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Research Article

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Suppression of Prostate Cancer Cell Growth by Mid-Infrared Rays

Junko Shima[1*], Seiji Igarashi[1]

[1]*SHIMA Institution for Quantum Medicine*

[*]Corresponding Author
Junko Shima, 2-chōme-10-2 Sakuragaoka, Minoo, Osaka 562-0046, Japan. Tel: 072-720-0550, Fax: 072-720-0520.

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Abstract

Experiments using rubber/resin foam (RB) have already shown that far-infrared rays suppress the proliferation of cancer cells. We will report as a follow up to these findings. When RB was used, the temperature of the culture solution increased by about 0.4°C. This time, with a focus on this temperature rise, a new heating plate was manufactured, and culture experiments were conducted without using RB. It was determined that the suppression of growth was because of heat transfer, not due to the special action of RB. The distribution of heat was calculated using Planck's formula, and it was speculated that mid-infrared rays around 4.8 μm are involved in the inhibition of cancer cell proliferation. Furthermore, irradiation with a 4.8 μm mid-infrared free electron laser confirmed that the ratio of α-helix to β-sheet changed, providing corroborative evidence for growth suppression.

Keywords: Prostate Cancer, Mid-Infrared Rays, Suppression of Proliferation of Prostate Cancer Cells

Introduction

In a culture experiment of prostate cancer cells in which RB was placed above and below the culture dish, it was reported that the apoptotic pathway of the prostate cancer cells was activated, leading to inhibition of proliferation and death of the prostate cancer cells1.When human prostate cancer cells (DU145, PC-3 and LNCaP) were cultured with the temperature in the incubator set at 37°C, the temperature of the culture medium without RB was 37.32°C and the temperature of the culture medium with RB was 37.68°C. The temperature difference of 0.36°C was confirmed [1].

It was suggested that the inhibition of cancer cell growth and death was a specific action of RB, but it was difficult to directly confirm this, so we focused on the temperature difference that occurred at that time.

It was thought that there was heat generation during cancer cell proliferation, and that heat was reflected by RB, resulting in a temperature difference in the culture solution. It was to be confirmed whether cancer cell growth could be suppressed by realizing this temperature difference without using RB.

Culture Experiment Focusing On Temperature Difference

One of the incubators was set to 37.0°C and the other to 37.4°C to grow cancer cells and epithelial cells. Since the temperature of the incubator can only be set in units of 0.1°C, the cells were cultured at 37.4°C. Culture temperature was measured using a digital thermometer (OMRON, Kyoto, Japan).

For both culture experiments, counting was performed by the XTT method on the 28th day of culture. As a result, compared with the cells cultured at 37.0°C, the numbers of both cancer cells and epithelial cells were significantly increased in the cultured cells at 37.4°C, and no suppression of cancer cell growth was confirmed.

Culture Experiments using a Heating Plate

A heating plate (length, width, height: 30x30x10cm³) was manufactured to maintain the temperature of the culture solution at 37.68°C. The heating plate consists of an aluminum plate with a nichrome wire attached to the back and an automatic temperature control device. The temperature uniformity of the heating plate is ±0.2°C. As a result of trial and error, it was possible to keep the culture solution at 37.68°C by setting the temperature of the incubator to 35.0°C and the temperature of the heating plate to 40.1°C.

First, cancer cells (DU145, PC-3 and LNCaP) were cultured at 37.0°C, 5% CO2 for 7 days, and then some cancer cells were placed in an incubator (35.0°C) equipped with the heating plate (40.1°C). The culture dish was placed on a heating plate, and culture was continued for 5 days. Other cancer cells were similarly cultured for 5 days at 37.0°C and 5% CO2.

The number of surviving cells was compared by the XTT method before the cancer cells were migrated (day 7) and on the 5th day after the migration. The cell number is expressed as the absorbance of the XTT method. As a result, the growth inhibition rate of cancer cells reached an average of 77.4% (DU145: 74.0%, PC-3: 85.3%, LNCaP: 71.8%) (Fig. 1).

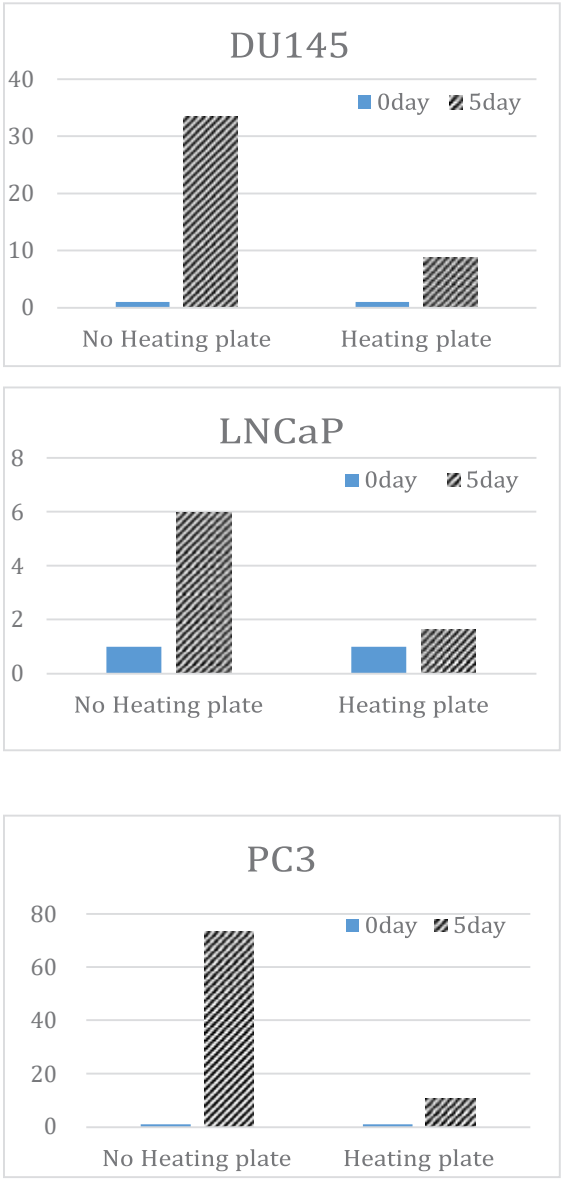

Figure 1: Growth rate of cancer cells when using a heating plate

Vertical axis: ratio of number of cells on day 5 based on the number(1) of cells on day 0. Horizontal axis: without heating plate and with heating plate, DU145: The number of cells increased by 33.52 and 8.72 LNCaP: The number of cells increased by 5.99 and 1.62 PC3: The number of cells increased by 73.23 and 10.80

Mid-Infrared Free Electron Laser Irradiation

In the culture experiment[1] using RB, it is thought that the temperature of the culture solution rose due to the reflection of heat (electromagnetic waves) by RB. In culture experiments using a heating plate, the transfer of heat from the heating plate was considered to be related to inhibition of growth. Therefore, we decided to conduct an experiment in which heat (energy) is directly applied to cancer cells using a mid-infrared laser. Since heat (energy) is transferred through the culture dish, the electromagnetic wave characteristics of the culture dish were confirmed in advance (Fig. 2). Based on the characteristics of the graph, we decided to irradiate at wavelengths of 4.8 μm and around that (4.5 μm, 5.4 μm, 5.9 μm).

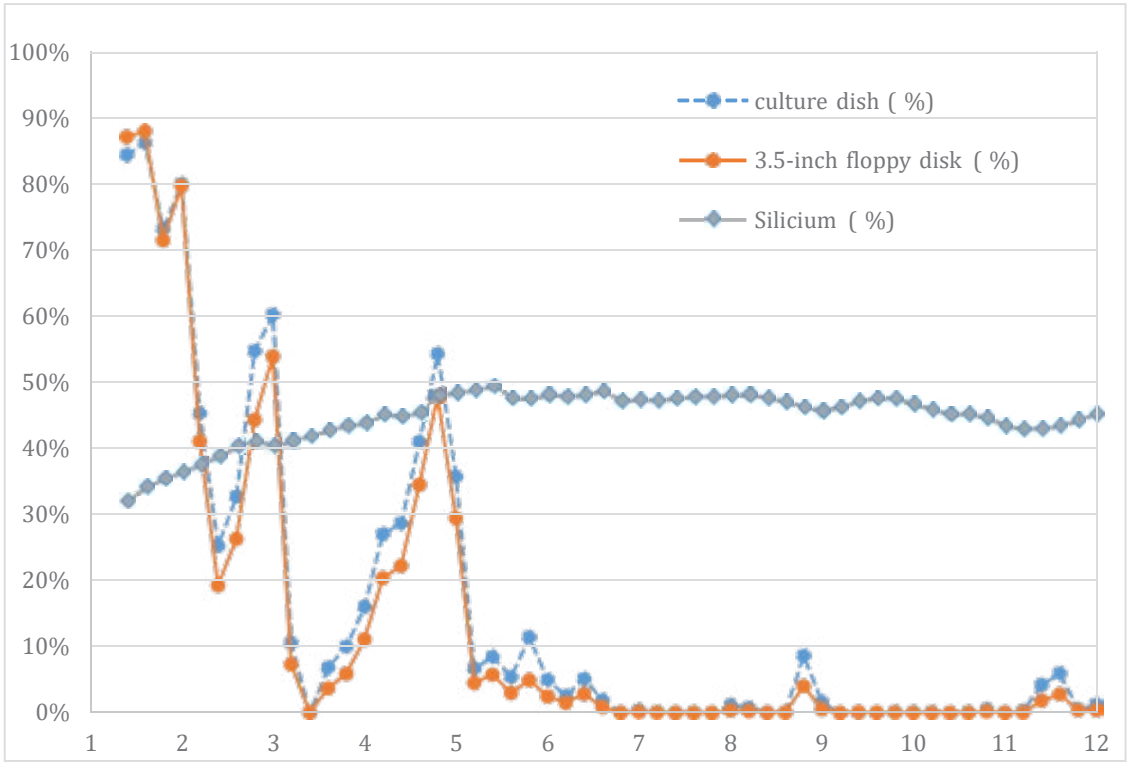

Figure 2: Transmittance of culture dish (polystyrene)
Vertical axis: transmittance (%), Horizontal axis: wavelength (μm)

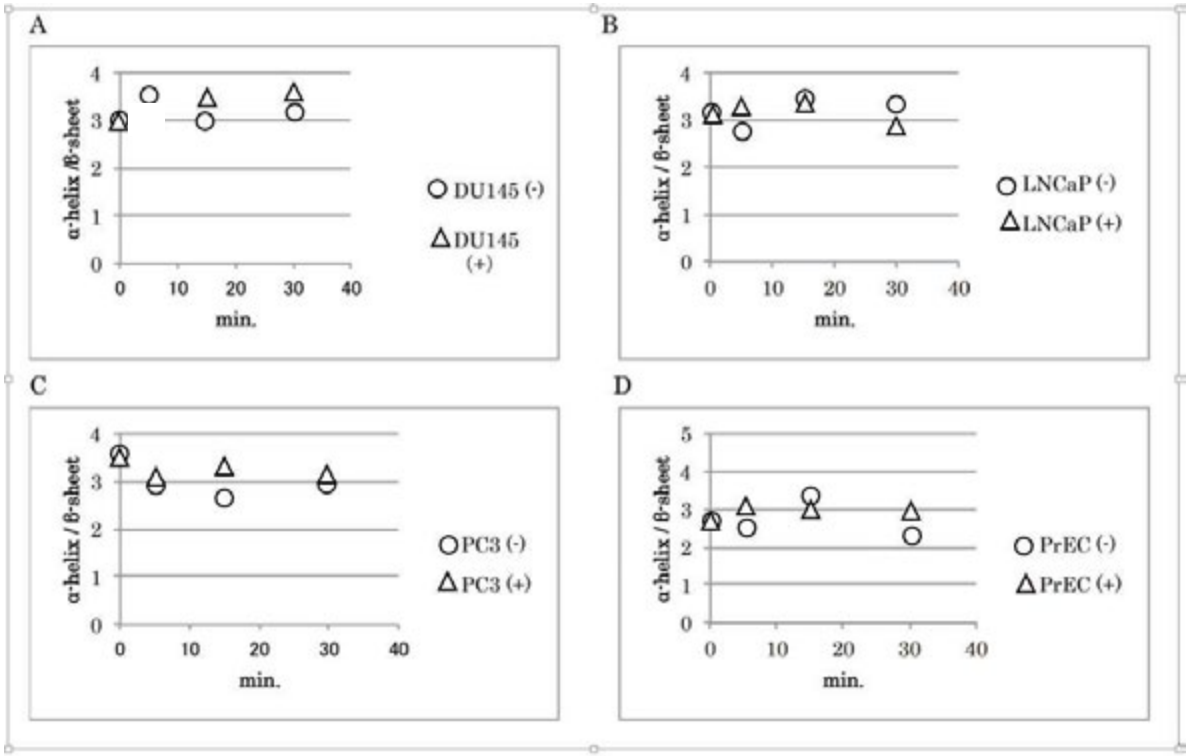

Figure 3: The ratio of α-helix to β-sheet of DU145, LNCaP, PC3 and PrEC Plus (+) stands for irradiated samples and minus (-) stands for samples that weren't irradiated.
Vertical axis: ratio Horizontal axis: irradiation time

A mid-infrared free electron laser (mid-infrared FEL)-TUS (Tokyo University of Science) was used to irradiate human prostate cancer cells (DU145, LNCaP, PC3) and epithelial cells (PrEC) at 4.8 μm (2100 cm^{-1}, 5 pulses per second, typically 40 mW) and was applied for 5, 15 and 30 minutes. After irradiating each set of cells (typically 5×10^7 cells) individually, each Eppendorf tube (SIGMA-ALDRICH, St. Louis, USA) was centrifuged at 500 g for 5 minutes, and 300 μl of medium was carefully replaced with isotonic D2O containing 0.9% NaCl. The tube was then centrifuged again, the supernatant was removed, and the precipitate was extracted onto a CaF2 glass. Cell clumps on CaF2 glass were focused under a transmission microscope, 100 scans were made at a resolution of 4 cm, and Fourier transform infrared spectra were analyzed with an FT/IR spectrophotometer (FT/IR-615) (Jasco International Co. Ltd. Tokyo, Japan) (Fig. 3). Similarly, cancer cells were irradiated with mid-infrared lasers with wavelengths of 4.5 μm, 5.4 μm, and 5.9 μm for 5, 15, and 30 minutes, respectively, and measured in the same manner as the 4.8 μm irradiation.

In experiments irradiated with a 4.8 μm mid-infrared laser, the α-helix to β-sheet ratio in both DU145 and PC3 was greater than that in the non-irradiated control at 15 and 30 minutes. Both LNCaP and PrEC ratios were greater at 5 minutes. The α-helix to β-sheet ratio in DU145, LNCaP and PC3 did not change after 30 min irradiation with FEL at other oscillation wavelengths (4.5, 5.4, 5.9 μm) (data not shown). This fact suggests that mid- infrared laser irradiation at around 4.8 μm changes the protein structure and indirectly supports the apoptotic event in these cancer cells and suppresses their growth.

Discussion

Factors of Inhibitory Effect on Cancer Cell Growth

It can be seen that the growth of cancer cells increases the temperature by 0.32° C. relative to the ambient temperature (incubator, 37.0° C.). Considering the effect of the RB, it is thought that the thermal radiation due to this temperature rise was reflected by the upper and lower RBs and was constantly irradiated to the cancer cells, which affected their proliferation.

In the case of the heating plate, the ambient temperature of the culture dish was 35.0°C, but continuous heat transfer from the bottom maintained the culture solution at 37.68°C which is believed to have affected the growth of the cancer cells.

In the culture experiment where the incubator was set to 37.4°C, the temperature of the culture dish increased, but it was found that there was no thermal radiation (heat absorption by the cancer cells) from the surroundings of the culture dish (incubator, 37.4°C).

Based on the above considerations, it is believed that continuously applying heat to the cancer cells in the culture medium will have an effect on the cancer cells.

In experiments using RB, the continuous thermal radiation to cancer cells in the culture dish is thought to be influenced by the transparency of the thermal radiation of the culture dish itself, in addition to the reflection by RB. In the case of heating plate, heat is not only transferred to the culture dish by thermal conduction, but, we can consider, also by the effect of thermal radiation penetrating the culture dish.

Effects of electromagnetic waves

Since heat (energy) is transmitted (radiated) as electromagnetic waves, when RB is used, it is thought that the reflectance of electromagnetic waves by RB and the electromagnetic wave transmittance of the culture dish (polystyrene) are related. The reflectance of RB has already been measured [1]. The transmittance of the culture dish was as shown in Fig.2. When using RB, the heat generated by the cancer cells themselves penetrates the culture dish, is reflected by the RB, penetrates the culture dish, and irradiates the cancer cells themselves.

When using a heating plate, the heat of the heating plate is not only transferred to the culture medium via the culture dish by heat conduction, but also electromagnetic waves from the heating plate are transferred to the cancer cells through the culture dish.

So, Planck's formula was used to investigate the spectral characteristics of both.

$$E(T,\lambda) = \frac{8*\pi*h*c}{h^5}\left(\frac{1}{\exp\left(\frac{n*c}{\lambda*k*T}\right)-1}\right)*10^2 \text{【nJ/m}^3\text{/0.1μm】}$$

h	Planck's constant	$6.62607004*10^{-34}$J*s
c	Speed of light	299792458 m/s
K	Boltzmann constant	$1.38064852*10^{-23}$J/K
T	temperature (K)	
λ	Wavelength (μm)	

When using RB

The culture medium was mostly water, and the emissivity of water was assumed to be 0.96. The heat distribution of the culture solution is as follows.

$$E(37.68, \lambda)*0.96 \qquad (F1.1)$$

Radiation from the culture medium is radiated into the space within the culture dish above. Heat is transferred downward to the culture dish by thermal conduction.

The culture dish (upper) (37.0°C) receives radiation from the culture medium (37.68°C) at 96% intensity, A portion of this radiation that has passed through the culture dish (polystyrene) (transmittance = α) is reflected by RB (reflectance = δ), passes through the culture dish again, and is radiated to the culture solution. (δ = 0.95.)

$$E(37.68,\lambda)*0.96*\alpha*0.95*\alpha \qquad (F1.2)$$

The culture dish (bottom) is at 37.68°C due to heat conduction from the culture solution (37.68°C).

Then radiant heat is emitted to the gap (space) between the RB and the culture dish and is reflected by the RB.
Assuming that the transmittance of the culture dish (polystyrene) is α and the reflectance is 0, the emissivity is (1-α). (Reflectance + Transmittance + Absorptivity = 1.0, Absorptivity = Emissivity)

Radiation from the culture dish (bottom) to the RB below is reflected by the RB, transmitted through the culture dish (bottom), and radiated into the culture medium.

$$E(37.68,\lambda)*(1-\alpha)*0.95*\alpha \quad (F1.3)$$

The ratio of the total heat distribution reflected by RB (F1.2 + F1.3) to the heat distribution of the culture medium (F1.1) was calculated. The results of this calculation are shown in Fig.4 (vertical axis: energy intensity and ratio, horizontal axis: wavelength in μm).

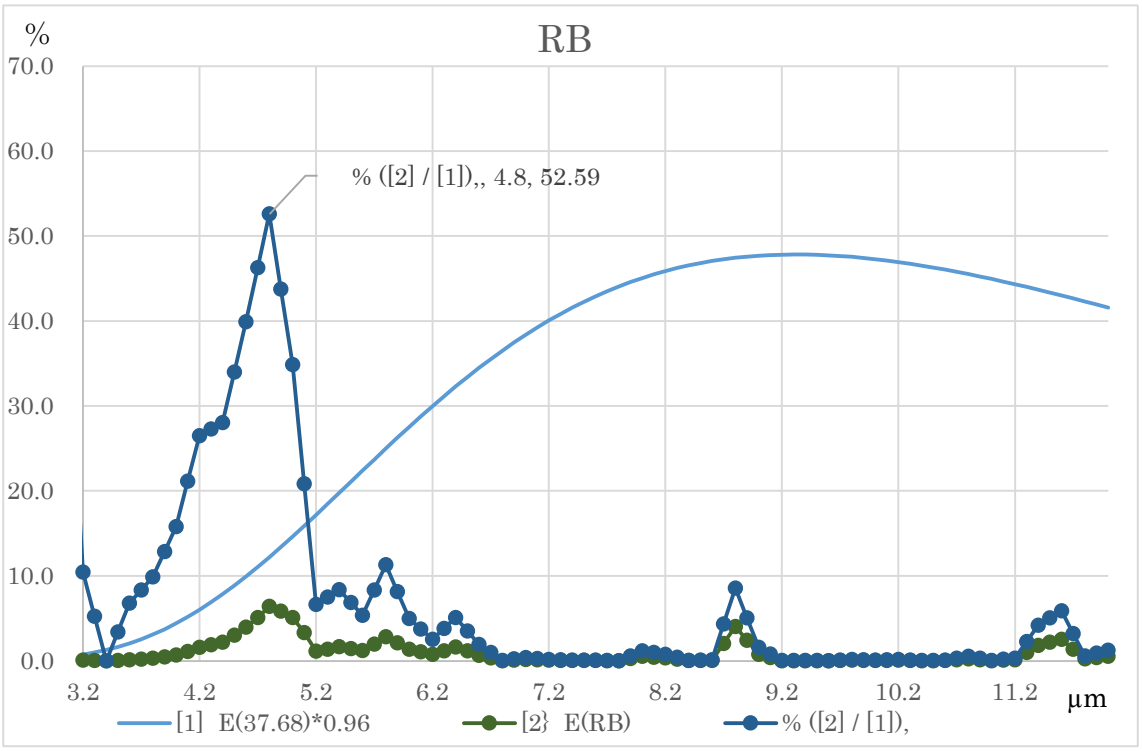

Figure 4: Energy distribution when using RB
Vertical axis: Energy intensity (nJ / m^3 / 0.1 μm) and ratio (%) Horizontal axis: wavelength: (μm)

When using a Heating Plate

At equilibrium, the heat from the heating plate (40.1°C) is transferred through the culture dish to the culture, resulting in the culture at 37.68°C. Since the absorption rate of the culture dish is (1-α), it is thought that the culture solution also absorbs heat at that ratio, so the heat distribution of the culture solution was assumed as follows.

$$E(37.68,\lambda)*(1-\alpha) \quad (F2.1)$$

Radiation from the heating plate (aluminum plate) and radiation from the culture dish (bottom) are considered. The emissivity of the heating plate (aluminum) is generally 0.06, and the culture medium is irradiated through the culture dish.

$$E(40.1,\lambda)*0.06*\alpha \quad (F2.2)$$

The emissivity of the culture dish (40.1°C) changes from transmittance (α) to (1-α), and the heat is reflected by the aluminum plate (reflectance = 0.9), passes through the culture dish, and irradiates the culture solution.

$$E(40.1)*(1-\alpha)*0.9*\alpha \quad (F2.3)$$

In addition, the ratio of the total heat distribution transferred from the heating plate (F2.2 + F2.3) to the heat distribution of the culture medium (F2.1) was calculated. The results of this calculation are shown in Fig. 5 (vertical axis: energy intensity and ratio, horizontal axis: wavelength: μm).

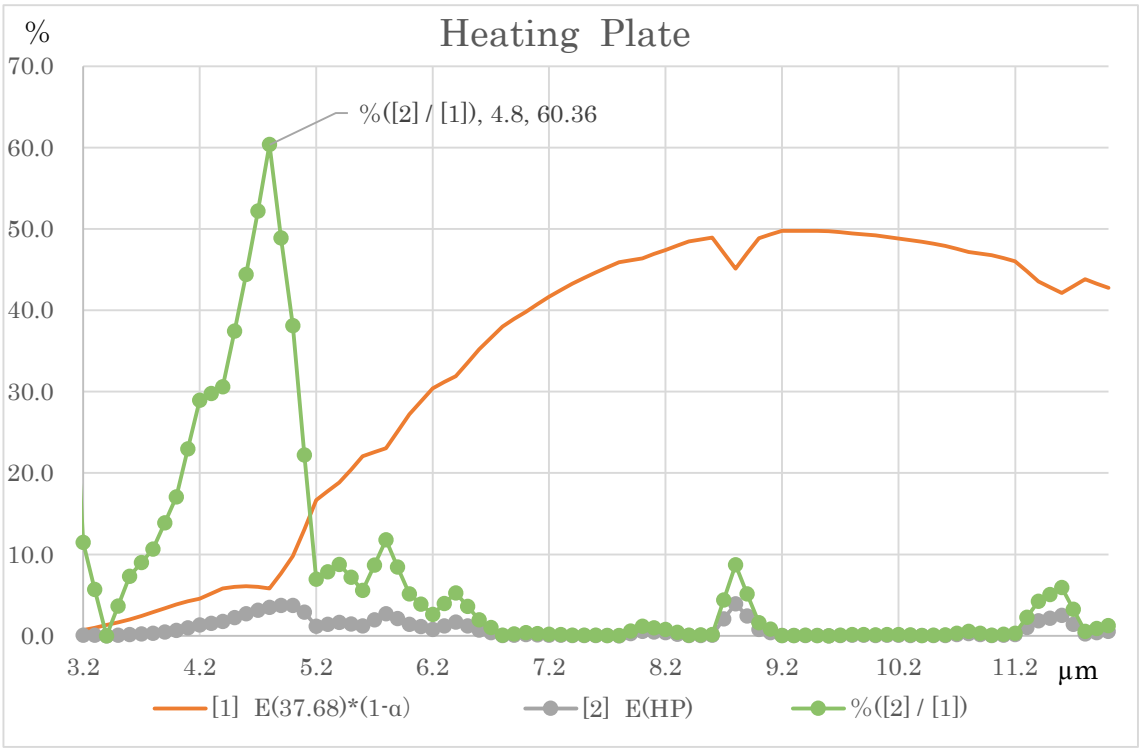

Figure 5: Energy distribution when using heating plate
Vertical axis: Energy intensity (nJ / m^3 / 0.1 μm) and ratio (%) Horizontal axis: wavelength: (μm)

Mid-Infrared Wavelength That Inhibits Cancer Cell Growth

The ratio peaks (52.59%, 60.36%) at 4.8 μm both with RB and with the hot plate. In addition, in experiments using mid-infrared irradiation, electromagnetic waves of 4.5, 4.8, 5.4, and 5.9 μm were irradiated, and the effect on cancer cells was confirmed only at 4.8 μm. (4.8μm of mid-infrared laser is 2100cm-1, so it is 4.7619μm.)

Also, polystyrene is measured in units of 2 μm, and even in the case of RB/heating plate calculation, it cannot be said that the peak is exactly 4.8 μm.

However, from the results of these experiments and calculations, it is considered reasonable that the effects of electromagnetic waves around 4.8 μm suppressed the growth of cancer cells.

In the previous report[1], it was pointed out that the effects of far-infrared rays in the range of 4 to 25 μm are due to the structural characteristics of RB, but the suppression of prostate cancer cell growth is not due to the structural characteristics of RB. It is presumed that there is a difference in the heat distribution (mid-infrared around 4.8 μm) of cancer cells due to the function of the culture dish (polystyrene) as a filter.

Materials and Methods

Cell Culture

Human prostate cancer cell lines (DU145, PC-3 and LNCaP) were obtained from the American Type Culture Collection (Manassas, VA, USA). A prostate epithelial cell line (PrEC) was obtained from Lonza Inc (Conshohocken, PA, USA).

Culture Dish

Falcon culture dishes (polystyrene, VWR, Pennsylvania, USA) were used.

Transmittance of culture dish (polystyrene) Transmittance of radiation by culture dish (polystyrene) (Falcon culture dish, VWR, Pennsylvania, USA) is shown in comparison with that of floppy disk (polystyrene) and silicone, at 20 °C and 50% humidity. Measured with C5-25GTM (JASCO Corporation, Tokyo, Japan) (Fig. 2).

Measurement of Cell Proliferation (XTT method)

Cell Proliferation Kit II (Roche Diagnostics, Mannheim, Germany) was used to measure cell proliferation. Medium in each well was measured at OD450 nm and OD650 nm on a SpectraMax microplate reader (Molecular Devices, Kobe, Japan). A standard curve was obtained by assaying a known number of viable cells of each cell line. Each assay was repeated 4 times.

IR Irradiation by a Mid-Infrared free Electron Laser

The experiment was conducted as an off-campus user at the Infrared Free Electron Laser Research Center, Institute of Science, Tokyo University of Science. (Since the activity was suspended on March 31, 2021, it is no longer possible to refer to external usage records from the FEL Liaison website.)

Acknowledgments

This paper is based on paper1 by the late Dr. Hiroki Shima and his own experiments, research, and considerations after submitting his thesis. In addition he developed an experimental device for cancer treatment and confirmed that mid-infrared rays were generated using a near-infrared camera (3.5-6 μm). Then, it was

confirmed that human prostate cancer cell proliferation was suppressed in an incubator. He then began a clinical study on patients who were referred for treatment.

We would like to express the deep gratitude to Dr. Shima for his knowledge and achievements.

Author Contributions

J.S. wrote this report. S.I. performed numerical calculations using Planck's formula.

Author Information

The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to J.S. (junko.shima@siqm.org)

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