Radiosensitization effect of novel cancer therapy, mEHT ~Toward overcoming treatment resistance~

Yoshikata Matsumoto [1,2], Junki Hayshi[3], Yuta Senkino[1,2], Nobuyoshi Fukumitsu[1,2], Takashi Saito[1,2], Hitoshi Ishikawa[1,2] and Hideyuki Sakurai [1,2]

[1]Faculty of Medicine, University of Tsukuba
[2] Proton Medical Research Center, University of Tsukuba
[3]Academic Service Office for the Medical Sciences Area, University of Tsukuba

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Radiosensitization effect of novel cancer therapy, oncothermia ~Toward overcoming treatment resistance~

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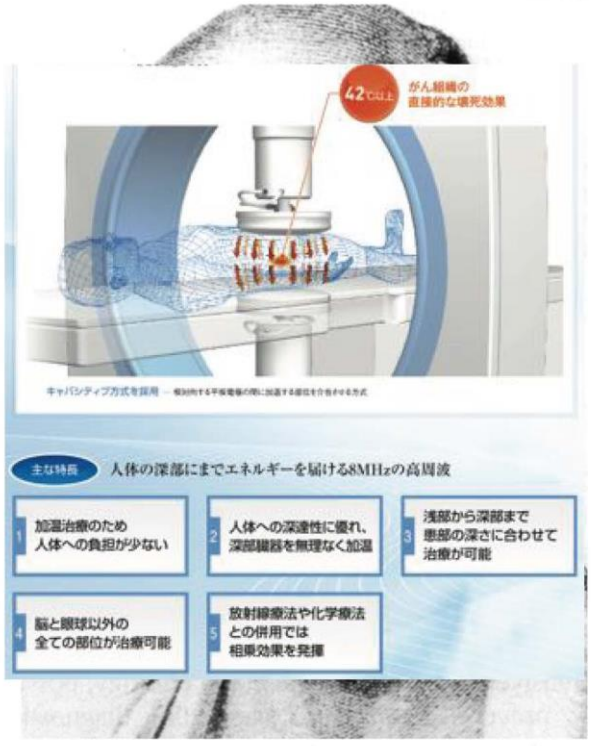
The history of hyperthermia in the cancer therapy



Hippocrates (BC 460-370)

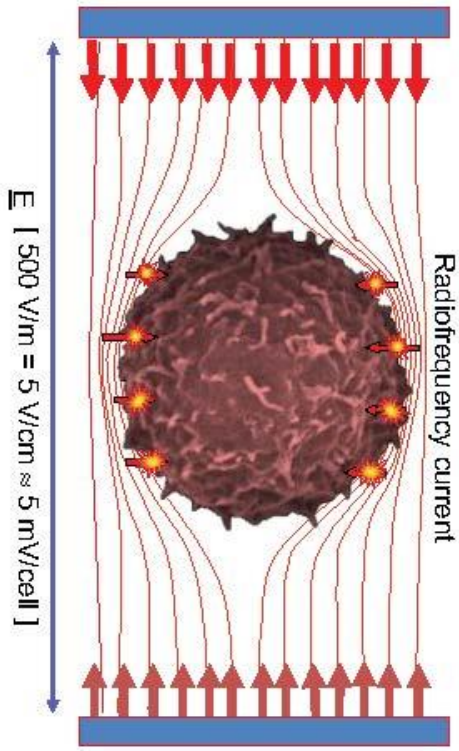
"Those who cannot be cured by medicine can be cured by surgery.
Those who cannot be cured by surgery can be cured by fire (hyperthermia).
Those who cannot be cured by fire, they are indeed incurable"

薬で治らなければ切り取ってしまえ。
それでもダメなら**火を使え。**
それでも治らないものは、決して治るものではない。（焼灼療法）

Heating by RF wave)
(Hyperthermia)

Medical treatment!

What is oncothermia?

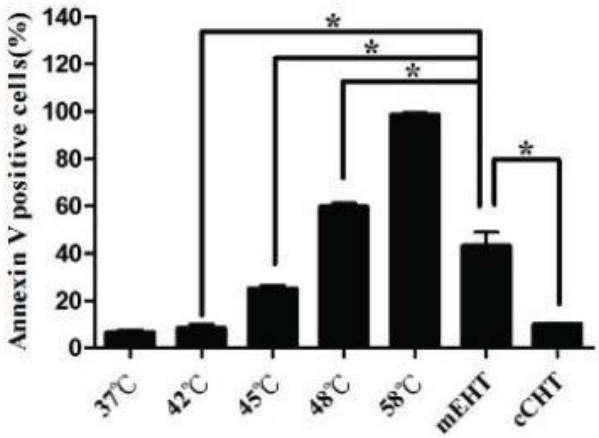


【Concepts and Features】

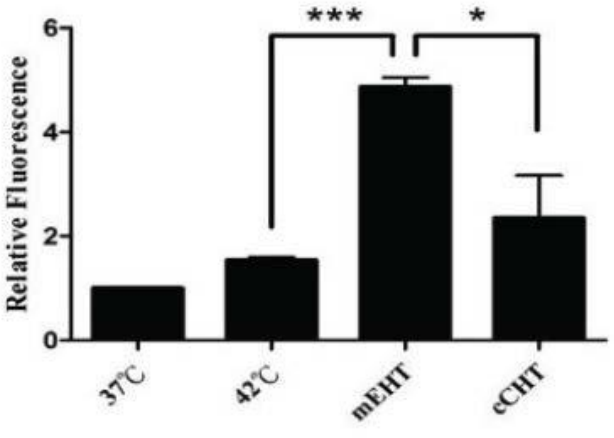
- **Attention to impedance matching**
  - ⇨ **Modulated 13.56 MHz** electromagnetic waves
  - ⇨ **Make thermal spot on the membrane of cancer cells (ununiform structure)**
- **Temperature independent factor (electromagnetic wave) is important**
- Therapeutic effects independent with high power
- **Max 150W** ⇨ Easy to control of patients
  - ⇨ Weak side effect
- Shield room is not necessary
  - ⇨ Easy to set up of machine
- **Potential of combination therapy**
  - ⇨ Immune activation affect

Biological effects by oncothermia

Induction of apoptosis



Production of ROS



Yang et al., Oncotarget 2016

Today's contents

Biological effects by **combination of oncothermia and radiation**

① **Cell killing effect** and **antitumor effect** (*in vitro* & *in vivo*)

② Effect on **radioresistant cells**

⇨ **Cyclic (Intermittent) hypoxic experience cells**

Oncothermia
EHY-2000

Oncotherm HP

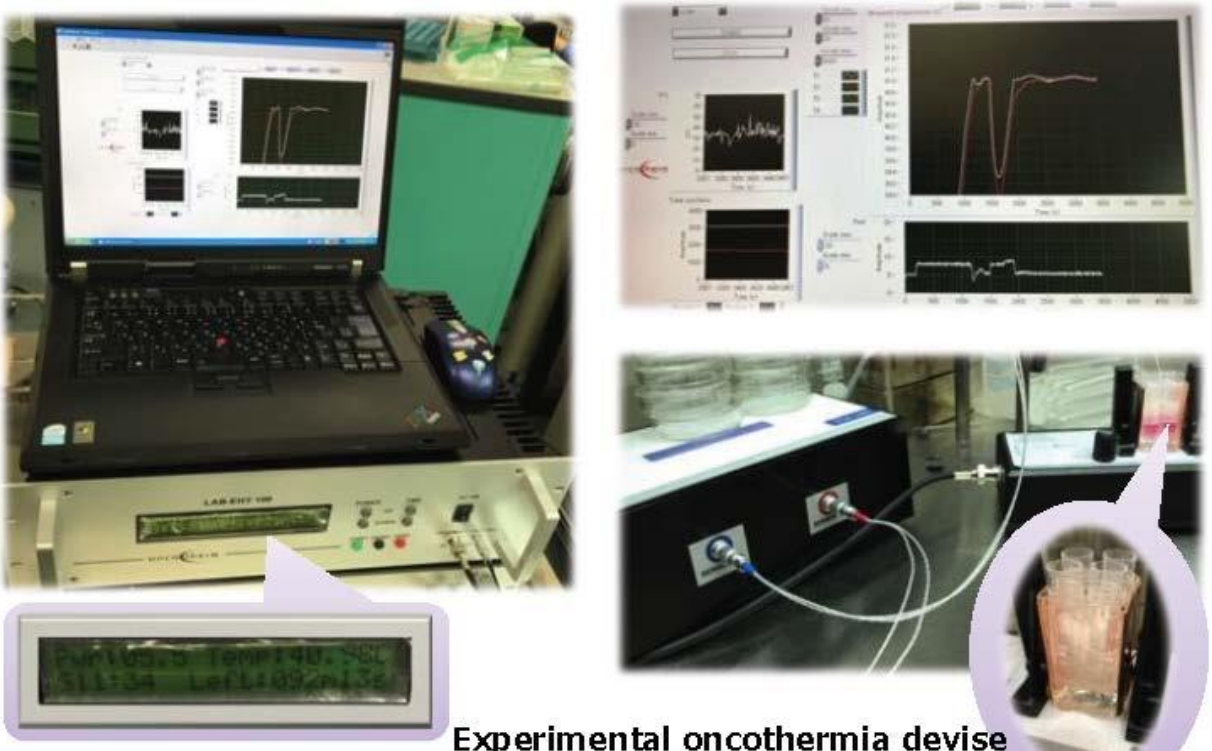
Purpose of this research

- **Comparison of the cell killing effect by Oncothermia (OT) with Water bass (WB)**
  - Time dependency
  - Temperature dependency
- **Radiosensitizing effect**
  - Cell killing effect
  - Cell death morphology
    - Apoptosis? Autophagy?
  - Cell cycle change
  - Antitumor effect (*in vivo*)

Materials and Methods *in vitro*

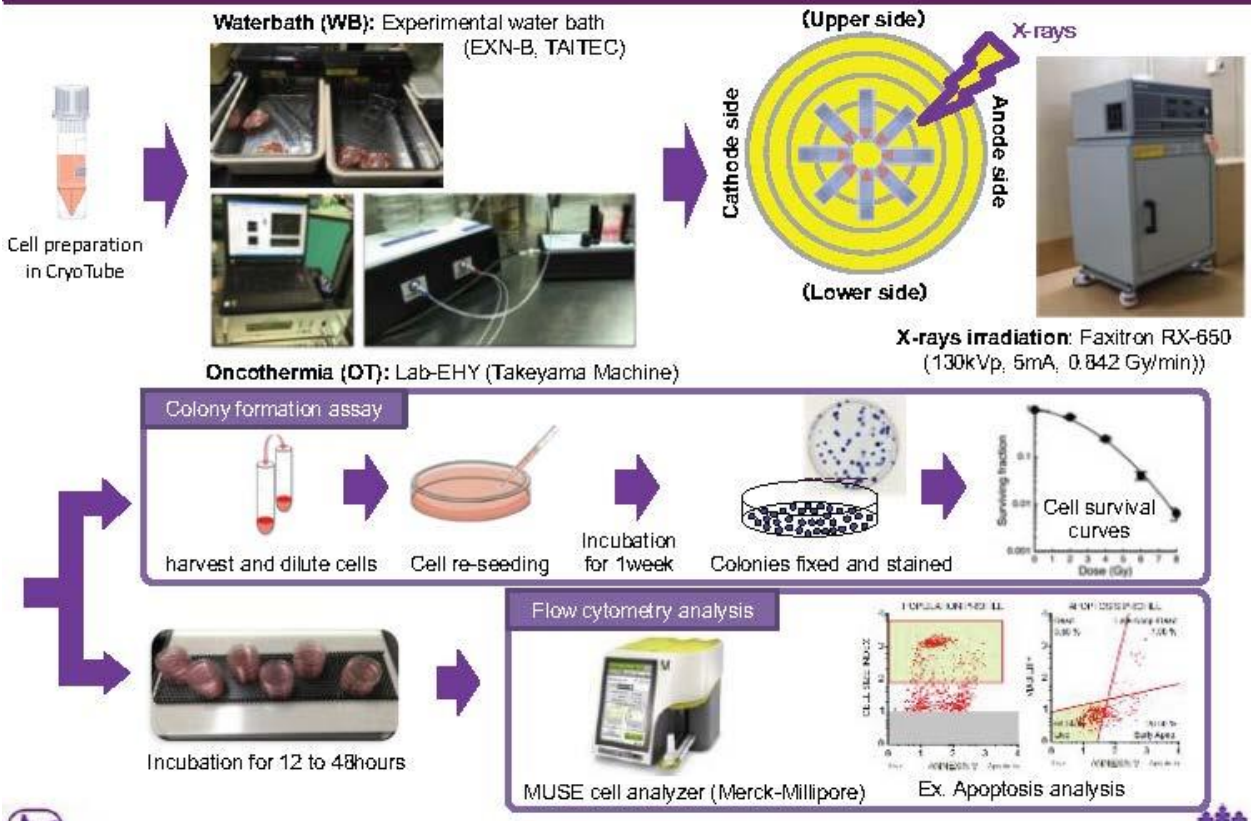
- **Cells** (frequently used in radiation research)
  - SCCVII: Chinese hamster skin squamous cell carcinoma cells
  - SAS: human oral squamous cell carcinoma cells
- **Culture condition**
  - Medium: E-MEM + 10% FBS + antibiotics
  - Incubator: 37℃, 5% CO_2
- **Treatment**
  - Water bath (WB): Experimental water bath
  - Oncothermia (OT): Lab-EHY 100 (Tateyama machine Corp.)
  - X-ray irradiation: Experimental X-ray generator (130 kV, 5mA)
- **Methods**
  - Cell death: Colony formation assay
  - Cell death morphology: Flow-cytometry technique
  - Cell cycle change: Flow-cytometry technique

Oncothermia treatment (*in vitro*)

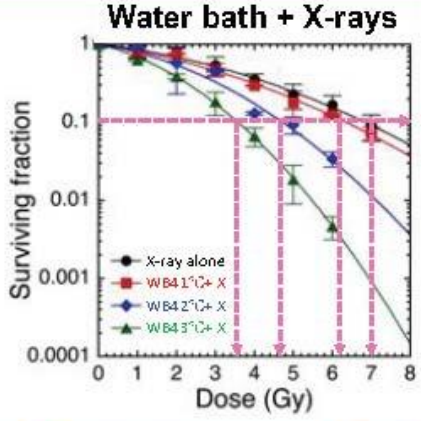


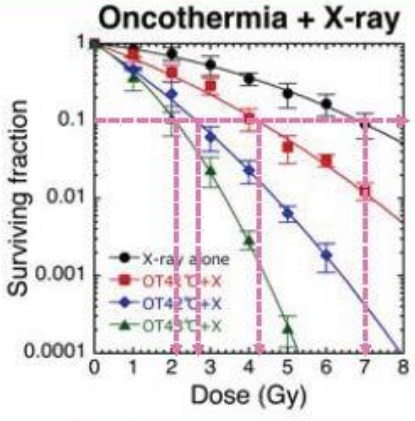
Experimental oncothermia devise
LAB-EHY 100

Experimental procedure (*in vitro*)



Result 2: Radiosensitizing effect of each thermal therapy





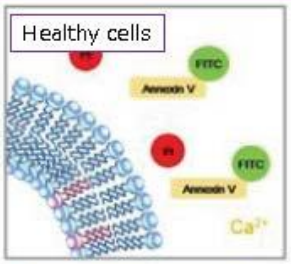
Treatment	D_{10} (Gy)*	TER**
X-ray	6.86	1.00
WB41℃+X-ray	6.35	1.08
WB42℃+X-ray	4.73	1.45
WB43℃+X-ray	3.58	1.91
OT41℃+X-ray	4.23	1.62
OT42℃+X-ray	2.64	2.59
OT43℃+X-ray	2.09	3.27

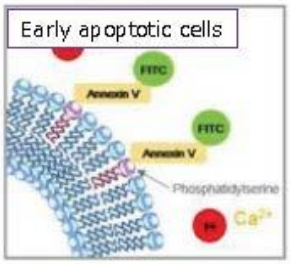
1.5倍
1.8倍
1.7倍

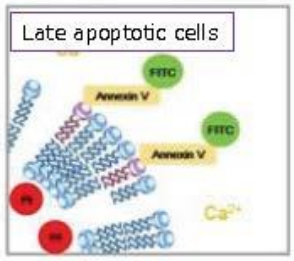
#*P*<0.01 (Student *t*-test)

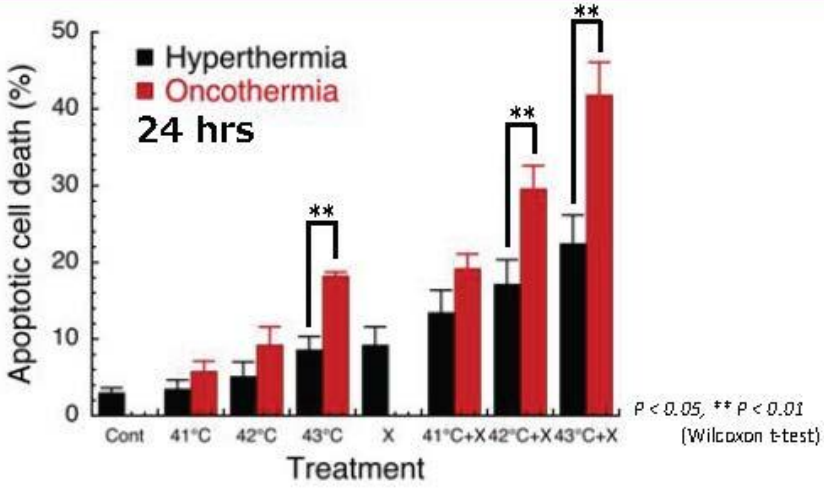
*D_{10}: biological equivalent dose introduce the 10% survival
**TER: Thermal Enhancement Ratio calculated with D_{10}(X-ray) / D_{10}(objective)

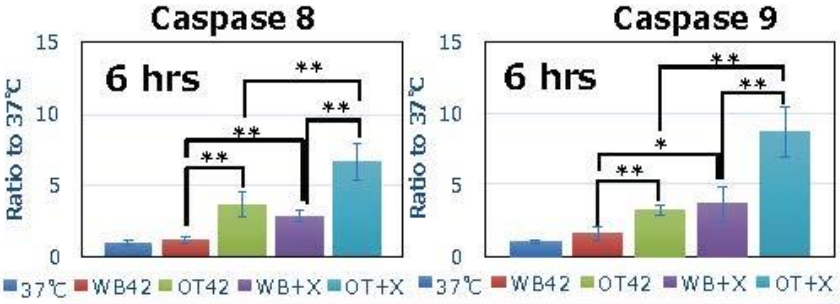
Result 3-1: Apoptosis frequency









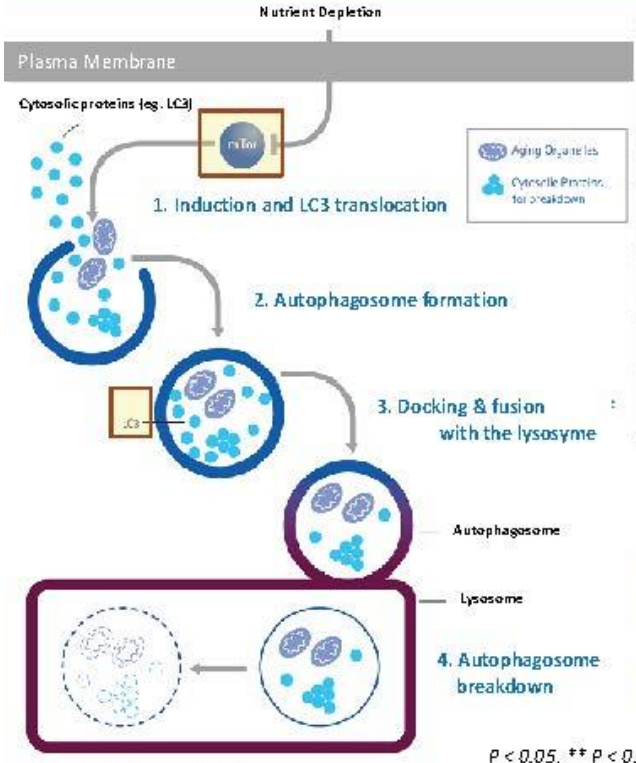


PMRC 2018/8/31

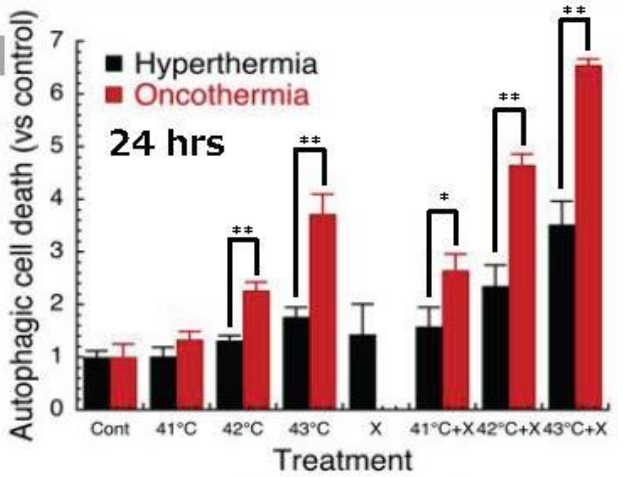
WB: Water bath 42℃, OT: Oncothermia 42℃, X-ray: 5 Gy

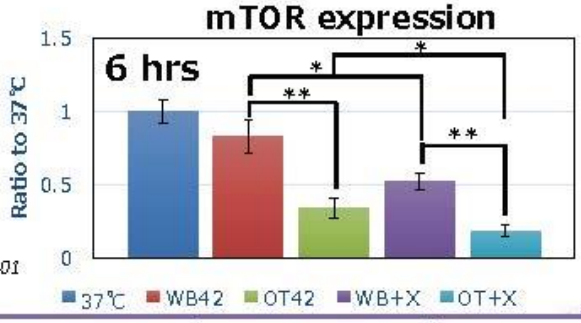
12

Result 3-2: Autophagy frequency



Merck-Millipore HPより





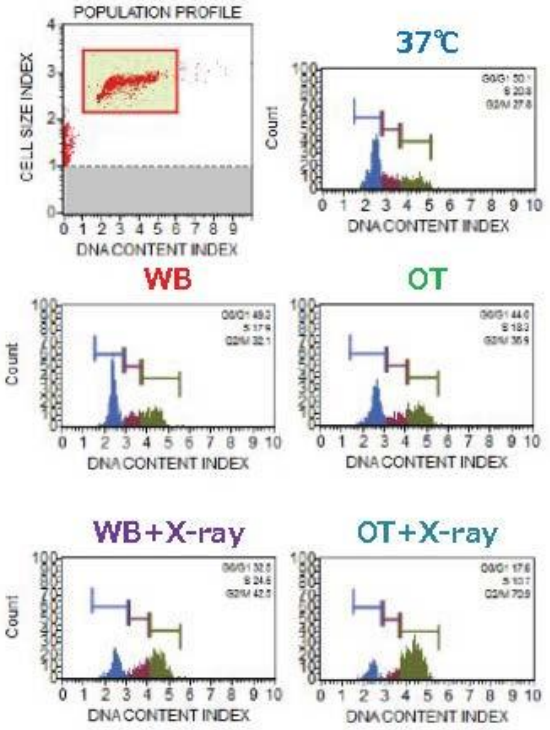
P < 0.05, ** P < 0.01
(Wilcoxon t-test)

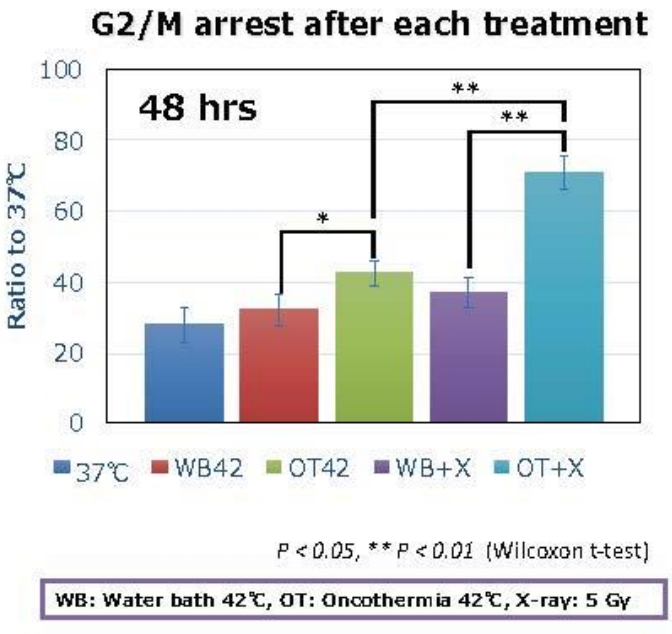
PMRC 2018/8/31

WB: Water bath 42℃, OT: Oncothermia 42℃, X-ray: 5 Gy

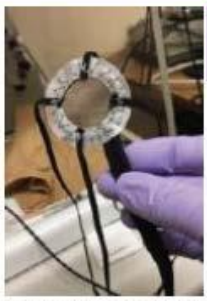
13

Result 4: Cell cycle change





Oncothermia treatment (*in vivo*)

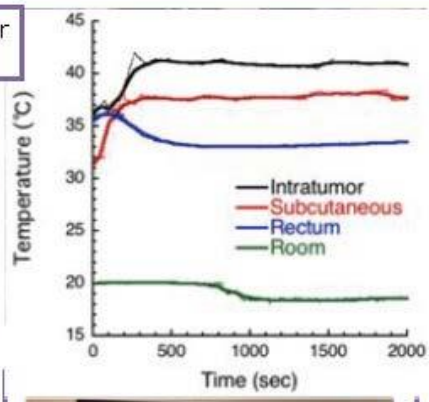


Electrode for small animal

Mouthpiece for inhalation for anesthesia

Anesthetic vaporizer (Isoflurane)

Temperature of subcutaneo directly above the tumo



Oncothermia machine LAB-EHY 100

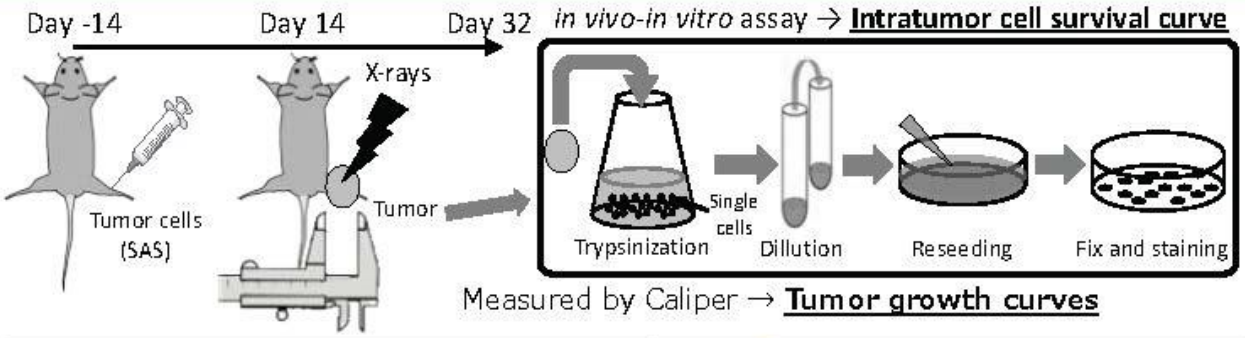
Fiber Optic Temperature Sensors

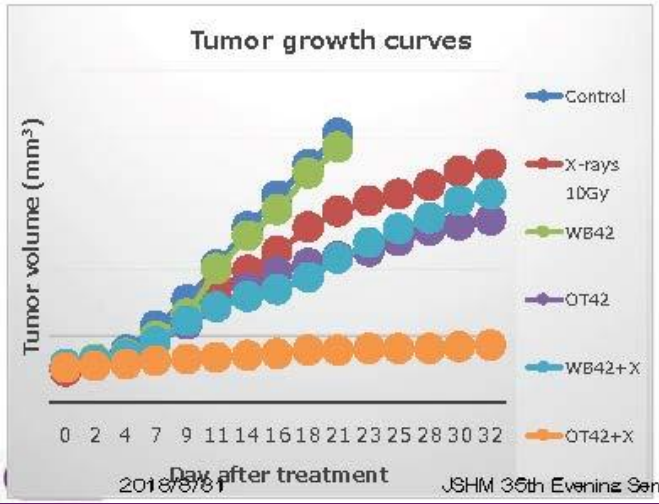
Temperature of tumor inside

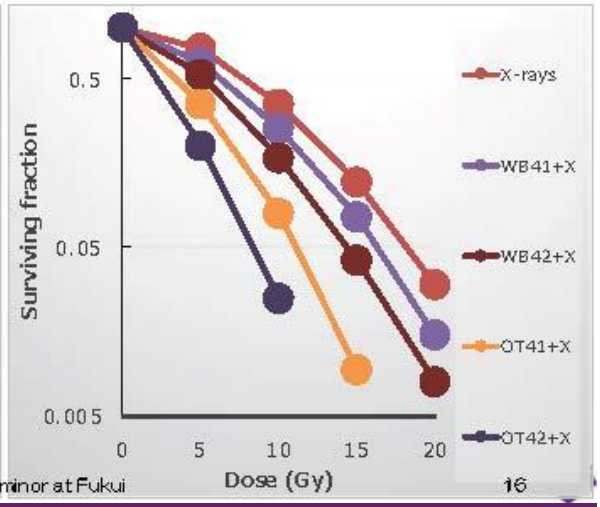
Heat retention of mouse (32-34℃)

Temperature of rectum

Result 5: Tumor growth and cell death *in vivo*







Summary 1

<Thermal therapy alone>

- Oncothermia induced cell death depending on the heating time and temperature.
- Oncothermia showed significant cytotoxic effects (at all temperature used in this study).

<Thermal therapy + radiation>

- Oncothermia showed remarkable radiosensitization effects.

<Cell death morphology, cell cycle>

- Apoptosis, autophagy frequency and G2/M arrest was significantly increased by OT and OT+X-rays.

<Antitumor effect>

- Tumor growth was suppressed and intratumor cell death was increased significantly by the OT and/or OT+X-ray

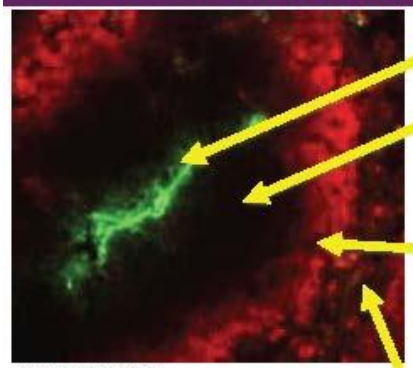
Today's contents

- Biological effects by **combination of oncothermia and radiation**
  - ① Cell killing effect and antitumor effect (*in vitro* & *in vivo*)
  - ② Effect on **radioresistant cells**
    - ⇨ **Cyclic (Intermittent) hypoxic experience cells**

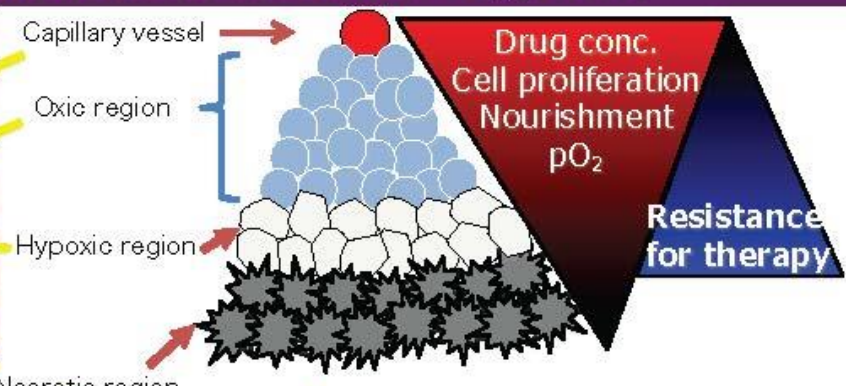
Oncothermia
EHY-2000

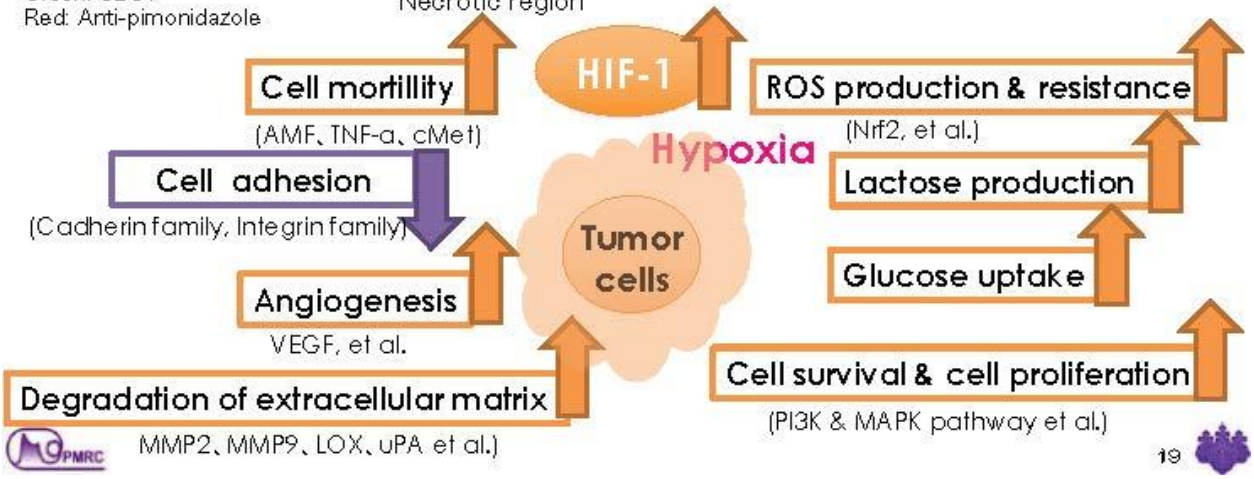
Oncotherm HP

Microenvironment in tumor: Hypoxia



Green: CD31
Red: Anti-pimonidazole



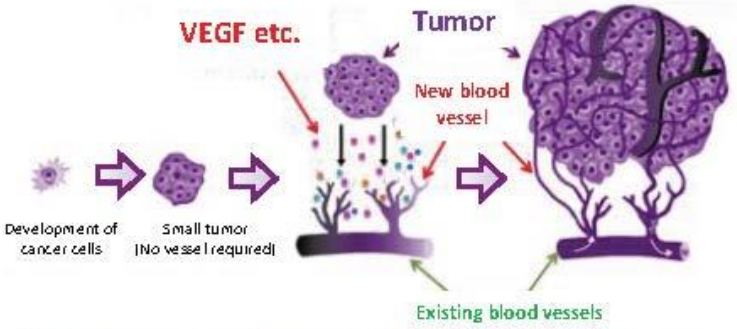


Intratumor vessel and hypoxia

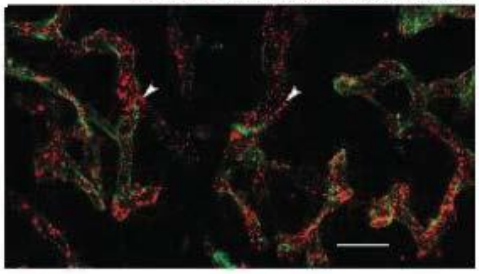
Tumors release angiogenic factors for proliferation and promote angiogenesis

Growth factors that promote angiogenesis are secreted from tumors

Tumor vessels ⇨ promote tumor growth and metastasis



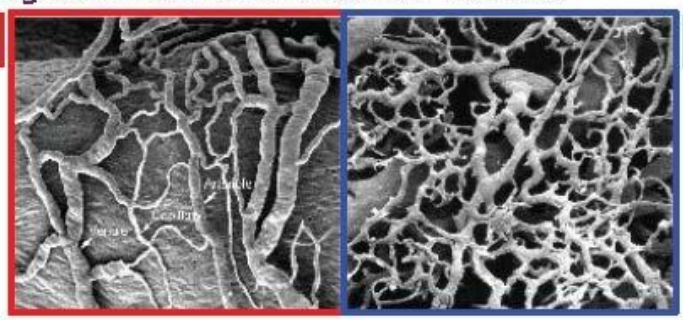
Tumor vessels (Red)
Vascular endothelial cells(Green)



D. McDonald et al., NatMed 2013

SEM images of normal and tumor vessels

Normal



Tumor

Vulnerable structure

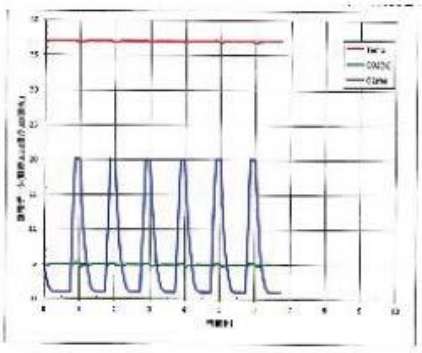
Repeated destruction and rebirth

Cyclic hypoxia

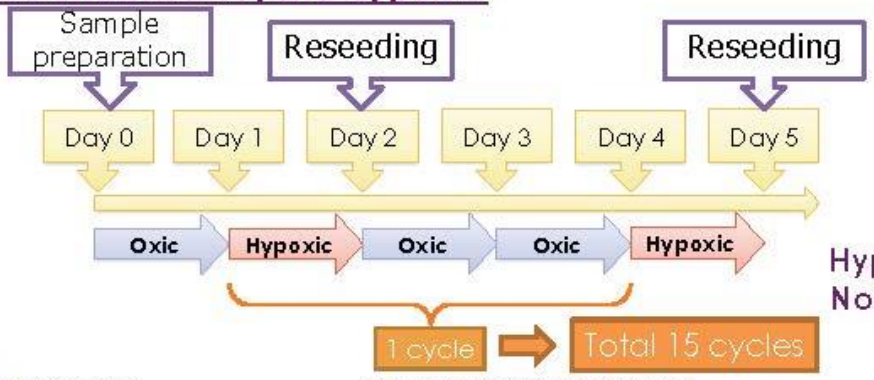
D. McDonald et al., NatMed 2013

Cyclic hypoxia treatment experiment

Cyclic hypoxia treatment



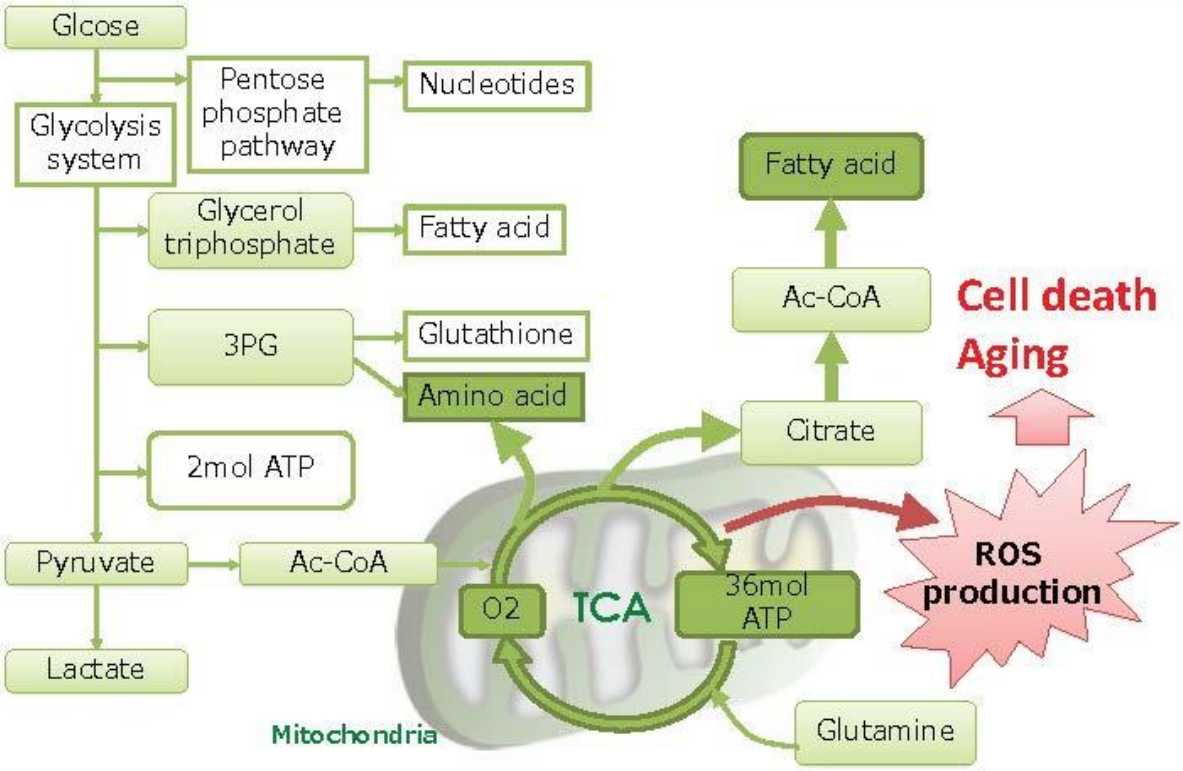
Exp. Scheme of cyclic hypoxia



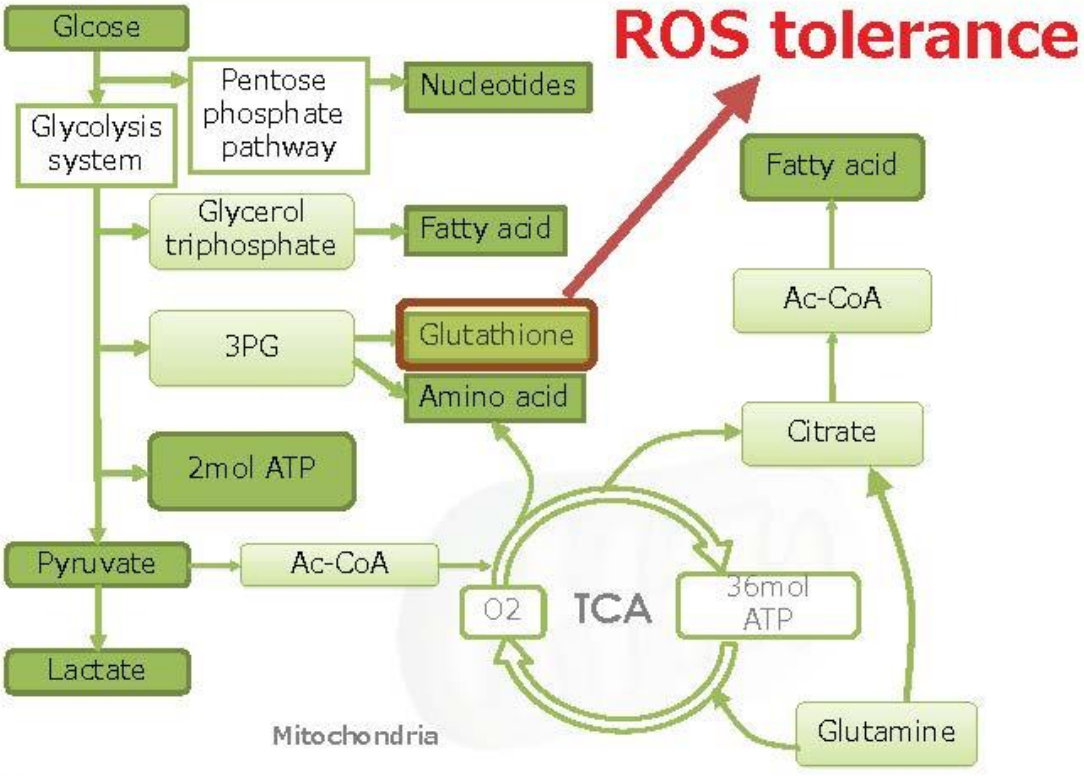
Hypoxia : 0.1% O_2

Normoxia : 20.9% O_2

Energy metabolism of "normal cells"

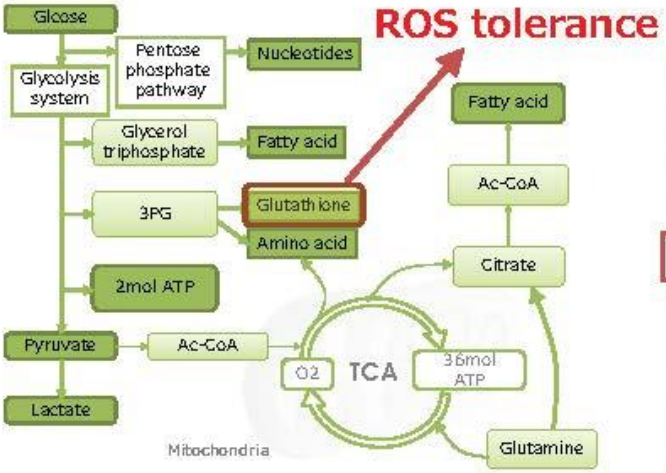


Energy metabolism of "cancer cells"

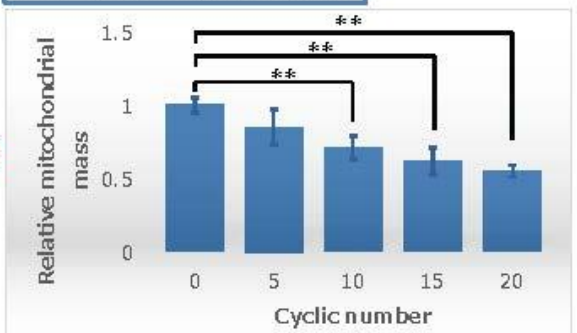


Acquisition of anaerobic glycolysis phenotype

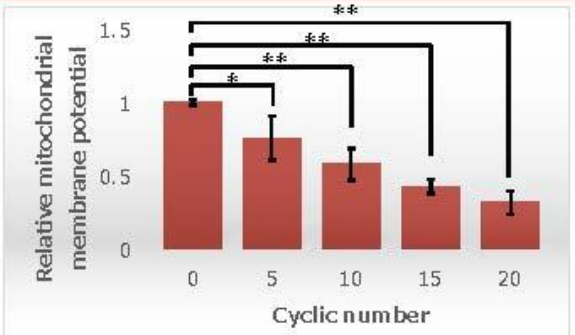
Dependency for mitochondria (TCA cycle)



Ⅰ. Mitochondrial mass

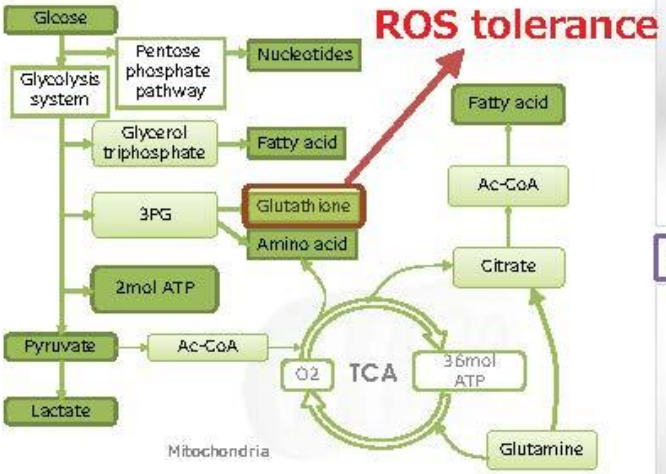


Ⅱ. Mitochondrial membrane potential

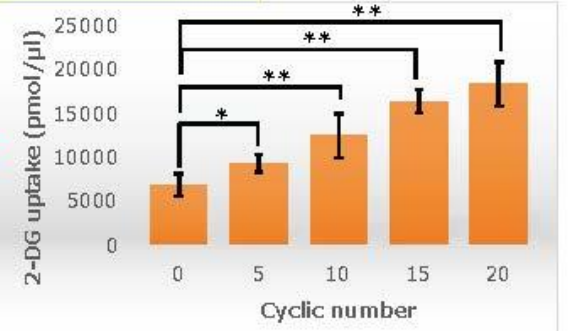


Acquisition of anaerobic glycolysis phenotype

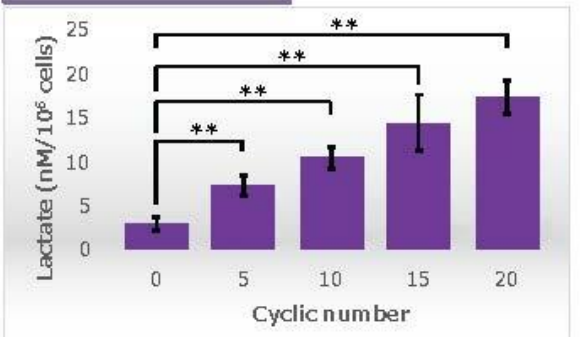
Dependency for anaerobic glycolysis system



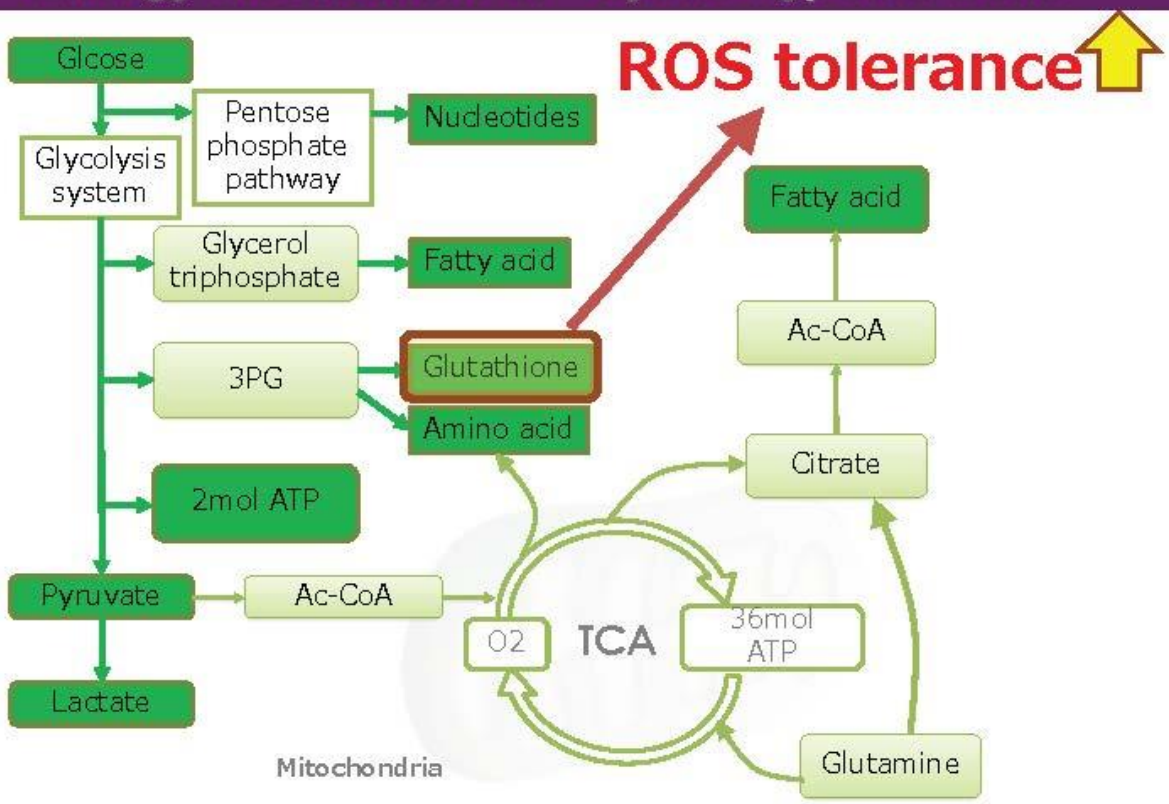
Ⅲ. Glucose uptake



Ⅳ. Lactate emission

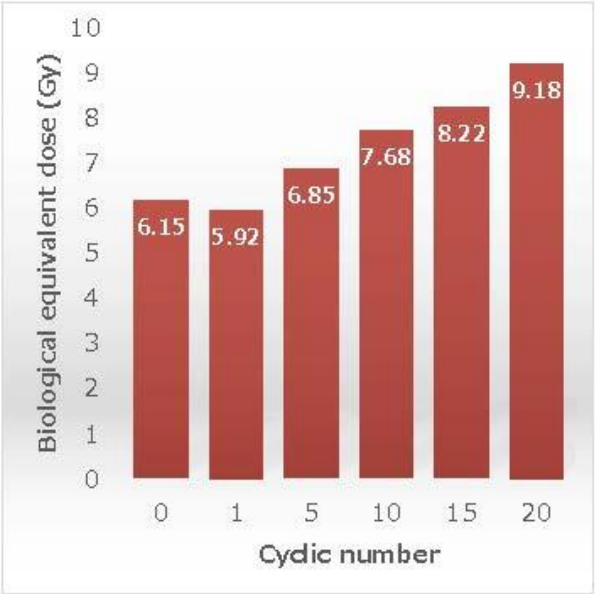


Energy metabolism of "cyclic hypoxia cells"

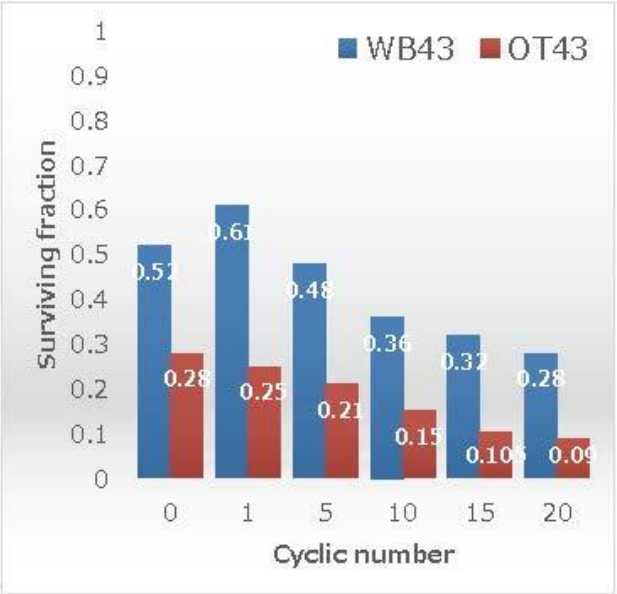


Resistance of cyclic hypoxia cells to radiation and heat

Radio-resistance



Thermal resistance



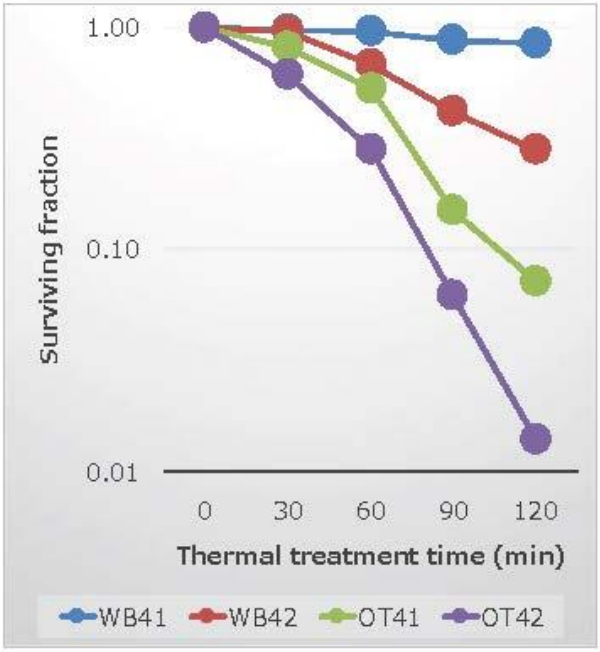
Cells: SAS (Human squamous cell carcinoma cell line)
X-ray: 130 kVp, 5 mA

Thermal therapy: 43℃-60min by WB & OT

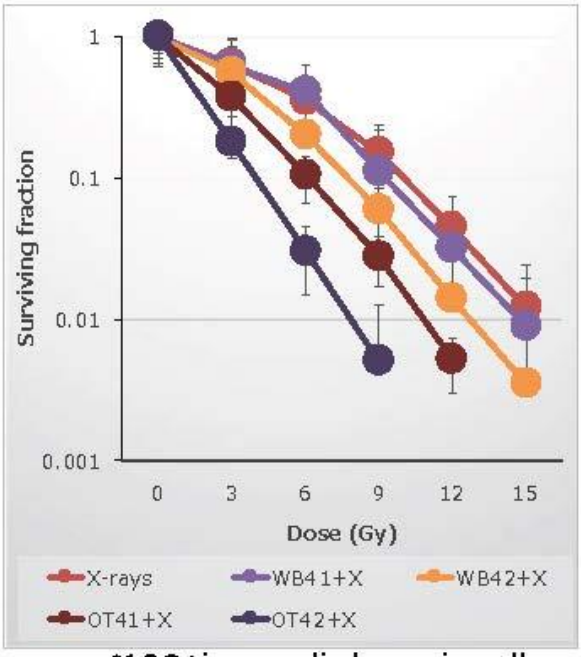
Thermal therapy is useful to radioresistant tumor cells!?

Cytotoxic effect of OT to cyclic hypoxia cells

Survival curves of cyclic hypoxia cells*1 after thermal therapy alone



Survival curves of cyclic hypoxia cells*1 after thermal and X-rays



*1 **20** time cyclic hypoxia cells
WB and **OT** treated for **60** min

Summary 2

<Cyclic hypoxia condition>

- Cyclic hypoxia enhanced the dependency to anaerobic glycolytic pathway.
- Decrease of mitochondrial mass and membrane potential.
- Increase of glucose uptake and lactose production.
- Increase of resistance to radiation but not thermal therapy.

<Biological effect of thermal to cyclic hypoxia cells>

- OT showed remarkable cytotoxic effect.
- OT showed remarkable radiosensitization effect compared with WB.

Conclusion and Future plans

- The cyototoxic effect, antitumor effect of a novel thermal therapy, **oncothermia (OT) was stronger** than water bath (WB) hyperthermia therapy.
- **OT may be effective** for cells that acquired treatment resistance by cyclic (intermittent) hypoxic environment involving recurrence etc..
- Elucidation of the **mechanism** of antitumor effect and analysis of **normal tissue response** is the most important issue in clinic. We must examine the oncothermal effect to normal tissue using in vivo model.
- It is necessary to **compare** the difference between OT and **conventional RF-hayperthermia** (ex. RF-8).

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Thank you for your attention!!
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