Published in final edited form as:

Proc SPIE Int Soc Opt Eng. 2020 February ; 11251: . doi:10.1117/12.2544113.

Prostate Cancer Epithelial Mesenchymal Transition by TGF- β 1 exposure monitored with a Photonic Crystal Biosensor

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Abstract

During prostate cancer progression, cancerous epithelial cells can undergo epithelial-mesenchymal transition (EMT). EMT is a crucial mechanism for the invasion and metastasis of epithelial tumors characterized by the loss of cell-cell adhesion and increased cell mobility. It is associated with biochemical changes such as epithelial cell markers E-cadherin and occludins being down-regulated, and mesenchymal markers vimentin and N-cadherin being upregulated. These changes in protein expression, specifically in the cell membrane, may be monitored via biophysical principles, such as changes in the refractive index (RI) of the cell membrane. In our previous research, we demonstrated the feasibility of using cellular RI as a unique contrast parameter to accomplish label-free detection of prostate cancer cells. In this paper, we report the use of our Photonic-Crystal biosensor in a Total-Internal-Reflection (PC-TIR) configuration to construct a label-free biosensing system, which allows for ultra-sensitive quantification of the changes in cellular RI due to EMT. We induced prostate cancer cells to undergo EMT by exposing these cells to soluble Transforming Growth Factor Beta 1 (TGF- β 1). The biophysical characteristics of the cellular RI were quantified extensively in comparison to non-induced cancer cells. Our study shows promising clinical potential in utilizing the PC-TIR biosensing system not only to detect prostate cancer cells, but also to evaluate changes in prostate cancer cells due to EMT.

Keywords

Prostate Cancer; Label-Free Biosensor; Cellular Refractive Index; Optical Biosensor; Photonic Crystal Biosensor; Cancer Diagnostics; Transforming Growth Factor Beta 1; Epithelial-mesenchymal transition

INTRODUCTION

Prostate cancer staging is essential in determining the correct treatment plan and predicting the patient's prognosis. Prostate diagnosis and prognosis require several methods and variables to accurately determine the stage of cancer, rendering it a challenge issue for medical professionals. The current screening methods for the prostate cancer diagnosis and staging include digital rectal exams, prostate-specific antigen (PSA) levels, transrectal ultrasound, magnetic resonance imaging, and computerized tomography scans [1–4]. The

stage of the prostate cancer is based on the level of metastasis. Metastasis occurs when the cancer cells acquire the ability to no longer be cell-to-cell adhesion dependent, and are therefore able to migrate across the human body^[5]. This is often caused by cells undergoing epithelial-mesenchymal-transition (EMT). EMT is the process in which epithelial cells transition from having epithelial features to a mesenchymal phenotype through internal molecular and genetic alternations, and in response to external microenvironment stimulus^[6, 7]. Changes in the surface proteins of the cells allow the malignant cells to detach from the extracellular matrix, and migrate to other areas of the body^[5]. At the new sites, they re-anchor and lead to the formation of new tumors. Loss of E-cadherin, a cell-to-cell adhesion molecule, plays a major role in the initiation of EMT by causing intercellular mechanical communication breakdown^[6, 8]. Conversely, mesenchymal markers, such as N-cadherin and vimentin expression upregulates, lead to increased mesenchymal phenotypes and thus increased cell mobility^[6, 8]. Quantification of changes in E-cadherin and N-cadherin can be used to monitor EMT and the level of metastasis. As the protein expression changes when the cells undergo EMT, we believe these biochemical changes can be correlated to changes in refractive index of the cell membrane. We have previously demonstrated the ability to use cellular membrane RI, as a novel contrast parameter to allow for label-free detection of prostate cancer cells^[9–11]

In this paper, we report a novel method to characterize prostate cancer cells undergoing EMT based on the cell's membrane RI via the use of a label-free biosensing system constructed with a Photonic Crystal Biosensor in a TotalInternal Reflection (PC-TIR) configuration. EMT in prostate cells lines was induced by treating the cells with Transforming Growth Factor Beta 1 (TGF- β 1). Using this model in conjunction with the PC-TIR biosensing system, we hope to correlate cellular RI with EMT as a potential method of prostate cancer prognosis.

MATERIALS &METHODS

2.1 Cells and Cell Culture

The Human prostate cancer cells (DU-145) were obtained from American Type Culture Collection (ATCC), Manassas, VA. Du-145 cells were cultured in Eagle's modified essential media (EMEM) medium containing 10 % Fetal bovine serum and 1% Antibiotic-Antimycotic. The prostate cancer cells lines were incubated at sterile conditions: 5% CO₂ atmosphere at 37° C. the cell lines passage was from 2 to a maximum of 15 times (P2-P15).

2.2 Chemical and Reagents

Recombinant human TGF- β 1 was obtained from Dr. Lu-Zhe Sun's laboratory at The University of Texas Health San Antonio. E-Cadherin *in vitro* SimpleStep Enzyme-Linked Immunosorbent Assay (ELISA) kit (#ab233611) and Human N-Cadherin *in vitro* SimpleStep ELISA Kit (#ab254512) were purchased from Abcam. Pierce BCA Protein Assay Kit (#23227) and RIPA Lysis and Extraction Buffer (# 89900) were purchased from ThermoFisher Scientific.

2.3 Inducing EMT with TGF- β 1

For the cell migration assay, the prostate cells with a density of 1.50×10^3 cells/well were grown in a 12-well plate until 80% confluency in regular medium and allowed to adhere for the first 24 hrs. The cells were then treated with appropriate TGF- β 1 concentration (0, 5, and 10 ng/mL) in serum free culture medium. After 24 hours, the cells were treated with trypsin and detached cells were centrifugated into a pellet. Cell pellet were used for downstream testing immediately.

2.3 Enzyme-linked immunosorbent assay (ELISA) for N-Cadherin and E-Cadherin

Pelleted Du-145 cells were lysed with RIPA lysis, an extraction buffer, according to the manufacturer's instructions. Prior to the ELISA assay, the protein concentration was normalized by the total overall protein count given from the Pierce BCA Protein Assay Kit (#23227). The samples were then diluted using RIPA according the normalized protein concentration. The samples were then tested with both E-Cadherin and N-Cadherin ELISA kits according to the manufacturer's instructions.

2.4 TGF- β 1 Cell viability

The Du-145 cells were treated with different TGF- β 1 concentrations (0, 5 and 10 ng/mL) for 24 hrs. The cells were subsequently detached with trypsin and pelleted by centrifugation. The Tali™ Viability Kit - Dead Cell Red (ThermoFisher, A10786) was used to measure the cell viability of the Du-145 cells.

2.5 Experimental Set-up for Quantifying Bulk Cell RIs

We have demonstrated, in previous studies^[12–18], the use of the PC-TIR biosensor to quantify RI changes. The PC-TIR possesses a unique working mechanism that utilizes a photonic crystal (PC) in a total internal reflection configuration, which creates an open sensing surface. This allows for simplistic placement of cells atop of the biosensor. The PC-TIR biosensor poceeses a sharper resonant dip of ~ 1 nm^[19], as compared to surface plasmon resonance (SPR) sensors, which have a bandwidth of ~ 40 nm^[20, 21]. Utilizing this sharp resonant dip, the PC-TIR biosensor can accurately monitor the changes in RI of the cells placed on the open sensing surface.

The PC-TIR biosensing system was constructed by utilizing a broadband white light source coupled into a single-mode optical fiber as a probe beam (Fig. 3). The output beam from the optical fiber was polarized and collimated. A nonpolarized beam splitter was utilized to split the output beam into two separate beams, which propagated in parallel through a prism and reached two distinct areas on the PC-TIR sensor surface with a constant incident angle. The two reflected probe beams from the sensor were combined and focused onto the entrance slit of a high-resolution spectrometry (Ocean Optics HR 4000) to measure the reflectance spectra of the sensor.

A polydimethylsiloxane (PDMS) layer was attached onto the PC biosensor surface to create two wells. One well served as the sample well, and the second well served as a control filled with deionized water. The PC-TIR sensor was placed onto the prism and air gap was removed by utilizing a refractive index matching liquid. Water was placed onto the sensor

wells for 20 minutes to establish the baseline. After 20 minutes, the water in the sample well was replaced with DPBS. The DPBS was allowed to settle for 20 mins and a reading was taken. The DPBS was immediately removed and replaced with approximately 1×10^6 DU-145 cells previously exposed to TGF- β 1 and the refractive index was measured.

2.7 Statistical Analysis

Origin Pro 2020(Originlab) was utilized to performed data analysis. A one-way ANOVA with a Post hoc test was used for statistical comparisons. Each experiment concentration was represented by the mean and standard error of the mean. The significance of the difference in means were represented by the p-values of less than .05*, .01**, or .001***.

RESULTS

3.1 Cell Viability

The cell viability after the treatment of TGF- β 1 at different concentrations were measured using the Tali™ Viability Kit - Dead Cell Red. Table 1 demonstrates the cell viability percentage which varied between 95% and 99%, over the course of 24 hrs. of TGF- β 1 exposure.

3.2 Enzyme-linked immunosorbent assay (ELISA) for N-Cadherin and E-Cadherin

The expression of E-cadherin and N-cadherin after the cells were exposed to varying levels of TGF- β 1 for 24 hrs. were measured by utilizing an ELISA kit. The expression of N-cadherin in DU-145 showed no significant change when exposed to 5 ng/mL of TGF- β 1, but it significantly increased when exposed to 10 ng/mL of TGF- β 1. In contrast, the E-cadherin expression showed no significant decrease nor increase when the DU-145 cells were exposed to both concentrations of TGF- β 1 (Figure 5).

3.3 RI measurement of Epithelial Mesenchymal Transition at different TGF- β 1 concentrations

Figure 5 shows the measured raw reflectance spectra of DU-145 cells in the absence and presence of TGF- β 1(5 and 10 ng/mL). A blue shift of approximately 1.2 nm was measured when DU-145 cells were exposed to 5 ng/mL for 24 hrs., while a ~0.9-nm red shift was observed when exposed to 10 ng/mL (Figure 6).

DISCUSSION

In this study, we have utilized TGF- β 1 to induce EMT in DU-145 cells and measured the subsequent shift of the resonant wavelength of the sensor (Figure 6), which is due to the changes in RI of the cells. The DU-145 cells were able to undergo EMT within 24 hrs. of being exposed to TGF- β 1 as confirmed by the expression of EMT makers, shown in Figure 4. Although N-cadherin expression did not show a significant increase when exposed to 5 ng/mL of TGF- β 1, the DU-145 cells showed a significant increase N-cadherin levels when exposed to a higher dose of 10 ng/mL. The elevated N-cadherin expression was a confirmation of EMT progression. Conversely, the E-cadherin expression did not show

significant changes when exposed to TGF- β 1 for 24hrs. This was to be expected, as DU-145 has been reported not to express high levels of E-cadherin^[22].

We have utilized the PC-TIR biosensor to measure the RI of the DU-145 cells undergoing EMT at various concentrations of TGF- β 1. We observed a clear blue shift in the reflectance spectra between untreated DU-145 cells and the induced DU-145 cells exposed to 5 ng/mL of TGF- β 1, with a significant difference $p < 0.05$. Additionally, we found a clear red shift when the DU-145 cells were induced with 10 ng/mL of TGF- β 1. Following the ELISA and the cells RI results, a direct correlation between cellular RI and expression levels of EMT makers was demonstrated. Both monitoring techniques showed decreased and increased readings when the cells were exposed to 5 and 10 ng/mL TGF- β 1, respectively. The data shows a possible correlation between EMT surface protein markers' expression and RI value of prostate cancer cells. Our results demonstrate the potential for utilizing cellular RIs to monitor surface protein expression and thus EMT in cancer cells. Further research will be aimed at optimizing the EMT model through longer exposure times for TGF- β 1, as well as showing how changes of surface proteins expression of other prostate cancer cells (i.e. LNCaP and PC-3) undergoing EMT affect cellular refractive indices.

ACKNOWLEDGEMENTS

This research was support by the National Institute of Health (NIH) grants R21CA198389, T34 GM060655 and R25GM060655. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH.

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Figure 1.
Illustration of cells exposed to TGF- β 1, and the relative expression of EMT Markers.

Figure 2.
(A). PC-TIR biosensor structure. (B) Illustration of cells on a PC-TIR biosensor coupled with a prism.

Figure 3:
Illustration of the PC-TIR biosensing system to quantify cell RIs.

Figure 4.

ELISA results showing the relative expression levels EMT markers in DU-145 cells after being treated with different concentrations of TGF-β1. DU-145 cells were treated with TGF-β1 (0, 5 and 10 ng/mL) for 24 hours before being trypsinized, lysed, and tested. **A.** Relative expression of N-cadherin of Du-145 cells after being exposed to TGF-β1(0, 5 and 10 ng/mL). **B.** Relative expression of E-cadherin of Du-145 cells after being exposed to TGF-β (0, 5 and 10 ng/mL).

Figure 5.

DU-145 reflectance spectra (green) compared to DU-145 exposed at 5ng/mL (blue) and 10 ng/mL (red). The refractive indices of the induced and non-induced cells with TGF- β 1 were measured based on the changes of wavelength from water (gray). Due to the sharp resonance dip of the biosensor, the EMT of DU-145 induced with TGF- β 1 was well identified.

Figure 6.

Resonant wavelength shifts of DU-145 cells after the being exposed to TGF-β1. The average wavelength shift from water for the TGF-β1-treated cells was 8.29, 7.26, 9.19 nm for the TGF-β1 concentrations at 0, 5, and 10 ng/mL, respectively. The * indicates significance ($p < .05$) difference between the 0 ng/mL and 5 ng/mL. The *** indicated significance ($p < .001$) difference between the 5 ng/mL and 10 ng/mL.

Table 1.

Cell viability of DU-145 cells after being exposed to TGF- β 1 (0, 5, and 10 ng/mL) for 24 hrs.

Prostate Cancer Cell Line	Cell Viability (%)		
	TGF- β Concentrations		
	0 ng/ml	5 ng/ml	10 ng/ml
DU-145	98.66 $\pm$ 2.00	97.58 $\pm$ 2.38	99.17 $\pm$ 1.15