MICROFLUIDIC SINGLE CELL BIOANALYSIS TO MEASURE DRUG UPTAKE ON SINGLE MULTIDRUG RESISTANT CANCER CELL AS ENHANCED BY GINSENOSIDE RG3

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Abstract- This paper is to demonstrate that the microfluidic-based single cell chip can be applicable to study micro-gram level amount of ginsenosides on daunorubicin uptake in multidrug resistant (MDR) leukemia cells. Pure ginseng compounds are very hard to obtain and can only be obtained at a trace amount (mini grams level). This small amount sometimes is not enough for traditional methods to evaluate the MDR effect of these compounds. Here, the microfluidic single cell analysis method was used to evaluate the MDR effect of several ginsenosides. In this paper, we tested two ginsenoside compounds: 20(S)-Ginsenoside Rg3 (Rg3-S) and 20(R)-Ginsenoside Rg3 (Rg3-R)and found that Rg3-S provided a stronger effect on enhancing the intracellular accumulation of anticancer drug in the single MDR cancer cells..

Keywords- Cancer cell, Fluorescence, Ginsenosides, Microfluidics.

I. INTRODUCTION

One of the properties of cancer that poses a substantial obstacle to treatment of cancer is called Multi-drug Resistance (MDR). In previous research, MDR cells, characterized by the over-expression of the P-glycoprotein trans-membrane protein, have been correlated with lower uptake of chemotherapeutic drug due to its efflux out of the cell.

We have previously reported the Same-Single-Cell Approach (SASCA) used to study the modulation effect on the drug efflux in the MDR cancer cells. In 2011, we further reported an approach to measure drug accumulation rather than drug efflux in order to study the drug modulation effect (same-single-cell analysis by accumulation (denoted as SASCA-A)). Comparing with the previous SASCA method, the SASCA-A is faster and simpler.

In recent years, there has been a revived attempt to study traditional Chinese medicine in anticancer research. One group of compounds that recently attracted a lot of attention is called ginsenosides, compounds that are derived from ginseng. Furthermore, several researchers have reported that MDR can be reversed by ginsenosides, active compounds of Panax ginseng. Ginsenosides have anti-cancer properties that work by inhibiting the Pgp pump and reversing the efflux of chemotherapeutic drugs such as daunorubicin (DNR).

Pure ginseng compounds are very hard to obtain and can only be obtained at trace amounts (i.e. at sub-milligrams level) This small amount sometimes is not enough for testing using traditional methods such as flow cytometry method to evaluate the MDR effect of these compounds.

The goal of our research was to apply the microfluidic-based single-cell analysis techniques to demonstrate that the uptake of DNR in MDR cells can be measured using sub-milligram amounts of ginsenosides.

There were two ginsenosides studied in this paper: 20(S)-Ginsenoside Rg3 (Rg3-S) and 20(R)-Ginsenoside Rg3 (Rg3-R). These compounds have very similar structures, but we manage to find that Rg3-S is more potent in terms of modulating the MDR in leukemia cells. There are three advantages of the microfluidic SASCA-A method. First, we extended this method further to study multiple concentrations using the same cell, which is even faster and simpler than SASCA. The second advantage is that this method can be used to study sub-milligram amounts (less than 100 μg compound) to for their MDR reversal potency, which the traditional method such as flow cytometry cannot achieve with such small amount of the compound. The third advantage is by using the same single cell method, the cell-to-cell variation can be ruled out, and thus more conclusive result can be achieved. We envision this method will have a high potential for use to study the multidrug resistance in patient cells, and to look for potent MDR inhibitors to offer thee patient the personalized treatment.

II. PROCEDURE

A. Cell cultures
The drug-sensitive human leukemia CCRF-CEM cell line (CEM/WT) and the multi-drug resistant

vinblastine (VLB) subline (CEM/VLB 1000) were obtained from BC Cancer Agency. The CEM/WT and the resistant subline, CEM/VLB 1000, were grown in □-MEM cell medium which contains 10% fetal bovine serum (FBS) and 5% penicillin. The cell lines were maintained in 37°C and a 5.0% CO_2 humidity and were passaged once a week. In addition, the drug-resistant subline, CEM/VLB1000, was sub-cultured with 100 □g/ml vinblastine solution to maintain resistance of 1000 □g/ml (CEM/VLB).
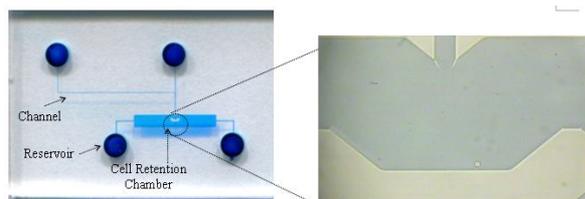

Figure 1. The Microfluidic Single-Cell Chip. (A) A photographic image of the microchip colored with blue food dye. The microchip consists of 4 solution reservoirs and 1 cell retention chamber. The dimensions of the microchip are 1.10 cm x 2.20 cm. (B) An image of the retained CEM/VLB1000 cell under 20x magnification, with chamber and channel dimensions shown in μm.

B. Microchip fabrication:

Figure 1 depicts the microfluidic single-cell chip. As shown in Figure 1(A), the microfluidic chip has 4 reservoirs and 4 channels that connect to 1 chamber in the center of the microchip. In the chamber, there is a V-shape structure, which can retain a single cell. Reservoir 1 is the □-MEM solution inlet, reservoir 2 is the reagent inlet, reservoir 3 is the cell inlet and reservoir 4 is the waste inlet. A microfluidic chip was prepared and cleaned with LiquiNOX, purified water and 90% ethanol, successively.

C. Drug accumulation:

A small sample vial (about 100 uL) of CEM/VLB1000 cell culture suspension was taken for each of the experiments. The microfluidic chip was mounted on microscope stage of an inverted fluorescent microscope, and the equipment was connected to a CCD camera and a computer. Before introducing the cells, 70% ethanol was introduced to reservoir 4; after it was pipetted out of reservoir 4, □-MEM was added to the other reservoirs. Next, about 10 □L of the cell sample was drawn by a micropipette and added into a reservoir 3 of the microfluidic chip reservoir. By manually adjusting the liquid pressure inside the microfluidic chip, one cell was selected and retained inside the microfluidic chip chamber for the experiment. A measurement window was set up, and the accumulation data were collected by bringing the cell in and out of the measuring window over a period of 3100 seconds (first 100 seconds is background measurement and the drug solutions were introduced every 1000 seconds afterwards). Drug accumulation data were processed and the accumulation curves

constructed. Fold increases were computed by dividing the initial DNR accumulation by the in the presence of the inhibitors.
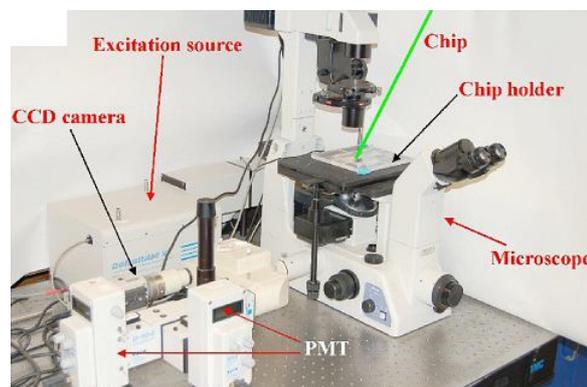

Figure 2. An inverted fluorescence microscope coupled with a photometric and imaging measurement system for microfluidic single cell bioanalysis

D. Instrumentation

An inverted fluorescence microscope coupled with a photometric and imaging measurement system was employed for conducting microfluidic single cell bioanalysis, see Figure 2.

III. RESULTS AND DISCUSSION

A. MDR reversal effect of Rg3 on DNR accumulation in the MDR cancer cells

We first tested the effect of Rg3-R on DNR accumulation in the MDR cancer cells, see Figure 3. When only DNR (35 □M) was given to the cell, the fluorescence of the cell increased, indicating that DNR was being accumulated in the cell. After ~700 s, the fluorescent intensity reached a steady state, because of the equilibrium of DNR going in and that being removed by the Pgp pumps. As soon as Rg3-R (50 μM) was co-administered with DNR, the intracellular DNR started to increase again. A higher Rg3-R concentration (100 μM) resulted in an even higher DNR accumulation.
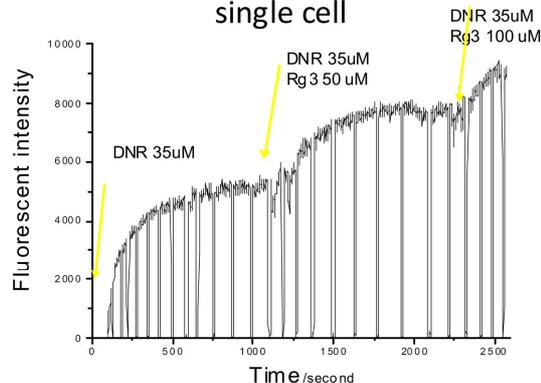
50 uM Rg3 results in 1.5 times enhancement, 100 uM: 2.0 times and more…..
Figure 3. Effect of Rg3 on DNR accumulation in single CEM cell.

Next, we applied the same procedure to another ginsenoside, Rg3-S, which is an diastereomers of Rg3-R. The results of the uptakes are summarized in Table 1. It is found he ginsenosides have a dose-dependent effect on CEM/VLB1000 cells. Moreover, the amount of intracellular DNR increased as the concentration of ginsenoside is doubled.

Table 1: Mean and SD of the fold-increase of DNR accumulation in single CEM/VLB cells due to ginsenosides

DNR 35 uM	Conc. of ginsenoside	Rg3-S & DNR 35uM	Rg3-R & DNR 35uM
1.35 ±0.06 (n=4)	50 □M	2.05 ±0.07 (n=2)	2.07 ±0.29 (n=3)
1.38 ±0.22 (n=4)	100 □M	5.55 ± 0.35 (n=2)	3.67 ± 1.16 (n=3)

B. DNR accumulation control experiments inside the MDR cancer cells

In order to confirm that the increased drug accumulation was not artifacts which might be caused by switching solutions during the experiments, we used DNR only solution to replace DNR/Rg3 solution. That is, we first added DNR solution to the cell, then repeat a second addition of DNR solution, then a third addition. We noticed that switching drugs (DNR 35 uM) did not increase intracellular accumulation of the drug further.

When we compared the ginsenosides between two cell lines, CEM/VLB1000 and CEM/wt, it was evident that the dose-dependent relationship is not as strong in the CEM/wt. This may be due to the evidence that CEM/wt does not possess as many Pgp pumps as the CEM/VLB1000 subline.

CONCLUSION

We measured uptake on the following compounds: Rg3-S and Rg3-R. In this study, we examined the accumulation of intracellular DNR on CEM/VLB 1000 leukemia cell line, alone and in combination with several ginsenosides such as Rg3-R and Rg3-S - for the for the purposes of comparing the relative efficacy of reversing the efflux. The results indicate that the enhancement of DNR without inhibitors was minor (1.3 fold increase). On the other hand, with addition of 100 □M of Rg3S, a fold increase of 5.55 was obtained, which is greater than that obtained by Rg3R (i.e. 3.67 fold increase). In this paper, we demonstrated that the microfluidic single cell analysis method is applicable for evaluating the MDR reversal effect of compounds of limited quantity (~2 mg). This evaluation cannot be done using traditional methods such as flow cytometry. Furthermore, we found that higher DNR accumulation can be enhanced in the presence of ginsenosides. And the MDR reversal effect is concentration dependent, i.e., higher DNR accumulation can be achieved at higher concentration of ginsenosides. However, different compounds have different MDR reversal capacities. For example, Rg3-S has higher MDR reversal effect than Rg3-R.

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