PHD THESIS SUMMARY
„THE EXOSOMES` ROLES IN THE PERSONALIZED MANAGEMENT OF GLIOBLASTOMAS"

PhD Student: Georgiana-Mihaela Müller-Șerban
Scientific Supervisor: Prof. Dr. Adrian Florian Bălașa

Background

Glioblastoma, defined as a grade 4 glioma according to the latest World Health Organization classification (2021), is the most aggressive primary brain tumour occurring in adult patients. It is associated with important morbidity and mortality despite rapid diagnosis and early treatment; the current *gold-standard* treatment consists of Stupp protocol – exhaustive tumour resection, followed by adjuvant chemo- and radiotherapy. This pathology singles out due to a marked intra- and inter-individual heterogeneity; thus, the identification of reliable diagnostic and prognostic markers through the histopathological, immunohistochemical and molecular analysis of tissue biopsies has been futile so far. Lately, the liquid biopsy, a novel, minimally invasive technique, has emerged, allowing among others the isolation of glioblastoma-derived extracellular vesicles from the patients' peripheral blood. These nanostructures present various molecules at their surface and into their cargo which are typical to the donor cells and mirror their behaviour. Therefore, extracellular vesicles characterization would allow the identification of reliable and reproducible biomarkers to accurately predict the glioblastomas` evolution and to guide a more personalized treatment.

Study 1 – Prognostic factors of survival in glioblastoma patients – A retrospective study

This retrospective study aimed to characterize the glioblastoma patients admitted in the Neurosurgery Clinic of The Emergency Clinical County Hospital of Târgu Mureș from a social, demographic, clinical and paraclinical perspective, and to identify potential diagnostic and prognostic markers. Our analysis took into consideration various factors related to the patient (age, sex, pre- and postoperative clinical status), to the tumour itself (localization, dimension, histological and immunohistochemical properties – Ki67, GFAP), to the treatment (the extent of surgical resection, adjuvant therapies) and to the body`s immune response to the tumour aggressiveness mirrored by the absolute counts of immune-inflammatory cells from the peripheral circulation and their respective ratios. Similarly to other publications, our study demonstrated that the tumour size at the moment of diagnosis and the postoperative worse clinical status predict an unfavourable evolution, and that the age is

negatively correlated with both pre- and postoperative clinical status. Moreover, the preoperative leucocytosis was linked to a poor survival rate. The other results confirm the contradictory conclusions of the existent publications regarding the efficacy of these factors to diagnose and to predict the glioblastomas` behaviour.

Study 2 – Identification of distinct profiles of glioblastomas through the immunocapture of extracellular vesicles

This study characterised the glioblastoma-derived extracellular vesicles via flow cytometry method and aimed to identify an exosomal profile to predict the evolution of these patients based on the extracellular vesicles surface markers. The results demonstrated a vast interindividual heterogeneity of the epitopes` expressions. The first clustering analysis related to specific tumour biomarkers established four subgroups characterised by a stem cell phenotype, a tumour cell phenotype and an endothelial cell phenotype, respectively. The only marker capable to distinguish between a glioblastoma patient and a non-cancerous control was CD29, a molecule recognised for its main involvement in all the mechanisms favouring the aggressiveness of glioblastomas. The second clusterization focused on the immune response and highlighted two different patterns: a potential lymphocyte activation and a potential platelet activation, respectively. Thus, our study confirmed the heterogeneity of the antigenic profiles of glioblastoma patients and emphasised the necessity of a personalized therapeutical approach.

Study 3 – Extracellular vesicles-based characterisation of stem cell phenotypes in patients with glioblastomas

The scope of this study was to validate an evolutionary pattern of stem cell biomarkers associated with glioblastoma-released extracellular vesicles (e.g. NANOGP8 – molecule within the cargo of extracellular vesicles; CD44, SSEA4, CD133, CD326/EpCAM – surface biomarkers) and to link it to the behaviour of this malignancy. The expression of all molecules significantly decreased one week after surgery. Nonetheless, the long-term evolution illustrated by the markers' expression at three weeks postoperatively demonstrated extremely variable tendencies and highlighted once again the extraordinary inter- and intraindividual variability of this pathology.

Study 4 – Identification of various markers of aggressiveness in glioblastomas` patients through flow cytometry and Western blot analyses

The last study followed the dynamic evolution of the molecules associated with the glioblastoma-derived extracellular vesicles, both at their surface and within their cargo. We made a broad selection of molecules to cover all the molecular mechanisms underlying the aggressiveness of this malignancy: cellular proliferation (EGFRvIII, MCSP), tumour invasion (Annexin A1, EGFRvIII, ROR1, CD24), promoters of a stem cell phenotype (ROR1, CD24), eluding the immune response (Annexin A1, PDL1, HLA-ABC, HLA-DRDPDQ), chemo- and radioresistance (Annexin A1). These pathways are often interlinked; thus, a molecular profile

comprising all these markers allows a multifactorial characterisation of glioblastoma. Our study demonstrated an important intra- and interindividual heterogeneity in the expression of these molecules over time.

PhD Thesis Originality

The originality of this PhD thesis consists in the holistic and modern approach to the glioblastoma tumorigenesis by isolating and analysing the tumour cell-derived extracellular vesicles, following a wide range of markers on the surface and inside these vesicles with a recognised role in the pathogenesis of this malignancy; it is the first study of this kind in Romania and among the few worldwide.

In addition, our study allowed, in a national first, the optimization of a protocol for the isolation of extracellular vesicles from peripheral blood by the density gradient ultracentrifugation method, as well as a protocol for the quantification of different epitopes using flow cytometry method and of proteins from the cargo of extracellular vesicles via Western blot technique.