Experimental Evaluation of Cytotoxicity Effects in Cancer and Normal Cells Exposed to Far Infrared Radiation

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Abstract— It has been proven that many of biological processes are frequency selective processes that relate to quantum energy state of photosensitive molecules. It was shown that light-activated changes in protein energy states can induce or modulate biological processes. Various up-to-date methodologies that incorporate low-intensity light into therapeutic procedures have been integrated into modern medicine. Here we have studied experimentally the hypothesis of the Resonant Recognition Model (RRM) that selectivity of protein activities is based on specific resonant electromagnetic interactions [1]. The RRM theory proposes that an external electromagnetic field at a particular activation frequency would produce resonant effects on protein biological activity, and this activation frequency can be determined computationally [1].

In our previous study [2] it was proposed that the wavelengths of the applied electromagnetic radiation (EMR) in a range of 3500–6400 nm are expected to affect biological activity of oncogene and proto-oncogene proteins [2, 3]. Thus, in this study we designed an exposure system based on IR-LED to irradiate the selected cancer and normal cells in the wavelength range predicted computationally by the RRM. The experimental evaluation of the attained far infrared wavelengths of 3400 nm, 3600 nm, 3800 nm, 3900 nm, 4100 nm and 4300 nm was conducted on a mouse melanoma (B16F0) and Chinese Hamster Ovary (CHO) cell lines. CHO cells are normal cells and used here as a control and B16F0 is cancer cell line.

A comprehensive quantitative analysis of the exposed and sham-exposed B16F0 and CHO cells has been carried out. The results obtained from LDH cytotoxicity test of B16F0 and CHO cells exposed to the computationally predicted wavelengths of far IR light presented and discussed here. In addition qualitative analysis of the effects of applied radiation on cancer and normal cells was performed using the light microscopy. The significance of the findings obtained from the cytotoxicity effects measured by LDH test as well as light microscopy's results is discussed and compared with the computational predictions.

1. INTRODUCTION

Cancer is one of the top ten disease with the highest mortality rate according to World Health Organization, and has a second highest death rate in the developed countries [4]. Cancer develops due to permutations in DNA of a somatic cell resulted from the functional changes in some of its genes. Genes are small coding sequences along a strand of DNA, which control the functionality of cells and human body in general. The functionality of a gene depends on the combination of amino acids present and active in the cell structure. Low intensity light therapy is an external irradiation exposure method which showed to be able to affect biological processes. The effects of low intensity light radiation on cells and molecules have been extensively studied recently.

Amongst different modelling approaches proposed in previous years, we found that the Resonant Recognition Model (RRM) presents an efficient tool for computation of frequencies which have resonant effects on proteins biological activity [5, 6]. Protein interactions are highly selective, and this selectivity is defined within a protein's structure. In our previous work [2] a relationship between the RRM spectra of some protein groups and their interaction with visible light has been established.

In this study, the RRM approach was used to predict the activation frequency of EMR that would modulate the function of proto-oncogene proteins. We have designed and presented the exposure system that can emit light at the selected frequencies [2]. This study investigates the effect of non-coherent low intensity light exposures on B16F0 mouse melanoma cancer cells and CHO, normal Chinese Hamster Ovarian cell line.

2. MATERIAL AND METHODS

2.1. Determination of the RRM Characteristic Frequency

It was shown in our previous studies that all protein sequences with a common biological function have a common frequency component in the free energy distribution of electrons along the protein backbone. This characteristic frequency was shown to be related to protein biological function [2, 3]. It was also shown that proteins and their targets share a characteristic frequency. Thus, it can be postulated that RRM frequencies characterize not only a general function but also a recognition/interaction between the particular proteins and their target at a distance. Thus, protein interactions can be viewed as a resonant energy transfer between the interacting molecules. This energy can be transferred through oscillations of a physical field, possibly electromagnetic in nature [2]. Since there is evidence that proteins have certain conducting or semi-conducting properties, a charge moving through the protein backbone and passing different energy stages caused by different amino acid side groups can produce sufficient conditions for a specific electromagnetic radiation or absorption [2]. A strong linear correlation exists between the predicted and experimentally determined frequencies corresponding to the absorption of electromagnetic radiation of such proteins [2]. It is inferred that approximate wavelengths in real frequency space can be calculated from the RRM characteristic frequencies for each biologically related group of sequences. These calculations can be used to predict the wavelength of the light irradiation, which might affect the biological activity of exposed proteins. The frequency range predicted for protein interactions is from 10^{13} Hz to 10^{15} Hz. This estimated range includes IR, visible and UV light. These computational predictions were confirmed by comparison of: (i) absorption characteristics of light absorbing proteins and their characteristic RRM frequencies [2]; (ii) frequency selective light effects on cell growth and characteristic RRM frequencies of growth factors [2]; and (iii) activation of enzymes by laser radiation [2]. These results indicate that the specificity of protein interaction is based on a resonant electromagnetic energy transfer at the frequency specific for each interaction. A linear correlation between the absorption spectra of proteins and their RRM spectra with a regression coefficient of $K = 201$ was established. Using RRM postulates, a computationally identified characteristic frequency for a protein functional group can be used to calculate the wavelength of applied irradiation, λ, defined as $\lambda = 201/f_{RRM}$, which could activate this protein sequence and modify its bioactivity [2, 3].

Here we employed the RRM for analysis of 28 proto-oncogene proteins. The RRM characteristic frequency was determined at $f_{RRM} = 0.0576$. This frequency is then converted into the wavelength of the applied irradiation using the scaling factor $K = 201$ to define the range of activation frequency, λ, that would modulate the activity of the proto-oncogene proteins. The predicted wavelength is defined at $\lambda = 3489$ nm. The exposure LED-based system was developed and the experimental evaluation of the attained far infrared wavelengths of 3400 nm, 3600 nm, 3800 nm, 3900 nm, 4100 nm and 4300 nm was conducted on B16F0 and CHO cell lines.

2.2. Materials and Cell Lines

LDH diagnosis kit (Roche Australia). The culture media: clear DMEM (Invitrogen Australia). Each bottle of 500 ml had 10ml of HEPES (buffer for Media) with 10% of Fetal Bovine Serum and 1% of Antibiotics (streptomycin). Three different patches of B16F0 and CHO have been used for the experiments.

2.3. Experimental Procedures

In our experimental set up, exposure and post exposure conducted inside incubator with constanct-intensity for a better comparison factor. All experiments were done three times in triplicate for the accuracy of the results.

All the cell lines were seeded in the plate for 24 hrs before the start of experiments. Three types of experiments were conducted: (i) 1.5 hrs of exposure +0 hrs of post exposure; (ii) 1.5 hrs of exposure +24 hrs of post exposure; and (iii) 1.5 hrs of exposure +24 hrs of post exposure.

In addition, to eliminate any effect induced by the heat generated by the LEDs used, we have used a heat shield gel purchased from Inventables, USA. More importantly, we have eliminated any cross talk between the LED frequencies and effect of two frequencies on the same well by having empty wells around each well that we are running the experiments.

3. RESULTS AND DISCUSSION

Irradiation of the selected cancer and normal cells was conducted with the results shown in Figure 1 which presents the cell viability tests conducted on B16F0 and CHO cells.

It can be clearly seen from Figure 1 that the cell viability of murine cancer cells B16F0 is reduced when the time of exposure and the time of post exposure are increased. The difference between the cell viability of exposed and non-exposed (untreated) cells is increased by increasing the post

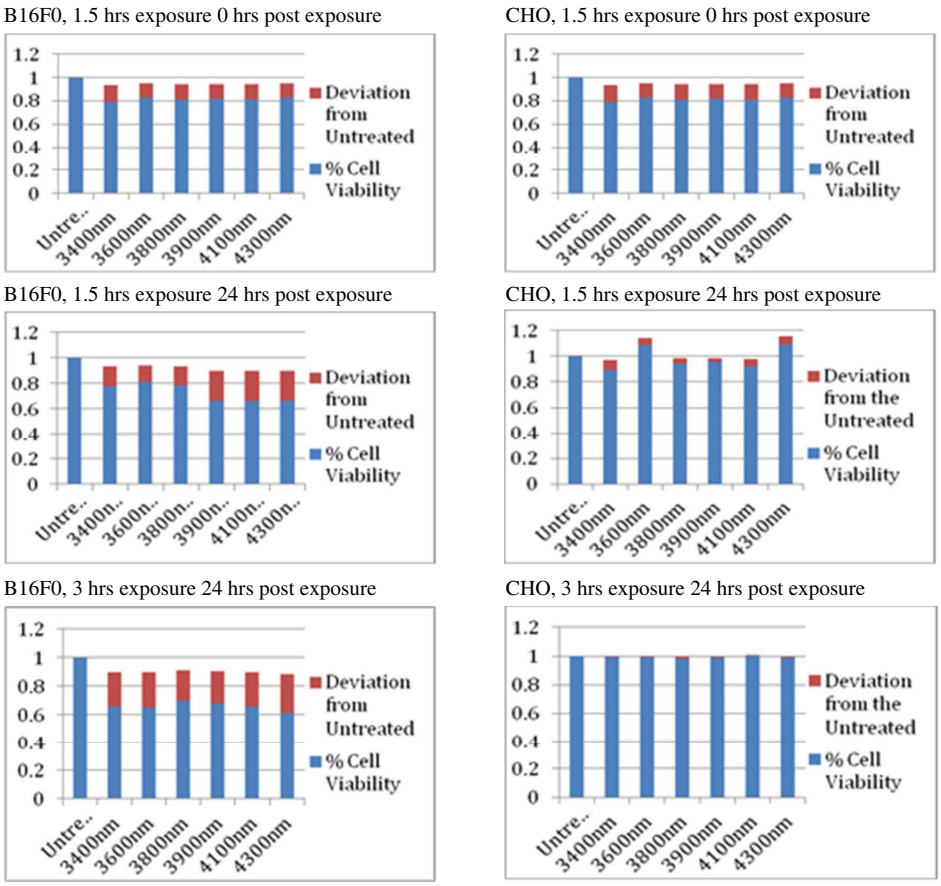

Figure 1: Cell viability of B16F0 and CHO cells for different exposure and post exposure times.

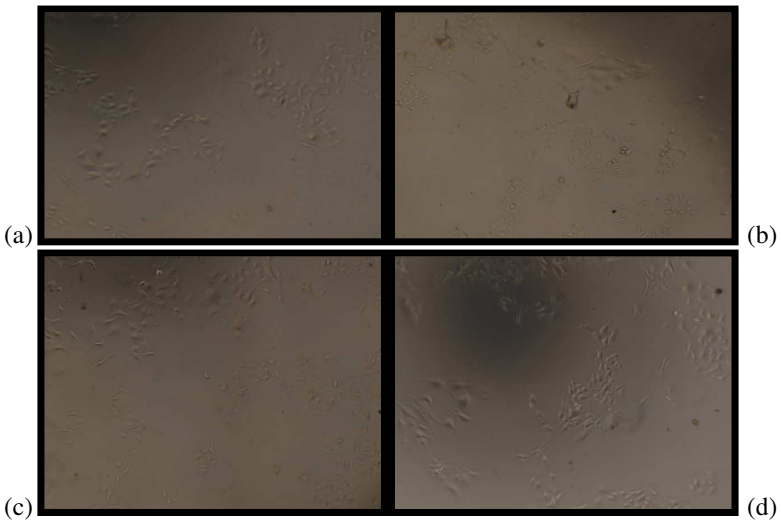

Figure 2: Light microscopy images of B16F0, murine melanoma cells, before and after the far infrared light exposures: (a) before 1.5 hr of exposure, (b) before 3 hrs of exposure, (c) after 1.5 hr of exposure, and (d) after 3 hrs of exposure.

exposure duration. However, in the case of CHO cells, the difference in cell viability for exposed vs. non-exposed cells is not significant. Irradiation of CHO cells at all studied wavelengths has not induced any effects on their viability.

Figure 2 shows the images obtained by light microscopy which do not indicate any changes in the morphology of the cells while LDH results clearly demonstrate reduction in the cell viability upon irradiation.

4. CONCLUSIONS

The experiments conducted in this study showed that far infrared light at the specific frequencies predicted computationally can induce changes in cell viability of the selected murine melanoma cells B16F0. These results support the hypothesis that external electromagnetic radiation can modulate biological process. The exposure system based on LEDs was developed and its efficiency was evaluated experimentally. Quantitative analysis of the experimental data with LDH cytotoxicity test showed the reduced cell viability observed for cancerous cell line while the normal cells were not affected by light exposures. However, light microscopy images of cancer cells taken before and after the exposures to far infrared light do not show any changes in the cells morphology.

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