The surface nanostructure features of ovarian cancer cells by atomic force microscopy

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ABSTRACT

Ovarian cancer is a disease with a high mortality rate in women. The important reasons for high mortality rate of ovarian cancer is the difficulty in early detection. The process of cell carcinogenesis is often accompanied by changes in surface nanostructure of cell membrane. In this study, atomic force microscopy (AFM) was used to obtain the nanostructure features of ovarian cancer cells. IOSE-80 (human ovarian normal cells) and Caov3 (human ovarian cancer cells) cell lines were selected and the morphology of the cell nuclear regions were measured using AFM Quantitative Imaging (QI) mode, which can offer information of hight, adhesion and slope channels. The surface parameters of the cell obtained from the three channels were analyzed. The results showed that there were significant statistical differences in parameters Root-mean-square height (Sq), Skewness (Ssk), Maximum height (Sz) and Arithemetic mean height (Sa) of adhesion channel, Sq, Ssk and Sa of hight channel. These findings indicate that the three channel in AFM imaging can offer different information of the surface nanostructure and the combination of these feature parameters may improve the identification accuracy of cancer. Our study will provide a new idea for the early diagnosis of ovarian cancer based on the nanostructure features of cell surface at the single-cell level.

Keywords: nanostructure features, ovarian cancer cell, atomic force microscopy, early detection

1. INTRODUCTION

Ovarian cancer is the leading cause of gynecologic cancer mortality, due to the difficulty of early detection. Early detection is the only way to achieve a high cure rate in women with ovarian cancer, unfortunately, there is no effective strategy for early detection, despite rapidly emerging biomarkers[1]. Approximately 207,000 people die of ovarian cancer each year[2]. Therefore, it is of great significance for early detection of ovarian cancer.

Atomic force microscopy (AFM) is a technique that allows visualizing surfaces down to the nanoscale[3]. Beyond pure microscopy, AFM can be used to measure the mechanical properties of samples, in particular[4-7]. It has been shown that the analysis of fractal dimension of cell surface imaged with atomic force microscopy (AFM) showed a strong segregation between normal, immortal (precancerous), and malignant human cervical epithelial cells, as unconstrained and rapidly dividing cells, the physicochemical properties of cancer cells have changed in comparison with the normal cells from which they are derived [8, 9, 10]. AFM can highlight the significant differences in cell membrane morphology between normal cells and tumor cells, and can determine whether the cells are malignant or not, compared with tumor cells from the same source[11]. During the invasion and metastasis of cancer cells, the adhesion between cells is reduced, and the shape and hardness of the cells changes according to the surrounding environment[12-14]. A new approach was recently reported in diagnostic imaging based on nanoscaleresolution scanning of surfaces of cells collected from body fluids using a recent modality of atomic force microscopy (AFM) and machine-leaning analysis, the surface parameters, which are typically used in engineering to describe surfaces, are used to classify cells[15]. The machine learning algorithm working with the surface parameters of different channels is capable of differentiating individual two or more cells with hight accuracy[3, 15]. Therefore, cell surface parameters are critical for distinguishing cancer cells from normal cells, but have not yet been used to differentiate ovarian cancer.

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In this study, the nuclear regions of human normal ovarian cells and human ovarian cancer cells were scanned by the QI mode of AFM. The cell surface parameters obtained in the scans of different channels were statistically analyzed.

2. MATERIALS AND METHODS

2.1 Cell culture

Two ovarian cell lines were used in this experiment, including normal human ovarian cells (IOSE-80) and cancerous ovarian cells (Caov3). IOSE-80 and Caov3 were cultured respectively in Roswell Park Memorial Institute (RPMI-1640) medium and Dulbecco's Modified Eagle Medium(DMEM) medium 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin solution at 37℃ in a 95% air/5% CO_2 incubator. Before AFM experiments, the normal cells (IOSE-80) and cancerous cells (Caov3) with the density of 50,000 cell were culture in 35 mm plastic petri dishes for 1 day.

2.2 AFM Imaging

JPK Nano Wizard III (JPK instrument, Berlin, Germany) AFM and optical microscope (Leica, Germany) in experiments were implemented to cell measurement. The cantilevers used were MLCT (Bruker) probes with a spring constant of 0.01N/m in all AFM experimentations. The cantilever tip is pyramidal in shape. Furthermore, the cantilever spring constant was verified by the thermal noise method. Before the experiment, the cells were fixed by 4% paraformaldehyde for 15min and replaced with 2mL PBS, then subjected to AFM imaging in QI working mode with Setpoint 1nN, Z Length 2500nm and Pixel Time 25 ms. After selecting the cells to be tested, then, conducting topography scanning at each pixel position (128×128) of the cell nuclear regions (5 μm×5 μm) to obtain high-resolution surface topography features of adhesion, slope and hight channels of cells.

2.3 Extraction of cell surface parameters

Cell surface parameters of three channels including adhesion, slope and hight were extracted by Gwyddion software, and the five parameters of Root-mean-square height (Sq), Skewness (Ssk), Maximum pit height (Sv), Maximum height (Sz) and Arithemetic mean height (Sa) were selected for subsequent data analysis. The data were obtained using 30 IOSE-80 and 30 Caov3 cells.

2.4 Data analysis

The data were analyzed using GraphPad Prism6. All values are represented by mean SD. Independent sample t test was used for statistical analysis, and p value was used to evaluate the statistical difference between groups.

3. RESULTS AND DISCUSSION

3.1 AFM Imaging of ovarian cells

Two ovarian cell lines were used in this study, including normal human ovarian cells (IOSE-80) and cancerous ovarian cells (Caov3). As shown in Figure 1, AFM probe scanned the cell surface of IOSE-80 and Caov3 cells at 5μm×5μm nuclear region, and obtained three cell surface images of adhesion, slope and hight channels. Each pixel in the imaging image contains information.

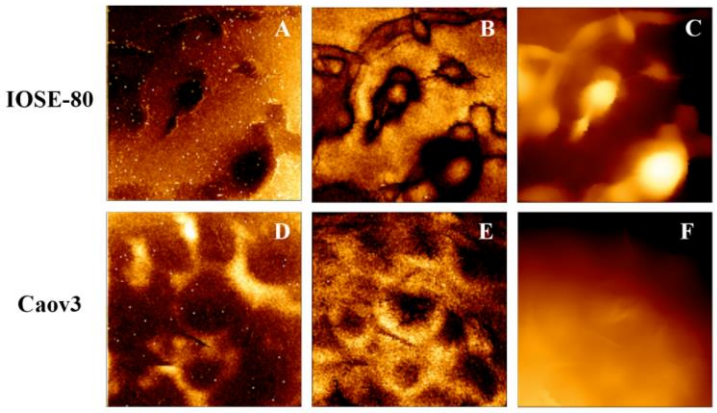

Figure 1. AFM images of cells. (A-C) are the adhesion channel imaging image, slope channel imaging image and hight channel imaging image of IOSE-80, respectively. (D-F) are the adhesion channel imaging image, slope channel imaging image and hight channel imaging image of Caov3, respectively.

3.2 Data analysis of adhesion channel

As shown in Figure 2, the parameters Sq, Ssk, Sz and Sa showed statistical differences in the adhesion channel. The Sq of IOSE-80 and Caov3 were 61.86±42.16 and 40.188±8.71pN. Sq is defined in the adhesion channel as the root mean square of adhesion at each point in the range, which is equivalent to the standard deviation of adhesion. Ssk were 2.518±3.36 and 11.94±8.9 for the two cells. Ssk is used to represent the distribution of adhesion. Ssk<0 is defined that the adhesion distribution is skewed upward relative to the mean surface (crest). The smaller the value of Ssk, the more adhesion values are greater than the average value. Ssk>0 is defined that lower adhesion distribution relative to the mean surface (trough). The larger the value of Ssk, the more adhesion values are smaller than the average value. Sz were 1.16±0.499 and 1.78±0.736 nN. Sz is defined as the sum of the maximum wave peak and the maximum valley depth in the range. Sa were 48.36±37.067 and 24.114±8.46 pN. Sa denotes the average absolute value of the difference in adhesion of each point relative to the mean surface.

Cell surface adhesion is known to be effective in distinguishing between normal cells and cancer cells [16]. Research has shown that the AFM imaging of fixed cells is capable of detecting different stages of progression toward cancer[3]. Our study found that many surface parameters can be obtained through adhesion channel that can be used to effectively distinguish between cancerous and normal cells. Combining these parameters may lead to a more effective way to distinguish cancer cells from normal cells, possibly detecting cells that are becoming cancerous and improving the possibility of early cancer detection.

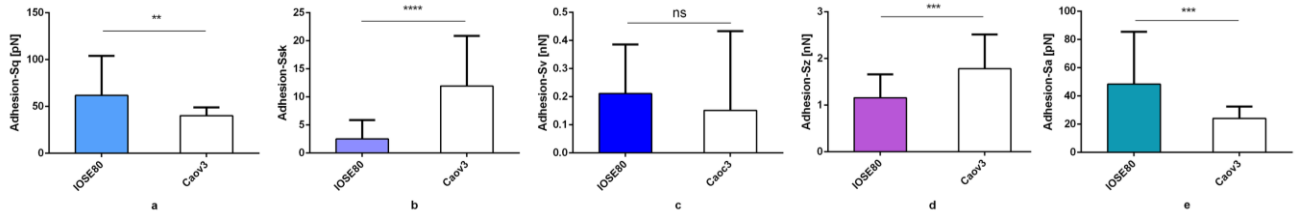

Figure 2. Nanostructure feature parameters of adhesion channel. The left side of the bar chart is IOSE-80 and the right side is Caov3. (a) Sq (b) Ssk (c) Sv (d) Sz (e) Sa. (Mean ± SD, ns meaning no statistical difference, ** meaning p<0.01, ***meaning p<0.001, ****meaning p<0.0001, n=30)

3.3 Data analysis of slope channel

As shown in Figure 3, there was no statistical differences in the five parameters of slope channel, which may be related to the use of fixed cells. Further study can be done on the living cells and the combination of the parameter in the three channels may improve the identification accuracy of cancer.

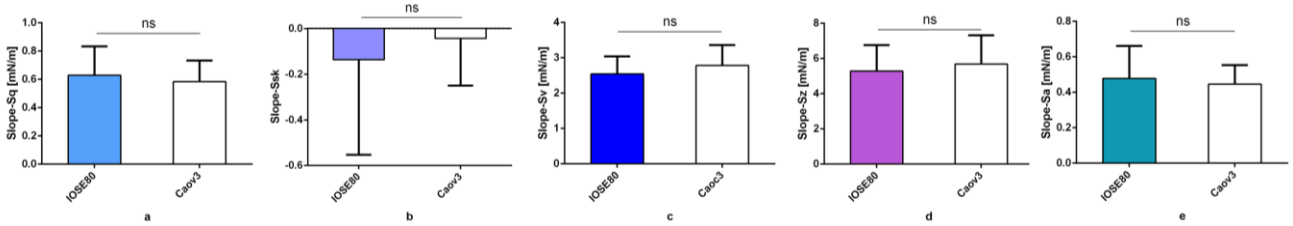

Figure 3. Nanostructure feature parameters of slope channel. The left side of the bar chart is IOSE-80 and the right side is Caov3. (a) Sq (b) Ssk (c) Sv (d) Sz (e) Sa. (Mean ± SD, ns meaning no statistical difference, n=30)

3.4 Data analysis of hight channel

As shown in Figure 4, the parameters Sq, Ssk and Sa showed statistical differences in the hight channel. The Sq of IOSE-80 and Caov3 were 429.537±219.715 and 308.8±171.287 nm. Sa were 353.96±190.78 and 254.94±147.08 nm for the two cells. Sq and Sa are commonly used to represent roughness. The results showed that IOSE-80 is rougher than Caov3. Ssk were -0.0329±0.541 and -0.467±0.4056. The parameter of the roughness shape (concave-convex) trend can be judged by the value of Ssk. Ssk<0 is defined that the height distribution is skewed upward relative to the mean surface (crest). The smaller the value of Ssk, the more hight values are greater than the average value. Ssk>0 is defined that lower height distribution relative to the mean surface (trough). The larger the value of Ssk, the more height values are smaller than the average value. The results showed that the surface height distribution of IOSE-80 cells and Caov3 cells is more wave peaks.

Our research showed a statistically significant difference in roughness between IOSE-80 and Caov3. However, the numerical difference is not particularly significant and the values overlap. If only the surface parameters of the hight channel are used, cancer cells cannot be detected very accurately at the single cell level. Therefore, we can combine multi-channel parameters to distinguish cancer cells form normal cells. Our experiments were carried out based on the QI mode of AFM, which allows simultaneous imaging of multiple channels, thus enabling multi-channel parameters extraction in a single experiment. It provides a new direction for early detection of ovarian cancer from the single cell level.

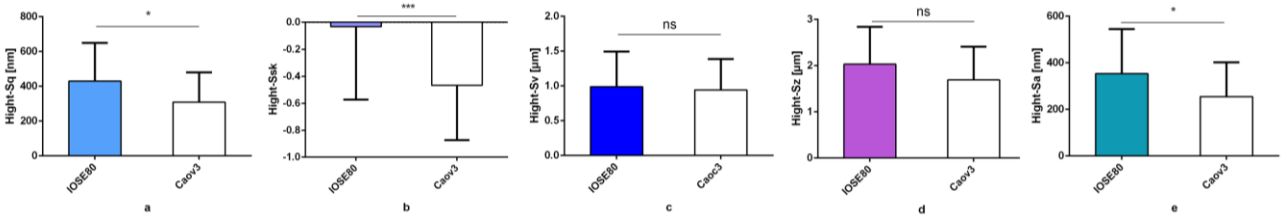

Figure 4. Nanostructure feature parameters of hight channel. The left side of the bar chart is IOSE-80 and the right side is Caov3. (a) Sq (b) Ssk (c) Sv (d) Sz (e) Sa. (Mean ± SD, ns meaning no statistical difference, * meaning p<0.05, ***meaning p<0.001, n=30)

4. CONCLUSION

In summary, we used AFM to image the nuclear region of human ovarian normal cells and ovarian cancer cells. The results showed that there were significant statistical differences in parameters Root-mean-square height (Sq), Skewness (Ssk), Maximum height (Sz) and Arithemetic mean height (Sa) of adhesion channel, Sq, Ssk and Sa of hight channel. The statistical parameters were less in the slope channel and the possible reason is the fixed cell used in the experiment. Further study can be done on the living cells and the combination of the parameter in the three channels may improve the identification accuracy of cancer.

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