ABSTRACT

In this Ph.D. thesis, we aimed to focus on molecular mechanisms that underlie the roles of hexokinases in health and disease. First, we focused on the molecular basis of *GCK*-MODY and possibilities how to predict effects of variations in genes causing Mendelian disorders in general. We performed *in vitro* experiments on GCK and its variants carrying activating, neutral or inactivating variations. Subsequently, we compared these experimental results with outcomes from the state-of-the-art prediction algorithms with distinct backgrounds. As a result of analyses, we realized that the prediction algorithms commonly suffered from low specificity. Therefore, we suggested a method how to tailor numerical outcomes of these prediction algorithms in order to increase specificity. Furthermore, we determined pH optimum of human GCK and HK2 and investigated the influence of ATP concentrations on buffering capacity of commonly used buffers in hexokinase assays.

In the part concerning the role of HKs in tumorigenesis, we studied *in vitro* somatic cancer-associated variations in GCK, which did not give meaningful evidence for a role of GCK in tumorigenesis, although a subset of somatic cancer-associated variations were activating, thus potentially advantageous for tumors. Therefore, we rather moved to the study of HK1 and HK2, which have been reported as important isoenzymes for cancer cells, on the model of ovarian cancer cell line. We have prepared HK1 and HK2 knockout cell lines using CRISPR/Cas9 system. Afterwards, we studied changes of expression levels of proteins involved in metabolic and signaling pathways. We have observed changes indicating that the HK1 KO cells trigger cell survival and proliferation. Nevertheless, HK2 KO cells remain to be studied in a similar manner and further supportive experiments are about to be conducted in a near future.