1/ Research project objectives/Research hypothesis. Tumor cells should be recognized and eliminated by the immune system. Yet, cancers that are detected clinically must have evaded antitumor immune responses to grow progressively. The involvement of the immune system in the host response to tumors is complex and involves both lymphocytes and lymphocyte-derived mediators, as well as tissue macrophages. Microenvironment-specific signals activate and polarize macrophages (tissue sentinels) to perform dedicated functions. In tumor microenvironment, macrophages accumulate and instead of fighting tumor, they are polarized into cells supporting tumor progression. The signals they send immobilize antitumor immunity, which is difficult to overcome and could be a big obstacle in immunotherapy. Central nervous system has own resident macrophages (called microglia) that support homeostasis, participate in neuroprotection and repair, and are effective guardians against infection and injury. However, microglia and peripheral macrophages accumulate in malignant brain tumors and undergo polarization to the pro-invasive, immunosuppressive phenotype in which these cells support tumor progression. We found that cultured microglia pre-exposed to glioma cells acquire a "*trained memory"* and display reduced responses after subsequent pro-inflammatory stimulation suggesting the existence of "*memory of tumor encounter*". Our preliminary studies show that this memory is fixed by epigenetic mechanisms that control opening or closing of chromatin structure giving or denying access of the transcriptional machinery to the genes. **We propose that tumor derived molecules induce changes in responsiveness to subsequent stimuli and create "*trained memory of brain macrophages"* resulting in blockade of antitumor responses.** Molecular mechanisms of this *trained memory* are largely unknown. So, in this project we would like study molecular mechanisms underlying this phenomenon in two animal models of experimental gliomas studying in the whole genome scale changes in the chromatin structure that allow gene expression to be upregulated or silenced.

2/ Research project methodology. We will analyze profiles of histone modifications and open chromatin marking accessible regions using molecular biology methods best suited for such analyses (ATAC-seq, ChIP-seq, RNA seq) and based on efficient, parallel sequencing of all genes. We will study those changes in microglia/ macrophages (GAMs) infiltrating murine gliomas to understand molecular mechanism governing gene expression regulation We will use two most frequently used experimental glioma models: murine Gl216 glioma cells implanted to brains of C57BL/6 mice and human U87 glioma cells implanted to immunocompromised mice. The use of two models will inform us if the mechanisms are universal or cell line specific. The epigenetic changes are based on either DNA methylation or chemical modifications of histones, structural proteins around which genomic DNA is wrapped. Mapping those modifications in whole genome scale we will obtained a full picture of complex events. Based on the distribution of histone modifications and openness of chromatin, we will map the regulatory elements in tumor infiltrating brain macrophages. We will assess the impact of tumor microenvironment on the immune system and identify several potential regulators tumor induced trained memory. We test some drugs that are supposes to block epigenetic enzymes with expectation to erase epigenetic marks and block pro-tumorigenic polarization of the immune system. We will verify if the observed changes appear in human patient samples from malignant brain tumors.

3/ Expected impact of the research project on the development of science.

Collectively, the planned experiments will show which regions control gene expression crucial for polarization of brain macrophages into pro-tumorigenic cells blocking antitumor immunity. We will provide description of mechanisms that blockade of antitumor immunity in malignant gliomas, and define how chromatin state dynamics allows for polarization of immune cells within tumors. These are pioneering studies as epigenetic mechanisms of tumor trained innate immunity are unknown. We will show how local tumor microenvironment likely contributes to shaping chromatin openness and active transcription in brain macrophages and evokes *trained immune memory*. Our experiments with histone modifying enzyme inhibitors will show if pharmacological interference may help to erase tumor *trained immune memory* and modulate immune responses in tumor microenvironment and host immunity. The means by which the dysregulation of chromatin landscape and transcription factors in tumor polarized macrophages can lead to impairment in their sentinel functions may direct the exploration of new therapeutic strategies to stimulate or inhibit the appropriate macrophage response. Finally, performing a similar study in humans will be highly beneficial for uncovering the mechanisms of regulatory aberrations in antitumor immunity.