Abstract

Specific interactions of annexins AnxA5 and AnxA6 with collagen X may be crucial for the initiation of matrix vesicle mediated mineralisation within the growth plate cartilage. However, the *in vivo* proof is still pending. Skeletal development appears to be normal in single deficient AnxA5 and AnxA6 mice suggesting that both annexins may fulfil redundant functions due to their highly conserved functional and structural similarities. To study the relevance of annexin-collagen interactions *in vivo* we now generated *Anxa5*$^{(-/-)}$/*Anxa6*$^{(-/-)}$ and *Anxa5*$^{(-/-)}$/*Anxa6*$^{(-/-)}$/*Col10*$^{(-/-)}$ deficient mice and analysed the skeletal development. Surprisingly, mutant mice were viable, fertile and showed no obvious abnormalities. Assessment of the growth plates architecture by histological, immunofluorescence and ultrastructural analysis indicated that endochondral ossification and mineralisation was not affected in newborn, 13, 5 days and 1 month old mutant mice. However, *Col10*$^{(-/-)}$ and *Anxa5*$^{(-/-)}$/*Anxa6*$^{(-/-)}$/*Col10*$^{(-/-)}$ newborns displayed an enlarged prehypertrophic/hypertrophic growth plate zone compared to wildtype controls. In peripheral quantitative computed tomography studies no changes in the degree of biomineralisation were found in femora of 1 month and 1 year old mutants, but the endosteal and periosteal circumference of the middiaphysial cortical bone was significantly decreased in 1 month old *Col10*$^{(-/-)}$ and *Anxa5*$^{(-/-)}$/*Anxa6*$^{(-/-)}$/*Col10*$^{(-/-)}$ mice. This effect became even more pronounced in 1 year old animals. In these mice changes in lymphocyte differentiation within the bone marrow were seen. The amount of IgM+/IgD+ B-cells was increased in bone marrow and spleen of *Col10*$^{(-/-)}$ and *Anxa5*$^{(-/-)}$/*Anxa6*$^{(-/-)}$/*Col10*$^{(-/-)}$ mice. Moreover the number of CD4+ and CD8+ spleenocytes was increased in collagen X deficient mice. Hence, genetic ablation of AnxA5 and AnxA6 in mice has no major impact on mineralisation and cartilage maturation whereas collagen X promotes endochondral ossification and hematopoiesis. These results challenge the concept of annexin-collagen X mediated biomineralisation *in vivo* and demonstrate the importance of collagen X for skeletal development.