# The Impact of Low Intensity Laser Irradiation on Lung Cancer Stem Cell Viability and Proliferation

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**Abstract.** *Background*: Cancer stem cells or tumour initiating cells are cells that have been attributed to metastatic drive and tumour genesis. These cells contribute to cancer recurrence, metastasis, aggressiveness and resistance to therapy. Laser irradiation has been shown to have a diverse range of clinical applications including wound healing and photo dynamic therapy (PDT). Middle infrared (MIR) radiation has shown to inhibit cellular proliferation and induce morphological changes to the cytoskeletal dynamics of A549 lung cancer cells. Recent studies done using Low Intensity Laser Irradiation (LILI) using visible red light with a wavelength of 636 nm and fluence between 5- 15 J/cm$^2$ on adipose derived stem cells (ADSCs) which are adult mesenchymal stem cells (MSCs), have shown to have an increase in proliferation, viability and differentiation into smooth muscle cells. The effects of LILI on cancer stem cells have yet to be elucidated. *Methodology:* Isolated A549 lung cancer stem cells were exposed to a wavelength of 636 nm and a fluence of 5 or 10 J/cm$^2$, where after cellular responses were monitored after several time intervals to evaluate proliferation and viability with the view to establish the wavelength and fluence at which LILI causes an effect on proliferation and viability after a specific incubation time. *Discussion*: This study assists in the understanding of the effects of LILI on isolated lung cancer stem cells by evaluating the results produced and comparing the different effects of different laser parameters on these cells.

## 1. Introduction

Lung cancer is a leading threat of cancer-related deaths world-wide [1]. Contributing factors include the deficiency in treatment modalities, local recurrence and systemic metastasis. Advancements in these areas are likely to improve overall outcome. Lung cancer is represented by tumour formation consisting of atypical cells; the cancer is then classified according to the cell types it consists of [2]. Research confirmed that these tumours also contain a subpopulation of cells, thought to drive tumour genesis, called cancer stem cells (CSCs) or tumour initiating cells (TICs) displaying stem like properties such as rapid proliferation, are clonal in origin and can self-renew [3]. These CSCs are partly held responsible for therapeutic resistance of cancer as it is said to regenerate the tumour after treatment [4,5]. This information poses the strategy to target CSCs with different treatment options [5]. Current cancer treatment strategies include Low Intensity Laser Irradiation (LILI) in conjunction with a photosensitiser called photodynamic therapy (PDT).

LILI is a technique that uses optical waves at a specific wavelength and no heat is produced [6]. LILI has been shown to promote viability and proliferation in various types of stem cells, especially in the red to near infrared spectrum (600-700 nm). This is most likely due to the mitochondrial respiratory chain components being able to absorb light at this wavelength resulting in the increase of reactive oxygen species (ROS), and adenosine triphosphate (ATP) or cyclic adenosine monophosphate (cAMP), and initiating a signalling cascade which promotes cellular proliferation and cytoprotection [7]. In previous LILI studies conducted using diabetic induced human skin fibroblast cells (WS1) at wavelengths of 632.8, 830 and 1064 nm at 5 and 16 J/cm$^2$ showed both a dose and wavelength dependent response, showing that cells respond best using visible red irradiation at a fluence of 5 J/cm$^2$ and a

wavelength of 632.8 nm [8]. Similar results were obtained in a study using adipose derived stem cells (ADSCs), both studies indicating that higher fluences such as 10 -16 J/cm$^2$ induced cell damage and decrease cell viability and proliferation [8,9].

In this study cells were characterised using cell surface marker CD133+ (prominin-1), which is a five-transmembrane glycoprotein and one of the most frequently demonstrated markers for CSCs in multiple organs/tissues including the lung [10,11]. Cells were treated with LILI using visible red light at a wavelength of 636 nm and fluences of 5 or 10 J/cm$^2$ to identify the dosage levels at which the cells indicate a response in cell proliferation and viability.

## 2. Materials and Methods

*Characterisation:* Isolated A549 CSCs were characterised using fluorescent microscopy and cell surface marker CD 133+. CaCo2 cells were used as a positive control and SKUT-1 as a negative control. *Culture:* The isolated cells were seeded into 3.4 cm$^2$ diameter culture dishes and incubated over night to allow cells to attach. Cell cultures were irradiated once in the dark from the top with a 636 nm diode with a fluence of, 5 or 10 J/cm$^2$. Non-irradiated cells used as a control were treated similar to irradiated cells, without the irradiation. Post-irradiation, cultures were incubated for 24, 48 and 72 hours. Post-incubation cells were trypsinysed for detachment and re-suspended in 1 ml complete medium. All tests were performed on different populations (n=4) of cells for each sample group and each biochemical assay was performed in duplicate [9]. *Cell viability:* All cell populations were treated with CellTiter-Glo® and ATP luminescence was recorded using a spectrophotometer measuring the ATP present in each sample. *Cell proliferation:* All samples were treated with 10% alamarBlue® where absorbance was measured quantitatively using an excitation wavelength of 560 nm and emission wavelength of 590 nm. Statistical significant differences between groups were indicated as $p<0.05$ (*), $p<0.01$ (**) and $p<0.001$(***) and considered as 95 percent confidence interval for the population mean.

## 3. Results

To characterise the isolated stem cells, they were labelled with a lung CSC marker CD133$^+$ as indicated by Figure 1.

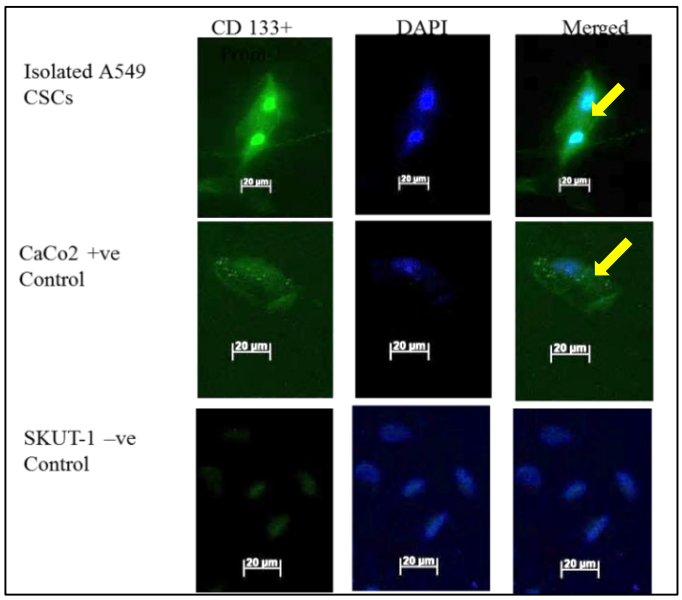


**Figure 1:** Characterisation of isolated A549 CSCs. $1^0$ unconjugated antibody CD133$^+$ and $2^0$ FITC conjugated antibody staining the cell surface green and DAPI staining the nucleus blue. Results indicate A549 CSCs and CaCo2 are both positive for CD 133, whereas SKUT-1 is negative.

CaCo2 cells were used as the positive control cell line and SKUT-1 the negative control. Positive results were indicated by green fluorescence and a counterstain of DAPI, making the cell nucleus fluoresce blue. Negative results only indicated the blue fluorescence.

Cell viability was determined by ATP luminescence (Fig 2.), showing an increase in viability as time elapses from 24 to 72 h. A similar trend is seen with proliferation (Fig.3) when Alamar blue is read as an absorbance value and an increase is seen in all samples measured over a period of time.

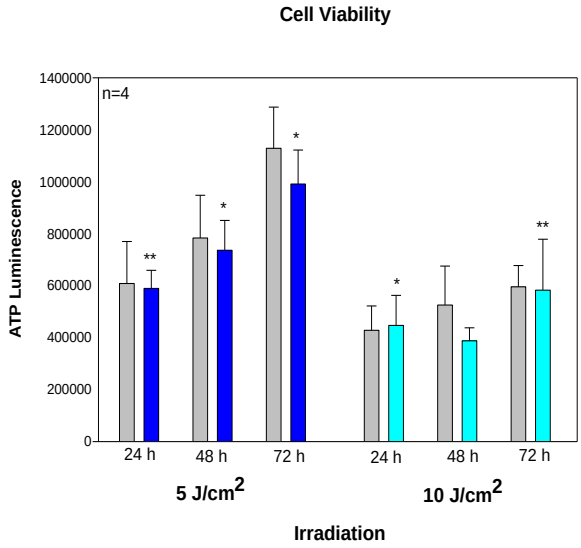


**Figure 2:** Increase in ATP luminescence is seen in all the samples as time elapses from 24- 72h. No significant change was observed with irradiation when comparing 5 J/cm$^2$ to 10 J/cm$^2$. Statistical significant differences were expressed as p<0.05 (*), p<0.01 (**) and p<0.001(***). All samples were compared to their control groups. Experiments were repeated four times (n=4).

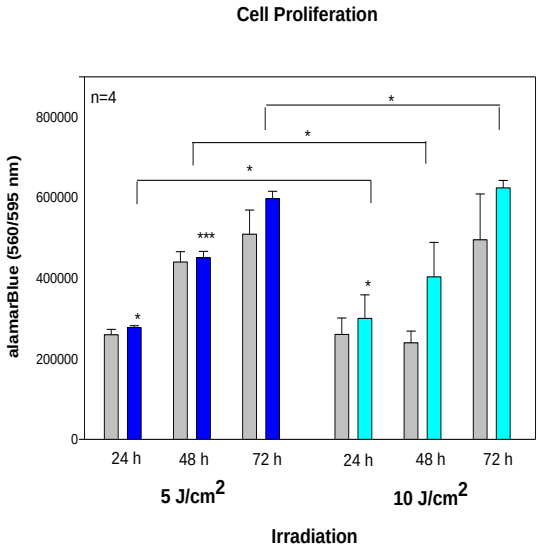


**Figure 3:** Irradiated samples all show a momentous increase in proliferation over a period of time. All samples are compared to their control counterparts as a statistical difference of p<0.05 (*), p<0.01 (**) and p<0.001(***). Statistical significant differences in time intervals when comparing 5 J/cm$^2$ to 10 J/cm$^2$ irradiation are indicated as p<0.05 (*) for 24h, 48h and 72h. Experiments were repeated four times (n=4).

## 4. Discussion and Conclusion

Fluorescence microscopy reveals that the isolated lung A549 CSCs were positive for the CD133 antigen used to characterise lung CSCs as well as the CaCo2 cells used as a positive control. This work concurs with research published in previous studies [10,11]. The negative results obtained for the SKUT-1 smooth muscle cells used as a negative control correlates with previous studies done [12]. These results obtained indicate that the isolated cells were CSCs.

An increase was observed in both ATP viability and alamarBlue® proliferation in the isolated A549 human lung CSCs. The rise in ATP level correlates with the increase in proliferation of the CSCs. Both the increase in viability and proliferation using a fluence of 5 J/cm$^2$ and wavelength of 636nm can be attributed to photobiostimulation which has a positive effect on cells at that specific dose and wavelength. This increase in cellular viability and proliferation can be made due to intracellular chromophores absorbing the light, activating the cells' electron transport chain, having an increased

effect on ATP production [13]. These effects have also been demonstrated in previous studies conducted on diabetic induced WS1 and ADSCs [8,9]. There was no significant decrease observed when comparing irradiation at 5 J/cm$^2$ and 10 J/cm$^2$. Results indicated yet another increase in viability and proliferation over time when irradiating the CSCs at 10 J/cm$^2$. These findings are contradictory to those observed in previous studies showing a decline in cellular proliferation and viability when using fluences between 10 and 16 J/cm$^2$ on ADSCs [9]. This effect can be attributed to CSCs sharing essential capabilities similar to those found in normal stem cells. These include self-renewal and differentiation potential; other CSC characteristics also include resistance to cell death as compared to normal cell death rates and failure of current treatment methodologies such as chemo and radiation therapy that should reduce proliferation potential [14]. The results obtained indicate both a dose and wavelength dependent response on cells treated. A higher fluence of irradiation should be considered when wanting the desired effect of decreasing cell viability and proliferation of CSCs. It should be considered that current studies being conducted using a fluence of 20 J/cm$^2$ should demonstrate to be an effective inducer of CSC death.

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