Albertí Servera and von Muenchow, DBM PhD student retreat, University of Basel, Hasliberg, Switzerland, January 17th 2014

LINEAGE-AFFILIATED SPECIFICATION EVENTS DURING EARLY STAGES OF HAEMATOPOIETIC DEVELOPMENT

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PURPOSE

LONG-TERM GOALS

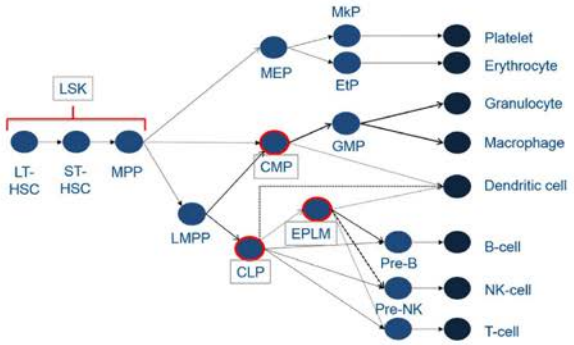
1. Contribute towards consolidating/modifying the model of haematopoiesis proposed by Brown, Ceredig and Rolink and towards resolving the debate about haematopoiesis.
2. Provide a clearer picture of early haematopoietic development at the molecular level.
3. Offer new insights in diseases caused by deregulated haematopoietic development.

SHORT-TERM GOALS

1. Transcriptome profiling of primitive haematopoietic progenitor cells such as the LSK, CLP, CMP populations and EPLM subpopulations
2. Identify the genes that play crucial roles in early stage of haematopoietic development.
3. Address the function of the by us identified genes.
4. Characterise the 3 EPLM subpopulations.

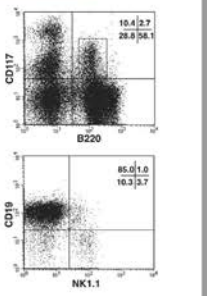
BACKGROUND

Haematopoietic hierarchical tree model



EPLM identification

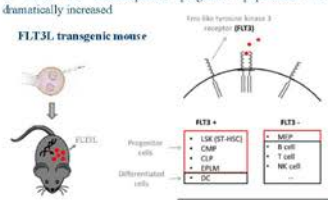
In 2005, Rolink's laboratory identified an early haematopoietic progenitor in the BM of wild-type adult mice which is B220^{+}, CD117^{+}, CD19^{-} and NK1.1^{+} with lymphoid and myeloid developmental potential. These cells represent 0.1 – 0.2% of nucleated BM cells.

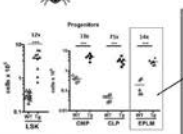


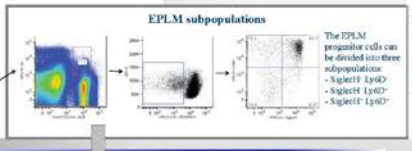
MATERIALS AND METHODS

TOOL TO STUDY THE PROGENITORS

- To overcome the low number of progenitor cells that can be isolate *ex vivo*, our laboratory generated FLT3L transgenic mice in which the haematopoietic progenitor populations are dramatically increased







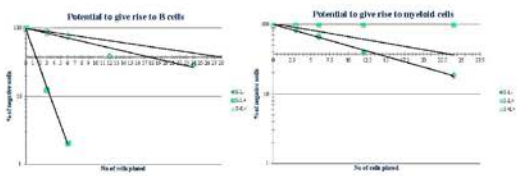
FIRST GOAL: Transcriptome profiling of the primitive haematopoietic progenitor cells: LSK, CLP, CMP populations and EPLM subpopulations.

SECOND GOAL: Identify the genes that play crucial roles in early stage of haematopoietic development.

Special emphasis will be put on differentially expressed transcription factors.

RESULTS

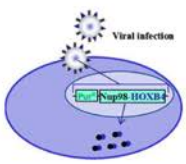
FOURTH GOAL: Characterise the 3 EPLM subpopulations.



EPLM subpopulations potential to give rise to B cells (left) or myeloid cells (right) by performing a limiting dilution assay. Cultures (48 replicates) containing graded numbers of sorted cells were plated in 96 -well plates on OP9 stromal cells in the presence of IL-7 (left) or on ST2 stromal cells without cytokines (right). After 7 days, the number of wells containing colonies was counted under an inverted microscope. S-L+ can efficiently differenciate into B cells but not into myeloid cells. S-L- and S-L+ have moderate to low potential to give rise to B cells and myeloid cells.

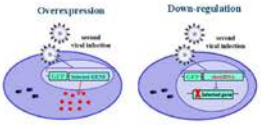
THIRD GOAL: Address the function of the by us identified genes.

Mouse bone marrow cell culture using the NUP98-HOXB4 system



HSC and progenitor cells can be extensively amplified when over-express NUP98-HOXB4 fusion protein

Stable overexpression and down-regulation of our selected genes in the progenitor cells.



Analyse the effect of the experimentally modulated expression of our genes *in vivo* (quantitatively and phenotypically).

REFERENCES

1. Balciunaite G, Ceredig R, Massa S, Rolink AG. A B220+ CD117+ CD19- hematopoietic progenitor with potent lymphoid and myeloid developmental potential. Eur J Immunol. 2005; 35(7): 2019-2030.
2. Panagiotis Tsapogas, Rolink AG. In vivo evidence for an instructive role of fms-like tyrosine kinase 3 in hematopoietic development (not published).

This research is supported by FP7 Marie Curie fellowships