SUMMARY

Osteoporosis is a common disorder affecting more than 200 million people worldwide. With rising treatment costs and increasing socioeconomic issues, disease prevention and effective therapy options become more important by the day. As of today, almost three-quarter of elderly people worldwide are affected by low bone mass and increased fracture risk. Interestingly, genetic predisposition was shown to be relevant in 60 – 80 % of osteoporosis patients.

Mutations in plastin 3 (PLS3) cause X-linked primary osteoporosis with fractures in men and a variable phenotype, ranging from unaffected to mild fractures, in women. Additionally, a single nucleotide polymorphism (SNP) in PLS3 shows the strongest association for the increased risk of developing postmenopausal osteoporosis in women up to date. PLS3 is an F-actin bundling and binding protein which is overexpressed in around 5% of the general population, with yet unknown consequences. Furthermore, PLS3 is a protective modifier of motor neuron disorder spinal muscular atrophy (SMA) when overexpressed. Hitherto, the role of PLS3 in bone homeostasis and its implications in osteoporosis remain elusive. However, PLS3 is expressed in all essential cell types in bone, namely osteoblasts, osteocytes and osteoclasts. Remarkably, morpholino-mediated pls3 knock-down in zebrafish causes a malformed craniofacial morphology, especially present in the jaw. Additional overexpression of human PLS3 mRNA in zebrafish was able to rescue the phenotype, emphasizing the role of PLS3 in bone development.

This thesis aims to unravel the function of PLS3 in skeletal health by studying genetically engineered mice in which PIs3 has been ubiquitously knocked-out (KO) or a human PLS3 transgene is overexpressed (OE). Hence, the following research questions were applied: 1) What is the pathophysiological effect on bone morphology and strength in PIs3 KO and PLS3 OE animals? 2) Which bone cell type is functionally or morphologically affected by PIs3 KO and PLS3 OE? 3) What is the underlying molecular mechanism causing the observed phenotype? 4) Which other bone related pathways might be affected by the loss or overexpression of PLS3?

Here, we examined bone morphology in ubiquitous PIs3 KO mice by micro-CT and 3-point-bending-test. PIs3 KO causes an osteoporotic phenotype, which was more pronounced in males than females, resembling the human phenotype. Contrarily, ubiquitous PLS3 OE mice exhibit an increased cortical thickness in males and females as well as augmented bone strength in females. Immunohistological stainings of femoral sections revealed that osteoclast numbers were significantly reduced in 5-day-old PLS3 OE animals but restored in 3-month-old mice. Osteoclast number was unaffected in PIs3 KO mice and early mineralization as well as chondrocyte differentiation was unaffected in both mouse models. Thus, osteoclast function was further examined in primary osteoclast cultures, which were differentiated from femoral
bone marrow of 3-month-old mice, and displayed an increase in resorptive activity in Pls3 KO and a decrease in PLS3 OE mice. Furthermore, immunofluorescence stainings of osteoclast cultures revealed that podosome structures in osteoclasts of both Pls3 KO and PLS3 OE was affected, presenting rather podosome clusters than rings. Interestingly, PLS3 OE osteoclasts were almost devoid of any podosome structures at all. To unravel the molecular mechanism underlying osteoclast dysfunctions, essential protein players of the NFκB pathway, essential in osteoclast differentiation, were investigated by western blotting of extracted proteins from primary osteoclast cultures. Remarkably, RELA (NFκB subunit p65) was highly increased in PLS3 OE osteoclasts, but unchanged in Pls3 KO. Concomitantly, NFκB repressing factor (NKRF) was found to be a novel interaction partner of PLS3 in mass-spectrometry as well as co-immunoprecipitation experiments. Interestingly, we demonstrated by quantitative real-time PCR that mRNA expression of master regulator of osteoclastogenesis nuclear factor of activated T cells cytosplasmic 1 (Nfatc1) was downregulated in PLS3 OE and upregulated in Pls3 KO osteoclasts. Immunofluorescence stainings in cell cultures revealed that this change was correlating with the nuclear translocation of NKRF. Precisely, NKRF nuclear localization was significantly diminished in Pls3 KO osteoclasts thereby causing increased Nfatc1 expression. Contrarily, decreased Nfatc1 mRNA levels were caused upon increased NKRF nuclear translocation in PLS3 OE osteoclasts. Thus, we hypothesized that transcriptional repression of Nfatc1 by PLS3-mediated NKRF translocation modifies osteoclast function, thereby causing either osteoporosis in Pls3 KO or an augmented bone phenotype in PLS3 OE. Taken together, PLS3 is a novel and crucial regulator of osteoclast function through alterations in the NFκB-NKRF-NFATC1 pathway.

Finally, transcriptome analysis of primary osteoclasts from male and female 3-month-old Pls3 KO and PLS3 OE mice was studied to unravel differentially expressed genes. We discovered changes in several candidate genes involved in the development of osteoporosis as well as osteoarthritis. Furthermore, differentially expressed genes were found pointing at a potential dysregulation of intracellular trafficking in Pls3 KO and PLS3 OE osteoclasts possibly contributing to the bone phenotype. While these are preliminary data, an in depth analysis is required in future to better understand the entire interactome of PLS3 and its impact on bone homeostasis.