

HEP internship project 5

CRISPR based manipulation of fetal hemoglobin expression in adult cells and generation of an immortal erythroblast cell line

Introduction: Erythrocytes (red blood cells) make up the majority of our blood. In adults production of erythrocytes occurs in the bone marrow in a process called erythropoiesis. Here erythrocyte precursors (pro-erythroblast) undergo significant changes among which are hemoglobinization, cytoskeleton and membrane remodeling, expulsion of the nuclei and degeneration of organelles like mitochondria and the golgi apparatus. While many of these processes are uniformly regulated, hemoglobinization is a process that differs throughout erythroid ontogeny; there is a switch in expression from fetal to adult hemoglobin chains that occurs around childbirth.

In diseases like sickle cell anemia and β -thalassemia, defects in the adult hemoglobin gene cause impaired hemoglobinization that results in a lifelong dependency on blood transfusions upon switching from fetal to adult hemoglobin. Interestingly, studies of individuals with hereditary persistence of fetal hemoglobin demonstrate that this requirement of recurrent transfusion can be alleviated by increasing expression of fetal hemoglobin in adults. Consequently a broader understanding of the hemoglobin might lead to means of manipulating the switch in favor of fetal hemoglobin expression. Even though, various components of the switch have been described, the exact mechanisms remains elusive.

Aim: The aim of this study is to broaden our understanding of the hemoglobin switch. We will use CRISPR genome editing to investigate the role of KLF1 and determine if we can alter fetal hemoglobin expression by interfering with this transcription factor in adult cells. Furthermore, we will generate an immortal cell line from adult pre-erythroblasts that will have to be characterized to confirm its validity as a model for erythropoiesis.

Techniques: This project involves cell culture, molecular cloning, CRISPR genome editing, protein gels, flow cytometry.

Duration: 6-9 months. Students from the University or HLO who are looking for a dynamic and interesting internship and are interested in the above project are encouraged to contact the group leader, Emile van den Akker, by e-mail: e.vandenakker@sanquin.nl