APOPTOTIC RESPONSE OF OVARIAN CANCER CELLS IN HYPOXIC CONDITIONS

Claus R. Poulsen[1], Bryan Lincoln[1], Ivan Dimov[1], Jose L. Garcia-Cordero[1], Sharon O'Toole[2], Marek Radomski[3], John O'Leary[2], and Luke P. Lee[1, 4]

[1]Biomedical Diagnostics Institute, Dublin City University, IRELAND; [2]Obstetrics & Gynaecology/Histopathology, Trinity Centre for Health Sciences, St. James's Hospital, Dublin, IRELAND; [3]School of Pharmacy, Trinity College Dublin, IRELAND, [4]Biomolecular Nanotechnology Center, Berkeley Sensor & Actuator Center, University of California, Berkeley, USA

ABSTRACT

A cell culture device is presented that has a daily consumption of only 20 µl media. The device is used for testing the response of ovarian cancer cells to the chemotherapy drug Paclitaxel at hypoxic conditions. In hypoxic conditions, the mortality is increased 3.2 times at 34 µg/ml Paclitaxel. However, this might not be due to apoptosis as the mitochondria activity and nuclei size is unchanged compared to in the absence of Paclitaxel.

KEYWORDS: Hypoxia, cell culture, apoptosis, chemotherapeutic drug

INTRODUCTION

In recent years, the importance of hypoxia in the treatment of tumors has become increasingly evident, as patients with hypoxic tumors have a poor prognosis due to increased resistance to radiation and chemotherapy [1, 2]. It is indicated that hypoxic conditions could inhibit the effect of the chemotherapeutic drug Paclitaxel by phosphorylation of the protein family Bcl-2, involved in apoptosis [3] (Fig. 1). For studying the relationship between hypoxia and the apoptotic response to chemotherapeutic drugs, a cell culture device allowing multiple tests of different drugs is designed and fabricated in order to characterize the impact of hypoxic conditions on human ovarian cancer cells.

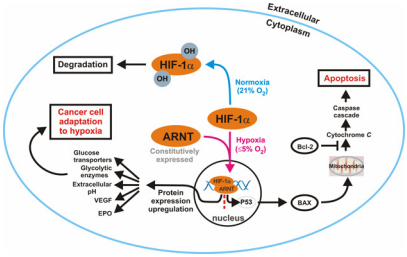

Figure 1. The signaling pathways regulating the hypoxic response and apoptosis in cells. The dotted line in the nucleus section indicates that in some instances cells can escape apoptosis if p53 is mutated or absent, and thereby can adapt to hypoxia.

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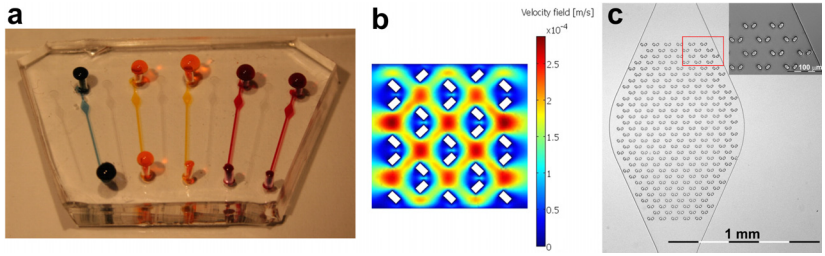

Figure 2. (a) Chip design with 5 individual culture chambers. (b) Simulation of fluid velocity near the cell traps in the culture chamber. (c) Image of single culture chamber. Insert shows close-up of 11 cell traps (area indicated by box).

EXPERIMENTAL

The PDMS device consists of a series of culture chambers with individual inlets and outlets in order to avoid cross-contamination between channels (Fig. 2). Traps retain the cells as they flow through the chamber. Simulation shows the low fluid velocity close to the traps helps the cells settle and protect from high shear (Fig. 2b). The chip is manufactured using standard soft lithography techniques.

After cell loading and attachment, the chemotherapeutic drug was administered to the cells via the media. Paclitaxel, a chemotherapeutic drug that prevents normal cell division by blocking microtubule depolymerization, causes the phosphorylation of the pro-apoptosis signal protein Bcl-2. Paclitaxel is tested in the concentrations 6.8 µg/ml and 34 µg/ml, the former being the standard final concentration in patients blood-flow. The cellular response to Paclitaxel is measured at normoxic concentration (21% O_2) and at hypoxic conditions (1% O_2), the latter being generated in a tri-gas incubator. The cells' apoptotic condition was determined after 24 hours using fluorescent dyes (Fig. 3 and 4). Propidium iodide (PI) fluorescence reflects the membrane integrity, as it is membrane impermeable. Hoechst 33342 dye (Hoechst) is a membrane permeable intercalating DNA stain and used as a reference stain to deduce cell coverage in chambers. Rhodamine 123 (Rh 123) stains active mitochondria. The area of PI fluorescence (representing number of cells with a compromised membrane, i.e. dead cells) is divided by the area of Hoechst fluorescence (representing total number of cells) for normalization in order to compare samples of varying cell density ("Normalized area" in Y-axis of Fig. 5). Hoechst and Rh 123 are normalized by the fluorescence area in the condition of no Paclitaxel.

RESULTS AND DISCUSSION

The results show that Paclitaxel increases the cancer cell mortality 2.3 and 3.2 times at normoxic and hypoxic conditions (Fig. 5a and c), respectively; both mortalities measured at 34 µg/ml. At normoxic condition a slight increasing trend can be observed in the number of dead cells at increasing Paclitaxel concentrations. In hypoxic conditions the mortality immediately increases at the low drug concentration. These results indicate that the hypoxic environment does change the effect on the cancer cells, although, it is in contrast to previous observations in Glioblastoma cells [3], where Paclitaxel protected against apoptosis in hypoxia. Fig. 5b and d indicate

that the apoptotic pathway might not be activated since neither active mitochondria nor the nuclei size is diminished, as represented by Hoechst and Rh 123 staining.

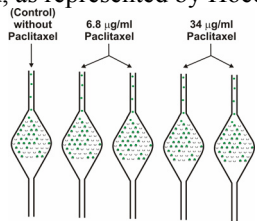

Figure 3. Schematic representation of chip operation. The cells are allowed to settle for 24 hours; hereafter they are exposed to 0, 6.8, and 34 μg/ml Paclitaxel. After 24 hours the cells are stained with fluorescent dyes to assess the apoptotic condition.

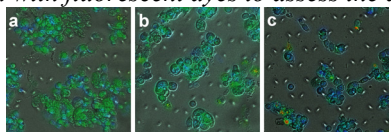

Figure 4. Human ovarian cancer 59M cells trapped in culture chamber stained with Hoechst 33342 (blue), Rhodamine 123 (green), and propidium iodide (red). (a) 59M cells not exposed to Paclitaxel, (b) cells exposed to 6.8 μg/ml Paclitaxel, and (c) cells exposed to 34 μg/ml Paclitaxel.

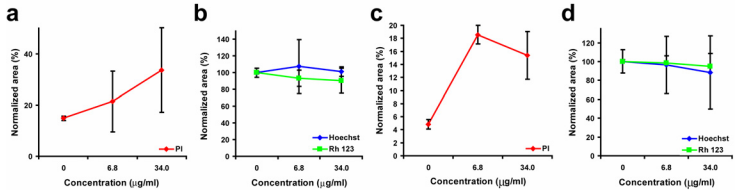

Figure 5. Response of 59M cells to 24 hours exposure of 0 to 34 μg/ml Paclitaxel at normoxic conditions (a and b), and hypoxic conditions (c and d). Error bars indicate standard deviation at both conditions for n=4 (0 μg/ml) and n=6 (6.8 and 34 μg/ml).

CONCLUSIONS

The results indicate that this initial platform can be used for drug testing on cells at different oxygen concentrations. Since the device uses ~20 μl media per day, the drug consumption is very low. This makes the device an ideal microfluidic platform for testing the cellular response to novel chemotherapeutic drugs.

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