Study on morphology of human ovarian cancer cells by the AFM and cell imprints technique

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Atomic Force Microscopy (AFM) technology is currently used to detect surface morphology of cells. Most cell samples are artificially cultured cells, and measured in the atmosphere or in liquid. The sample cell does not really coming from the ill tissue of some human body. There are some differences between the sample cells and the diseased cells in human body. Moreover, AFM test results so far, but only for basic research are difficult to directly be used in the clinical diagnosis. The cell imprints technology, however, can be directly used to obtain fresh tissue cells of the human body. It also has the following advantages, such as simple cell preparation; little or no damage to the cell structure; clear cell structure features, and so on. Therefore, in order to promote the use of AFM nano-measurement technology in the clinical diagnosis, in the present study, the cell imprints technology is combined with the AFM imaging technique to study micro-morphology of living human ovarian cancer cells. Based on pathological analysis on traditional optical micrographs of ovarian cancer cells, comparison of cultured ovarian cancer cells and cells prepared by the imprint technique is carried out using the AFM nano-measurement technique. Micro topography including geometry of cells, interaction of multiple cells, and other topographic features of samples prepared by the imprints technique are studied in detail. Comparative analysis with the pathological conclusion is consistent with the clinical diagnosis and it verifies the feasibility of combination the cell imprint technology and the AFM nano-measurement technique.

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1. Introduction

Among malignant tumors in the female reproductive system, diagnosis and treatment of the ovarian cancer is a huge problem for gynecologist. Because of the deep physical location of the ovaries in the pelvis, and the incidence of occult, 70% of patients with ovarian cancer are late for treatment. The effects of treatment and prognosis are also poor. Up to now, there is currently no effective method for the early diagnosis and treatment [1-2]. Recently studies have shown that the microstructures of the cancer and normal cell surface have shown a great difference. For example, Sokolov et al. found that the cell surface owns the micro-ridge and microvilli brush type structures. Such structures respond differently to micro particles and nano particles. This difference can be used to develop cancer detecting and therapy systems [3]. Thus, imaging cancer cells morphology is to gain an insight into the pathogenesis of ovarian cancer, which can perform early diagnosis and treatment and provide a reference for clinical treatment.

Since the invention of the Atomic Force Microscope (AFM), this technique has been widely applied in physics, chemistry, mechanical engineering, and biology because of its nanometer measurement accuracy. AFM can image samples in air or water, and the sample does not need to be specially prepared. It does not damage the sample surface. All these advantages make AFM widely be used in biology [4-11]. In the process of applying AFM to detect cell surface micro-structure, the majority of cell samples are cultured on the glass slide, and fixed by glutaraldehyde. They are often imaged in the atmosphere or in PBS liquids by the AFM [10 -11]. This kind of cell sample is not really ill and not the tissue from the human body. The cultured cell sample and the ill sample from the human body have significant differences. So far, the most reliable diagnostic method is still the pathological diagnosis. This is because the tissue samples of pathological diagnosis are obtained directly through the surgery, which owns the properties of originality for the cell morphology. The cell imprints technology is the basic method which is used for the pathologic diagnosis, which has the following advantages: simple and fast preparation of cell sample, little effect on the cells, little damage to the cell surface structure, maintaining the cell structure clearly, and high accuracy. Cell samples prepared by this

way can reflect the true situation of the cells in ill organizations. Up to now, however, the relevant reports on adopting this technology to preparing the ovarian cancer cell sample from the patient's body lesions, and using AFM to detect the micro topography have not been found yet. This kind of cell preparation method for direct observation of ovarian cancer will provide more useful information, and is conducive to the diagnosis of ovarian cancer.

Therefore, in the present study, living human serous ovarian cancer samples is prepared by the cell imprints technique. AFM is used to measure the morphology of the microstructure of cell surface. Different kinds of surface micro structure of the cell prepared by the cell imprints technique are studied, which are compared with the sample cells prepared by the cultured approach.

2. Experiments and methods

In the present study, the serous ovarian cancer cells of human are selected as the sample. The reason is as follows: the incidence of epithelial ovarian cancer is the first place among all kinds of the ovarian cancers. And the serous ovarian cancer is the most common. This will make the results of our study have a wider representation. The selected 13 cases samples are all gynecological surgery specimens carried out by Affiliated Tumor Hospital of Harbin Medical University.

Fig. 1 shows the typical light microscopic histological picture for the pathological diagnostic. The magnifications of Fig. 1 (a) and Fig. 1(b) are 10 times and 40 times, respectively. Fig. 2 shows the high magnification images of the serous ovarian cancer cells prepared by using the imprints technology for the pathological diagnosis during the operation. As shown in the figure, the cell aggregation can be found, but the cell's internal structure and morphology can not be observed very clearly. Samples used in the present study are drawn from the same person after the serous ovarian cancer from the pathological diagnosis is confirmed.

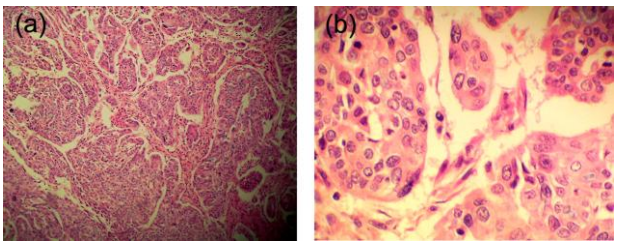

Fig. 1 The HE stained optical graph of the serous ovarian cancer pathology for the pathological diagnosis

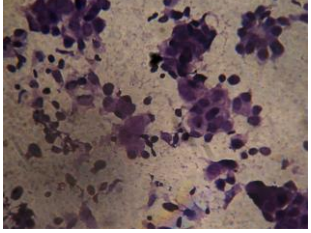

Fig. 2 The stained optical graph of the serous ovarian cancer pathology samples prepared by the imprints technique for the pathological diagnosis

By the optical microscopy it is very difficult to achieve the cell micro morphology. AFM is used in the present study to detect single cell morphology. The sample prepared process using the cell imprints technique for AFM to image in the atmospheric environment is expressed as follows: After the surgery ovarian cancer samples leave the human body. In a sterile environment, the sample is immediately cut along the surface of the largest axis of the tumor. Then it is pressed onto a clean glass slide gently with the cross section. This process will make cells to be printed on the glass slide. Then the cell sample is fixed for 3 minutes with the alcohol. Then the cell can be imaged by AFM. This process is the same with the sample prepared observed by the optical microscopy.

The AFM (Dimension 3100, Veeco Company, USA) is provided by the Center of Precision Engineering of Harbin Institute of Technology. Triangular Si_3N_4 cantilevers and tips are used to measure cells in contact mode in air. The temperature is 20℃, scan rate is 2 Hz. The normal load must be set as small as possible to reduce the effects of the AFM sharp tip on cell morphology. Thus, the normal load is set about 10 nN. By using the CCD of the AFM system, we can locate the cell roughly, and then AFM image can distinguish the cell precisely.

3. Results and discussions

Fig. 3 shows AFM images of the cell samples using different preparation methods in air. Fig. 3 (a) shows the AFM image of a single ovarian cancer cell that is prepared by the cell culture method. The rectangular shape cell adheres to the glass slip with a sucker-like structure at both ends. The height of the cell is about 2-3 μm. The overall length of the cell including the suction part is about 60-70μm. The existence of short fibronectin fibrils can be found around the cell. The previous studies showed that some cells and other tissues or cells connected with other cells through the fiber [10]. Fig. 3 (b) shows the AFM image of single ovarian cancer cell prepared by the imprints technique. As the figure shown, the morphology of the cell is quite different from that shown in Fig. 3(a). The cell exists in isolation with an irregular polygon shape. The oval shape nucleus of the cell is clearly visible. There is no ciliated and sucker-like structure surrounding the cell. Meanwhile, Fig. 4 shows the section analysis of the single ovarian cancer cell presented in Fig. 3 (b). It can be seen from the figure: The height of the nucleus is about 1μm or so. The average diameter of the nucleus is about 20μm. And the relatively flat top of the nucleus can be found. Structures of the surrounding cytoplasm are about 200nm higher than the glass substrate. All the data are processed and the average diameter and height of cells are $17.5^{+2.5}_{-4.5}\mu m$ and $921^{+480}_{-436}nm$, respectively. Here, the average value of the long axis and short axis of the oval nucleus is thought as the average diameter.

The difference in cell morphology by two methods may be due to as follows: When using the cell culture method for sample preparation, cells adheres and grows on the glass slide. Induced by the effect of the culture medium, the cell can grow freely. And cells in the vertical direction on the glass slide are not subject to any external force. Thus, cells are not deformed or broken. The morphology of cancer cells are shown in Fig. 3 (a). When using the cell imprints technique to prepare cell samples, the section of the cancer tissue is gently pressed on the glass slide. Then the cell adheres to the glass surface for the future observation. Such process actually applies an extra vertical force on the cell surface leading to cell deformation and a reduced cell height in the vertical direction. As shown in Fig. 4, the

height is less than 1μm, far less than the cell height of 2-3μm in the Fig. 3 (a). The external force even leads to cell rupture and the organization of the cell scatters around the nucleus. Moreover, the growth of cancer cells in the tumor depends on the human body. The growth environment is very complex, resulting in a huge difference between the true shape of the cell in the human body and the shape of the cell by the cultured method. Therefore, there exists a great error for judging the types and stages of the cancer only though the cell sample prepared by the culturing method.

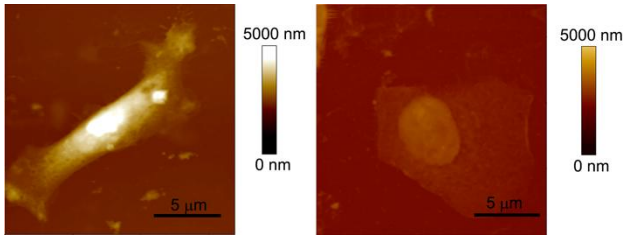

Fig. 3 AFM images of the cell samples using different preparation methods

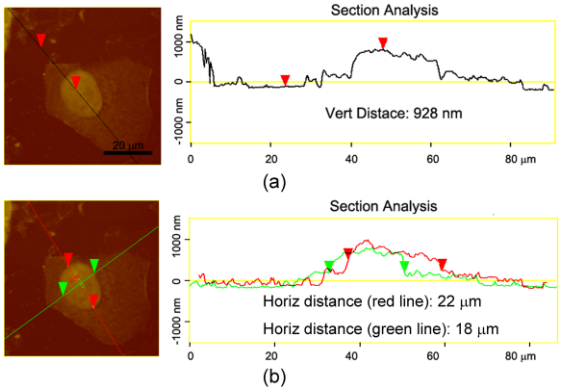

Fig. 4 The section analysis of the cell shown in Fig. 3(b)

Fig. 5 (a) shows AFM images of two adjacent ovarian cancer cells. Compared with Fig. 4 (b), the nuclear area density is significantly higher than that of the cytoplasm, the nuclear plasma is much larger, and the nucleus membrane is almost complete. No significant connection structures between the two cells can be found. In order to distinguish the connection between the two cells, cell junctions in Fig. 5 (a) is magnified and shown in Fig. 5 (b). The figure shows no obvious cilia class structure connecting both cells. While using

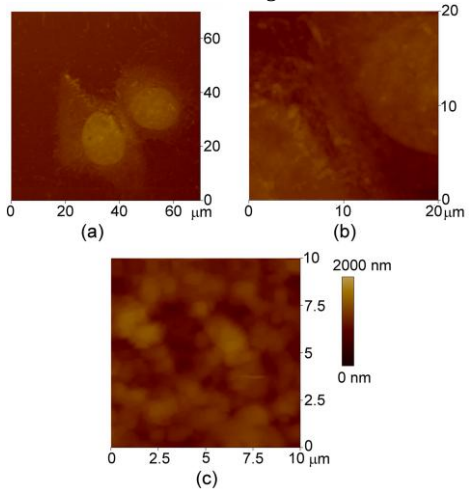

Fig. 5 AFM images of two adjacent ovarian cancer cells

cultured cell sample, there exists the short or long cilia class structure around the cell. Especially the observed cell is surrounded by other cells, or some other organizations. There will be a link between these structures [10]. Conclusions obtained by using the cell imprints technique and the cultured approach are not consistent. In addition, the nucleus is not a smooth flat surface. It has a property of owning rich, dense granular nuclear chromatin structures. A further amplified figure is presented in Fig. 5 (c). The granular structure on the nucleus can be seen clearly. Lighter areas in the figure should reflect the phenomenon of increased DNA replication, chromatin dysplasia, increasing protein synthesis at the micro scale. Nucleus area is brighter with the height increasing. These characteristics are in agreement with proliferation properties of the cancer cell.

Fig. 6 shows overlap of some ovarian cancer cells. The height of the cell is 1.6μm from the cross-section, which is much larger than the average value of 921nm. It shows that a overlap arises in the height direction. As the figure shown clearly, several nucleuses are overlapped. And cytoplasm is mutual integrated. The whole structure likes a complete cell. This phenomenon is induced by the cell aggregation, piles, disorder of the nucleus, which is in full compliance with the characteristics of malignant cells. When people judge the type and period of the ovarian cancer with the optical microscopy, there are a lot of similar structures. This kind of structure can improve the diagnostic accuracy, but there are some false judgments simultaneously. Two adjacent cells as shown in Fig. 5 observed by the low-magnification light microscopy may also be judged as the result shown in Fig. 1. Namely, they are looked on as a single cell. Therefore, AFM testing results can improve the diagnostic accuracy indicating that this method has important significance.

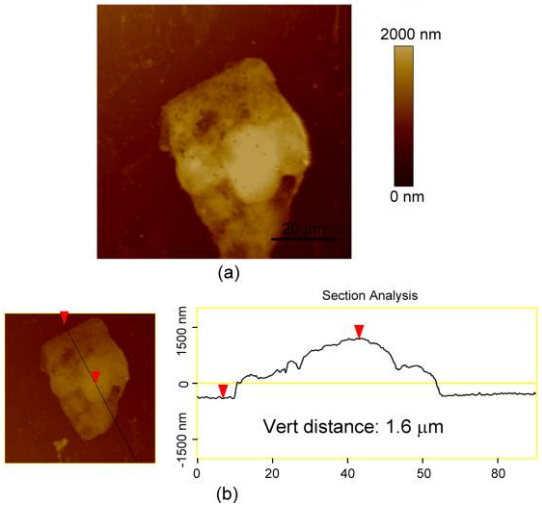

Fig. 6 AFM images of overlap of some ovarian cancer cells

Sometimes there is the structure shown in Fig. 7. The ovarian cancer cell appears the bare nuclear state. As the Fig. 7 (a) shown, the nuclear membrane is integrated, and nuclear matter density is increased. The imprints of red blood cells about 10 microns in diameter can be seen clearly. Malignant tumor is usually associated with necrosis. Thus, a lot of red blood cells are prone to arise in the cancer imprints. In the Fig. 7(a), the size of the mark is consistent with the size of the red blood cell, which is likely to be washed during the fixed time. Thus, this result also indirectly proves that the cells are malignant. Fig. 7 (b) presents another state of naked nuclear. Obviously, it owns the following properties of nuclear deformity,

membrane retraction, uneven, existing of fissures, and increasing mitotic activity which are typical features of cancer cells. Therefore, by AFM results we can clearly see the microscopic properties of the heterogeneous density structure within the nucleus.

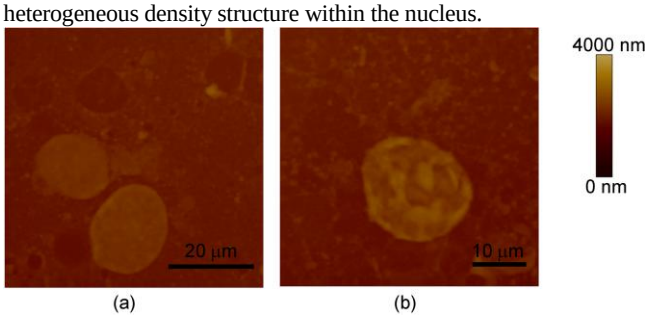

Fig. 7 AFM images of the bare ovarian cancer cell

4. Conclusions

In the present study, the ovarian cancer samples were prepared using the cell imprints technique. Based on the traditional analysis of cancer tissue by the optical microscopy, AFM is used to obtain micro morphology of cancer cells. Experimental results show that this technique can clearly distinguish a variety of cell super microstructures. And by comparing AFM images differences of the cells prepared by the culture approach and the cell imprints technique, following conclusions are obtained. The dimensions of the cell prepared by the cell imprints technique are determined by the sample preparation and the real situation of the body. The average diameter and height are $17.5^{+2.5}_{-4.5}\mu m$ and $921^{+480}_{-436}nm$, respectively, which are less than the dimensions of the cells prepared by the culture method. In addition, there are no obvious links between two adjacent cells. There is also the phenomenon of a large number of cell overlaps. On the one hand the macro diagnosis is verified; on the other hand it also brings a certain judge error. There is also a bare nucleus phenomenon, showing the typical characteristics of cancer cells. The cell imprints technology can obtain the sample cells directly from surgical specimens, which can ensure the organization's fresh, and almost no big change in cell morphology characteristics. Therefore, the use of AFM technique to observe the cell morphology, from the point of diagnosis of pathological cell tumor, has important clinical significance for the diagnosis of ovarian cancer.

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