A *Pax3*-expressing progenitor gives rise to embryonic haematopoietic cells.

Giovanni Canu[1], Rosamaria Correra[1], Raphaël F. P. Castellan[1], Laura Denti[1], Alessandro Fantin[1,2] and Christiana Ruhrberg[1]

[1] UCL Institute of Ophthalmology, University College London, 11-43 Bath Street, London EC1V 9EL, UK
[2] University of Milan, Department of Biosciences, Via G. Celoria 26, 20133, Milan, Italy

Haematopoiesis in vertebrate embryos occurs in temporally and spatially overlapping waves, thereby producing distinct haematopoietic progenitors that collectively sustain foetal development until birth. Primitive haematopoietic progenitors are generated by yolk sac mesoderm to contribute primitive erythrocytes and macrophages, followed by multipotent haematopoietic progenitors that are generated from both extra-embryonic and intra-embryonic endothelial intermediates. These progenitors include KIT-expressing erythro-myeloid progenitors (EMPs) that bud from the yolk sac endothelium, derived from extra-embryonic mesoderm, and KIT-expressing haematopoietic stem cells (HSCs) that bud from the dorsal aorta endothelium, derived from intra-embryonic lateral plate mesoderm. By contrast, intra-embryonic paraxial mesoderm has not been shown to have haematopoietic potential, and instead gives rise to muscle and skin cells. It was therefore unexpected when a recent bulk RNAseq transcriptomic study of mouse embryo limbs showed that Cre-LoxP mediated genetic lineage tracing with the paraxial mesoderm marker *Pax3* included cell populations with a haematopoietic signature alongside the expected neural, neural crest and muscle signatures. To date, the identity of these *Pax3*-expressing cell lineage(s) remains unknown. Here, we report that *Pax3*-mediated lineage tracing labels 5% of blood cells in the circulation of midgestation mouse embryos and 20% of blood cells in the mouse foetal liver, which at midgestation becomes the main haematopoietic site, mostly fuelled by EMPs. Notably, a subset of foetal liver KIT+ CD45+ haematopoietic progenitors, which include EMPs, was *Pax3*-lineage traced and expressed the EMP marker *Csf1r.* Furthermore, the KIT+ CD45+ *Pax3*-lineage traced population contained cells with the potential to differentiate into blood cells *ex vivo*. Together with the finding that *Pax3* transcripts were detected in the blood during midgestation, our results raise the possibility that a novel and transient subset of haematopoietic progenitors akin to EMPs actively expresses *Pax3* and may contribute to foetal haematopoiesis. Our findings suggest that *Pax3*-mediated lineage tracing may be insufficient to unequivocally assign cell populations to a paraxial mesoderm origin. Further, *Pax3* should be considered as a candidate gene for mutation screening in congenital haematopoietic malignancies.