The Prox1–Vegfr3 feedback loop maintains the identity and the number of lymphatic endothelial cell progenitors

R. Sathish Srinivasan,¹,⁵,⁷ Noelia Escobedo,¹,⁷ Ying Yang,¹,⁶,⁷ Ashley Interiano,¹ Miriam E. Dillard,¹ David Finkelstein,² Suraj Mukatira,² Hyea Jin Gil,¹ Harri Nurmi,³,⁴ Kari Alitalo,³,⁴ and Guillermo Oliver¹
Introduction

Embryonic veins (>E8.5) → COUP-TFII

Competence (E9.0) → Sox18

Commitment (E9.75-E14.5)
Specification (E9.75-E12.5) → Prox1

Determination (E12.5-E14.5)
Nrp2, Pdpn, COUP-TFII, Vegfr3

Differentiation and maturation (E14.5-postnatal)
Elnb2, Foxc2, Ang2, Aspp1, T-synthase

Key
- Venous endothelial cell (EC)
- Venous EC competent for lymphatic EC (LEC) fate
- Specified LECs

Mesenchyme

Formation of lymph sacs
Lymphovenous separation (Slp76, Syk, Plcg2)

LEC sprouting
Lymphatic plexus remodeling and maturation

OLIVER G. (2010). Endothelial cell plasticity: how to become and remain a lymphatic endothelial cell
Introduction

**Prox1 - a master switch of LEC determination**

- In Prox1-null embryos LECs are absent.
- Conditional deletion of Prox1 results in the loss of LEC identity.
- Ectopic expression of Prox1 in BECs leads to activation of LEC-specific genes.
- Dose-dependent autoregulation of Prox1 expression.

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**Introduction**

**Vegfr3 - a target of Prox1**

*Vegfc* being a ligand for the receptor tyrosine kinase *Vegfr3*

expressed in all BECs until E10.5, later on it is restricted to LECs

number of LEC progenitors and LECs is reduced in *Vegfr3*+/− and *Vegfc*+/− embryos

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Methods

retroviral transduction

transfection of 293T cells with the retroviral vector (empty or containing avitag-Prox-1)

retroviral transduction of H5V cells by incubation with supernatant of transfected 293T cells

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Methods

siRNA analysis

human dermal LECs

target RNA:  *Prox 1*

*Vegfr3*

knockdown was examined by

western blot

immunofluorescence

qPCR

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Methods

Chromatin immunoprecipitation

mouse primary LECs from E 14.5 were isolated by flow cytometry
Lyve-1⁺, CD31⁺ and CD45⁻ population
real-time PCR

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Vegfr3 is a dosage-dependent target of Prox1
other BEC- and LEC-specific genes such as Foxc2, Integrin α9, Coup-TFII, Reelin, Tie2, Nrp1, Nrp2, PECAM1, VE-Cadherin and Lyve1 showed no correlation with Prox1 expression

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Lymphatic vasculature is defective in \( \text{Prox1}^{+/\text{GFPCre}\cdot\text{Vegfr3}^{+/\text{LacZ}}} \) embryos.

The number of LEC progenitors and differentiating LECs is severely reduced in \( \text{Prox1}^{+/\text{GFPCre}\cdot\text{Vegfr3}^{+/\text{LacZ}}} \) embryos.

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LEC progenitor identity is lost in 

\( \text{Prox}^+/\text{GFPCre} \cdot \text{Vegfr}^+/\text{LacZ} \) embryos
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Vegfr3 regulates Prox1 expression in LEC progenitors and LECs

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Vegfc signaling helps maintain LEC progenitor identity
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Conclusion

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Conclusion

*Prox1*^+^ LEC progenitors require *Vegfr3* to maintain their identity and bud from the CV

this regulation is mediated through the activation of *Vegfr3* by *Vegfc*

alterations in *Vegfc/Vegfr3* signalling leads to the loss of *Prox1* expression in LEC progenitors and their reversal to venous EC fate