The Role of Promyelocytic Leukemia Protein in Pathological Developmental Pathways in Brain Cancer

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INTRODUCTION

The Promyelocytic Leukemia Protein (PML) is a regulator of cell growth and apoptosis, expressed in all tissues implicated in various ways to cancer biology [1]. However, the specific effects of this protein in cancer are not clear because it can have either tumor suppressing or tumor promoting effects regarding the type of the cancer [1]. In brain, PML participates in the physiological migration of the neural progenitor cells (NPCs)[2], which are also hypothesized to serve as the cell of origin of glioblastoma (GB) [2]. Therefore, we study the role of PML in GB physiology using 3D *in vitro* biological models. We used Optical Microscopy (OM), confocal and Light Sheet Fluorescence Microscopy (LSFM) to study the U87MG GB cell line PML-related growth, cell death and invasion over time.

METHODS

The U87MG GB cell line (U87MG-wt) was lentivirusly transfected with the PML isoform IV (U87MG-PML), which was fused with the fluorophore DsRed. 3D spheroids were generated via the hanging drop technique and cultured with and without an extracellular matrix-like substrate. Brightfield photo-micrographs were captured every 24h to monitor the growth and invasive pattern. The sensitivity to the DZNeP, an EZH2 histone methyltransferase inhibitor, was also tested both in 2D, using the MTT viability assay and in 3D, by estimating the growth elimination. LSFM and confocal imaging was used to visualize the PML expression, using the DsRed distribution and the cell death pattern, using the Draq7 nuclear probe.

RESULTS

The U87MG-PML cells exhibited significant differences compared to the U87MG-wt regarding their growth and invasive properties. The U87MG-PML spheroids were smaller in size, indicating different aggregative and proliferative capacity [2]. The invasive pattern was common in both cell lines adopting the typical starburst morphology [3], yet with altered migration dynamics. The spheroid core of the U87MG-PML spheroids was smaller than the U87MG-wt, though the invasive rim was the same. The LSFM and confocal scans showed a uniform distribution of the PML in both conditions, while the cell death pattern was not considerably affected. The DZNeP effect on tumor growth expansion was not significantly altered by the presence of PML. However, the

migration capacity was significantly inhibited in the U87MG-PML compared to the U87MG-wt, indicating a PML-EZH2 mediated migratory pathway. This is in line with previous findings, suggesting that EZH2 is mainly involved in tumor migration in human malignancies [2], while PML contributes to tumor growth inhibition by decelerating the cell cycle [1], [2].

CONCLUSIONS

GB expansion is attributed to both excessive proliferation and local spreading. Our results are in line with previous findings [1], [2] indicating that these two functions are modulated by distinct cellular mechanisms. We plan to extend these studies using an ex vivo experimental setup to monitor the progression of the tumor in conditions that better mimic the natural microenvironment of GB. Unravelling the role of PML in GB invasion could set PML as a therapeutic target aiming at eliminating multiple sub-clones depending on their proliferative and/or invasive phenotype within the heterogeneous GB tumor.

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