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ROLE OF TRIM28 MULTIDOMAIN PROTEIN IN THE MAINTENANCE OF CANCER STEM CELL POPULATION

Overcoming resistance to chemotherapy and radiotherapy in solid tumors is one of the fundamental issues of clinical oncology. Considerable responsibility for resistance to conventional treatments, as well as the processes of metastasis and relapse, has been attributed to the existence of cancer stem cells (CSCs). These cells, also known as tumor-initiating cells (TICs) are rare within the tumor and exhibit stem cell properties such as the capacity of self-renewal, pluripotency, highly tumorigenic potential and resistance to therapies. Therefore, it is essential to find therapeutic approaches that would eradicate CSC population.

The main objective of the study is to analyze the role of TRIM28 protein in regulation of cancer stem cell (CSC) population and consequently, to point at TRIM28 domain/-s that is/are responsible for the maintenance of stem-like phenotype. As previously reported, TRIM28 is indispensable for cancer stem cell self-renewal and therefore, may emerge as a potential target for anti-cancer therapy. To date, the exact mechanism of TRIM28-dependant regulation of CSC population has not been determined. Due to complex structure of TRIM28 and possessed enzymatic activity mediated through RING and PHD domains, the main purpose of the project is to find the mechanistic explanation of TRIM28-mediated cancer stem cell safeguard.

To identify the TRIM28 domain that is indispensable to maintain pluripotency of CSCs, selected techniques of genetic engineering, molecular biology as well as cell cultures *in vitro* followed by *in vivo* experiments with mice models of subcutaneous tumor growth and *in silico* analyses of high-throughput transcriptomic and proteomic data will be utilized.

Utilization of genetic modification system that enables for downregulation of endogenous TRIM28 with simultaneous expression of exogenous *TRIM28* cDNAs that encode specific domain-disruption mutants in melanoma and glioma cell lines cultured in non-adherent conditions to form 3D spheres will enable to clarify the pro-tumorigenic role of TRIM28 in cancer development and progression. Realization of planned experiments will point at the specific TRIM28 domain and, therefore, at TRIM28 enzymatic activity that is indispensable for CSC self-renewal. Utilization of melanoma and glioma cell line models *in vitro* in addition to hitherto published data with other cell and tumor types may help to verify the versality of TRIM28-dependant mechanism safeguarding the pluripotency of stem cells. Moreover, performed high-throughput transcriptomic and proteomic analyses with subsequent correlation of obtained results with available databases will expand our understanding of specific molecular mechanisms regulating cancer stem cell maintenance. Furthermore, the success of this project may in the future contribute to the design of specific inhibitor that blocks the pro-tumorigenic role of TRIM28. Results of this project will be presented at scientific conferences and published in scientific journals with the international scope, enhancing the competitiveness and innovation of Polish science.