Universität Zürich[UZH]

UNIVERSITY CHILDREN'S HOSPITAL ZURICH
*Children's Research Center CRC*

Master Thesis Project:

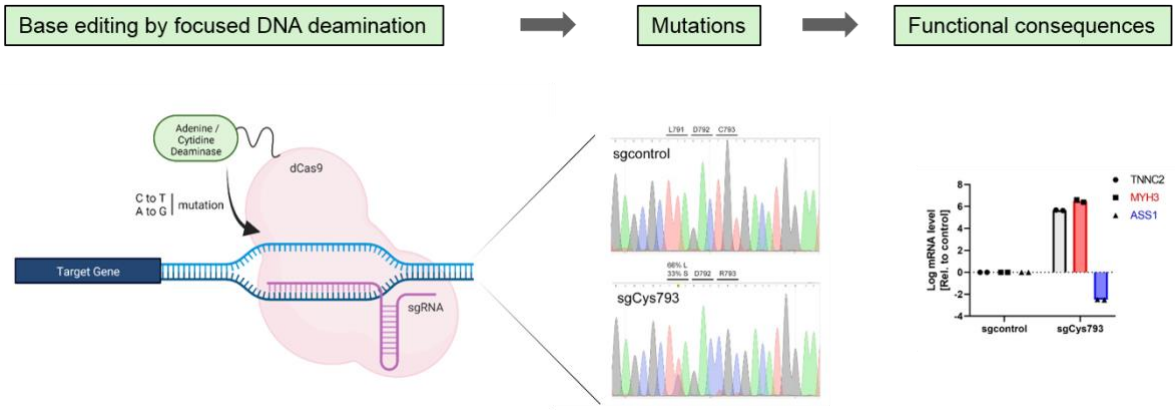
**"Base editing to define structure-function relationship in fusion transcription factors"**

Background

Fusion transcription factors (fTFs) represent a major class of oncogenes, especially in sarcoma and leukemia. The focus of our lab are pediatric sarcoma including alveolar rhabdomyosarcoma that is driven by the PAX3-FOXO1 or PAX7-FOXO1 fTF, respectively. These proteins are the main drivers of aRMS tumorigenesis and their inhibition represents a promising therapeutic approach. However, TFs in general represent challenging drug targets due to the lack of enzymatic activity and druggable structural pockets. In depth characterization of their biology, including synthesis/stability, activation, co-regulation and downstream effects might reveal possibilities for indirect therapeutic interference.

Project

In the master project offered here, we will use a CRISPR/Cas9-based base editing approach to mutate individual amino acids in the genes coding for PAX3-FOXO1 or PAX7-FOXO1 in aRMS cells. With this approach we aim to measure the contribution of each amino acid to the functionality of these proteins and identify relevant domains and sites.




We will apply a gene scanning format using an sgRNA library covering the whole gene and allowing mutation of the majority of the amino acids. sgRNAs affecting functionality of the gene will affect cell survival and drop out from the library over time, which can be detected by next generation sequencing. In parallel, amino acids of special interest such as targets for posttranslational modifications, will be mutated individually. Effects of mutations on the functionality of the TFs will be measured by gene expression profiling using qRT-PCR assays. Functional test will be used to evaluate the effect at the physiological level of the cells.

Methods

Cell culture, cloning, virus generation and transduction, pooled library screening, PCR and qRT-PCR, cell viability and cell proliferation assays, Western blot, flow cytometry.

Lab location: Balgrist Campus (https://www.balgristcampus.ch/)
The lab is part of the department of oncology of the University Children`s Hospital Zurich.

Starting date: 2023, upon agreement

Contact: PD Dr. Marco Wachtel (marco.wachtel@kispi.uzh.ch)