

Photobiomodulation of Isolated Lung Cancer Stem cells

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Abstract. Research has uncovered that one of the plausible reasons for cancer relapse is the existence of stem like cells, possessing cancer properties, called cancer stem cells (CSCs). Cancer research is highly focused on improving current cancer treatments. One method of targeted cancer therapy is Photodynamic therapy (PDT), where Low Intensity Laser Irradiation (LILI), along with a photochemical compound, is used. When implementing a mechanism by which CSCs are targeted, LILI might pose as a viable treatment option. Studies have shown that using high fluences of LILI (HF-LILI) can induce cell death in normal and neoplastic cells. Further investigations on cell death induced by HF-LILI of CSCs still needs to be explored. Lung CSCs were isolated using the stem cell marker CD 133 and were exposed to LILI at wavelengths of 636, 825 and 1060 nm at fluences ranging from 5 J/cm² to 40 J/cm². Post irradiation biochemical assays were conducted to monitor cellular responses including: viability, proliferation and cytotoxicity, after 24 hours incubation. Studies have indicated that LILI, when treating lung CSCs, can induce either a bio-stimulatory or bio-inhibitory effect depending on the wavelength and fluence used. This study indicated successful cell damage of lung CSCs when using HF-LILI, as well as, stimulation of ATP production, when using lower fluences of LILI.

1. Introduction

Cancer is the term used to describe a disease where there is abnormal proliferation of cells, which can affect any part of the body. Cancer is one of the primary diseases contributing to mortality rates globally. Lung cancer is the most commonly diagnosed cancer in both men and women [1]. Poor prognosis of lung cancer can be attributed to deficient treatment modalities and relapse caused by its metastatic capabilities [2]. Research have found that a subpopulation of tumour initiating cells referred to as cancer stem cells (CSCs) drive tumour genesis and relapse as it has been said to regenerate tumour formation after treatment thus being accountable for therapeutic resistance [3, 4]. This subpopulation of cells residing within a malignant tumour display a variety of stem-like properties; as they are clonal in origin, can regenerate and proliferate exponentially [5, 6]; as well as tumorigenic properties such as drug resistance, evading apoptosis, tumour initiation and metastatic potential [7]. CSCs reside within a niche that keeps them quiescent and enhance DNA repair, which contribute to their therapeutic resistance [8].

Normal lung tissue is maintained by stem cells (SCs) that are controlled by several pathways controlling these pulmonary precursors enabling them to develop into their different lineages. Abnormal pulmonary SC development can lead to lung CSCs arising from these lineages causing tumour formation [9]. CSCs have been identified and characterised using SC markers [10]. Promonin-1 (CD 133) is a gene encoding for a pentaspan transmembrane glycoprotein localized to membrane protrusions. It is an adult stem cell marker maintaining stem cell properties by suppressing differentiation. It is considered a primary marker for CSCs as its high expression is said to be an adverse prognostic factor [11].

Photobiomodulation is a form of phototherapy which uses Low Intensity Laser Irradiation (LILI) with wavelengths ranging from visible to near infrared light (600 – 1070 nm) which allows for optimal tissue penetration [12]. The effects seen are generated at a mitochondrial level where photobiological responses are generated from the intracellular chromophores causing different metabolic reactions depending on the wavelengths and energy output of the incident light [13]. Studies conducted on different cell lines found that when using LILI with low fluences (LF-LILI) ranging from 1 – 15 J/cm² and wavelengths of 600 nm – 700 nm it stimulated biological processes by increasing proliferation and viability [14-18], but have an inhibitory effect when using increased wavelengths of 800–830 nm and fluences larger than 10 J/cm² [15, 19,20]. An innovative therapy currently under investigation is photodynamic therapy (PDT), which uses the activation of a photo chemotherapeutic chemical by low level light emitting lasers. Reasons for new therapies being under investigation is to avoid current therapies failing to reject recurrence of cancer and having viewer side effects.

This exploratory study evaluated the effects of LF-LILI (5 – 20 J/cm²) and high fluence LILI (HF-LILI) (40 J/cm²), where different fluences were calculated through laser time exposure and output (mW), with wavelengths of 636 nm, 825 nm and 1060 nm on isolated lung CSCs. Biochemical analysis of irradiated lung CSCs included viability, proliferation and cytotoxicity.

2. Methodology

Lung CSCs were cultured in complete media consisting of Rosewell Park Memorial Institute 1640 medium (RPMI), with additional supplements consisting of 10% foetal bovine serum (FBS) and 1% antibiotics consisting of 0.5% penicillin/ streptomycin and 0.5% amphotericin B. Incubation took place at 37°C with 5% CO₂ in an 85% humidifying incubator.

Prior to irradiation lung CSCs were seeded at a number of 1 x 10⁵ cells in culture plates of 35 mm in diameter along with 3 ml complete media and incubated for 24 hours allowing attachment to the culture dish. After 24 h incubation the culture dish was rinsed 3 times using Hanks Balanced Salt Solution (HBSS) and replaced with 3 ml complete media before placing the culture dish underneath a fibre optic irradiating the cells with a semiconductor diode laser in the dark with a fluence of 5 – 20 J/cm² and 40 J/cm². Control cells received no irradiation. This procedure was used for all wavelengths used of 636 nm, 825 nm and 1060 nm. All lasers were kept at a constant power output of 85 mW with a continuous pulse.

Post irradiation biochemical assays were conducted to monitor cellular responses including: viability, proliferation and cytotoxicity, after 24 hours incubation. Cell viability was determined using the dye exclusion test. Trypan blue is a diazo dye which is expelled by live cells which still have intact membranes. Cells undergoing cell death or damage will take up the dye when added to the cell suspension. Viability was measured as a percentage value. Cell proliferation only takes place in metabolically active cells. To measure cellular metabolism we looked at the amount of ATP present. This was measured using an ATP luminescent assay where the luminescent signal measured is proportional to the amount of ATP present. Cytotoxicity was calculated by measuring the amount of LDH in the media. LDH release indicates membrane damage and that cells are not metabolically active.

Statistical significant differences between groups were indicated as p<0.05 (*), p<0.01 (**), and p<0.001 (***).

3. Results

All assay results are combined and indicated as stimulation (↑) or inhibition (↓) of viability and proliferation of lung CSCs and cytotoxicity as in increase (↑) in cell membrane damage that was statistically significant. Results indicated with an (↑/↓) were not statistically significant and (--) no difference was observed.

Table 1. Photobiostimulation or inhibition of lung CSCs at different irradiation levels.

		Viability	Proliferation	Cytotoxicity
636 nm	5 J/cm ²	↑	↑	---
	10 J/cm ²	↑	↑*	---
	20 J/cm ²	---	---	---
	40 J/cm ²	↓	↓*	↑*
825 nm	5 J/cm ²	↑	↑	---
	10 J/cm ²	↑*	↑**	---
	20 J/cm ²	---	---	---
	40 J/cm ²	↓***	↓*	↑*
1060 nm	5 J/cm ²	↑	↑	---
	10 J/cm ²	↑	↑	---
	20 J/cm ²	---	↑	---
	40 J/cm ²	↓	↓	↑*

4. Discussion and Conclusion

Potential outcomes of LILI on lung CSCs were explored in this study. There was an increase seen in both viability and proliferation when using LF-LILI on the lung CSCs. The increase in cell viability correlates with similar proliferation results seen. A statistical significance was seen when using LF-LILI at a wavelength of 825 nm and fluence of 10 J/ cm² for both viability and proliferation. Significant stimulation in proliferation was also seen when using a wavelength of 636 nm and 10 J/cm². No cytotoxicity was observed when using LF-LILI for all respective wavelengths. These findings are in accordance to similar studies conducted on SCs [14,18].

This indicates that photobiostimulation is achieved when irradiating lung CSCs with LF-LILI. When using HF-LILI of 40 J/cm² photobioinhibition is achieved as there was a decrease seen in proliferation when using wavelengths of 636 nm and 825 nm. Cytotoxicity results revealed that cell membrane damage was induced when irradiating lung CSCs with all respective wavelengths and HF-LILI of 40 J/cm². Similar results were seen in a study where replication inhibition was demonstrated [21].

The photobiomodulatory effects seen can be attributed to intracellular chromophores found in organelles such as the mitochondrion of a cell. The response triggered by LILI is due to these chromophores absorbing the light, having either a biostimulatory or bioinhibitory effect depending on the wavelength and fluence used. Photobiomodulation relies on specific parameters such as wavelength, fluence, power density, pulse structure, and treatment time when applied to biological tissue. This allows for targeting of specific light-absorbing molecules in specific tissues, operating on the principle of photochemistry, as opposed to photo thermogenesis. The light energy absorbed causes singlet state excitation of oxygen molecules, leading to triplet state excitation causing an energy transfer to ground state molecular oxygen (a triplet) to form the reactive species, singlet oxygen. Alternatively superoxide may be formed as a result of electron reduction. LILI operates at an exact wavelength of light, which

influences the depth of tissue penetration. Similar to normal cells, cancer cells also contain with intracellular chromophores. Different cellular chromophores are stimulated at different wavelengths. Therefore, the prediction can be made that in targeting cancerous cells, the outcome expected can be controlled by the wavelength as well as by the energy output that will lead to either stimulation or inhibition. The exact mechanism behind the stimulation of the light-absorbing molecules producing these two different effects is still being investigated [22,23].

Cell death studies conducted using lung cancer cells and PDT indicated that cell membrane damage and apoptosis was induced [2]. This type of photochemical therapy has shown to be a promising treatment for lung cancer. Further studies should include whether similar results are achieved when using PDT on lung CSCs as well as the mechanism behind the cell death induced.

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