ABSTRACT

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Title of diploma thesis: Preparation of STAT3-knockout glioblastoma multiforme cell line using CRISPR-Cas9 editing

Glioblastoma multiforme (GBM) is one of the most common primary brain tumours in adults and one of the most fatal cancers in humans A key oncogene in GBM development and progression is the signal transducer and activator of transcription 3 (STAT3). It is one of the mediators of tumorigenesis, plays a strong *pro-survival* role in GBM, and is permanently activated in GBM, leading to increased cell proliferation, invasiveness, and angiogenesis.

The aim of this work was to establish a GBM STAT3-knockout (STAT3-KO) cell line. The U87MG cell line and the CRISPR-Cas9 gene knockout method were used for this purpose.

In a first step, we investigated the possibility of silencing STAT3 expression by siRNA and the suitability of this cell type to pinpoint the mechanism for CRISPR gene editing into the cell. In the second step, we transfected the cells using the STAT3 gene knockout kit (CRISPR) and tested the knockout success. In the established STAT3-KO GBM cell line, we confirmed STAT3-KO at the DNA, RNA and protein levels. We then treated the U87MG and U87MG-STAT3-KO cell line with temozolomide (a chemotherapeutic drug used for GBM) and compared their sensitivity.

Finally, U87MG-STAT3-KO cells were successfully implanted into the model organism and a decrease in STAT3 marker expression was again observed.