Study of lung-metastasized prostate cancer cell line chemotaxis to epidermal growth factor with a BIOMEMS device

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Abstract
Understanding the effects of different growth factors on cancer metastasis will enable researchers to develop effective post-surgery therapeutic strategies to stop the spread of cancer. Conventional Boyden chamber assays to evaluate cell motility in metastasis studies require high volumes of reagents and are impractical for high-throughput analysis. A microfluidic device was designed for arrayed assaying of prostate cancer cell migration towards different growth factors. The device was created with polydimethylsiloxane (PDMS) and featured two wells connected by 10 micro channels. One well was for cell seeding and the other well for specific growth factors. Each channel has a width of 20 µm, a length of 1 mm and a depth of 10 µm. The device was placed on a culture dish and primed with growth media. Lung-metastasized cells in suspension of RPMI 1640 media\textsuperscript{1} supplemented with 2% of fetal bovine serum (FBS) were seeded in the cell wells. Cell culture media with epidermal growth factor (EGF) of 25, 50, 75, 100 and 125 ng ml\textsuperscript{-1} concentrations were individually added in the respective growth factor wells. A 5-day time-lapsed study of cell migration towards the chemoattractant was performed. The average numbers of cells per device in the microchannels were obtained for each attractant condition. The results indicated migration of cells increased from 50 to 100 ng ml\textsuperscript{-1} of EGF and significantly decreased at 125 ng ml\textsuperscript{-1} of EGF, as compared to control.

Keywords: cancer, cell migration, microfluidics

Classification numbers: 2.04, 6.12, 6.13

1. Introduction
The metastasis of cancer from primary to distant secondary sites often makes the cancer incurable \cite{1-3}. Migration of the cancer cells as an important step in metastasis involves chemokines that attract the cancer cells from the primary to secondary sites. Literature suggests that there are several chemokines found to be at elevated levels in patients with advanced prostate carcinoma \cite{4}. These chemokines are also believed to attract the prostate cancer cells to distant secondary sites such as bone or lung \cite{5}. One of the primary limitations for the study of cancer cell migration has been the lack of a suitable cost-effective assay for cell migration with easy translatability to clinical situations. Conventional assays using Boyden chamber models require a high volume of expensive reagents and are limited in their scopes of cell migration evaluation. They also do not lend themselves to high-throughput analyses. These measurements provide only end-point results. Time-lapsed study of the cell deformation and migration, which is of interest to understand the various cellular interactions and responses in real time, cannot be performed. One of the methods used is to study the temporal responses of cells in controllable, steady concentration gradients and determining the migration

\textsuperscript{1} Developed by scientists at Roswell Park Memorial Institute (RPMI).
patterns. Microfluidic devices have been demonstrated to have the capability to generate controllable and steady gradients of chemokines [6]. Several studies using such devices have been presented in the past for quantitative evaluation of neutrophil chemotaxis [7–10]. They can also repeatedly generate and maintain concentration gradients. However, these systems require continuous flow of the reagents, which might not be cost effective. Some of the studies on microfluidic devices include array configurations for parallel testing [11], investigation of neutrophil [10] and breast cancer cell chemotaxis [12]. Our previous work demonstrated a microfluidic platform that can generate steady gradients without a need for continuous flow of reagents and is also suitable for cell migration studies [13]. The design could be further refined to lower chemokine consumption and improve high throughput capabilities. In this study, we have successfully designed a high throughput microfluidic device in an arrayed configuration to measure cancer cell responses and their movement to chemical stimulation in a time-lapsed fashion. The device was characterized using a lung-metastasized prostate cancer cell line.

2. Device design

The microfluidic array consisted of 24 individual devices for 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 growth factor 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 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 temporary bond with the culture dish preventing the media from leaking. 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 used for the commercial tissue culture substratum were employed. Thus, the cell culture conditions remained unaltered during the experiments.

3. Experiments

Several different growth factors have been shown to increase the metastatic activity of cells [14]. One such factor is epidermal growth factor (EGF) [15]. 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$^{-1}$ of EGF. The concentration range was chosen according to literature suggesting practical physiological conditions [15]. First, the primed devices were seeded with commercial lung metastasized prostate cancer (PC3-ML) cells suspended in culture media RPMI 1640 supplemented with 2% FBS. The cells were allowed to attach for 6–8 h. Then the spent media from the cell well was carefully removed and fresh media was added. At the same time, media from the chemoattractant well was replaced with 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 h intervals for a period of five days. Figure 3 shows the images of the cells in the devices captured on Day 1, Day 3 and Day 5 for different concentrations of EGF as attractant. The cells under migration (marked in green) were tracked as they entered and exited the microchannels.

4. Results

The average numbers of cells in the channels on Day 1 to Day 5 are shown in figure 4. The migration in response to EGF was compared to the response to RPMI 1640 supplemented with 2% FBS, as control. From Day 1 to Day 5, the number of cells in the channels 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, 50, 75, 100 and 125 ng ml$^{-1}$ EGF, respectively. This indicates
Figure 3. PC-3 cells migrate in response to EGF stimulation in a concentration-dependent manner.

Figure 4. Responses 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 100 ng ml\(^{-1}\) on Day 5.

that there is a steady increase in the average cell number towards the chemoattractant in both control and with different EGF concentrations from Day 1 to Day 5. Comparing the cell numbers on a specific day of Day 5, results indicated that 25, 50 and 125 ng ml\(^{-1}\) 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 and 100 ng ml\(^{-1}\) as compared to control. On Day 5, normalized to the migration number of the control, there are 14%, 65% and 97% increases in the numbers of cells migrating at 50, 75 and 100 ng ml\(^{-1}\) of EGF, respectively. This indicated that there was a higher potential for cells to migrate in response to 100 ng ml\(^{-1}\) than the control. The migration of individual cells can also be observed helping to understand how cells move on the substrate across narrow confined tunnels that mimic bone or lung microenvironment.
Figure 5. Measurement of cell travelling distances inside the channels.

Figure 6. The average migration distance of the cells per day on Day 1 to Day 5 toward different concentrations of EGF as attractant.

Our device has the capability to track the cell movement in a time lapsed fashion which enables us to calculate the migration speeds of the cells towards the attractant. The distances travelled by the leading cells in the channels within a particular period of time were obtained using Axiovision 4.7 (Zeiss) software with the images as indicated in figure 5. The average migration distance of the cells increased from 103 to 224 µm, 44 to 394 µm, 85 to 460 µm, 226 to 573 µm, 120 to 682 µm and 161 to 561 µm in response to control, 25, 50, 75, 100 and 125 ng ml\(^{-1}\) EGF, respectively, from the Day 1 to Day 5. This indicates that there is a steady increase in the average cell migration distance towards the chemoattractant in both control and at different EGF concentrations with time as shown in figure 6. Migration speed could be calculated as the ratio of the distance travelled by cells to the total period of the experiment. On comparing the migration speeds at different concentrations of EGF as attractant, the highest migration speed of 112 µm day\(^{-1}\) occurred at the concentration of 100 ng ml\(^{-1}\).

5. Discussion

Our assay for cell migration study design combines the advantages in microfluidic devices and commercially available standard substratum with objectives aiming to establish the chemoattractant gradients in a confined micro-environment that provides (i) cell anchorage on a regular substratum, (ii) a well-defined guiding pathway for cell growth and migration, (iii) a means to observe cell behaviors in a time-lapsed fashion and (iv) a high throughput screening platform. We have fabricated, assembled and characterized such a microfluidic device using optically transparent PDMS with micro-molding techniques. The uniqueness of our methodology is that the seeded cells attach, grow and migrate on a standard culture dish along migration paths defined by barriers in the PDMS structures that are bonded onto the culture dish. The characteristics reflect cell migration on standard substratum, yet with a defined migration pathway. The data presented here demonstrated the increase in migration of PC3-ML cells under the influence of EGF. As per cell models, growth factors are often isolated by the extracellular matrix, as well as being bound by the cell membrane [16]. Therefore, when an EGF gradient is formed inside the device, in the local cell environment, there is potential increase in the available EGF molecules which affect the cell behavior. In this study, maximum stimulation for prostate cancer cell migration was achieved at 100 ng ml\(^{-1}\). Higher concentrations failed to further stimulate the cell migration. It is possible that receptor saturation might have occurred above 100 ng ml\(^{-1}\) concentration.

Our devices design, using small amounts of reagents with in-parallel and time-lapsed fashion can be used to investigate and discover potential chemoattractant factors and the combined effects of several different factors with varied concentrations in prostate cancer patients’ sera. The device and study method can serve as a screening tool for better diagnosis of cancer progression and metastasis risk.
6. Conclusion

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

Acknowledgments

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