Photobiomodulation of Isolated Lung Cancer Stem cells

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Abstract. *Background*: 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. *Methodology:* Lung CSCs positive for the stem cell marker CD 133 were exposed to LILI at wavelengths of 636, 825 and 1060 nm at fluences ranging from 5 J/cm^2 to 40 J/cm^2. Twenty four hours post irradiation biochemical assays were conducted to monitor cellular responses including: viability, proliferation and cytotoxicity. *Discussion*: 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 [13]. Studies conducted on different cell lines found that when using LILI with low fluences (LF-LILI) ranging from 1 – 15 J/cm^2 and wavelengths of 600 nm – 700 nm it stimulated biological processes [14-18], but have an inhibitory effect when using high fluence LILI (HF-LILI) of > 10 J/cm^2 and wavelength of 800–830 nm [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/cm^2) and HF-LILI (40 J/cm^2) 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 concentration of 1 x 10^5 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/cm^2 and 40 J/cm^2. Control cells received no irradiation. This procedure was used for all wavelengths used of 636 nm, 825 nm and 1060 nm. All lasers had a power output of ±85 mW with a continuous pulse (table 1.).

Table 1. Laser Parameters used in this study

	Parameters		
Laser type	Semiconductor (Diode)		
Wavelength (nm)	636	825	1060
Wave emission	Continuous	Continuous	Continuous
Power output (mW)	±85	±85	±85
Power density (mW/cm^2)	9.36	9.36	9.36
Spot size (cm^2)	9.1	9.1	9.1
Fluence (J/cm^2)	5, 10, 20 and 40	5, 10, 20 and 40	5, 10, 20 and 40
Duration of irradiation ± min, sec	8min 54sec, 17min 48sec, 35min 36sec and 71min 13sec	8min 54sec, 17min 48sec, 35min 36sec and 71min 13sec	8min 54sec, 17min 48sec, 35min 36sec and 71min 13sec

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 in table 2. 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.

Table 2. Photobiostimulation or inhibition of lung CSCs at different irradiation levels.

		Viability	Proliferation	Cytotoxicity
636 nm	5 J/cm²			
	10 J/cm²		↑ *	
	20 J/cm²			
	40 J/cm²		↓ *	↑ *
825 nm	5 J/cm²			
	10 J/cm²	↑ *	↑ **	
	20 J/cm²			
	40 J/cm²	↓ ***	↓ *	↑ *
1060 nm	5 J/cm²			
	10 J/cm²			
	20 J/cm²			
	40 J/cm²			↑ *

$p<0.05$ (*), $p<0.01$ (**) and $p<0.001$ (***)

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/ cm^2 for both viability and proliferation. Significant stimulation in proliferation was also seen when using a wavelength of 636 nm and 10 J/cm^2.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/cm^2 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/cm^2. 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 [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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References
[1] World Health Organization 2015 Media Centre, Cancer. Available:http://www.who.int/mediacentre/factsheets/fs297/en/
[2] Manoto S L, Sekhejane P R, Houreld N N, Abrahamse H 2012 *Photodiagnosis Photodyn. Ther.* **9** 52–59
[3] Lu B, Chiou S, Deutsch E and Loric A 2011 *J. Clin. Oncol* Article ID 269437, doi:10.1155/2011/269437
[4] Michael H 2009 *J. Cell. Biochem.* **106** 745–749
[5] Baiguera S Kaiathur M and Macchiarini P 2011 *CML Lung Cancer* **4**(3) 69-78
[6] Plaks V Kong N and Werb Z 2015 *Cell Stem Cell* **16**(3) 225-38
[7] Mohr M Zänker K S and Dittmar T 2015 *Med. Hypotheses* **85**(6) 1012-8
[8] Kosovsky M 2012 Culture and Assay Systems Utilized for Cancer Stem Cell Research. Available: http://www.flowcytometri.dk/literature/Kosovsky%202012%20BD%20ecm_cancer_stem_cell.pdf
[9] Natarajan T G and FitzGerald K T 2007 *Cancer Biomark.* **3** 211–231
[10] Bin Bao, Aamir Ahmad, Asfar S. Azmi Shadan Ali, and Fazlul H Sarkar 2013 *Curr. Protoc .Pharmacol.* **14** Unit–14.25
[11] Qiu Z X, Zhao S, Mo X M and Li W M 2015 *Int. J. Clin. Exp. Pathol.* **8**(6) 6589-95
[12] Huang Y Y, Chen A and Hamblin M 2009 *SPIE Newsroom*, doi:10.1117/2.1200906.1669
[13] Crous A and Abrahamse H 2016 *Photomed. Laser Surg.* 2015 Dec 21. doi:10.1089/pho.2015.3979
[14] de Villiers J A, Houreld N N and Abrahamse H 2011 *Stem Cell Rev* **7** 869–882
[15] Moore P, Ridgway T D, Higbee R G, Howard E W and Lucroy M D 2005 *Lasers Surg. Med.* **36** 8– 125
[16] Fonseca A S, Moreira T O, Paixa˜o, D L, et al. 2010 *Lasers Surg. Med.* **42** 481–488
[17] Hu W P, Wang J J, Yu C L, Lan C C, Chen G S and Yu H S 2007 *J. Invest. Dermatol.* **127** 2048–2057
[18] Mvula B, Mathope T, Moore, T J and Abrahamse H 2008 *Laser Med. Sci.* **23** 277–282
[19] Abrahamse H, Houreld N N, Muller S and Ndlovu L 2010 *Med.l Tech. SA* **24** 9–14
[20] Chow R T, David M A and Armati P J 2007 *J. Peripher. Nerv. Syst.* **12** 28–39
[21] Ocana-Quero J M, Perez de la Lastra J, Gomez-Villamandos R and Moreno-Millan M 1998 *Lasers Med. Sci.* **13**(3) 214-218
[22] Wan-Ping H, Jeh-Jeng W, Chia-Li Y, Cheng-Che E L, Gow-Shing C and Hsin-Su Y 2007 *J. Invest. Dermatol.* **127** 2048–2057
[23] Abrahamse H and Crous A 2016 *Proceedings of SPIE 9695* 96950J; doi:10.1117/12.2228902