C-type lectin domain family 3 member A (CLEC3A) is a poorly characterized protein belonging to the C-type lectin superfamily. According to its domain structure CLEC3A was assigned to the tetranectin IX group, together with tetranectin (CLEC3B) and stem cell growth factor (SCGF). CLEC3A carries a N-terminal positively charged sequence, followed by α-helical potential oligomerization domain and a C-terminal globular carbohydrate recognition domäne (CTLD/CRD). The aim of this study was to analyze the structure, tissue distribution and function of CLEC3A.

Recent structure analysis were mainly based on gene sequence analysis of CLEC3A. It was predicted that CLEC3A is able to form mono-, di-, or trimers due to an α-helical coiled-coil structure. Here, we recombinantly expressed murine and human full-length CLEC3A as well as truncated forms of CLEC3A in HEK-293-EBNA cells and generated a specific antiserum against CLEC3A full-length protein. Wecompared the properties of the recombinant CLEC3A protein with those of CLEC3A extracted from cartilage; analyzed the structure of CLEC3A by SDS-PAGE, immunoblot, matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI), native-PAGE, composite/agarose gelelectrophoresis, size-exclusion chromatography, circular dichroism spectroscopy, and electron microscopy. We found that CLEC3A mainly occurs as a monomer, but also forms dimers and trimers, potentially via a coiled-coil -helixand tends to oligomerize to form higher aggregates. Sequence analysis predicted two potential O-linked glycosylation sides for CLEC3A. Enzymatic analysis showed that CLEC3A can be N-terminal modified with chondroitin/dermatan sulfate side chains. So far, tissue distribution of CLEC3A was only investigated by northern-blot and RT-PCR experiments. For a systematic study we investigated CLEC3A tissue distribution and extracellular assembly by RT-PCR on organ extracts, immunohistochemistry and immunofluorescence microscopy. On protein levels we could show a cartilage specific expression of CLEC3A. We showed that CLEC3A is present in resting, proliferating, and hypertrophic growth-plate cartilage and assembled into an extended extracellular network in cultures of rat chondrosarcoma cells. RT-PCR analysis indicated also an ectopic expression of CLEC3A in some other organs, these results however could not be confirmed on protein levels.

Although the exact biological function of CLEC3A is not known. Recent results showed that CLEC3A promotes tumor cell adhesion onto a laminin or fibronectin substrate. We could show that CLEC3A is involved in promoting cell adhesion and forming fokal adhesion sides on rat chondrosarcoma cells. It is also known that CLEC3A closest

homologue tetranectin binds to the kringle 4 domain of plasminogen and enhances its association with tissue plasminogen activator (tPA) thereby enhancing plasmin production. Three specific amino acid residues that are involved in plasminogen binding were identified and are conserved in human and murine CLEC3A. But whether CLEC3A contributes to plasminogen activation, was still unknown at the beginning of this work. However, here we found that CLEC3A specifically binds to plasminogen and enhances tPA-mediated plasminogen activation. In summary, we have determined the structure, tissue distribution and molecular function of the cartilage-specific lectin CLEC3A. We could show that CLEC3A has an effect on cell adhesion, binds specifically to plasminogen and participates in tPA-mediated plasminogen activation. We propose that CLEC3A is involved in regulation of tissue remodeling processes in the ECM of cartilage due to enhancing tPA-mediated plasminogen activation.