ATOMIC FORCE MICROSCOPY STUDY OF IRRADIATED FIBROBLASTS OF FANCONI ANEMIA PATIENT USING FORCE MODULATION MODE

Kukharenko L.V.[1], Schimmel T.[2], Fuchs H.[3], Barczewski M.[2], Shman T.V.[4], Tarasova A.V.[4]

[1]Belarussian State Medical University, Minsk, Belarus, lvk@europe.com
[2]Instiut für AngewandtePhysik und Centrum fürFunktionelle Nanostrukturen, Karlsruhe, Germany
[3]Westfälische Wilhelms-Universität, Münster,Germany
[4]Belarussian Center for Pediatric Oncology and Hematology, Minsk., Belarus

The atomic force microscopy (AFM) is becoming a promising method of studying fibroblasts, including both molecular level visualization of cytoplasmic submembranous structure and measuring the mechanical properties (local stiffness, hardness, elasticity) of the fibroblasts membrane. In this study both membrane surface morphological changes and membrane mechanical properties of Fanconi anemia (FA) fibroblasts occurring after exposure to γ–radiation were investigated by atomic force microscopy. The reorganization of the FA fibroblasts cytoskeleton structure after exposure to γ–radiation leads to change in the mechanical properties of cells, therefore the cell mechanical parameters can be used as certain markers of the pathology state.

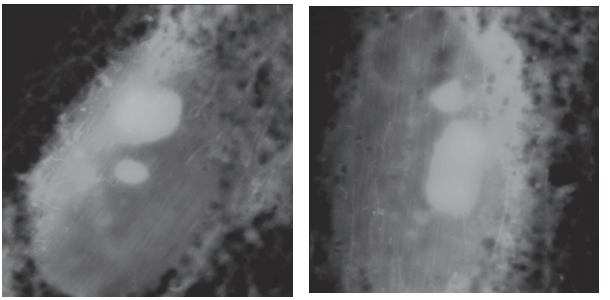
A MultiMode Nanoscope (R) IIIa AFM (Digital Instruments/Veeco) was used in all experiments. The AFM imaging was performed under ambient air conditions using tapping-mode. Silicon nitride cantilevers (NSC12/50) with a nominal force constant of 0.65 N/m (NT-MDT, Zelenograd, Russia) were used. The AFM capabilities were extended by using force modulation mode (FMM), which enables to obtain information about relative difference in fibroblasts surface elasticity with nanometer-scale resolution. In this mode, dynamic changes of both elastic and viscous properties of the FA fibroblasts cytoplasmic membrane were imaged simultaneously by detecting the membrane deformation caused by external vibration of the cantilever. The images of adhesion and stiffness 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.

Primary skin fibroblasts were obtained from Fanconi anemia patients by minimal invasive 3-mm punch biopsy. Small pieces of skin were incubated in the appropriate medium (Dulbecco's Modified Earle's minimal essential me-

dium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic in 6-well plates under the cover slides at 37˚C. Fibroblast's growth was observed after 10-14 days of cultivation. Monolayer of fibroblasts was detached using 0,25% trypsin-EDTA. To expose FA cells to ionizing radiation 50,000 fibroblast were transferred into the culture dishes with glass slides at the bottom and incubated 24 hours at 37˚C. Then several plates containing attached FA fibroblasts were exposed to γ radiation at 5 Gy, then incubated 30 minutes or 24 hours at 37˚C and fixed with 2% glutaraldehyde for 30 min. Then FA fibroblasts were washed five times in phosphate-buffered saline, dehydrated in a graded series of ethanol and air dried.

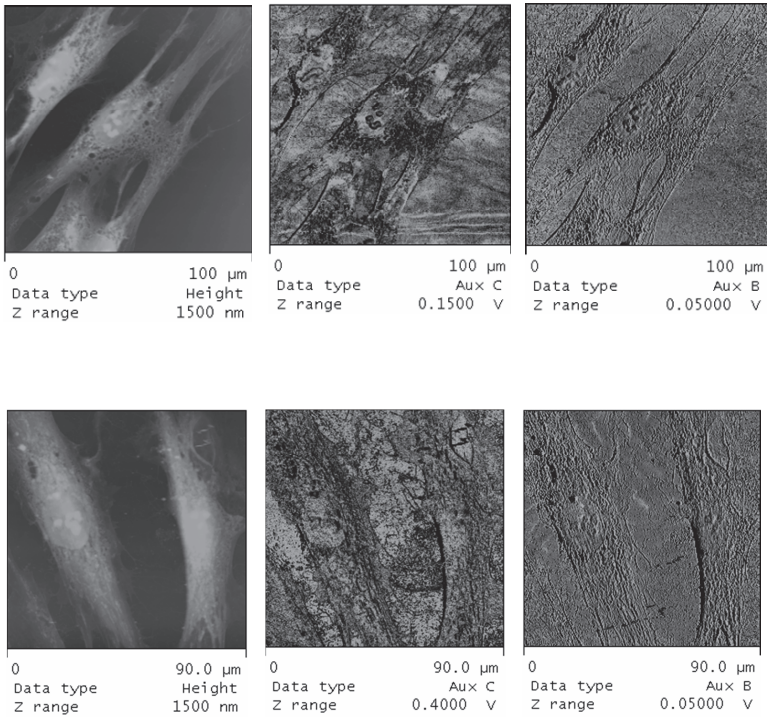
Two strains of skin fibroblasts isolated from an FA patient were evaluated for their in vitro radiosensitivity using AFM and foci immunofluorescence staining. While one set of cells left untreated (control cells), the other one was exposed to γ–radiation at 5 Gy.

The AFM images of zoomed area on the nucleus of FA fibroblast demonstrate that actin stress fibers formed one or few densely packed parallel arrays traversing the nucleus area (fig. 1(a)). The lateral size of densely packed parallel arrays of actin stress fibers varies from 30 to 200 nm. AFM images of FA fibroblasts in 30 minutes after exposure to γ–radiation at 5 Gy showed the actin filaments breaks, fragmented and disorganized actin stress fibers in irradiated FA fibroblasts (fig. 1(b)).



The topographic, adhesion and stiffness images of the FA fibroblasts before exposure to γ-radiation are presented in figure 2. Darker parts in the adhesion and stiffness images correspond to low adhesion and stiffness value on fibroblast membrane. As follows from the AFM images their nuclei are more adhesive and less rigid than the surrounding nucleus region and the peripheral

(lamellipodial) regions. The stiffest part of the control 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 in 30 minutes after exposure to γ–radiation at 5 Gy are given in figure 3. 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 (fig.3).

The AFM measurement of cellular topography, adhesion and stiffness was employed as a method of the mechanical properties investigation of the FA fibroblasts before and after exposure to γ–radiation in relation with their cytoskeleton organization.