Role of integrin adhesions in cellular mechanotransduction

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Summary

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The ability of cells to translate between extracellular mechanical cues and intracellular signals is called cellular mechanotransduction. Cellular mechanotransduction plays an important role in cell survival, differentiation, migration and cancer progression. Mechanotransduction consists of inside-out and outside-in signaling. Outside-in signaling consists of cells sensing the mechanical cues from the environment and regulating intracellular signaling pathways accordingly. Whereas inside-out signaling is cells changing the mechanical properties of their environment through application of traction forces or secretion of extracellular material. Through these mechanisms cells are able to maintain a dynamic mechanical equilibrium with their environment. The misregulation of this equilibrium is observed frequently in pathologies such as cancer and fibrosis.

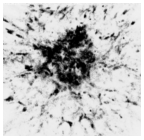
Integrin transmembrane proteins physically connect the extracellular matrix to intracellular multimolecular complexes called cell-matrix adhesions. Cell-matrix adhesions and associated proteins have been widely studied in close relation to cellular mechanotransduction. Some of these proteins take part in intracellular-extracellular force transduction by connecting integrins to the actin cytoskeleton and others respond to mechanical stress. These proteins change their activities or open cryptic interaction sites and hence induce mechanotransduction by changing their chemical activities upon physical stimuli. In this thesis I worked on the role of cellular mechanotransduction in cancer and proteins that affect mechanotransduction. I specifically focused on integrins and other integrin associated proteins.

In **chapter 2** my findings on role of mechanotransduction in cancer progression are shown. This was studied *in vitro* by microprinting tumor

cells to form spheroids (tumoroids) in 3D collagen gel and monitoring both tumor expansion and collagen organization over a course of several days. You may observe the expansion of such a tumoroid at the left bottom of the even pages of this thesis. The studies where I used more than 20 different tumor cell lines indicated that the ability to expand and locally invade into the collagen correlated with an ability to remotely reorganize the surrounding collagen. Remote organization of collagen was observed up to five times the tumoroid expansion area. Vascular cells that were printed in these areas of collagen organization showed directional migration towards the tumoroid. Ablation of the physical contact between the tumoroid and the oriented collagen network abolished this response. In conclusion, in this chapter I have shown that the physical communication of tumor cells with their environment can affect both tumor expansion and tumor angiogenesis.

In **chapter 3** the effect of integrin expression profiles on cellular mechanotransduction is studied. Cells binding to the extracellular matrix protein fibronectin showed differential inside-out and outside-in mechanotransduction depending on the predominant receptor being $\alpha5\beta1$ or $\alpha v\beta3$. Expression of the latter integrin emerges during active cellular processes, e.g. on endothelial cells during angiogenesis. Even though cells were able to apply traction forces of similar magnitude and increase their areas comparably with increasing environment stiffness, cells that expressed higher levels of $\alpha5\beta1$ prominently showed longer actin fibers and a higher fraction of centripetally oriented forces. Upon treatment with inhibitors that resulted in reduced actin fiber length, the force orientation was also reduced, which resembled the phenotype of the cells expressing higher levels of $\alpha v\beta3$. In contrast, cells binding to fibronectin mainly through $\alpha v\beta3$ showed more effective actin cytoskeleton reorganization upon application of extracellular forces as well as cell-matrix adhesion formation on softer substrates.

In **chapter 4** the relationship between the amounts of cell-matrix adhesion proteins with the applied forces is studied. In previous studies several proteins that affect cellular traction forces have been identified, but to my knowledge there are no studies on the abundance of these proteins in the adhesions. In this chapter, studies on talin, vinculin, paxillin and focal adhesion kinase are shown. These proteins have been shown to be important for integrin-actin cytoskeleton connections and biochemical signals controlling the actin cytoskeleton. These proteins



were immunostained, and studied in nanoscale resolution with super resolution techniques. From these high-resolution images, the interlocalization spacing was statistically analyzed and information with regard to number of molecules in a designated area was obtained. Using this technique, the abundance of proteins in cell-matrix adhesions was calculated. By combining this technology with traction force microscopy, this information was related to local forces applied by these adhesions on substrates of varying rigidities. No relation between the number of focal adhesion kinase molecules and force application was observed. However, higher numbers of talin, vinculin and paxillin molecules were related with larger forces. An increase of ∼60 pN on a substrate of ∼50 kPa was related with recruitment of 1 talin, 2 vinculin and 2 paxillin molecules in the adhesion. On a substrate of four times lower stiffness, the same increase in force was associated with 2 talin, 12 vinculin and 6 paxillin molecules. Here, the marked change in vinculin recruitment points to a stiffness-dependent switch in function. In conclusion, in this chapter the relation of the local abundance of cell matrix adhesion proteins and force applied by that adhesion is shown for the first time.

In **chapter 5**, studies on proteins that affect cancer cell migration, cell-matrix adhesions and cellular force application are shown. For this purpose, siRNA knockdowns were performed and the resulting effects on breast cancer cells were studied. More than 200 proteins were identified to influence cell-matrix adhesion dynamics and size. The most effective 64 proteins were tested for their effect on cell migration and 11 were shown to significantly influence cell migration. Finally, four of these proteins, encoded by genes *TPM1*, *PPP1R12B*, *HIPK3* and *RAC2*, were further studied for their role in cellular force application. Silencing *PPP1R12B*, *HIPK3* and *RAC2* resulted in increased cellular force application and reduced force turnover at adhesions. Silencing of *TPM1* did not significantly affect cellular forces. Taken together, genes encoding proteins that regulate cancer cell migration, cell-matrix adhesions and cellular force application have been identified.

Overall, the work described in this thesis unravels the role of cellular mechanotransduction in different aspects of cancer progression and reveals how the molecular composition of cell-matrix adhesions relates to traction force generation.