Inhibition of Lung Cancer Migration and Invasion Using a Gold Nano Photosensitizer Conjugate

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Abstract. Despite advances in cancer treatment, lung cancer remains one of the leading causes of cancer deaths worldwide. Lung cancer can spread through the blood and lymphatic systems, as well as infiltrate healthy tissues underlying the lung, resulting in both distant and local metastasis. The most common causes of death are cancer metastasis and the threat of secondary tumours. The ability of cells to invade, which is largely controlled by cell motility, is an essential aspect of metastases. Photodynamic therapy (PDT), a minimally invasive cancer treatment, is based on the concept of light stimulation of a photosensitizing agent at a certain wavelength, which, combined with an optimum energy density of light activation, induces the photosensitizer (PS) to reach their triplet state, where oxidants causing tumour cell death can form in the presence of molecular oxygen. Due to their physicochemical and optical properties, gold nanoparticles have been shown to improve the effectivity of PDT by increasing the loading potential of the PS within cancer cells, are biocompatible and non-toxic, and give improved permeability and retention. The use of gold nanoparticles in nano-mediated PDT has been shown to cause lung cancer cell death. Several physiological studies, including migration, cell cycle analysis and the extracellular matrix cell invasion assay were carried out in this study to determine whether PDT using a gold nano sensitizer inhibits lung cancer migration and invasion. The results show that nano mediated PDT treatment of lung cancer inhibits lung cancer migration and invasion, causes cell cycle arrest, and reduces lung cancer proliferative abilities, elaborating on the efficacy of nano mediated PDT treatment of lung cancer.

1. Introduction

Lung cancer continues to be the top cause of cancer death globally, with an estimated 1.8 million fatalities [1]. Despite considerable diagnostic and therapeutic improvements, overall survival remains poor. About 70% of lung cancer patients have advanced-stage illness, which contributes to its high death rate [2]. Cancer metastasis is the spread of cancer cells to different organs and tissues, as well as the creation of new tumours [3]. Lung cancer treatment options vary based on the cancer's stage, size, and location in the lung. Treatment is also based on whether the cancer has spread and the patient's general condition. However, most conventional lung cancer treatments have drawbacks [4].

Photodynamic therapy (PDT), is a non-invasive, sophisticated technique of cancer treatment that is generally conducted as an outpatient operation [5]. PDT is founded on the notion of a photoexcited photosensitizer (PS) interacting with oxygen to create oxidants, resulting in the death of tumour cells. PDT follows a Type I and Type II mechanism. In type I interactions, the PS interacts with biomolecules in its excited triplet state, exchanging hydrogen atoms through the radical process.

It produces free radicals and radical ions, which combine with oxygen to produce reactive oxygen species (ROS). Triplet–triplet annihilation is the basis for Type II reactions. The excited PS interacts with oxygen resulting in the formation of reactive and cytotoxic singlet oxygen [6]. The PS's characteristics affects its pharmacokinetics and biodistribution allowing for either selective accumulation and/or selective retention preferentially in cancerous cells [7]. PDT advantages over conventional therapy includes repeatability, decreased adverse effects, no PDT-related morbidity or mortality, bio interactivity and compatibility and low cost [8]. Aluminium (III) Phthalocyanine Chloride Tetra sulphonate ($AlPcS_4Cl$) has been extensively studied and proven to be a PS with excellent PDT properties including high molar absorption coefficient in the red to NIR spectrum [9]. Its sulphate functional groups allow for thermal stability and solubility, along with increased oxidant production [10], is amphophilic and has little or no dark toxicity [9].

There is still opportunity for development in PDT by improving PS delivery and making the PS target selective. Incentives for using nanoparticles (NPs) include their polydispersity, stability, and biocompatibility. Gold nanoparticles (AuNPs) have exact physicochemical and optical characteristics, are biocompatible and non-toxic, and have increased permeability and retention (EPR) [11]. AuNPs loaded with $AlPcS_4$ can enhance PS binding affinity and tumour selectivity, as well as EPR-induced singlet oxygen generation. It also inhibits non-specific protein binding through PEGylation of AuNPs [12]. In this study we aimed to evaluate whether nanoPDT, using a gold nano sensitizer, inhibits lung cancer metastatic abilities.

2. Materials and methods

2.1. Cell culture

Complete media composed of Rosewell Park Memorial Institute 1640 medium (RPMI), supplemented with 10% foetal bovine serum and antibiotics consisting of 0.5% penicillin/ streptomycin and 0.5% amphotericin B were used to grow lung cancer cells (A549; ATCC® CCL-185™). Cells were placed in a humidifying incubator set at RH 85%, 37°C and CO_2 of 5%.

2.2. Photodynamic treatment

An $AlPcS_4Cl$-goldnano bioconjugate was used where the PS using a predetermined [IC50] concentration of 20 μM $AlPcS_4Cl$ (Frontier Scientific, AlPcS-834) [13] was adsorbed onto 20 ppm AuNP-PEG-COOH (Sigma-Aldrich, 765465) [14]. The cells were divided into four experimental groups, a control receiving no treatment, cells receiving photobiomodulation (PBM)/ irradiation alone, PDT and nanoPDT groups. The cells were irradiated with a 660 nm Light Emitting Diode (LED) plate illuminator, using the Keithley 2200-32-3 power supply (PSU). To eliminate nuisance variables, all experiments were done in the dark at ambient temperature. The parameters of irradiation are listed in Table 1 below.

Table 1. LED well plate illuminator parameters.

ʎ nm	V (volts)	A (amps)	Power (mW)	Intensity (mW/cm2)	Fluence (J/cm^2)
659.4	22.913	3.02	33.130	117.17	10

2.3. Migration

The migration of cells was determined using the 'central scratch' technique. Cells were grown overnight in 6-well cell culture plates and incubated. A central scratch was made using a sterile P-200 pipette tip prior to irradiation. At 0, 24, and 48 hours after irradiation, migration was examined using an inverted light microscope (Wirsam, Olympus CKX41) and captured using a SC30 Olympus Camera.

2.4. Cell cycle analysis

DNA content was quantified using flow cytometry as a measure of cell cycle. Cells were fixed using 70% ethanol and washed, followed by treatment with 50 µl RNase (stock 100 µg/ml) and stained with 200 µl Propidium iodide (PI) (stock solution 50 µg/ml). For analysis using a flow cytometer (BD Accuri Flow Cytometer C6) the cell population was gated and applied to a scatter plot, where the gated samples were then applied to a PI histogram plot describing the cell phases.

2.5. Proliferation and Toxicity

The effect of PDT and nanoPDT on the metabolism of lung cancer cells was evaluated by the quantity of ATP present in the cells following treatment. Intracellular ATP was quantified using a Multilabel Counter (Perkin Elmer, VICTOR3TM, 1420) in conjunction with the CellTiter-Glo® luminous cell proliferation assay (Promega, G7570). To ascertain the degree of toxicity, the quantity of LDH produced from cells because of membrane damage and cell death was determined. Where formazan generation was determined using a multilabel counter at 490 nm, the CytoTox96® nonradioactive cytotoxicity assay (Promega, G400) was employed.

3. Results

3.1. Migration

The scratch test is conducted by creating a "scratch" in a monolayer of cells and photographing the cells at the start and at regular intervals throughout cell migration as the scratch closes. Figure 1 shows the migration of lung epithelial carcinoma cells (A549) at 0, 24 and 48 hours post PDT treatment. Untreated a) control cells and b) cells receiving PBM show a similar trend in migration where the cells move towards the central scratch and the cell density increases. Cells treated with c) PDT and d) nanoPDT show a decreased migration rate and were not able to close the scratch over time, where dead cells and cell debris were seen floating in the culture medium due to cell arrest.

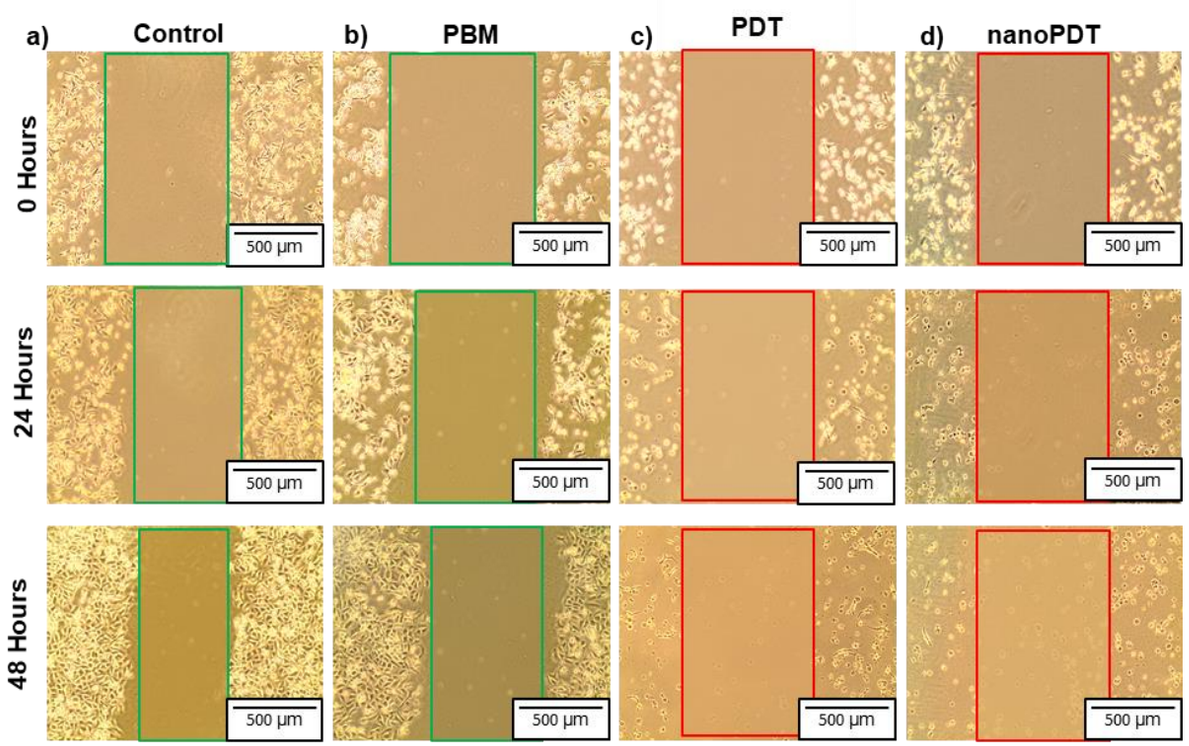

Figure 1. Migration of lung epithelial carcinoma cells (A549) at 0, 24 and 48 hours post PDT treatment.

3.2. Cell cycle analysis

The cell cycle phases were identified by quantifying cellular DNA. The cell cycle is a multiple process during which cells proliferate. It consists of the gap 1, or G1, stage where cells mature. The S phase where the cell copies its DNA, the G2 phase where cells prepare to divide and does DNA checks and the M phase where cells undergo mitoses. Cells can also move in to the G0 phase indicative of cell cycle arrest or cell death. Results in figure 2 show that the a) control cells and b) PBM treated cells are predominantly in the G1 and S phase. Whereas c) PDT treated samples show many cells entering G0, where the percentage of cells in G1 decreased, as well as the cells moving out of S phase. Cell treated using d) nanoPDT shows an even greater cell cycle arrest with no cells seen in S phase and less cells in G1, compared to PDT.

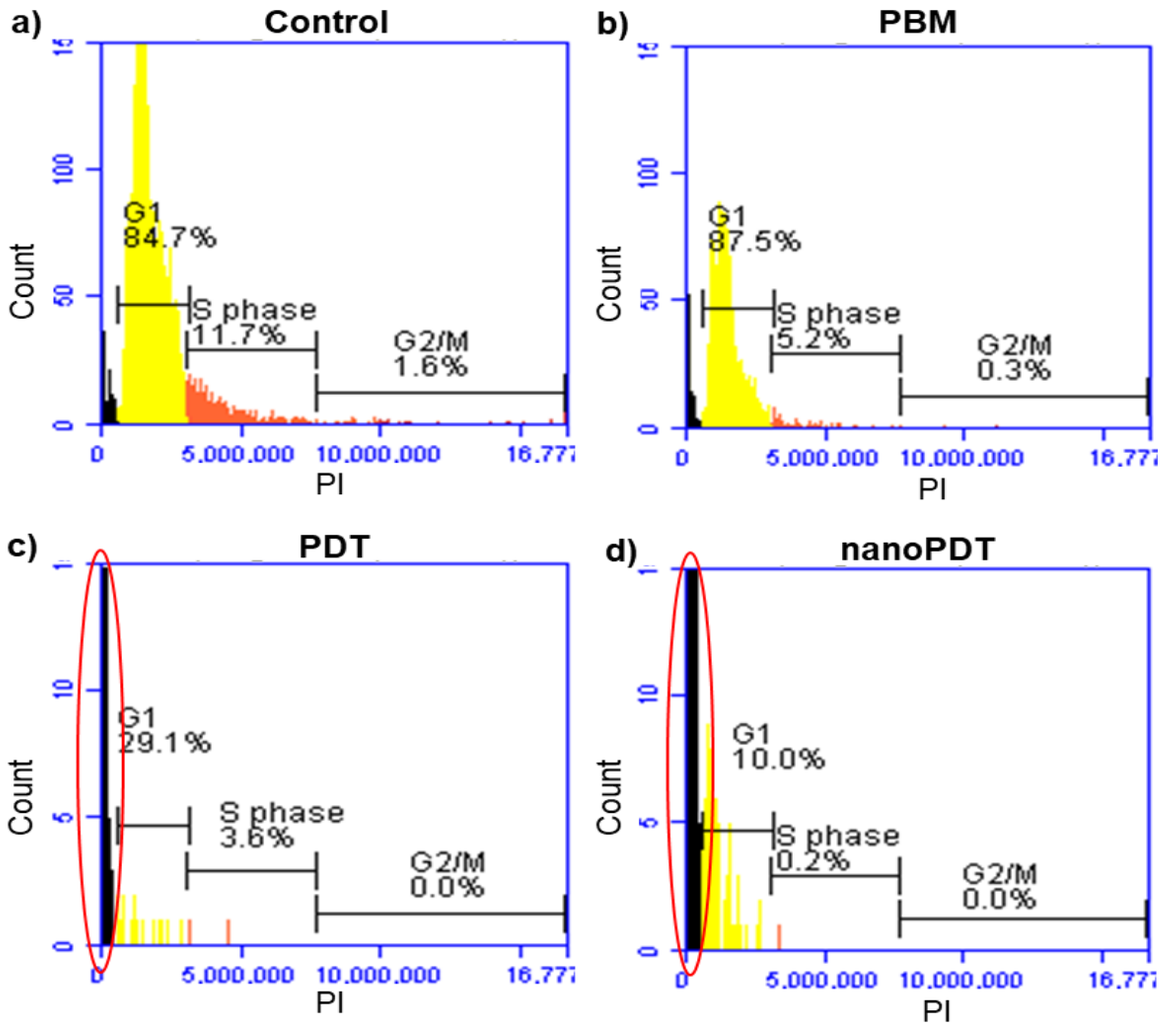

Figure 2. Cell cycle analysis through DNA quantification of lung epithelial carcinoma cells (A549) 24 hours post PDT treatment.

3.3. Proliferation and toxicity

Cell proliferation was measured using ATP luminescence in relative light units and cytotoxicity was measured as the amount of LDH leakage caused by membrane damage and cell death, where it was

read using an absorbance of 490 nm. Proliferation results (figure 3 a) show that control cells and PBM treated cells had an increased proliferation rate with PBM cells having a significant increase in proliferation. Cells treated with PDT and nanoPDT had a significant decrease in proliferation compared to the control and cells treated with PBM alone. There is a slight decrease seen in cells treated with nanoPDT compared to PDT, however this decrease is not significant. Cytotoxicity results (figure 3 b) show that control cells and PBM-treated samples released little LDH into the environment, cells treated with PDT and nanoPDT both released significant amounts of LDH. Additionally, nanoPDT treated samples show a higher increase in LDH release when compared to PDT-treated samples.

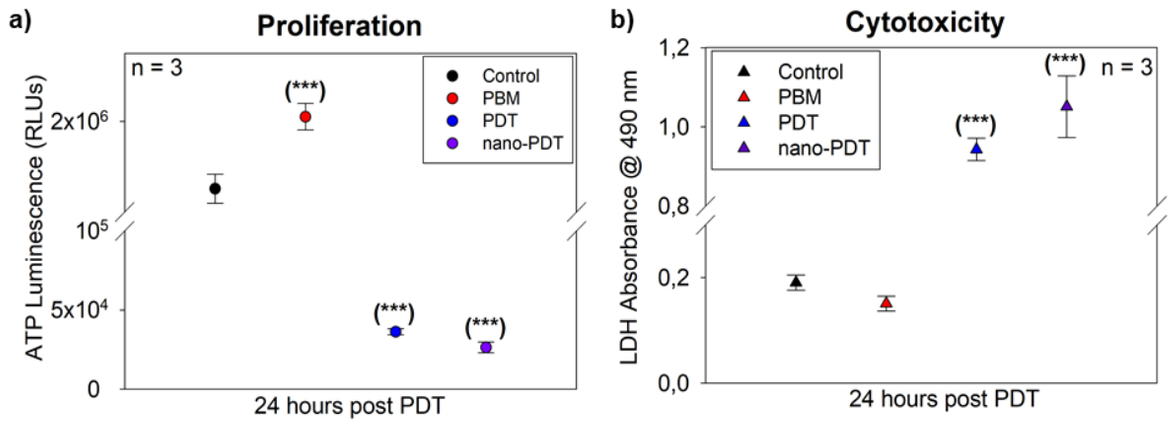

Figure 3. Proliferation and cytotoxicity of lung epithelial carcinoma cells (A549) 24 hours post PDT treatment.

4. Discussion and Conclusion

Considering lung cancers ability to metastasize, cancer treatments need to be effective at reducing cell motility and their proliferative abilities, which can reduce cancer relapse and enhance prognosis. The interruption of normal biological function followed by disseminating tumour cells causes mortality and morbidity in individuals with cancers. Cancer metastasis is thought to be caused by tumour cell motility. The importance of tumour cell migration in metastatic development has been demonstrated experimentally and empirically through fundamental and clinical studies. Cell motility is seldom targeted clinically, and adjuvant treatment to prevent cancer cell spread is severely restricted [15]. The purpose of this study was to determine the effect of nanoPDT on lung cancer cells. NanoPDT was shown to limit migration because of cell death. Cancer cells must be both proliferative and invasive to infiltrate and metastasize. Whereas lung cancer's proliferative, invasive, and migratory properties are correlated to the cell cycle phase they are in [16]. Cancer cell cycle arrest in the G1 phase is required for cell invasion [17] and cells in the S phase are indicative of proliferative activity [18]. Cells that are in the G0 phase or are senescent are unable to begin the cycle due to DNA damage and self-destruction [19]. NanoPDT, more than PDT, induces lung cancer cell cycle arrest, in which cells exit the G1/S stages of invasion and proliferation and enter irreversible G0 owing to DNA damage and degradation. The regulatory criteria for cancer treatment include the need that the drug be cytostatic, producing antiproliferation and toxicity in the cancer, as research indicates that therapy-induced senescence offers a unique functional target that may enhance cancer therapy [20]. The results of this study suggest that nanoPDT is an effective cytostatic treatment for lung cancer cells, as demonstrated by a significant reduction in proliferation and an increase in toxicity. In conclusion, the impacts of PDT and nanoPDT on lung cancer cell motility demonstrated substantial reductions in cell migration and proliferation, an increase in cell cycle arrest, and a large rise in membrane damage, all of which

facilitated lung cancer cells' anti-metastatic capacities. In general, nanoPDT demonstrated increased anti-metastatic activity.

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