Summary
Muscle LIM Protein and Nesprin-1 in Mechanotransduction

Striated muscle cells are constantly confronted with differing physical stimuli like changes in the stiffness of their surroundings, and stretch because of the movement of the muscles. Physical stimuli are translated into a biochemical signal by mechanotransduction. If mechanotransduction is disturbed in muscle cells and their precursors, cardiac or skeletal muscle diseases may develop. In this thesis, I studied three proteins that are participating in two different pathways of mechanotransduction.

Muscle LIM Protein (MLP) is a small striated muscle specific cytoplasmic protein. When cardiomyocytes in 2D cell culture are stretched, MLP shuttles to the nucleus. Without shuttling MLP, isolated cardiomyocytes fail to respond to the stretch stimulus. Although several interaction partners of MLP are known, its overall function is not completely understood. Human patients with mutations in the gene coding for MLP develop cardiomyopathies and have a high risk of sudden cardiac death. Mice with a functional knock-out of MLP develop a phenotype similar to dilated cardiomyopathy in humans.
I wanted to elucidate the role of MLP in these cardiomyopathies by expressing mutated MLP in isolated neonatal cardiomyocytes of mice without endogenous MLP ($MLP^{-/-}$ mice). I established cell culture of freshly isolated neonatal cardiomyocytes in 2D and 3D culture conditions and prepared viruses to transduce the isolated cardiomyocytes with mutated MLP. Surprisingly, I found that in 3D cultures of cardiomyocytes, MLP did not shuttle to the nucleus after stretching of the cells. Although I could not solve this issue during the time given, I prepared the setup for subsequent experiments in 2D.

Nesprin proteins, together with SUN proteins, form a nuclear envelope-spanning protein complex, the LINC complex. Inside the nucleus, the LINC complex interacts with Emerin and Lamin proteins. Outside the nucleus, it binds different parts of the cytoskeleton. Thus, information can be carried from the cytoskeleton directly into the nucleus. A patient with congenital muscular dystrophy harboring a nonsense mutation in the gene coding for Nesprin-1 should express a truncated protein Nesprin1-ΔKASH lacking the SUN binding domain, probably disturbing the LINC complex.
Nesprin1-ΔKASH was not present in isolated myoblasts from this patient. These cells displayed deformed nuclei and had defects in mechanosensitive responses similar to myoblasts from a second patient with congenital muscular dystrophy who lacks aminoacid K32 in A-type lamins (LMNA- ΔK32).
When the cells were cultured on soft tissue culture dishes that resemble the stiffness of muscle fibers, both patient cell lines displayed an elevated level of stress fibers and focal adhesions, and the cells spread further than WT myoblasts in these conditions. I present data that this was not due to MLC kinase but because of activity of ROCK and SRC. A knockdown of the formin FHOD, a downstream target of ROCK and SRC, reduced the phenotype of ectopic stress fiber formation in the mutant cell lines.

While it is hypothesized that mutations in Nesprin and Lamin proteins lead to a mechanical instability of the nuclear envelope, these results indicate that signalling pathways through the nuclear envelope are disturbed as well.