STUDY OF PROSTATE CANCER CELL LINE PC3-ML CHEMOTAXIS TO EPIDERMAL GROWTH FACTOR WITH A BIOMEMS DEVICE

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ABSTRACT

Understanding the effects of different growth factors on the prostate cancer metastasis will enable researchers to develop different therapeutic strategies to stop the advance of cancer form primary to a secondary site. A microfluidic device was designed for high throughput assaying of prostate cancer cell migration to different growth factors. The device design was created with polydimethylsiloxane (PDMS) and featured two wells connected by 10 microchannels. Each channel has a width of 20 μm and a length of 1 mm. One well is for cell seeding and other well contains chemoattractant factor. The device was placed on a culture dish and primed with growth media. PC3-ML cells in suspension of RPMI 1640 media supplemented with 2% of FBS were seeded in the cell wells. EGF (Epidermal Growth Factor) with 25, 50, 75, 100 and 125 ng/ml concentrations in cell culture media were individually added in the respective attractant wells. A 5-day time-lapsed study of cell migrating toward the chemoattractant was performed. The average numbers of cells per device in the microchannels were obtained for each attractant condition. The results indicate migration of cells increased from 50 ng/ml to 100 ng/ml of EGF and significantly decreased at 125 ng/ml of EGF, as compared to control.

Keywords: Cancer, Cell migration and Microfluidics

INTRODUCTION

The prognosis for prostate cancer is important to avoid metastasis which may be incurable. Migration of the cancer cells as an important step in metastasis involves chemokines that attract the cancer cells from the prostate to secondary sites. Literature suggests that there are several chemokines found to be at elevated levels in patients with advanced prostate carcinoma. These chemokines are also believed to influence the metastasis of prostate cancer to the bone. Cell motility studies using conventional transwell assays require high volumes of reagents [1]. The measurement provides only end-point results. Time-lapsed study of the cell deformation and migration cannot be performed which is of interest to understand the various cellular interactions and responses in real time for better understanding of cellular migration. One of the methods used is to study the response of cells in controllable, steady concentration gradients and determining the migration patterns. Microfluidic devices have been demonstrated to have capability to generate controllable and steady gradients of chemokines [1]. Several studies using such devices have been presented in the past for quantitative evaluation of neutrophil chemotaxis [2-5]. Microfluidic devices lower the cost of testing by reducing the amount of chemokines required. They can also repeatedly generate and maintain concentration gradients. Some of the studies on microfluidic devices include array configurations for parallel testing [6], investigation of neutrophil [5] and breast cancer cell chemotaxis [7]. We have demonstrated a microfluidic platform suitable for cell migration studies [8-9] and its feasibility for assaying cell migration toward different chemokines.

DEVICE DESIGN

The microfluidic array device consisted of 48 individual devices each comprised of two chambers connected by 10 microchannels as shown in Figure. 1. The chamber on the left was designated the cell well with the one on the right as chemoattractant well. The channels were 10 μm high, 20 μm wide and 1 mm in length. The device was molded from polydimethylsiloxane (PDMS) by soft lithography. The PDMS structures formed the confinement chambers and channels of the microfluidic device on culture
dishes. PDMS is optically transparent allowing us for detailed optical interrogation and documentation.

The cleaned and prepared PDMS array device was carefully placed on a standard tissue culture substratum as shown in Figure 2. The clean surface of the device forms a leak free bond with the culture dish. In our experiments, the media introduced does not exert any force on the temporary bond formed by the PDMS and the culture dish and the device remains leak-free. The cells were seeded in the cell wells in which they proliferated and migrated across the channels on the standard tissue culture substratum. Standard cell culture techniques were employed. Thus, the cell culture conditions remained unaltered during the experiments.

**EXPERIMENTS**

Several different growth factors have been shown to increase the metabolic activity of cells [10]. One such factor is epidermal growth factor (EGF) [11]. In this study, we chose five different concentrations of EGF mixed with RPMI 1640 supplemented with 2% FBS. The concentrations chosen were 25, 50, 75, 100 and 125 ng/ml of EGF. The concentration range was chosen according to the literature [11]. First, the primed devices were seeded with lung metastasized prostate cancer (PC3-ML) cells suspended in culture media RPMI 1640 supplemented with 2% FBS, and allowed to attach for 6-8 hours. Then the spent media from the cell well was carefully removed and fresh media was added. At the same time, media containing the desired growth factor concentrations.

Each test condition was repeated five times (n=5). Devices containing culture media with 2% FBS in the chemoattractant well served as the control. Images were captured at 24 hour intervals for a period of five days. The cells under migration were tracked as they entered and exited the microchannels.

**RESULTS**

The average number of cells in the channels on Day 1 to Day 5 is shown in Figure 3. The migration in response to EGF was compared to the response to RPMI 1640 supplemented with 2% FBS, as control. The number of cells increases from 4 to 37, 4 to 42, 2 to 42, 2 to 61, 3 to 73 and 4 to 53 in response to control, 25 ng/ml, 50 ng/ml, 75 ng/ml, 100 ng/ml and 125 ng/ml EGF, respectively, from the Day 1 to Day 5. This indicates that there is a steady increase in the average cell number towards the chemoattractant in both control and at different EGF concentrations from Day 1 to Day 5. Comparing the cell numbers on a specific day after Day 5, results indicate that 25 ng/ml, 50 ng/ml and 125 ng/ml did not enhance the migration significantly as compared to control. There is a clear increase in the migration of cells toward EGF with concentrations at 75 ng/ml and 100ng/ml as compared to control. On Day 5, normalized to the migration number of control, there are 14%, 65% and 97% increases in the numbers of cells migrating at 50, 75 and 100 ng/ml of EGF, respectively. This indicates that there is a higher potential for cells to migrate in response to 100ng/ml than the
control. The migration of individual cells can also be observed helping to understand how cells move on the substrate across small confined tunnels that mimic bone or lung microenvironment.

Figure 3. Response of PC3-ML cells to different concentrations of EGF monitored over a period of five days. The number of cells under migration in the microchannels was high at a concentration of 100ng/ml.

CONCLUSION

A microfluidic device was designed, fabricated and used to quantitatively assess the migration of PC3-ML cells to specific concentrations of EGF. The results indicated the capability of assaying chemoattractants and variation of concentrations to investigate the chemotactic effects for specific types of cancer cells.

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References


