

# Study of Phototoxicity of LED Light for Colon Cancer



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## Abstract

Colon cancer is very important cancer worldwide. Although many therapies and therapeutic strategies are applied in the patients with cancer, however, the deaths of patients with cancer maintain in the top of the dead people worldwide. The visible light at a wavelength corresponding to the drug absorption activates these agents and induces highly cytotoxic products was verified. Application of the visible light at a wavelength is a potential for cancer therapy. In this study, *in vitro* and *in vivo* data were demonstrated that LED light at 450 nm induces highly cytotoxic ability and suppresses the growth of colon cancer in the CT-26 bearing mice. Taken together, LED light at 450 nm has the potential to be developed as a new therapeutic method for colon cancer in the future.

**Keywords:** Colon cancer; *In vitro*; *In vivo*; LED light; Phototoxicity

## Mini Review

### Phototoxicity on Colon Cancer

Cancer may be viewed as a disease that progresses through multiple stages that involve initiation, promotion, progression, and metastasis [1]. Colon cancer is very important cancer worldwide. Although many therapies and therapeutic strategies are applied in the patients with cancer, however, the deaths of patients with cancer maintain in the top of the dead people worldwide [2]. Previous researchers have demonstrated that photodynamic therapy using cancer designed photosensitizers and laser beams has been increasingly used in clinical medicine. The visible light at a wavelength corresponding to the drug absorption activates these agents and induces highly cytotoxic products: singlet molecular oxygen and oxygen radicals. The LED light induced cell damage was wavelength dependent, but not energy dependent [3]. Therefore, novel therapies and therapeutic strategies are needed for the cancer therapy in the future. The objective of this investigation was to R&D of the novel therapies and therapeutic strategies against

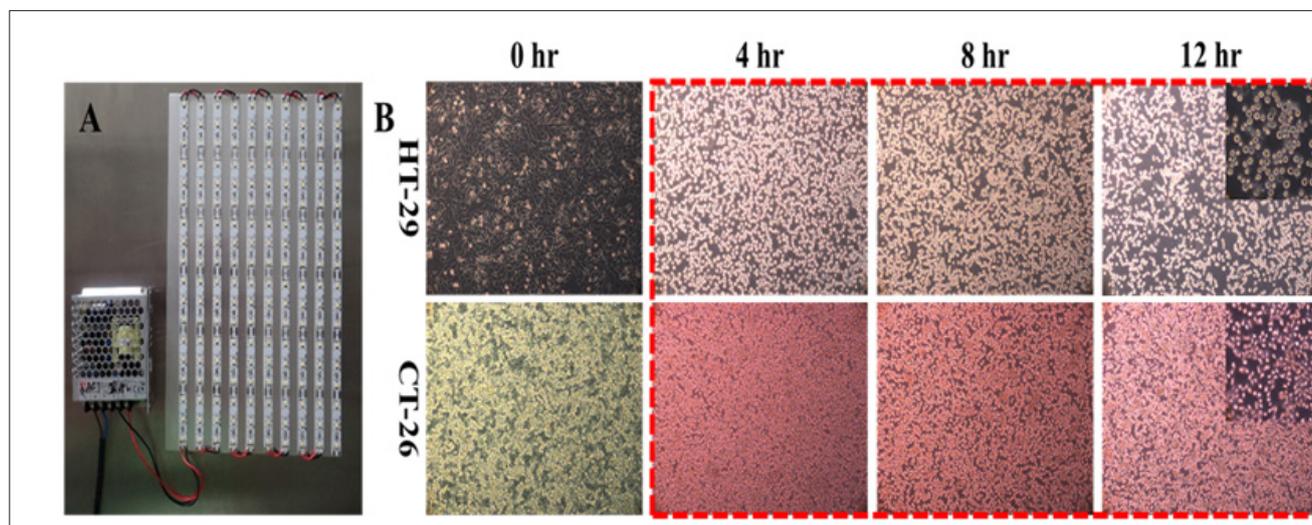
colon cancer via this novel therapy. In addition, we will wish to apply this phototoxic therapeutic method against the tumor growth in the deep body cavities in the future. Finally, we hope this novel therapies also can be applied to suppress tumor growth, metastasis, and recrudescence.

### Effect of LED Light at 450 nm Wavelength with on Colon Cancer Growth *In vitro* and *In vivo*

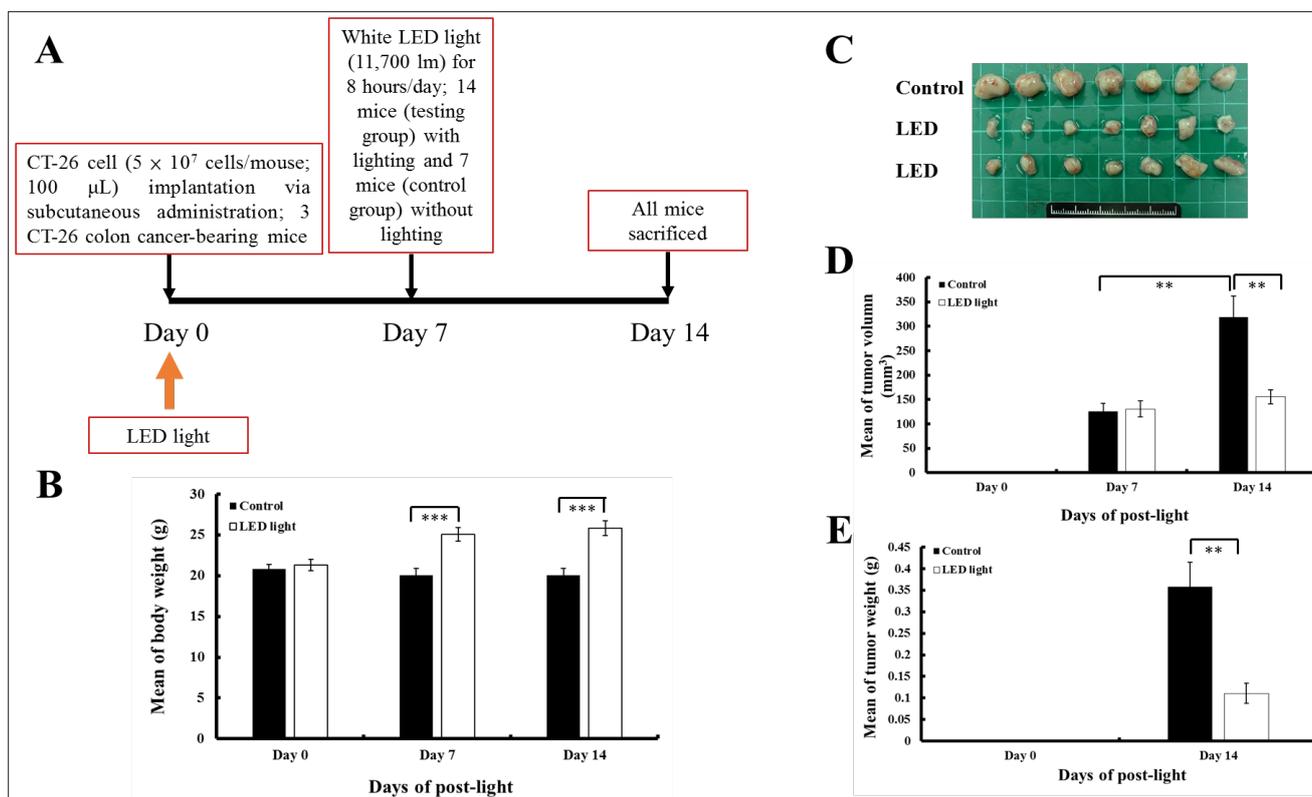
Human and murine colon carcinoma cell lines used were HT-29 (ATCC® number: HTB-38™) and CT-26 (ATCC® number: CRL-2638™). HT-29 and CT-26 were respectively cultured in McCoy's 5a medium modified and RPMI-1640 medium (GIBCO®) and were maintained in its specific media supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 mg/ml streptomycin (Invitrogen), in a humidified 5% CO<sub>2</sub> incubator at 37°C. Effect of phototoxicity under LED lighting for human and murine colon cancer cell lines *in vitro* was evaluated. Cell morphology was observed between the *in vitro* experiments. Cells

were treated with LED lighting 8 hr per day for 14 days in the 6 well culture plate. Observation of cell morphology with/without LED lighting (11,700 lm; Figure 1A) was by light microscope (Axiovert 200 M, Zeiss). Data were shown that LED light significantly induce

cell death of HT-29 and CT-26 cells after 4 hours LED light exposure. Between 4 to 12 hours LED light exposure, all HT-29 and CT-26 cells were died according to their cell morphology (Figure 1B).



**Figure 1:** Phototoxic device and *in vitro* effects. (A) Appearance of LED light. (B) LED light is phototoxic for colon cancer cell lines, HT-29 and CT-26, by direct cytotoxic effect *in vitro*. HT-29: Human colon cancer cell line; CT-26: Murine colon cancer cell line.



**Figure 2:** Design of this study and phototoxic device and *in vivo* effects. (A) Design of this study. (B) LED light is benefit for the increase of body weight of mice. \*\*\**p* < 0.001. (C) Gross appearance of CT-26 colon tumor mass. (D) LED light is photo suppression for the tumor volume of CT-26 murine colon carcinoma in an ectopic allograft murine model *in vivo*. \*\**p* < 0.01. (E) LED light is photo suppression for the tumor weight of CT-26 murine colon carcinoma in an ectopic allograft murine model *in vivo*. \*\**p* < 0.01. No. of mice in LED group is 14; No. of mice in Control group is 7.

Animal care and all *in vivo* experiments were performed in compliance with the guidelines of the Agricultural Technology Research Institute (ATRI): Institutional Animal Care & Utilization Committee. BALB/c mice (6 to 8 weeks old) were purchased from National Laboratory Animal Center, Taipei, Taiwan. Mice were provided a standard laboratory diet and distilled water and kept on a 12 h light/dark cycle at  $25 \pm 2^\circ\text{C}$  in the GLP Animal Test Room, Animal Technology Laboratories, ATRI, Miaoli, Taiwan. The experimental design *in vivo* was performed. To test the effects of LED light on CT-26 cells *in vivo*, CT-26 was established by subcutaneous implantation of CT-26 cells into BALB/c mice. BALB/c mice ( $n = 7$  mice/control group; 14 mice/LED group) were randomly assigned to two groups: (1) a control group, vehicle with CT-26 cell implantation and without LED lighting; (2) a LED groups, CT-26 cell implantation with LED lighting (8 hr/day for 2 weeks). After 7 days CT-26 cell implantation, LED lighting was performed (Figure 2A). All animal experiments were approved by the Institutional Animal Care and Use Committee of ATRI, Hsinchu, Taiwan (approval No.: 107025). CT-26 cells ( $5 \times 10^6$  cells per mouse) were suspended in 100 ml of serum free RPMI-1640 medium and injected subcutaneously into mice, aged 6 to 8 weeks. When tumors were detected, tumor volume was measured using the formula:  $1/2 \times \text{the largest diameter} \times (\text{the smallest diameter})^2$ , as reported in previous cancer studies [4,5]. Measurement of tumor

volume was periodically performed with calipers. At the end of the experiment, all mice in each group were sacrificed and tumor mass were collected. Herein, LED light with wavelength at 450 nm significantly elevated body weight of mice (Figure 2B). *In vivo* data were demonstrated that LED light with wavelength at 450 nm significantly suppressed CT-26 tumor weight and tumor volume in BALB/c mice with an ectopic allograft model (Figure 2C-2E).

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