Popular Scientific Summary
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Original title of master thesis: Exploring the Potential of CRISPRa and CRISPRi to Systematically Dissect Fate Options in Hematopoiesis

Generating CRISPR Gene Manipulation Systems to Understand the Development of Blood Cells

A bacterial immune system called CRISPR can be modified to overexpress or repress any gene of interest, which makes it possible to study why some blood cells develop normally while others become diseased.

The bacterial immune system called CRISPR normally protects bacteria from viruses that want to transfer their DNA and take over the bacterial cell. This immune system uses a protein termed Cas9, which acts like a scissor and cuts out the foreign DNA that the virus inserted. A short DNA sequence called guide RNA (gRNA) that matches the foreign DNA guides the scissor to the location that needs to be cut. Therefore, the sequence of the gRNA can be changed to direct Cas9 to another target. The Cas9 protein can also be modified so that it cannot cut DNA anymore but it can still be guided to a location determined by the gRNA. This 'dead' Cas9 (dCas9) can be paired with a repressor to make genes silent so that they are not expressed anymore. The dCas9 can also be paired with an enhancer, which increases the expression of the target gene. To use these systems for research, these modified bacterial immune systems can be introduced in mice which then can work as models. These mouse models make it possible to study the development of the blood, for instance why some blood cells develop normally while others become diseased, which can lead to for example leukemia.

We generated these mouse models that have the dCas9 systems that could be used to overexpress or repress any gene of interest. To check if these models work, we tried to target genes whose effects when overexpressing or repressing them are known. Thereby, we know what are supposed to happen to the cells if the systems are working. To direct the dCas9, a gRNA targeting the chosen gene is needed. Therefore, we produced viruses that can deliver these gRNA to the cells of the mice. This process needed to be optimized to produce good viruses that can infect a lot of cells. Therefore, we tested different ways to produce virus, which could affect how many cells the virus is able to infect. These experiments led to an optimized protocol where we could produce better viruses than the ones we produced at start. However, further improvements could be done to obtain viruses that infects even more cells.

When trying to infect cells from one of the mouse models using these viruses, we found that it was not possible to overexpress our chosen gene. This is probably because the gRNA is blocked from reaching the target gene and thereby not able to guide the dCas9 to the location. To be able to use these mouse models, one must make sure that the gRNA can find and bind to its target. This is a problem that has to be solved for the system to work. Due to time constrains, we were not able to test the mouse model for silencing of genes, and this is something that will have to be done. In conclusion, these mouse models that we generated needs to be further tested and could then be a valuable tool to give insights in the development and formation of blood cells.