

**Haematopoietic stem cells derive directly from aortic endothelium during development.**

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**Authors:** Julien Y Bertrand, Neil C Chi, Buyung Santoso, Shutian Teng, Didier Y R Stainier, David Traver

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**Public Summary:**

**Scientific Abstract:**

A major goal of regenerative medicine is to instruct formation of multipotent, tissue-specific stem cells from induced pluripotent stem cells (iPSCs) for cell replacement therapies. Generation of haematopoietic stem cells (HSCs) from iPSCs or embryonic stem cells (ESCs) is not currently possible, however, necessitating a better understanding of how HSCs normally arise during embryonic development. We previously showed that haematopoiesis occurs through four distinct waves during zebrafish development, with HSCs arising in the final wave in close association with the dorsal aorta. Recent reports have suggested that murine HSCs derive from haemogenic endothelial cells (ECs) lining the aortic floor. Additional in vitro studies have similarly indicated that the haematopoietic progeny of ESCs arise through intermediates with endothelial potential. Here we have used the unique strengths of the zebrafish embryo to image directly the generation of HSCs from the ventral wall of the dorsal aorta. Using combinations of fluorescent reporter transgenes, confocal time-lapse microscopy and flow cytometry, we have identified and isolated the stepwise intermediates as aortic haemogenic endothelium transitions to nascent HSCs. Finally, using a permanent lineage tracing strategy, we demonstrate that the HSCs generated from haemogenic endothelium are the lineal founders of the adult haematopoietic system.

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