On the Mechanism of Plasma Inducing Cell Apoptosis

Article *in* IEEE Transactions on Plasma Science · September 2010

DOI: 10.1109/TPS.2010.2056393

11 authors, including:

Xu Yan
Beijing Neurosurgical Institute
21 PUBLICATIONS **336** CITATIONS

SEE PROFILE

Xinpei Lu
Huazhong University of Science and Technology
170 PUBLICATIONS **6,465** CITATIONS

SEE PROFILE

Guangyuan He
Huazhong University of Science and Technology
230 PUBLICATIONS **3,001** CITATIONS

SEE PROFILE

Zilan Xiong
University of California, Berkeley
32 PUBLICATIONS **759** CITATIONS

SEE PROFILE

On the Mechanism of Plasma Inducing Cell Apoptosis

Xu Yan, Fei Zou, Shasha Zhao, XinPei Lu, *Senior Member, IEEE*, Guangyuan He, Zilan Xiong, Qing Xiong, Qiangqiang Zhao, Pengyi Deng, Jianguo Huang, and Guangxiao Yang

***Abstract*—A cold atmospheric-pressure plasma, which has been widely used for biomedical applications, may potentially affect the cell cycle and cause cell apoptosis. In this paper, a human hepatocellular carcinoma cell (HepG2) is treated by a single-electrode plasma jet device. Further investigation by using flow cytometric analysis demonstrates that plasma treatment increases the percentage of apoptotic cells being associated with cell cycle arrest at the G2/M phase. Moreover, the reverse transcription polymerase chain reaction assay shows that the cyclin B1 and Cdc2 are decreased at the transcription level after plasma treatment, while the expression of the p21 Cdk inhibitor, as well as that of tumor suppressor p53, is enhanced. On the other hand, the levels of certain pro- or antiapoptotic genes are checked, and the experimental results suggest that the plasma induces apoptosis by shifting the Bax/Bcl-2 ratio to trigger HepG2 cell apoptosis. The results of the present investigation indicate that the plasma jet device may have potential therapeutic activities, such as sterilization of living tissue and tumor therapy of the postoperative treatment process.**

***Index Terms*—Atmospheric-pressure nonequilibrium plasmas (APNPs), cell apoptosis, cell cycle, flow cytometry, plasma jet.**

I. Introduction

CELL death can either be necrotic or apoptotic. Necrosis is typically described as a nonspecific form of cell death [1]. Apoptosis, or programmed cell death, is an active form of cell death that plays a crucial role in the normal development and differentiation of multicellular organisms. It is characterized by a highly stereotypical series of morphological and biological changes, such as cytoplasmic shrinkage, chromatin condensation, and DNA degradation [2].

Manuscript received May 5, 2010; revised June 18, 2010; accepted June 19, 2010. Date of publication July 23, 2010; date of current version September 10, 2010. This work was supported in part by the National Natural Science Foundation of China under Grant 10875048, by the Chang Jiang Scholars Program, by the Ministry of Education of China, and by the Genetically Modified New Varieties of Major Projects of China under Grants 2008ZX08002-004 and 2009ZX08002-013B.

X. Yan, S. Zhao, G. He, Q. Zhao, P. Deng, J. Huang, and G. Yang are with The Genetic Engineering International Cooperation Base of Ministry of Science and Technology, Key Laboratory of Molecular Biophysics of the Ministry of Education, China–U.K. HUST–RRes Genetic Engineering and Genomics Joint Laboratory, College of Life Science and Technology, Huazhong University of Science and Technology (HUST), Wuhan 430074, China (e-mail: hegy@hust.edu.cn).

F. Zou, X. Lu, Z. Xiong, and Q. Xiong are with the College of Electrical and Electronic Engineering, HUST, Wuhan 430074, China (e-mail: luxinpei@hotmail.com).

Color versions of one or more of the figures in this paper are available online at http://ieeexplore.ieee.org.

Digital Object Identifier 10.1109/TPS.2010.2056393

A cell cycle is composed of a tightly regulated sequence of events, whose main purpose is to ensure that the genomic material is ready to be replicated, faithfully copied, and properly segregated into daughter cells. The integrity of genomic DNA is continually monitored, and DNA repair is coordinated with the cell cycle via the G1/S, intra-S phase, and G2/M checkpoints [3].

Liver cancer is the most frequent cause of cancer-related deaths in Asia and is currently being treated with a wide variety of drugs [4]. These drugs may inhibit the growth of tumor cells, induce apoptosis *in vitro* and *in vivo* in a variety of cells, and act as antiproliferative agents. However, severe side effects for the therapeutic treatment (chemotherapy and radiotherapy) of patients suffering from cancer would appear simultaneously.

In recent years, atmospheric-pressure nonequilibrium plasmas (APNPs) have been used in many biomedical applications, such as bacterium, yeast, fungus, and alga inactivation; surface treatment for cell sheet engineering to controlled cell cultivation; blood coagulation promotion; and tooth root canal sterilization [5]–[22]. APNPs can operate at room temperature, and they can form numerous short-lived but highly active chemical particles, such as reactive oxygen species (O and O_3), hydroxyl radical (OH), and other excited species (N_2^+, NO, etc.). These active chemical particles are essential for various biological processes in cells and human body; reactive oxygen species are involved in the initiation, progression, and metastasis of cancers; and nitric oxide (NO) can induce apoptosis and necrosis or protect cells from death, depending on the cell type and the amount, duration, and site of NO production. Therefore, it is important to study the impact of plasma on cancer cells [23]–[29]. Recently, Stoffels *et al.* [14] and Fridman *et al.* [16] found that APNPs can induce the cell apoptosis process. However, the mechanism of APNPs inducing cell apoptosis is not well understood. In this paper, the mechanism of cancer cell apoptosis induced by APNPs is investigated. It is found that the plasma induces apoptosis through shifting the Bax/Bcl-2 ratio to trigger HepG2 cell apoptosis.

II. Experimental Setup

A. Cell Culture and In Vitro Plasma Treatment

A specially designed single-electrode plasma jet device is used to generate the nonequilibrium plasma plume. A high-voltage (HV) wire electrode, which is made of a copper wire with a diameter of 2 mm, is inserted into a 4-cm-long quartz tube with one end closed. The inner and outer diameters of

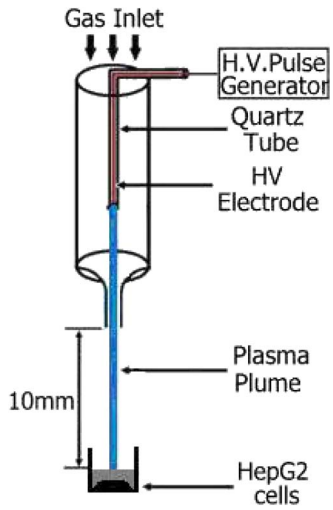


Fig. 1. Schematic of the experimental setup.

the quartz tube are 2 and 4 mm, respectively. The quartz tube, along with the HV electrode, is inserted into a hollow barrel of a syringe. The diameter of the hollow barrel is about 6 mm, and the diameter of the syringe nozzle is about 1.2 mm. The distance between the tip of the HV electrode and the nozzle is 1 cm. When helium with a flow rate of 2 L/min is injected into the hollow barrel and the HV pulsed dc voltage (amplitudes of up to 10 kV, repetition rate of up to 10 kHz, and pulsewidth variable from 200 ns to dc) is applied to the HV electrodes, a homogeneous plasma is generated in front of the end of the quartz tube, along the nozzle, and in the surrounding air. The length of the plasma plume can be adjusted by the gas flow rate and the applied voltage (amplitude, frequency, and pulsewidth). A detailed description of the experimental setup can be found in [17]. The schematic of the device is shown in Fig. 1. For all the experiments reported in this paper, a pulse frequency of 8 kHz, a pulsewidth $t_{\rm pw}$ of 1.6 μs, and an applied voltage of 8 kV are fixed.

Cells are maintained in high-glucose Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% (v/v) fetal calf serum (FCS) at 37 °C in a humidified atmosphere containing 5% (v/v) CO_2.

The HepG2 cells are grown in a six-well plate at a concentration of 1×10^5 cells per well. The cells are allowed to attach for 12 h. Before plasma treatment, the medium is changed to 800-μL phosphate-buffered saline (PBS). Five wells of the six-well plate are placed right under the nozzle for plasma treatment, and one well is left for control. Plasma treatment is performed for one well at a time. Five plasma treatment durations of 120, 240, 480, 720, and 960 s are used. The distance between the nozzle and the surface of PBS is fixed to 10 mm. The working gas He/O_2 (1%) with a flow rate of 1 L/min is used. The experiment is carried out at room temperature. Immediately after treatment, the PBS of the wells is removed, and 1-mL DMEM, supplemented with 10% (v/v) FCS, is added to the dishes and returned to the CO_2 incubator. The control cells are subjected to an identical procedure without plasma treatment. The cells are harvested at the indicated time points.

B. Flow Cytometric Analysis

The treated cells are continuously cultured for 24 and 48 h at the same condition. Then, the cells are collected and fixed in 75% (v/v) ethanol for 1 h at 4 °C when cultured to the indicated time. The cells are washed once with PBS and resuspended in cold propidium iodide solution (50 μg/mL), a commonly used fluorescent dye, containing RNase A (0.1 mg/mL) in PBS (pH 7.4) for 30 min in the dark, and cell cycle phase analysis is performed by flow cytometry (BDLSR II) and analyzed by BD FACSDiva software. The DNA contents of the cells are stained with propidium iodide (PI). Cell cycle phase analysis is performed on the flow cytometer by analyzing the DNA contents with the fluorescence-activated cell sorting (FACS) technique. The number and the percentage of cells in each cell cycle are analyzed and calculated by the software.

C. RT-PCR of mRNA for Cycle- and Apoptosis-Related Genes

Genes in cells with different cell cycle or status are selectively transcribed into mRNA and then translated into protein to perform different biological functions. Reverse transcription polymerase chain reaction (RT-PCR) is the most sensitive technique currently available for mRNA detection and quantization. In RT-PCR, however, an RNA strand is first isolated and reversely transcribed into its DNA complement (cDNA) using the enzyme RT. The cDNA can be used as the template for PCR amplification of a particular gene relied on the specific primers for the gene. The PCR products are analyzed and imaged by agarose gel electrophoresis, which can separate DNA fragments of different lengths. The band in the agarose gel electrophoresis image indicates the DNA fragments amplified by the PCR reaction with specific length. The brightness of the band reflects the quantity of the PCR products.

In this paper, the HepG2 cells are treated as mentioned earlier. The total RNA is isolated by a TRIZOL method (Life Technologies, GibcoBRL) according to the manufacturer's protocol and quantitated by a spectrophotometer (U0080D, HITACHI). After preparing the cDNA from the extracted RNA using oligo d(T) [16] as an RT primer, PCR amplification is performed using a GeneAmp Kit (PerkinElmer, MA) according to the manufacturer's instructions with the indicated primers shown in Table I. The amplification conditions are 94 °C (60 s), annealing (60 s), and 72 °C (60 s) for 30 cycles. The PCR products are analyzed by electrophoresis (Bio-Rad Gel Doc 2000) on 1% agarose gel stained with ethidium bromide.

III. Experimental Results

A. G2/M Arrest of the Cell Cycle by Plasma

Flow cytometry enables the identification of cell distribution during the various phases of the cell cycle. Four distinct phases can be recognized in a proliferating cell population: G1, S (DNA synthesis phase), G2, and M phase (mitosis). Thus, the DNA content of the G2 phase doubles that of the G1 phase. However, the G2 and M phases have an identical DNA content, and they could not be discriminated based on their differences in DNA content. Furthermore, the M phase is relatively very

TABLE I
SEQUENCES OF THE PRIMERS USED FOR RT-PCR

Name	Sequence of primers		Amplicon length(bp)
GAPDH (NM_002046.3)	sense	5'-cttctgagtggcagtgatgg-3'	457
	antisense	5'-tggcacagtcaaggctgaga-3'	
CDC2 (NM_ 001786.3)	sense	5'-cttatgcaggattccaggtt -3'	380
	antisense	5'-ggtgcctatactccaaatgtc-3'	
CyclinB1 (NM_031966.2)	sense	5'-cagtcagaccaaaatacctactgggt-3'	190
	antisense	5'-acaccaaccagctgcagcatcttctt-3'	
P21 (NM_000389.3)	sense	5'-ctcagaggaggcgccatg-3'	517
	antisense	5'-gggcggattagggcttcc-3'	
P53 (NM_000546.4)	sense	5'-gctctgactgtaccaccatcc-3'	413
	antisense	5'-ctctcggaacatctcgaagcg-3'	
Bcl-2 (NM_000633.2)	sense	5'-tcgccctgtggatgactgag-3'	143
	antisense	5'-cagagtcttcagagacagccagga-3'	
Bax (NM_138763.3)	sense	5'-gggctggacattggacttcc-3'	152
	antisense	5'-agatggtgagtgaggcggtg-3'	

brief, so it is often written as G2/M phase in biology. Thus, a one-parameter histogram displays the distribution of cell contents or, in other words, how many cells contain a given quantity of DNA. In it, such cell content is assigned to one of the many classes or channels and is represented on the x-axis, whereas the number of cells being assigned to a given channel is referred to as channel content or simply count and is shown on the y-axis. All cells having about equal quantities of the cell content, e.g., DNA, form a peak. For a typical DNA histogram, one peak represents the G1, and another (with twice the channel value) represents the G2/M phase of the cell cycle.

The cell cycle analysis of HepG2 cells treated with plasma is shown in Figs. 2 and 3. An accumulation of cells in G2/M is observed after 240 s of treatment and 24 h of culture [Fig. 2(b)]. When the cells are treated for 480 s and cultured for 24 h, the distribution of the G2/M phase cells in the population increased from 13.4% [Fig. 2(a)] to 49.6% [Fig. 2(c)], and the apoptotic cells appear [Fig. 2(c)]. When the cells are incubated for 48 h, more apoptotic cells are observed, and the percentage rose to 33.35% [Fig. 2(f)]. It is evident that the G2/M phase became the dominant phase in cells treated with plasma in a time-dependent manner with late apoptosis. These findings indicate that, at the ranges of the time of treatment studied, the antiproliferative effect of plasma on HepG2 cells could be attributed primarily to the induction of G2/M arrest, with less contribution to the induction of apoptosis.

B. Effects of Plasma Treatment on the Levels of Cell Cycle Regulatory Genes in HepG2 Cells

The cell cycle is mediated by the activation of a highly conserved family of protein kinases, i.e., the cyclin-dependent kinases (Cdks). Activation of a Cdk requires binding to a specific regulatory submit, termed a cyclin. Together, these cyclin/Cdk complexes are the cell cycle regulators. The entry into the M phase is under the control of B-type cyclins, which also associate with Cdc2. Regulation of cyclin B1/Cdc2 complexes at multiple levels ensures tight control of the timing of mitotic entry and cell division. Without synthesis of cyclin B1 prior to G2–M transition, Cdc2 remains inactive, the cell cannot enter mitosis, and the cell cycle will arrest at the G2 phase. P21 is known to inhibit the activities of many cyclin/Cdk complexes. The common pathway reported is that p53 upregulating p21 resulted in cyclin B1/Cdc2 complex decrease, and then, G2/M phase arrest happened. Constitutively expressed housekeeping genes, such as GAPDH, are often used as internal standards for RT-PCR analysis.

In an attempt to understand the effect of molecular events involved in cell cycle progression, we next investigate the effect of plasma on the expression of genes that are pivotal for G2/M transition, including Cdc2, cyclin B1, and P21 [Fig. 4(a)]. Fig. 4 is part of the agarose gel electrophoresis image. The PCR products of a specific gene can be analyzed and imaged by agarose gel electrophoresis and be separated by their different lengths. The intense whiter band in the agarose gel electrophoresis image indicates the DNA fragments amplified by the PCR reaction with specific length. The brightness of the band reflects the quantity of the PCR products. The mRNA levels of cyclin B1 and Cdc2 are dose-dependently downregulated by plasma treatment, while those of P21, a potent inhibitor of cell cycle kinase, are markedly upregulated in plasma-treated HepG2 cells.

C. Alteration of P53 Gene and BCL-2 Family by Plasma Treatment in HepG2 Cells

To further elucidate the mechanisms of cell cycle arrest and apoptosis induced by plasma in HepG2 cells, the expression level of related genes in cells treated with plasma was

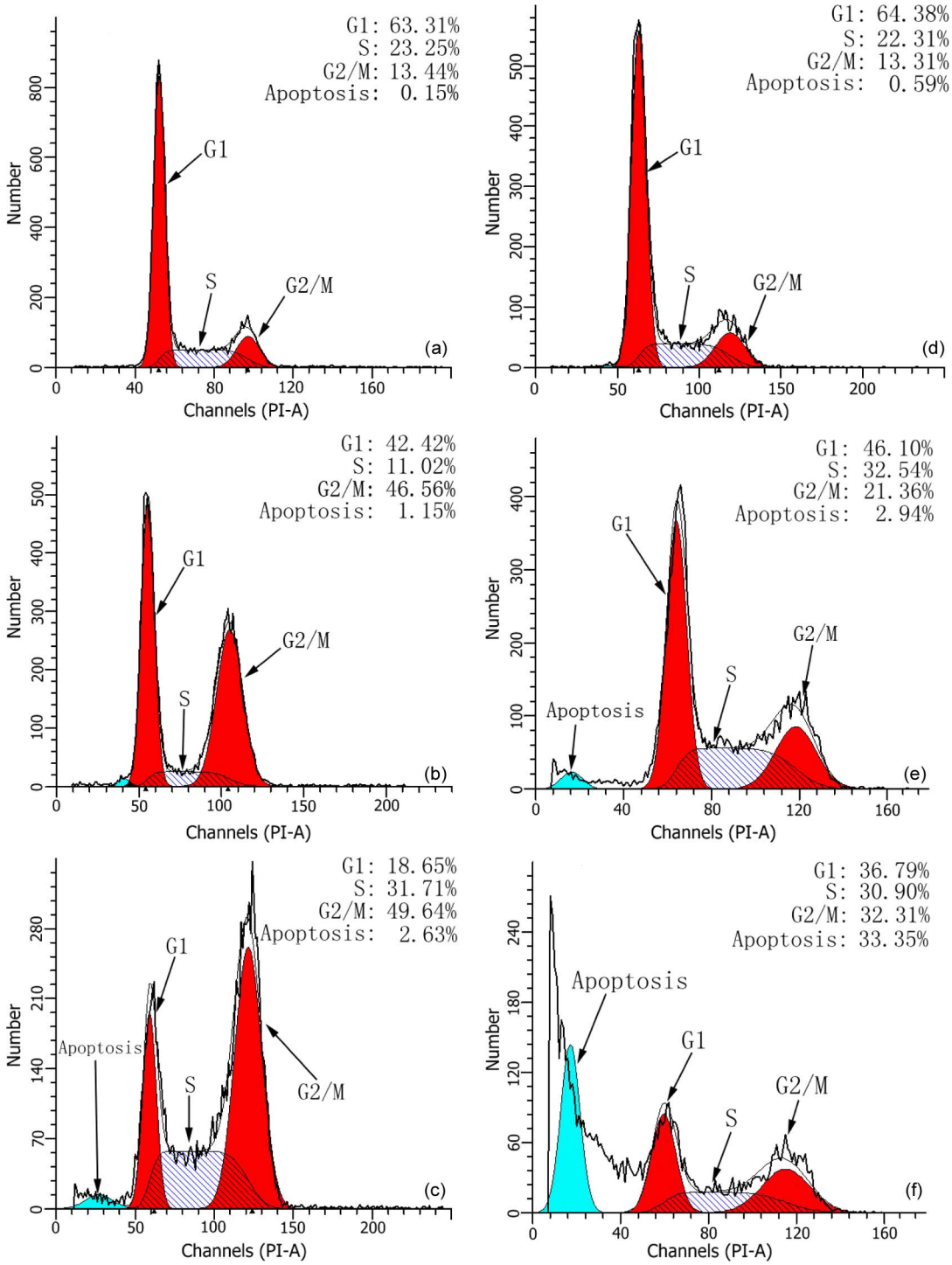


Fig. 2. Cellular DNA content histograms showing the cell cycle phase distribution and apoptosis of HepG2 cells following plasma treatments. The results from one of the three independent experiments that produced similar results are shown. (a–c) Cells were treated by plasma for 0, 240, and 480 s, respectively, and then cultured for 24 h. (d–f) Cells were treated by plasma for 0, 240, and 480 s, respectively, and then cultured for 48 h.

investigated [Fig. 4(b)]. The tumor suppressor gene P53 is known to be a member of the DNA damage-response pathway. It has been proved that the P53 protein increases at the early stages of cellular damage in response to a variety of stress-inducing agents [30]. The P53 gene was analyzed by RT-PCR after plasma treatments, which showed that the plasma could induce an upregulation of P53.

The Bcl-2/Bax family is critical for cell survival and cell apoptosis. The expression of Bcl-2 can block some gene products, which are induced by some apoptosis-inducing factors, such as ionizing radiations. Therefore, the trend of cell apoptosis is reduced. On the contrary, the upregulation of Bax gene expression promotes cell apoptosis significantly. Therefore, the decrease of the Bcl-2/Bax ratio can accelerate cell apoptosis,

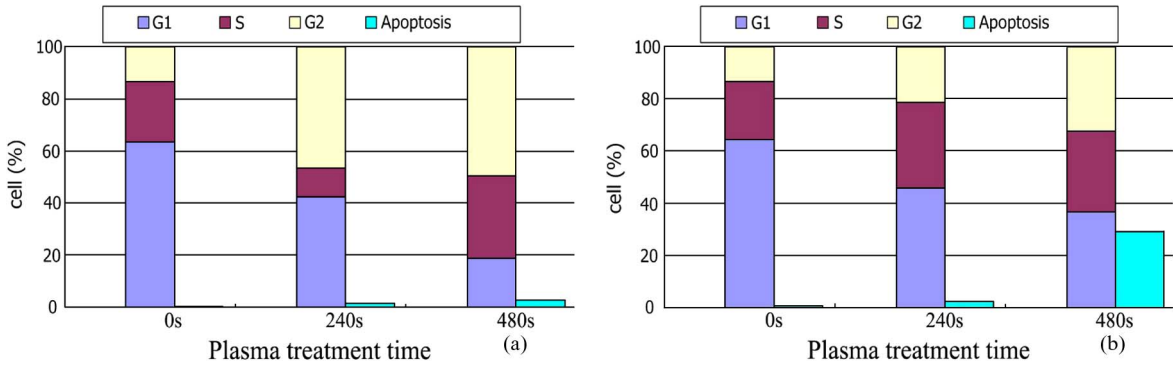


Fig. 3. Analysis of the cell cycle of HepG2 cells by FACS after plasma treatment and incubation for different periods. All cells were classified into apoptotic and cycling cells, and then, cell cycle analysis was carried out in the cycling cells. The cell distributions at each phase of the cell cycle were listed. (a) Cultured for 24 h after plasma treatment. (b) Cultured for 48 h after plasma treatment.

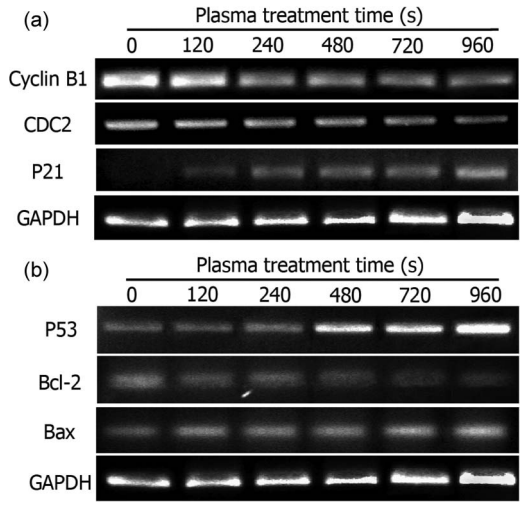


Fig. 4. Effect of plasma on the mRNA expression of (a) cell-cycle-related genes and (b) apoptosis-related genes in HepG2 cells. Cells were treated with plasma for 0, 120, 240, 480, 720, and 960 s and cultured for 48 h and then harvested. The total RNA was extracted, and then, the cDNA was synthesized from the total RNA, which was subjected to PCR with the indicated primers. The reaction products were subjected to electrophoresis in 1% agarose gel and visualized by ethidium bromide staining. GAPDH was used as the internal control.

while the increase of the Bcl-2/Bax ratio will inhibit apoptosis of the cells.

Since a plasma could also induce HepG2 cell apoptosis, the levels of certain pro- or antiapoptotic molecules were checked, and their levels correlated well with the apoptotic trend of HepG2 cells induced by treatment with plasma. The result showed that the expression level of antiapoptotic molecules, such as Bcl-2, decreased gradually, whereas the level of a proapoptotic molecule, namely, Bax, which opposes the action of Bcl-2, was increased by treatment with plasma in a dose-dependent manner.

IV. Discussion and Conclusion

Shi *et al.* [15] found that APNP treatment can increase the percentage of apoptosis in lymphocytes cells, and further substantiated it by flow cytometric analysis after the annexin V-FITC/propidium iodide (PI) staining. In the present studies, we have observed that plasma treatment *in vitro* increases the percentage of apoptotic cells actually associated with cell cycle arrest at the G2/M phase. Plasma treatment does not cause immediate necrosis but initiates complex cascade of biochemical processes, leading to cell death many hours following plasma treatment. These observations also suggest the complexity of intracellular events after plasma treatment.

To further analyze the molecular mechanism by which plasma treatment causes the cell cycle arrest, we have evaluated cycle- and apoptosis-related genes at the transcription level. Cyclin B1, together with Cdc2 kinase, creates a well-known complex, which is one of the major regulatory elements governing the G2 to M progression [31]. In this paper, we have found that cyclin B1 and Cdc2 are decreased at the transcription level by semiquantitative RT-PCR assay, suggesting that the plasma might exert a certain effect on the cyclin B1/Cdc2 complex. This suggests that the plasma arrests the cells at the G2 phase, delaying them to enter the M phase, by the limitation of the mRNA of cyclin B1 and Cdc2 to the cyclin B1/Cdc2 complex formation. The activity of Cdks is negatively regulated by binding to Cdk inhibitors in response to a variety of stimuli.

P21 is an important mediator of cell cycle arrest imposed by the p53 tumor suppressor in response to DNA damage [32]. The present results have clearly indicated that plasma treatment enhances the expression of the p21 Cdk inhibitor, as well as p53, at the transcription level, suggesting that the Cdk inhibitor, namely, p21, also plays an important role in the maintenance of G2/M cell cycle arrest.

Apoptosis is a genetically regulated biological process with two major pathways: the death-receptor-induced extrinsic pathway and the mitochondria-apoptosome-mediated apoptotic intrinsic pathway [33]. The Bcl-2 family has a central role in controlling the mitochondrial pathway. The pro- and antiapoptotic proteins of the Bcl-2 family may turn apoptosis on and off because of the formation of heterodimers among these proteins. The data have demonstrated that the level of Bax is increased, and that of Bcl-2 is concomitantly decreased in plasma-treated HepG2 cells, which suggests that the plasma induced apoptosis through shifting the Bax/Bcl-2 ratio in favor of apoptosis.

In summary, we have demonstrated that plasma treatment *in vitro* has been shown to increase the percentage of apoptotic cells actually associated with cell cycle arrest at the G2/M phase in a dose-dependent manner, probably by modulating the cyclin B1 and Cdc2 levels, and this modulation is associated with the

induction of p21. Furthermore, the plasma also induce concomitant apoptosis by increasing the Bax/Bcl-2 ratios, which is accompanied by the upregulation of the p53 tumor suppressor. Although more study is needed to examine the detailed mechanisms, the results provide important new insights into the possible molecular mechanisms of the anticancer activity of plasma.

Furthermore, atmospheric plasmas have been operated in open air at room temperature, so this device may have potential therapeutic activities in cancer therapy, particularly if, by manipulation of plasma parameters, the treatment could be made selective to cancerous cells over healthy cells, and it may be used in the postoperative treatment process.

ACKNOWLEDGMENT

X. Yan, S. Zhao, G. He, F. Zou, and X. Lu contributed equally to this paper.

REFERENCES

[1] J. Bose, V. Gan, R. Prakash, S. Jain, and S. Manna, "A dihydrobenzofuran lignan induces cell death by modulating mitochondrial pathway and G2/M cell cycle arrest," *J. Med. Chem.*, vol. 52, no. 10, pp. 3184–3190, May 2009.

[2] J. Li, H. Cheung, Z. Zhang, G. Chan, and W. Fong, "Andrographolide induces cell cycle arrest at G2/M phase and cell death in HepG2 cells via alteration of reactive oxygen species," *Eur. J. Pharmacol.*, vol. 568, no. 1–3, pp. 31–44, Jul. 2007.

[3] M. S. Chaurushiya and M. D. Weitzman, "Viral manipulation of DNA repair and cell cycle checkpoints," *DNA Repair*, vol. 8, no. 9, pp. 1166–1176, Sep. 2009.

[4] J. Paeng, J. Jeong, C. Yoon, Y. Lee, Y. Suh, J. Chung, J. Park, J. Chung, M. Son, and M. Lee, "Lipiodol solution of Re-188-HDD as a new therapeutic agent for transhepatic arterial embolization in liver cancer: Preclinical study in a rabbit liver cancer model," *J. Nucl. Med.*, vol. 44, no. 12, pp. 2033–2038, Dec. 2003.

[5] M. Laroussi and X. Lu, "Room-temperature atmospheric pressure plasma plume for biomedical applications," *Appl. Phys. Lett.*, vol. 87, no. 11, p. 113 902, Sep. 2005.

[6] M. Laroussi, "Low temperature plasma-based sterilization: Overview and state-of-the-art," *Plasma Process. Polym.*, vol. 2, no. 5, pp. 391–400, Jun. 2005.

[7] J. L. Walsh and M. G. Kong, "Contrasting characteristics of linear-field and cross-field atmospheric plasma jets," *Appl. Phys. Lett.*, vol. 93, no. 11, p. 111 501, Sep. 2008.

[8] D. Mariotti, "Nonequilibrium and effects of gas mixtures in an atmospheric microplasma," *Appl. Phys. Lett.*, vol. 92, no. 15, p. 151 505, Apr. 2008.

[9] P. Bruggeman and C. Leys, "Non-thermal plasmas in and in contact with liquids," *J. Phys. D, Appl. Phys.*, vol. 42, no. 5, p. 053 001, Mar. 2009.

[10] G. Fridman, A. Brooks, M. Galasubramanian, A. Fridman, A. Gutsol, V. Vasilets, H. Ayan, and G. Friedman, "Comparison of direct and indirect effects of non-thermal atmospheric-pressure plasma on bacteria," *Plasma Process. Polym.*, vol. 4, no. 4, pp. 370–375, May 2007.

[11] X. Lu, T. Ye, Y. Cao, Z. Sun, Q. Xiong, Z. Tang, Z. Xiong, J. Hu, Z. Jiang, and Y. Pan, "The roles of the various plasma agents in the inactivation of bacteria," *J. Appl. Phys.*, vol. 104, no. 5, p. 053 309, Sep. 2008.

[12] M. Keidar and I. Belis, "Sheath and boundary conditions for plasma simulations of a Hall thruster discharge with magnetic lenses," *Appl. Phys. Lett.*, vol. 94, no. 19, p. 191 501, May 2009.

[13] M. Laroussi, "Low-temperature plasmas for medicine," *IEEE Trans. Plasma Sci.*, vol. 37, no. 6, pp. 714–725, Jun. 2009.

[14] E. Stoffels, I. E. Kieft, R. E. J. Sladek, L. J. M. van den Bedem, E. P. van der Laan, and M. Steinbuch, "Plasma needle for *in vivo* medical treatment: Recent developments and perspectives," *Plasma Sources Sci. Technol.*, vol. 15, no. 4, pp. S169–S180, Nov. 2006.

[15] X. Shi, G. Zhang, Y. Yuan, Y. Ma, G. Xu, and Y. Yang, "Effects of low-temperature atmospheric air plasmas on the activity and function of human lymphocytes," *Plasma Process. Polym.*, vol. 5, no. 5, pp. 482–488, Jul. 2008.

[16] G. Fridman, G. Friedman, A. Gutsol, A. B. Shekhter, V. N. Vasilets, and A. Fridman, "Applied plasma medicine," *Plasma Process. Polym.*, vol. 5, no. 6, pp. 503–533, Aug. 2008.

[17] X. Lu, Z. Jiang, Q. Xiong, Z. Tang, and Y. Pan, "A single electrode room-temperature plasma jet device," *Appl. Phys. Lett.*, vol. 92, no. 15, p. 151 504, Apr. 2008.

[18] J. Kolb, A. Mohamed, R. Price, R. Swanson, A. Bowman, R. Chiavarini, M. Stacey, and K. Schoenbach, "Cold atmospheric pressure air plasma jet for medical applications," *Appl. Phys. Lett.*, vol. 92, no. 24, p. 241 501, Jun. 2008.

[19] R. Sladek, E. Stoffels, R. Walraven, P. Tielbeek, and R. Koolhoven, "Plasma treatment of dental cavities: A feasibility study," *IEEE Trans. Plasma Sci.*, vol. 32, no. 4, pp. 1540–1543, Aug. 2004.

[20] D. Mariotti, V. Svrcek, and D. G. Kim, "Self-organized nanostructures on atmospheric microplasma exposed surfaces," *Appl. Phys. Lett.*, vol. 91, no. 18, p. 183 111, Oct. 2007.

[21] A. Shashurin, M. Keidar, S. Bronnikov, R. A. Jurjus, and M. A. Stepp, "Living tissue under treatment of cold plasma atmospheric jet," *Appl. Phys. Lett.*, vol. 93, no. 18, p. 181 501, Nov. 2008.

[22] X. Lu, Y. Cao, P. Yang, Q. Xiong, Z. Xiong, Y. Xian, and Y. Pan, "An RC plasma device for sterilization of root canal of teeth," *IEEE Trans. Plasma Sci.*, vol. 37, no. 5, pp. 668–673, May 2009.

[23] R. Franco, O. Schoneveld, A. Georgakilas, and M. Panayiotidis, "Oxidative stress, DNA methylation and carcinogenesis," *Cancer Lett.*, vol. 266, no. 1, pp. 6–11, Jul. 2008.

[24] A. Galanis, A. Pappa, A. Giannakakis, E. Lanitis, D. Dangaj, and R. Sandaltzopoulos, "Reactive oxygen species and HIF-1 signaling in cancer," *Cancer Lett.*, vol. 266, no. 1, pp. 12–20, Jul. 2008.

[25] L. Rowe, N. Degtyareva, and P. Doetsch, "DNA damage-induced reactive oxygen species (ROS) stress response in *Saccharomyces cerevisiae*," *Free Radical Biol. Med.*, vol. 45, no. 8, pp. 1167–1177, Oct. 2008.

[26] W. Pong and W. Eldred, "Interactions of the gaseous neuromodulators nitric oxide, carbon monoxide, and hydrogen sulfide in the salamander retina," *J. Neurosci. Res.*, vol. 87, no. 10, pp. 2356–2364, Aug. 2009.

[27] V. Rettori, J. Fernandez-Solari, C. Mohn, M. Zubilete, C. de la Cal, J. Prestifilippo, and A. De Laurentiis, "Nitric oxide at the crossroad of immunoneuroendocrine interactions," *Ann. N. Y. Acad. Sci.*, vol. 1153, pp. 35–47, Apr. 2009.

[28] D. Shi, F. Xie, C. Zhai, J. Stern, Y. Liu, and S. Liu, "The role of cellular oxidative stress in regulating glycolysis energy metabolism in hepatoma cells," *Mol. Cancer*, vol. 8, no. 1, p. 32, Jun. 2009.

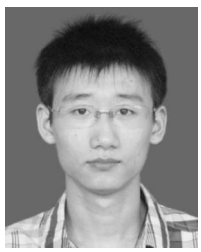
[29] Y. Chirino and J. Pedraza-Chaverri, "Role of oxidative and nitrosative stress in cisplatin-induced nephrotoxicity," *Exp. Toxicol. Pathol.*, vol. 61, no. 3, pp. 223–242, May 2009.

[30] J. Wang, A. Wu, Y. Xu, J. Liu, and X. Qian, "M-2-A induces apoptosis and G(2)-M arrest via inhibiting PI3 K/Akt pathway in HL60 cells," *Cancer Lett.*, vol. 283, no. 2, pp. 193–202, Oct. 2009.

[31] N. Paul, "Universal control mechanism regulating onset of M-phase," *Nature*, vol. 344, no. 6266, pp. 503–508, Apr. 2009.

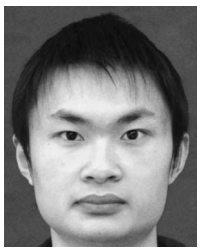
[32] W. S. Deiry, J. Harper, P. O'Connor, V. Velculescu, C. Canman, J. Jackman, J. Pietenpol, M. Burrell, D. Hill, and Y. Wang, "WAF1/CIP1 is induced in p53-mediated G1 arrest and apoptosis," *Cancer Res.*, vol. 54, no. 5, pp. 1169–1174, Mar. 1994.

[33] W. Hu and J. Kavanagh, "Anticancer therapy targeting the apoptotic pathway," *Lancet Oncol.*, vol. 4, no. 12, pp. 721–729, Dec. 2003.



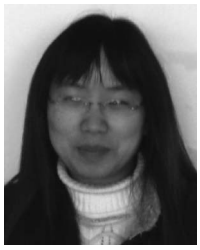
Xu Yan received the M.S. degree from the Huazhong University of Science and Technology (HUST), Wuhan, China, in 2007, where he is currently working toward the Ph.D. degree in The Genetic Engineering International Cooperation Base of the Ministry of Science and Technology, Key Laboratory of Molecular Biophysics of the Ministry of Education, China–U.K. HUST–RRes Genetic Engineering and Genomics Joint Laboratory, College of Life Science and Technology.

He is devoted to the study of the applications of atmospheric-pressure nonequilibrium plasma sources and the mechanism on cancer therapy.



Fei Zou received the B.S. degree from the Huazhong University of Science and Technology, Wuhan, China, in 2007, where he is currently working toward the M.S. degree in the Nuclear Fusion and Advanced Electromagnetic Technology Key Laboratory, Ministry of Education, and the College of Electrical and Electronic Engineering.

His research interests include plasma medicine and the applications of atmospheric-pressure nonequilibrium plasma sources.



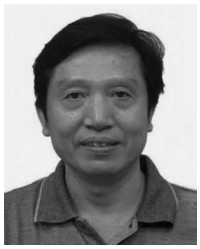
Shasha Zhao received the B.S. degree from Henan Normal University, Xinxiang, China, in 2009. She is currently working toward the M.S. degree in The Genetic Engineering International Cooperation Base of the Ministry of Science and Technology, Key Laboratory of Molecular Biophysics of the Ministry of Education, China–U.K. HUST–RRes Genetic Engineering and Genomics Joint Laboratory, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan, China.

She is devoted to the study of the applications of atmospheric-pressure nonequilibrium plasma sources on cancer therapy.

XinPei Lu (M'06–SM'07) received the Ph.D. degree in electrical engineering from the Huazhong University of Science and Technology, Wuhan, China, in 2001.

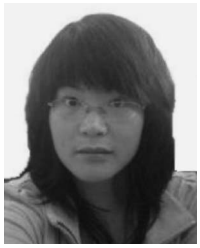
From 2002 to 2006, he was with the Applied Plasma Technology Laboratory, Old Dominion University, Norfolk, VA, as a Research Associate. In 2007, he joined the Huazhong University of Science and Technology, where he is currently a Professor (Chang Jiang Scholar) with the College of Electrical and Electronic Engineering. He is the author or coauthor of about 60 scientific articles in these areas. His research interests include low-temperature plasma sources and their applications, modeling of low-temperature plasmas, plasma diagnostics, and pulsed-power technology.

Dr. Lu has served as Guest Editor for the IEEE TRANSACTIONS ON PLASMA SCIENCE and as Session Chair at the IEEE International Conference on Plasma Science for several years.



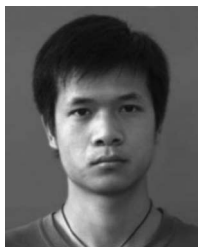
Guangyuan He received the Ph.D. degree from the Agricultural Research Institute, Hungarian Academy of Sciences, Martonvásár, Hungary, in 1993.

He was with the Department of Plant Biotechnology, Wuhan Institute of Botany, Chinese Academy of Sciences, Wuhan, China, and also with the Department of Biochemistry and Physiology, Institute of Arable Crops Research, Rothamsted Experimental Station, Harpenden, Herts, U.K. He is currently a Professor with and the Head of the China–U.K. HUST–RRes Genetic Engineering and Genomics Joint Laboratory, and the Vice Dean of the College of Science, HUST, majors in plant genetic engineering and molecular biology. He is the author or coauthor of about 100 scientific articles. His main research interests include optimization of plant tissue and cell culture systems, cereal transformation and crop improvement, plant molecular breeding, and the action mechanism of anticancer substances from Chinese herbs.



Zilan Xiong received the B.E. degree in electrical engineering from the Huazhong University of Science and Technology, Wuhan, China, in 2008, where she is currently working toward the M.S. degree in the College of Electrical and Electronic Engineering.

She is devoted to the study of plasma medicine and the applications of atmospheric-pressure nonequilibrium plasma sources.

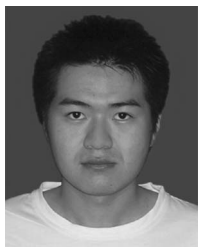


Qing Xiong received the B.E. degree in electrical engineering from Southwest Jiaotong University, Chengdu, China, in 2007. He is currently working toward the Ph.D. degree in the College of Electrical and Electronic Engineering, Huazhong University of Science and Technology, Wuhan, China.

His research interests are focused on the diagnostics and applications of atmospheric-pressure nonequilibrium plasma sources.

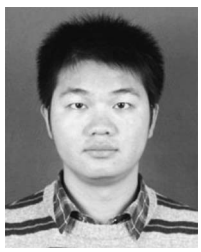
Qiangqiang Zhao received the B.E degree in biology science from Nanchang University, Nanchang, China, in 2008. He is currently working toward the M.S. degree in the College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan, China.

His research interests are focused on the inhibition of inflammatory cytokines of polysaccharides from *Poria cocos*.



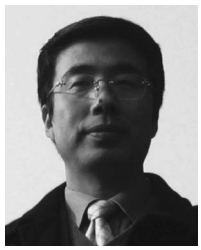
Pengyi Deng received the B.S. degree from the Huazhong University of Science and Technology (HUST), Wuhan, China, in 2007, where he is currently working toward the Ph.D. degree in the China–U.K. HUST–RRes Crop Genetic Engineering and Genomics Laboratory, Ministry of Education, School of Life Science and Technology.

His research interests include the anti-inflammation and antitumor mechanisms of sesamin.



Jianguo Huang received the M.S. degree in biomedical engineering from the Huazhong University of Science and Technology, Wuhan, China, in 2010. He is currently working toward the Ph.D. degree in the School of Medicine, Southern Illinois University, Carbondale.

His research interests include cancer research and cell and molecular biology.



Guangxiao Yang received the Ph.D. degree from the College of Bioscience, China Agricultural University, Beijing, China, in 1994.

From 1996 to 1999, he was with the Institute of Biological Sciences, University of Tsukuba, Tsukuba, Japan, as a Postdoctoral Fellow. From 1999 to 2005, he was with the National Institute of Agrobiological Resources, Tsukuba, as a Research Associate. In 2005, he joined the Huazhong University of Science and Technology, Wuhan, China, where he is currently a Professor with the College of Life Science and Technology. He is the author or coauthor of about 40 scientific articles. His research interests focus on understanding how plants adapt to environmental stresses and the mechanisms involved in abiotic stress tolerance in cereals, such as wheat and rice. His research interests also include phytohormone signaling and regulated gene expression, the relationships between the structure of storage protein and wheat-milling–bread-making quality, and screening and studying the action mechanism of anticancer substances from Chinese herbs.