

Nonvascular pathways of aqueous humor outflow in the choroid of the human eye

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The question about presence of lymphatic outflow from human eye is uncertain until now. For a long time it was supposed that there were no lymphatics in the eye. But after the revelation of molecular markers of lymphatic endothelial cells it became possible to detect the lymphatic capillaries and vessels in the various structures of the human eye in normal and pathological conditions. Nowadays there are some data about lymphatic structures in the anterior eye segment, including ciliary body. This structures possibly takes a part in unconventional pathway of aqueous humor drainage.

Currently, the question of the presence of lymphatic vessels in the posterior eye segment remains debatable.

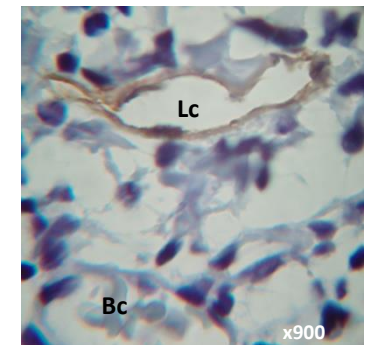
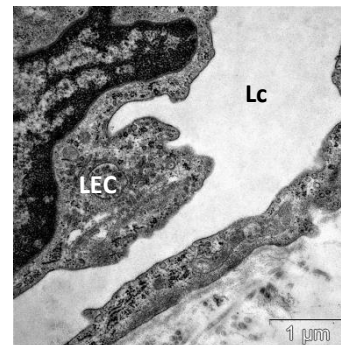
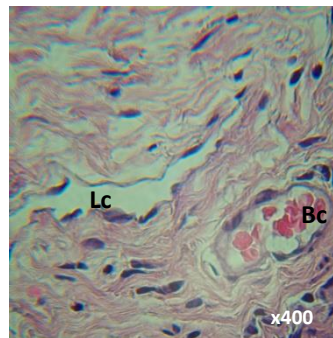
Purpose: to study the structural organization of the lymphatic pathways of aqueous humor outflow in the choroid.

Methods and Algorithms: The object of study were fragments of enucleated eyes (n = 10), harvested from patients with normal intraocular pressure. For morphological study samples were treated according to standard procedures for light and transmission electron microscopy.

Immunostaining:

- 1) lymphatic specific endothelial markers Podoplanin (Monosan, Netherlands), PROX-1 (Covance, Germany), and LYVE -1 (Abcam, England),
- 2) the vascular specific endothelial marker CD31 (Abcam, England),
- 3) a marker for fibroblast growth factor receptors FGFR-1 (Abcam, England) and fibroblast marker TE-7 (Novus Biologicals, USA).

The presence of lymphatic vessels in conjunctiva was verified and it have typical organization, so we used conjunctiva as a control tissue.



Bc – blood capillaries
Lc – lymphatic channels
LEC -lymphatic endothelial cells

Podoplanin+

RESULTS

1. Immunohistochemical staining

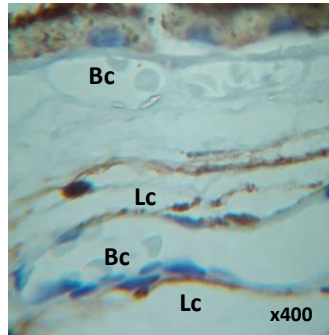
The choroid was found to contain lymphatic channels in choroidal stroma and layer of choriocapillaris.

The channels were limited by narrow elongated cells, positively stained for markers of lymphatic vessels Prox-1, LYVE-1, Podoplanin and were shown negative CD31 staining; therefore we consider that this channels are lymphatic structures.

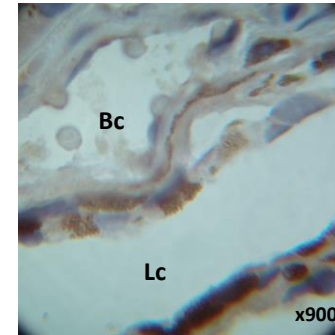
Positively stained lymphatic-like structures in the choroid:

Lc – lymphatic channels

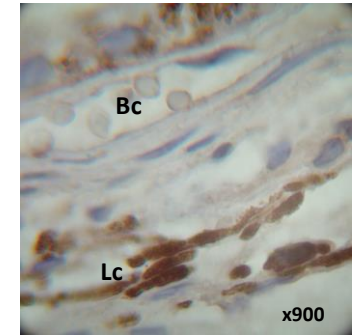
Bc – blood capillaries



Podoplanin+



Prox-1+

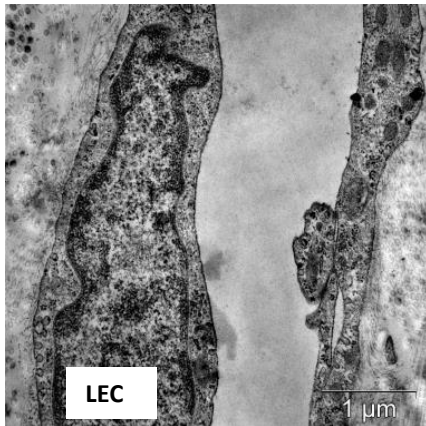


LYVE-1+

2. Transmission electron microscopy, Morphometric processing and Statistical analysis

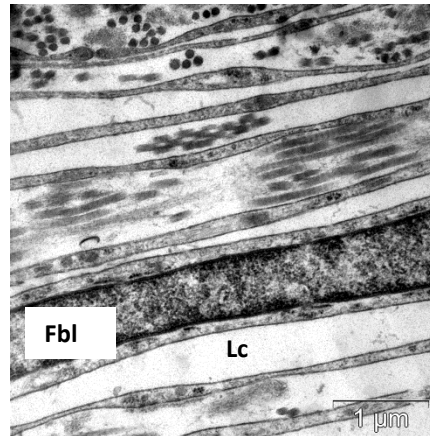
Using transmission electron microscopy we identified that lymphatic channels did not have a typical vascular structure. We shown that these channels circumscribed by narrow process cells that have ultrastructural differences from lymphatic endothelial cells: they do not have stropic filaments, contain a greater volume density of the granular endoplasmic reticulum membranes, and a lower volume density of mitochondria and caveolae.

conjunctiva:

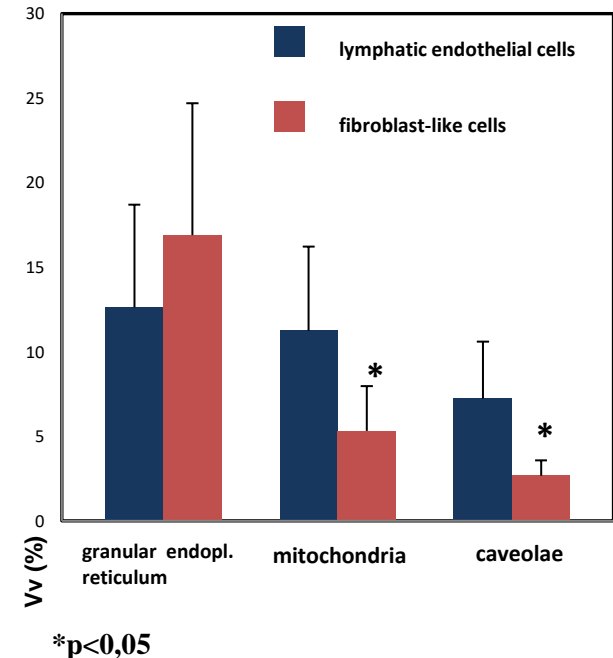


LEC -lymphatic endothelial cells

choroid:

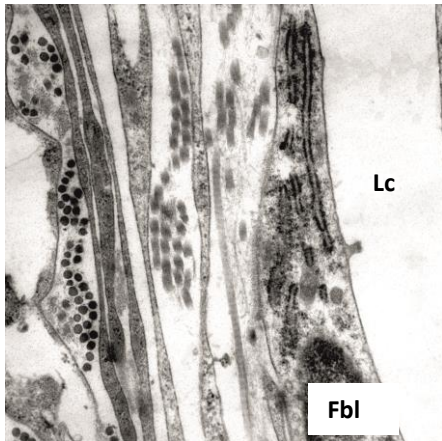


Lc – lymphatic channels Fbl – fibroblast-like cells.



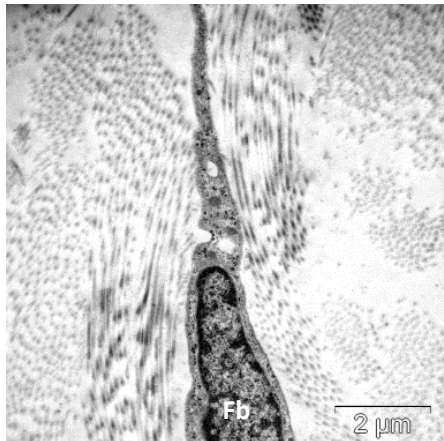
The cells lining the lymphatic channels are similar to fibroblasts, but differ from them in the lower bulk density of the granular endoplasmic reticulum and the lower content of fibrous structures in the microenvironment.

choroid:

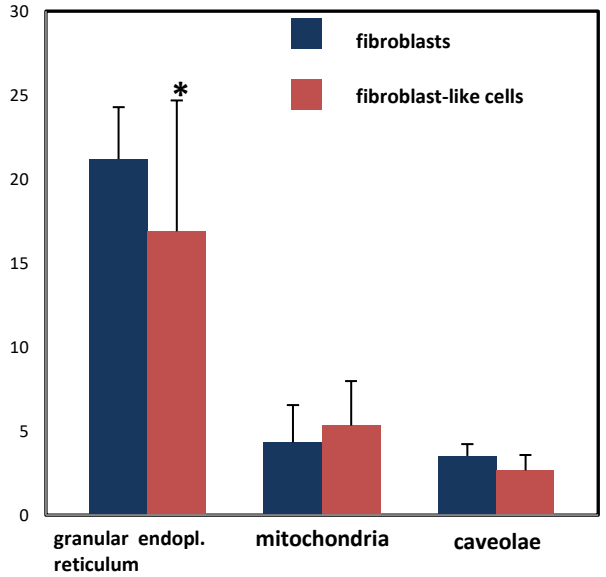


Lc – lymphatic channels
Fbl – fibroblast-like cells.

conjunctiva:

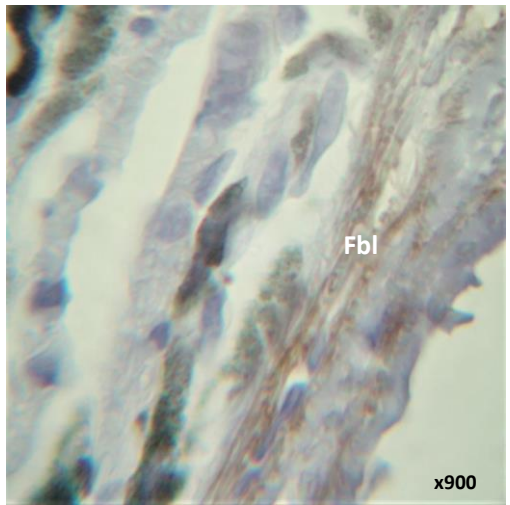


Fb - fibroblast



*p<0,05

We carried out an immunohistochemical study of the samples using fibroblast markers FGFR-1 and TE-7. During immunohistochemical staining of samples using the TE-7 marker, rare positively colored elongated narrow cells were detected in the choroid of the human eye. As a result, staining of the same cells was noted as when staining for lymphatic endothelial markers. However, not all cells lining the channels studied by us were stained for fibroblast markers TE – 7 and FGFR – 1.



Considering that these cells have ultrastructural differences from fibroblasts and lymphatic endothelial cells, they express lymphatic endothelial markers and do not always have fibroblast markers, i.e. these cells are fibroblast-like cells.

CONCLUSION
Thus, in the structure of the choroid of the human eye, there are non-vascular pathways of aqueous humor outflow, represented by atypical lymphatic channels confined by fibroblast-like cells that have markers of the endothelium of the lymphatic vessels.