Characterization of new functional interactions of hemicentins within the elastin/ fibrillin microfibril network

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Abstract

The extracellular matrix represents a crucial cellular microenvironment for the regulation of tissue structure and function. The glycoproteins fibrillin-1 and fibrillin-2 (FBN1 and FBN2) assemble into microfibrillar networks that not only define biomechanical properties of tissues, but also control the bioavailability of growth factors within the extracellular space. Members of the fibulin family have been described as essential factors in elastogenesis that are targeted to FBNs via specific interactions. Hemicentin-1 (HMCN1) and Hemicentin-2 (HMCN2) are large 600 kDa proteins that belong to the fibulin family, however, little is known about the binding repertoire of HMCNs within the elastin/ FBN fiber network.

Murine tissues were investigated by immunofluorescence and immunogold electron microscopy employing newly raised antibodies against recombinant HMCN1 and HMCN2 protein fragments. Immunofluorescence analyses revealed that HMCN1 predominantly localizes to FBN positive fibers which transverse the dermis and engulf hair follicle bulbs in a basket like fashion. A similar co-distribution of HMCN1 was observed with members of the fibulin and latent TGF-β binding protein (LTBP) family as well as with elastin confirming HMCN1 as an interconnected constituent of the elastin/ FBN microfibril network. HMCN2 was not detected in dermis but was found to co-localize with basement membrane markers of the endomysium of skeletal muscle where HMCN1 was absent. Immunofluorescence analysis also demonstrated that HMCN1 altered its dermal localization in absence of FBN2 suggesting that proper tissue localization depends on FBN microfibrils.

Further, immunogold labeling of skin showed that HMCN1 is directly targeted to FBN microfibrils. Solid-phase interaction assays showed that the fibulin-like module of HMCNs mediates the interaction with the N-terminal EGF3-hybrid1 region of FBNs and that HMCNs are able to limit LTBP binding to FBNs.

Analysis of *Hmcn1*$^{-/-}$, *Hmcn2*$^{-/-}$ and *Hmcn1*$^{-/-}$;*Hmcn2*$^{-/-}$ mice showed no deficit in overall growth or FBN microfibril deposition. However, transmission electron microscopy and echocardiography of adult aortas suggested elastic fiber breaks and dilatations of the ascending aorta.

The findings of this thesis show HMCN1 and HMCN2 as new interaction partners of proteins of the elastin/ FBN microfibril network. These results reveal new insights regarding HMCNs and the elastin/ FBN microfibril network as they might play important roles in elastogenesis and modulate growth factor targeting.