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$CD34^{+}VEGFR\text{-}3^{+}$ progenitor cells have a potential to differentiate towards lymphatic endothelial cells

Tan YZ et al.

J Cell Mol Med. (2014 Mar;18(3):422-33)

Denise Traxler-Weidenauer

April 2014

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Introduction

Lymphatic vessels

no basement membrane
no pericytes
functions: draining excess fluid from extracellular space
absorbing & transportation of lipids
transporting leukocytes & APC
removal of cell debris, dust particles & microorganism
tumour metastasis

Lymphangiogenesis

occurs in wound healing
embryonic development
tissue regeneration
inflammation
tumour metastasis

newly formed lymphatic vessels sprout from pre-existing vessels by proliferation, migration and tube formation of endothlial cells

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Introduction

Endothelial progenitor cells

are circulating cells that promote neovascularization at sites of ischemia, injury, hypoxia or tumour formation

majority resides in bone marrow

number of EPCs in human cord blood is much higher than in peripheral blood

were already detected at growing lymphatic vessels in the cornea of mouse & transplanted human kidney

Tan YZ (2014). CD34+VEGFR-3+ progenitor cells have a potential to differentiate towards lymphatic endothelial cells
De Biase C (2013). Effects of physical activity on endothelial progenitor cells

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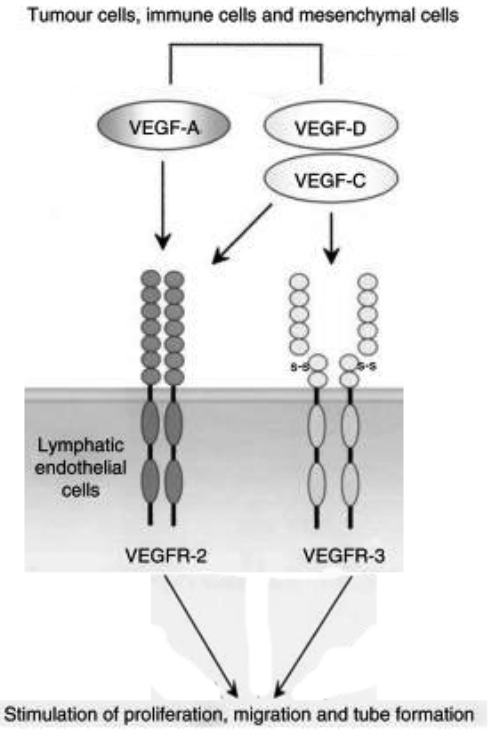
Introduction



CD34	haematopoetic cells, EPCs
CD133	haematopoetic stem/progenitor cell marker
VEGF-C	
VEGFR-3	receptor of VEGF-C/-D , expressing specifically on lymphatic endothelial
Prox-1	a lymphatic specific marker expressed in nuclei
5'Nase	marker for lymphatics
LYVE-1	marker or lymphatics, may play a role in lymphatic hyaluronan transport
CD44	is a receptor for hyaluronic acid

Tan YZ (2014). CD34+VEGFR-3+ progenitor cells have a potential to differentiate towards lymphatic endothelial cells
Van Der Auwera (2006). First international consensus on the methodology of lymphangiogenesis quantification in solid human tumours.

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Methods

Harvesting of mononuclear cells

40-60 ml human umbilical cord blood

mononuclear cell isolation by density-gradient centrifugation (percoll)

examination of cells under confocal laser microscope

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Methods

Isolation & identification of EPCs

detection of $CD34^{+}VEGFR\text{-}3^{+}$ and $CD133^{+}VEGFR\text{-}3^{+}$ cells by fluorescence-activated cell sorting

examination of CD133 expression on $CD34^{+}VEGFR\text{-}3^{+}$ cells

evaluation of uptake of acetylated low-density lipoprotein (Dil-Ac-LDL) and Ulex Europaeus agglutinin-1 (UEA-1)

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Methods

Induction of cell differentiation

seeding of $CD34^{+}VEGFR\text{-}3^{+}$ cells on dishes (pre-coated with fibronectin)

incubation in medium enriched with VEGF-C for at least 14 days

observation of morphological changes at day 1, 7, 10, 14 & 21

for following experiments only cells which were incubated in VEGF-C enriched medium for 14 days were used

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Methods

Immunocytochemistry

to evaluated if VEGF-C induced EPCs differentiated towards lymphatic endothelial cells, they were seeded on coverslips (pre-coated with polylysine) and stained for

- 5'Nase
- LYVE-1
- Prox-1
- CD44

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Methods

Quantitative real-time PCR

extraction of total RNA from $CD34^{+}VEGFR\text{-}3^{+}$ cells treated with VEGF-C at different time-points

determination of VEGFR-3 mRNA level

control: $CD34^{+}VEGFR\text{-}3^{+}$ cells without VEGF-C treatment

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Methods

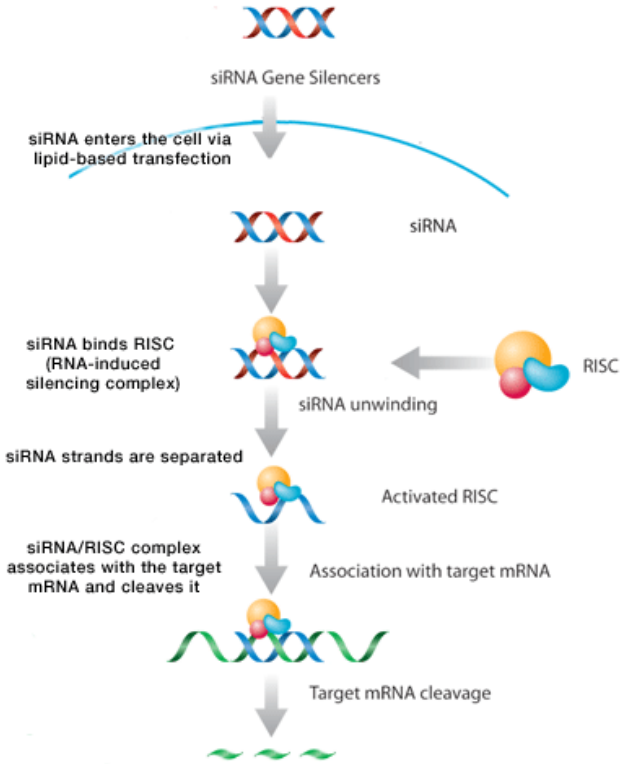
RNA interference

to evaluate the effect of VEGF-C/VEGFR-3 pathway on lymphangiogenesis

3 different siRNAs targeting human VEGFR-3 and 1 irrelevant siRNA (control) were designed

transfection of cells with Lipofectamine 2000 → incubation in VEGF-C enriched medium

assessment of VEGFR-3 mRNA levels by RT-PCR



Tan YZ (2014). CD34+VEGFR-3+ progenitor cells have a potential to differentiate towards lymphatic endothelial cells
http://www.scbio.de/gene_silencers.html (April 27th, 2014)

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Methods

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4 groups
vehicle
VEGF-C
VEGF-C + VEGFR-3 siRNA
VEGF-C + irrelevant siRNA

Proliferation assay

cells were seeded on fibronectin pre-coated dishes

after 24 h incubation cells were counted with a haemocytometer

assays were repeated 5 times

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Methods

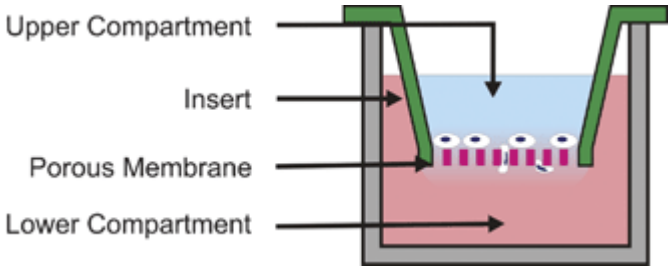
4 groups
vehicle
VEGF-C
VEGF-C + VEGFR-3 siRNA
VEGF-C + irrelevant siRNA

Transmigrating assay

VEGF-C was places in lower chamber

after 12 h incubation, cells in the lower chamber were counted

assays were repeated 3 times



Tan YZ (2014). CD34+VEGFR-3+ progenitor cells have a potential to differentiate towards lymphatic endothelial cells
Keenan TM (2008). Biomolecular gradients in cell culture systems

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Methods

5 groups
vehicle
bFGF
VEGF
VEGF-C
VEGF-C + VEGFR-3 siRNA

Wounding assay

cells in dishes were scraped on 4 different sides

cells were counted in successive 100 µm section of 300 µm wide

maximal distance of cell migration from the wounded edge was measured

assays were repeated 3 times

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Methods

4 groups
vehicle
VEGF-C
VEGF-C + VEGFR-3 siRNA
VEGF-C + irrelevant siRNA

Tube formation in 3D collagen gel & Matrigel

collagen gel: collagen type I from rat tail, dissolved in acedic acid
DMEM
$NaHCO_3$

cells were seeded on dishes (pre-coated with collagen), when cells grew to monolayer they were treated and afterwards a layer of collagen was made

tube formation & area + length of tubes were assessed using TEM

LYVE-1 staining for identifying as lymphatic capillary like structures

assays were repeated 3 times

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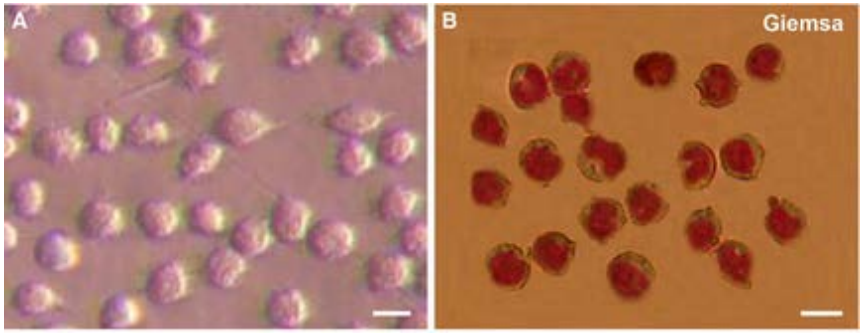
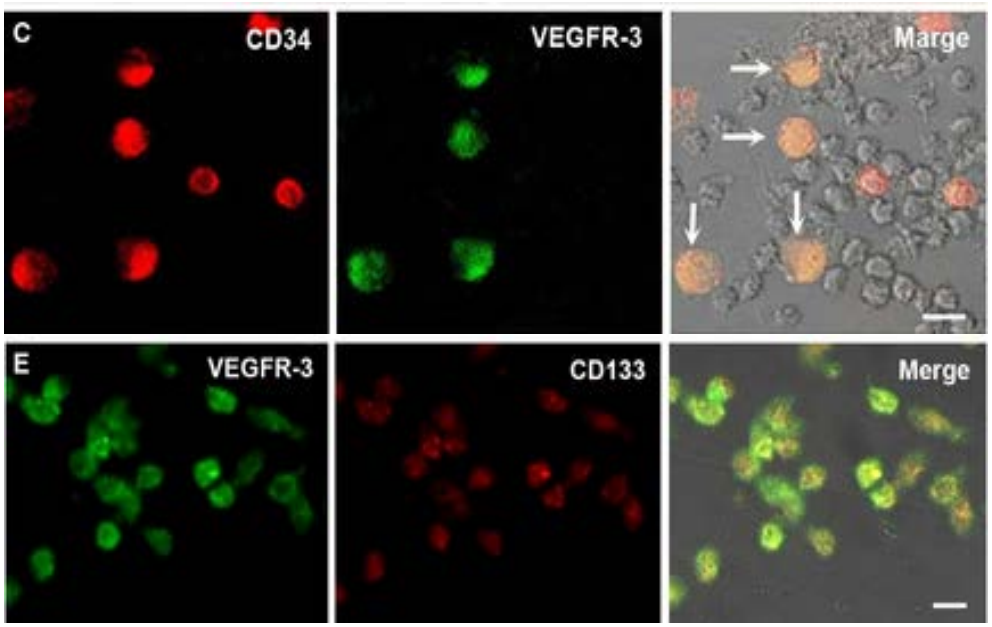
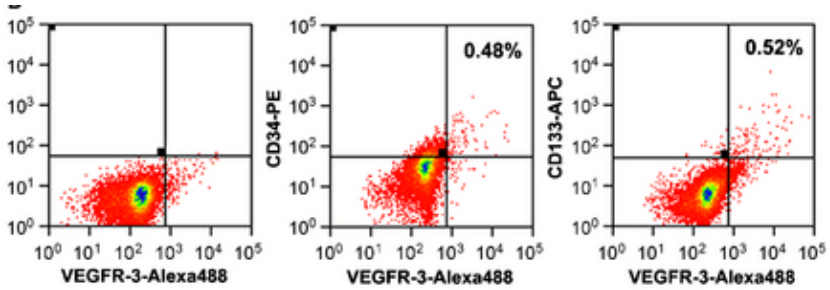
Results







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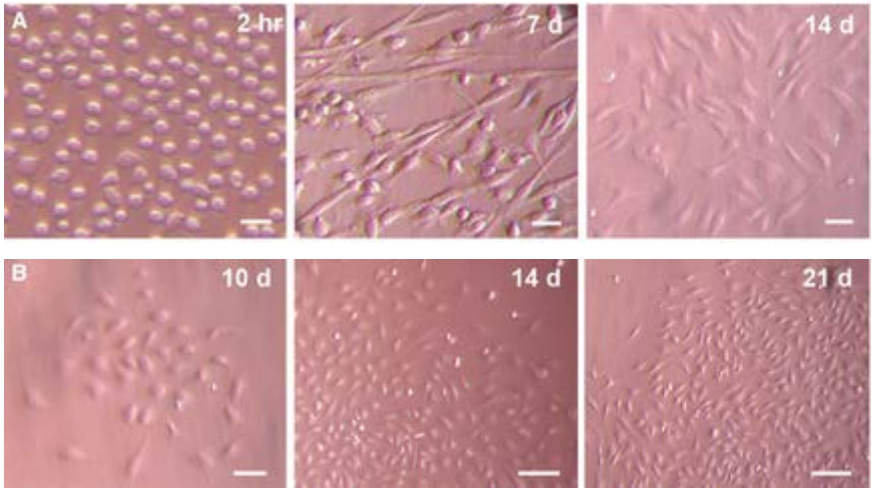

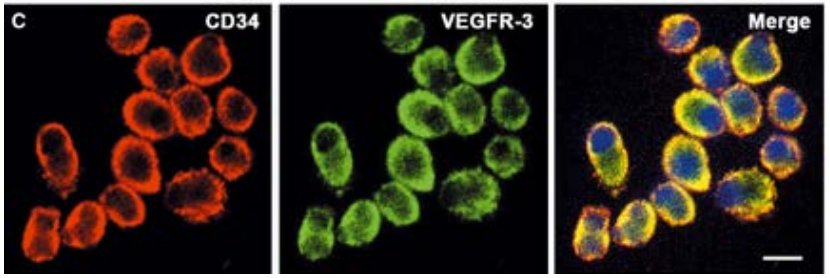

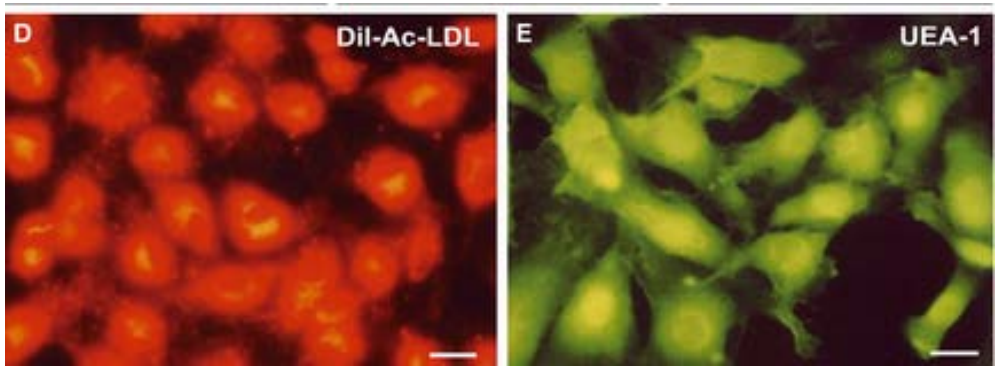

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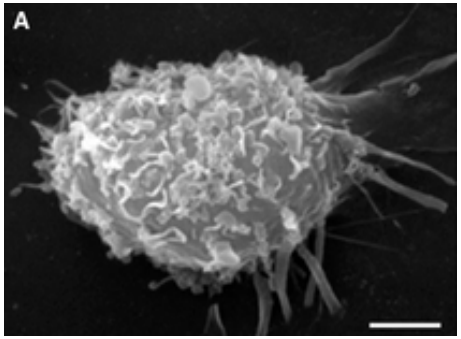

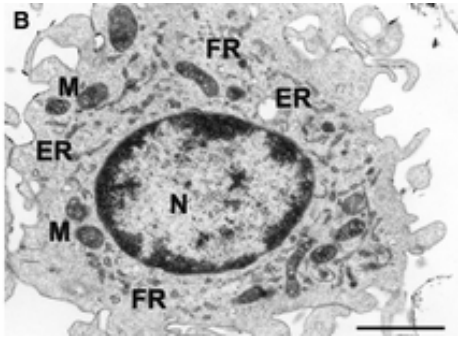

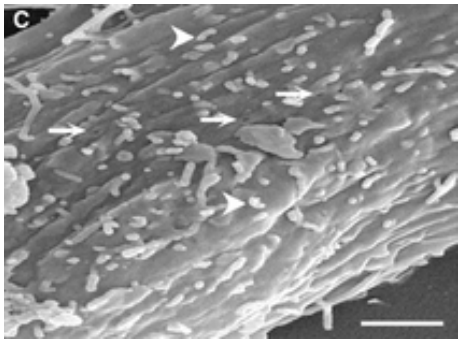

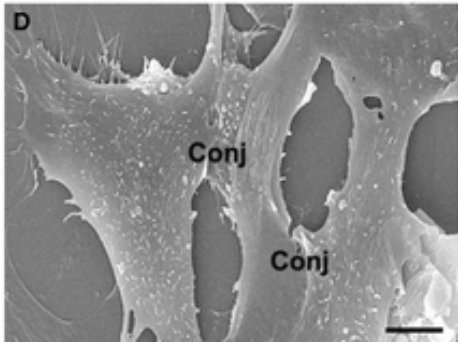

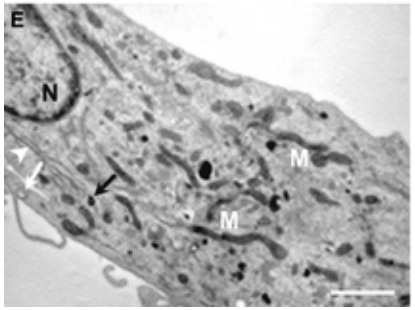

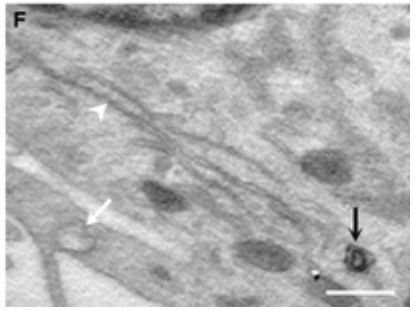

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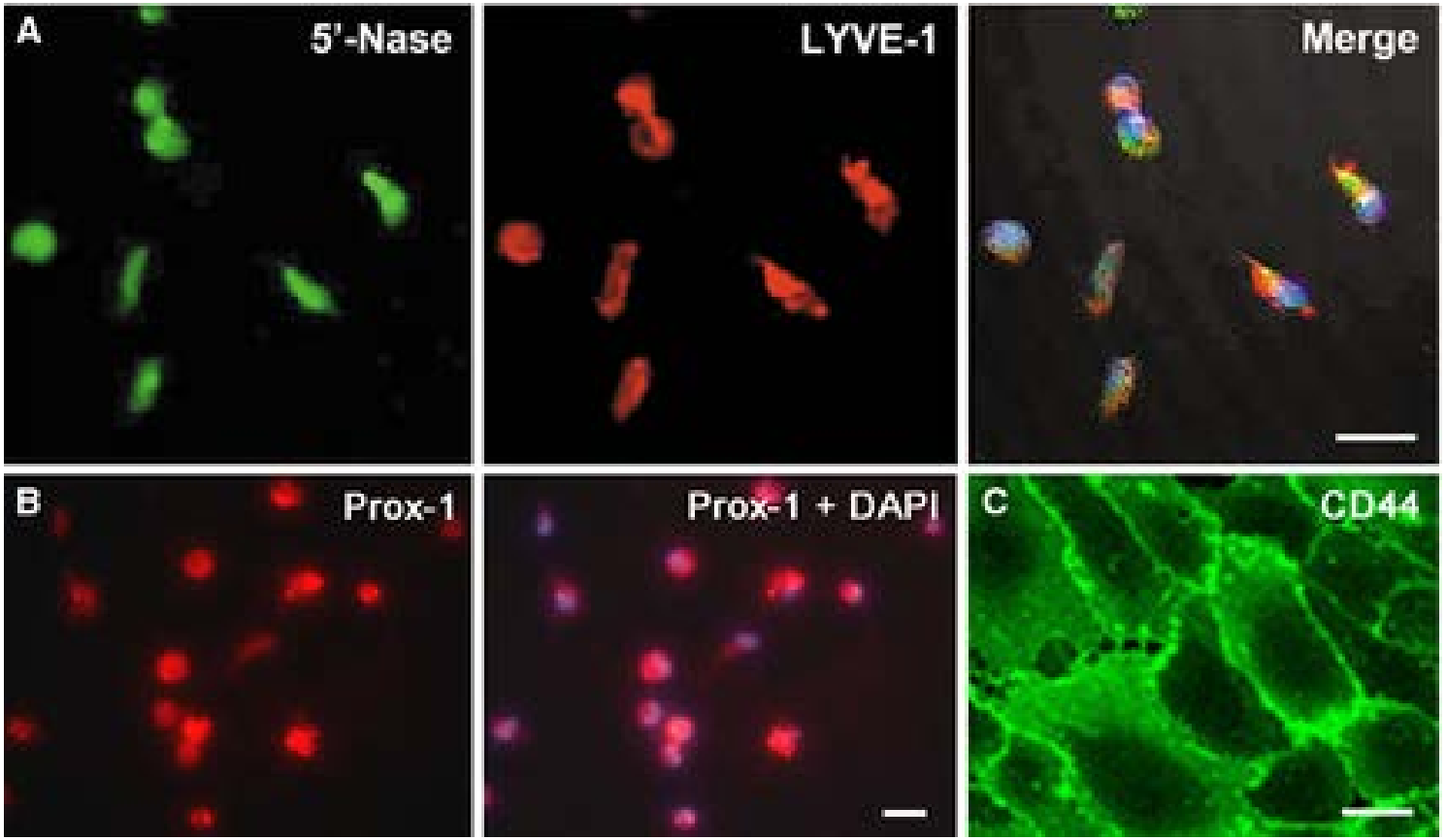

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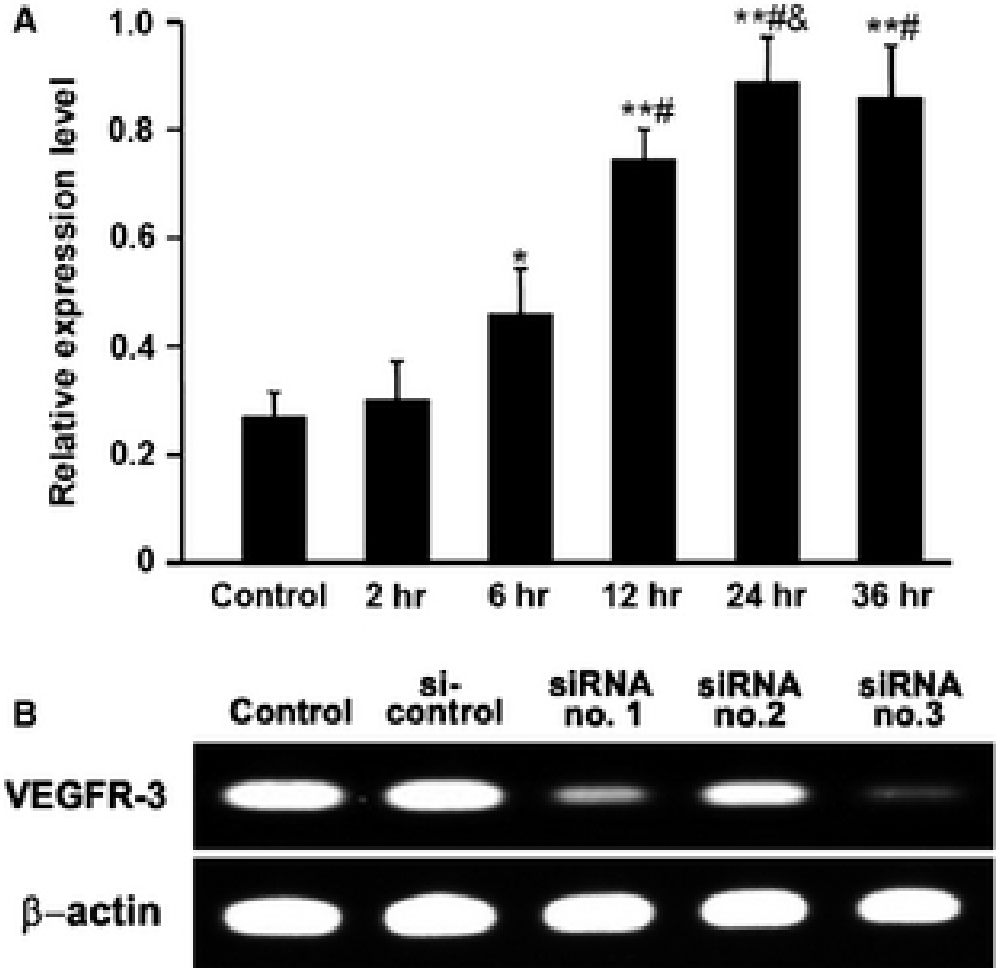

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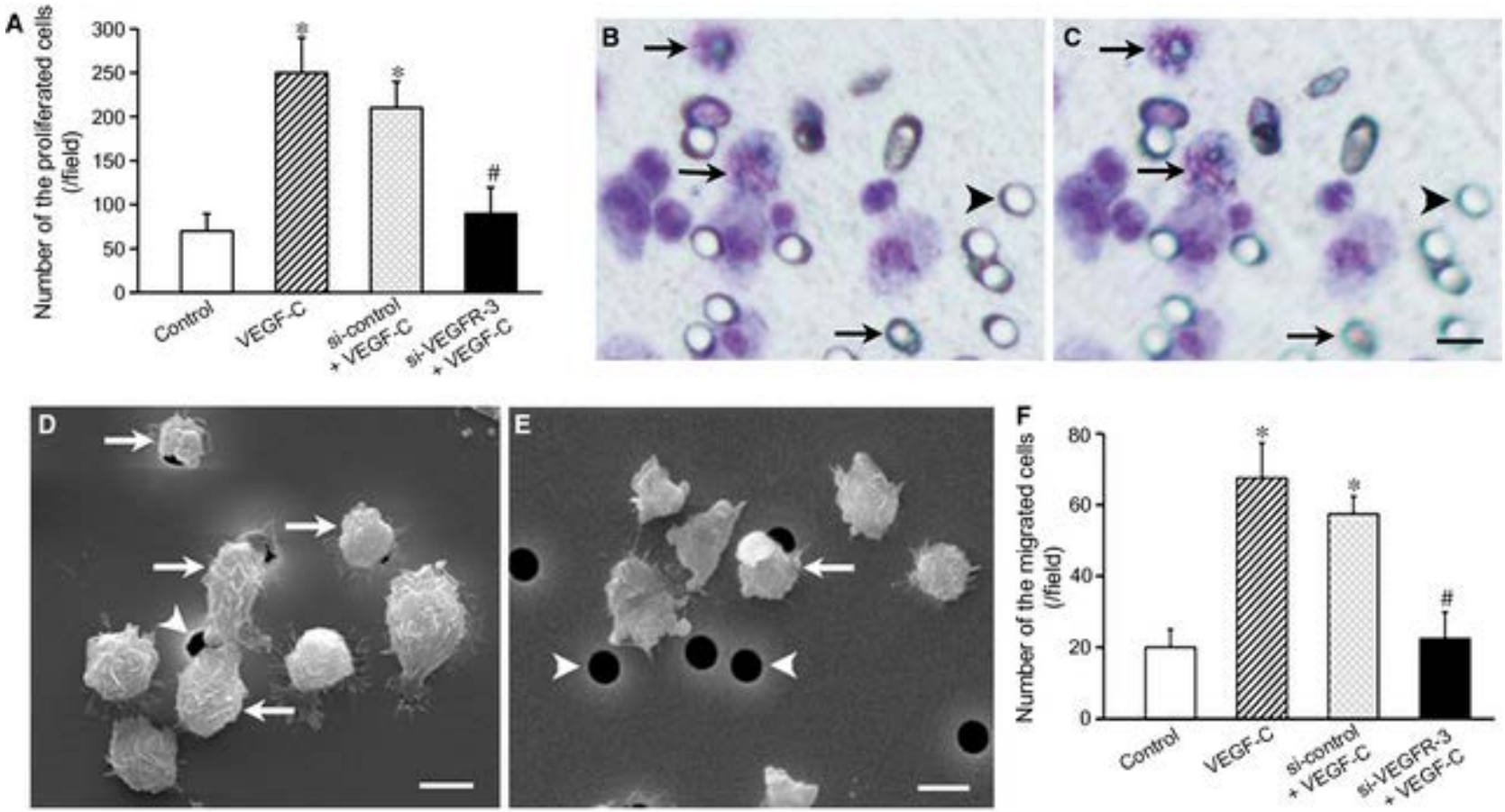

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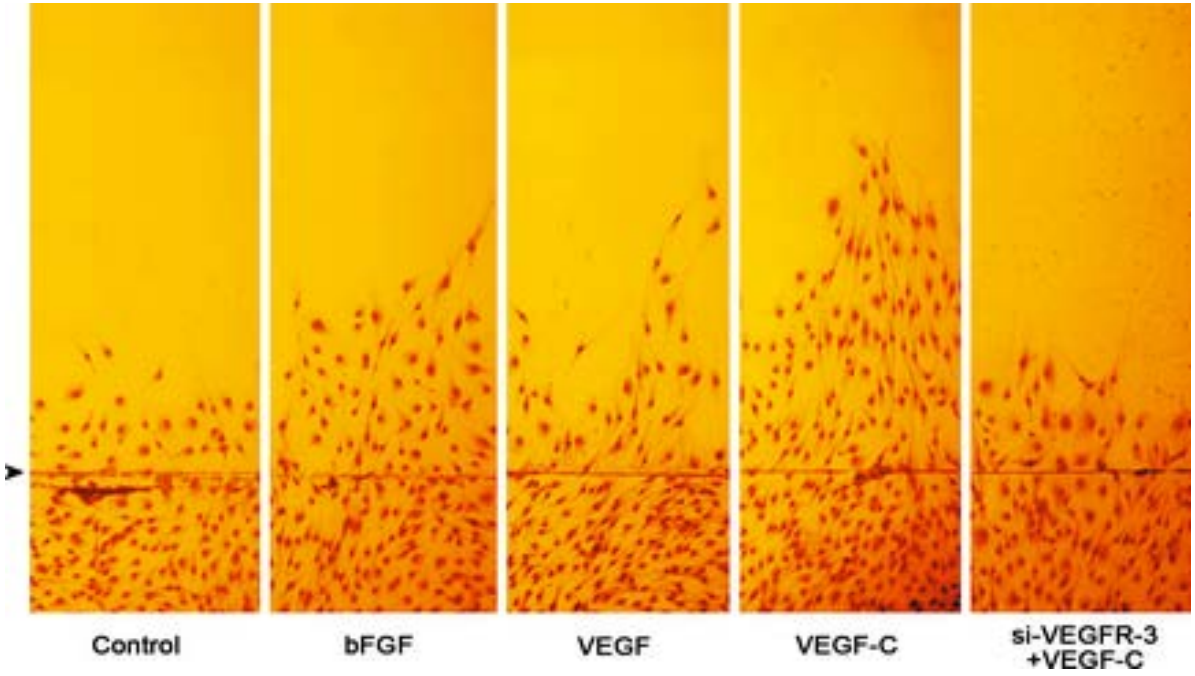

Table 1 Effects of bFGF, VEGF and VEGF-C on migration of the EPC-derived cells

Groups	Cell numbers	Maximal distance (μm)
Control	14 ± 3	239 ± 36
bFGF	36 ± 8*	398 ± 28*
VEGF	30 ± 6*	386 ± 42*
VEGF-C	48 ± 10*,†	578 ± 48*,†
RNAi	16 ± 5#	242 ± 39#

The values are mean ± SD.
*$P < 0.01$ *versus* control group, †$P < 0.01$ *versus* bFGF and VEGF groups, #$P < 0.01$ *versus* VEGF-C group. $n = 16$.

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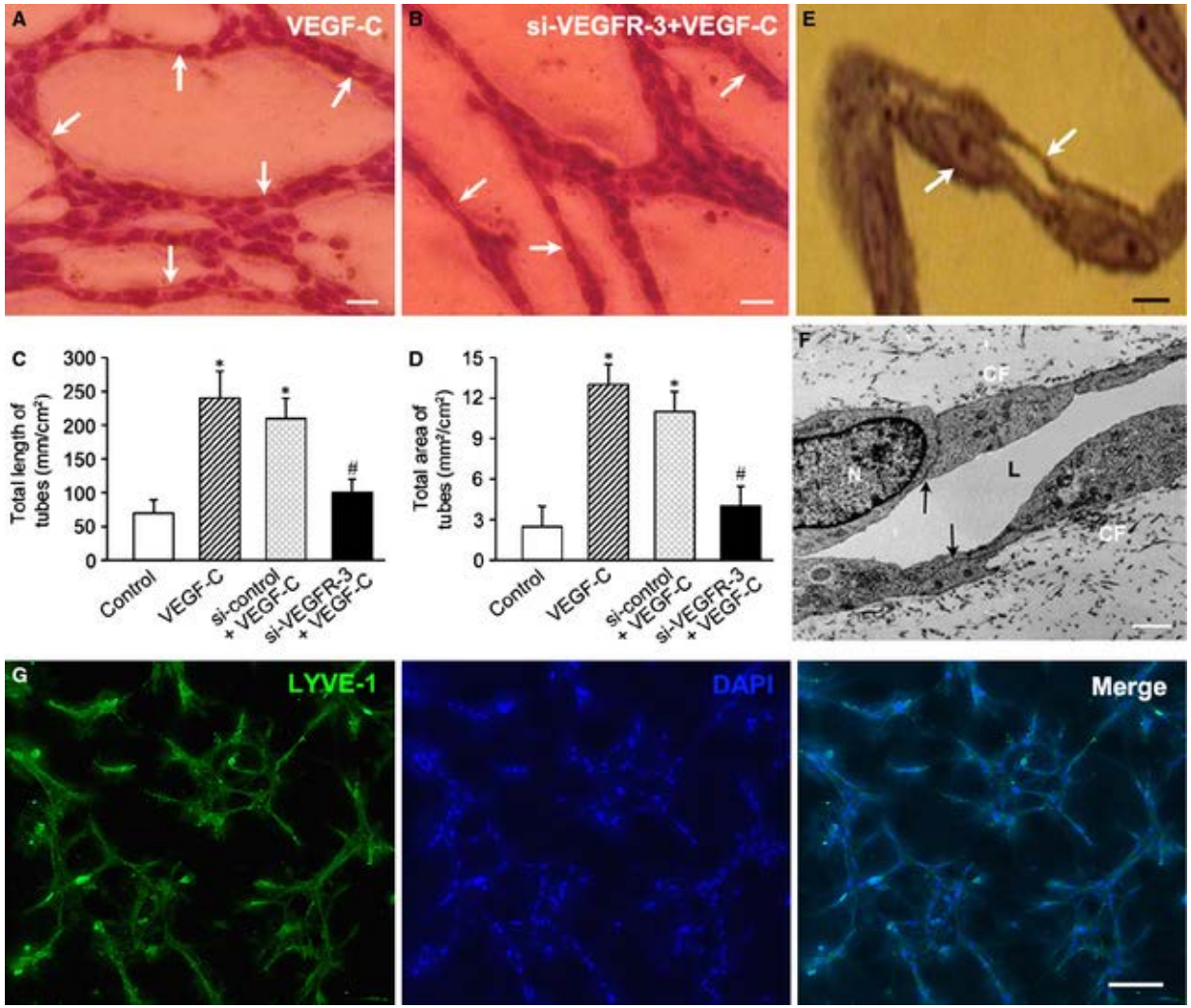

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Conclusion

VEGFR-3^{+}CD34^{+} cells were found & isolated in human cord blood

VEGF-C induction lead to differentiation into LECs

proliferation of LECs

migration of LECs

LECs could form tubes which connected and formed a network in 3D collagen gel

VEGF-C/VEGFR-3 signalling pathway may be crucial in lymphangiogenesis

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