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Cyclic GMP signalling in human ES and iPS-derived vascular cells

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Background

Previously we demonstrated the differentiation pathway of mouse, monkey and human ES cells to vascular cell components [1-3]. In addition, we showed the therapeutic potential of these ES-derived vascular cells. When transplanted in mouse hind-limb ischemia model, these cells were incorporated in host vessels in the ischemic lesion and contributed to host neovascular formations [4]. In these our studies, however, primary cultured human adult vascular endothelial cells were not incorporated in host vessels and did not have same therapeutic effect as ES-derived vascular endothelial cells (See Figure 1). In addition, ES-derived vascular endothelial cells had higher viability against oxidative stress and higher ability to re-endothelization in wound-healing model than adult vascular endothelial cells. We supposed that ES-derived young vascular endothelial cells have higher ability as the cell source for vascular regeneration than adult aged vascular endothelial cells. On the other hand, we previously reported significance and therapeutic potential of the natriuretic peptides/cGMP/cGMP-dependent protein kinase pathway in vascular regeneration. Natriuretic peptides (NP) significantly stimulated capillary network formation of cultured endothelial cells by cGK stimulation and subsequent Erk12 activation [5]. Therefore, we are now investigating the difference of NO/cGMP and NP/cGMP pathway between ES-derived vascular cells and adult vascular cells. In addition, human induced pluripotent stem (iPS) cells are a novel stem cell population derived from human adult somatic cells through reprogramming using a defined set of transcription factors [6]. Our aim was to determine the features of the directed differentiation of human iPS cells into vascular endothelial cells and mural cells, and to compare that process with human ES cells.

Results

We previously established a system for differentiating hES cells into vascular cells. We applied this system to human

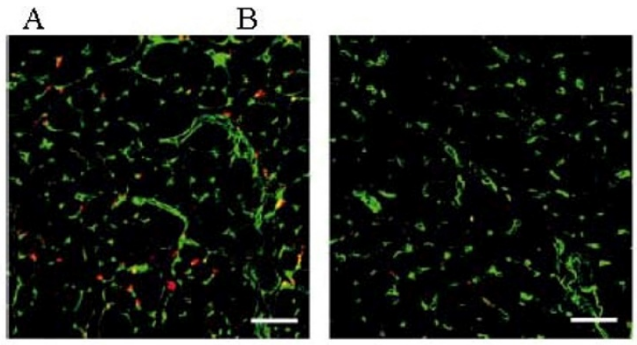

Figure 1
Incorporated human ES-derived endothelial cells at the sites of regeneration. **A**: Transplanted human ES-derived endothelial cells in mouse ischemic hindlimbs were detected by fluorescence stereomicroscope. **B**: Transplanted human adult endothelial cells. Red: human CD31 Green: host mouse CD31. Scale Bar: 50 μm.

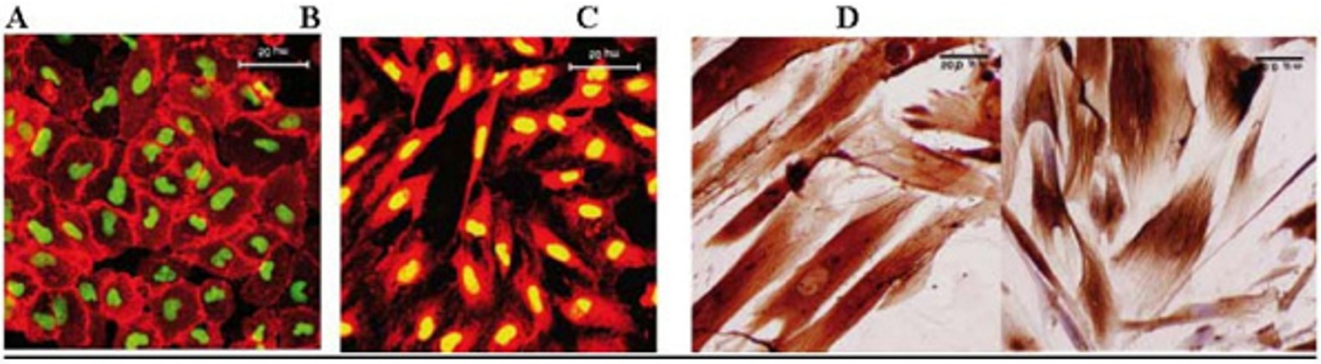

Figure 2
A, B: Immunostaining of human iPS-derived VEcadherin$^+$ cells: red, CD31 (**A**) or eNOS (**B**) **C**, **D**: Immunostaining of human iPS-derived VEcadherin$^-$ Flk1$^+$ cells for mural cell markers: **C**, αSMA; **D**, calponin Scale bar: 50 μm.

iPS cells and examined their directed differentiation. After differentiation, TRA1-60$^-$ Flk1$^+$ cells emerged and divided into VE-cadherin-positive and -negative populations. The former were also positive for CD34, CD31 and eNOS, and were consistent with ECs. The latter differentiated into MCs, which expressed smooth muscle α-actin and calponin after further differentiation (See Figure 2). The efficiency of the differentiation was comparable to that of human ES cells. In addition, we compared the gene expressions such as eNOS, CNP and CGK of human ES-derived or human iPS-derived endothelial cells with those of human adult endothelial cells to clarify the difference of characters between them.

Conclusion

We succeeded in inducing and isolating human vascular cells from iPS cells and indicate that the properties of human iPS cell differentiation into vascular cells are nearly identical to those of hES cells. We also compared them with human adult endothelial cells in relation to the difference of NO/cGMP and NP/cGMP pathway. This work will contribute to our understanding of human vascular differentiation/development and to the development of vascular regenerative medicine.

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