Data Article

Differential data on the responsiveness of multiple cell types to cell death induced by non-thermal atmospheric pressure plasma-activated solutions

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\section*{ABSTRACT}

A discovery that cells die of a novel and distinctive process, along with some characteristic events, such as cellular shrinkage and Programmed cell death 4 disappearance, has been done by using non-thermal atmospheric pressure plasma-activated solutions \cite{1}. Data on the responsiveness of multiple cell types to the induction of cellular shrinkage and cell death and the loss of Programmed cell death 4 by exposure to the non-thermal atmospheric pressure plasma-activated solutions were collected. Human neuroblastoma SH-SY5Y cells, murine myoblast C2C12 cells, and murine embryonic fibroblasts were cultured for various periods in each of the non-thermal atmospheric pressure plasma-activated solutions and then examined by light field microscopic observation for their effects on cell morphology, by Trypan blue dye exclusion assay for those on cell death, and by Western blotting for those on Programmed cell death 4 disappearance. The data clarified some differences in the responsiveness to the induction of cellular shrinkage, cell death, and Pdcd4 disappearance by all the non-thermal atmospheric pressure plasma-activated solutions among the cells.

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Specifications Table

| Subject                          | Cell Biology                      |
|---------------------------------|-----------------------------------|
| Specific subject area           | Cell death biochemistry in cancer research |
| Type of data                    | Figure                            |
| How data were acquired          | Microscopic observation, Trypan blue dye exclusion assay, Western blot analyses. |
| Data format                     | Raw                               |
| Analyzed                        |                                   |
| Parameters for data collection  | The data were collected under conditions, where several kinds of cells were cultured at 37°C under an atmosphere of 5% CO₂ for various periods in each of non-thermal atmospheric pressure plasma-activated solutions supplemented with 10% heat-inactivated fetal bovine serum and then analyzed with cell biological, such as microscopic observation and Trypan blue dye exclusion assay, and biochemical methods, such as Western blot analyses. The non-thermal atmospheric pressure plasma-activated solutions, referred to as plasma-activate medium (PAM), plasma-activated saline (PAS), and plasma-activated Krebs-Ringer solution (PAK), were used for the experiments. |
| Description of data collection  | Cells were cultured until each of the indicated periods in non-thermal atmospheric pressure plasma-activated solutions and then examined by microscopic observation for cellular shrinkage, followed by Trypan blue dye exclusion assay for cell death and Western blot analyses for Programmed cell death 4 disappearance. |
| Data source location            | Institution: Kumamoto University |
| City/Town/Region                | Kumamoto/Kurokami/2-39-1          |
| Country                         | Japan                             |
| Latitude and longitude (and GPS coordinates, if possible) for collected samples/data: | 32.798612 and 130.705814 |
| Data accessibility              | With the article                  |
| Related research article        | K. Eto, C. Ishinada, T. Suemoto, K. Hyakutake, H. Tanaka, M. Hori, A novel and distinctive mode of cell death revealed by using non-thermal atmospheric pressure plasma: the involvements of reactive oxygen species and the translation inhibitor Pdcd4, Chem.-Biol. Interact. 338 (2021) 109403. https://doi.org/10.1016/j.cbi.2021.109403. |

Value of the Data

- The data indicate some differences in the responsiveness of multiple cell types to the induction of cellular shrinkage, cell death, and Programmed cell death 4 disappearance by exposure to the non-thermal atmospheric pressure plasma-activated solutions.
- The data can be benefited to researchers in the fields for the biology of cell death, cell biology, developmental biology, and cancer biology.
- The data may provide insight into the differences in the susceptibility to cell death induced by non-thermal atmospheric pressure plasma-activated solutions between cancer and non-cancerous, i.e. healthy, cells; cancer cells appeared to be more susceptible than healthy cells to the non-thermal atmospheric pressure plasma-activated solutions.
- The data may contribute to understanding the pathophysiology of cancers and to helping the development of their specific treatment with non-thermal atmospheric pressure plasma-activated solutions.
- The data may demonstrate that various types of cells can die of “spoptosis”, a novel and distinctive mode different from other known ones, such as apoptosis, upon necessity.
1. Data Description

Recently we have reported the existence of a novel and distinctive mode of cell death that was revealed by using non-thermal atmospheric pressure plasma-activated solutions [1]. Here we present differential data on the responsiveness of multiple cell types to cell death, along with cellular shrinkage and Programmed cell death 4 disappearance, that is induced by non-thermal atmospheric pressure plasma-activated solutions. Fig. 1 shows raw and analyzed data on the susceptibility of multiple cell types to the induction of cellular shrinkage and cell death by non-thermal atmospheric pressure plasma-activated solutions. Fig. 2 shows raw and analyzed data on the susceptibility of multiple cell types to the loss of Programmed cell death 4 by non-thermal atmospheric pressure plasma-activated solutions. The detail raw data are deposited to a link with DOI. The data provided in this article are related to the published article in [1].

2. Experimental Design, Materials and Methods

2.1. Preparation of non-thermal atmospheric pressure plasma-activated solutions

The non-thermal atmospheric pressure plasma-activated solutions, termed as PAM, PAS, and PAK, were produced by non-thermal atmospheric pressure plasma irradiation to Dulbecco’s modified Eagle’s medium (D-MEM, Wako), to a saline (pH 7.4) comprising (in g/L) 0.2 CaCl₂, 0.4 KCl, 0.01 MgSO₄, 6.4 NaCl, 3.7 NaHCO₃, and 0.11 NaH₂PO₄, which includes minerals at their concentrations same as D-MEM and excludes amino acids and vitamins from D-MEM, and to another saline Krebs-Ringer solution (pH 7.4) comprising (in g/L) 0.15 CaCl₂, 0.36 KCl, 0.14 MgSO₄, 7 NaCl, 2.1 NaHCO₃, and 0.16 KH₂PO₄, respectively. The experimental setup to prepare the non-thermal atmospheric pressure plasma-activated solutions has been previously described [3]. While argon gas was flowing, non-thermal atmospheric pressure plasma in the main discharge region was excited by applying 10 kV from a 60-Hz commercial power supply to two electrodes 20 mm apart. The flow rate of argon gas was set at 2 standard L/m, and the distance between the plasma source and each of the medium and the two kinds of saline was fixed at 3 mm. The medium and the two kinds of saline of 8 mL in a 60 mm dish were irradiated for 5 min with non-thermal atmospheric pressure plasma.

2.2. Cell culture and treatments

Experiments were performed by culturing some cancer and non-cancerous, namely healthy, cells in each of the non-thermal atmospheric pressure plasma. SH-SYSY cells, which had been established from human neuroblastomas, were used as one of cancer cells. C2C12 cells, which had been established from mammalian myoblasts, were used as one of non-cancerous cells. These cell lines were obtained from the American Type Culture Collection. Murine embryonic fibroblasts (MEF) were also used as one of non-cancerous cells. The primary cultured cells were prepared with a conventional method. Briefly, embryos were collected from pregnant mice (ICR) at the embryonic day 13 under the deep anesthesia, and then the torsos were taken quickly. Their skin was washed in phosphate-buffered saline (PBS) free of Ca²⁺ and Mg²⁺ ions, minced finely, and treated with 0.1% trypsin and 0.2 mM EDTA while being stirred for 1 h at 37 °C. The cells were suspended, filtrated through a 70-μm nylon cell strainer (BD Falcon), and centrifuged at 400 × g for 5 min at room-temperature. After the pellets were resuspended, dissociated cells were cultured for 2 to 3 days until confluent, proliferated through two subsequent passages, and then utilized for experiments, as described below. The Institutional Animal Care and Use committee of Kumamoto University approved protocols for the animal experiments. All the cells were seeded at the confluency of approx. 2 × 10⁵/well onto 24-well plates and maintained in D-MEM supplemented with 10% heat-inactivated fetal bovine serum (FBS) at 37 °C under an
**Fig. 1.** Differential susceptibility to the induction of cellular shrinkage and cell death in different cells treated with non-thermal atmospheric pressure plasma-activated solutions. SH-SY5Y cells, C2C12 cells, and murine embryonic fibroblasts (MEF) were cultured for the indicated periods in each of the non-thermal atmospheric pressure plasma-activated solutions (PAM, PAS, and PAK) or for 3, 8, and 9 h, respectively, in each of their corresponding controls (0) and subjected to light field microscopic observation for cellular shrinkage (A), followed by Trypan blue dye exclusion assay for cell death (B). A, data were shown in the respective cells cultured for 3, 8, and 9 h in PAM or the corresponding control and representative of three independent experiments; scale bars in upper and lower panels (enlarged figures of the randomly selected microscopic fields, which are indicated by squares in upper panels, each) are 200 and 50 μm, respectively; a minimum of 300 cells was scored under each condition for quantification of shrunk cells, and the frequency of cellular shrinkage was presented as a percentage (means ± S.E.) of the morphologically changed cells among total ones, derived from three independent experiments (a lower graph). B, a minimum of 300 cells was scored under each condition for quantification of dead cells, and the frequency of cell death was presented as a percentage (means ± S.E.) of the Trypan blue dye-included cells among total ones, derived from three independent experiments. *, p<0.05, **, p<0.01, ***, p<0.005.
atmosphere of 5% CO₂, and 20 h later substituted with each of the non-thermal atmospheric pressure plasma-activated solutions (PAM, PAS, and PAK) containing 10% heat-inactivated FBS or each of the corresponding controls (D-MEM, a saline, and Krebs-Ringer solution) containing 10% heat-inactivated FBS and then cultured for various periods at 37°C under an atmosphere of 5% CO₂. The culture periods set for the respective cell types were determined as the conditions optimal to compare the temporal differences among the multiple cell types in the induction of cellular shrinkage, cell death, and Programmed cell death 4 disappearance. The cells were subjected to light field microscopic observation for the induction of cellular shrinkage, followed by Trypan blue dye exclusion assay for the incidence of cell death and Western blot analyses for the loss of Pdcd4 and the cleavage of PARP-1.

2.3. Trypan blue dye exclusion assay

Cells cultured in each well of 24-well plates were washed in PBS, harvested by trypsinization, and added with 0.5% Trypan blue dye in PBS. The numbers of dead cells were measured by microscopic examination with Trypan blue dye inclusion.

2.4. Western blot analyses

Whole protein extracts were prepared from cells cultured in each well of 24-well plates by using 50 μl of SDS-PAGE sample buffer (125 mM Tris–HCl, pH 6.8, 4% SDS, 10%
β-mercaptoethanol, 10% sucrose, 1% bromophenol blue), and 10 μl of the respective extracts were applied to and resolved on SDS-7.5% PAGE. Proteins were transferred to a polyvinylidene difluoride membrane (Immobilon, Millipore) and blocked with 5% non-fat milk in Tris-buffered saline (pH 7.4) containing 0.1% Tween20 (TBS-T). The membrane was probed with a primary antibody, diluted with TBS-T containing 5% non-fat milk, specific for Pdcd4 (1:8000, Rockland), PARP-1 (1:2000, Enzo Life Sciences), or β-actin (1:10,000, Millipore), detected with the appropriate secondary antibodies conjugated to horseradish peroxidase, and developed with an enhanced chemiluminescence system (Immobilon Western Chemiluminescent HRP substrate, Millipore).

**Ethics Statement**

All the animal experiments comply with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines and were performed in accordance with the protocols approved by the Institutional Animal Care and Use committee of Kumamoto University (ethics approval number: A30–092).

**CRediT Author Statement**

**Ko Eto:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Validation, Visualization, Writing - original draft, Writing - review & editing; **Chiaki Ishinada:** Data curation, Formal analysis, Investigation, Validation, Visualization, Writing - original draft; **Takuya Suemoto:** Data curation, Formal analysis, Investigation, Validation, Visualization; **Keiichiro Hyakutake:** Data curation, Formal analysis, Visualization, Writing - original draft; **Hiromasa Tanaka:** Resources, Writing - original draft; **Masaru Hori:** Resources.

**Declaration of Competing Interest**

All the authors declare that they have no known competing financial interests or personal relationships, which have, or could be perceived to have, influenced the work reported in this article.

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**References**

[1] K. Eto, C. Ishinada, T. Suemoto, K. Hyakutake, H. Tanaka, M. Hori, A novel and distinctive mode of cell death revealed by using non-thermal atmospheric pressure plasma: the involvements of reactive oxygen species and the translation inhibitor Pdcd4, Chem. Biol. Interact. 338 (2021) 109403, doi:10.1016/j.cbi.2021.109403.

[2] K. Eto, S. Goto, W. Nakushima, Y. Ura, S. Abe, Loss of programmed cell death 4 induces apoptosis by promoting the translation of procaspase-3 mRNA, Cell Death Differ. 19 (2012) 573–581, doi:10.1038/cdd.2011.126.

[3] H. Tanaka, M. Mizuno, K. Ishikawa, K. Nakamura, H. Kajiyama, H. Kano, F. Kikkawa, M. Hori, Plasma-activated medium selectively kills glioblastoma brain tumor cells by down-regulating a survival signaling molecule, AKT kinase, Plasma Med. 1 (2013) 265–277, doi:10.1615/PlasmaMed.2012006275.