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ORIGINAL ARTICLE

Utilizing 808 nm laser for sensitizing of melanoma tumors to megavoltage radiation therapy

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Abstract

Melanotic melanoma has high content of melanin and laser can destroy melanin-containing cells through thermal effect. In this study, the therapeutic effect of 808 nm laser therapy was investigated on B16-F10 melanoma tumor growth and tumor-bearing mice survival time. In addition, as laser can destroy melanin as the main cause of melanoma radioresistance, the effect of laser administration to enhance radiation therapy efficacy at B16-F10 cancer cells was evaluated in vitro and in vivo. Laser therapy (1 W/cm^2 × 4 min) could cause significant ($P < 0.05$) inhibition of melanoma tumors' growth (~ 61%) and about three times increase of the tumor-bearing mice survival time in comparison with no-treatment group. In addition, the mice which were treated with 1 W/cm^2 × 4 min laser administration plus 6 Gy megavoltage radiation therapy exhibited ~ 68% lesser tumors' volume and 27 days increase of survival time in comparison with 6 Gy irradiated tumor-bearing mice. Also, significantly higher ($P < 0.05$) tumor necrosis percentage was observed at the histopathological slides of 1 W/cm^2 × 4 min laser + RT treated mice tumors (57 ± 12%) in comparison with radiation therapy group (31 ± 10%). Therefore, not only laser therapy can inhibit melanoma tumors' growth per se but also its combination with radiation therapy can cause a significant enhancement of radiation therapy efficacy. The laser administration can be used as a radiosensitizing method for melanotic melanoma radiation therapy.

Keywords Melanoma · 808 nm laser · Radiation therapy · B16-F10

Abbreviations

KX	Ketamine–xylazine
RT	Radiation therapy
MV	Megavoltage

Introduction

The human epidermis consists of keratinocytes, Langerhans cells, and melanocytes [1]. Melanocytes are located among the basal layer of the epidermis and also can be found in hair bulb [2]. Malignant transformation of melanocytes causes arising of an aggressive neoplasm which is known as melanoma [3, 4]. It is the most lethal form of skin tumors [5]. Although melanoma accounts for less than 5% of all cutaneous malignancies, majority of skin cancer deaths are related to this neoplasia [6]. Nowadays, researchers are focusing on introducing new treatments for melanoma and improving current therapeutic and palliative methods [7].

Like many other solid tumors, the most effective therapeutic modality for primary melanoma is surgical excision [8]. Also, other therapeutic approaches like radiation therapy (RT) have been utilized for melanoma treatment. RT utilizes high-energy radiation beams for damaging cancer cells [7, 8]. Although it is one of the most well-known anti-cancer therapies, it has long been negatively marked by the lack of considerable effectiveness with melanoma due to radioresistant properties of this tumor [8, 9].

Melanocytes owe their name to their specific pigment which is known as melanin. These cells can produce melanin and store it at the specific cytoplasmic organelles called melanosomes. The main role of melanin is cell protection against

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solar radiation damages [10]. It forms a shield around the nucleus of basal cells and prevents the ultraviolet injurious effects on the nucleus and consequent DNA damage. Also, melanin exhibits antioxidant effects and can neutralize reactive oxygen species [11, 12]. Despite these advantages, the black side of melanin is its protective role for melanoma cells. These malignant cells contain melanosomes and their melanin content has a direct relationship with their aggressiveness, radioresistance, and poor prognosis [12, 13]. Melanin protects melanoma cells against chemo-, radio-, and phototherapies [14–16].

Laser means light amplification by stimulated emission of radiation. It consists of monochromatic and coherent light which is emitted in a parallel manner [17]. According to applied laser properties, it may be absorbed, reflected, transmitted, or scattered within tissue [18]. Water, melanin, and hemoglobin as tissue chromophores are the main agents to absorb the directed light to the tissue [19]. Each of them has its specific wavelength absorption profiles. Laser has been utilized as an effective agent for skin lightening or to remove unwanted hair. Upon high absorption of laser energy by melanin, thermal effects will occur at the target tissue and destroy the melanin and its containing cells [20, 21]. Therefore, high melanin content of melanoma can be its Achilles heel and leads to significant therapeutic effects in melanoma tumors by laser therapy. Also, many researchers have employed laser for eradication of melanin and its surrounding cells in the normal tissue [20, 22].

In this study, the therapeutic effect of 808 nm laser therapy was investigated on B16-F10 melanoma tumor growth and tumor-bearing mice survival time. At the next step, the 808 nm laser efficacy for radiosensitizing of B16-F10 cells to the megavoltage radiation beams was evaluated in vitro and in vivo. In addition, histopathological exams were investigated to access laser and radiation therapy effects on melanoma tumors. According to the best of our knowledge, this is the first time to employ laser as a radiosensitizer for enhancement of melanoma tumors radiation therapy efficacy.

Materials and methods

Cell culture and preparation

Murine melanoma cell line (B16-F10) was purchased from Pasteur Institute of Tehran, Iran. The cells were cultured in DMEM medium (Sigma, USA) containing 10% fetal bovine serum (FBS) (Sigma, USA) and 1% antibiotics mixture containing penicillin (Sigma, USA) and streptomycin (Sigma, USA). The cells were incubated at 37 °C in a humidified incubator in 5% CO_2 atmosphere [7].

Animal husbandry, handling, and tumor implantation

This study was approved by the institutional review committee of Isfahan University of Medical Sciences, and all procedures were reviewed and approved by Institutional Animal Care and Ethics Committee of Isfahan University of Medical Sciences according to their guidelines for care and use of the laboratory animal. Female C57BL/6J mice (age 6–8 weeks, weight 23 ± 2 g) were purchased from the Pasteur Institute of Tehran, Iran. The mice were maintained at 24 ± 2 °C temperature, 50 ± 10% relative humidity, and 12 h light/12 h dark cycle condition with complete access to standard mouse chow and water. The mice were acclimated for at least 1 week before the start of the study. The mice were injected subcutaneously with 1.5×10^6 cells suspended in 50 μL of DMEM-F12 (Sigma, USA) into their left flank. The injection site was shaved and sterilized before injection. For anesthetizing of the mice during laser therapy or radiation therapy, they were intraperitoneally injected with a ketamine–xylazine (KX) solution (ketamine 191.25 mg/kg, xylazine 4.25 mg/kg). To manage post-laser therapy pain, ketoprofen (5 mg/kg) was administered subcutaneously until next 72 h after laser administration. If any signs of pain, skin burn, wounds, massive necrosis and hemorrhage, diffuse metastasis were observed during any steps of the study, the mice were sacrificed. The neck dislocation was used for scarifying the mice. In order to determine tumors' growth progression, the greatest longitudinal diameter (length) and the greatest transverse diameter (width) of the tumors were determined every 3 days for 18 days after treatment administration. Then, the tumor's volume was calculated by the tumor volume Eq. (1). For survival analysis, the tumor-bearing mice were observed for 80 days after treatment administration. The animals' death was recorded every day. Standardized humane end point used to euthanize animals was failure to eat and drink for over 3 days and without any limb movement.

$$\text{Tumor volume} = (\text{Tumor length}) \times (\text{Tumor width})^2 \times 0.52 \quad (1)$$

Laser therapy and radiation therapy

At first, 40 female C57BL/6J mice were purchased and injected with the cancer cells according to the abovementioned methods. When the tumors' volume reached 50–100 mm^3, the tumor-bearing mice were randomly divided into five groups ($n = 8$). The groups included the following: (1) no treatment, (2) 1 W/cm^2 × 1 min laser therapy, (3) 1 W/cm^2 × 2 min laser therapy, (4) 1 W/cm^2 × 4 min laser therapy, and (5) 1 W/cm^2 × 8 min laser therapy. The laser (808 nm, 1 W/cm^2) was directed to the tumor sites for 1, 2, 4, and 8 min

according to the treatment groups. Laser light was manually directed to the tumor sites with rotational movements of the device's applicator over the tumor sites to prevent severe damages and burns. To minimize mice movement during laser therapy, they were anesthetized with the KX solution. Also, one group of the mice did not receive any treatments as no-treatment group. The tumors' volumes were estimated every 3 days until 18 days after laser administration. Also, the tumor-bearing mice survival time was evaluated for 80 days after laser administration. At the next step, 48 new C57BL/6J mice were purchased and injected with the cancer cells for evaluation of the radiosensitizing effect of the best selected laser therapy regime (according to the results, the 1 W/cm^2 × 4 min regime was selected). When the tumors' volumes reached 50–100 mm^3, the tumor-bearing mice were randomly divided into four groups ($n = 12$). The groups included (1) no treatment, (2) radiation therapy (RT), (3) laser therapy, and (4) laser therapy + RT. The 1st groups did not receive any treatment. The 2nd group of mice (Radiation therapy) was treated just with 6 Gy megavoltage radiation. The 3rd and 4th groups were anesthetized with the KX solution and the laser light was manually directed to the tumor sites with rotational movements of the device's applicator over the tumor sites. The laser (808 nm, 1 W/cm^2) was directed to the tumor sites for 4 min. Twelve hours after laser administration, the mice of 4th group were irradiated with 6 Gy megavoltage (MV) radiation. The radiation therapy was performed using a Compact linear accelerator (Primus, Siemens Ltd., Germany) with a source-surface distance (SSD) of 100 cm and a field size of 20 × 20 cm^2. To minimize mice movement during irradiation, they were anesthetized the KX solution. The tumors' volume was estimated every 3 days until 18 days after radiation therapy. Six days after the treatments' administration, four mice from each group were randomly selected and their tumors were harvested for histopathological evaluation of tumors' necrosis.

Histopathological evaluation of tumor's necrosis

Four mice from each group were sacrificed 6 days after treatments administration, and their tumors were harvested. The tumors were fixed in 10% formalin neutral buffer solution, and the fixed specimens were processed overnight for dehydration, clearing, and impregnation using an automatic tissue processor (Sakura, Japan). Then, the specimens were embedded in paraffin blocks and serial sections of 4 μm thickness were cut using a microtome (Leica Biosystems, Germany). The sections were stained by hematoxylin and eosin (H&E) [23]. The histopathology of blindly labeled slides was reviewed independently by two expert pathologists under dual-head light microscope (Olympus, Japan). Both low-power and high-power fields were examined. A minimum of ten random microscopic fields were scored for the area of necrosis as a percentage of total areas viewed. The final score of the percentage of tumor necrosis was agreed upon by both pathologists [24].

MTT assay

B16-F10 cells (10^5) were seeded in 96-well plates and incubated for 24 h at 37 °C under 5% CO_2 atmosphere. Three therapeutic regimes were used in this experiment, and one group did not undergo any treatment as control (no treatment). Each group contained at least six wells, and the experiment was repeated three times. The 2nd group contained six wells in which 1 W/cm^2 laser (808 nm) was directed to them for 4 min. The 3rd group contained six wells which were irradiated with 6 Gy MV radiation. The plates were placed under a Compact linear accelerator (Primus, Siemens Ltd., Germany). Source-to-surface distance (SSD) of 100 cm and field size of 25 × 25 cm^2 were set. The total delivered dose was 6 Gy with a dose rate of 200 MU/min. For the 4th group, at first, 1 W/cm^2 laser (808 nm) was administered to the wells for 4 min and after 12 h, they were irradiated with 6 Gy MV radiation. After 24 h, cell survival was evaluated using MTT assay protocol [7].

Statistical analysis

In this study, the obtained data were analyzed for their normality by Lilliford's test. The statistical analyzes were performed using one-way analysis of variance (ANOVA) with Tukey's post-hoc test by JMP 11.0 software (SAS Institute, Japan). The results were statistically significant at $P < 0.05$ (*P <0.05, ns: not significant). All values were expressed as the mean ± standard deviation.

Results

Different regimes of 808 nm laser therapy for inhibition of B16-F10 melanoma tumors' growth in vivo

In this study, four different regimes of 808 nm laser therapy (1 W/cm^2 × 1 min, 1 W/cm^2 × 2 min, 1 W/cm^2 × 4 min, and 1 W/cm^2 × 8 min) were employed to treat the melanoma tumors in vivo. The 1 W/cm^2 × 8 min treated mice were sacrificed and excluded from the experiment due to severe skin damage and wound formation at laser administration sites. As is illustrated in Fig. 1a, no sign of skin damage was appeared at the other treatment groups. 1 W/cm^2 × 1 min and 1 W/cm^2 × 2 min regimes did not cause significant tumor growth inhibition ($P > 0.05$) in comparison with control (Fig. 1b). However, 1 W/cm^2 × 4 min laser treatment could significantly ($P < 0.05$) inhibit

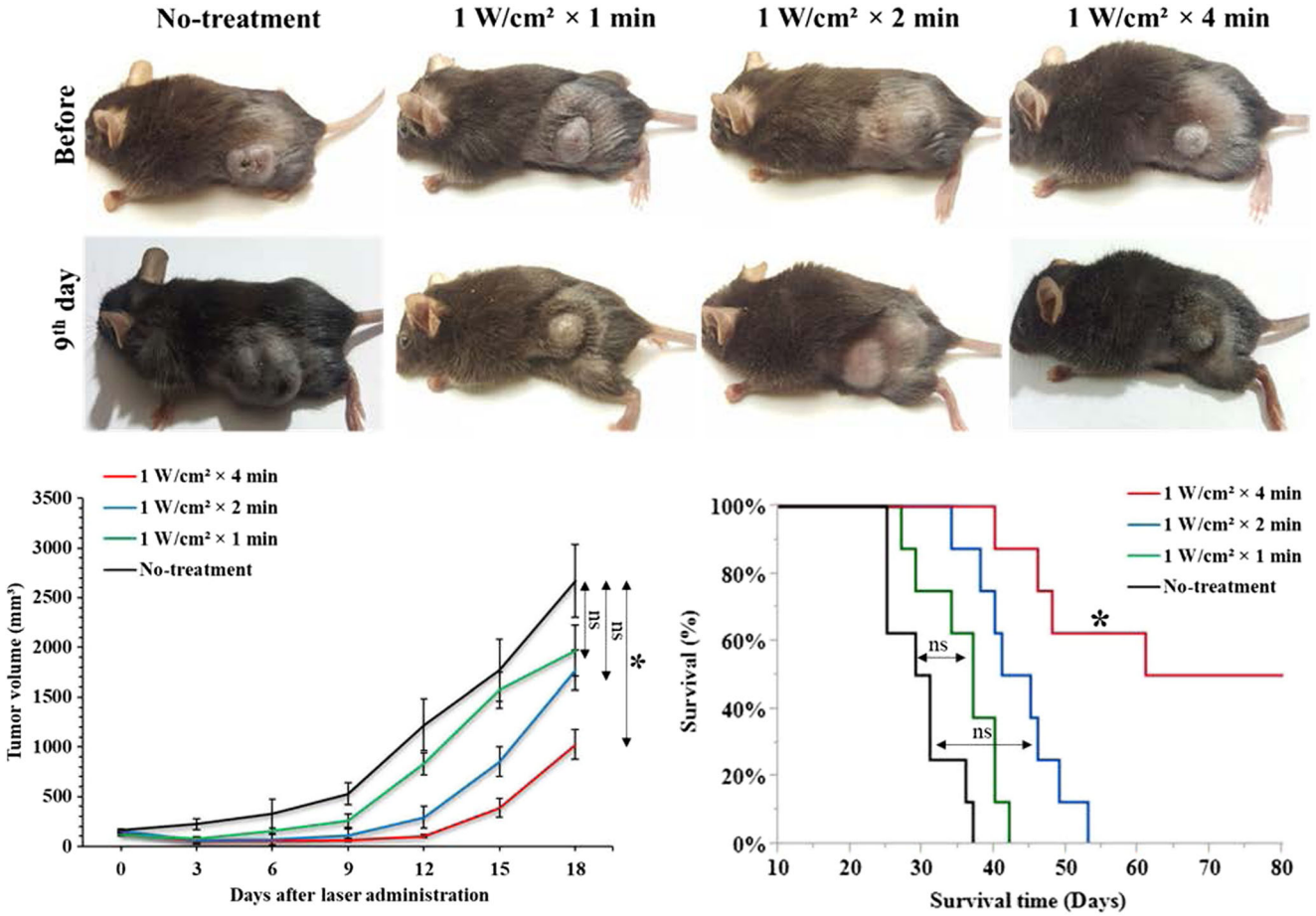

Fig. 1 Different laser therapy regimes effect on the B16-F10 melanoma tumors. **a** Images of B16-F10 tumors before and 9 days after laser administration at different groups. **b** B16-F10 melanoma tumors' growth progression at different groups ($n = 8$). **c** The B16-F10 tumor-bearing mice survival time in different treatment groups, after laser administration ($n = 8$). (*$P < 0.05$, ns: not significant)

melanoma tumors' growth. To assess the effect of these laser therapy regimes on the tumor-bearing mice survival, the mice were followed for 80 days after laser administration. The groups exhibited a various range of survival time (Fig. 1c). The 1 W/cm^2 × 4 min group had significantly longer survival time in comparison with other groups. Based on these observations, the 1 W/cm^2 × 4 min regime could significantly ($P < 0.05$) inhibit melanoma tumors' growth in vivo and subsequently increase tumor-bearing mice survival.

Macroscopic effects of the 1 W/cm^2 × 4 min laser therapy regime on B16-F10 melanoma tumors

The macroscopic changes at the melanoma tumors were evaluated 12, 24, and 48 h after 1 W/cm^2 × 4 min laser administration (Fig. 2). Considerable changes were observed after laser therapy. As Fig. 2 illustrated, vessels became prominent in macroscopic view. In addition, a white aura started to engulf all over the tumor.

Employment of 1 W/cm^2 × 4 min laser therapy for enhancement of melanoma radiation therapy efficacy in vitro and in vivo

The 1 W/cm^2 × 4 min regime exhibited the most therapeutic effect for B16-F10 melanoma tumors. Therefore, this regime was selected for the next series of experiments. To evaluate the radiosensitizing effect of the laser, in vitro and in vivo investigations were done. At first, the radiosensitizing ability of 808 nm laser was evaluated on B16-F10 cells in vitro (Fig. 3a). The cells were treated with 1 W/cm^2 × 4 min laser therapy, 6 Gy radiation therapy, or their combination. For the 1 W/cm^2 × 4 min + RT therapeutic regime, the cells were undergone 1 W/cm^2 × 4 min laser therapy and then irradiated with 6 Gy MV radiation. The cell viability was significantly lower in the 1 W/cm^2 × 4 min + RT in comparison to RT and laser therapy. Therefore, the laser therapy could significantly increase the radiation therapy efficacy in vitro. Also, the radiosensitizing efficacy of 1 W/cm^2 × 4 min 808 nm laser therapy was evaluated in vivo (Fig. 3b). The 6 Gy MV

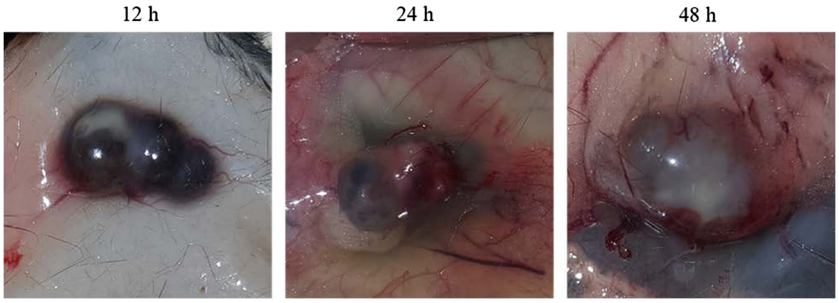

Fig. 2 Macroscopic view of the 1 W/cm^2 × 4 min laser treated tumors 12, 24, and 48 h after laser administration

radiation therapy and 1 W/cm^2 × 4 min laser therapy could significantly inhibit tumors' growth in comparison with no-treatment group. However, the smallest tumors were observed at the 1 W/cm^2 × 4 min + RT group in comparison with other groups. In this group, the laser (808 nm, 1 W/cm^2 × 4 min) was directed to the tumor sites and, then, the tumors were irradiated with 6 Gy MV radiation, 12 h after laser administration. In addition, the 1 W/cm^2 × 4 min + RT treatment could significantly ($P < 0.05$) increase the tumor-bearing mice survival time in comparison with no-treatment, RT, laser therapy groups (Table 1). Moreover, histopathological evaluations exhibited more significant tumor necrosis at the 1 W/cm^2 × 4 min + RT treated group in comparison with others (Table 2). Therefore, melanoma tumors became more vulnerable to megavoltage radiation beams after laser administration which can be attributed to melanin destruction by laser.

Discussion

RT is one of the most common therapeutic approaches for cancer treatment. More than half of cancer patients benefit from this therapeutic approach [25]. The main goal of RT is to deliver high-energy ionizing radiation beams to the tumor sites for eradicating the malignant cells. Therefore, the radiation dose has direct relation with the destructive effect of RT on the tumor. However, the presence of normal tissues in the field of treatment can extremely limit the usable range of RT dose [26]. Therefore, vulnerability of normal cells and resistance of cancer cells to the radiation beams can considerably decrease the RT efficacy. Therefore, many studies have focused on radiosensitizing of the tumors to the ionizing radiation beams like inhibition of hypoxia, targeting topoisomerases, microtubules, caspases, etc. [27].

Melanotic melanoma is one of the most radioresistance tumors [28]. This tumor not only exhibits the common radioresistance related properties of cancer cells but also contains a specific chromophore which is a powerful radioprotective agent [8]. This chromophore is melanin which is synthesized from L-tyrosine through series of oxidoreduction reactions. Melanin scavenges free radicals and chelates metal cations and cellular toxins including chemotherapy agents [13]. Also, melanin pigment serves as a radioprotective agent which makes melanoma cells resistant to radiotherapy [14, 29]. Therefore, many studies have focused on the decrease of the melanin content of melanoma cells to make them more vulnerable to radiation therapy [14].

Melanin as a tissue chromophore has a significant ability to absorb light. Each chromophore has its specific wavelength absorption profile which is 700–1000 nm for melanin. Therefore, within this wavelength range, most of the directed light will be absorbed by melanin [19]. In this study, 808 nm

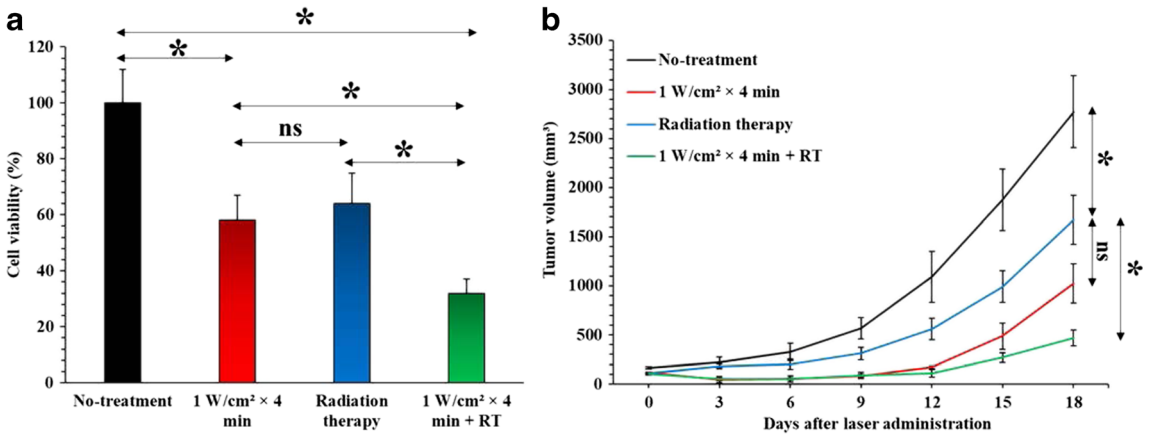

Fig. 3 Radiosensitizing effect of 1 W/cm^2 × 4 min laser therapy for enhancement of B16-F10 cancer cells radiation therapy efficacy at **a** in vitro and **b** in vivo evaluations

Table 1 The tumor-bearing mice survival times at different groups during 80 days follow-up, after treatments administration

Groups ($n = 8$)	Mean survival time (days)
No-treatment	30 ± 5
Radiation therapy	47 ± 7
1 W/cm^2 × 4 min	59 ± 14
1 W/cm^2 × 4 min + RT	74 ± 6*

RT radiation therapy, *n* number of mice
*$P < 0.05$

laser was selected to deliver the maximum amount of heat to melanin and cause tumor cells' damage due to their high content of melanin. Melanin can absorb the 808 nm laser energy which, subsequently, cause destruction of the melanin and the surrounding cells by thermal effect [20–22, 30–32]. In this study, the 808 nm laser (1 W/cm^2) was directed to the melanoma tumors' site for 1, 2, 4, and 8 min. The 1 W/cm^2 × 4 min laser therapy could significantly inhibit B16-F10 melanoma tumors' growth progression (Fig. 1b) and caused significant increase in the tumor-bearing mice survival time in comparison with other regimes (Fig. 1c). Also, no sign of massive skin damage was observed at the 1 W/cm^2 × 4 min 808 nm laser treated mice (Fig. 1a). Therefore, this safe and effective regime was selected for the next series of experiments. Administration of this laser therapy regime before 6 Gy MV radiation therapy could significantly increase the RT efficacy. The 1 W/cm^2 × 4 min + RT regime exhibited significantly more destructive effects on the B16-F10 cells in vitro in comparison with radiation therapy alone (Fig. 3a). Also, the radiosensitizing effect of 1 W/cm^2 × 4 min 808 nm laser administration was apparent at inhibition of B16-F10 melanoma tumors' growth (Fig. 3b) and increase of the tumor-bearing mice survival time in comparison with radiation therapy (Table 1). These results are inconsistent with the observing of more tumor necrosis at the 1 W/cm^2 × 4 min + RT treated tumors' tissue in comparison with radiation therapy group which only underwent 6 Gy MV irradiation. So, 808 nm laser can be a safe agent for radiosensitizing of melanotic melanoma tumors and enhance melanoma tumor radiation therapy efficacy.

Table 2 Histopathological evaluation of tumor necrosis at different groups

Groups ($n = 4$)	Necrosis ± SD (%)
No-treatment	12 ± 7%
Radiation therapy	31 ± 10%
1 W/cm^2 × 4 min	36 ± 5%
1 W/cm^2 × 4 min + RT	57 ± 12%*

SD standard deviation, *RT* radiation therapy, *n* number of mice
*$P < 0.05$

Conclusion

In this study, 808 nm laser therapy was applied in combination with megavoltage radiation therapy for melanoma treatment. Four different laser therapy regimes including 1 W/cm^2 × 1 min, 1 W/cm^2 × 2 min, 1 W/cm^2 × 4 min, and 1 W/cm^2 × 8 min were employed to treat the B16-F10 melanoma tumors in C57BL/6J mice. The value 1 W/cm^2 × 4 min caused significant tumors' growth inhibition and increase of tumor-bearing mice survival time in comparison with no-treatment group. Also, this laser therapy regime was safe and did not cause any significant skin damage. In addition, utilizing of this laser therapy regime for radiosensitizing of melanoma tumors caused significant enhancement of radiation therapy efficacy according to in vitro and in vivo evaluations. Therefore, laser can be employed for radiosensitizing of primary melanoma tumors to MV radiation therapy.

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Authors' contributions Dr. A. Kefayat and Dr. F. Ghahremani conceived all the experiments, analyzed the results, and wrote the manuscript. The radiation therapy was done under the guidance of Dr. A. Amouheidari. Also, the laser administration was supervised by Dr. N. Taheri and Dr. S.M. Okhravi.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study was approved by the institutional review committee of Isfahan University of Medical Sciences, and all procedures were reviewed and approved by Institutional Animal Care and Ethics Committee of Isfahan University of Medical Sciences according to their guidelines for care and use of the laboratory animal.

References

1. Regazzetti C, De Donatis GM, Ghorbel HH, Cardot-Leccia N, Ambrosetti D, Bahadoran P et al (2015) Endothelial cells promote pigmentation through endothelin receptor B activation. J Investig Dermatol 135(12):3096–3104
2. Cichorek M, Wachulska M, Stasiewicz A, Tymińska A (2013) Skin melanocytes: biology and development. Adv Dermatol Allergol 30(1):30

3. Bandarchi B, Ma L, Navab R, Seth A, Rasty G (2010) From melanocyte to metastatic malignant melanoma. Dermatol Res Pract 2010
4. Bandarchi B, Jabbari CA, Vedadi A, Navab R (2013) Molecular biology of normal melanocytes and melanoma cells. J Clin Pathol 66(8):644–648
5. Guy GP Jr, Thomas CC, Thompson T, Watson M, Massetti GM, Richardson LC (2015) Vital signs: melanoma incidence and mortality trends and projections—United States, 1982–2030. MMWR Morb Mortal Wkly Rep 64(21):591
6. Linos E, Swetter SM, Cockburn MG, Colditz GA, Clarke CA (2009) Increasing burden of melanoma in the United States. J Investig Dermatol 129(7):1666–1674
7. Ghahremani F, Shahbazi-Gahrouei D, Kefayat A, Motaghi H, Mehrgardi MA, Javanmard SH (2018) AS1411 aptamer conjugated gold nanoclusters as a targeted radiosensitizer for megavoltage radiation therapy of 4T1 breast cancer cells. RSC Adv 8(8):4249–4258
8. Khan MK, Khan N, Almasan A, Macklis R (2011) Future of radiation therapy for malignant melanoma in an era of newer, more effective biological agents. OncoTargets Ther 4:137
9. Strojan P (2010) Role of radiotherapy in melanoma management. Radiol Oncol 44(1):1–12
10. Feller L, Masilana A, Khammissa RA, Altini M, Jadwat Y, Lemmer J (2014) Melanin: the biophysiology of oral melanocytes and physiological oral pigmentation. Head Face Med 10(1):8
11. Slominski A, Tobin DJ, Shibahara S, Wortsman J (2004) Melanin pigmentation in mammalian skin and its hormonal regulation. Physiol Rev 84(4):1155–1228
12. Michael HT, Merlino G (2017) A topical solution to the sunless tanning problem. Trends Mol Med 23(9):771–773
13. Brożyna AA, Jóźwicki W, Roszkowski K, Filipiak J, Slominski AT (2016) Melanin content in melanoma metastases affects the outcome of radiotherapy. Oncotarget 7(14):17844
14. Brożyna AA, VanMiddlesworth L, Slominski AT (2008) Inhibition of melanogenesis as a radiation sensitizer for melanoma therapy. Int J Cancer 123(6):1448–1456
15. Urbanska K, Romanowska-Dixon B, Elas M, Pajak S, Paziewski E, Bryk J et al (2000) Experimental ruthenium plaque therapy of amelanotic and melanotic melanomas in the hamster eye. Melanoma Res 10(1):26–35
16. Sharma SK, Huang Y-Y, Hamblin MR (2015) Melanoma resistance to photodynamic therapy. Resistance to photodynamic therapy in cancer. Springer, pp 229–246
17. Tanzi EL, Lupton JR, Alster TS (2003) Lasers in dermatology: four decades of progress. J Am Acad Dermatol 49(1):1–34
18. Jawad MM, Qader STA, Zaidan A, Zaidan B, Naji A, Qader ITA (2011) An overview of laser principle, laser-tissue interaction mechanisms and laser safety precautions for medical laser users. Int J Pharmacol 7(2):149–160
19. Tseng S-H, Bargo P, Durkin A, Kollias N (2009) Chromophore concentrations, absorption and scattering properties of human skin in-vivo. Opt Express 17(17):14599–14617
20. Husain Z, Alster TS (2016) The role of lasers and intense pulsed light technology in dermatology. Clin Cosmet Investig Dermatol 9:29
21. Haywood RM, Linge C (2004) Differences in production of melanin radicals by 694 nm ruby laser and UVA radiation. Lasers Surg Med 35(1):77–83
22. Kilmer SL (2002) Laser eradication of pigmented lesions and tattoos. Dermatol Clin 20(1):37–53
23. Ibrahim K, Al-Mutary M, Bakhiet A, Khan HJM (2018) Histopathology of the liver, kidney, and spleen of mice exposed to gold nanoparticles. 23(8):1848
24. Chen Y, Taghian AG, Rosenberg AE, O'Connell J, Okunieff P, Suit HDJIjoc. Predictive value of histologic tumor necrosis after radiation. 2001; 96 (6):334–340
25. Haume K, Rosa S, Grellet S, Śmiałek MA, Butterworth KT, Solov'yov AV et al (2016) Gold nanoparticles for cancer radiotherapy: a review. Cancer Nanotechnol 7(1):8
26. Chithrani DB, Jelveh S, Jalali F, van Prooijen M, Allen C, Bristow RG et al (2010) Gold nanoparticles as radiation sensitizers in cancer therapy. Radiat Res 173(6):719–728
27. Linam J, Yang L-X (2015) Recent developments in radiosensitization. 35(5):2479–2485
28. Mileo AM, Mattei E, Fanuele M, Delpino A, Ferrini UJP (1989) Differential radiosensitivity in cultured B-16 melanoma cells following interrupted melanogenesis induced by glucosamine. 2(3):167–170
29. Slominski A, Zbytek B, Slominski RJIjoc. Inhibitors of melanogenesis increase toxicity of cyclophosphamide and lymphocytes against melanoma cells. 2009; 124 (6):1470–1477
30. Patil UA, Dhami LD (2008) Overview of lasers. Indian J Plast Surg 41 (Suppl:S101
31. Rosa DSA, Aranha ACC, de Paula Eduardo C, Aoki A (2007) Esthetic treatment of gingival melanin hyperpigmentation with Er:YAG laser: short-term clinical observations and patient follow-up. J Periodontol 78(10):2018–2025
32. Margolis RJ, Dover JS, Polla LL, Watanabe S, Shea CR, Hruza GJ et al (1989) Visible action spectrum for melanin-specific selective photothermolysis. Lasers Surg Med 9(4):389–397