

Collagen XVIII and endostatin are components of basement membrane networks and essential for retinal pigment epithelium function

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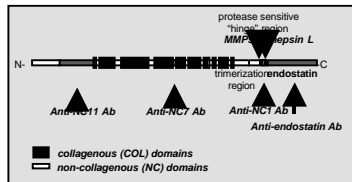
ABSTRACT

Endostatin is a proteolytically derived fragment of the C-terminal NC1 domain of collagen XVIII, a component of almost all basement membranes. In this study, ultrastructural immuno-labeling experiments demonstrate that the N-terminal NC1(XVIII) domain is localized in the matrix subjacent to the lamina densa of basement membranes (BMs), co-localizing to microfibrils in skin. In contrast, the C-terminal NC1(XVIII)/endostatin domain is anchored within the lamina densa, co-localizing to perlecan and laminin, and the intermediate NC7(XVIII) domain is localized in between. Rotary shadowing electron microscopy of recombinant collagen XVIII and of immunopurified material from vitreous, show a structural flexibility of this collagen. Additionally, it is demonstrated that endostatin is part of full-length collagen XVIII in the BM zone, and is released upon cathepsin L treatment. Immunogold labeling of suprastructural fragments isolated from superficial dermis shows that endostatin is part of BM networks with laminin, type IV collagen and perlecan. Therefore, collagen XVIII is anchored through its endostatin domain in basement membrane networks in vivo.

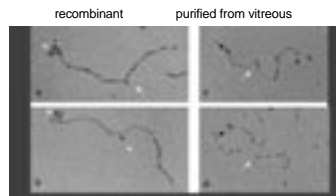
A role for collagen XVIII/endostatin in cell-matrix interactions is suggested through abnormal deposition of extracellular material underlying the retinal pigment epithelium (RPE) in Col18a1 KO mice. Deposit formation increases with age and acts as a barrier to sufficient retinoid supply in the eye, as measured by HPLC analysis of retinyl esters. RPE abnormalities result in pathological electroretinograms (ERGs), and may provide explanations for retinal degeneration in human patients with Knobloch syndrome and inactivating mutations in the COL18A1 gene.

RESULTS

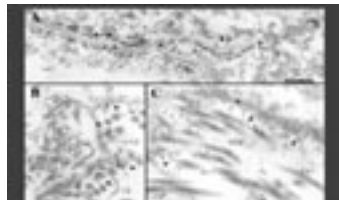
Immunolabeling using collagen XVIII domain-specific antibodies



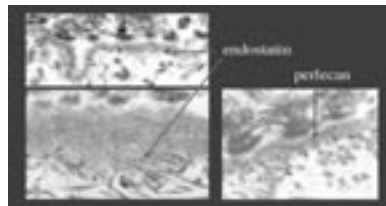
Rotary shadowing EM: kinks and bends of collagen XVIII



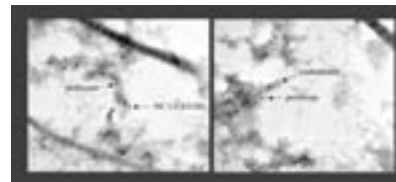
Collagen XVIII N-terminus localizes to the sublamina densa



Endostatin localizes to the lamina densa of basement membranes



Collagen XVIII/endostatin is part of the laminin/perlecan basement membrane network

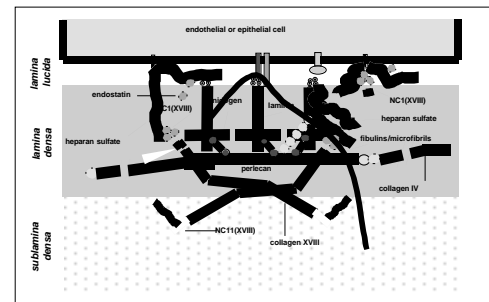


Endostatin is part of full-length collagen XVIII in basement membranes

Skin homogenates after high salt-epidermolysis: Cathepsin L releases endostatin from full-length collagen XVIII

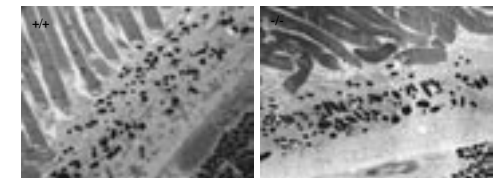
Lane 1: cathepsin L treatment
Lane 2: no cathepsin L
Lane 3: recombinant endostatin
A: anti-endostatin labeling
B: anti-NC7(XVIII) labeling

A model for the organization of collagen XVIII in the basement membrane



Age-dependent abnormalities of the retinal pigment epithelium (RPE) cause loss of visual sensitivity in Col18a1^{-/-} mice

Reduced interdigitation of apical RPE and photoreceptors in Col18a1^{-/-} mice

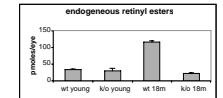


Col18a1^{-/-} mice show age-dependent accumulation of extracellular material underlying the retinal pigment epithelium (RPE)

Accumulation of extracellular material and lipid accumulation at the basal RPE



Extracellular deposits act as a barrier for retinoid transport to the RPE



Pathological electroretinogram (ERG):



Col18a1^{-/-} mice show age-dependent loss of visual sensitivity

DISCUSSION

Endostatin anchors collagen XVIII into the laminin/perlecan scaffold, whereas the N-terminus is co-localized to microfibrils in the sublamina densa. Thus collagen XVIII might have a role in cell anchorage to the underlying matrix. In skin strong adhesion complexes exist, and loss of collagen XVIII in Col18a1^{-/-} mice does not result in cell anchorage defects.

Endostatin is part of full-length collagen XVIII in the basement membrane zone. This implies that proteolytic release of endostatin-containing fragments locally at the BM zone allows endostatin to exert direct cellular effects.

Abnormal cell-matrix interaction is demonstrated in the eye of Col18a1^{-/-} mice, resembling loss of visual sensitivity in human patients with inactivating collagen XVIII mutations. Lack of collagen XVIII results in abnormal extracellular deposit formation underlying the retinal pigment epithelium in the eye, acting as a barrier for retinoid transport to the RPE. In conclusion, collagen XVIII/endostatin is a component of basement membrane networks with an essential function for the RPE.