

8. Summary

Nesprins (Nuclear envelope spectrin repeat proteins) are type II transmembrane proteins that localize to the nuclear envelope. Like other proteins, that are part of the LINC (Linker of Nucleus and Cytoskeleton) complex, they are involved in cancer and their loss leads to diseases termed laminopathies. Examples for laminopathies are Emery-Dreifuss muscular dystrophy and dilated cardiomyopathy. In Nesprin-2, recently a SMC-domain was found which had high sequence homology to SMC proteins. SMC proteins are essential components of condensin and cohesin, which associate with chromosomes and have important roles in mitosis. In this study, we identified SMC2 and SMC4 as novel interaction partners of Nesprin-2. SMC2 and SMC4 proteins are the core of the condensin complexes, which are needed for condensation and chromatid disentanglement in mitosis. Volume measurements revealed that the volume of chromosomes is significantly increased upon knock down of Nesprin-2. Furthermore, the number of chromatin bridges was increased in Nesprin-2 knockdown cells. Taken together, these results hint at a connection between Nesprin-2 and the function of SMC proteins in chromosome condensation. Since Nesprins have never been described in the context of mitosis these results expand the repertoire of Nesprin-2 functions.

Until now, Nesprin-2 knockdown mice have been reported in which the N-terminal actin binding domain or the KASH-domain of Nesprin-2 were targeted leading to loss of some but not all Nesprin-2 isoforms. These mice were viable and healthy. Here, an inducible N- and C-terminal knockdown using shRNAs was performed. However, the shRNA production occurred already without induction and therefore was not anymore conditional. This knockdown was embryonic lethal. Embryos, that were genotyped positive for the knockdown, were underdeveloped when compared to their wild type siblings. To characterize the knockdown, mouse embryonic fibroblasts were isolated from knockdown and wild type embryos. The comparison of knockdown and wild type fibroblasts showed that the sizes of the nuclei were increased in knockdown fibroblasts and they also exhibited more DNA-damage, their migration speed was reduced and several proteins such as NUP107, Lamin A/C, SUN2 and LAP2 displayed lower levels. This might explain why the loss of Nesprins in humans leads to laminopathies and might contribute to a better understanding of the various functions of Nesprin-2.