Abstract

C-Type Lectin Domain Family 3 Member A (CLEC3A) is a cartilage-specific protein found in both articular cartilage and the growth plate. Due to its ability to accelerate tissue-type plasminogen activator-mediated activation of plasminogen, CLEC3A may play a crucial role in tissue remodeling and endochondral ossification. Recent *in vitro* experiments revealed that exogenous CLEC3A stimulates osteoblastogenesis of mesenchymal stem cells and promotes chondrogenesis. To elucidate its underlying physiological function, in this study CRISPR/Cas9-mediated CLEC3A knockout (KO) mice were generated. Conducted studies focused on examinations of the general and skeletal phenotype of mice with an age of six weeks and six months. In addition, cartilage extracts from the femora of newborn mice were examined by proteomic analysis. When compared with wild-typ (WT), KO mice showed no abnormalities regarding their behavior and general phenotype. Female KO mice with an age of six weeks, exhibited a slightly reduction in length of the femur and cortex area. As a result, bones withstood lower force until fracture and showed lower stiffness when compared to the WT. This effects, however, normalized with increasing age of the mice. CLEC3A was confirmed to be present in the growth plate of knee joints and sternum and articular cartilage. However, expression decreased with increasing age. In male mice, KO of CLEC3A did not result in differences in morphology of the growth plate and articular cartilage. Furthermore, no increase in susceptibility for osteoarthritis was detected in CLEC3A KO mice. CLEC3A KO mice showed an increased extractability of tissue-type plasminogen activator, but proteomic analysis revealed no changes in composition of the cartilage extracellular matrix. In summary, in female mice at an age of six weeks KO of CLEC3A led to a minor growth impairment of the femur which became compensated by increasing age. When combined with the observed decrease in expression of the protein with increasing age, the conducted experiments support the assumption, that CLEC3A plays a role in skeletal development.

In context of the Severe Acute Respiratory Syndrome Coronavirus Type 2 (SARS-CoV-2) pandemic, first evidence indicated that vitamin D may positively influence the progression of Coronavirus Disease 2019 (COVID-19). This effect was assumed to be related to an increased expression of the antimicrobial peptide (AMP) LL-37. An antiviral effect of AMPs is often mediated by binding to viral surface proteins. Therefore, within this study binding of LL-37 to recombinantly expressed SARS-CoV-2 spike glycoprotein (Spike) and open reading frame 8 protein (ORF8) was investigated. Using surface plasmon resonance spectroscopy, and co-affinity purification it is demonstrated that LL-37 binds to Spike and that *in vitro* LL-37 can inhibit its binding to the cellular receptor human Angiotensin Converting Enzyme 2 (hACE2). By carrying

out electron microscopy it was confirmed that up to seven LL-37 molecules can bind to Spike. In addition, it was shown that two LL-37 molecules were also able to bind to ORF8. Since ORF8 is associated with efficient pathogen transmission, binding of LL-37 to ORF8 could attenuate its effect, possibly leading to a reduced infectivity of SARS-CoV-2 and a less severe course of COVID-19.

The CLEC3A-based AMPs Ex1 and Ex12 show structural similarity to LL-37. For this reason, Ex1 and Ex12 were also studied regarding its binding capacity to Spike and ORF8 using surface plasmon resonance spectroscopy and electron microscopy. Both proteins were able to bind to Spike. In addition, using electron microscopy it is demonstrated that several molecules of Ex1 and Ex12 bind to Spike. Comparison of the results with the control peptides gives first clues about the underlying binding mechanism. Apparently, the positive charge of the peptides is essential for binding to Spike and the additional amphipathic alpha-helix of Ex12 seems to positively influence its binding properties. The binding affinities for binding of Ex1 or Ex12 with Spike are comparable to the affinity of LL-37 and Spike. For LL-37, it is demonstrated that the binding of LL-37 to Spike is strong enough to inhibit binding of Spike to hACE2. Thus, it can be assumed that Ex1 and Ex12 are also able to inhibit the binding of Spike to hACE2. Furthermore, Ex1 and Ex12 were also able to bind to ORF8. Since Spike and ORF8 are highly glycosylated proteins, binding of the positively charged peptides to the glycans of Spike and ORF8 is conceivable. Since many viral proteins are highly glycosylated, binding of AMPs to glycans of viral proteins are discussed as a general mechanism of action for antiviral properties of AMPs.