ImmunoTools *special* Award 2014

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In vitro differentiation of human induced pluripotent stem cells into hematopoietic stem & lineage cells for leukemia research

Leukemia is the most common malignancy in children and accounts for 25-35% of all childhood cancers. Current research is hampered by the limited availability of patient samples and lack of appropriate model systems. Conventional model systems utilize immortalized and transformed cell lines or animal models where the degree of comparability to humans is unknown and maybe not as high as hoped for.

Thus, the recent advance in generating human induced pluripotent stem cells (hiPSC) might provide an important new source of model systems that resemble human biology more closely.

Human induced pluripotent stem cells can be generated from adult somatic cells of any tissue origin via direct reprogramming. Using non-viral based transfection strategies, integration free, transient expression of the necessary transcription factors is possible.

This provides the opportunity to obtain pluripotent stem cells, from patients as well as healthy donors, which are as close to their natural state as possible and still represent the genetic background that might decide about health or disease.

If those cells can be differentiated in vitro into the cell type affected by the disease this would allow new insights into the pathogenesis and progression of the disease as well as to test future therapeutic regimen. Candidate oncogenes found in patient material could be validated and their function elucidated using those in vitro models. Furthermore, if the in vitro differentiation model system is successful, we will further

generate mutant cells for ectopic expression of candidate oncogenes to investigate whether and if so how they alter the in vitro differentiation process.

In general, hematopoiesis is quite well understood in mouse development, but less so in humans. Therefore, also in this field, valuable insights can be obtained that also might help to advance cellular therapies like stem cell transplantation in the future.

The in vitro hematopoietic differentiation of hiPSC requires tight control of the cell culture conditions as the cells need to be provided with the necessary cues to differentiate into the desired lineage. Addition of a cytokine cocktail to the cell culture medium is one of the most promising ways to do so. Therefore, the cytokines rh FGF-b / FGF-2, rh Flt3L /CD135, rh IL-3, rh IL-6, rh IL-7, rh SCF, rh TPO and rh VEGF-A/VEGF-165 provided by ImmunoTools offer us the possibility to reach our goal of differentiating hiPSCs towards blood cells and investigating leukemic oncogenes.

Furthermore, we need to monitor the differentiation status of the hiPSC to distinguish between pluripotent progenitor cells, committed progenitor cells and lineage specific cells. This is possible with multicolor flow cytometry. As we expect a high demand of antibodies specific for hematopoietic cells such as CD3, CD10, CD11b, CD14, CD19, CD20, CD21, CD29, CD31, CD34, CD38, CD43, CD45, CD45RA, CD62L, CD147 and CD235a provided by ImmunoTools it is a perfect opportunity for us to establish routine protocols for evaluating the differentiation status on a regular basis.

ImmunoTools *special* AWARD for **Anna-Maria Husa** includes 25 reagents

FITC - conjugated anti-human CD19, CD29, CD43, CD45RA,

PE - conjugated anti-human CD11b, CD21, CD34, CD45, CD235ab,

PerCP - conjugated anti-human CD20,

APC - conjugated anti-human CD3, CD10, CD14, CD31, CD38, CD62L, CD147,

recombinant human cytokines: rh FGF-b / FGF-2, rh Flt3L /CD135, rh IL-3, rh IL-6, rh IL-7, rh SCF, rh TPO, rh VEGF-A/VEGF-165 DETAILS more AWARDS