Sequential roles of BMP-4, VEGF and FGF-2 in generation of CD34⁺ vascular progenitor cells from human embryonic stem cells under animal product-free condition

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Human embryonic stem cells (hESCs) not only have enormous potential as a source of therapeutic tissues, but also provide a unique system for studying human embryonic development, including hematopoietic and cardiovascular lineage commitment. However, there are scientific obstacles that need to be overcome before clinical applications, such as animal product contamination, isolation of desired cell types, and availability of suitable animal models to test cell function. Recently, we established an efficient hESC system for the generation of CD34⁺ progenitor cells (*Nat Biotechnol*, 2007). Under specific conditions, CD34⁺ cells generated from hESCs differentiated into cells with a molecular and functional phenotype of hematopoietic and endothelial cells. In an *in vivo* SCID mouse model, we demonstrated that these hESC-derived endothelial cells self-assemble into functional blood vessels, and integrate into the recipient's circulatory system. A major roadblock toward the goal of human clinical therapies using hESCs is establishing culture conditions that do not contain animal products that may contain pathogens to humans. To advance the potential clinical application, we established an animal product-free condition to obtain CD34⁺ vascular progenitor cells from hESCs. hESCs were cultured on human feeder cells and induced to generate CD34⁺ progenitor cells in a serum-free medium. The addition of VEGF and FGF-2, or BMP-4 alone in serum-free medium was insufficient to induce CD34⁺ cells, whereas the combination of VEGF, FGF-2 and BMP-4 increased CD34⁺ cells synergistically. Our studies demonstrated that BMP-4 was important to promote CD34⁺ progenitor cells at early differentiation stage, mainly before day 6, whereas VEGF and FGF-2 had effects on CD34⁺ vascular progenitor cell development in the later stage. The addition of BMP-2 and BMP-7 promoted CD34⁺ cells in less effectiveness than BMP-4, whereas BMP-9 had no effect on CD34⁺ progenitor cell development. In addition, TGF- β or Activin promoted hESC differentiation to CD34⁺/CD31⁻ cells that were unable to give rise to endothelial cells. These data demonstrate the distinct roles of BMPs and TGF-beta in directing hESC differentiation to CD34⁺ vascular progenitor cells in animal product-free condition, and provide a platform of potential clinical application for transplantation.

Keywords: human embryonic stem cells, vascular progenitor cells, CD34

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