^{-1}$ of catalase was added to both PAM-treated (t_{exp} = 120 seconds, t_{trans} = 15minutes) and control (untreated) culture media to analyze a possible direct effect of catalase on the spheroids. As shown in Fig. 4, during the first days the volume of the control and PAM-treated spheroids have the same kinetics. No growth inhibition is correlated with a lack of DNA damage in MCTSs in the presence of catalase in the culture medium (data not shown). However, a moderate growth inhibitory effect was observed for the first time 5 days after transfer in PAM (Fig. 4).

B. Assessment of H_2O_2 Concentration in PAM

From the analysis of cryosections obtained by adding different concentrations of exogenous H_2O_2 (Sigma) to the culture media without plasma treatment, it is possible to obtain a calibration curve indicating the concentration of H_2O_2 required in the culture medium to achieve a particular rate of DNA damage. This curve is shown in Fig. 5, which displays the proportion of DNA damage as a function of different concentrations of exogenous H_2O_2. As observed the deepest layers of the MCTS, it is difficult to access for H_2O_2 molecules in cells.

The concentration of H_2O_2 generated by the plasma in the culture medium can be estimated by comparing the proportion of DNA damage achieved using both PAM and the culture media involving exogenous H_2O_2(Fig. 5). Our results indicate that a 60-second exposure to the plasma jet yielded approximately 0.27 ± 0.09 mmol/L of H_2O_2 in culture medium. In addition, 120- and 240-second exposures to the plasma jet produced H_2O_2 concentrations of approximately 0.74 ± 0.19 and 3.16 ± 0.59 mmol/L, respectively. These results confirm that the increase in exposure time leads to an increase in the number of active species produced in culture media. To validate the H_2O_2 quantification method using DNA damage on MCTSs, H_2O_2 in PAM was evaluated with a Fluorimetric Hydrogen Peroxide Assay Kit (Sigma). This measurement indicates that a 60-second exposure to the plasma jet yielded approximately 0.41 ± 0.1 mmol/L of H_2O_2 in the culture medium**.** With a *P* value of 0.36, the difference between both methods is considered to be not statistically significant.

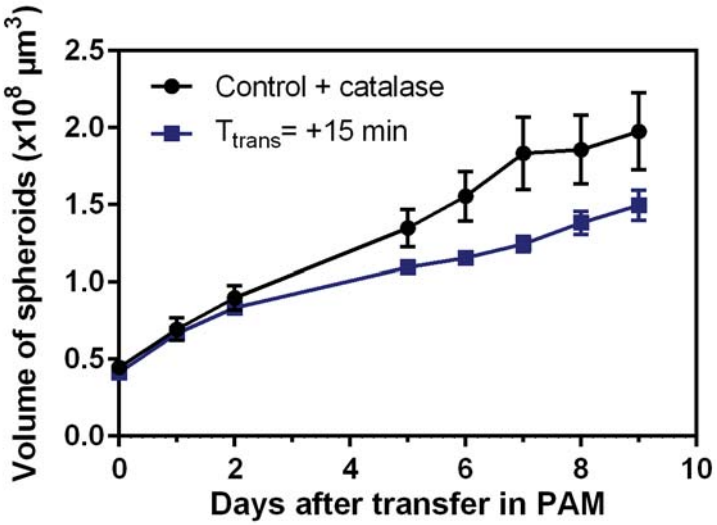

FIG. 4: Evolution of the volume of HCT116 cells after plasma-activated media (PAM) treatment including catalase (t_{exp} = 120 seconds). Data are shown as the means ± standard deviations from 4 independent experiments.

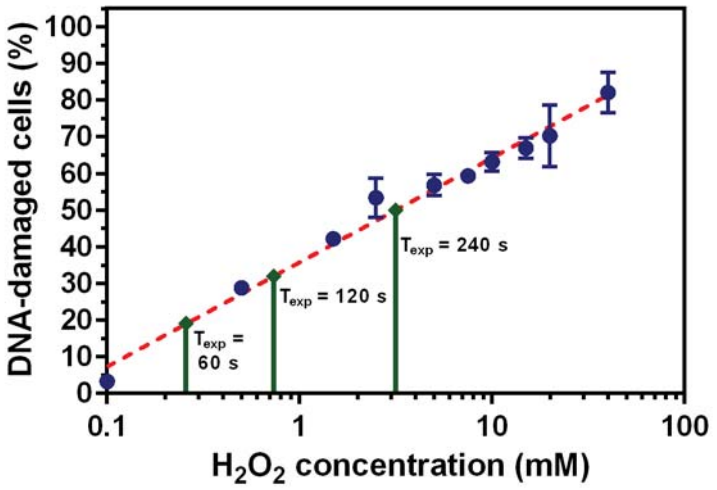

FIG. 5: Influence of hydrogen peroxide (H_2O_2) concentration in the culture media on the percentage of cells in the multicellular tumor spheroid with DNA damage. Data are shown as the means ± standard deviations from 4 independent experiments.

C. Selective DNA Damage by PAM on HCT 116 Spheroids Compared with Fibroblast GM637 Spheroids

We also analyzed the DNA damage in the case of healthy human fibroblast GM637 cells. The corresponding fibroblast spheroids (150 μm in diameter) were prepared in a similar way as the HCT116 spheroids, then immersed inside the PAM before being exposed to the helium plasma jet (t_{exp} = 120 seconds). The t_{trans} of GM637 spheroids in PAM varied between 15 minutes and 4 hours. The DNA damage induced by PAM treatment of fibroblast spheroids was then analyzed by immunodetection of the phosphorylated form of histone H2AX. Figure 6 displays the cryosections of the GM637 fibroblast spheroid. In all tested conditions, phosphorylated form of histone H2AX was detected, and weak staining indicated that no significant DNA damage was detected in the fibroblasts. These results mean that PAM does not induce any observable DNA damage to fibroblast GM637 spheroids, contrary to HCT116 tumor spheroids.

This interesting selective effect of PAM, which was previously observed in the literature (see, e.g., Ref. 28), may be the result of the significant difference in signaling networks between cancer cells and healthy cells. In addition, there is a threshold of H_2O_2 amount above which any cell cannot survive; cancer cells are sensitive to lower concentrations of H_2O_2 than healthy cells.[29] Therefore, low-temperature plasma induces a therapeutic concentration of H_2O_2 in PAM, which can cause the selective death of cancer cells without affecting healthy cells.

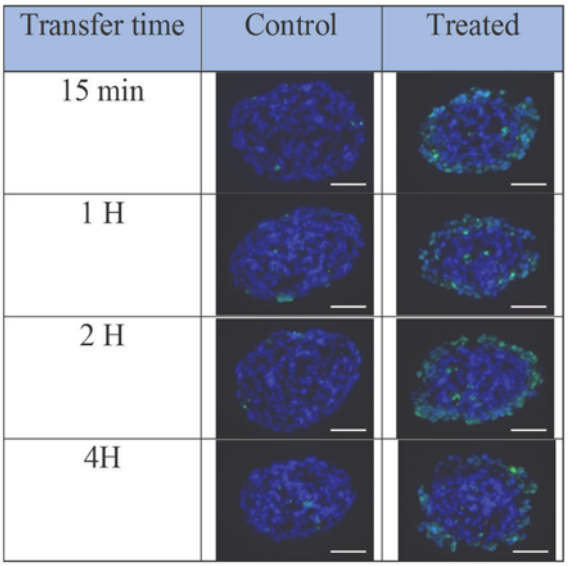

FIG. 6: The genotoxic effect of plasma-activated media (PAM) on healthy (or nontumor) spheroids detected 4 hours after transfer time by immunodetection of the phosphorylation of the histone variant H2AX (lightest patches) (t_{exp} = 120 seconds). Nuclei were stained using DAPI (darker patches). Displayed images are representative of 4 independent experiments. Scale bar, 50 μm.

IV. CONCLUSIONS

We confirm here that media exposed to a low-temperature plasma jet have genotoxic and cytotoxic effects on the immersed spheroids of cancer cells (MCTSs). We also demonstrated that the observed DNA damage in and growth inhibition of the MCTSs are dependent on both the exposure time needed to generate the PAM and the transfer time. A single treatment with PAM (t_{exp} = 240 seconds) total inhibits the growth of spheroids over 9 days, with a rate of DNA damage of about 50%; this damage is localized in the outermost layers of the MCTS volume. During the first days after treatment, catalase in the culture medium inhibits all DNA damage and primary growth inhibition. This shows that endogenous H_2O_2 produced in PAM plays an important role in MCTS DNA damage and initial growth inhibition. However, the late growth inhibitory effects in the presence of catalase (5 days after the treatment) are likely to be associated with other active, long-lived aqueous plasma species. The latter are formed during the generation of PAM after exposure to the plasma jet. Such actives species can directly or indirectly affect spheroid growth following reactions with amino acids.[30]

In comparison to a liquid involving only H_2O_2, PAM has shown clear action on cancer cell inactivation; PAM involves not only H_2O_2 but also other long-lived aqueous plasma by-products that play an antiproliferative role.

In addition, analysis of DNA damage caused by exogenous H_2O_2 in the culture medium without plasma treatment was used to estimate the proportion of H_2O_2 in the PAM after different durations of exposure to the plasma jet. This was in good accordance with the results of the H_2O_2 Fluorimetric Assay Kit.

PAM has also an interesting selective effect in that DNA damage was observed on MCTSs, but not in the case of the fibroblast spheroids treated with PAM under the same conditions. Finally, because the pharmacologic concentration involves H_2O_2 in particular, as well as other active plasma species that are able to induce DNA damage and inhibit the growth of cancer cells, but not healthy cells, PAM can be considered potential novel and effective tools for cancer therapy.

ACKNOWLEDGMENTS

The authors are grateful for their biologist colleagues B. Ducommun and V. Lobjois, from ITAV (University of Toulouse), for fruitful discussions and for access to the different experimental tools available in their laboratory, which were used for the biological analyses.

REFERENCES

1. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. Ca Cancer J Clin. 2014;64(1):9–29.
2. Institut national du cancer (INCa). Les cancers en France. Boulogne-Billancourt, France: INCa; 2014.
3. Scottish Intercollegiate Guidelines Network (SIGN). Diagnosis and management of colorectal cancer. Edinburgh: SIGN; 2011.
4. Frank RE, Saclarides TJ, Leurgans S, Speziale NJ, Drab EA, Rubin DB. Tumor angiogenesis

as a predictor of recurrence and survival in patients with node-negative colon cancer. Ann Surg. 1995;222(6):695–9.
5. Keidar M. Plasma for cancer treatment. Plasma Sources Sci Technol. 2015;24(3):033001
6. Utsumi F, Kajiyama H, Nakamura K, Tanaka H, Mizuno M, Ishikawa K, Kondo H, Kano H, Hori M, Kikkawa F. Effect of indirect nonequilibrium atmospheric pressure plasma on anti-proliferative activity against chronic chemo-resistant ovarian cancer cells in vitro and in vivo. PloS One. 2013;18:e81576.
7. Chang JW, Kang SU, Shin YS, Kim KI, Seo SJ, Yang SS, Lee J-S, Moon E, Baek SJ, Lee K, Kim CH. Non-thermal atmospheric pressure plasma induces apoptosis in oral cavity squamous cell carcinoma: involvement of DNA-damage-triggering sub-G1 arrest via the ATM/p53 pathway. Arch Biochem Biophys. 2014;545:133–40 .
8. Hirst AM, Simms MS, Mann VM, Maitland NJ, O'Connell D, Frame FM. Low-temperature plasma treatment induces DNA damage leading to necrotic cell death in primary prostate epithelial cells. Br J Cancer. 2015;112(9):1536–45.
9. Keidar M, Shashurin A, Volotskova O, Stepp MA, Srinivasan P, Sandler A, Trink B. Cold atmospheric plasma in cancer therapy. Phys Plasmas. 2013;20:057101.
10. Gibson AR, McCarthy HO, Ahalm AA, O'Connell D, Graham WG. Interactions of a non-thermal atmospheric pressure plasma effluent with PC-3 prostate cancer cells. Plasma Process Polym. 2014;11(12):1142–9.
11. Ishaq M, Evans MD, Ostrikov KK. Atmospheric pressure gas plasma-induced colorectal cancer cell death is mediated by Nox2-ASK1 apoptosis pathways and oxidative stress is mitigated by Srx-Nrf2 anti-oxidant system. Biochim Biophys Acta. 2014;1843(12):2827–37.
12. Volotskova O, Hawley TS, Stepp MA, Keidar M. Targeting the cancer cell cycle by cold atmospheric plasma. Sci Rep. 2012;2:636.
13. Gherardi M, Turrini E, Laurita R, De Gianni E, Ferruzzi L, Liguori A, Stancampiano A, Colombo V, Fimognari C. Atmospheric non-equilibrium plasma promotes cell death and cell-cycle arrest in a lymphoma cell line. Plasma Process Polym. 2015;12(12):1354–63.
14. Kaushik N, Uddin N, Sim GB, Hong YJ, Baik KY, Kim CH, Lee SJ, Kaushik NK, Choi EH. Responses of solid tumor cells in DMEM to reactive oxygen species generated by non-thermal plasma and chemically induced ROS systems. Sci Rep. 2015;5:8587.
15. Adachi T, Tanaka H, Nonomura S, Hara H, Kondo SI, Hori M. Plasma-activated medium induces A549 cell injury via a spiral apoptotic cascade involving the mitochondrial-nuclear network. Free Rad Biol Med. 2014;79:28–44.
16. Walk RM, Snyder JA, Srinivasan P, Kirsch J, Diaz SO, Blanco FC, Shashurin A, Keidar M, Sandler AD. Cold atmospheric plasma for the ablative treatment of neuroblastoma. J Pediatr Surg. 2013;48(1): 67–73.
17. Hattori N, Yamada S, Torii K, Takeda S, Nakamura K, Tanaka H, Kajiyama H, Kanda M, Fujii T, Nakayama G, Sugimoto H, Koike M, Nomoto S, Fujiwara M, Mizuno M, Hori M, Kodera Y. Effectiveness of plasma treatment on pancreatic cancer cells. Int J Oncol. 2015;47(5):1655–62.
18. Vandamme M, Robert E, Lerondel S, Sarron V, Ries D, Dozias S, Sobilo J, Gosset D, Kieda C, Legrain B, Pouvesle JM, Pape AL. ROS implication in a new antitumor strategy based on non-thermal plasma. Int J Cancer. 2012;130(9):2185–94.
19. Kang SU, Cho JH, Chang JW, Shin YS, Kim KI, Park JK, Yang SS, Lee JS, Moon E, Lee K, Kim CH. Nonthermal plasma induces head and neck cancer cell death: the potential involvement of mitogen-activated protein kinase-dependent mitochondrial reactive oxygen species. Cell Death Dis. 2014;5:e1056.
20. Plewa J, Yousfi M, Frongia C, Eichwald O, Ducommun B, Merbahi N, Lobjois V. Low temperature plasma-induced antiproliferative effects on multicellular tumor spheroid. New J Phys. 2014;16:043027.
21. Yousfi M, Eichwald O, Merbahi N, Jomaa N. Analysis of ionization wave dynamics in low-temperature plasma jets from fluid modeling supported by experimental investigations. Plasma Sources Sci Technol. 2012;21:045003.

22. Laurent J, Frongia, C, Cazales M, Mondésert O, Ducommun B, Lobjois V. Multicellular tumor spheroid models to explore cell cycle checkpoints in 3D. BMC Cancer. 2013;13:73.
23. Hirschhaeuser F, Menne H, Dittfeld C, West J, Mueller-Klieser W, Kunz-Schughart LA. Multicellular tumor spheroids: an underestimated tool is catching up again. J Biotechnol. 2010;148(1):3–15.
24. Graves DB. The emerging role of reactive oxygen and nitrogen species in redox biology and some implications for plasma applications to medicine and biology. J Phys D Appl Phys. 2012;45:263001.
25. Xu D, Liu D, Wang B, Chen C, Chen Z, Li D, Yang Y, Chen H, Kong, MG. In situ OH generation from O2− and H2O2 plays a critical role in plasma-induced cell death. PloS One. 2015;10(6):e0128205.
26. Judée F, Wattieaux G, Merbahi N, Mansour M, Castanié-Cornet MP. The antibacterial activity of a microwave argon plasma jet at atmospheric pressure relies mainly on UV-cradiations. J Phys D Appl Phys. 2014;47:405201.
27. Chelikani P, Fita I, Loewen PC. Diversity of structures and properties among catalases. Cell Mol Life Sci. 2004;61(2):192–208.
28. Keidar M, Walk R, Shashurin A, Srinivasan P, Sandler, Dasgupta S, Ravi R, Guerrero-Preston R, Trink B. Cold plasma selectivity and possibility of a paradigm shift in cancer therapy. Br J Cancer. 2011;105(9):1295–301.
29. López-Lázaro M. Dual role of hydrogen peroxide in cancer: possible relevance to cancer chemoprevention and therapy. Cancer Lett. 2007;252(1):1–8.
30. Takai E, Kitamura T, Kuwabara J, Ikawa S, Yoshizawa S, Shiraki K, Kawasaki H, Arakawa R, Kitano K. Chemical modification of amino acids by atmospheric-pressure cold plasma in aqueous solution. J Phys D Appl Phys. 2014;47(28):285403.