ImmunoTools *special* Award 2015

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Receptor protein-tyrosine phosphatase controlling activity of the oncoprotein FLT3 ITD

Acute Myeloid Leukaemia (AML) is a heterogeneous group of diseases caused by combination of several genetic lesions. In 25 - 30 % of AML patients the oncoprotein FLT3 (Fms-like tyrosine kinase) carries internal tandem duplication mutations which lead to constitutive active kinase activity. Protein-tyrosine phosphatases (PTP) counteract the receptor protein tyrosine kinase (RPTK)-mediated signalling. Previous studies of my research group showed that the two transmembrane (receptor-like) protein-tyrosine phosphatases (RPTP) PTPRJ/DEP-1 and PTPRC/CD45 RPTP act as negative regulators of wild type FLT3 *in vitro.* Density enhanced phosphatase 1 (Dep1, encoded by *PTPRJ*) is present in all haematopoietic lineages. Positive and negative regulation of different signal transduction pathways was reported in several cell types including thymocytes, neutrophils, T-lymphocytes. CD45 is a well-established cell surface marker in the haematopoietic compartment. It has an essential role in regulation B and T cell antigen receptor-mediated signalling. Loss of functional CD45 results in immunodeficiency disorders (SCID). The knock down of DEP-1 in FLT3 ITD-transformed cells did, however, neither affect FLT3 ITD phosphorylation nor cell transformation. We could demonstrate that due FLT3 ITD mediated induction of reactive oxygen species (ROS) DEP-1 was partially inactivated by reversible oxidation.

The aim of my current studies is the further validation of the role of these RPTP on the signalling activity of FLT3 ITD. No one hand side we inactivate the RPTP encoding genes *Ptprj* and *Ptprc* using CRISPR/Cas9-mediated genomic editing of hematopoietic cells expressing FLT3 ITD. In parallel agonist-mediated activation of RPTP Dep-1 in combination with ROS quenching agents will be used to impair oncogenic FLT3 ITD activities.

For *in vivo* studies effects of RPTP deficiency and RPTK activation will be investigated in FLT3 ITD knockin mouse strains. FLT3 ITD expressing mice do not show severe haematopoietic aberrancies. Similarly, the depletion of PTPRJ or PTPRC does not result in leukeamic-like diseases. Our first data indicate that the depletion of PTPRJ in FLT3 ITD mice induces myoproliferative diseases of leukaemia-like nature. These data reveal that PTPRJ counteracts FLT3 ITD activity. Therefore, we are going to combine the genetic knock out of *Ptprj* and/or *Ptprc* with FLT3 ITD. To examine the consequences of RPTP deficiency and RPTK activation as well as combinatorial effects the genetic modified mice are monitored by analysis of peripheral blood each two weeks and body weight weekly. If anomalies and significant loss of weight are recognized mice will be analysed for their blood cell composition in peripheral blood as well as in bone marrow and spleen are analysed by flow cytometry. The number of B (B220) and T cells (CD3e), the amount of monocytes, granulocytes (CD11b, Gr-1) and lineage negative cells ($CD11b^-$, $Gr\text{-}1^-$, $CD45R^-$, $CD3e^-$, $Ly76^-$, $CD19^-$) will be determined. In addition, the percentage of haematopoietic stem cells and multipotent progenitors (c-kit, Sca-1) will be investigated. After the determination of hematopoietic abnormalities lineage specific analysis of cell will be carried out. In particular, the stage of development of B (CD19, CD40) and T (CD3δ CD4 CD8) cells for disturbances in differentiation processes will be characterised further.

To investigate the regulatory role of protein tyrosine phosphatases on the oncoprotein FLT3 ITD and the effect on lymphoid lineage differentiation the reagents from ImmunoTools would be very helpful.

ImmunoTools *special* AWARD for **Anne Kresinsky** includes 25 reagents

FITC - conjugated anti-mouse CD3e, CD4, CD8a, CD9, CD11b, CD18, CD19, CD25, CD44, CD62L

PE - conjugated anti-mouse CD3e, CD4, CD8a, CD11b, CD19, CD25, CD44, CD49d

APC - conjugated anti-mouse CD3e, CD8a, CD11b, CD19, CD25, CD44, NK-cells,

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