Resolving structure and functions of RECQL4 and BLM helicases to understand consequences of deleterious genetic alterations in glioma pathogenesis

1. **Research project objectives/ Research hypothesis.** Recent whole-genome and exome sequencing studies identified numerous somatic aberrations in cancer, and many new cancer genes, that frequently act in various signaling/regulatory pathways and protein complexes Each adult tumor carries ~20–300 genes with somatic alterations to single nucleotides, short insertions, deletions, gene copy number variants and translocations and these genomic changes are amplified after radio- and chemotherapy. Gliomas represent over 70% of all brain malignancies and the most common is glioblastoma (GBM), an aggressive, highly diffusive and vascularized astrocytic tumor and one of the most difficult human malignancies to treat due to frequent dysfunction of tumor suppressors and oncogenes. Despite advances in surgery, radiotherapy and chemotherapy current therapies against malignant gliomas are not effective. Using targeted NGS sequencing of 700 genes involved in cancer we analyzed low grade and high grade gliomas. Exclusively in GBM patients, we found novel, potentially deleterious genetic alterations in *RECQL4* and *BLM* genes coding for DNA helicases. RECQL4 belongs to RecQ family of ATP-dependent DNA helicases consisting RECQL1, BLM, WRN, RECQL4 and RECQL5, which are participate in many aspects of DNA metabolism: replication, recombination, transcription, DNA repair and telomere maintenance. A very little is known about the role of *RECQL4* mutations in cancerogenesis, despite several reports on involvement of RECQL4 in osteosarcoma, breast and prostate cancer, and their involvement in pathogenesis of human gliomas is unknown. **We hypothesized that these newly discovered mutations in the *RECQL4* and deletions of *BLM* genes may contribute to pathogenesis of human glioblastoma or modify tumor responses to therapy**. Our preliminary results indicate functional importance of both identified mutations that could affect RecQ4L function and lead to dysfunction.

2. **Research project methodology.** We plan to produce a wild type and mutated variants of RECQL4 and **s**olve the RECQL4 crystal structure, which would allow the definition of critical protein domains and potential partner binding domains. We will characterize the biological consequences of the newly discovered *RECQL4* mutations for cell biology using various model systems: cultured glioma cells and *Xenopus* embryos, testing DNA replication, replication of mitochondrial DNA, DNA repair processes and mitochondrial bioenergetics. Using CRISPR/Cas9 knock in system we will generate glioma cell lines carrying mutated RECQL4. Basal and stress-, chemiotherapeutics-induced DNA damage/repair processes will be analyzed Cells expressing wild type and mutated RECQL4 will be analyzed The newly discovered genetic alterations in the *BLM* gene affect its expression, therefore we plan to study the *BLM* mRNA and protein expression in a panel of glioma samples of different grades using cancer tissue microarrays. Since in some cellular processes during gliomagenesis RECQL4-BLM interactions may be critical, disturbances of the *BLM* gene can have a specific role in oncogenesis. We plan to test if RECQL4 and BLM may work in parallel with other protein implicated in DNA repair – PARP1 and test whether knockdown of PARP or its inhibition with specific inhibitors affect tumor cell growth and sensitize cells to chemotherapeutics. If mutated RECQL4 or upregulated BLM helicases contribute significantly to glioma pathogenesis, we will search for specific inhibitors would exert anticancer activity. We will test both NCBI library of compounds and newly synthesized inhibitors to select those with activity against RECQL4 and BLM helicases.

3. **Expected impact of the research project on the development of science, civilization and society.** The implementation of the proposed project and application of the knowledge obtained will undoubtedly provide new insights into glioma pathogenesis. Based on the roles of the RECQ proteins in non-transformed cells, namely their involvement in proliferation, the DNA damage response, DNA repair and telomere maintenance, there is growing interest in exploring roles or inhibiting these functions in susceptible cancer cells. Small molecule inhibitors to the RECQ helicases may be useful to enhance existing or develop novel anti-cancer strategies. Therefore, further investigation of RECQL4 and BLM dysfunctions in glioma pathogenesis will widen our knowledge and enhance potential for new targeted therapy of this malignancy.

4. **International collaboration.** In collaboration with **Partner 1** Prof. Matthew Guille (a head of European *Xenopus* Resource Center (EXRC) at the University of Portsmouth, UK, we will remove or replace respective exons of *RECQL4* in *Xenopus* embryos with the ones containing mutations and determine the influence of such genome editing on DNA replication, replication of mitochondrial DNA, helicase activity of embryos. We will take advantage of the libraries of compounds develop by the foreign **Partner 2 Prof. Waldemar Priebe** from University of Texas MD Anderson Cancer Center, Houston, USA and will test library of compounds and seek for novel inhibitors of dysfunctional RECQL4 and BLM in gliomas.