CHARACTERIZATION OF DRUG INDUCED AUTOPHAGY AND CYTOTOXICITY IN MCF7 CELLS ON MULTI-LAYER MICROFLUIDIC DEVICE

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ABSTRACT

In this paper, we show an improved method for cell-based drug screening which for the first time mimics the physiological drug concentration profile. Using a time-varying profile during drug screening may give more predictive data for cell cytotoxicity assessments. To explore the mechanisms of autophagy, we analyzed rapamycin (RAP) and tamoxifen (TAM) induced autophagy and effects on cell viability with time-varying drug concentration treatment. This microfluidic device is composed of multiple cell culture chambers which are connected by parallel diffusion channels. It can integrate cell loading, cell location, drug treatment and cell staining all in one chip.

KEYWORDS: Microfluidic chip, Autophagy, MCF7

INTRODUCTION

Autophagy is an evolutionarily conserved cellular process responsible for the routine degradation for long-lived proteins and the turnover of organelles [1]. To explore the mechanism of autophagy and identify additional small molecules that can activate it, we developed an image-based screen for autophagy analysis on microfluidic chip. For the first time, we mimics the physiological drug concentration profile and treat cells with time-varying drug profile. In many drug screening experiments, cells are exposed to a constant concentration which does not vary with time. However, physiologically, drug concentration in the serum peaks on average 3 to 6 hours after dosage and subsequently decreases within 24 hours post dose [2]. Using a time-varying profile during drug screening may give more predictive data for cell cytotoxicity assessments. Our device is composed of multiple cell culture chambers which are connected by parallel diffusion channels. This device is simple and easy to operate which allows for characterization of various cellular events on a single device. Using this microfluidic system, we analyzed rapamycin and tamoxifen induced autophagy and effects on cell viability with gradient concentration treatment.

EXPERIMENTAL

Microfluidic chip fabrication

The microfluidic chips and setup used for cell culture are shown in Figure 1a.

Cell culture and Staining

To assess if the microtubule-associated protein 1 light-chain 3 (LC3) is involved in TAM and RAP-induced autophagy, we used MCF7 cells transfected with LC3 fused to green fluorescent protein (GFP-LC3). The cell seeding sequence is shown in Fig. 1b: (1) An 80 µL suspension of ~5x10⁶/mL MCF-7 cells in a pipette tip was inserted into the inlet and the cells were introduced into the cell chamber under hydrostatic force with valve 1 closed. (2) Subsequently, cells in the flow channel were flushed out, and fresh medium was infused into the channel with valve 2 closed. (3) After cell loading, the chips were returned to the incubator overnight to permit cell attachment. Drug induced autophagosome formation were performed immediately by loading gradient concentration of RAP and TAM for 72 hours. After exposure to TAM and RAP, the cells were stained and analysed by 3 color confocal fluorescence microscopy.

Time-varying drug concentration profile

We used syringe pumps to produce and simulate the real drug concentration curve in vitro which is more predictive cell cytotoxicity assessments. In the first 6 hours, drug concentration increased from 2.5 µM to 20 µM and then decreased from 20 µM to 2.5 µM in the following 18 hours. Fig. 1d shows the time-varying concentration profile as verified using FITC and fluorescence intensity measurement. The MCF7 cells were fedded with this time-varying drug profile using another syringe at 0.35 µL/min flow for 3 days.

RESULTS AND DISCUSSION

Cell Cytotoxicity and Autophagy

Fig 1c shows microphotographs of MCF7 cells cultured following 72 hours treatment with 10 µM concentrations of RAP and TAM. Cells showed good adhesion and morphology.
Figure 1: Schematic diagram of multi-layer microfluidic cell culture chip. (a) Layout of the microfluidic device composed of two layers including cell chamber & flow layer and control layer. The chip was fabricated in PDMS using multilayer soft lithography. There are 4×3 cell culture chambers in each single chip. Each chamber was 600 µm in width, 100 µm in height, 1000 µm in length. (b) Cell loading steps. (c) Representative micro-photographs of control and drug-treated cells cultured on-chip for 72 hours. Cells showed good adhesion and morphology. (d) Time-varying concentration profile created in the chip as verified using FITC and fluorescence intensity measurement. The drug concentration curve was modeled in two parts: In the first 6 hours, the concentration \( y_1 \) was calculated according to: \( y_1 = \frac{(C_o \times V_0 + v_1 \times t \times C_i)}{(V_0 + v_1 \times t)} \); Between 6 to 24 hours, the concentration \( y_2 \) was calculated according to: \( y_2 = \frac{(V_0 + v_1 \times 360 \times C_f)}{(V_0 + v_1 \times 360 + v_2 \times (t-360))} \), where \( t, C_o, C_i, C_f, V_0, v_1, v_2 \) are time, original concentration, injected high concentration, final concentration, original volume, the flow rate of injected high concentration of drug during the first 6 hours, the flow rate of medium during the following 18 hours. This profile was repeated for 3 days. According to the calculated area under the curve, the average concentration was 10.5 µM.

Figure 2: Typical 3 color fluorescent images of MCF7 cells treated with time-varying concentration of RAP and TAM over 3 days (DAPI: total cell count; EthD-1: dead cells). The GFP puncta indicate autophagosomes. Tamoxifen can induce autophagy in breast cancer cells. [3]
Figure 2 shows 3-color fluorescent images of MCF7 cells treated with the time-varying drug concentrations over 3 days. Cells showed decreased cell viability and increased punctuate pattern in number and fluorescence intensity with time. To quantify the induction of cells expressing LC3 aggregation, we counted around 1000 GFP-positive cells for each treatment. The cells were separated into two groups of less than or equal to five (≤5) versus more than five (>5) GFP-puncta per cell. Figure 3(a) shows that cell viability decreased after treatment with time-varying concentration of drugs compared to that of constant concentration of 10 µM and 20 µM drugs. This indicates that cells are more sensitive to time varying gradient concentration treatment; using a time-varying profile may give more predictive data for cell cytotoxicity assessments. Figure 3(b) shows the percentage of MCF7 cells showing LC3 aggregation after treatment with time-varying concentration of TAM was comparatively lower than that of cells treated with constant concentration of 10 and 20 µM drugs for 3 days. This microfluidic platform can be used to further study cellular response to cancer therapy, and assist in understanding the function of autophagy as an indicator of cell death or a protective response.

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REFERENCES

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