In vitro generation of human hematopoietic cells

Created on: 21-08-2019 - Last modified on: 08-11-2019

Contact person

Tom Taghon

Organisation

Name of the organisation Ghent University (UGent)
Department Diagnostic Sciences
Country Belgium
Geographical Area Flemish Region

SCOPE OF THE METHOD

The Method relates to	Human health
The Method is situated in	Basic Research
Type of method	In vitro - Ex vivo
Specify the type of cells/tissues/organs	Human hematopietic stem and progenitor cells

DESCRIPTION

Method keywords

human HPCs
in vitro differentiation of hematopoietic cells
OP9-coculture
MS5-coculture
ATO system
organoid culture
FTOC

Scientific area keywords

immune deficiency
leukemia
human hematopoiesis
stem cells
gene editing

Method description

Better understanding of molecular mechanisms controlling both normal and malignant human hematopoiesis will lead to a more efficient therapy of immune deficiencies and

lymphoid leukemias. Therefore, human hematopoietic progenitor cells (HPCs) are differentiated *in vitro* towards distinct hematopoietic lineages, with or without perturbation conditions such as gene targeting, viral transductions, specific compounds or blocking antibodies. Our lab has a broad expertise in the differentiation of human T cell progenitors, for which 3 different *in vitro* techniques are available:
1) Fetal thymic organ cultures (FTOCs), using fetal thymic lobes from NOD/SCID mice as 3D-micro environment allowing human HPCs to differentiate towards T cells. ;
2) OP9-coculture system, using OP9 mouse stromal cells with or without specific Notch ligands as a 2D-layer to culture human HPCs on ;
3) Artificial Thymic Organoid (ATO) cultures, using Notch expressing MS5 mouse stromal cells in combination with human HPCs in 3D aggregates.
Furthermore, OP9- and MS5-cocultures are used in order to differentiate HPCs towards myeloid cells (dendritic cells, monocytes, granulocytes), B cells, NK cells and both erythrocytes and megakaryocytes. Differentiation of different cell types is determined using flow cytometry.

Lab equipment

Biosafety cabinet level 2 ;
Tissue culture incubator ;
Centrifuge ;
Flow cytometer.

Method status

Internally validated
Published in peer reviewed journal

PROS, CONS & FUTURE POTENTIAL

Advantages

These techniques allow to study normal or aberrant differentiation of human hematopoietic stem cells in conditions of genetic or other perturbations *in vitro*. It permits a kinetic and quantitative analysis of human hematopoietic differentiation which is difficult *in vivo*.

Challenges

The challenge of *in vitro* differentiation systems is reproducing the *in vivo* environment in which different hematopoietic cells arise. Although FTOCs and the ATO system offer a close physiological background, the use of OP9 or MS5 stromal cells also allows us to generate distinct hematopoietic cells resembling their *in vivo* counterparts. Gene targeting in human HSCs is still inefficient.

Modifications

More efficient gene targeting in human HSCs is still desired for genetic studies, as well as further modifications that lead to a closer resemblance of the *in vivo* environment.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References

Taghon T et al. Blood 2002; 99(4):1197-204.
Schmitt TM et al. Immunity 2002; 17(6):749-56.
Van de Walle I et al. Blood 2011; 117(17):4449-59.
Seet CS et al. Nat Methods 2017; 14(5):521-530.

Montel-Hagen A et al. Cell Stem Cell 2019; 24(3):376-389.

Links

lab website

Coordinated by

Financed by