

CRISPR/Cas9 – Mediated Production of Genetically Modified T Cells

Cristina Hefti

Master thesis in Medicine

The movement of immune cells plays a key role in the performance of the immune response. Cells must have the ability to adapt their movement capability based on the tissue they are in and based on the activation state. External stimuli are important influences on the cell kinetic, by that the cell creates protrusions or stays in the same area. Positive and negative regulators of the Rho family of small GTPases, including the so-called guanine exchange factors (GEFs) and GTPase-activating proteins (GAPs), play critical roles for immune cell migration. At the moment, little is known about the function and role of RhoGTPase regulators in cell movement. ArhGEF1 plays an important role in immune functions and in cell homeostasis of Marginal Zone B cells, whereas ArhGEF2 showed an impact in the regulation of the tail retraction during cell migration. Fam49b is an atypical Rac GTPase regulator highly expressed in T cells. Yet, which roles these factors play for antiviral CD8+ T cells is unknown to date, mainly owing to a lack of genetic models. Through mastering of the CRISPR/Cas9 method precise mutations and gene inactivation via deletions and insertions are made possible. Thus the aim of this work is to develop CRISPR/Cas9 modified CD8 T cells and determine the role in the cell movement of ArhGEF1, ArhGEF2 and Fam49b genes. Unfortunately, CRISPR/Cas9 modification did not result in effective inactivation of ArhGEF1, ArhGEF2 and Fam49b genes and even through adjusting conditions of the variables the result turned out non-effective. Hence CRISPR/Cas9 modified CD8 T cells were not successfully produced and the role of RhoGTPase regulators could not be assessed.

Director: Prof. Stein Jens, University of Fribourg