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COMBINATION OF CONFOCAL LASER SCANNING MICROSCOPY WITH SCANNING FORCE MICROSCOPY FOR K562 CELLS STUDY

L. V. Kukharenko[1], T. Koshikawa[2], O. V. Aleinikova[3], T. V. Shman[3], A. B. Krylov[1], A. A. Ivanov[1], N. G. Tsikunova[4]

[1] Belarussian State Medical University, 83 Dzerzhinskii Ave., Minsk, Belarus. E-mail: lvk@europe.com
[2] Osaka Electro-Communication University, Hatsu-cho 18-8, Neyagawa-shi, Osaka 572-8530, Japan
[3] Belarussian Center for Pediatric Oncology and Hematology, Pos. Lesnoe, Minski distr., Belarus
[4] Belarusian State University of Informatics and Radioelectronics, 6 P. Brovka Str., Minsk, Belarus

This study has briefly introduced some of the surface morphology features of K562 cells. Using scanning force microscopy and confocal laser scanning microscopy allows us to visualize K562 cell surface morphology details such as the membrane convexities with diameter ranged from 95 to 300 nm, different kind of surface microvilli with breadth from 45 to 160 nm extended from the cell body, the plasma membrane surface knobs with width ~890 nm and height ~280 nm.

1. Introduction

The scanning force microscope (SFM) is increasingly applied to biomedical objects because of the combination of extreme resolution, the ease of sample preparation and the ability to operate under physiological conditions [1, 2]. The SFM generates image contrast by a way completely different from optical and electron microscopy. It scans the sample surface with a very small sharp tip at the end of a soft cantilever. The combination of SFM and optical microscopy opens the greatest possibilities for visualization of biomedical objects. The aim of this study is to apply SFM, combined with confocal laser scanning microscopy to investigate K562 cells surface morphology details.

2. Material and method

In childhood acute leukemia prognosis has improved dramatically over the past decades. However acute myeloid leukemia, leukemia with Philadelphia (Ph+) chromosome are associated with a poor prognosis. Therefore K562 cell line is a good model for investigation of myeloid leukemia and Ph+ leukemia [3]. Moreover, recently K562 cells were used for bionanotechnological investigations [4].

K562 erythromyeloid cell line (obtained from human with chronic myeloid leukemia in blast crisis, Ph+) was grown in RPMI-1640 medium supplemented with 10 % fetal bovine serum, antibiotics, 2mM L-glutamin. Cell suspension was placed onto mica cover slip and spread to cover the entire surface. Cells were fixed with 2% glutaraldehyde for 1 hour. K562 cells on the mica cover slips were washed five times in phosphate-buffered saline (PBS), dehydrated through increasing concentrations of ethanol for 5 min each and air-dried.

Nanosearch microscope LSM/SPM (SFT-3500, Japan), where SFM (Shimadzu, Ja-

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pan) is integrated into scanning type confocal laser microscope (Olympus, Japan) with laser wave length 408 nm was used for most experiments. This technique allowed to widen range of questions to be explored. Surface morphology of K562 cells was also investigated by a SFM (SPM-9600, Shimadzu, Japan). SFM images were captured under ambient air conditions using SFM tapping-mode with commercially available silicon cantilevers (spring constants of 40 N/m, Nanoworld).

3. Results and discussion

K562 cells were high to be studied using SFM large scan areas. Therefore the visualization of whole cell body by laser scanning microscope was then combined with the potential for high resolution investigation of K562 cells surface morphology details using the SFM. Whole K562 cell was viewed on a laser scanning microscope and then it was possible to zoom into a defined location on the same cell for detailed analysis of subcellular structures at high resolution on the nanometer scale by SFM.

Confocal laser scanning microscope images of K562 cells are given in Fig. 1 a, b, showing general topography of the cells.

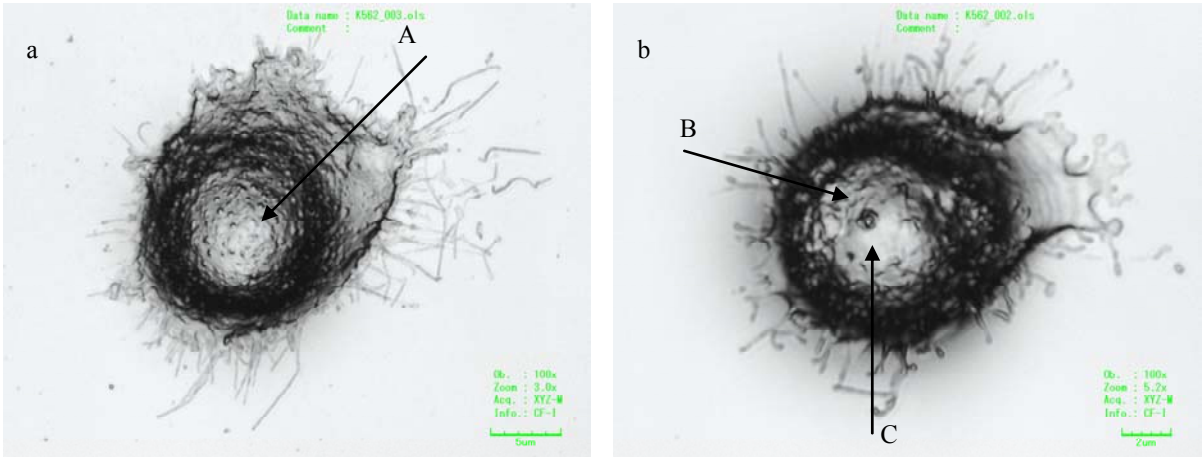

Fig. 1. Confocal laser scanning microscope images of K562 cells (a, b)

The K562 cells appeared to be spherical shaped with diameter, ranging from 10 to 19 μm. The height of cells varied from 6 to 8 μm. Adhesion to mica substrate leads to irregular cell shape with long pseudopodia and cytoplasmic protrusions. The cells had mixed surface morphology, with both smooth and encrusted forms. Some of the cells were relatively smooth with the local membrane convexities and didn't possess any microvilli. Figures 2 a, b show magnified SFM images of the upper right portion of the K562 cell indicated by the arrow A in Fig. 1 a. The high-magnification SFM image shows that membrane convexity height varied from 50 to 130 nm and its diameter ranged from 95 to 300 nm. Higher resolution scan of the membrane convexities by SFM revealed its granular structure with granule size of from 7 to 10 nm (Fig. 2 b).

Viewed by SFM, the majority of cells display a small-to-moderate number of surface microvilli. Two different types of microvilli could be distinguished morphologically with SFM: thin membrane protrusions of various length and short, blunt ones. Figure 3 shows magnified SFM image of the upper left portion of the K562 cell indicated by the arrow B in Fig. 1 b. As illustrated in Fig. 3, thin long surface microvilli of

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varying length (up to 4 μm) and from 100 to 160 nm in breadth extend from the cell body. A few holes in the plasma membrane with a diameter, ranging from 70 to 100 nm were observed.

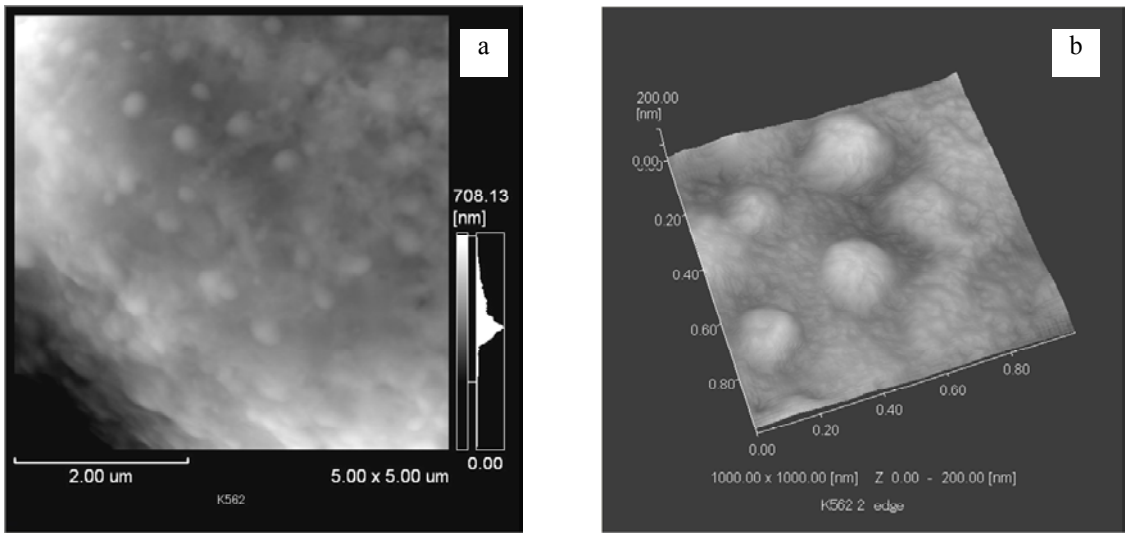

Fig. 2. a) SFM image of plasma membrane region of the K562 cell indicated by the arrow A in Fig. 1 a; b) three-dimensional magnified SFM image of the same region of plasma membrane

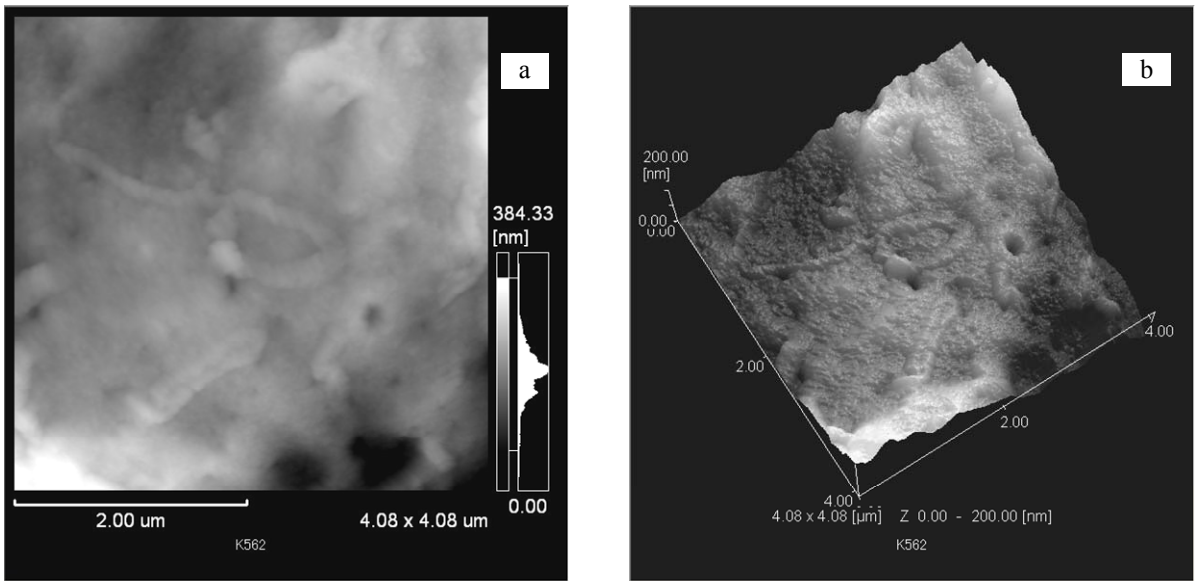

Fig. 3. SFM (a – two-dimensional; b – three-dimensional) images of plasma membrane region of the K562 cell indicated by the arrow B in Fig. 1 b

Figure 4 presents magnified SFM image of the central portion of the K562 cell indicated by the arrow C in Fig. 1 b. The surface knobs on the plasma membrane with width ~890 nm and height ~280 nm were visualized by SFM.

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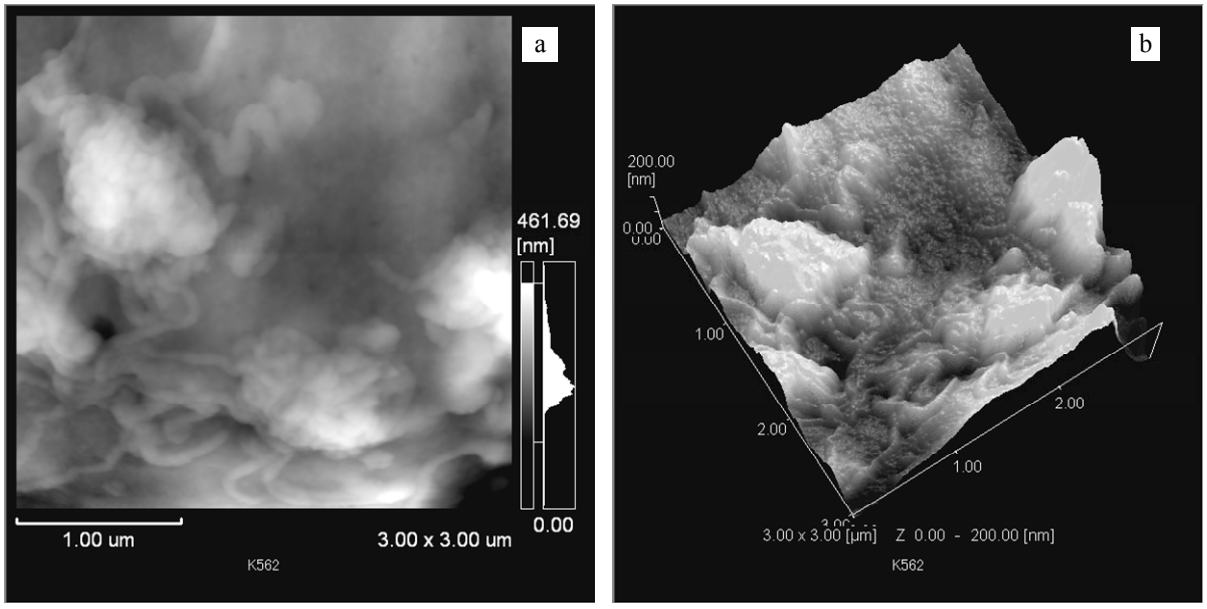

Fig. 4. SFM (a – two-dimensional; b – three-dimensional) images of plasma membrane central portion of the K562 cell indicated by the arrow C in Fig. 1 b

The knobs have granular structure with granule size of from 80 to 100 nm. Moreover, stublike (45–65 nm broad) and short microvilli (80–115 nm broad, and generally up to 1 µm long) projecting from knobs can be seen. The knob granules have approximately the same diameter as the microvilli.

A major goal of the present study was to characterize the K562 cell surface morphology with SFM and confocal laser scanning microscopy. Using SFM and confocal laser scanning microscopy allows us to visualize K562 cell surface morphology details such as the membrane convexities with diameter ranged from 95 to 300 nm, different kind of surface microvilli with breadth from 45 to 160 nm extended from the cell body, the plasma membrane surface knobs with width ~890 nm and height ~280 nm.

Conclusions

Using SFM and confocal laser scanning microscopy allows us to visualize K562 cell surface morphology details. As demonstrated by the presented results, the combined techniques open up unique opportunities to visualize biomedical objects at high resolution. The combination of SFM and confocal laser scanning microscopy has given complementary information about K562 cell surface morphology details. The combined techniques can be used in a correlative way to obtain a more detailed surface morphology images than is obtainable with either technique alone.

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