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# Hyaluronan derived from the limbus is a key Regulator of Corneal Lymphangiogenesis

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Footnotes

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## Abstract

**Purpose** :

Corneal lymphangiogenesis and angiogenesis leads to the loss of corneal transparency. We have recently shown that in the cornea hyaluronan (HA) is present primarily in the

limbal region and plays a key role regulating the limbal stem cell phenotype. Given the HA receptor LYVE-1 is highly expressed in corneal lymphatic vessels we investigated whether HA could play a role in regulating corneal lymphangiogenesis.

**Methods** : Wild-type (wt) and hyaluronan synthase (HAS) knockout mice - specifically combined *Has1*$^{-/-}$ and *Has3* $^{-/-}$ *null* mice (*HAS1*$^{-/-}$*;HAS3*$^{-/-}$) and conditional *Has2* knock-out mice (*HAS2*$^{D/DCorEpi}$), were used. The mice were subjected to injury, alkali burn or suture placement, to investigate the role of HA on corneal lymphangiogenesis. Corneal buttons were also obtained from different developmental time-points to study the role of HA in lymphatic vessel development. The corneas were analyzed by whole mount immunohistochemistry and entire corneas were imaged under an LSM 800 confocal microscope using the both the z-stack and tiling mode. Primary lymphatic vessel endothelial cells from human dermis (hDLECs) and lymph node (hLLECs) were used for tube formation assay and cell proliferation assay *in vitro*.

**Results** : After injury both wild-type and *HAS1*$^{-/-}$*;HAS3*$^{-/-}$ mice presented both an increase in HA expression and lymphangiogenesis. Interestingly, lymphatic vessels extended exclusively into HA rich areas. In stark contrast, *HAS2*$^{D/DCorEpi}$ mice did not upregulate HA synthesis after injury and, in turn, did not present lymphangiogenesis. Our developmental studies revealed first HA is expressed in the corneal limbus and thereafter lymphatic vessels invade this region. Our *in vitro* studies corroborated our *in vivo* data, with both HA increasing the proliferation and tube formation ability of hDLECs and hLLECs.

**Conclusions** :
HA regulates corneal lymphangiogenesis, both during development and after injury. These findings raise the possibility that therapeutic blockade of HA-mediated lymphangiogenesis could be used to reduce corneal scarring and also prevent rejection after corneal transplantation.

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