Gadolinium Chloride (GdCl₃) Inhibits Cell Proliferation and Cell Motility in Cisplatin-Resistant Human Oral Cancer CAR Cells: A Novel Screening System for Real Time Imaging Using incuCyte™ Kinetic Live Cell Imaging System

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ABSTRACT

Gadolinium chloride (GdCl₃) is a contrast medium for Magnetic Resonance Imaging (MRI) use. Previously, our study has demonstrated that GdCl₃ significantly reduced cell viability and triggered apoptosis in human osteosarcoma cells by death receptor, mitochondria-dependent and Endoplasmic Reticulum (ER) stress pathways. However, no report showed the anticancer effects of GdCl₃ on drug-resistant oral cancer cells. In the present study, we investigated the anti-proliferation and anti-motility effects of GdCl₃ on the cisplatin-resistant human oral cancer CAR cells. Our results showed that GdCl₃ inhibited cell proliferation and cell motility in a time-dependent manner by using IncuCyte™ Kinetic Live Cell Imaging System. Our finding provides direct evidence of real-time imaging analysis to support the oral anti-cancer effects of GdCl₃ on drug-resistant oral cancer cells in vitro.

Introduction

Oral cancer is the fifth leading cause of death in cancer from the annual report of the Ministry of Health and Welfare, R.O.C. (Taiwan) in 2014 [1]. Chemotherapy, radiotherapy, and surgery are major treatments for oral cancer [1-4]. However, drug resistance to the chemotherapeutic agents is a major impediment in clinical medical treatment [4,5]. It is urgent for the discovery of a new compound to overcome the resistance of chemotherapeutic drugs in oral cancer. Gadolinium (Gd), a member of Lanthanides family, has multi-biological effects and applications in Magnetic Resonance Imaging (MRI) contrast medium or the potential anti-cancer agents [6,7]. Gadolinium chloride (GdCl₃) has demonstrated to inhibit cell proliferation and to induce mitochondria-dependent apoptosis in human hepatoblastoma HepG2 cells [7]. Our previous study has shown that GdCl₃...
triggered U-2 OS cell apoptosis through death receptor, mitochondria and ER stress-dependent pathways [6]. This study is to investigate the anti-cancer effects of GdCl3 on cell proliferation and cell motility of cisplatin-resistant human oral cancer CAR cells.

**Materials and Methods**

**1. Chemicals and reagents**

Gadolinium chloride (GdCl3) was obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). Fetal bovine serum (FBS), Dulbecco’s modified Eagle’s medium (DMEM), L-glutamine, and penicillin/streptomycin were obtained from Thermo Fisher Scientific (Waltham, MA, USA).

**2. Cell culture**

The cisplatin-resistant cells (CAR) was developed by treating CAL 27 cell line, a parental human tongue squamous cell carcinoma (American Type Culture Collection, Manassas, VA, USA) with 10 to 80 μM of cisplatin. The cells were cultured in DMEM fortified with 10% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine at 37°C with a humidified 5% CO2 air. The cisplatin-resistant CAR cells were constantly cultured in complement medium containing 80 μM cisplatin, unless otherwise indicated [1,2,4,5].

**3. Cell proliferation assay by IncuCyte™ kinetic live cell imaging system**

To measure the cell proliferation, CAR cells (2×10^4 cells) were plated into a 96-well plate and then incubated with or without 100 μM of GdCl3. Cell proliferation was determined by the IncuCyte™ Kinetic Live Cell Imaging System (Essen BioScience, Ann Arbor, MI, USA). Imaging was photographed every 2 h for a 48-h period [1,8].

**4. Cell motility assay by IncuCyte™ Kinetic Live Cell Imaging System**

Cell motility assay was analyzed using wound healing assay and by IncuCyte™ Kinetic Live Cell Imaging System. CAR cells were plated in 96-well plates (~1×10^5 cells/well) and incubated to 90% confluence. After scratch wounds were made using the Incucyte Scratch instrument (Essen Bio Science), cells were treated with or without 50 μM of GdCl3. Cell motility was determined by the IncuCyte™ Kinetic Live Cell Imaging System (Essen Bio Science). Imaging was photographed every 3 h for a 48-h period [4,9].

**Results and Discussion**

After CAR cells were treated with GdCl3 at 100 μM, cell proliferation was assessed by IncuCyte™ Kinetic Live Cell Imaging System. Cell proliferation was decreased in GdCl3-treated CAR cells in a concentration-dependent manner (Figure 1 and Supplementary video 1).

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and some cell debris were observed in CAR cells. Our data suggested that GdCl3 inhibited cell proliferation in CAR cells. In addition, CAR cells were exposed to 50 μM of GdCl3, cell motility was reduced in CAR cells, and this effect was in a time-dependent manner (Figure 2 and Supplementary video 2).

Our results indicated that high concentration of GdCl3 caused growth inhibition and low concentration of GdCl3 caused antimotility in CAR cells. Lanthanides (Lns) compounds have anti-cancer activities such as anti-proliferation, promotion of cell cycle progression, antimotility and induction of cell apoptosis [6,10-13]. This study is first to report that GdCl3 can be successfully functional to inhibit cell proliferation and motility in cisplatin-resistant human oral cancer CAR cells. It is important and essential for anti-cancer drug discovery to measure the cell proliferation rate in an in vitro study [14-17]. Many strategies and methods have been proposed to detect cell proliferation rates. MTT and WST-1 have been widely used to detect the overall metabolic activity in cells [18-20]. However, those methods cannot provide direct evidence of real-time imaging analysis [1]. In the current study, we are the first time to use the IncuCyte™ Kinetic Live Cell Imaging System for characterizing cell proliferation and cell motility in GdCl3-treated CAR cells. In the future, the anti-proliferation and antimotility activities of GdCl3 will be further studied by using this powerful tool IncuCyte™ Kinetic Live Cell Imaging System in our group.

Conclusion

In conclusion, we herein demonstrated that GdCl3 exhibits direct anti-cancer activity by suppression of cell proliferation and cell motility in cisplatin-resistant human oral cancer CAR cells.

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