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Short communication

Application of microfluidic gradient chip in the analysis of lung cancer chemotherapy resistance

Wang Siyan[a,c], Yue Feng[b], Zhang Lichuan[c], Wang Jiarui[c], Wang Yingyan[d], Jiang Li[c], Lin Bingcheng[e], Wang Qi[f,*]

[a] Department of Emergencyt, The Second Hospital Affiliated to Dalian Medical University, 467 Zhongshan Road, Dalian 116023, China
[b] Department of Urology, Dalian Municipal Friendship Hospital, 47 Renmin Road, Dalian 116001, China
[c] Graduate School of Dalian Medical University, 9 West Part, Lvshun Southern Road, Dalian 116044, China
[d] Diagnostics Laboratory Center, Dalian Medical University, 9 West Part, Lvshun Southern Road, Dalian 116044, China
[e] Dalian Institute of Chemical Physics, Chinese Academy of Sciences, 457 Zhongshan Road, Dalian 116023, China
[f] Department of Respiratory Medicine, The Second Hospital Affiliated to Dalian Medical University, 467 Zhongshan Road, Dalian 116023, China

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ABSTRACT

The major challenge of chemotherapy is the disease resistance for many lung cancer patients. Integrated microfluidic systems offer many desirable characteristics and can be used in cellular biological analysis. This work aimed to study the correlation between the expression of Glucose Regulated Protein-78 (GRP78) and the resistance to anticancer drug VP-16 in human lung squamous carcinoma cell line SK-MES-1 using an integrated microfluidic gradient chip device. We used A23187, a GRP78 inducer, with a gradient concentration in the upstream network of the device to induce the expression of GRP78 in the cells cultured in the downstream before the addition of VP-16. The expression of GRP78 was detected by immunofluorescence, the apoptosis for the cells treated by VP-16 was assessed morphologically by 4′,6-diamidino-2-phenylindole (DAPI) staining. The results indicated that the expressions of GRP78 increased greatly for the cells under the induction of A23187 with a dose-depended manner, while the percentage of apoptotic cells decreased significantly after being treated by VP-16. Our results from this study confirmed the role of GRP78 played in the chemotherapy resistance to VP-16 in SK-MES-1 cell line, suggesting that the integrated microfluidic systems may be an unique approach for characterizing the cellular responses.

1. Introduction

Lung cancer is the leading cause of cancer death in human beings all over the world. The major clinical strategy for administration of lung cancer with the advanced stages is chemotherapy. However, the success of the treatment is limited by the intrinsic or acquired resistance of cancer cells to chemotherapy. The stress conditions that induce glucose regulated proteins (GRPs), a major family of stress proteins, include glucose starvation, hypoxia and low pH, all of which are normally observed in solid tumors [1–3]. These conditions could be an important mechanism for the resistance to anticancer drug in solid tumors.

Glucose Regulated Protein-78 (GRP78), a representative member of GRPs family in the tumor cell niche, plays an important role in the chemotherapy resistance in many tumors including breast, ovarian, colon and bladder cancer. However, little is known about the function of GRP78 in human lung cancer chemotherapy resistance. The conventional methods for the in vitro detection of GRP78 mainly include immunoprecipitation, Western blotting, and gel electrophoretisis on the cells cultured in flasks [4,5]. These processes involve a relatively long time, troublesome liquid-handling procedures and large quantities consumption of reagents; some require labor-intensive purification of the protein and complex analysis steps.

Microchip-based systems widely known as micro-total-analysis-systems (μ-TAS) or lab-on-a-chip have been spreading rapidly [6,7]. Many applications, including flow-injection analyses, solvent extractions, and microreactors have been demonstrated [8,9]. In these systems, full advantage was taken of the scale merits of the microspace, such as a short diffusion distance, a large specific interface area, a rapid and efficient reaction. Microchip techniques also appear to provide some advantages for cellular biological analysis systems [10,11], because the scale of the liquid microspace inside a microchip is fitted to the size of the cells. Besides, rapid and sensitive immunoassay systems for protein analyses with these systems have been demonstrated as well [12,13].

* Corresponding author at: Department of Respiratory Medicine, The Second Hospital Affiliated to Dalian Medical University, 467 Zhongshan Road, Dalian 116027, China. Tel.: +86 411 86110151; fax: +86 411 86110151.
E-mail address: wqdmu@yahoo.com.cn (W. Qi).

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In this work, an integrated microfluidic gradient chip was used as a platform to analyze the correlation between the expression of GRP78 and the resistance to anti-cancer drug VP-16 (etoposide) in human squamous carcinoma cell line SK-MES-1. Herein, A23187, an agent of calcium ionophore, a highly potent endoplasmic reticulum stress inducer, was used to induce the expression of GRP78. Also, the potential applications of the microchip system on the research of cell culture and cell function have been investigated.

2. Materials and methods

2.1. Microfluidic gradient chip fabrication

The microfluidic gradient chip was composed of an upstream concentration gradient generator (CGG) and a downstream cell culture module (Fig. 1). The design of the CGG was based on the work previously presented by Jeon et al. [14]. The formula of the concentration interval from channel 1 to channel 8 which generated by CGG in theory is (drug concentration$_{max}$ − drug concentration$_{mix}$)/7. The cell culture module, composed of an array of cell culture chambers, was integrated with the CGG unit, which was similar to the work described by Thompson et al. [15]. Three chambers in row were connected by channels for the performance of three independent cell groups. The microfluidic gradient chip was fabricated in polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning, Midland, MI, USA) by using standard soft lithography methods and rapid prototyping techniques [16,17]. The PDMS microfluidic network was irreversibly bonded to a glass slide assisted by oxygen plasma surface treatment (150 mTorr, 50 W, 20 s), creating an optically transparent device for cell culture and protein expression profiling. Dimensions and layout of the chip was shown in Fig. 1.

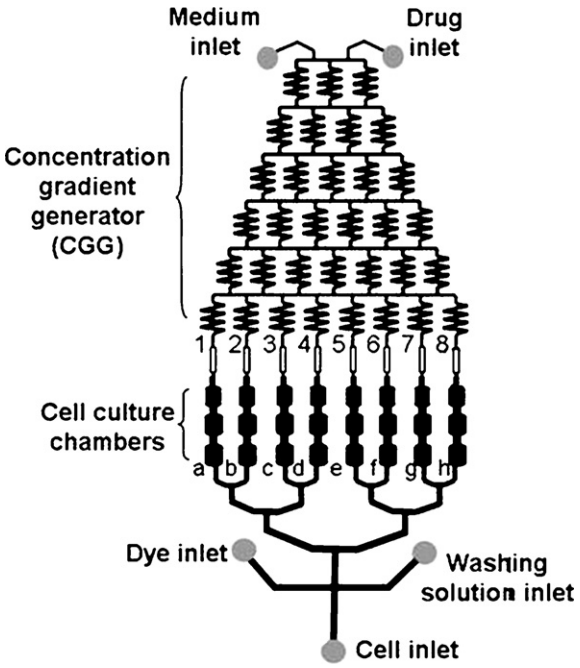

Fig. 1. Layout of the integrated microfluidic device mainly composed of an upstream gradient network (CGG) and the downstream cell culture chambers. The microfluidic channels in the upstream dilution module are 400 μm in length, 50 μm in width and 50 μm in height and generate several concentrations of the stimulus by continuous-flow diffusive mixing of adjacent laminar flow streams. The various concentrations are delivered to the downstream array of culture chambers, each 800 μm long, 500 μm wide and 100 μm in height.

2.2. Cell culture

Prior to cell culture, the PDMS microchip was treated as follows: First, the chamber was washed with dehydrated alcohol, and water, successively. Next, the chip was autoclaved at 120 °C for 15 min. The culture chamber was filled with poly-L-lysine solution (0.01%, m/v) (Sigma–Aldrich Co., St. Louis, MO, USA) for 1 h in order to coat its inner surface. The human lung squamous carcinoma cell line SK-MES-1 was obtained from ATCC (Manassas, VA, USA). The cells were maintained in minimum essential medium (MEM) with sodium pyruvate (1 mM) supplemented with fetal bovine serum (FBS) (10%, v/v)–penicillin (0.2 mM)–streptomycin (0.2 mM) (200:1:1, v/v/v) (pH* 7.4) in a humidified atmosphere of 95% air, 5% CO_2 at 37 °C. After being cultured in flasks at 60–70% confluence, the cells were digested with trypsin–PBS and resuspended, then they were injected into the microfluidic device at 5×10^6 cells/mL via the downstream cell inlet by syringe pump. The total injected volume was 10 μL. After incubated for 24 h, the viabilities of the cells were detected by a trypan blue exclusive assay [18]. All the experiments were repeated at least three times.

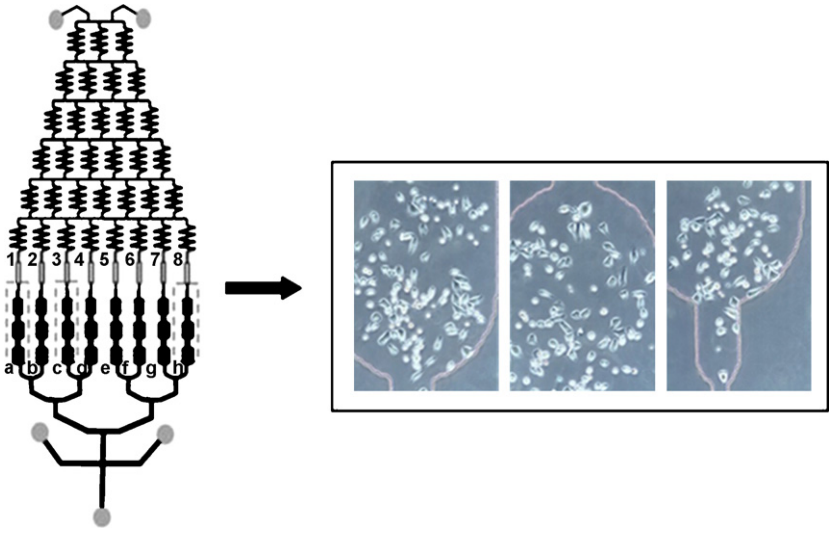

Fig. 2. The SK-MES-1 cells in cell culture chambers were incubated under stable conditions for 24 h. The SK-MES-1 cells could be successfully maintained in the microfluidic device. The viability of cells maintained in the culture chambers was almost unaffected prior to the treatment.