Multifactorial Optimizations for Directing Endothelial Fate from Stem Cells.

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Public Summary:
Embryonic stem cells (ESC) and induced pluripotent stem (iPS) cells are attractive in vitro models of vascular development, therapeutic angiogenesis, and tissue engineering. However, distinct ESC and iPS cell lines respond differentially to the same microenvironmental factors. Developing improved/optimized differentiation methodologies tailored/applicable in a number of distinct iPS and ESC lines remains a challenge in the field. Currently published methods for deriving endothelial cells (EC) robustly generate high numbers of endothlelial progenitor cells (EPC) within a week, but their maturation to definitive EC is much more difficult, taking up to 2 months and requiring additional purification. Therefore, we set out to examine combinations/levels of putative EC induction factors-utilizing our stage-specific chemically-defined derivation methodology in 4 ESC lines including: kinetics, cell seeding density, matrix signaling, as well as medium treatment with vascular endothelial growth factor (VEGF), and basic fibroblast growth factor (bFGF). The results indicate that temporal development in both early and late stages is the most significant factor generating the desired cells. The generation of early Flk-1+/KDR+ vascular progenitor cells (VPC) from pluripotent ESC is directed predominantly by high cell seeding density and matrix signaling from fibronectin, while VEGF supplementation was NOT statistically significant in more than one cell line, especially with fibronectin matrix which sequesters autocrine VEGF production by the differentiating stem cells. Although some groups have shown that the GSK3-kinase inhibitor (CHIR) can facilitate EPC fate, it hindered the generation of KDR+ cells in our preoptimized medium formulations. The methods summarized here significantly increased the production of mature vascular endothelial (VE)-cadherin+ EC, with up to 93% and 57% purity from mouse and human ESC, respectively, before VE-cadherin+ EC purification.

Scientific Abstract:
Embryonic stem cells (ESC) and induced pluripotent stem (iPS) cells are attractive in vitro models of vascular development, therapeutic angiogenesis, and tissue engineering. However, distinct ESC and iPS cell lines respond differentially to the same microenvironmental factors. Developing improved/optimized differentiation methodologies tailored/applicable in a number of distinct iPS and ESC lines remains a challenge in the field. Currently published methods for deriving endothelial cells (EC) robustly generate high numbers of endothlelial progenitor cells (EPC) within a week, but their maturation to definitive EC is much more difficult, taking up to 2 months and requiring additional purification. Therefore, we set out to examine combinations/levels of putative EC induction factors-utilizing our stage-specific chemically-defined derivation methodology in 4 ESC lines including: kinetics, cell seeding density, matrix signaling, as well as medium treatment with vascular endothelial growth factor (VEGF), and basic fibroblast growth factor (bFGF). The results indicate that temporal development in both early and late stages is the most significant factor generating the desired cells. The generation of early Flk-1+/KDR+ vascular progenitor cells (VPC) from pluripotent ESC is directed predominantly by high cell seeding density and matrix signaling from fibronectin, while VEGF supplementation was NOT statistically significant in more than one cell line, especially with fibronectin matrix which sequesters autocrine VEGF production by the differentiating stem cells. Although some groups have shown that the GSK3-kinase inhibitor (CHIR) can facilitate EPC fate, it hindered the generation of KDR+ cells in our preoptimized medium formulations. The methods summarized here significantly increased the production of mature vascular endothelial (VE)-cadherin+ EC, with up to 93% and 57% purity from mouse and human ESC, respectively, before VE-cadherin+ EC purification.