Fatty tissue as a modulator of cancer cell mechanics

Eliane Blauth[1], Hans Kubitschke[1], Benjamin Wolf[2], Peter Kovacs[2], Matthias Blüher[3,4], Nadja Dornhöfer[2], Bahriye Aktas[2] and Josef A. Käs[1]

[1]Peter Debye Institute for Soft Matter Physics, Leipzig University, Leipzig, Germany
[2]Department of Gynecology, Women's and Children's Centre, University Hospital Leipzig, Leipzig, Germany
[3]Medical Department III - Endocrinology, Nephrology, Rheumatology, University of Leipzig Medical Center, Leipzig
[4]Helmholtz Institute for Metabolism, Obesity and Vascular Research, Helmholtz Munich, Leipzig, Germany

ABSTRACT

The viscoelastic properties of cells are an essential physical parameter in many biological processes. A crucial example is the softening of cancer cells during the metastatic cascade. The drivers behind the change in cell mechanics are still not fully understood and the mechanical properties of the substrate, the ECM, and crosstalk with other cells often influence measurements of cell mechanics.We used the optical stretcher (OS), a dual laser beam trap, to measure the active and passive viscoelastic properties of cancer cells in suspension. We compare cancerous cells with and without co-culturing them with adipose tissue cells. With this assay, we can investigate the impact of the cellular crosstalk between the cancerous and adipose tissue cells on the physical properties of cells, thereby disentangling it from any substrate effects. Our goal is to understand how cancer cells are able to migrate through soft fatty tissue and what mechanical properties are essential during this process.

Keywords: Cell mechanics, Physics of cancer, Optical Stretcher

1. INTRODUCTION

The crucial role of the tumor microenvironment has been brought more and more in the focus of research in the last few years. While already a lot is known about the influence of the extracellular matrix (ECM) and cell-cell interaction on cancer progression and the mechanics of cells [1]–[7], the role of other connective tissues, such as fatty tissues, remains mostly unclear. Recent studies showed that cancer cells can activate adipocytes to release triacylglycerol via lipolysis. The cancer cell absorb these free fatty acids and start to accumulate lipid droplets [8]. They further adapt their metabolism to predominantly mitochondrial fatty acid oxidation when needed [9]–[12]. This metabolic switch promotes tumor invasion [11]. However, how these metabolic changes affect cancer cells from a biophysical point of view is still under investigation. Here we try to shed light on this point and investigate the mechanical properties of cancer cells when they grow in the vicinity of fatty tissue. Therefore, we use two cervical cancer cell lines (HeLa (adenocarcinoma) and CaSki (squamous cell carcinoma)) and co-culture them with different primary adipose tissues, which we obtain directly from surgery. While CaSki cells are considered to have a predominantly epithelial phenotype, HeLa cells display a more mesenchymal behavior.

2. RESULTS

2.1 The optical stretcher: a suitable tool to measure active and passive cell properties

We measure their mechanical properties with the optical stretcher (OS), a dual laser trap, that can assess the cell mechanics of single suspended cells. This provides the advantage that substrate effects do not influence the cells and we can measure them in their "ground state". We recently showed that cells can behave differently in the optical stretcher, depending on their morphology and thus dominant contractility mode **[13]**. Mesenchymal cells typically behave like a classical viscoelastic material, while epithelial cells show a clear distinct, more active, behavior. The external force stimulates the cells, which in turn start to contract themselves. This results in a first increasing but then decreasing deformation curve. We attribute this behavior to a strong contraction of the actin cortex and name the related cell property thus cortical contractility [13]. To extract different passive (elasticity and viscosity) and active (pretension and cortical contractility) mechanical cell properties from the deformation curves, we fit a standard linear solid model (SLS) to them.

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2.2 Fatty tissue softens epithelial cells with predominantly mesenchymal phenotype

The phenotypical mesenchymal HeLa cells become softer in each individual experiment that we performed. We observe significant changes in the passive and active cell properties. The elasticity decreases while the viscosity increases after being cocultured with fatty tissue. Both active properties, pretension and cortical contractility, decrease in co-cultured cells. In three out of four cases paracolic adipose tissue influenced the HeLa cells less than adipose tissue from the abdominal region. However, we observe drastic changes in one measurement, where the HeLa cells become highly deformable. To investigate what might be causing this difference we correlated our findings with the clinical and pathological records of the patients from which we received the four paracolic fatty tissue samples. It should be noted that we obtain all paracolic fatty tissues samples from cervical cancer patients undergoing primary surgical treatment. Interestingly, the patient whose adipose tissue induced the largest changes had an adenocarcinoma, while the other three had squamous cell carcinoma. How exactly this influences the mechanics of cancer cells needs further investigation. Still, all used fatty tissues influenced the HeLa cells in the same direction. They became softer and had lower cortical contractility, suggesting a higher invasive potential **[13], [14]**.

2.3 Fatty tissues change the mechanics of epithelial cells with predominantly epithelial phenotype

As a second cervical cancer cell line we used the epithelial CaSki cells. As already mentioned, epithelial cells show a distinct behavior in the OS compared to mesenchymal cells. When co-cultured with primary adipose tissue the cells become less deformable in 6 out of 9 measurements. The cells increase their elasticity and viscosity while their cortical contractility changes only slightly. In 3 of the 9 measurements the epithelial CaSki cells changed their mechanics in the opposite direction and become softer (see figure 2). As we did not change any steps in our coculture protocol or in the OS measurement, we speculate that these changes originate from the primary fatty tissues. Further investigations are needed here to find the reason for these changes.

3. CONCLUSION

Our experiments with two cocultured cervical cancer cell lines demonstrate that fatty tissue does not only influence cells on a molecular and metabolic level, but also on a mechanical one. Phenotypically mesenchymal HeLa cells become softer which suggests that fatty tissue acts here as a tumor promoter. For the epithelial, cancerous CaSki cells we observe mostly opposing behavior. Single cells become stiffer, which points towards a tumor suppressing role of fatty tissue in this case. However, some fatty tissue samples changed the mechanical properties in the other direction, making the cells softer. As we used primary fatty tissue samples for our measurements, we have a high sample to sample variability. Future research should focus on this variability to find out what features are important to switch the mechanical properties of cancer cells in a such a drastic way.

Figures and captions

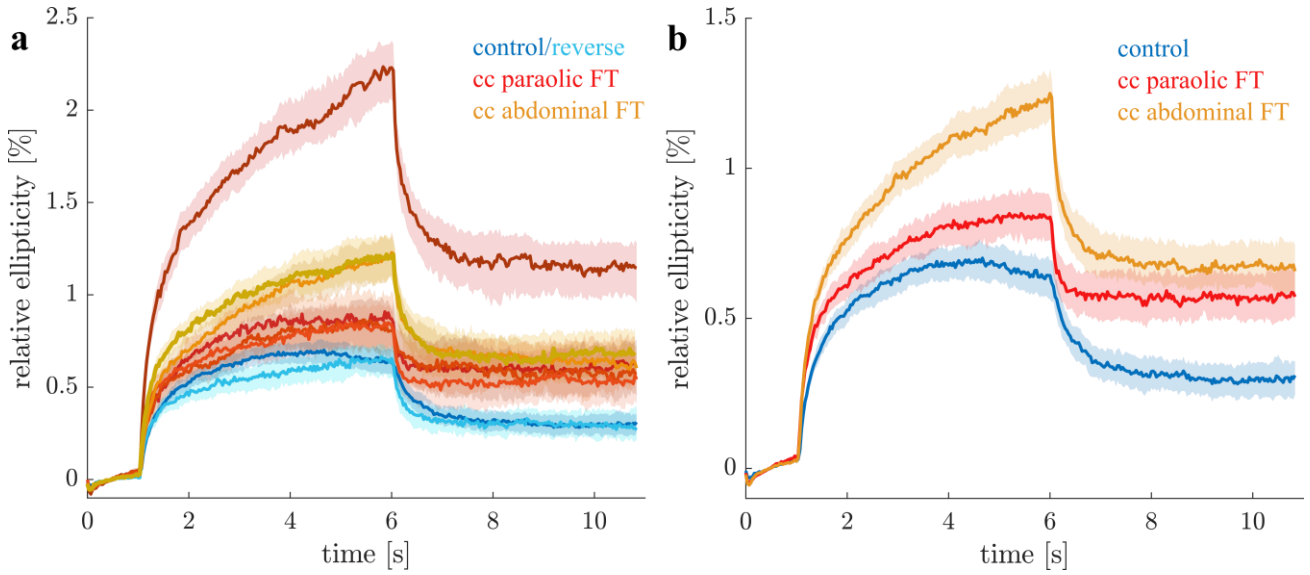

Figure 1. HeLa cervical cancer cells change their active and passive viscoelastic properties after being co-cultured (cc) with primary fatty tissue (FT). **a)** deformation curves of each individual experiment. All experiments with paracolic fatty tissue are color-coded in red. One paracolic fatty tissue has much more drastic effects on the HeLa cells than the others. Measurements with abdominal fatty tissues are color-coded in yellow. The dark blue curve shows that the effect is reversible when the cells are cultured again without fatty tissue. **b)** Co-cultured phenotypically mesenchymal HeLa cells become softer and less contractile. The three paracolic measurements and the two abdominal ones are merged here in a single curve for further analysis. Control n = 3040 (4 independent experiments), coculture with paracolic fatty tissue n = 2257 (3 independent experiments), coculture with abdominal fatty tissue n = 2153 (two independent experiments).

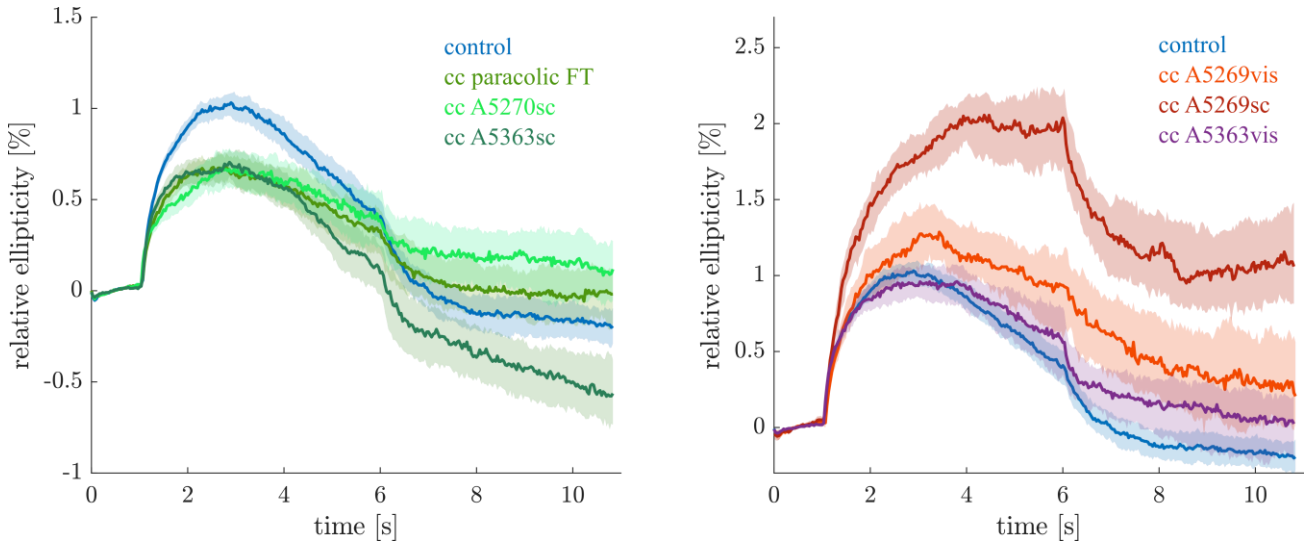

Figure 2: Fatty tissues have different effects on epithelial CaSki cervical cancer cells. **a)** Most CaSki cells become less deformable after being co-cultured (cc) with primary fatty tissue (FT). Especially all experiments with paracolic fatty tissue show the same effect on CaSki cells. Control n = 2852, co-culture with paracolic fatty tissue n = 1721, co-culture with A5270sc n = 864, co-culture with A5363sc n = 1147. **b)** Some fatty tissues change the mechanics of epithelial CaSki cells in the opposite direction. Fatty tissue samples A5269 viscerale (n = 627) and subcutaneous (n = 572), which originate from the same patient, show drastic differences. Sample A5363 viscerale (n = 844) induces only slight changes.

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