DRUG RESISTANCE OF A SINGLE MOUSE MELANOMA CELL AND ITS INTERACTION WITH A MOUSE DENDRITIC CELL STUDIED USING THE MICROFLUIDIC SINGLE CELL BIOANALYZER

基于微流控芯片的单细胞生物分析方法用于鼠黑色素瘤细胞的耐药性研究及其与鼠树突细胞相互作用的免疫研究初探

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ABSTRACT

The melanoma cell is relatively drug resistant and one of the mechanisms is drug efflux from the cancer cell. We have developed a single cell bioanalyzer to confirm the reversal of drug efflux by an inhibitor compound, leading to the enhancement of drug accumulation, on the murine melanoma cell (B16OVA). A second therapeutic approach is the tumor-targeted immunotherapy, based on the use of the dendritic cell (DC), which has been used to treat melanoma patients. Here, the B16OVA cell has been used as a tumor cell model to determine in what way the murine dendritic cells (DC2114) interact with it.

摘要

癌细胞的多药耐药性已成为癌症化疗的一大瓶颈。黑色素瘤癌是一种最具侵袭性的皮肤癌，其复发和转移率、死亡率高，预后差的特点，与癌细胞的多药耐药性正相关。我们以小鼠黑色素瘤癌细胞（B16OVA）为模板，应用自行研发的单细胞生物分析芯片，在细胞通道抑制剂作用下，实现了抗癌药物在黑色素瘤癌细胞内的药物蓄积，一定程度逆转了癌细胞耐药性。应用单细胞生物分析仪，进一步在线监测了小鼠树突细胞（DC2114）与 B16OVA 的相互融合过程，初步建立了生物免疫治疗黑色素瘤癌的实验室模型。

INTRODUCTION

Queensland in Australia has the highest incidence rate of melanoma in the world and novel treatment approaches are needed [1]. Melanoma is highly drug resistant, and the drug efflux from the cancer cells leads to low drug accumulation, a phenomenon termed as multidrug resistance (MDR). The efflux is due to the drug-pumping action of transporter proteins on cell membranes, and MDR inhibitor compounds have been applied to reverse efflux, thus enhancing the drug accumulation [2]. Another therapeutic strategy is to make use of dendritic cells (DC), which have a unique antigen presenting ability, for presenting tumor-specific antigens to the patient's immune system so that specifically activated T cells can kill the cancer cells [3].

In this study, we first measure the drug accumulation in a single melanoma cell (B16OVA) and employ an MDR inhibitor to reverse the efflux. Recently, we developed a microfluidic approach, termed as same single cell analysis in drug accumulation mode (*SASCA-A*) for the study of MDR inhibition [4, 5]. Second, we monitored the interaction between the mouse melanoma cell and the dendritic cell (DC). In this regard, we first selected the DC (*i.e.* DC2114), and then brought a second type of cell, the tumor cell (*i.e.* B16OVA) in proximity to the first one. The cell-to-cell interaction is then imaged to show the time-course changes.

前言

居住在澳大利亚昆士兰州的白种人中，黑色素瘤癌的发病率全球最高 [1]。如何提高化疗有效率并延长患者生存时间、制定合理的癌症诊断和治疗规划，逆转黑色素瘤癌的抗药耐药性是关键[2]。生物免疫疗法涉及自身免疫，是将病患体内采集的免疫细胞进行体外培养和扩增后回输到病人体内，来激发、增强机体自身免疫功能，从而达到治疗肿瘤的目的[3]。黑色素瘤癌是一种免疫原性较高的肿瘤，化疗联合免疫疗法治疗恶性黑色素瘤将是合理的的综合治疗方法。

我们已应用微流控芯片，检测了多药耐药抑制剂协同抗癌药物在单个B16OVA细胞中的药物蓄积[4,5]；最近，我们在微流控芯片基础上进一步发展了单细胞分析技术，用于化疗过程中逆转癌细胞的多药耐药研究。同时，在单细胞生物分析仪上，我们在线观察了树突细胞（DC2114）和B16OVA的相互作用过程，为下一步化疗及生物免疫联合治疗癌症的实验室研究建立了医学模型。

MATERIALS AND METHODS

试剂和方法

Fabrication of the microfluidic biochip

The biochip was made of a polydimethylsiloxane (PDMS) slab (Fig. 1) that was sealed to a 0.17-mm glass cover slip [6]. Reservoirs 1 & 2 serve as the inlet & waste ports, respectively; whereas, reservoirs

3-5 are used for drug delivery. The cells were selected by hydrodynamic liquid flow [7].

微流控生物芯片的构建

生物芯片由 PDMS 厚片倒模制成，用 0.17 ㎜盖玻片密封[6]。储液池 1 和 2 分别为注入池和废液池；3-5 储液池用于药物的导入。应用流体动力学原理选择细胞[7](Fig.1)。

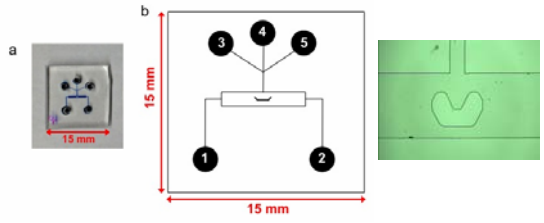

Fig. 1 (a) The microfluidic biochip. (b) The microchip consists of 5 solution reservoirs & 1 central chamber with a cell retention structure shown on the right inset image.

Cell lines and reagents

B16OVA is a murine melanoma cell line developed to include the artificial tumour antigen ovalbumin [8]. The murine DC2114 cell line was developed and used as the dendritic cell model [9]. Daunorubicin (DNR) and cyclosporine A (CsA) were obtained from Sigma-Aldrich (St Louis, MO).

细胞培养和试剂

B16OVA 是修饰了卵清蛋白抗原的小鼠黑色素瘤细胞系[8]，鼠树突细胞选择 DC2114 细胞系模型[9]。道诺霉素（DNR）和环孢霉素 A（CsA）均购自 Sigma-Aldrich。

On-Chip Drug Accumulation Study

After the retention of a B16OVA cell, simultaneous optical observation & fluorescent measurement were conducted [10]. During this, the chip was moved up and down across the detection aperture window to get the signals for the cell and the background (when inside the detection window, the cell's total fluorescence was measured; the background signal was measured when it was outside the window) [11].

芯片上的药物蓄积实验

芯片负载 B16OVA 细胞之后，机械臂规律移动该芯片[10]。当芯片处于单细胞生物监测装置的光路中，药物蓄积的荧光信号和细胞影像被记录；移出光路时，仪器记录背景信号[11]。

RESULTS AND DISCUSSION

结果和讨论

Drug accumulation study

Drug accumulation of DNR in a single B16OVA cell was measured. In order to overcome the issue of cellular variations by measuring many cells, the drug accumulation study was performed in which the same cell was used as both the control and test cell using *SASCA-A*. Fig. 2a shows DNR accumulation measured in a single B16OVA cell treated with the drug in the absence and presence of CsA. The initial drug accumulation after adding DNR alone was observed in a low fluorescent intensity, an obvious slope transition in the curve occurred after adding DNR solution containing CsA to the same single cell, causing the single cell fluorescence to increase by 2.2 fold. The morphologies of this MDR cell were shown, indicating some changes in the cell membrane integrity (Fig. 2b). Such an experiment had been repeated a few more times.

药物蓄积实验

药物蓄积实验中，机械臂带动芯片定时移入、移出光路的设计，以及数据处理时导入细胞荧光和背景荧光的比值计算，不仅显著降低药物荧光信号的背景干扰，而且进药前后的细胞荧光强度变化的自行对照，更避免了单细胞研究中细胞个体差异性导致的数据困扰。如图 2a 所示，道诺霉素在单个 B16OVA 细胞的荧光强度，在环孢霉素 A 的协同作用下，比细胞荧光初值增大了 2.2 倍。图 2b 是同步的细胞形态学影像。

Cell interaction study

After the DC2114 cell was trapped, the melanoma cell B16OVA was brought in close proximity to it. Fig. 3 shows the B16OVA stained in red by the PKH26 membrane dye. It was observed B16OVA interacted with multiple DC2114 cells (green). The time-course changes of the interactions of an attached DC2114 cell and a stained B16OVA cell have been followed.

细胞相互作用研究

绿色的 DC2114 细胞负载之后，导入已被 PKH26 染色的红色 B16OVA 细胞，二者相互作用过程如图 3 所示。

CONCLUSION

The microfluidic *SASCA-A* method has provided time-dependent drug transport in single B16OVA cells as well as the cell morphological information. The B16OVA cell was found to be multidrug resistant, with low initial daunorubicin (DNR) accumulation. Treatment of the melanoma cell with DNR as the anti-cancer drug in the presence of CsA enhanced drug accumulation notably. Furthermore, only a small amount of cells and reagents are needed to confirm the findings. Selection and attachment of mouse melanoma (B16OVA) cells was achieved in a

microchip. Selection and attachment of mouse dendritic cells (DC2114) was observed. Initial interaction between two cells were observed. Such a cell interaction study may provide useful information about the antigen-presentation of dendritic cells.

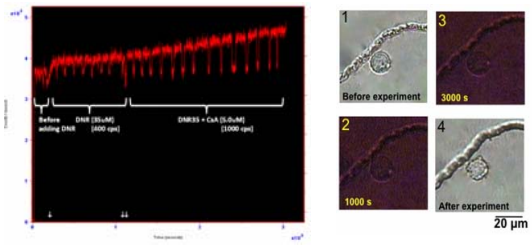

Fig. 2. Drug accumulation on a single B16OVA cell. (a) Slope transition occurred after using CsA as an inhibitor. (b) Cell images before experiment (b1) and after treating cell with only DNR (b2), with DNR and CsA (b3), after experiment (b4).

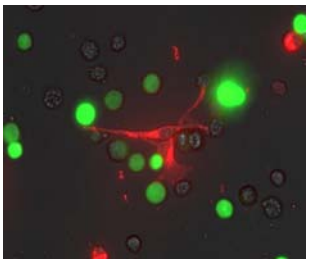

Fig. 3. Images of B16OVA cells (stained by PKH26 in red) interacting with DC2114 cells, which constitutively expressed green fluorescent protein (GFP).

结论

基于微流控芯片的单细胞分析方法可以实时在线的同时给出 B16OVA 单细胞上的药物蓄积量的变化以及细胞形态影像。实验证实环孢菌素 A 协同抗癌药物道诺霉素作用，可逆转 B16OVA 癌细胞的多药耐药性。该方法还可应用于探索联合化疗及生物免疫法治疗癌症。

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