Evolutionary Conservation of Human CD34⁺ Cell Endothelial Differentiation in the Zebrafish Embryo

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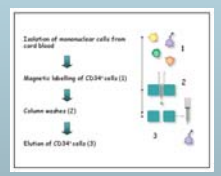
BACKGROUND AND AIM: Zebrafish is a well established model to study the molecular mechanisms that regulate vertebrate angiogenesis. In the present study, we used the zebrafish embryo as a novel tool to investigate both the angiogenic potential and the evolutionary conservation of human endothelial progenitor cells (EPCs) fate. Circulating EPCs differentiate into mature endothelial cells and give rise to blood vessels sprouts by improving angiogenesis, both *in vitro* and *in vivo*, showing a specific endothelial commitment. Hematopoietic and vascular cells arise from a common progenitor cell named the "hemangioblast". In the zebrafish gastrula, as well as in other vertebrates, bipotential hematopoietic stem cells (HSCs) arise from the ventral mesoderm, migrate, colonize the hematopoietic sites, and differentiate into both vascular and blood cells.

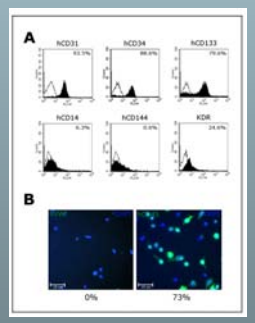
METHODS AND RESULTS: We performed a cell transplantation assay by using the transgenic TG(*fli1*:*EGFP*)^{y1} zebrafish embryo at 48 hpf and blastula stages, to test the differentiation potential of hCD34⁺ stem cells isolated from human umbilical cord blood. In the 48 hpf zebrafish embryo, hCD34⁺ cells injected into the vascular system circulate and differentiate into hCD31⁺ and hVWf⁺ cells; furthermore, these cells induce ectopic blood vessels development.
To investigate whether hCD34⁺ cells are integrated into the embryo vasculature and function as the zebrafish hemangioblast, we transplanted these cells at blastula stage before the onset of gastrulation. Under these conditions, hCD34⁺ cells migrate and co-segregate at tailbud stage with zebrafish HSCs, and subsequently populate the cardiovascular system. Furthermore, hCD34⁺ derived-cells are detected in the blood flow as well as in the caudal vein plexus, the embryonic hematopoietic site, supporting the hypothesis that hCD34⁺ progenitors might have the potential to give rise to both vascular and blood cells.

CONCLUSIONS: We show that circulating hCD34⁺-derived cells maintain their endothelial commitment in the zebrafish circulatory system, and behave as the embryonic hemangioblast after blastula stage transplantation. Our results provide the first *in vivo* evidence of an evolutionary conservation of hCD34⁺ progenitor cells fate in the developing zebrafish embryo. Furthermore, they indicate the zebrafish model as a powerful and unique screening system for the analysis of human stem cells plasticity.

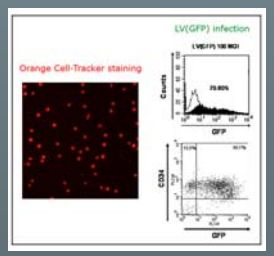
1. Isolation of endothelial progenitor cells hCD34⁺ from human cord blood



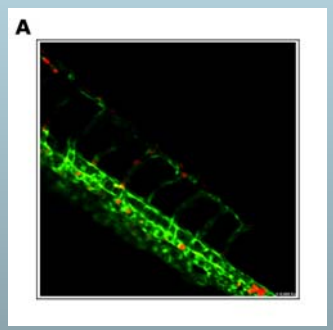
hCD34⁺ stem cells immunophenotype



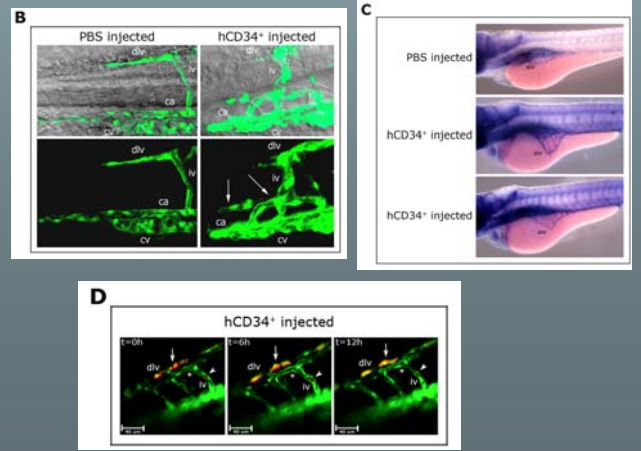
hCD34⁺ labelling prior transplantation into the zebrafish embryo



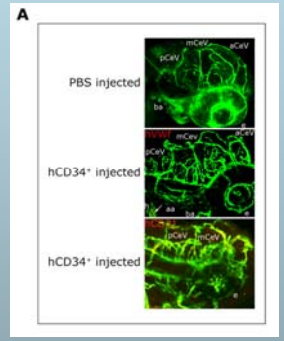
2. hCD34⁺ cells injected into the sinus venosus at 48 hpf circulate and show homing events

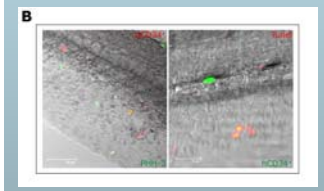


Transplanted hCD34⁺ cells induce ectopic blood vessels development 24 hours after injection

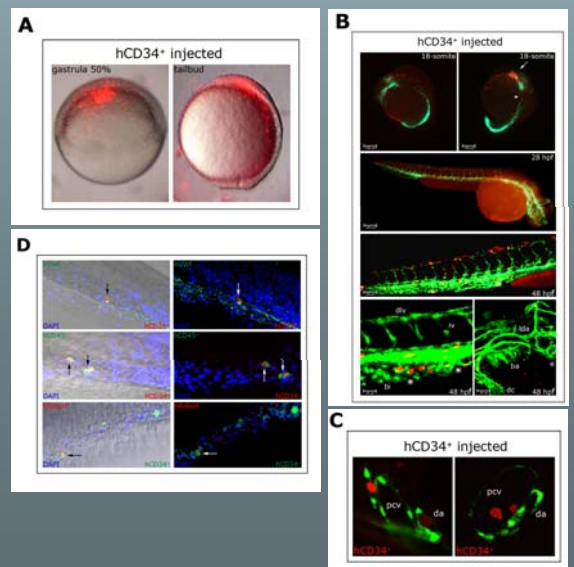


3. Transplanted hCD34⁺ cells differentiate into hCD31⁺ and hVWf⁺ cells 24 hours after injection

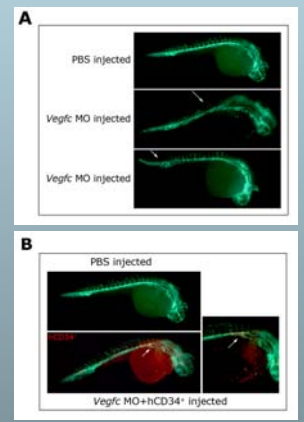




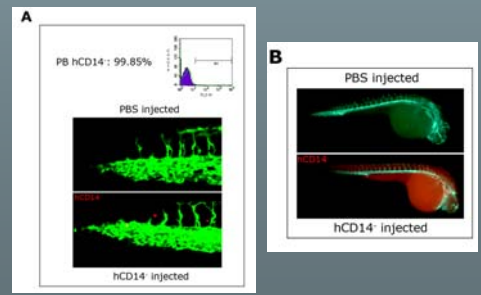
4. hCD34⁺ cells injected into the zebrafish blastula differentiate, are incorporated into the cardiovascular system, and function as the zebrafish hemangioblast



5. Blastula-transplanted hCD34⁺ cells show an angiogenic activity that partially rescue the *Vegfc* MO-induced vascular phenotype



6. A negative control: injection of hCD14⁻ cells from PB into the zebrafish blastula



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