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A. V. Khokhlova[1], D. R. Dolgova[2], L. V. Poludnyakova[3], A. K. Gilmutdinova[1], I. O. Zolotovskii[1], Yu. V. Saenko[4], S. G. Sokolovskii[5], E. U. Rafailov[5], D. A. Stoliarov[5], E. S. Pogodina[4], V. A. Ribenek[1], I. I. Antoneeva[2], and A. A. Fotiadi[6,7]
[1]Laboratory of Nonlinear and Microwave Photonics, Ulyanovsk state university, Ulyanovsk, Russia
[2]Department of Physiology, Ulyanovsk state university, Ulyanovsk, Russia
[3]Murmansk Medical College, Murmansk, Russia
[4]Laboratory of Molecular and Cell Biology, Ulyanovsk state university, Ulyanovsk, Russia
[5]Aston Institute of Photonic Technologies, Aston University, Birmingham, UK
[6]Optoelectronics and Measurement Techniques Unit, University of Oulu, 90570 Oulu, Finland
[7]Electromagnetism and Telecommunication Department, University of Mons, B-7000 Mons, Belgium

Email: avhohlova@gmail.com

Abstract
Melanoma is the most dangerous type of cancer, with a high rate of metastasis. The conventional method of treating skin melanoma is photodynamic therapy, yet this type of phototherapy has several side effects. In addition, the photosensitizers used are relatively expensive and toxic. Thus, developing methods of treating melanoma cancer using laser only is a promising area of research.
Here we present *in vitro* effects in melanoma cell culture after 1265 nm laser irradiation exposure.

1. Introduction

Today, skin melanoma is considered one of the most aggressive types of cancer, with a high rate of metastasis and a high level of mutagenesis [1-4]. This rate of metastasis is due to the spread of melanoma cells not only through the lymphatic vessels but also through the blood vessels [5, 6].

According to the International Agency for Research on Cancer, in 2020, there were 325,000 cases of skin melanoma in the world, including 57,000 deaths. According to forecasts, by 2040, the incidence will increase by 50% and amount to 510 thousand people, and the death rate will increase by 68% affecting 96 thousand people [7]. At the same time, the central part of the world human population susceptible to skin melanoma is the fair-skinned population of European origin, primarily people with skin phototypes I and II [8].

Treatment of skin melanoma at its early stages has two approaches:

1. Surgical removal of tumor tissue with a small area of healthy tissue to reduce the risk of metastasis. This method is reliable but highly traumatic due to the relatively sizeable surgical field [1, 5].

2. Photodynamic therapy, that is, the use of a photosensitizer (sensitive to light at a specific wavelength) introduced into the tumor tissue and a light source, which, when interacting, cause the transition of molecular oxygen from the triplet state to the singlet state and the development of oxidative stress leading to cell death [9, 10].

This method is quite convenient and causes less damage than surgery, but its use is limited by the photosensitizers, their cost, and their side effects [9].

Surprisingly, the laser irradiation at 1264-1270 nm enables a similar effect on cells and tissue [11, 12]. It causes singlet oxygen generation without a xenobiotic employing laser radiation intensities that do not cause significant tissue damage [13-17].

Most low-intensity laser radiation studies show stimulation of melanoma cell growth [18, 19]. Suppression of growth and induction of cell death requires an accurate choice of irradiation energy and new therapeutic approaches.

2. Methods

2.1. Cell cultures

Our study used B16 mouse melanoma cells (ATCC® CRL-6475™) and normal CHO-K1 Chinese hamster ovary cells (ATCC® CCL-61™) (figures 1, 2).

The B16 cell line is a standard model for the in vitro study of melanoma and transplantation into a laboratory animal.

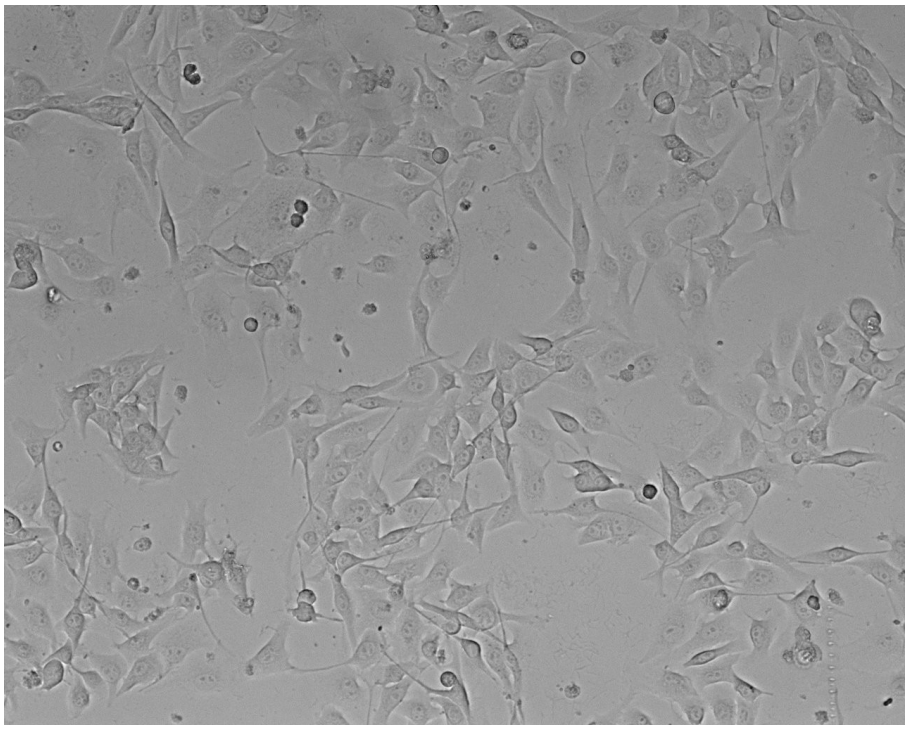

Figure 1. Exponential growth stage of B16 cells before the exposure to laser irradiation. ×100

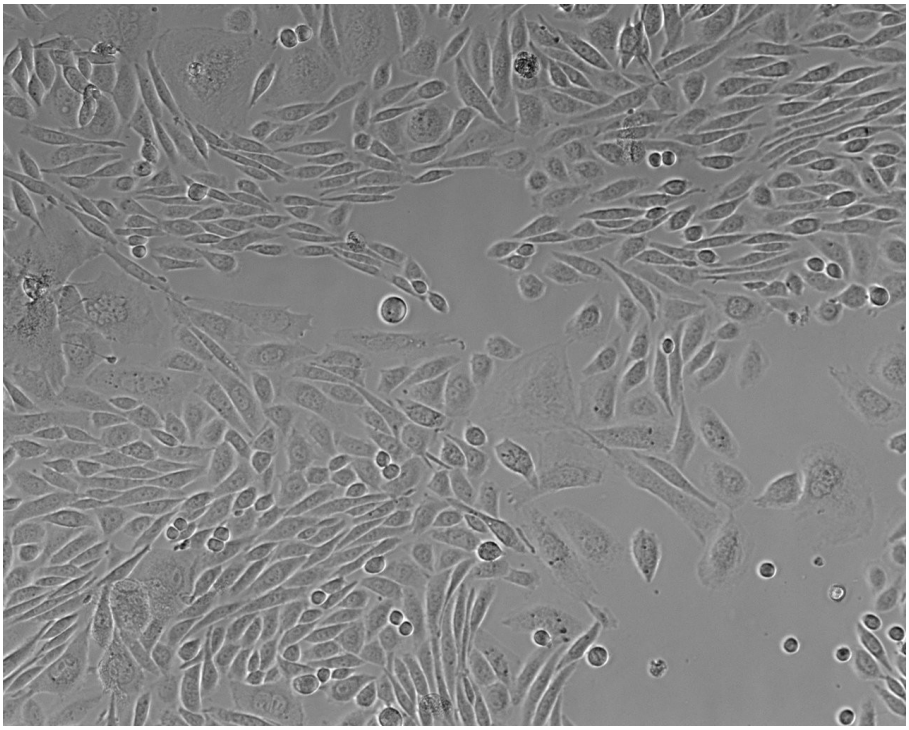

Figure 2. Exponential growth stage of CHO-K1 cells before the exposure to laser irradiation. ×100

2.2. Laser Sources

The semiconductor laser diode with an external fiber Bragg grating LD-1265.5-FBG-350 (Innolume, Germany) operating at a wavelength of 1265±1.5 nm has been used as an irradiation source.

Laser irradiation was carried out inside the tabletop CO_2 incubator (UNO Okolab, USA) with an 8-well slide chamber (SPL Lifesciences, South Korea) (figure 3).

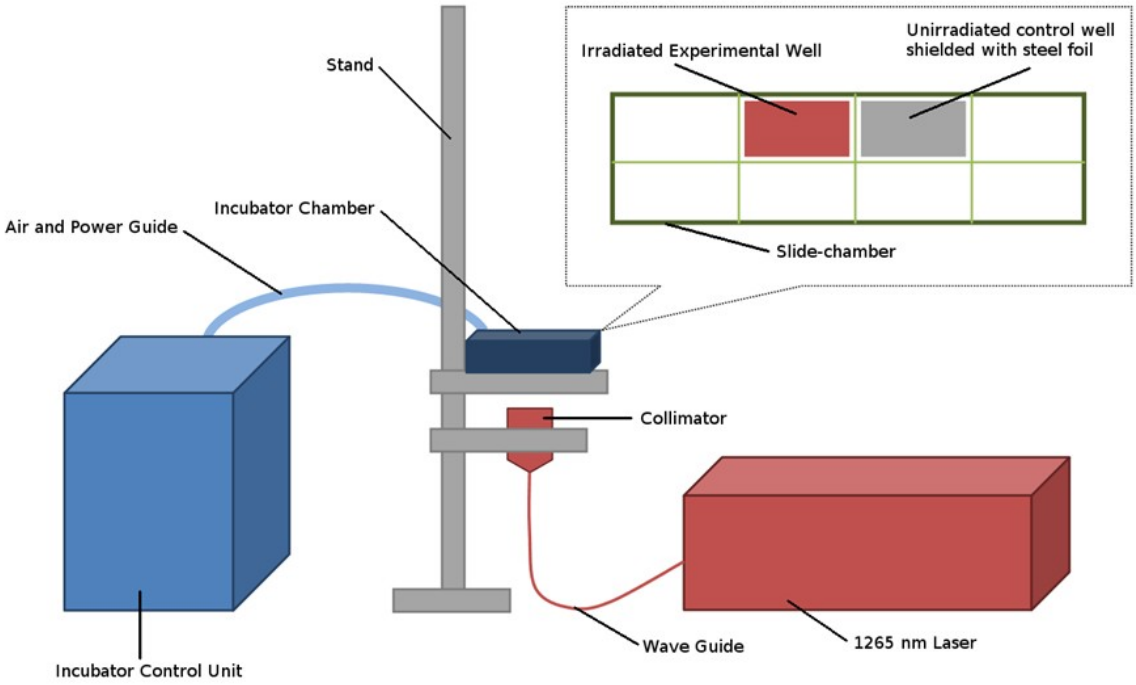

Figure 3. Design of the experiment on cell culture laser radiation.

2.3. Experiment

Cells throughout the experiment were kept under standard conditions; the experimental and control wells' temperature was 37±0.1˚C.

The choice of laser source power and laser irradiation energy density was based on the intracellular reactive oxygen species (ROS) level.

The energy density of laser radiation was calculated as follows:

$$E = P \times t / S, \quad (1)$$

where P is the average output power (W),

t is the exposure time (sec),

S is the laser spot area on the cell culture (cm^2).

2.4. Fluorescent staining of cells

After irradiation, the cells were stained for fluorescence microscopy with dichloro-dihydro-fluorescein diacetate to evaluate intracellular concentration of the reactive oxygen species as described [15].

2.5. Enzyme activity

After irradiation, cells were trypsinized and ddH_2O was added. The ELISA assay was conducted with all samples for determination of superoxide dismutase and glutathione S-transferase activity.

3. Results and Discussion

For normal CHO-K1 cells, P = 10 mW and E = 22.5 J/cm^2. Figure 4 shows the increase in ROS level immediately and 1 hour after laser irradiation.

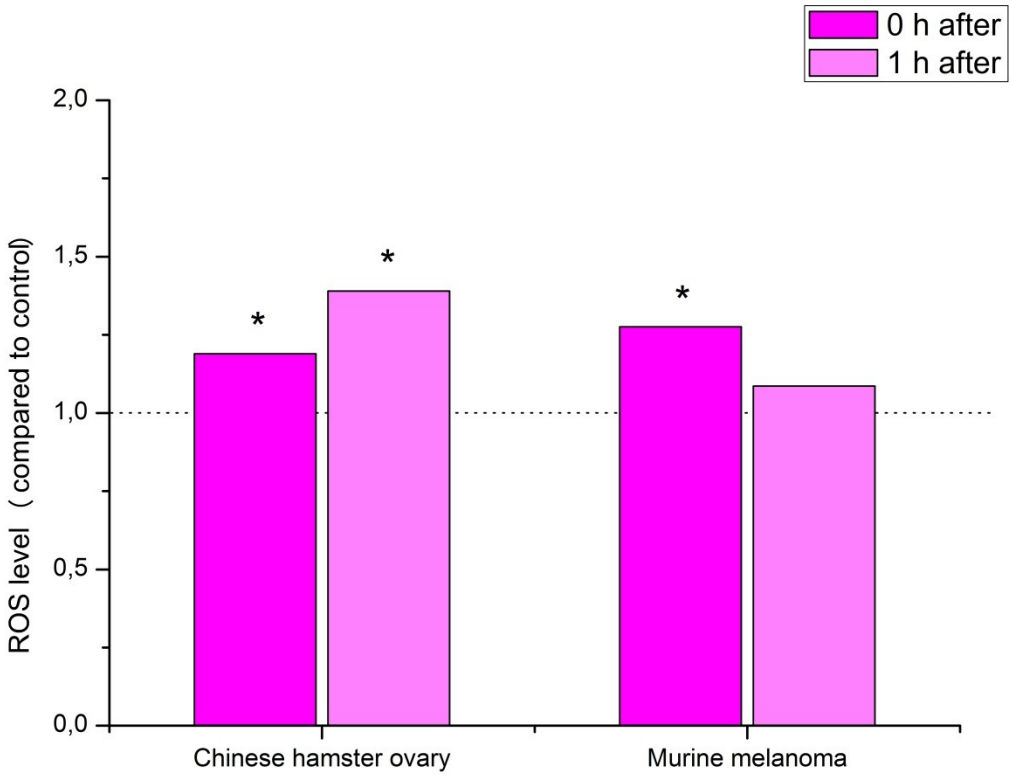

Figure 4. Level of ROS in the CHO-K1 (Chinese hamster ovary) and B16/F10 (murine melanoma) cell lines exposed to 1265 nm laser diode irradiation (t=30 min); P=250 mW; S=0.8 cm^2; E=562.5 J/cm^2.
* - statistically significant difference between experiment and control.

The laser power for B16 melanoma cancer cells was 250 mW, and the energy density was 562.5 J/cm2. One hour after irradiation, the level of ROS decreases to the control value in this cell line. This may be explained by different metabolism in two cell cultures.

The activity of antioxidant defense enzymes after laser irradiation was determined.

Superoxide dismutase (SOD) is the enzyme involved in the neutralization reaction of superoxide anion, one of the reactive oxygen species inside the cell.

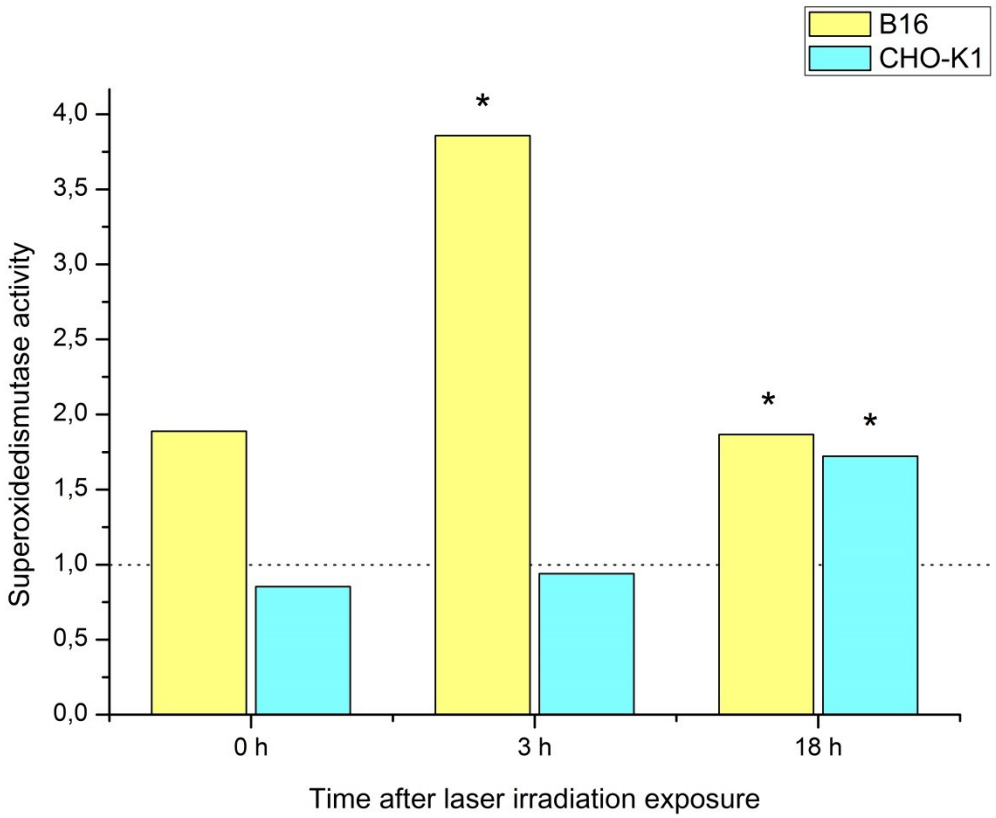

Figure 5. Superoxide dismutase activity in the CHO-K1 (Chinese hamster ovary) and B16/F10 (murine melanoma) cell lines exposed to 1265 nm laser diode irradiation (t=30 min); P=250 mW; S=0.8 cm^2; E=562.5 J/cm^2.
* - statistically significant difference between experiment and control.

Immediately after laser irradiation, the activity of SOD in both cell lines remains unchanged compared to the control (figure 5). Three hours after laser irradiation, SOD activity in melanoma cells increased by four times. Eighteen hours after laser irradiation, SOD activity was increased in both cell lines.

Glutathione S-transferase (GST) is the enzyme that catalyzes the conjugation of the reduced form of glutathione (GSH) to xenobiotic substrates for detoxification inside the cell.

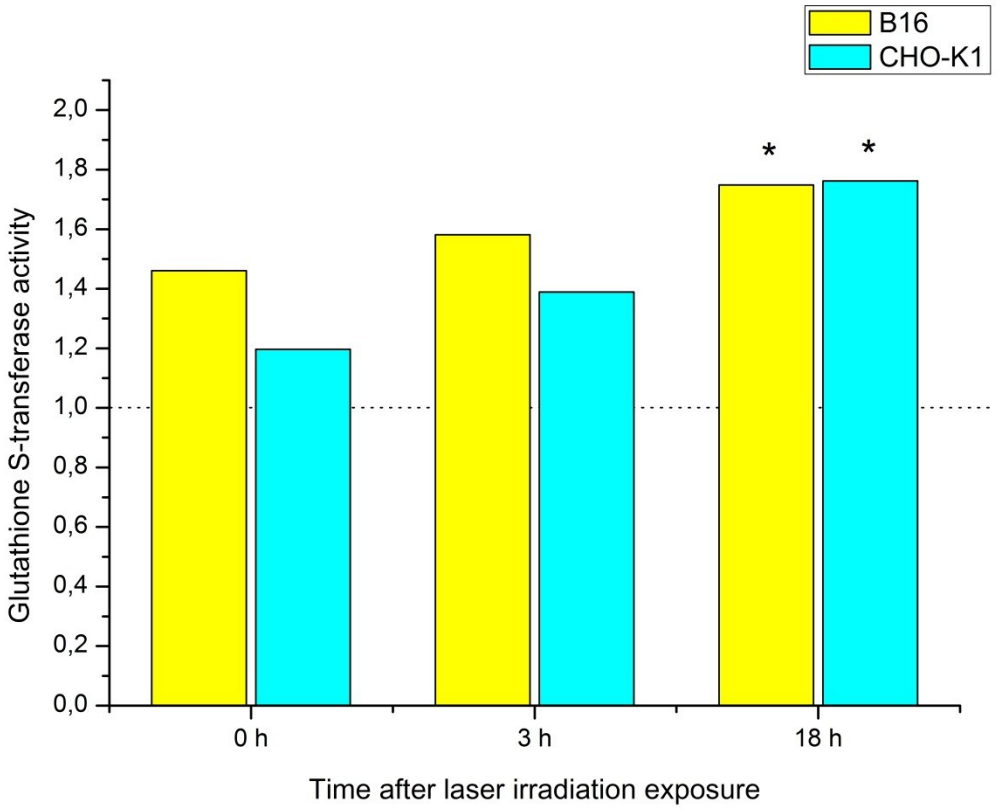

Figure 6. Glutathione S-transferase activity in the CHO-K1 (Chinese hamster ovary) and B16/F10 (murine melanoma) cell lines exposed to 1265 nm laser diode irradiation (t=30 min); P=250 mW; S=0.8 cm^2; E=562.5 J/cm^2
* - statistically significant difference between experiment and control.

Immediately and three hours after laser irradiation, the GST activity in both cell lines remains unchanged compared to the control (figure 6). Eighteen hours after, GST activity is increased in both cell lines.

The activity of enzymes involved in the cell antioxidant defense in cancer and normal cell lines suggests melanoma cells have defense mechanisms through more effective superoxide dismutase production, allowing them to cope with oxidative stress caused by laser radiation at a wavelength of 1265 nm [20-26].

This also can explain the effect of melanoma growth stimulation induced by low doses of laser radiation observed by other researchers, but this assumption requires further research.

4. Conclusions

Our study shows that the choice of doses for inhibiting melanoma growth requires a more objective approach and further investigation of intracellular mechanisms that would explain the resistance of this type of cancer to laser radiation at different wavelengths.

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Conflict of interests

The authors declare no conflict of interests.

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