

P3 – Changing of Cellular Mechanics During Aging

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Aging is closely correlated with drastic changes on the cellular level. Many details about aging from the molecular side of view are known, for example about changes in gene expression or the reduction of telomeres protecting DNA. How the cytoskeleton changes its structure and function and how aging alters the mechanical properties of the cell is still under investigation.

For our experiments fibroblasts from human donors differing in age were chosen. Their age lies in the range between 10 and 67 years. Initial experiments demonstrated an age-specific difference in mechano-sensitivity if cyclic stretched. Senescent cells orient faster perpendicular towards the stretching direction than cells from young donors. To correlate that age-specific difference in mechano-sensitivity to mechanical cell properties the Young-modulus of the cells was measured using atomic force microscopy (AFM). Large cell areas (100x100 μm^2) were mapped with 5x5 μm per pixel resolution to get an insight of the local cell properties. At each point a force-distant curve was acquired and the Young-modulus was calculated via Hertz fit.

A significant difference for the Young-Modulus values of young and senescent cells was found. To affirm the data given an actin assay was performed via G-actin / F-actin assay kit (Cytoskeleton). Here the total amounts of G- and F-actin were determined, indicating that senescent cells have a decreased total amount of actin. The ratio G/F actin seems to be independently from age. Further experiments were then performed with siRNA transfected cells. The siRNA was used to decrease the amount of actin aiming for the value for senescent cells. Using this method it was shown that the elasticity of the transfected cells lays in the same range as the elasticity of senescent cells.

In conclusion, our results suggest that actin expression decreases with aging and thus softening of the cells takes place. This may also explain the faster mechano-response of the older cells since a smaller amount of actin needs to be reorganised.

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Introduction

Aging is closely correlated with drastic changes on the cellular level. Many details about aging from the molecular side of view are known, for example about changes in gene expression or the reduction of telomeres protecting DNA. How the cytoskeleton changes its structure and function and how aging alters the mechanical properties of the cell is still under investigation.

For the experiments presented here fibroblasts from human donors of different age were chosen. Their age ranged from 10 to 67 years. Initial experiments demonstrated an age-specific difference in mechano-sensing, when cyclically stretched. Senescent cells orient faster perpendicular to the stretching direction than cells derived from young donors. Further on, cells orient in a different way on microstructured surfaces, so sensing and translation of forces towards the cytoskeleton must be different.



Fig 1. Examples of fibroblasts of different aged donors on microstructured surfaces.

To correlate age-specific differences in mechano-sensing to mechanical properties of the cells, the Young-modulus was measured using atomic force microscopy (AFM). As main component of the cytoskeleton, the amount of actin was determined. To study the mechano-sensing of cells with a reduced amount of actin, cells of a young donor were chosen for actin-siRNA transfection.

Materials and Methods

Atomic force microscopy (AFM)

The elasticity of cells under cell culture conditions was determined by using an atomic force microscopy (AFM). The JPK Nanoscope III was used. Force indentation curves were obtained with a conically shaped tip with full opening angles of around 38°. Cantilevers with a spring constant of around 0,09 N/m were used. Prior to the experiments cells were allowed to adhere on the glass substrates for 6 h.

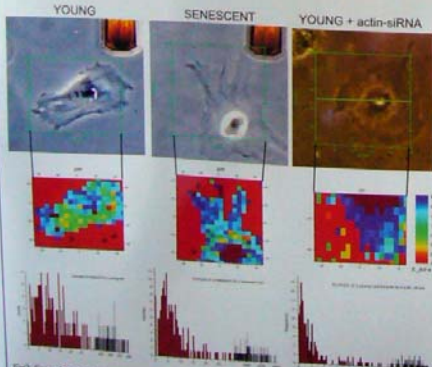


Fig2. Examples of optical calibration and force histograms and force mappings of a young, senescent and transfected (with siRNA to lower the actin amount) cell. CF each subgroup only one example was chosen.

To achieve cells similar in the elastic properties as cells derived from a senescent donor, young cells were mimicked. The amount of actin was reduced using siRNA transfection. These cells were transfected three days before the AFM measurements took place. To quantify the transfection efficiency a cotransfection with GFP was performed.

G-actin/F-actin

For all cells derived from different donors, the total amounts of G- and F-actin were determined. Therefore, around 300.000 cells were lysed and both kinds of actin were separated by ultra-centrifugation (under physiological conditions (37°C, ATP, protease inhibitor), separated and purified by SDS-PAGE. To quantify the ratio of both forms of actin an immunoblot and antibody coupling was used.

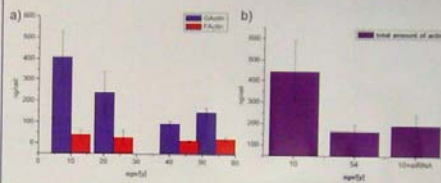


Fig 3. a) Amounts of G-actin and F-actin in cell lines of differently aged donors b) Whole amounts of actin in cells of a young donor, a senescent donor and transfected cells.

Results and Discussion

AFM experiments showed that younger cells appear softer than senescent cells, independent from the donors. The major differences were found in the cytosol. The first guess was that this may result from cellular structures like actin. The performed actin assays showed that the amount of actin decreases during aging, independent from the donors. Around one third of actin got lost. But as the percentage between G-actin and F-actin, and the value of G-actin compared to the whole amount of actin, stayed constant it might have been a change in molecular expression.

The amount of actin present in cells derived from young donors could be reduced by transfecting these cells with actin-siRNA. The actin expression level could be tuned that it mimics the amount of senescent cells. When these modified cells were probed by AFM it could be shown that elasticity values were in the same regime as those of senescent cells.

Next to that, observing the reaction of cells, when mechanically stressed by cyclic stretching, it was shown that senescent cells orient faster perpendicular to the stretching direction than cells derived from a younger donor. In this experiment siRNA transfected cells showed a behavior similar to that of cells derived from a senescent donor.

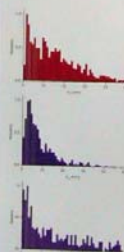


Fig4. Normalized frequency counts of elasticity of donor groups of young and senescent cells and young cells, transfected with actin-siRNA.

Outlook

To see whether the cells that are transfected with siRNA react the same in response to mechanical stress, transfected cells will be cyclically stretched using the same show, as described before, that senescent cells orient faster perpendicular to the stretching direction as cells from young donors do.

To provide a basis for the results from a translational perspective of view a genetic screening using superarrays will take place. Moreover, the changes in morphology (differential interference-contrast) will be performed to observe the cytoskeleton, focal adhesions as well as changes in formfactors like convexity and roundness.

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