doi:10.1017/S1431927618006670
Microsc. Microanal. 24 (Suppl 1), 2018

The ASM Study of Elastic Properties of Rat Fibroblasts, Lacking Vimentin

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Vimentin, a protein of intermediate filaments (IF) type III, is present in mesenchymal cells and is involved in many cellular processes, such as cell adhesion, migration and signalling. It participates in vesicular transport, in the anchoring of many cellular organelles, including mitochondria [1].

To investigate the relationship between the presence of vimentin IF, elastic properties and motility of cells, we utilize the model system of rat fibroblasts REF52 that are characterized by a remarkably high motility rate.

First, the rat fibroblast line REF52(Vim -/-) lacking a *vimentin* gene, using the CRISPR system Cas9, was generated. Than we analysed the migration of REF52(Vim -/-) cells, and compared it to the migration of normal REF52 cells. The average migration rate of cells lacking vimentin does not differ from the normal cells (0.6 um/min). However, using the computer program, DiPer [5], we have found that the directional persistence of migration of the cells lacking vimentin was significantly violated (Figure 1). We assume that one of the roles of vimentin IF in the cell is the stabilizing the cellular polarity. Earlier, we demonstrated that the elastic properties of cells can play a major role in their motility [2] and could be used as a marker for metastatic potential [3].

To characterize the elastic properties of the fibroblasts we measured the Young's moduli at the leading edge of migrating cells. The experiments were performed on an atomic force microscope Solver BIO Olympus (NT-MDT, Russia) combined with an inverted optical microscope. The microscope was equipped with a system of capacitive sensors (Closed-loop feedback) to compensate for nonlinearity, creep and hysteresis of piezoceramics. In experiments on power spectroscopy, the vertical displacement of the scanner was in the range between 2 to 9 um, the time per one curve was from 2 to 9 sec, and the velocity of the probe 2 um/s in all experiments. Cantilevers were modified with microspheres as described in [4].

The Young's moduli for normal REF52 cells were in the range of 1600 Pa (table 1), which was consistent with previous results for non-malignant cells [2, 4]. When measuring the Young's modulus of the REF52 (-/-) line, we found that their elasticity is higher. The cells lacking vimentin were almost 200 Pa softer than normal cells (table 1). It should be noted that Young's moduli of both REF52 and REF52 (-/-) cells were close to lognormal (Figure 2). Lognormal distribution is very common in nature, in particular in the measurement of various intracellular parameters [2].

Altogether, these results demonstrate that vimentin IP are strongly affect the fibroblasts motile state and influence cell mechanical properties [6].

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[6] The authors acknowledge funding from the Russian Science Foundation (Grant # 14-14-00234 to O.S.S.) and Russian Foundation for Basic Research (Grant # 17-04-01775 to A.A.M.).

Table 1. Values of the Young's modulus of cells

Parameters	REF52	REF52(-/-)
Mean, Pa	1910	1840
Geometric mean, Pa	1650	1540
Median, Pa	1640	1470

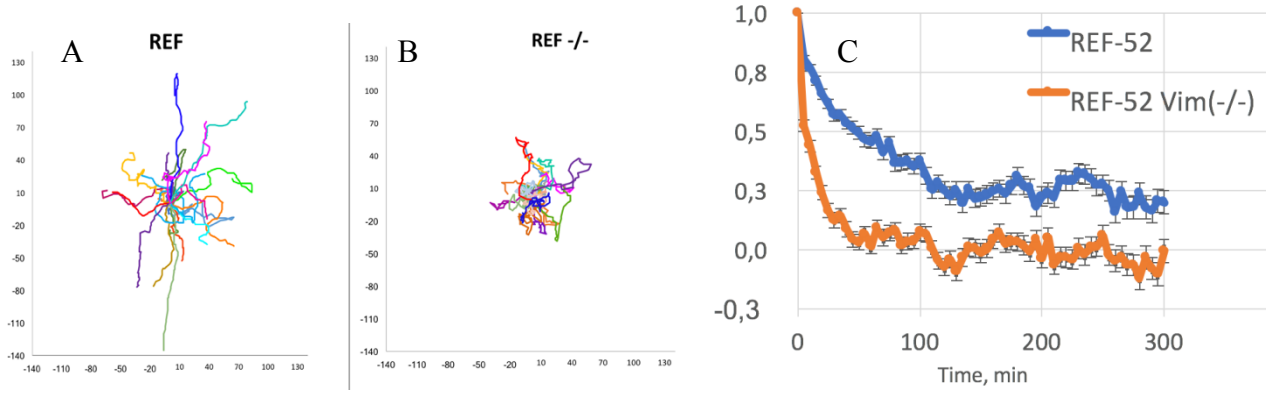

Figure 1. Trajectories of the movement of REF52 (A) and REF52(-/-) (B) cells. The starts of the movement are shifted to one point in the center. (C) Comparison of the direction autocorrelation of cellular migration.

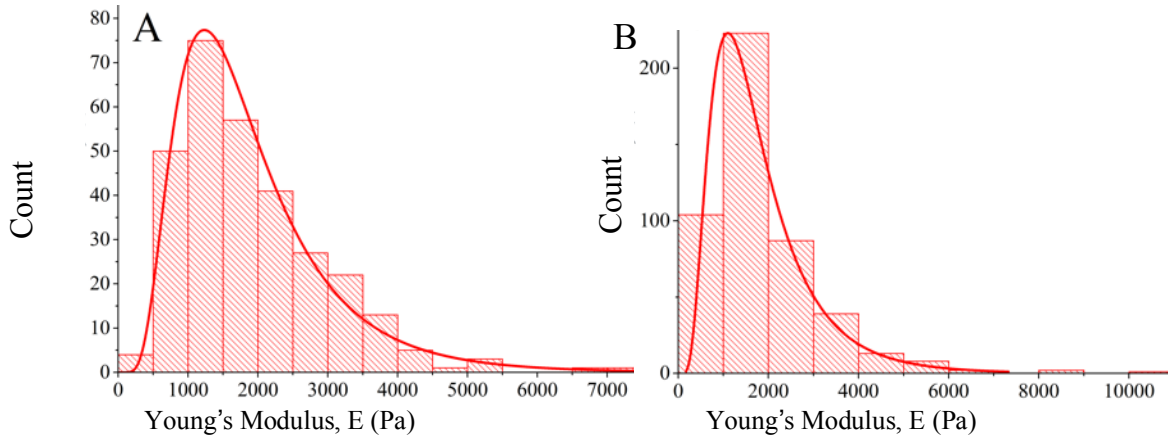

Figure 2. Histograms of the distribution of Young's moduli of cells (A) REF52 and (B) REF52(-/-). Solid line - data approximation using the lognormal distribution.

https://doi.org/10.1017/S1431927618006670 Published online by Cambridge University Press