Abstract

Collagen VI is a heterotrimeric protein of the extracellular matrix. The longest known form consists of the three distinct α-chains α1, α2 and α3. Each chain contains a triple helix forming collagenous domain and N- as well as C-terminal non-collagenous domains, mainly made up from von Willebrand factor A like modules. The assembly starts inside the cell where the three different chains form a collagen VI monomer, two monomers form a dimer in an anti-parallel alignment and two dimers form a tetramer. The tetramers are secreted into the extracellular space where they associate head to head to form microfibrils that in turn interact with a variety of other extracellular matrix proteins. In 2008 three additional chains, the so called "new chains" α4, α5 and α6 were identified. Their domain structure is highly homologous to that of the α3-chain and it is assumed that the new chains replace the α3-chain in the collagen VI molecule in certain tissue locations.

To perform studies on the structure and the macromolecular organisation of collagen VI, fragments of the single chains were recombinantly expressed. For the expression of triple helical regions several methods were tested. All of the N- and C-terminal fragments of the non-collagenous domains were successfully cloned, expressed in cell culture and affinity purified. Specific antibodies were raised against almost all fragments. Both the recombinant fragments and the specific antibodies are important tools to study collagen VI function.

The N-terminal domains of α3, α4, α5 and α6 were studied by single particle electron microscopy and the results used to generate structural models. In addition, SAXS measurements of the N-terminal domains of the new chains were performed, which allowed the design of homology based rigid body models. The models obtained by the two methods show good agreement and also great similarity with the published model of the α3 chain N1-N9 domains. Also the C-terminal domains of α1, α2, α3 and α4 were investigated by SAXS and the derived models indicate dimer formation for both α1 and α2.

The interactions between the non-collagenous domains and with other candidate binding partners in the extracellular matrix were studied by ELISA style binding assays. Only few and mostly weak interactions between the different collagen VI fragments were detected. However, these may be potentiated by cooperative effects in the fully assembled molecule. The screening of potential binding partners of collagen VI revealed the strongest interactions with decorin and nidogen-1. Weak interactions were found with collagen IV, nidogen-2, laminin-γ1, thrombospondin-1 and the fibulins and no binding was detected with matrilins

and COMP. The interaction between collagen VI and nidogen-1 was confirmed by SPR measurements and a broad affinity of collagen VI VWA domains for nidogen was detected. This interaction is novel and could serve to link the basement membrane and the collagen VI microfibrillar network.

Altogether, with regard to both structure and interactions a great similarity between the new chains and the α3 chain was found. This supports the assumption that the new chains replace the α3 chain in specific tissue locations. Subtle differences point to the possibility of a functional specialization of each chain.