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TOPICAL REVIEW

# Nonthermal-plasma-mediated animal cell death

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**Abstract**
Animal cell death comprising necrosis and apoptosis occurred in a well-regulated manner upon specific stimuli. The physiological meanings and detailed molecular mechanisms of cell death have been continuously investigated over several decades. Necrotic cell death has typical morphological changes, such as cell swelling and cell lysis followed by DNA degradation, whereas apoptosis shows blebbing formation and regular DNA fragmentation. Cell death is usually adopted to terminate cancer cells *in vivo*. The current strategies against tumour are based on the induction of cell death by adopting various methods, including radiotherapy and chemotherapeutics. Among these, radiotherapy is the most frequently used treatment method, but it still has obvious limitations. Recent studies have suggested that the use of nonthermal air plasma can be a prominent method for inducing cancer cell death. Plasma-irradiated cells showed the loss of genomic integrity, mitochondrial dysfunction, plasma membrane damage, etc. Tumour elimination with plasma irradiation is an emerging concept in cancer therapy and can be accelerated by targeting certain tumour-specific proteins with gold nanoparticles. Here, some recent developments are described so that the mechanisms related to plasma-mediated cell death and its perspectives in cancer treatment can be understood.

(Some figures in this article are in colour only in the electronic version)

## 1. Introduction

The comprehensive term 'plasma medicine' pertains to an emerging field in the interdisciplinary study of biomedical physics [1]. It treats nonthermal air plasma as a novel method applicable in various medical issues containing sterilization, wound healing, tissue regeneration and disease treatment [2, 3]. Despite its short history, the biomedical applications of a nonthermal air plasma have been actively proposed of late. These suggested clear advances in various biomedical issues compared with the previous methods. Among these, the most implicative part may be the application to anticancer therapeutics. Several reports have shown nonthermal-air-plasma-mediated animal cell death [4–6]. Animal cell death consists of three morphologically distinct types: apoptosis, necrosis and autophagic cell death (ACD) [7, 8]. Several reports have given clear evidence that plasma-mediated cell death was elicited by inducing apoptosis and necrosis [5, 6, 9]. This provides a possibility suggesting that a nonthermal air plasma can be adopted as a promising method for terminating abnormal cell growth.

The strategies for anticancer therapies commonly include surgical operation, radiotherapy (radio radiation),

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chemotherapy, etc. Most primitive cancer tissues can be removed by simple surgical operation, but there still exists a risk of recurrence because of the small number of residual cancer cells. Additionally, in the case of a malignant tumour encroaching on a large part of tissue, not enough diseased area can be cut off, and there is an increased crisis of recurrence. As curative or adjuvant treatments, radiotherapy and chemotherapy are frequently adopted, but as these conventional methods have limitations, the development of a novel therapeutic method is strongly encouraged. In this review, the possibility of the successful use of a nonthermal plasma in treating cancer and its perspectives are discussed.

## 2. Animal cell death -apoptosis and necrosis

Cell death is generally classified into three types: apoptosis (type I cell death), autophagic cell death (ACD, type II cell death) and necrosis (type III cell death) [8]. ACD is distinguishable among the cell death types with a large-scale sequestration of portions of the cytoplasm in autophagosomes, giving the cell a characteristic vacuolated appearance. In addition, ACD does not occur in the presence of chromatin condensation. Simply put, ACD is cell execution with autophagy. Even though the actual occurrence of ACD in some model organisms has been known (e.g. *Drosophila melanogaster*) [10], there is no report so far that mammalian cells can be killed via ACD *in vivo*.

In the mammalian system, the remaining two cell death types commonly occur under various physiological conditions. People tend to compare those two cell death pathways. The distinction between cell death types is important particularly because necrosis is often associated with unwarranted cell loss in human pathologies [11, 12] and can lead to local inflammation, presumably through the liberation of factors from dead cells that alert the innate immune system [13, 14]. In addition, it seems that the clearance of necrotic cells operates differently from that of apoptotic cells [15]. In general, necrosis has been known as a type of cell demise that involves the rupture of the plasma membrane. The principal features of necrosis include a gain in cell volume (oncosis) that finally culminates in the rupture of the plasma membrane. Furthermore, necrotic cell death is usually defined in a negative way when it is compared with apoptosis, which accompanies various apoptotic hallmarks, such as pyknosis [16], karyorhexis [17], cell shrinkage and the formation of apoptotic bodies. Even if cell death begins with apoptosis of cells, it can become necrotic cell death when the apoptotic pathway is blocked by specific inhibitors, or through the elimination of the key regulator of apoptosis, such as Apaf-1 [18, 19]. Similarly, the inhibition of ACD at an early stage can also shift to necrosis [20]. The term 'programmed cell death' has been used in a generalized fashion, as a near-synonym of apoptosis in *Caenorhabditis elegans* and mammals. In contrast, necrosis has been considered a merely 'accidental' consequence of nonphysiological stress. Even if necrosis has been known to occur with independent signalling, there have also been some reports of necrosis driven by death signalling.

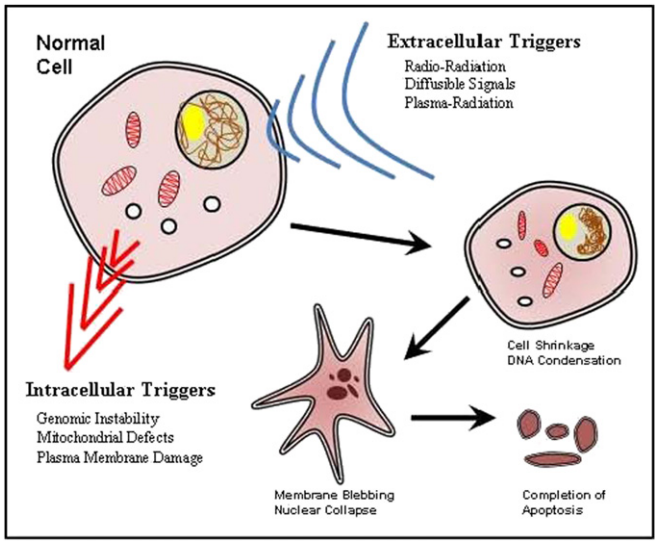


**Figure 1.** Causes and processes of animal cell apoptosis. The apoptosis can be triggered both by extracellular (extrinsic) and intracellular (intrinsic) triggers. Following apoptotic pathways results in morphological and physiological changes such as cell shrinkage, DNA condensation, membrane blebbing and nuclear collapses. Completion of apoptosis is made by phagocytosis.

Under normal physiological conditions, apoptosis can manage organism homeostasis and normal development. Apoptosis occurs when a cell is damaged beyond repair, is infected with a virus or is undergoing stressful conditions (e.g. starvation), achieving the elimination of the damaged cell. In the pathological aspect, apoptosis plays an important role in preventing cancer. In other words, forcing cancer cells into apoptosis can be used in curing malignancy. Actually, this strategy has been developed in clinics.

## 3. Recent progress in and general mechanism of animal cell apoptosis

In this section, more details of apoptosis will be discussed. Apoptosis is a well-known cell death type caused by extracellular death signals (extrinsic) mediated through the plasma membrane, as well as irreversible damages of intracellular components (intrinsic) induced by various effectors (figure 1). After its pioneer investigations in *C. elegans* [21], several model organisms were determined to have apoptosis, and intensive studies were conducted. These include *D. melanogaster*, *Mus muculus* and *Homo sapiens*. A number of papers discussed the importance of apoptosis and reported serious problems induced by an impediment in apoptotic gene expression [22, 23]. In practice, knock-out mice against essential genes regulating apoptosis clearly showed embryonic lethality [24, 25]. Although questions about the physiological needs of apoptosis were constantly raised, a level has now been reached where the inevitability of such phenomenon can be determined.

After the recognition of the importance of apoptosis, many studies were conducted focusing on its genetic pathways. Many pro- and anti-apoptotic gene families were discovered in several model organisms, and their functions were extensively

investigated [26]. The pro-apoptotic gene families include *Bax*, *Bak* and *Bad* and play certain roles in facilitating the apoptotic process, but the anti-apoptotic *Bcl* family performs opposed roles [27]. A gene family of caspase (the abbreviated form of cysteinyl aspartic acid-specific protease) consists of more than ten genes in humans alone and plays major roles in apoptosis, as a final effector [28]. Apoptotic caspases are composed of two subgroups: initiators and executioners [29]. Initiator caspases are activated under apoptotic conditions and turn pro-caspases into active executioner caspases [30]. Fully activated executioner caspases degrade crucial cellular proteins, including structural and functional units [31]. The caspases are essential components in the apoptotic 'no-return' processes, and much effort has been exerted to elucidate their physiological roles.

There are two theories that may directly trigger apoptotic mechanisms in mammals. These are the TNF-(tumour necrosis factor)-induced and Fas-Fas-ligand (FasL)-mediated models, both involving receptors of the TNF receptor family [32]. TNF is a cytokine produced by immune cells, such as activated macrophages, and plays a major role as an extrinsic inducer of apoptosis. TNF binding to TNF-R1 has been shown to initiate the pathway followed by caspase activation via the TNF-receptor-associated death domain (TRADD) and the Fas-associated death domain protein (FADD) [33]. The interaction between Fas and FasL results in the formation of the death-inducing signalling complex (DISC), which contains FADD, caspase-8 and caspase-10. In some cell types, processed caspase-8 directly activates other caspases and triggers the apoptosis of the cell. In other types of cells, Fas-DISC begins a feedback loop that goes into the enhanced release of pro-apoptotic factors from mitochondria as well as the amplified activation of caspase-8. Following receptor level activation in cells, a balance between the pro- and anti-apoptotic members of the Bcl-2 family is established (see figure 2). This balance pertains to the proportion of pro-apoptotic homodimers that form in the outer membrane of the mitochondrion. The pro-apoptotic homodimers, such as *Bax* or *Bak*, are needed to make the mitochondrial membrane permeable for the release of cytochrome c and SMAC (second-mitochondria-derived activator of caspases). Under normal conditions, the control of pro-apoptotic proteins has not been fully understood. There has been a report of a caspase-independent apoptotic pathway mediated by AIF (apoptosis-inducing factor) [18, 34].

In the meantime, apoptotic proteins that target the mitochondria affect the latter in different ways. They may cause mitochondrial swelling through the formation of membrane pores, or they may increase the permeability of the mitochondrial membrane and may cause the apoptotic effectors to leak out. There is also a growing body of evidence indicating that nitric oxide (NO) is able to induce apoptosis by helping to dissipate the membrane potential of mitochondria [35]. Mitochondrial proteins known as SMACs are released into the cytosol following an increase in permeability. SMAC binds to the inhibitor of apoptosis proteins (IAPs) and deactivates them, preventing the IAPs, which normally suppress the activity of a group of cysteine proteases called 'caspases', from arresting the apoptotic process, and therefore allowing apoptosis to proceed [36]. As caspases degrade the cell, the actual degradation enzymes can be seen to be indirectly regulated by mitochondrial permeability. Cytochrome c is also released from the mitochondria due to the formation of permeabilization pores in the outer mitochondrial membrane [37], and fulfils a regulatory function as it precedes the morphological change associated with apoptosis. Once cytochrome c is released, it binds with Apaf-1 and ATP, which then binds to pro-caspase-9 to create a protein complex known as 'apoptosome'. The apoptosome cleaves the pro-caspase into its active form of caspase-9, which in turn activates the effector caspase-3. The mitochondrial outer permeabilization pore (MAC) is regulated by various proteins, such as those encoded by the mammalian *Bcl*-2 family of anti-apoptotic genes, the homologues of the ced-9 gene found in *C. elegans* [38]. *Bcl*-2 proteins are able to promote or inhibit apoptosis by direct action on MAC. *Bax* and/or *Bak* form the pore, while *Bcl*-2, *Bcl*-xL or *Mcl*-1 inhibits its formation. After a cell receives a stimulus, it undergoes organized degradation of the cellular organelles by the activated proteolytic caspases. A cell undergoing apoptosis shows various apoptotic hallmarks, such as pyknosis [16],

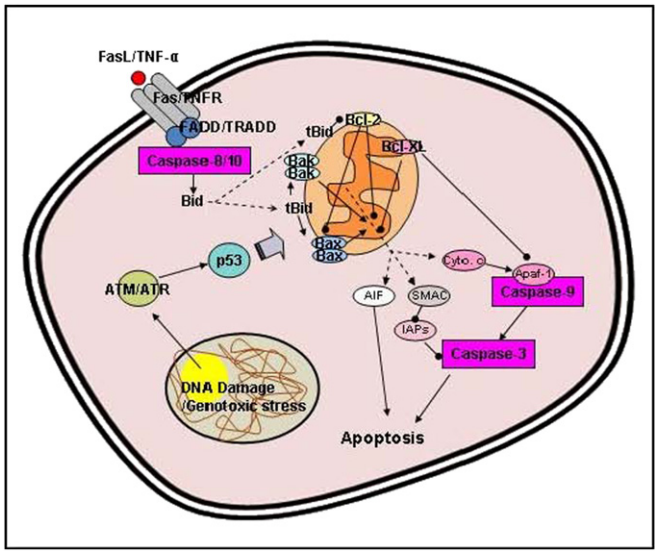


**Figure 2.** Mitochondria-centred apoptotic molecular pathways. The extracellular trigger FasL or TNF-$\alpha$ can induce FADD/TRADD-mediated upstream caspases (caspase-8 and caspase-10) activation. Following Bid translocation to mitochondria facilitates multimerization of *Bak* or *Bax* which form pores at the outer membrane. Through those pores cytochrome c or SMAC is released to the cytoplasm. Anti-apoptotic protein *Bcl*-2 functions as inhibitor to pore formations and protein releases. Caspase-3 activation is an important hallmark for mitochondria mediated apoptosis. Caspase-3 is activated by caspase-9 but inhibited by IAPs. Caspase-9 can be activated by binding of Apaf-1, which is activated by cytochrome c released from mitochondria but inhibited by anti-apoptotic mitochondrial protein *Bcl*-XL. *Bcl*-XL also inhibits the release of SMAC and AIF from mitochondria. SMAC suppresses IAPs' inhibitory action to caspase-3, and AIF induces caspase-3/9-independent apoptosis. In the meantime, genotoxic stress or DNA damage can induce apoptosis intrinsically, which is mediated by ATM/ATR and p53 proteins implying the close relationship to cell cycle regulation.

the chromatin condensation into compact patches, karyorhexis [17], which indicates DNA fragmentation, cell shrinkage and rounding due to the breakdown of the cytoskeletal structure, and the formation of apoptotic bodies, which are finally phagocytosed.

Apoptosis is needed for the faithful completion of several developmental processes. These functions in development are responsible for processes from *C. elegans* development to the organization of the mammalian body systems. Apoptosis regulates systemic cell number homeostasis and neuronal-network formation in *C. elegans* [39]. In particular, vertebrate fingers and toes are developed with extensive and well-regulated apoptotic processes [40]. The importance of apoptosis in developmental processes is also related to the immune system, the most complex system in mammals. The establishment of a relevant T lymphocytes population is achieved via serial apoptosis called 'thymic selection' [41].

From a different standpoint, apoptosis is often adopted as a defense mechanism preventing severe problems. For example, viral infected cells secrete a diffusible factor called 'interferon' (IFN) to prevent a virus from spreading throughout the body [42]. The radiated interferon is taken up by the cells near it to induce apoptosis; thus, viruses can no longer proliferate. Moreover, apoptosis removes cells suffering irreversible loss of genomic integrity to prevent cancer from developing in advance [43]. Mature cancer cells are even targeted by the immune surveillance system and are induced to apoptosis [44]. Based on these facts, it can be inferred that apoptosis is an inevitable process for treating cancer cells *in vivo*, and that it can be developed by various cues, including radio irradiation and chemotherapeutics. This actually exists in the strategies of many drug companies that want to terminate proliferation of cancer cells through the induction of apoptosis. Recently, plasma-mediated apoptosis has been highlighted and discussed for the possibility of effective treatment as a cancer medicine.

## 4. Mechanism of plasma-mediated cell apoptosis

Cumulated plasma studies have achieved much in terms of the generation of a nonthermal air plasma device, and active discussion of this issue is in progress [45]. The concept of 'plasma medicine,' which is frequently bandied about in connection with biomedical application, implies that nonthermal air plasmas can modulate various biological processes and can thus be used as a substitute for conventional therapeutics. This has a comprehensive meaning, including cancer treatment, wound healing, tooth bleaching and sterilization.

The speeding up of blood coagulation is very helpful and useful in particular situations. According to a recent report, it can be made possible by the use of a portable microwave-excited atmospheric-pressure plasma jet (APPJ) that employs a coaxial transmission line resonator operating at 900 MHz with low power [46]. These authors also identified the generation of highly reactive species, including OH, NO and O radicals. A nonthermal plasma also seems to be an alternative solution for inactivation of a biofilm. A biofilm means a matrix-enclosed accretion of micro-organisms combined with various extracellular polymeric substances [62]. This is usually hard to destroy and thus triggers some severe problems under particular circumstances. Nonthermal plasma-mediated biofilm inactivation could treat dental cavity as an alternative to conventional lasers [63]. In this study, nonthermal plasma successfully inhibited growth of *Streptococcus mutans,* one of the major causative tooth decaying anaerobic bacteria. A nonthermal plasma also effectively killed endospores of *Bacillus atrophaeus* [47], and its effect in direct radiation was determined [48]. Further, a nonthermal plasma-induced topology changes in double-stranded circular bacterial plasmids [49]. Recently, the effect of nonthermal plasmas on a biofilm formed by nonsporing coccobacillus was also analysed and showed significant decrease in biofilm mass [64]. Interestingly, plasma can also be used to assist tooth whitening [50]. Taken together, nonthermal plasmas certainly have a broad spectrum in various applications and still have great possibilities to be used for other purposes. Among these, the most interesting and promising application may fall under cancer treatment. As mentioned above, apoptosis governs various life phenomena and was thus adopted as the most efficient method of eliminating cancer cells early. A small number of cells escape this kind of surveillance system, however, and this produces a malignant tumour that threatens an organism's life. Complex and diverse therapeutics have been chosen in the struggle against cancer, including surgical operation, radio radiation and chemotherapeutics, but it seems that cancer still cannot be overcome. Thus, much effort was concentrated on developing a way of using nonthermal plasmas for cancer treatment. Plasma medicine implies the possibility of the use of nonthermal plasmas as a novel therapeutic tool against cancer, through apoptosis (see figure 3). In particular, plasma-irradiated cells have lost their attaching ability [5], clearly showing that mammalian cells respond to nonthermal air plasmas, and that some cellular changes occur. This

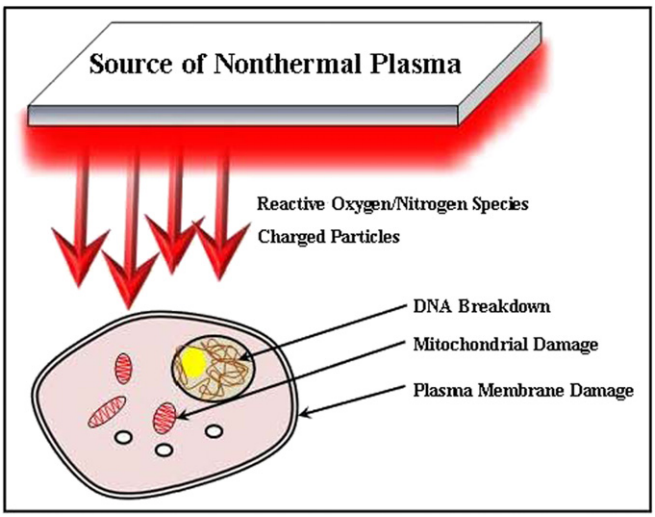


**Figure 3.** Generation of apoptotic factors by plasma irradiation. Various sources of nonthermal plasma produce highly reactive oxygen/nitrogen species and charged particles. These elements can intrinsically or extrinsically induce cellular apoptosis. This kind of plasma application shows promise for anticancer therapeutics.

attracted some researchers to this field, and several reports followed.

Cellular changes induced by nonthermal plasmas were reported, showing surface detachment and the loss of cell–cell interaction in CHO-(Chinese hamster ovary)-K1 cells [5]. In this report, the authors designed a plasma needle device and analysed its effects. A low dose of plasma detached the CHO-K1 cells from the surface and their neighbouring cells. A high dose of plasma, on the other hand, triggered necrotic cell death. An interesting study was also performed, using the colorectal cancer cell line of HCT 116 [4]. In this paper, HCT116 showed apoptotic responses regardless of the p53 gene expression. A nanosecond-pulsed electric field was used, and DNA fragmentation and caspase activation were found to have occurred. In particular, changes in the levels of cytochrome c and *Bax* were found, clearly showing that mitochondrial damage is one of the causes of plasma-mediated cell apoptosis. The plasma's effects on the melanoma cells were also determined [6]. TUNEL (terminal-deoxynucleotidyl-transferase-mediated dUTP nick end labelling) assay was used to measure the apoptotic death of plasma-irradiated cells. Additionally, the hepatocytic responses against plasma were also analysed, and changes in specific subcellular structures were shown, which was followed by necrotic death [51]. The nonthermal-plasma-mediated induction of apoptosis has been clearly demonstrated by a recent paper that investigated cellular signalling related to an apoptotic process [52]. The plasma-treated cells showed a dramatic increase in the ratio of the subG1 phase and $\gamma$-H2A.X level. This was also supported by an increase in the levels of p53 and caspase, two of the final executors of apoptosis. Considering these facts, it can certainly be accepted that a nonthermal air plasma induces the apoptotic death of mammalian cells, although it still introduces necrotic cell death. Due to the increase in the level of $\gamma$-H2A.X, one of the representative signals against double-strand break (DSB) in the genomic DNA, it can be assumed that plasma-mediated apoptosis starts with severe disturbances in genomic integrity. That apoptosis originates from mitochondrial defects has been suggested, with considerable evidence for mitochondrial dysfunction. The plasma membrane is a fairly vulnerable structure in mammalian cells and can thus also be a major target of plasma attack. As such, it is certainly acceptable that the nonthermal plasma causes certain changes to occur in several cellular organelles, followed by cell apoptosis. The actual player triggering these responses, however, should be investigated. Reactive oxygen species (ROS) or charged particles can be responsible for this, but there is still the possibility of the presence of other authentic players. There is a need to determine the priority of contribution and characterization of the authentic players in plasma-mediated apoptosis.

## 5. Antitumoural chemotherapy and mechanisms

Cancer is the uncontrolled growth of cells coupled with malignant behaviour (invasion and metastasis). It is thought to be caused by the interaction between genetic susceptibility and environmental toxins. Chemotherapy, in its most general sense, is the treatment of a disease using chemicals, particularly by killing the microorganisms or cancerous cells. In popular usage, it refers to antineoplastic drugs used to treat cancer, or to the combination of these drugs into a cytotoxic standardized treatment regimen. In a broad sense, most chemotherapeutic drugs work by impairing mitosis (cell division), effectively targeting fast-dividing cells. As these drugs damage the cells, they are termed 'cytotoxic.' Some drugs cause cells to undergo apoptosis.

Advances in the molecular and genetic approaches for understanding cell biology have unveiled signalling networks that regulate cellular activities such as proliferation and survival. Many of these networks were found to be radically altered in cancer cells, and these alterations had a genetic basis caused by chance somatic mutation.

For the excavation of therapeutic agents, in the 1960s, scientists discovered that an extract from the bark of the Pacific yew tree can be used to fight cancer. The substance taxol, for example, one of the hundreds of naturally occurring substances that people have used for centuries to treat diseases and to promote good health, is a novel antimitotic agent that promotes microtubule assembly. It was then found to be effective in ovarian or breast cancer therapy two decades after its initial discovery. The majority of chemotherapeutic drugs can be divided into alkylating agents, antimetabolites, anthracyclines, plant alkaloids, topoisomerase inhibitors and other antitumour agents. All these drugs affect cell division or DNA synthesis and function in some way. There are some newer agents, though, that do not directly interfere with the DNA. These include monoclonal antibodies and the new tyrosine kinase inhibitors, such as imatinib mesylate (*Gleevec*), which directly targets a molecular abnormality in certain types of cancer (chronic myelogenous leukemia and gastrointestinal stromal tumours). These are examples of targeted therapies. In addition, some hormone treatments that modulate tumour cell behaviour without directly attacking those cells may be used.

In another aspect of cancer therapeutics, cancer cells manifest a series of metabolic changes, one of which is the so-called 'Warburg effect'. Accordingly, even in the presence of oxygen, cancer cells tend to synthesize ATP mainly through anaerobic glycolysis, a phenomenon that requires high glucose uptake and that causes local acidification owing to lactate production [53]. Those phenomena are closely related to the malfunction of the mitochondria, which are generally known as energy plants in cells. As such, some chemotherapeutic trials have dealt with mitochondrial abnormality, enhancing the pro-apoptotic protein action called 'chemosensitization', which means liberating the apoptotic caspase cascade by sequestering the caspase-inhibiting protein XIAPs via Smac/DIABLO [54].

For chemical-treatment strategies, there are several ways of administering the agents. Combined-modality chemotherapy is the use of drugs along with other cancer treatments, such as radiation therapy or surgery. Most cancers are now treated in this way. Combination chemotherapy is a similar practice that involves treating a patient with a number of different drugs simultaneously. The drugs differ in their mechanisms and

side effects. The biggest advantage is minimizing the chances that resistance to any one agent will develop. In neoadjuvant chemotherapy (preoperative treatment), initial chemotherapy is designed to shrink the primary tumour, thereby rendering local therapy (surgery or radiotherapy) less destructive or more effective. Adjuvant chemotherapy (postoperative treatment) can be used when there is little evidence of cancer present, but it presents a risk of recurrence. This can help reduce the chances of developing resistance if the tumour does develop. It is also useful in killing cancerous cells that have spread to other parts of the body. This is often most effective when the newly growing tumours are fast dividing and are thus very susceptible. Lastly, palliative chemotherapy is given without curative intent but simply to decrease the tumour load and to increase the life expectancy. For these regimens, a better toxicity profile is generally expected.

In advanced ovarian cancer, hyperthermic intraperitoneal chemotherapy (HIPEC) is an example of a new treatment strategy aimed to improve the treatment outcome in cancer patients. Based on theoretical and experimental evidence, HIPEC can be said to be an effective treatment for ovarian cancer. Undoubtedly, cancer therapists have perpetually been interested in the development of more effective chemotherapeutics that would elicit tumour (stem) cell death, in spite of the fact that there has been some success in the development of cell-death-inducing regimens for cancer chemotherapy. Combined treatments with chemotherapy and plasma-mediated cancer therapy would have a good possibility of treating cancer effectively.

## 6. Possible selective cancer treatments and future prospects

As discussed above, the use of nonthermal plasmas can be one of the clear solutions against cancer. The concept of plasma-mediated cancer treatment is similar to conventional radiotherapy (radiation therapy), a representative therapeutic strategy that includes surgical operation and chemotherapy. Radiotherapy uses strong ionizing radiation and has thus been adopted by only half of the cancer patients [55]. Irradiated tissues show phenotypes of global damages on all the cellular components. Among these, the most well-known landmark of radiation is DNA damage, followed by apoptosis with irreversible loss of genomic integrity [56]. Additionally, the nonirradiated neighbouring cells are also induced to apoptosis even in low-dose radiation. 'Bystander effect' is the term commonly used to describe this phenomenon; it makes radiotherapy a treatment with more powerful side effects [57]. Radiation severely impairs genomic integrity by ionizing oxygens, but most solid tumours grow under hypoxic conditions and thus meet with intratumoural anaemia [58]. This shows a clear limitation of radiotherapy, which uses oxygen as a major source for remediation. The cancer stem cell theory implies that all tumours have a stem-cell-like population; this can be another reason for the difficulty of tumour elimination [59]. Notably, cancer stem cells show remarkable radio-resistance [60]. Radiation therapy is one of the most powerful cancer treatment methods, but it manifests certain limitations.

As mentioned above, the cells irradiated by nonthermal plasmas behaved as the radio-irradiated cells did. They clearly showed several apoptotic responses [52]. In addition to this, a recent paper has reported that plasma-induced cytotoxicity can be accelerated by introducing gold nanoparticles (GNPs) [9]. The authors of the paper wanted to target tumour cells specifically. Towards this end, GNPs were conjugated with a monoclonal antibody against focal adhesion kinase (FAK), a well-known oncogene. FAK is also known as protein tyrosine kinase 2 (PTK2) and plays roles in cell migration and adhesion; it is thus strongly related to cancer cell metastasis [61]. Interestingly, FAK-targeted cancer cells showed a fivefold higher increase in plasma-mediated cell apoptosis. This suggests that the irradiation of nonthermal plasmas can be a novel solution for cancer therapy. Moreover, GNPs were previously cited as having no flaws in biocompatibility and as providing certain merits, unlike radionuclide therapy, which uses radioactive materials. Plasma-mediated cancer therapy would be more desirable if proper ways are developed to apply it selectively to cancer cells.

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