Chapter 1

Introduction

1.1 Background

Glioblastoma (GBM) is the most aggressive form of glioma, a group of primary brain tumors in adults that originate from glial cells (Hanif et al., 2017). GBM is characterized by rapid proliferation, diffuse invasion into surrounding brain tissue, and a strong resistance to current therapeutic strategies. Despite advances in surgery, radiation, and chemotherapy, the GBM patient prognosis remains poor, with a median survival of only 15 months (Poyuan et al., 2025; Kanderi et al., 2024). A key mechanism underlying GBM aggressiveness is the aberrant reactivation of developmental transcriptional programs, particularly neural development transcription factors, which promote sustained proliferation and phenotypic plasticity (Silva et al., 2023; Mehta & Lo Cascio, 2018). As transcription factors act as central regulators of gene expression networks, dysregulation of these developmental regulators can have profound effects on tumor cell behavior. Identifying how individual developmental transcription factors function in GBM is therefore critical for understanding the molecular mechanisms that drive tumor progression and may reveal new therapeutic targets.

Transcription Factor 241 (TF241; placeholder designation used for anonymization purposes) is a homeobox-containing transcription factor classically involved in neural patterning, lineage specification, and cellular identity (Jia et al., 2026). Beyond its developmental functions, TF241 has increasingly been implicated in cancer progression, where its overexpression is associated with aggressive tumor behavior and poor clinical outcomes, including in glioma, although its direct downstream transcriptional targets remain poorly characterized (Xu et al., 2023; Jia et al., 2026). Interestingly, studies in other cancer types show that TF241 knockdown or knockout does not always suppress tumor progression; in some contexts such as pancreatic cancer, loss of TF241 is associated with increased proliferation and enhanced survival through compensatory signaling pathways that can worsen outcomes (Xu et al., 2023). These observations highlight the complex and

context-dependent role of TF241 in cancer (Beltran et al., 2013; Peluffo et al., 2020). Consequently, broad therapeutic strategies aimed at either complete TF241 overexpression or inhibition may produce unpredictable outcomes.

Accumulating evidence suggests that TF241 may function as either a transcriptional activator or repressor depending on cellular context and interacting cofactors (Beltran et al., 2013; Peluffo et al., 2020). While suppression of TF241 could reduce oncogenic transcriptional programs, it may also unintentionally inhibit tumor-suppressive or differentiation-associated functions. Conversely, TF241 overexpression may restore beneficial regulatory pathways in some contexts while simultaneously enhancing malignant phenotypes in others. This functional duality is thought to be mediated in part through conserved repression domains, including Transcription Factor 241 Homology Domain 1 (TFH1; placeholder designation used for anonymization purposes) and Transcription Factor 241 Homology Domain 5 (TFH5; placeholder designation used for anonymization purposes), which are implicated in the recruitment of co-repressor complexes to target genes (Fröbius, 2026). However, the functional significance of TFH1 and TFH5 and their subsequent biological effects in GBM is still unknown. Therefore, deletion of TFH1 and TFH5 provides a targeted approach to dissect the domain-specific functions of TF241 in GBM and may provide insight into how selective modulation of TF241 activity could be therapeutically leveraged while minimizing unintended effects associated with complete TF241 loss or overexpression.

1.2 Objective

To investigate how wild-type (WT) TF241 and TFH domain deletions influence transcriptional regulation in U87-MG cells.

- To identify potential direct target genes of TF241 for downstream transcriptional analysis.
- To generate TF241 construct variants containing deletions of the conserved repression domains TFH1 and TFH5.

- To express wild-type and TFH1/TFH5-deleted TF241 constructs in U87-MG GBM cells.
- To evaluate the effects of TFH1 and TFH5 deletion on TF241-mediated transcriptional regulation of candidate target genes using quantitative PCR (qPCR).
- To investigate whether TFH1- and TFH5-mediated repression contributes to the oncogenic transcriptional functions of TF241 in GBM

1.3 Hypothesis

Because TFH1 and TFH5 are predicted repression domains involved in co-repressor recruitment, deletion of these domains is hypothesized to disrupt TF241-mediated transcriptional repression in GBM cells. Consequently, TFH1/TFH5 deletion is expected to alter the expression of candidate TF241 target genes in U87-MG cells, resulting in transcriptional profiles distinct from those induced by the wild-type TF241. These changes may indicate a contribution of TFH1- and TFH5-mediated repression to the oncogenic transcriptional functions of TF241 in GBM.