The effects of microwave normothermic irradiation on cultured cancer cells

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Introduction

Microwaves (frequency: 0.3–300 GHz) have long been used in cancer therapies such as microwave coagulation therapy and hyperthermia therapy. In these therapies, microwave irradiation is used to kill tumour cells by raising cellular temperature. These therapies have been used for treatment of various cancers for a long time. In recent years, microwave irradiation technology has been developed further, and it has been reported that the yield and reaction rate of many chemical reactions can be increased by microwave irradiation at a much lower temperature as compared to a conventional heating method such as water bath heating[1]. Therefore, we hypothesized that microwave normothermic irradiation might affect biological phenomena in cells.

Objectives

We previously developed a microwave irradiation system that could irradiate cells under normothermic conditions by controlling the outputs and frequency precisely[2]. We then investigated the cell death pathways in HL-60 cells, induced during microwave irradiation under normothermic conditions. After being exposed to our microwave irradiation system, the cells were killed through "caspase-independent apoptosis"[3]. In this study, we investigated the cell death of other cultured cancer cells by microwave irradiation such as T98G (for human glioblastoma cells), MDA-MB-231 (for human breast cancer cells), and KATO III (for human gastric cancer cells).

Material/Methods

T98G, MDA-MB-231, and KATO III cells were seeded in 35 mm culture dishes containing 2.5 mL of media, at a density of 1×10^5 cells/mL. Microwave irradiation (2.45 GHz) was applied for 1h, and the temperature of cells was maintained at 37 °C. However, the temperature inside the applicator, during these experiments, was set at 10 °C. Following irradiation, the cells were moved to a CO_2 incubator, where they were incubated for 6 h before used in the following assays; Caspase 3/7 assay carried out by using Caspase-3/7 Assay Kit (AnaSpec, San Jose, CA, USA) and Annexin V-PI assay performed by using an Annexin V-FITC Apoptosis Detection Kit (Nacalai Tesque).

Results

According to the microscopic observations, the number of late stage apoptotic cells (both Annexin V and PI positive) had increased in all cell types, while early apoptotic cells (Annexin V positive, PI negative) were not observed. The adherent cell types, T98G and MDA-MB-231, were cast off by microwave irradiation, further indicating that cells were near death. Moreover, after microwave irradiation, the activity of caspase 3/7 did not increase significantly in any of the cell types.

Conclusion

The results indicate that cell death pathways activated by microwave irradiation in the examined cells may be similar. However, further investigations should be performed to better understand the effects of irradiation on each cell type in detail.

References

[1] Sawada T. and Yamada T. (2018) J. Jpn. Petrol. Inst., 61(2), 121-128.

[2] Asano M., Sakaguchi M., Tanaka S., et al. (2017) Effects of normothermic conditioned microwave irradiation on cultured cells using an irradiation system with semiconductor oscillator and thermo-regulatory applicator, Sci Rep, 7, 41244.

[3] Asano M., Tanaka S., Sakaguchi M., et al. (2017) Normothermic microwave irradiation induces death of HL-60 cells through heat-independent apoptosis. Sci Rep, 7(1), 11406.

The Effects of microwave normothermic irradiation on cultured cancer cells

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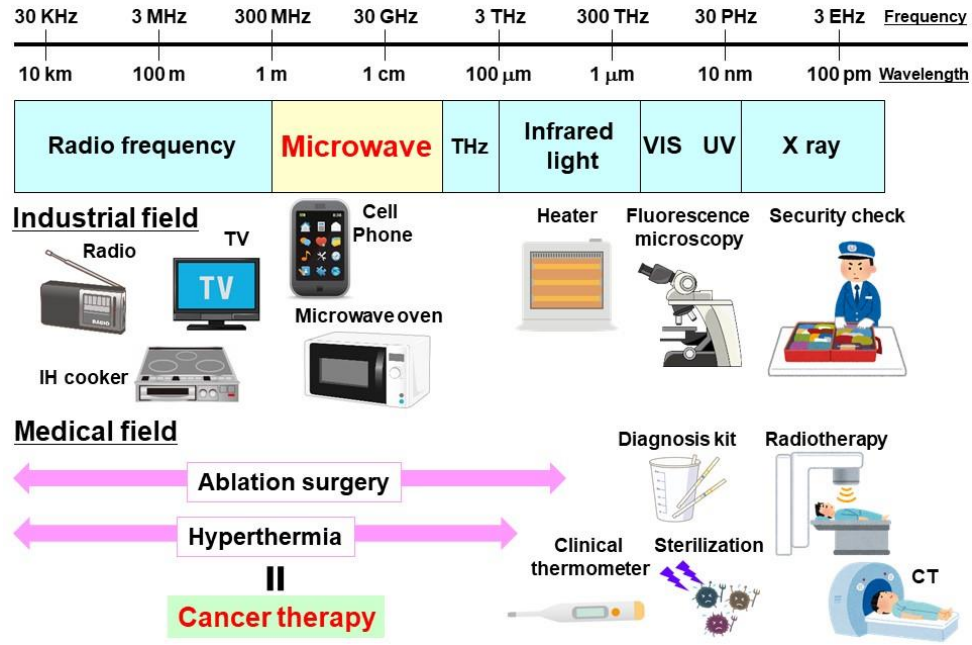
What are 'Microwaves'?



Outline of our presentation

Recent trends of microwave irradiation
- Spread of a semiconductor oscillator
- Development of simulation technology for electromagnetic field

We can control microwave energy for cells precisely.

Outline
1, Microwave irradiation system for cultured cells.

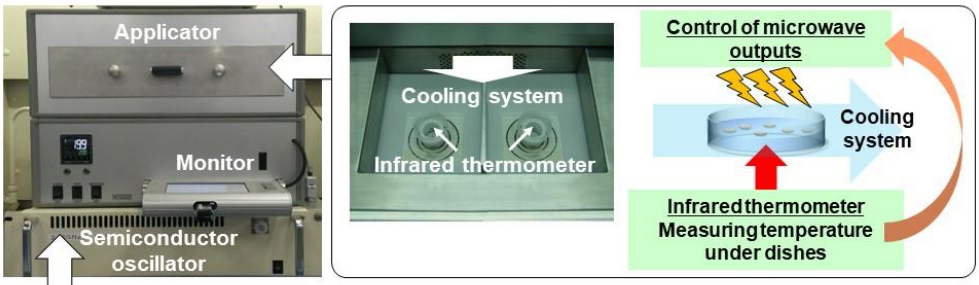
2, Cell death pathway by microwave normothermic irradiation.

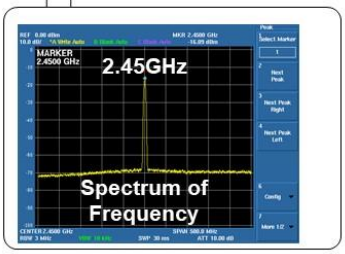
*normothermic: at normal body temperature

Microwave irradiation system

Microwave can be irradiated normothermically* in this system

*normothermal: at normal body temperature





Specifications
- Model number: MTS03(S)
  (Made by Sunny Engineering Co. Ltd.)
- Frequency: 2.45 GHz
- Outputs: 0-40 W
- Temperature in applicator: 10-45°C

Procedure: assays of cell death pathways

HL-60 cells (Human promyelocytic leukemia cells)
1×10^5 cells/mL

Negative control group
Without any treatments

Microwave irradiation group
Kept at 37°C for 1 h
(Applicator: 10°C)

Thermal treatment group
Incubation at 42.5°C for 1 h
(Most of cells are killed at 42.5°C.)

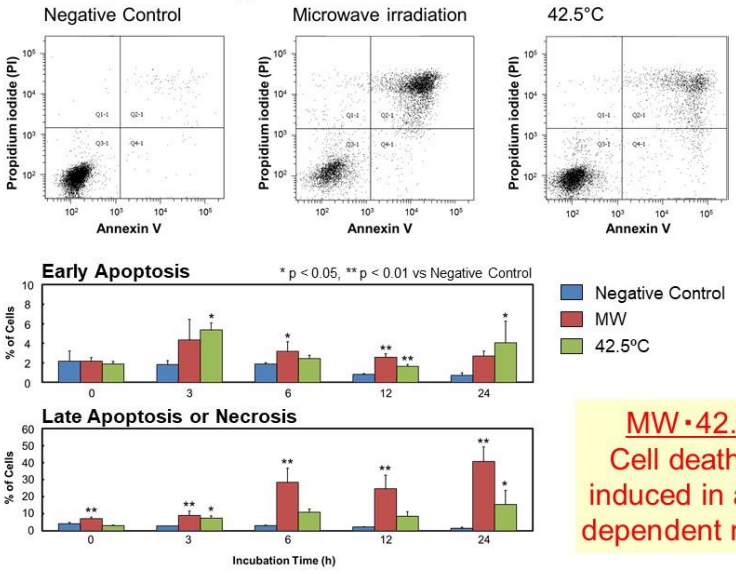
Incubation in CO_2 incubator for 0-24 h

Assays of cell death pathways
(Western blotting, ELISA assay, microscope observation, etc.)

Annexin V - Propidium Iodide (PI) assay

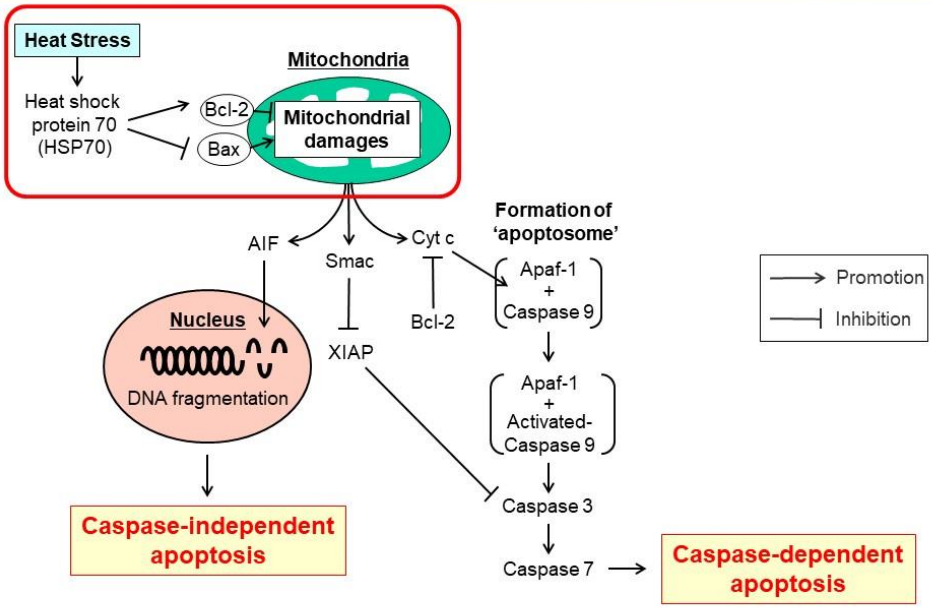
Asano M* et. al., *Sci. Rep.*, 7, 11406 (2017).

After 24 h following treatments



MW・42.5°C
Cell death was induced in a time-dependent manner.

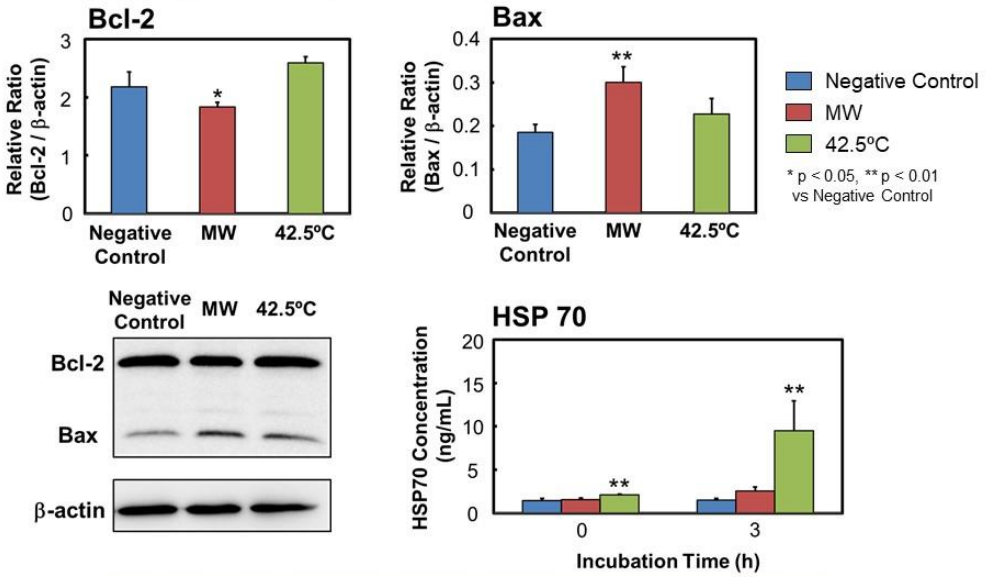
Caspases-related apoptotic pathways



Expression of Bcl-2 family and HSP 70 activation

After 3 h following treatments

Asano M* et. al., *Sci. Rep.*, 7, 11406 (2017).

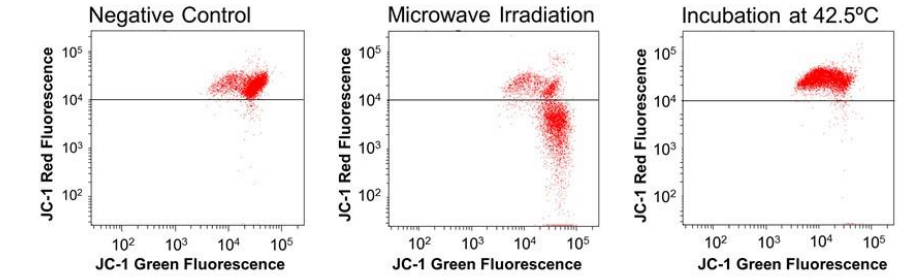


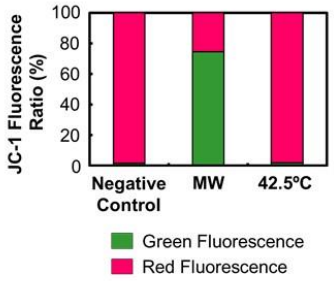
MW Bcl-2 family promoted mitochondrial damages.
42.5°C Thermal stress response was occurred.

Mitochondrial membrane potential (Δψm) assay

Asano M* et. al., *Sci. Rep.*, 7, 11406 (2017).

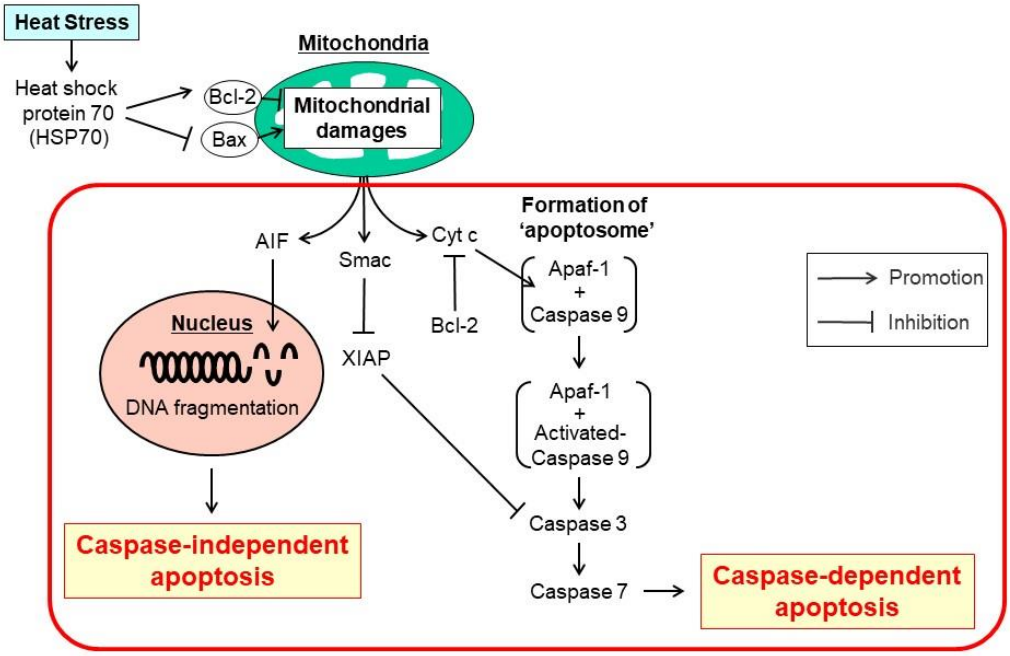
After 3 h following treatments





MW
Mitochondria was damaged.
42.5°C
No effect on Δψm

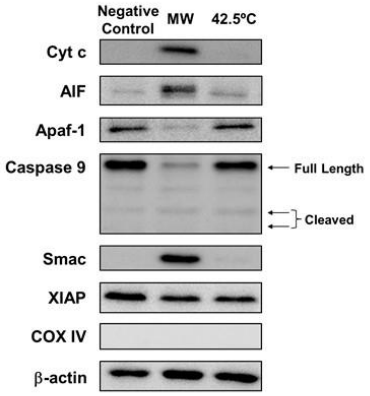
Caspases-related apoptotic pathways

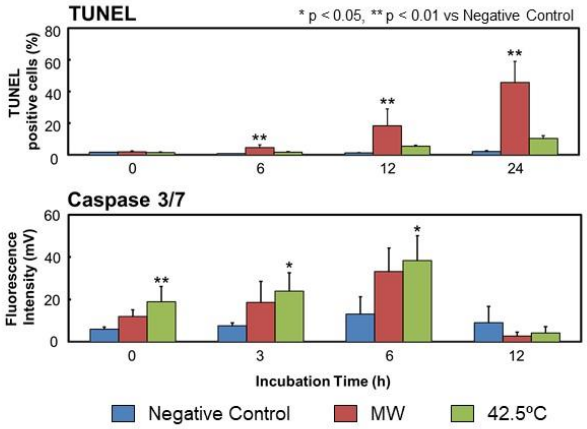


Caspase independent or dependent apoptosis

Asano M* et. al., *Sci. Rep.*, 7, 11406 (2017).

After 3 h following treatments (Cytosol extraction)

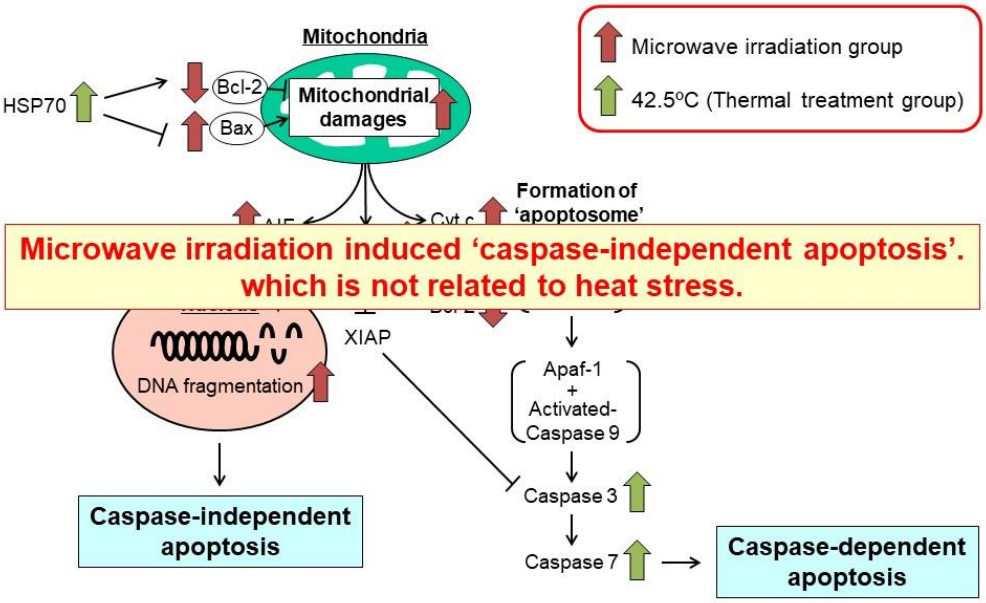




MW
AIF induced DNA fragmentation.
⇒Caspase independent apoptosis

Summary: apoptotic cell death pathways

*Asano M et. al., *Sci. Rep.*, 7, 11406 (2017).



Procedure: cell death assays

1×10^5 cells/mL

- T98G cells (Human glioblastoma multiforma tumor)
- MDA-MB-231 cells (Human breast adenocarcinoma)
- KATO III cells (Human gastric carcinoma)

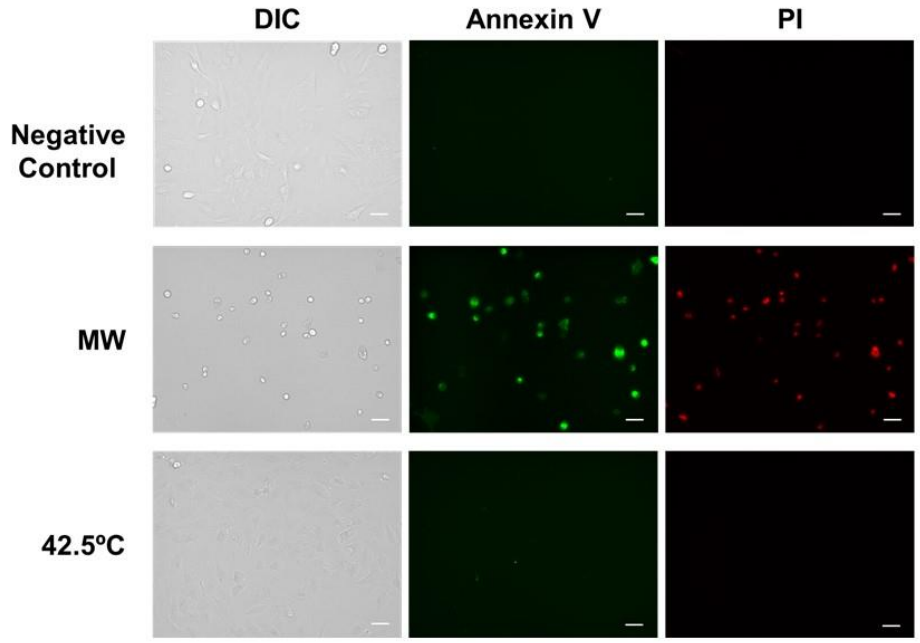
Negative control group	Microwave irradiation group	Thermal treatment group
Without any treatments	Kept at 37°C for 1 h (Applicator: 10°C)	Incubation at 42.5°C for 1 h (Most of cells are killed at 42.5°C.)

Incubation in CO_2 incubator for 0-24 h

Cell death assays
(Annexin V-PI assay, Caspase 3/7)

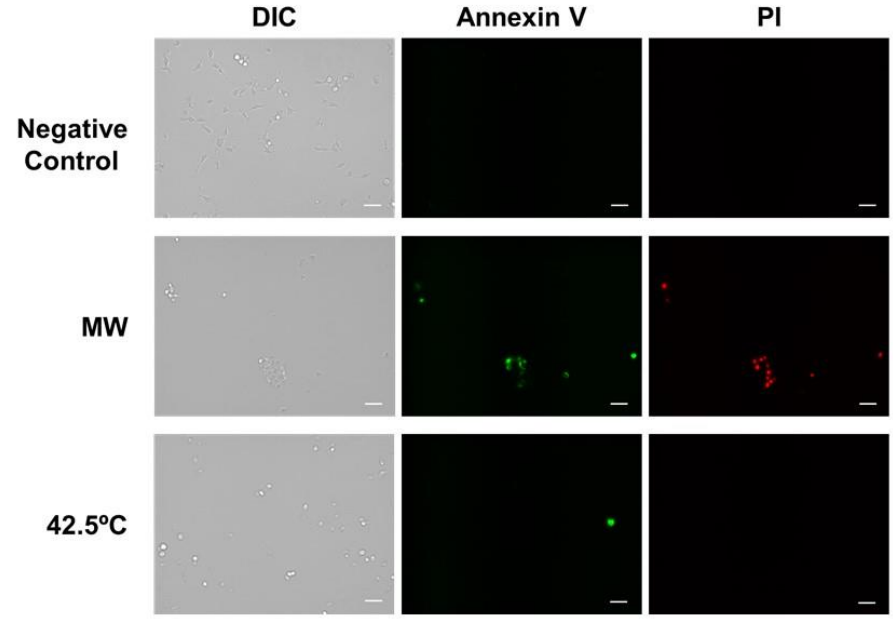
Annexin V-PI assay (T98G)

T98G



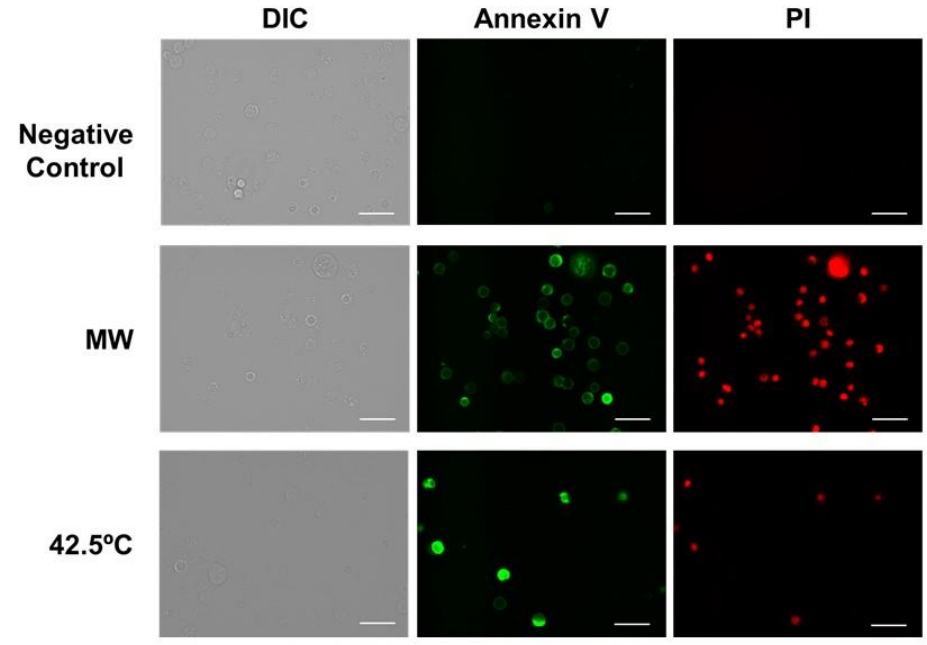
Annexin V-PI assay (MDA-MB-231)

MDA-MB-231



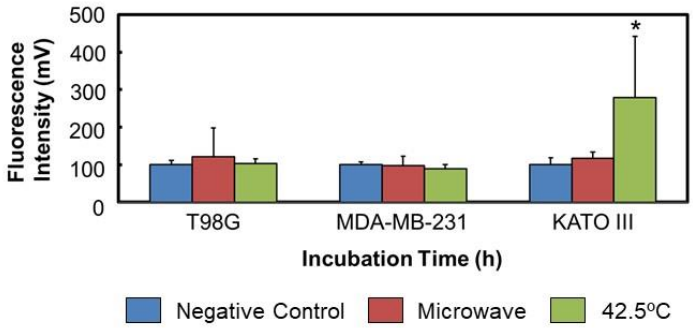
Annexin V-PI assay (KATO III)

KATO III



Caspase 3/7 assay

Caspase 3/7



Caspase 3/7 was…
- NOT activated by microwave irradiation.
- activated in KATO III cells only at 42.5°C.

Conclusion

Microwave irradiation

- HL-60 cells were killed through 'Caspase **independent** apoptosis'.
- T98G, MDA-MB-231 and KATO III cells might be killed through the similar pathway of HL-60 cells.

42.5°C (Thermal treatment group)

- Cells were killed through 'Caspase **dependent** apoptosis' .

Future works
- Cell death analysis for many types of cancer cells.
- Application to *in vivo* (e.g. xenograft mouse models).

Acknowledgements

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Thank you for your attention!