Atomic Force Microscopy as a Tool for Research in Oncocytology

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ABSTRACT

Most of investigations have studied specimens prepared by complex techniques [1,2] which are unusual for ordinary hospitals. In present paper Atomie Force Microscopy (AFM) has been used for searching signs of malignancy in specimens prepared identically to those used in traditional cytological studies.

Keywords: nanoparticles, AFM, semicontact mode, cytological specimens.

1 AIMS AND INNOVATIONS

Atomic Force Microscopy has been used to measure the local properties of biological systems, including living cells, DNA, antibody-antigen interactions. Most of investigations have studied specimens prepared by quite complex techniques like attaching ligands to AFM tips, design of hybrid cell/polyelectrolyte systems, taking measures in fluids and so on [1,2]. Those techniques are unusual for hospitals. In present paper AFM has been used for searching AFM signs of malignancy in specimens prepared identically to those used in traditional cytological studies in hospitals without any additional preparation.

2 DEVICES AND MATERIALS

We have investigated 35 cytological specimens of breast cancer and thyroid cancer, specimens of normal breast and thyroid tissues, 10 cervical smears .

The hybrid nanoparticle containing anti-c-erbB2 antibody labeled with effectors metal ion was synthesized by a chemical approach. Specimens of breast cancer bearing c-erbB2 oncogene were incubated with those nanoparticles before scanning.

Diagnostic procedures: Fine-Needle Aspiration Cytology/Biopsy (FNAC/FNAB) and Imprint Cytology Smears. We prepared liquid-based monolayer cytological specimen and routine specimens, half of them were stained by azure and eosin technique. All specimens were investigated by SPM (NTEGRA Prima, NT-MDT Co., Russia) and by optic microscopy to get access to our previous experience in clinical cytology while using quite new approach.

3 MEASUREMENTS

We used semicontact mode in air. Line scan frequency about 0,5 Hz. In order to observe the impact of metallic ions Kelvin Probe Microscopy, a technique for the study of distribution of surface electric potentials, was used. Finally, after SPM images were collected, they were treated with different Image analyses functions like filtering, flatten correction, equalization, statistics.

3.1 Breast Cancer Cells

The whole shape of three cancer cells is presented in figure 1. Some fine structures on the cell surface, including small holes and granules with minimum size of about 50 nm, can be resolved in figure. The rough microrelief of the surface of nuclei is observed in the breast cancer cells in contrast to normal cells (Figure 2).

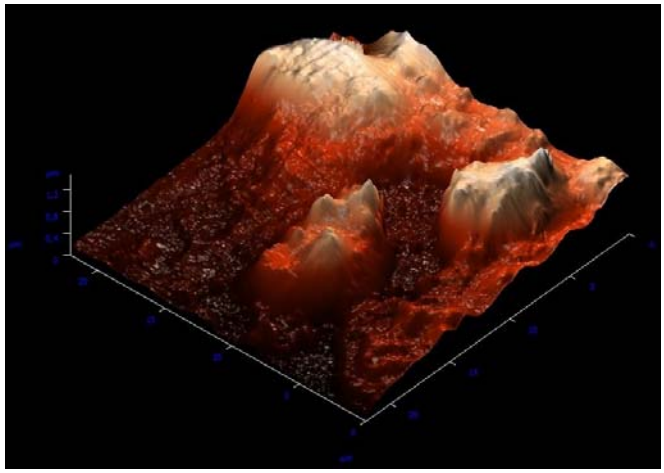

Figure 1. Three cells of breast cancer.

Nucleoli are seen as local elevations, nuclear-cytoplasmic relationship changed in the direction of its increase in cancer cells. Cells and nuclei boundaries are uneven.

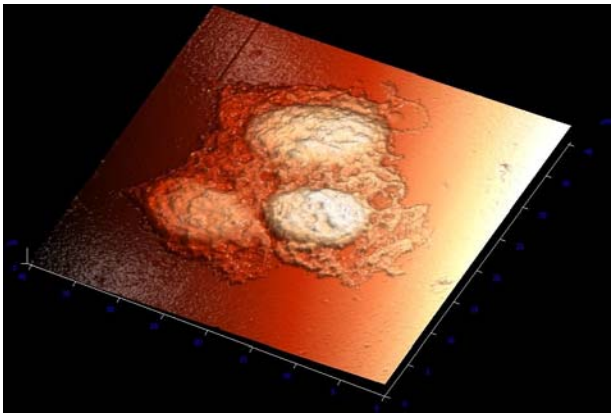

Figure 2. Three normal cells

In figures 3 and 4 are represented the results, obtained after immunocytochemical dyeing of cells overexpressing Her2/neu , which is evidenced by the intensive dyeing of the cytoplasmic membrane. Overexpression of this receptor in breast cancer is associated with increased disease recurrence and worse prognosis. Because of its prognostic role as well as its ability to predict response to trastuzumab, breast tumors are routinely checked for overexpression of HER2/neu.

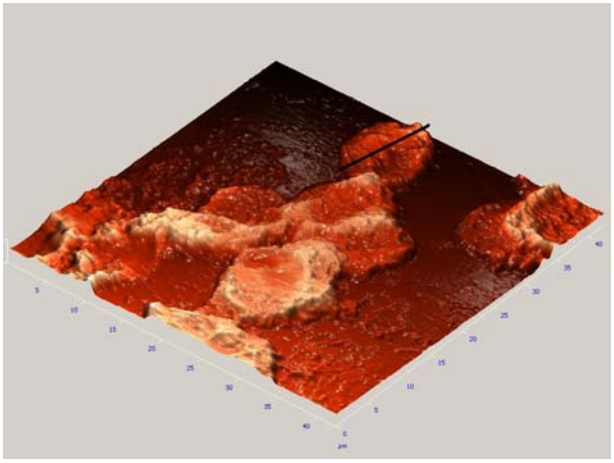

Figure 3. AFM image of Her2/neu overexpressing cells. Black line indicates the position of cross-section of the surface (figure 4).

AFM-images of Her2/neu overexpressed specimens demonstrate a packing of the membrane of tumor cells. The estimation of the intensity of immunocytochemical reaction is possible according to the height of the painted membrane (Figure 4). It is easy to evaluate the closure of staining of the membrane as well.

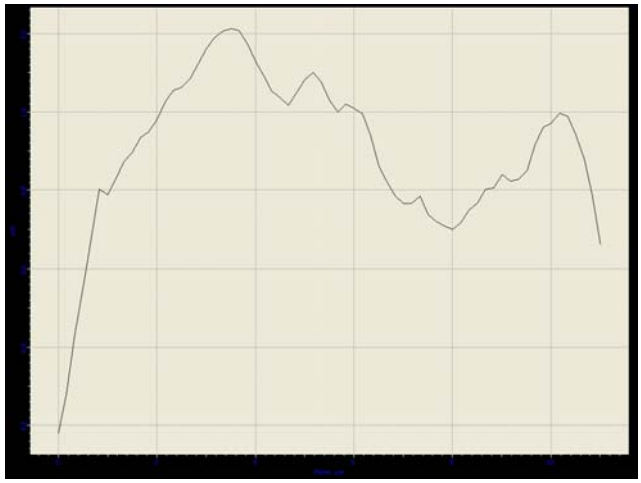

Figure 4. Cross-section of surface of the immunocytochemicaly stained cell.

We also found strong correlation between surface topography and spatial distribution of the surface potential over the surface of a specimens bearing c-erbB2 oncogene after incubation with hybrid nanoparticles abovementioned

3.2 Thyroid Cancer Cells

The changed rough microrelief of the surface of nuclei having irregular shape can be seen in AFM-image (Figure 5). Nucleoli are revealed in the form of the local elevations.

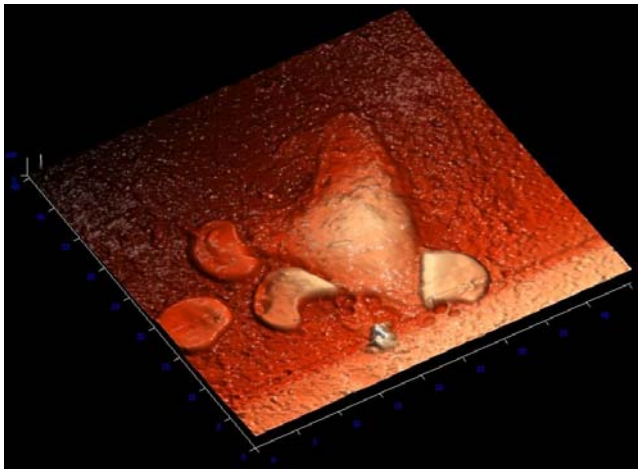

Figure 5. AFM-image of thyroid cancer cell.

Normal cells of the thyroid gland (figure 6) do have rounded shape with the smoothed microrelief of nuclei without visible nucleoli.

NSTI-Nanotech 2009, www.nsti.org, ISBN 978-1-4398-1783-4 Vol. 2, 2009

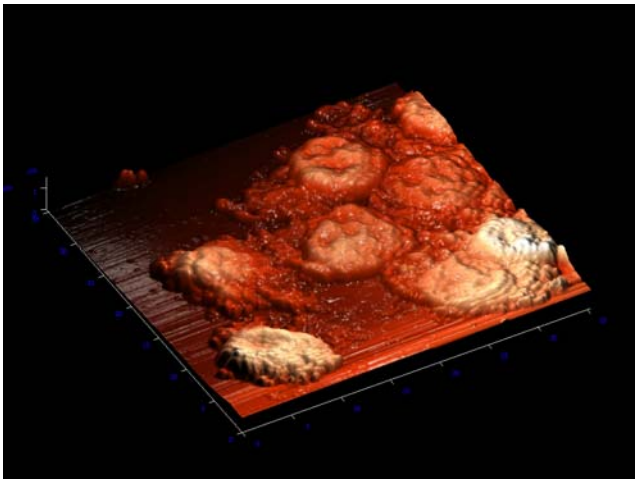

Figure 6. Normal cells of thyroid gland.

3.3 Epithelial Cells

The signs of planocellular keratotic cancer (Figure 7) are: cellular polymorphism, presence of the cells of the elongated and whimsical shape with the signs of hyperkeratosis. Nucleus is amalgamated and is located eccentrically. The nuclear chromatin is uneven and lumpy. The height of nucleus is significant and the height of cytoplasm in the zone of keratosis is approximating the height of nucleus. The microrelief of the surface of nuclei is rough.

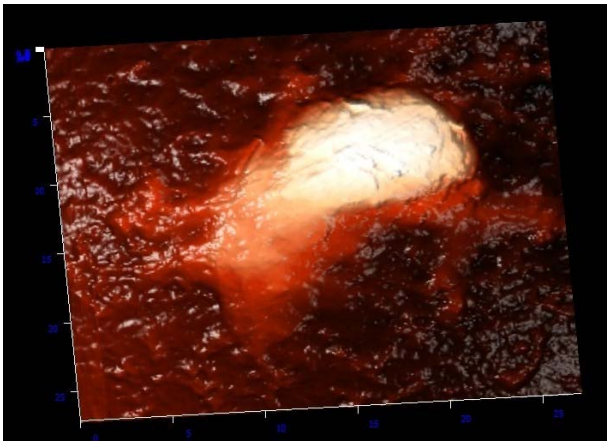

Figure 7. AFM-image of planocellular keratotic cancer.

Normal flat epithelium (Figure 8): quite large flat cells of polygonal form. Nuclei are oval or round, with the unstructured chromatin, pycnotic. Cells are located separately.

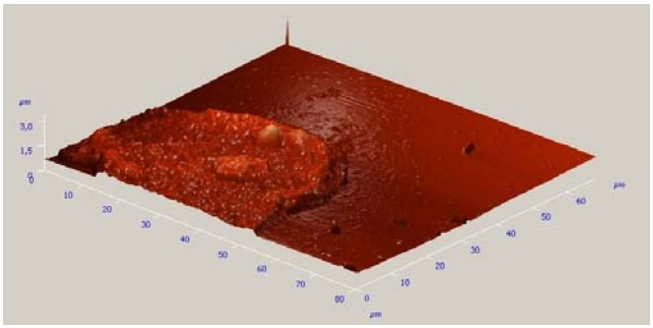

Figure 8. Normal flat epithelium.

One of important task of investigation was search of AFM-signs of human papilloma virus associated lesions of the cervix (Figure 9).

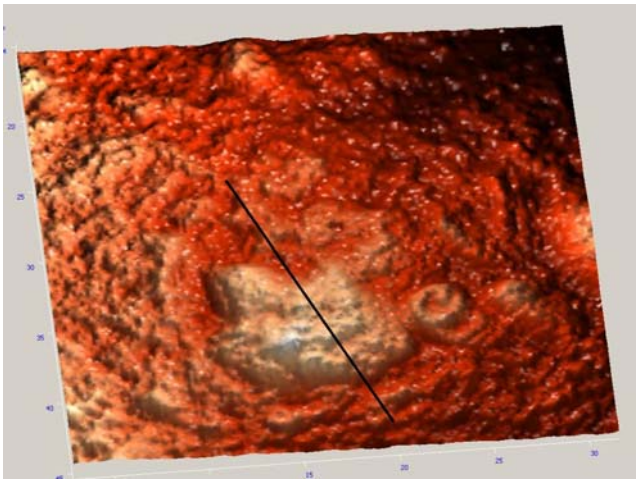

Figure 9. AFM-image of Koilocytes. Black line indicates the position of cross-section (figure 10).

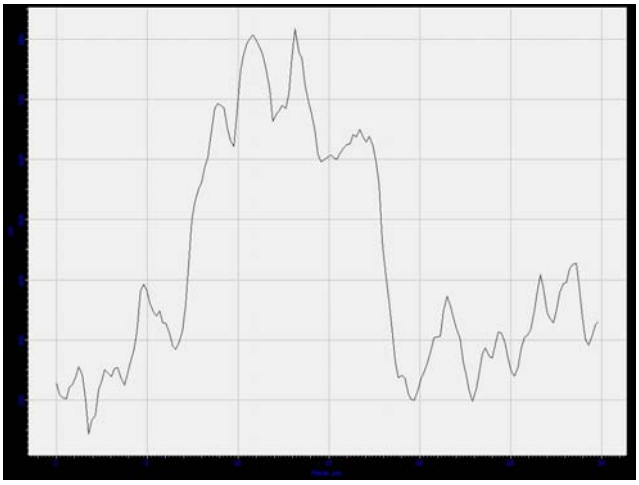

Figure 10. Koilocytes surface cross-section. Cells appear to have halo nuclei.

Presence of koilocytes is one of known signs of virus lesion (black line indicated cell in figure 9) . Koilocytes are a type of dysplastic squamous cell found in potentially precancerous cervical lesions. They have halo nuclei on cytologic examination. As seen in figure 10 morphologic appearance of halo is perinuclear groove about 200 nm depth.

4 RESULTS

Table 1 gives summary of measurements results.

Tissue	Nucl. hight nm	Sizes (micrometer) and Shape of nucleus	Summary: AFM signs of lesion
Breast cancer	500	9x5 irregular	Visible nucleoli 300 nm in cancer cells, irregular shape of nucleus
Intact breast	450	13, round	
Thyroid cancer	450	11x20 irregular	Visible nucleoli 200 nm in cancer cells, irregular shape of nucleus, nuclear enlargement
Intact thyroid gland	300	8, round	
Normal epithelium	500	12, round	Visible nucleoli 200 nm in cancer cells, irregular shape of erlanged nucleus, perinuclear grove in koylocytes
Cervical cancer	1700	16x20 irregular	
Virus lesion	400 Perinucl. groove 200 nm	14, irregular	

Table 1: Results of measurements.

5 CONCLUSIONS

1. SPM is capable of distinguishing tumor cells in vitro on actual cytological samples with teens of nanometer resolution of 3-D details.

The preparation of samples is quite simple and may exercised in ordinary hospitals, which should accelerate the adaptation of nanotechnology tolls (AFM) into everyday medical practice.

2. Results of investigation can be applied in a routine diagnostic setting for the detection of cancer cells.

3. The AFM approach for simultaneous imaging of specimen's topography and distribution of surface electric potentials can be a promising method for studying nanoparticles biodistribution.

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