Generating human artery and vein cells from pluripotent stem cells

Assoc. Prof Christine Cheung*
Mar Teng Chou, Nathan*
Soon Wei Ze*
Surya Varma*

**Lee Kong Chian School of Medicine, Nanyang Technological University*

Introduction

Endothelial cells (ECs) are core components of **vasculature** in states of both **health** and **disease**, specifically relating to **high-risk viruses** with vascular tropism[1].

Cultivation of arterial and venous cell lines from **human pluripotent stem cells (hPSCs)** will allow for *in vivo* investigations of the effects of certain pathogens (e.g. **Nipah** and **Hendra** viruses)[2].

Current methodologies in culturing endothelial cells do **not** retain **arterial or venous identities** *in vitro*, which would **limit our understanding** of diseases with **specific vascular tropisms**.

This research aims to **refine current methodology** in generating **pure** populations of **differentiated arterial and venous cells** from human pluripotent stem cells (**hPSCs**).

Hypothesis

Using **specific molecular signals**, we can induce the differentiation of arterial and venous cells from human pluripotent stem cells, with **appreciable functional differences** *in vivo*.

Methodology

Molecular signals were used to direct lateral mesodermal cell differentiation towards arterial and venous cell lines.

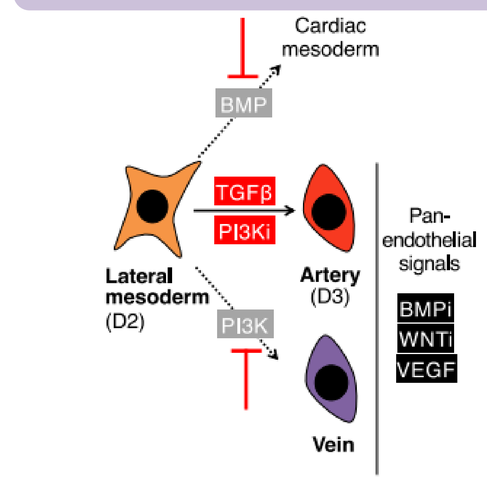

Fig 1a. Molecular signals involved in the differentiation of lateral mesodermal progenitor cells to arterial cells. *TGF-β and PI3K inhibitor direct the lateral mesodermal cell towards arterial differentiation. Concurrently, there is a suppression of signals encouraging cardiac (BMP) or venous (PI3K) differentiation.*

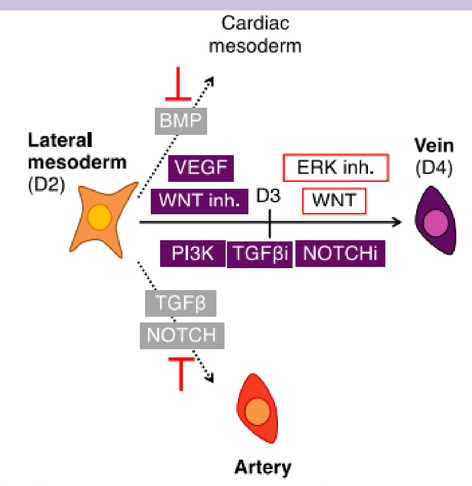

Fig 1b. Molecular signals involved in the differentiation of lateral mesodermal progenitor cells to venous cells. *A multitude of molecular signals (listed in purple boxes) direct the lateral mesodermal cell towards venous differentiation. Concurrently, there is a suppression of signals encouraging cardiac (BMP) or arterial (TGF-β, NOTCH) differentiation.*

Results

Human artery and vein ECs were efficiently generated from hPSCs.

The immunomarker **VE-cadherin** was used to identify differentiated ECs, while **SOX17** and ***NR2F2*** were used to identify arterial and venous cells respectively (Figure 2). Flow cytometry indicated a high percentage differentiation of hPSCs into the targeted ECs, with a **97.2%** differentiation into artery ECs by day 3 and a **88.3%** differentiation into vein ECs by day 5.

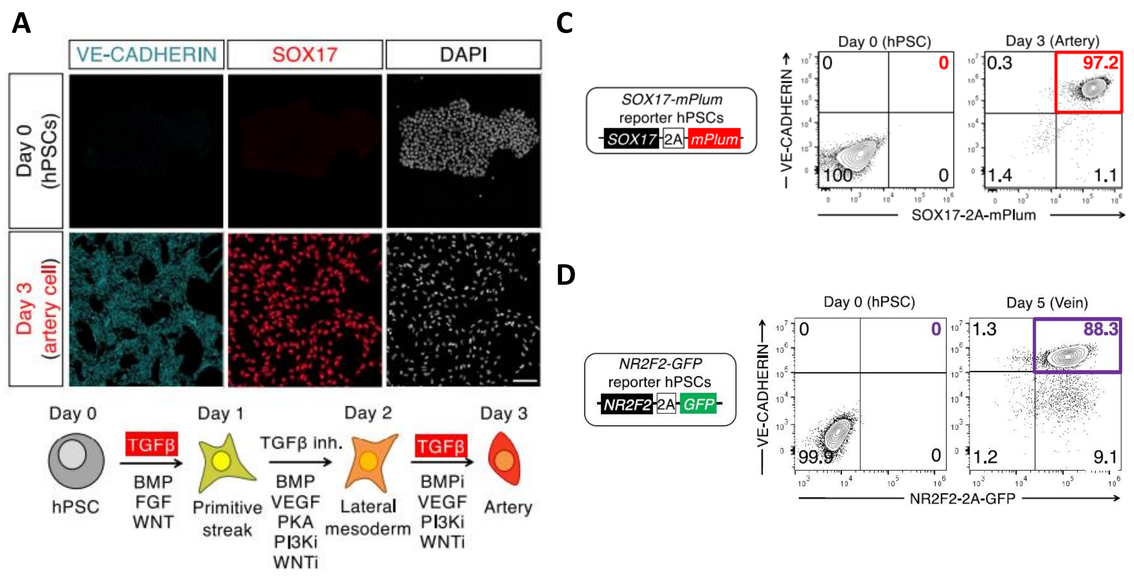

Fig 2. Efficient generation of human artery and vein endothelial cells.
(A) Immunostaining of H1 hPSCs either before or after differentiation into day 3 artery ECs; scale bars, 100 mm.
(B) (E) NR2F2 and VE-cadherin immunostaining of H1 hPSC-derived day 4 vein ECs; scale bars, 100 mm.
(C) Flow cytometry of SOX17-2A-mPlum hPSCs before or after differentiation into day 3 artery ECs.
(D) Flow cytometry of H1 NR2F2-2A-GFP hPSCs before or after differentiation into day 5 vein ECs.

hPSC-derived artery and vein ECs executed hallmark EC functions *in vivo*.

hPSC-derived endothelial cells were transplanted into **SCID mice** to test their viability ***in vivo***, rather than merely ***in vitro***. Within a month, it was found that **both** venous and arterial ECs were able to form **viable vascular networks**.

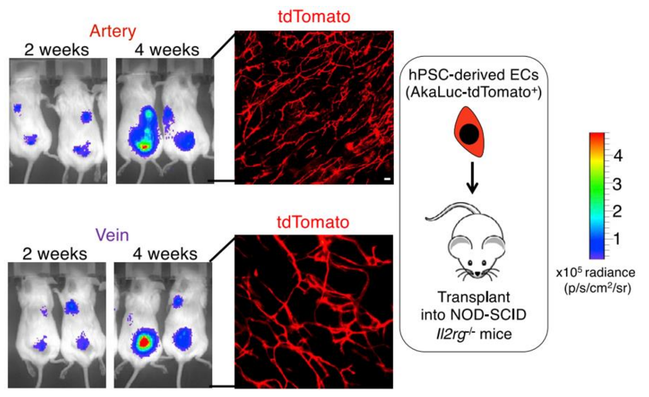

Fig 3. Visualization of vascular networks within SCID mice. *hPSC-derived ECs were transplanted into SCID mice, and were visualized via intravital imaging to determine successful proliferation of newly-implanted ECs. Microscopy done subsequently revealed that both venous and arterial cell lines were able to form vascular networks in vivo, thereby validating previous in vitro studies that showed the ability of hPSC-derived ECs to organize into viable vascular networks.*

hPSC-derived artery and vein ECs exhibited functional differences *in vivo*.

hPSC-derived endothelial cells exhibited **key functional differences** *in vivo* in SCID mice, in parameters such as **shear stress** (Fig. 4a) and **chemokine migration** (Fig. 4b).

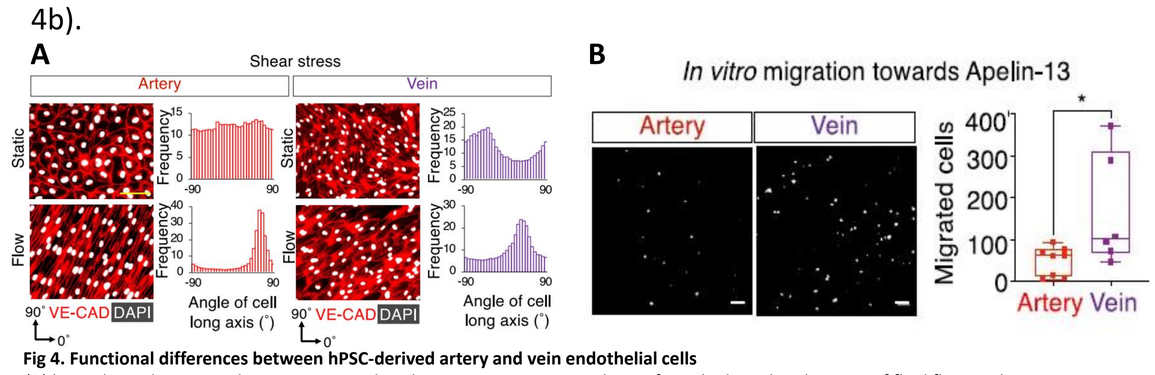

Fig 4. Functional differences between hPSC-derived artery and vein endothelial cells
(A) hPSC-derived artery and vein ECs exposed to shear stress or static conditions for 24h aligned to direction of fluid flow, with response more pronounced in arterial than venous ECs.
(B) hPSC-derived vein ECs preferentially migrate towards Apelin-13, a chemokine critical for vein migration in vivo.

Discussion

Our research charts a stepwise **road map for hPSC differentiation into artery and vein subtypes of ECs** using molecular signals. At the bifurcation between artery versus vein subtypes, we precisely induced artery ECs by repressing vein-specifying signals and vice versa. This approach generated >90% pure populations of artery- versus vein-specific ECs within 3-5 days of hPSC differentiation. Importantly, these hPSC-derived artery and vein ECs were distinguished by clear **transcriptional and functional differences**.

As an extension of this research, our laboratory is actively studying the **tropism of the Nipah and Hendra viruses** for artery versus vein ECs in vitro.

The ability to efficiently and rapidly generate artery and vein ECs ‘‘**on demand**’’ paves the way for exploring the viability of generating **full vascular parenchyma** *in vivo.* Such methodology would empower developments in **regenerative medicine, tissue engineering, and the modeling of human vascular diseases**.

References

1. Baseler, L., de Wit, E., Scott, D.P., Munster, V.J., and Feldmann, H. (2015). Syrian hamsters (Mesocricetus auratus) oronasally inoculated with a Nipah virus isolate from Bangladesh or Malaysia develop similar respiratory tract lesions. Vet. Pathol. 52, 38–45. https://doi.org/10.1177/0300985814556189.
2. Geisbert, T.W., Daddario-DiCaprio, K.M., Hickey, A.C., Smith, M.A., Chan, Y.- P., Wang, L.-F., Mattapallil, J.J., Geisbert, J.B., Bossart, K.N., and Broder, C.C. (2010). Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. PLoS One 5, e10690. https://doi.org/10.1371/journal.pone.0010690.