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# ИСПОЛЬЗОВАНИЕ АТОМНО-СИЛОВОЙ МИКРОСКОПИИ ДЛЯ ФИБРОБЛАСТОВ ПАЦИЕНТОВ С АНЕМИЕЙ ФАНКОНИ ПОСЛЕ ВОЗДЕЙСТВИЯ Г–ИЗЛУЧЕНИЯ

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**Аннотация.** В данной работе актиновый цитоскелет и механические свойства фибробластов пациента с анемией Фанкони были изучены с помощью флуоресцентной микроскопии и атомно-силовой микроскопии. Была исследована кинетика восстановления структуры цитоскелета фибробластов до и после воздействия γ–излучения. Детальное изучение механических свойств фибробластов таких как упругость, адгезия и жесткость, организация цитоскелета необходимо для диагностических целей.

**Ключевые слова:** АСМ; фибробласты; анемия Фанкони; γ–излучение.

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**Abstract.** In this work the actin cytoskeleton and mechanical properties of the Fanconi anemia patient fibroblasts were studied using fluorescence microscopy and atomic force microscopy. The repair kinetics of the fibroblasts cytoskeleton structure was investigated before and after exposure to γ–radiation. Study in details of the fibroblasts mechanical properties such as elasticity, adhesion and stiffness, cytoskeleton organization and cell shape is required to the diagnostic purposes.

**Keywords:** AFM; fibroblasts; Fanconi anemia; γ–radiation.

**Conflict of interests.** The author (-s) declare no conflict of interests.

**Gratitude.** Текст благодарности на английском языке (если необходимо).

## Introduction

Fanconi anemia (FA) is an autosomal recessive disorder characterized by chromosomal instability, bone marrow failure and a predisposition to cancer. It is known FA cells show elevated rates of chromatid breaks and chromatid exchanges. It is known that chromosome breakage by DNA cross-linkers is used as a diagnostic hallmark of FA. A less known and still disputable feature of FA cells is their radiosensitivity. Atomic force microscopy (AFM) offers great promise as an instrument for studying FA fibroblasts, including molecular level visualization of cytoplasmic submembranous structure, structural and morphological surface changes occurring after exposure of fibroblasts to γ–radiation [1,2]. In addition to topographical measurements, AFM is also capable of complementary tool that provide information on other fibroblasts surface properties, e.g. stiffness, hardness, elasticity. The reorganization of the fibroblasts cytoskeleton structure after exposure to γ–radiation leads to change in the mechanical properties of cells, so it is possible to use the cell mechanical parameters as certain markers of the pathology. The use of force modulation mode (FMM) of AFM provides information on the mechanical properties of FA fibroblasts surface before and after exposure of the cells to γ–radiation. Here the fibroblasts of Fanconi anemia patients and healthy donors were studied. The repair kinetics of fibroblasts cytoskeleton structure was studied before (untreated) and at different times after exposure to γ–radiation (30 min, 24 hours) using AFM and fluorescence microscopy.

## Materials and methods

Two strains of skin fibroblasts isolated from an FA patient and from an apparently healthy donor were evaluated for their in vitro radiosensitivity using AFM and foci immunofluorescence staining. While one set of cells (both from an FA patient and healthy donor) left untreated (control cells), the other one was exposed to γ–radiation at 5 Gy. Both FA fibroblasts and healthy donor fibroblasts (before and after exposure

to γ–radiation in 30 minutes and 24 hours) were studied by AFM and foci immunofluorescence staining. The cells were fixed with 2% glutaraldehyde for 30 min.

All data were obtained on a Nanoscope (R) IIIa MultiMode atomic force microscope (Digital Instruments/Veeco). FMM was used to study mechanical properties (local stiffness and adhesion) of the fibroblasts membrane. The FMM is a non-resonant, intermediate contact mode of AFM. When working in FMM, an additional sinusoidal modulation to the cantilever with user-selectable frequency, which is far below the resonance frequency of the cantilever is applied while the tip scans the surface. FMM enables to obtain information about relative difference in cell surface elasticity with nanometer-scale resolution. The images were acquired by using silicon nitride cantilevers (NSC12/50) with a nominal force constant of 0.65 N/m (NT-MDT, Zelenograd, Russia). The measurements were performed in air at room temperature. AFM images were processed with the Nanoscope software (Digital Instruments/Veeco).

## Results and discussion

The changes in fibroblast cytoskeletal organization after exposure to γ–radiation were reflected in the cellular mechanical properties. The topographic, adhesion and stiffness images of the FA fibroblasts are presented in fig.1.

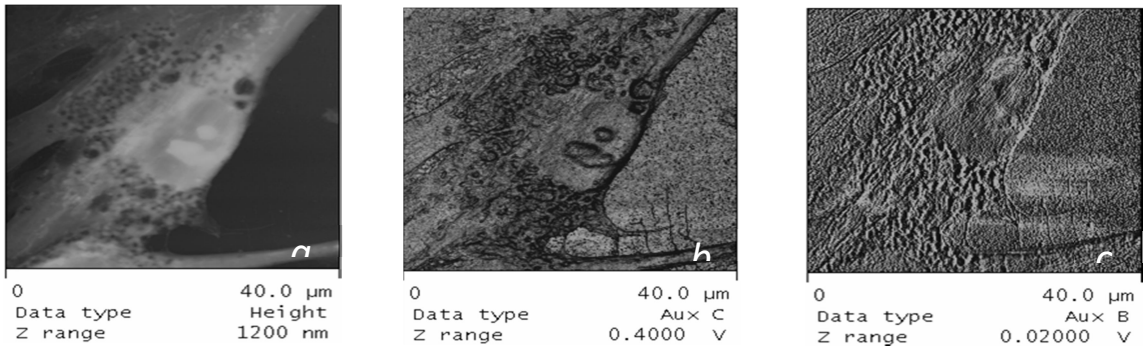


**Fig.1.** AFM images of FA fibroblast: *a*- height, *b*- adhesion, *c*- stiffness

Darker parts in the adhesion and stiffness images correspond to low adhesion and stiffness value on the fibroblast membrane. As follows from the AFM images of the control fibroblasts before exposure to γ–radiation (fig. 1) their nuclei are more adhesive and less rigid than the surrounding nucleus region and the peripheral (lamellipodial) regions. The stiffest part of the fibroblasts corresponds to the lamellipodial region of cell. Since the lamellipodium is very thin, probably the underlying substrate affects the fibroblast stiffness. The topographic, adhesion and stiffness images of the FA fibroblasts and healthy donor ones in 30 minutes after exposure to γ–radiation at 5 Gy are given in fig. 2, 3. The rearrangement of the actin cytoskeleton was observed for fibroblasts in 30 minutes after exposure to γ–radiation. FA fibroblasts in 30 minutes after exposure to γ–radiation have less adhesive nucleus region and the lamellipodial regions due to reorganization of the actin cytoskeleton. On the contrary, nucleus region and the lamellipodial regions are more adhesive for donor fibroblasts in 30 minutes after exposure to γ–radiation.

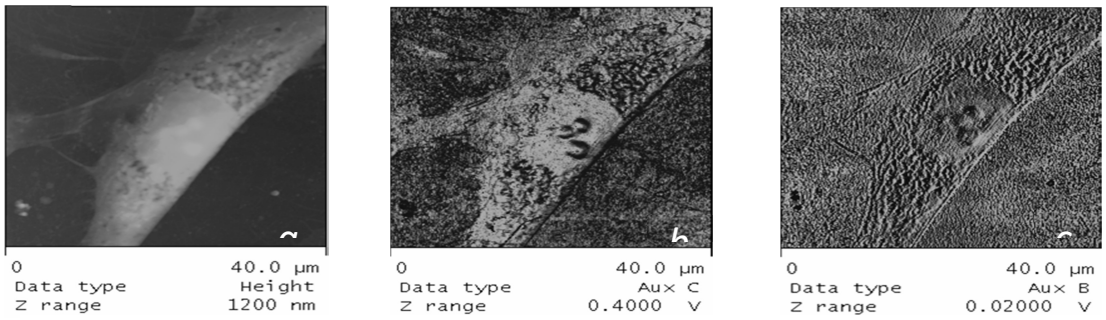


**Fig.2.** AFM images of control fibroblast in 30 minutes after exposure to γ–radiation at 5 Gy: *a*- height,

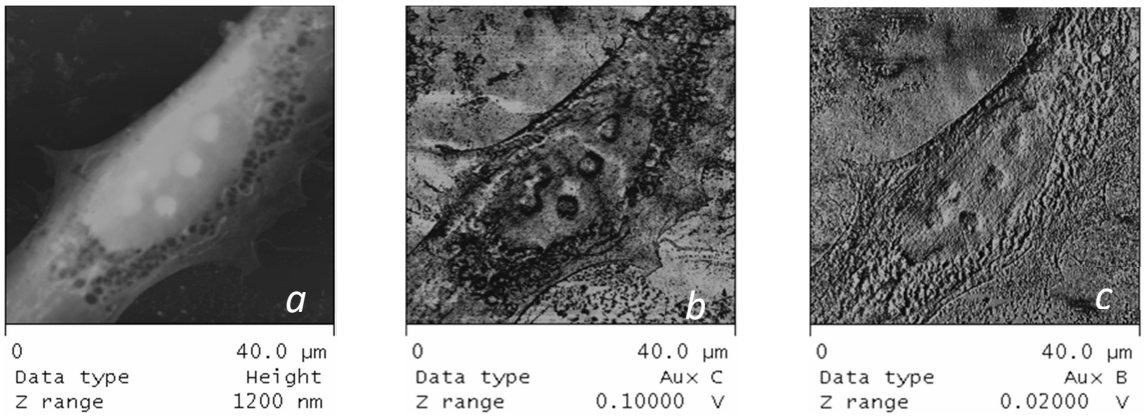


**Fig.3.** AFM images of FA fibroblast in 30 minutes after exposure to γ–radiation at 5 Gy: *a*- height,

The topographic, adhesion and stiffness images of healthy donor fibroblasts and FA ones in 24 hours after exposure to γ–radiation at 5 Gy are given in fig. 4, 5. In 24 hours after irradiation in donor fibroblasts and FA ones a reorganization of the actin cytoskeleton occurs, resulting in reduction of the cell membrane stiffness and adhesion increase in nuclear and lamellipodial regions of the cell. As seen from the AFM images the FA fibroblasts appear less stiff even in thinner lamellipodial regions.

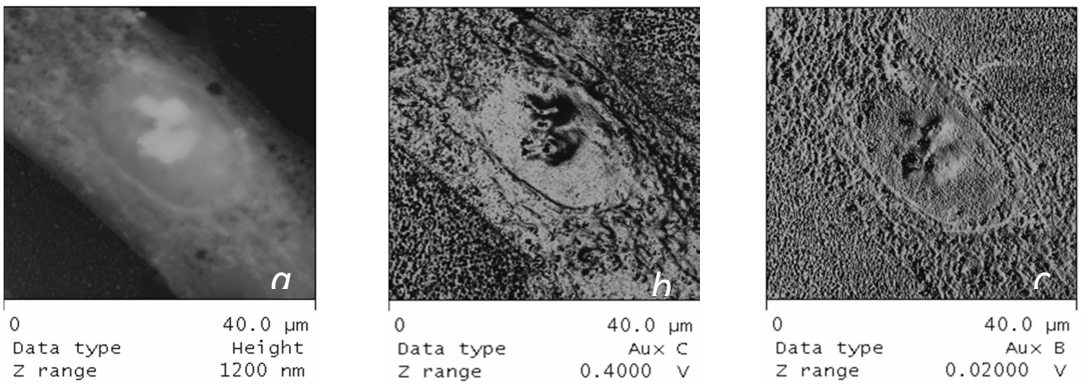


**Fig.4.** AFM images of control fibroblast in 24 hours after exposure to γ–radiation at 5 Gy: *a* - height, *b*- adhesion, *c*- stiffness)

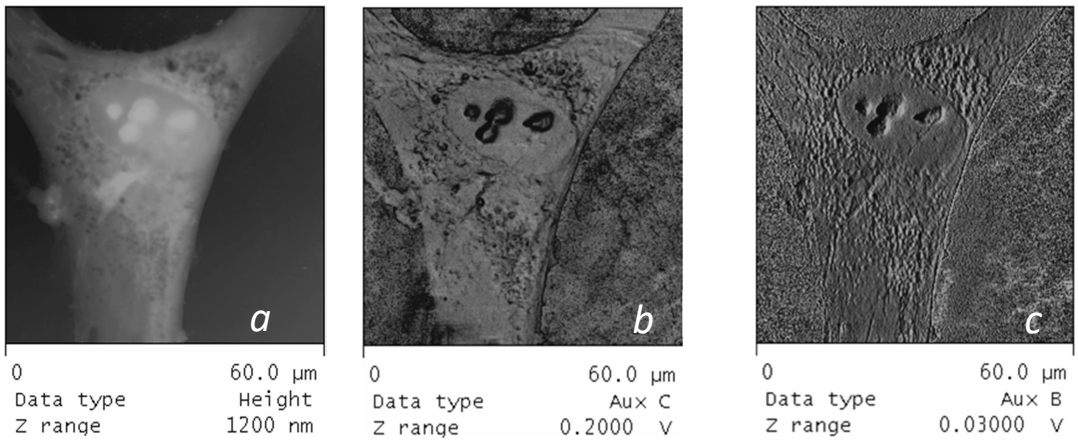


**Fig.5.** AFM images of FA fibroblast in 24 hours after exposure to γ–radiation at 5 Gy: *a*- height,

## Conclusions

AFM images of skin fibroblasts isolated from an FA patient and from an apparently healthy donor exhibited the characteristic spindle shaped cells with a well-developed cytoskeleton, comprising mainly arrays of parallel actin stress fibers extending the long axis of the cells and irregularly shaped flat lamellipods. The lateral size of densely packed parallel arrays of actin stress fibers varies from 30 to150 nm for donor fibroblasts, whereas for FA fibroblasts the size varies from 30 to 200 nm. Zooming in on the nucleus the granular structure of elongated bundles of actin filament with minimum measured granule size of 30 nm is visualized. The structure of actin stress fibers appears better defined in the error signal image. A rearrangement of the actin cytoskeleton was observed for FA fibroblasts in 30 minutes after exposure to γ–radiation. Many thick parallel actin stress fibers with the lateral size from 90 to 320 nm extending throughout the nucleus were visualized for FA fibroblasts in 30 minutes after exposure to γ–radiation. AFM images showed the actin filaments breaks, fragmented and disorganized actin stress fibers in irradiated FA fibroblasts. In contrast, irradiated healthy donor fibroblasts had fewer thick parallel actin stress fibers with lateral size of 200 nm and showed, predominantly, thin densely packed parallel actin stress fibers with the lateral size from 50 to 70 nm. The AFM images of the control fibroblasts from an FA patient and from an apparently healthy donor in 24 hours shows thin densely packed parallel actin stress fiber with the lateral size from 30 to 90 nm extending throughout the nucleus. In 24 hours after exposure to γ–radiation at 5 Gy both FA fibroblasts and healthy donor ones revealed densely packed parallel long, straight actin stress fibers with average fiber diameters in the range of 30-70 nm. Thick parallel actin stress fibers with the lateral size from 100 to 320 nm extending throughout the nucleus were also visualized for both FA fibroblasts and healthy donor ones in 24 hours after exposure to γ–radiation. The AFM study also showed a decreased height of nucleoli in the FA fibroblasts nucleus as compared to healthy donor nucleus.

Mechanical properties of fibroblasts most likely are regulated by the actin cytoskeleton structure. According to the fluorescent images of FA fibroblasts in 24 hours after irradiation microtubules originated from the center and formed a radiating network near the nucleus. Disrupting actin filaments and changing spatial organization of the actin cytoskeleton in 24 hours after exposure to γ–radiation lead to a softening of the FA fibroblasts membrane. This study demonstrates that the pulsed force mode for AFM combined with fluorescence microscopy opens up possibilities for investigating the mechanical properties of the FA fibroblasts membrane in relation with their cytoskeleton organization. Moreover, the study in details of the FA fibroblasts mechanical properties such as elasticity, adhesion and stiffness, cytoskeleton organization and cell shape is required to realize the accurate diagnosis.

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**Вклад авторов**

Кухаренко Л.В., Шиммель Т., Фукс Х., Барщевский М. – выполнение эксперимента по исследованию актинового цитоскелета и механических свойств фибробластов донора и пациента с анемией Фанкони с помощью атомно-силовой микроскопии. Шман Т.В., Тарасова А.В. получение и облучение фибробластов донора и пациента с анемией Фанкони, а также исследование цитоскелета фибробластов донора и пациента с анемией Фанкони с помощью флуоресцентной микроскопии. Кухаренко Л.В. –приготовленит фибробластов для проведения АСМ исследований.

**Authors contribution**

Kukharenko L.V., Shimmel Th., Fuchs H., Barczewski M. – carrying out an experiment to study the actin cytoskeleton and mechanical properties of fibroblasts of a donor and a patient with Fanconi anemia using atomic force microscopy. Shman T.V., Tarasova A.V. obtaining and irradiating fibroblasts from a donor and a patient with Fanconi's anemia, as well as studying the cytoskeleton of fibroblasts from a donor and a patient with Fanconi's anemia using fluorescence microscopy. L.V. Kukharenko - preparing the fibroblasts for AFM studies.

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