Inflammation Endows Benign Prostatic Hyperplasia Cells With Similar Physical Properties to Prostate Cancer Cells

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ABSTRACT Although inflammation is considered an important factor for promoting carcinogenesis, further evidence is still needed to draw definitive conclusions on its role in prostate cancer (PCa) development and progression. This study characterized the radius, specific membrane capacitance (SMC), and Young’s modulus of 20 patient-derived prostate cells, including 6 patients diagnosed with benign prostatic hyperplasia (BPH), 5 patients diagnosed with BPH accompanied with chronic inflammation (BCI), and 9 patients diagnosed with PCa. The characterized results show that the three groups of cells possess approximate radius value. Both BCI and PCa cells show larger SMC values than BPH cells. Only PCa cells possess lower Young’s modulus than BPH cells, the stiffness of which is approximate to that of BCI cells. Additionally, experiments have testified that inflammatory cytokine, (i.e. TNF-α) can induce the increase of cellular SMC values. The finds demonstrate that inflammation is linked to cancer promotion process and accompanied with cellular biophysical changes, providing a new insight into the effects of inflammation in promoting PCa.

INDEX TERMS Prostate cell, specific membrane capacitance (SMC), Young’s modulus, inflammation effect.

I. INTRODUCTION
Prostate cancer (PCa) is the most common form of cancer and the second leading cause of cancer death in men. Many factors contribute to the development of PCa, including age, family history, and diet. Recently, inflammation has been suggested as a potential key factor in the development and progression of benign prostatic hyperplasia (BPH) and PCa [1]–[3]. Based on a biopsy study, Gurel et al. reported that men who had at least one biopsy core with inflammation had 1.78 (95% confidence interval, 1.04–3.06) times the odds of PCa compared with men who had zero cores with inflammation [4]. A meta-analysis performed by Dennis et al. demonstrated a significant association between prostatic inflammation or infection and the risk of PCa [5]. Several lines of evidence also support the link between inflammation and PCa. For example, 1) epidemiological studies have shown that chronic inflammation predisposes individuals to various types of cancer (eg., liver cancer and breast cancer) [6]; 2) non-steroidal anti-inflammatory drugs reduce the risk of developing certain cancers and the mortality caused by these cancers [7]; 3) inflammatory cells, chemokines, and cytokines are present in almost all tumors during their development. Although these preclinical studies provide biological rationale for the association between inflammation and the risk of PCa, a direct relationship between inflammation and malignant transformation in the human prostate is still unclear due to limited evidence [3], [8].

Cells are the basic unit of life. Considerable pieces of evidence have demonstrated that cellular physical properties including morphology, and mechanical and electrical properties are efficient indicators in reflecting differential degree, tumor metastasis potential, and other cellular states [9]–[11]. For
example, cancer cells possess a larger size, lower elastic modulus, and larger specific membrane capacitance (SMC) than normal cells from comparable regions [12]–[16]. The SMC value is associated with the membrane micromorphology of cells and increases with increasing number and size of microvilli, ruffles, and folds on the plasma membrane [13], [14]. The cell stiffness (i.e., Young’s modulus) is decided by the microstructure and organization of the cell cytoskeleton [17], [18]. The cause of these physical changes on cancer cells is still unclear although these changes are considered to be beneficial to cancer aggression and invasion [19], [20]. Recently, inflammatory cytokines such as TNF-α and IL-8 have been reported to modulate cellular physical properties [21]–[23], suggesting a hypothesis that biophysical changes in cancer cells is a clue for linking inflammation with cancer, even much more evidence are still required.

In the present study, the radius, SMC, and Young’s modulus of 20 patient-derived prostate cells, including 6 patients diagnosed with BPH, 5 patients diagnosed with BPH accompanied with chronic inflammation (BCI), and 9 patients diagnosed with PCa (also accompanied with inflammation), were characterized and analyzed. Clinically, PCa is more aggressive than BCI, which is more serious than BPH. The following are the characterization results: 1) The radius of the three groups (i.e., BPH, BCI, and PCa) are approximate. 2) BCI and PCa cells possess approximate SMC values, both of which are larger than BPH cells, demonstrating that inflammation can cause changes in membrane micromorphology. 3) PCa cells show a lower stiffness than BPH cells, whereas the stiffness of BCI cells is close to BPH cells, which is consistent with the clinical fact that prostate cancer cells are more malignant than BCI and BPH cells. These sequential changes in biophysical properties among BPH, BCI and PCa suggest that inflammation promote cancer progression and aggression by modulating membrane micromorphology first and related signaling events. This hypothesis is consistent with the discoveries that changes in membrane electrical properties can lead to changes in stiffness [24] and that membrane dysregulation promotes the initiation of cancer-related signaling processes [25]. In summary, these changes in biophysical properties provide a new insight into understanding how inflammation promotes PCa.

II. METHODS AND PROCEDURES

A. CELL SAMPLE

All patient-derived cell samples were prepared using conditional reprogramming culture (CRC) method [26]. The tissue samples were collected from patients histopathologically diagnosed with BPH, BCI, or PCa via needle biopsy or surgery at Huashan Hospital (Shanghai, China). The protocol of the CRC method is as follows: 1) digest the tissue with digestion buffer containing the enzymes dispase, collagenase, and hyaluronidase overnight; 2) obtain the targeted cells through centrifugation at 1000 rpm for 5 min followed by removal of the digestion buffer (Trypsin/EDTA 0.05%, 1 mL); 3) resuspend the cell pellet into phosphate-buffered saline and a feeder culture after discarding the supernatant [27]; 4) culture the primary prostate cells in complete Dulbecco’s modified Eagle medium-F12 nutrient mixture supplemented with insulin (5 μg/mL), epidermal growth factor (0.125 ng/mL), hydrocortisone (25 ng/mL), and the ROCK inhibitor Y-27 632 (10 μM). Primary cells at passage 3–5 were utilized for characterization of physical properties. All above experimental protocols were approved by the Ethics Committee of Huashan Hospital (Shanghai, China; approval no. KY2011-009) and conducted in accordance with the tenets of the Declaration of Helsinki. The use of the resected tissue for research purposes has been consented by all patients.

B. CHARACTERIZATION OF ELECTRICAL PROPERTIES

The SMC values and radius of all clinical patient-derived cells were characterized using a self-developed optically induced electrokinetic (OEK) technique [28], [37], as shown in Fig. 1(a). For electrical characterization using OEK techniques, cultured cells were suspended into a 200 μL isotonic solution at a concentration of 10^6 cells/mL, forming a cell suspension with a conductivity of 2.4 × 10^-2 S/m. This cell suspension was injected into the microchamber of an OEK chip, which is the kernel component of OEK technique. The OEK chip was assembled through attaching a top ITO glass substrate with a photoconductive substrate using a double adhesive, forming a microchamber with thickness of 70 μm for containing cell suspension. The photoconductive substrate comprised a hydrogenated amorphous silicon layer (a-Si:H, 1
μm) deposited on an ITO-coated glass substrate via a plasma-enhanced chemical vapor deposition process. A sinusoidal voltage with an amplitude of 10 vpp and a sweeping frequency from 10 kHz to 30 kHz at a step of 2 kHz was applied between the top and bottom ITO electrodes. A designed optical pattern was projected onto the a-Si:H film through a projector. The a-Si:H film is a type of photoconductive material, whose conductivity can increase several orders from \(10^{-11}\) S/m to \(10^{-9}\) S/m when it is illuminated by light. Therefore, the projected light pattern can work as a virtual electrode due to this photoconductive effect of the a-Si:H film and generate a non-uniform electric field in the cell suspension atop itself.

The suspended cells can be polarized by the non-uniform electric field and exerted on a frequency-adjusted dielectrophoretic (DEP) force [29]. As shown in Fig. 1(b), the cell was exerted on a positive DEP force and moved toward the light pattern when the applied frequency was above its crossover frequency, whereas it was exerted on a negative DEP force and moved far away from the light pattern when the frequency was below its crossover frequency [30]. The crossover frequency of each cell corresponded to the swept frequency at which it turned. For each cell, the crossover frequency, conductivity of the suspending medium, and membrane capacitance (\(C_{\text{mem}}\)) of the cell can be expressed as follows (1).

\[
C_{\text{mem}} = \frac{\sigma_{\text{m}}}{\sqrt{2\pi r}} f_{\text{cross}}
\]

where \(\sigma_{\text{m}}\) is the conductivity of cells and suspension media, \(r\) denotes the cell radius, and \(f_{\text{cross}}\) denotes the dielectrophoresis crossover frequency. The SMC value of each cell can be calculated through extracting its crossover frequencies and radius via a real-time image analysis algorithm. Both SMC and radius values are intrinsic properties of cells.

C. CHARACTERIZATION OF MECHANICAL PROPERTIES

As shown in Fig. 2(a), the mechanical properties of cells were quantified using a commercial AFM instrument (BioScope Resolve; Bruker Corporation, Billerica, MA, USA). A PeakForce QNM-Live Cell probe (Bruker Corporation) with a tip length of 17 \(\mu\)m, tip radius of 65 nm, tip half angle of 18°, and spring constant of 0.076 N/m was applied to probe the cell surface using the force-volume model. The probing parameters were set as follows: constant loading force of 1 nN, probing array of \(32 \times 32\), and ramp rate of 10 Hz. Automatic gain control was applied to improve the feedback for surface tracking. The approach curves during indentation were used to calculate the cellular Young’s modulus using a Hertz-Sneddon model [31]. For each cell, a \(32 \times 32\) modulus map shown in Fig. 2(b) was obtained first, then a mean modulus was calculated by Gaussian fitting as shown in Fig. 2(c). This fitting modulus value was set as the apparent modulus of the probed cell. At least 20 cells were quantified for each type of cell.

III. RESULTS AND DISCUSSION

A. COMPARISON OF ELECTRICAL PROPERTIES

The measured radius of the three groups of cells is shown in Fig. 3(a). The radii of BPH, BCI, and PCa cells were 9.42 ± 1.24, 9.98 ± 1.04, and 9.43 ± 1.22 \(\mu\)m, respectively. The results of two-sample independent t-test showed no significant changes among the three groups of cells (\(p > 0.05\)).

As shown in Fig. 3(b), the measured SMC values of the six types of BPH cells were 23.4 ± 3.6, 26.6 ± 4.9, 27.3 ± 4.9, 28.3 ± 5.6, 28.8 ± 4.0, and 29.9 ± 2.7 mF/m². The SMC values of the five types of BCI cells were 33.8 ± 8.6, 33.7 ± 8.4, 33.9 ± 5.8, 35.2 ± 7.7, and 37.2 ± 6.2 mF/m². The SMC values of the nine types of PCa cells were 31.2 ± 5.6, 31.8 ± 4.1, 33.6 ± 9.2, 33.5 ± 7.7, 33.8 ± 8.4, 34.6 ± 7.5, 36.1 ± 8.3, 37.2 ± 6.8, and 38.7 ± 6.4 mF/m². The two-sample independent t-test was conducted among the three groups of cells. A significant difference in SMC values was demonstrated both in BCI vs. BPH cells (\(p < 0.001\)) and PCa vs. BPH cells (\(p < 0.001\)), whereas no significant difference in SMC values was observed between BCI and PCa cells (\(p = 0.99\)).

The SMC values of BCI cells were much larger than those of BPH cells (34.7 ± 7.4 vs. 27.6 ± 5.0 mF/m²), demonstrating that the inflammatory environment can induce the increase of SMC values of cell. Interestingly, the SMC values of PCa cells were approximate to those of BCI cells. A similar inflammatory environment in cancer and their similar SMC values suggest that the increase of SMC values of PCa cells is also due to the inflammatory environment. The inflammatory cytokines may contribute to prostatic epithelial and stromal...
cell growth directly by stimulating the production of prostatic growth factors, and indirectly through decreases in prostate cell death via downregulation of prostate cell apoptosis. This proliferation may increase the expressed proteins on the membrane and the roughness of cell morphology, inducing an increase in SMC value [13], [14]. To verify this hypothesis, the SMC value of a type of normal prostate cell named RWPE-1 was characterized after treatment with different concentrations of TNF-α for 12 h. As shown in Fig. 4, the SMC value of the cell increased from 25.9 ± 6.6 mF/m² to 33.5 ± 7.7 mF/m² when the TNF-α concentration was increased from 0 ng/mL to 30 ng/mL, which demonstrates that inflammatory cytokines can induce the increase of cellular SMC values.

**B. COMPARISON OF MECHANICAL PROPERTIES**

As shown in Fig. 5, the Young’s modulus values of the six types of BPH cells were 6.8 ± 3.6, 7.6 ± 4.1, 6.3 ± 2.6, 8.2 ± 5.8, 7.1 ± 2.5, and 6.7 ± 3.6 kPa. The Young’s modulus values of the five types of BCI cells were 7.6 ± 4.2, 5.8 ± 2.3, 9.0 ± 2.9, 5.1 ± 1.8, and 7.4 ± 3.1 kPa. The Young’s modulus values of the nine types of PCa cells were 2.0 ± 1.1, 2.3 ± 0.7, 1.7 ± 0.9, 2.5 ± 2.0, 1.8 ± 0.4, 1.3 ± 0.4, 1.6 ± 0.9, 2.6 ± 1.6, and 2.2 ± 0.8 kPa. As shown, the Young’s modulus values of PCa cells were much smaller than those of BPH cells (2.0 ± 1.2 vs. 7.1 ± 3.9 kPa, p < 0.001), which is consistent with a generally accepted knowledge that the stiffness of tumor cells decreases with the increase of their aggressiveness [12][13]. Compared with BPH cells, BCI cells possess a slightly smaller Young’s modulus, but not significantly (6.8 ± 3.2 vs. 7.1 ± 3.9 kPa, p > 0.1), demonstrating that the mechanical properties of prostate cells were not changed by the inflammation environment in BCI, which is consistent with a controversial phenomenon that inflammatory cytokines both decrease and increase the stiffness of cells [23], [32].
C. ROLE OF INFLAMMATION IN MODULATION BIOPHYSICAL PROPERTIES

The link between inflammation and cancer is related to the intrinsic and extrinsic pathways, both of which activate various important inflammation-inducing factors, such as TNF, NF-κB, IL-1β, IL-6, and IL-18 [33], [34]. These factors affect the expression of receptor proteins in the cell membrane, activate signaling pathways, and cause changes in cell membrane folding and protrusion [35][2], which induce the increase of SMC value. Another potential key factor in the relationship between inflammation and PCa is the expression of transforming growth factor beta, which has been found at increased levels in prostates with inflammation and might be involved in the epithelial-mesenchymal transition (EMT) [36]. The EMT is often accompanied with changes of mechanical properties. However, BCI cells only possess an increase in SMC value, but PCa cells possess both increase in SMC value and decrease in Young’s modulus, as shown in Fig. 6. This interesting phenomenon may be related to the fact that PCa is more aggressive than BCI, suggesting that the modulation of inflammation on cell state begins from cell membrane. This preferential change on membrane properties maybe due to the barrier role of the plasma membrane, which serves as a point of the first contact between cells and various signaling elements [35].

The role of inflammation in modulating cellular physical properties and promoting PCa process can be further figured out using a currently accepted model called proliferative inflammatory atrophy (PIA) [3]. In PIA, the inflammation environment including immune cells and their released cytokines can induce DNA damage, cell injury, and cell death, triggering the onset of epithelial cell regeneration and forming prostatic intraepithelial neoplasia (PIN). In PIN, gene mutations caused by inflammation can induce a dysregulation of plasma membrane homeostasis and generation of lipid/protein raft, and alter the degree of clustering and other biochemical and biophysical properties, thereby providing a suitable environment for the initiation of cancer-related signaling processes [25]. Additionally, inflammation may confer tumor progenitors with stem-cell-like properties and cause membrane depolarization [35]. All these processes contribute to an increase in SMC value. The PIN state has been proposed to be precursor of PCa, because the continued proliferation of genetically unstable luminal cells and the further accumulation of genomic changes in PIN may lead to a progression toward invasive carcinomas. It is worth noting that the decrease in stiffness of cancer cell is also related to the change in cell membrane properties. Membrane potential depolarization and SMC increase can affect the polymerization stratus of the submembrane action network and decrease cellular stiffness [24]. These findings suggest that inflammation promotes PCa by changing membrane micromorphology and related signaling events.

IV. CONCLUSION

This study characterized the SMC values, radius, and Young’s modulus of 20 prostate cells from BPH, BCI, and PCa patients. The results showed that all BPH, BCI, and PCa cells possess approximate radius. Both BCI and PCa cells possess larger SMC values than BPH cells. Only PCa cells show a lower Young’s modulus than BPH cells. The similarity in SMC values between BCI cells and PCa cells indicates that inflammation induces changes in the biophysical properties of BPH cells similar to PCa cells, demonstrating that inflammation may promote PCa. Additionally, the sequential changes in membrane electrical properties and mechanical properties from BPH to BCI and to PCa cells suggest that inflammation promotes cancer by changing membrane micromorphology and related signaling events, which may provide a new insight into the role of inflammation in cancer.

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