Photothermal Ablation of Glioblastoma Using Anti-CD133 Modified Carbon Nanotubes

Chung-Hao Wang[1], Yao-Jhang Huang[1], Ching-An Peng[1,2,*]
[1]Department of Chemical Engineering, National Taiwan University, Taipei, Taiwan
[2]Department of Chemical Engineering, Michigan Technological University, Houghton, Michigan

Statement of Purpose: Glioblastoma (GBM) is the most common primary brain tumor in adults, without efficient known therapy. Treatment with chemotherapy has been hampered by the apparent resistance of GBM in vivo to various agents and challenges in delivering agents to the tumor cells. The lack of progress in such treatments reflects the need for rapid assays to study the effect of cytotoxic agents on GBM in vitro. It appears now that a new approach for such an assay can be based on the existence of a small fraction of the GBM that can be identified as cancer stem-like cells. In malignant brain tumors, CD133 has been suggested to be a cancer stem cell marker since only CD133 positive cells from brain tumor biopsy material were able to initiate brain cancer in a mouse model. Prominin-1 (PROM-1), also called CD133, is a protein with several isoforms of unknown physiological or pathological function, and is localized both in the cytoplasm and at the cell surface. Among a plethora of nanomaterials designed and synthesized for biomedical applications, carbon nanotube (CNT) owing to its unique features has been demonstrated to be an alternative option for localized hyperthermia treatment in comparison with nanogold-based materials. In this study, our objective was to conjugate anti-CD133 onto CNT and destroy $CD133^+$ GBM cells by harnessing photothermal energy generated from CNT that are irradiated with near-infrared (NIR) light.

Methods: 10 mg of the carboxylated MWNTs dissolved into DMF were reacted with 4 mg of EDC in the presence of 6 mg of NHS for 1 h at room temperature. After centrifugation for 10 min at 7,690 × g, the modified MWNTs were collected from the bottom layer. Five mL of deionized water were used to suspend MWNTs under sonication for 30 min. Then, 0.5 μg of anti-CD133 were added into 10 μg of the MWNT-containing tube and reacted for 2 h. For fluorescence observation, 2 μg of rhodamine B dissolved in DMF were further used to conjugate anti-CD133 via carbodiimide chemistry at 4 ℃ for 8 h. After the reaction, the mixture was washed with deionized water for 3 times to remove excrescent rhodamine B. Glioblastoma cell line GBM S_1R_1 and DAOY obtained from institute of clinical medicine in National Yang-Ming University were cultured in α-MEM medium supplemented with 10% FBS, non-essential amino acids solution, sodium pyruvate, L-glutamine. All the cultivation was performed at 37°C in humidified air containing 5% CO_2. Cells (1×10^6 cells) were plated on T-25 flask for 24 h. Prior to MWNT challenge, GBM S_1R_1 and DAOY cells were separately detached from the T-25 flask by trypsinization and inoculated onto a coverslip-bottomed Petri dishes (MatTek, MA, USA). In each dish, 2.5 μg of rhodamine B labeled anti-CD133-MWNTs (CDMWNT) was added. Glioblastoma cell line GBM S_1R_1 cells were imaged by a confocal spectral microscope imaging system (Leica TCS SP5, Wetzlar, Germany). For nucleus staining, 20 μg DAPI was added to each dish and allowed to incubate for 1 hr at room temperature before taking confocal images. First, 1×10^5 cells of GBM S_1R_1 cells were cultured in 24-well tissue cultured plate for 24 h. GBM S_1R_1 cells with CDMWNT treatment were exposed to an 808-nm laser source (Opto Power Corporation, Tucson, AZ), a 15 watt fiber-coupled laser integrated unit. Note that all cells were washed with excess MWNTs in the solution removed and placed in fresh solutions after incubation in MWNT solutions and before any of the *in vitro* laser radiation experiments described in this work. The laser beam was delivered to the target through a 1.5m long, 600 μm single core fiber with a numerical aperture of 0.37, followed by a 25-mm focal length fused-silica biconvex lens. The focused spot size was 1.2 cm. After the treatment of laser, cells were stained by 2.5 μM calcein-AM. Cell death was determined by calcein-AM dying cells to determine cell viability.

Results: Our results showed that the rhodamine B labeled CDMWNTs were targeted toward the cell membrane of glioblastoma GBM S_1R_1 cells to express the high-intensity CD133 receptor. Comparing with DAOY cells, GBM S_1R_1 cells could internalize more CDMWNT into cell inside than DAOY cells did. Therefore, anti-CD133 labeled MWNT could selectively target on GBM S_1R_1 cells expressed with the CD133 receptors. After cells irradiated with NIR laser, calcein-AM was used to detect the cell viability. After dying with calcein-AM 496 nm blue light was used to irradiate the cell. If the cells were alive, it would emit 516 nm green light. If the cells were dead, it would not emit any light. After incubation of CDMWNT for 6 hr, the images of DAOY in the laser-beaming range, boundary and outside were taken. DAOY cells were green in all regions. By contrast, GBM S_1R_1 cells treated with CDMWNT were all dead in the region of laser beam. In Figure 1(d)-(e), the one-by-one ratio mixture of GBM S_1R_1 and DAOY cells did leave some green alive cells (i.e., DAOY), after 808 nm NIR beamed.

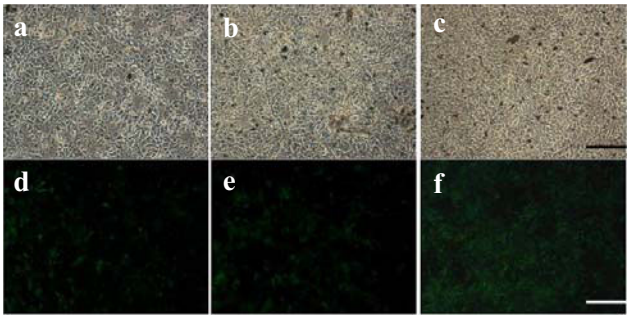

Figure 1. Photothermal therapy of mixture with GBM S_1R_1 and DAOY cells, ratio was 1 to 1, beamed 808-nm NIR laser. After incubation of 1:1 mixture of GBM S_1R_1 and DAOY cells with CDMWNT for 6 hr, 808-nm laser was used to beam the cells for 15 min. Then, the cells were dyed with calcein-AM. Fig (a-c) showed the bright view photos in the wells of laser beaming inside, boundary, and outside. Fig (d-f) showed the corresponding fluorescent photos. (Scale bar = 100 μm)

Conclusions: It was demonstrated that anti-CD133-tagged MWNTs can specifically target onto the surface of high $CD133^+$ GBM S_1R_1 cells, internalized into cells, and phototheraml ablated by NIR irradiation. The CD133- DAOY cells were able to survive after 15-min NIR laser exposure. With these two different types of GBM cells well mixed in equal cell amount, and cultured in plates, our result showed that green living cells (i.e., DAOY cells) can be observed in the NIR laser irradiated zone. This indicates that the anti-CD133 labeled CNT can be used as the functional nanomaterials targeting CD133-expressing stem-like cancer cells among a mixed neural cell population and photothermally ablating them by NIR laser light.