Identification and functional characteristics of circular RNAs in glioblastoma

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One of the most prominent types of non-coding RNA – circular RNAs (circRNAs) is proposed to be a significant factor in the development and progression of several disorders, especially suggested as a key factor involved in tumorigenesis. CircRNAs role is widely linked with brain neoplasm development due to its high abundance, especially in the human brain, followed by great diversity, and tissue- as well as development-specific expression patterns. The key feature of circRNAs, which could justify their importance in tumorigenesis is also their distinctive, covalently closed structure lacking 5′-to-3′ polarity, allowing it to exert its biological functions through binding to numerous types of molecules, including RNA, DNA, and protein.

Despite intensive scientific endeavors, glioblastoma (GBM) is a highly aggressive and malignant form of brain tumor, which remains a major challenge for clinicians and scientists. Notwithstanding recent discoveries regarding the GBM genetic characteristics, the conventional treatment of GBM involves only surgical tumor extraction, followed by temozolomide-based chemotherapy and further radiotherapy. Presented treatment methods emerge as ineffective in many cases due to the commonly observed treatment resistance, which ultimately leads to tumor recurrence. Several key GBM features support the failure of effective GBM therapy discovery. One of the major causes is high GBM heterogeneity, described at a cellular, molecular, histological, and clinical level, which potentially facilitates the very different responses to therapeutic agents and failure of targeted therapies. Additionally, a small population of glioma stem cells (GSCs) present within the specific tumor niche is frequently reported to be responsible for tumor growth, progression, and metastasis. Moreover, the tumor microenvironment (TME), a complex and dynamic ensemble of tumor cells that are surrounded by several types of non-tumor cells and the non-cellular components of extracellular matrix (ECM), is the key structural component, which rearrangement, supports the tumor invasion and metastasis. Furthermore, as a result of reciprocal cell-cell and cell-ECM interactions and tumor cell hijacking of non-malignant cells, stromal cells lose their functional phenotypes that support the growth and invasion of tumor cells. The acquisition of invasive phenotype in solid tumor tissue is frequently linked with the epithelial-to-mesenchymal transition (EMT) process, characterized by the loss of cell-cell adhesion and higher migratory and invasive potential of the tumor cells. As tumor development

and its progression are highly complex processes, which lead to high genomic instability and multiple rearrangements, advanced molecular studies are still required to comprehensively understand underlying mechanisms.

Therefore, the objective of this research is to gain a more comprehensive understanding of the circRNAs role in the development and invasion process of GBM. The first part of the dissertation is devoted to the in-depth analysis of circCLIP2 function, as several reports highlight its significance in GBM onset and progression. A variety of functional assays was applied to indicate the circCLIP2 potential involvement in GBM cells proliferation, migration, and invasion, which are, on the other hand, linked with the epithelial-to-mesenchymal transition and glioma stem cells population appearance. The second part of the work is devoted to the identification of circRNAs exhibiting deregulated expression patterns in primary and recurrent GBM by RNA sequencing of GBM tissues, which were analyzed in parallel to the circCLIP2 functional analysis. The last part of the research was designated to the development and characterization of complex, three-dimensional GBM models. In the course of the research, two GBM models were generated – the GBM organoid, derived from the GBM patient tissue, and the assembloid, which states the GBM invasion model into healthy tumor-surrounding tissues. The assembloid model is comprised of the GBM organoid and the cerebral organoid grown in coculture into the assembloid. Despite the delivery of the novel research models, the aim was to generate a substitute for commonly used two-dimensional cell lines in the research related to the neoplastic invasion processes. Moreover, this model could potentially serve as a diagnostic screening platform for GBM invasion-hindering therapies.