Gliomas are the most frequent tumors occurring in the central nervous system. Despite decades of advancement in both the understanding of their molecular pathogenesis and the clinical protocols available, malignant gliomas remain almost always fatal. None of the current state-of-the-art treatments for malignant glioma could be regarded as effective. The most promising in anti-glioma therapy is a sequence-specific inhibition of therapeutic targets, such as tenascin-C (TN-C), by RNA interference (RNAi) technology using double-stranded RNA (dsRNA) specific for TN-C mRNA. However, there are obstacles in the application of RNAi therapy in systemic administration resulting from negative charge of RNA and its susceptibility to degradation. Development of nucleic acid carrier that would deliver dsRNA cargo into tumor cells with high efficiency still remains a great challenge. Over the last few decades nanotechnology has developed from an intriguing science fiction idea to the present level. Currently nanotechnology facilities the development of innovative technologies and examples of objects designed and manufactured at the nanoscale, demonstrating the enormous potential of this science. The *nano-* refers to the size that nanoparticles reach, i.e. the order of 10^{-9} m. For comparison, 1 nm is one thousandth of the bacteria length or 10 carbon atoms arranged in one row. Human hair has a width of approx. 80 000 nm and blood cell approx. 7 000 nm. Nanotechnology offers multifunctional delivery systems which could not only cross the blood-brain barrier (BBB) but also overcome drawbacks of classical cancer therapy and could improve existing treatment methods. The latest generation of nanomaterials for nanomedicine allow also to combine conventional methods with other treatment approaches like photothermal therapy what results in synergistic effect of treatment. ***The aim of this project is the synthesis and characterization of new nanocomposites based on inorganic and polymeric nanomaterials for delivery of dsRNA to brain tumor cells as well as the assessment of their effectiveness in combined RNA interference gene silencing and photothermal therapy of glioblastoma multiform.***

The project will be realized at the NanoBioMedical Centre (NBMC) of Adam Mickiewicz University in cooperation with the Partner - the Institute of Bioorganic Chemistry Polish Academy of Sciences. Firstly, magnetic nanoparticles, gold nanoparticles and mere polydopamine particles will be synthesized. Inorganic core of the particles will be covered with polydopamine and the obtained nanomaterials will be functionalized with polyethylenimine (PEI). After each step nanomaterials will be characterized using advanced physical methods. The biological studies will involve assessment of nanomaterials cytotoxicity on glioblastoma cell line (metabolic activity measurements, live/dead cell assay, measurements of reactive oxygen species and DNA-double strand breaks), synthesis of double-stranded RNA (dsRNA), determination of binding capability of dsRNA, examination of the stability and release of dsRNA from complexes, the cellular uptake and localization of NPs@PEI/dsRNA complexes inside the cell using confocal microscopy and transmission electron microscopy. Further analysis will include profound molecular biology studies in order to evaluate the effectiveness of gene therapy using determination of TN-C level by RT-PCR, real time PCR and western blotting as well as to monitor epigenetic alterations by DNA-methylation changes and ROS level by HPLC-ECD assay. The permeability of nanocomposites through blood-brain barrier using established models will be also investigated. The final step will be the experiments on simultaneous application of photothermal and RNA interference therapy in order to determine effectiveness of proposed treatment method.

Glioblastoma multiform is one of the most infiltrating, aggressive, and poorly treated brain tumors. The highest incidence is in the older population and the 2 year survival rate is less than 3%. The median survival is only 12–15 months for patients with glioblastomas. Therefore, tremendous efforts have been made to develop effective therapeutic strategies for anti-tumor therapy in glioma treatments. ***The implementation of the therapy based on RNA interference may contribute to improvement of overall survival and quality of life of patients with brain tumor.*** The development of appropriate carriers is crucial for practical applications of dsRNAs as therapeutics. This project aims to develop highly efficient system based on multifunctional nanomaterials for delivery of double-stranded RNAs into glioblastoma cells. We propose here the silencing of a gene responsible for expression of ***extracellular matrix protein*** involved in tumor cell adhesion, invasion, migration and proliferation. This is relatively new approach as compared to inhibition of protein expression localized inside the cell. The studies included in this project will help answer the questions, which of the synthesized nanomaterials will be the most promising in *in vitro* gene silencing therapy and whether they can cross the blood-brain barrier. Furthermore, the presence of polydopamine which exhibits the photothermal properties will allow to improve the efficiency of the synergistic therapy in terms of cancer cell death. To our best knowledge, there is no report which would describe the combination of such experiments on glioblastoma.