The contribution of PML in cell-fate decision making

Sirago Spanou[1,2 #], Amalia P. Vogiatzoglou[1 #], Takis Makatounakis[1], Ioanna Zota[1,3], Ioannis Charalampopoulos[1,3], Joseph Papamatheakis[1], Androniki Kretsovali[1 *]

[1] IMBB, FORTH, Heraklion, Greece,
[2] Biology Department, University of Crete, Heraklion, Greece
[3] Department of Basic Sciences, Faculty of Medicine, University of Crete, Heraklion, Greece
Presenting author: Sirago Spanou, email: sirago_spanou@imbb.forth.gr & Amalia P. Vogiatzoglou, email: amalia_vogiatzoglou@imbb.forth.gr
* Corresponding author: Androniki Kretsovali, email: kretsova@imbb.forth.gr

ABSTRACT

The promyelocytic leukemia protein (PML) is the core organizer of the cognate nuclear bodies (PML-NBs). Through physical interaction or modification of diverse protein clients, PML-NBs regulate a multitude of -often antithetical- biological processes. PML was originally recognized as a tumor-suppressive factor however more recent studies revealed a “double faced” agent role for PML in cancer. Furthermore in the last decade, a number of publications have implicated PML in the physiology of normal or cancer stem cells. In the present report we study the divergent functions of PML in two different cell types namely: **a**. neuronal stem cells and **b.** breast cancer cells.

a. Although previous studies suggest that PML regulates neuronal plasticity, cognitive function and toxic poly Q protein aggregates clearance there is no conclusive evidence for a role in neuroprotection or neurodegeneration Embryonic Stem (ES) cells that are deficient in PML expression show impaired ability to differentiate along the neuronal lineage. To examine the role of PML in neuronal cell specification, survival and response to stress we employed embryonic Neuronal Stem Cells (eNSC) isolated from E13,5 embryos from WT and PML$^{-/-}$ mice. In eNSCs neuronal differentiation the absence of PML causes a reduction in the percentage of neurons and an increase in the percentage of glial cells. We examined cell survival of NSCs (WT and KO PML) following oxidative, genotoxic or amyloidic stress. PML$^{-/-}$ eNSCs showed an impaired response towards Hydrogen Peroxide and Etoposide. Moreover in the presence of beta-amyloids we observed a significant reduction in their viability. PML KO eNSCs present lower mitochondrial potential and higher ROS levels compared with the WT, reinforcing the hypothesis that key mitochondrial mechanisms are disrupted when PML is absent. Furthermore, the ablation of PML causes a reduction in the autophagic flux as measured by immunodetection of LC3-II. s. We also examined the expression of PML in prefrontal cortexes from control and mouse model (5XFAD) of familial Alzheimers Disease (AD). The reduction of PML staining in the 5XFAD mouse cortex sections could suggest a potential protective role for PML against AD degeneration. In conclusion PML regulates the response to oxidative, genotoxic and beta-amyloid stress of eNSCs and sustains their mitochondrial integrity and autophagic flux.

b. In order to understand the basis of divergent PML functions in cancer, we knocked down PML in breast cancer cell lines and studied the consequence in cell physiology and gene expression. While PML silencing of all PML isoforms in MDAMB231 cells caused a slight increase of the cell growth rate in monolayer culture, yet their tumor sphere ability (TSF) was compromised (25-30% of the control line) implying a role for PML in the maintenance of stemness. The ablation of PML caused a shift towards a more mesenchymal appearance that has been previously linked to increased cell invasiveness and tumor recurrence or metastasis. In accordance with this, PML deficient cells showed increased migration capacities. This observation contradicts the *in vitro* TSF assay suggesting that the growth defect in PML loss can be reversed during *in vivo* tumor growth. Indeed, upon sequential tumor sphere passaging, PML KD cells showed a gradual increase of their TSF ability concomitant with a decrease of the control TSF. These results show that PML KD cells retain higher long-term TSF ability especially when taken from primary tumor sites and/or hypoxic conditions in agreement with their higher *in vivo* tumor growth rate. Taken together these results suggest that PML KD cells acquire higher TIC properties *in vivo* and show a more aggressive phenotype. Tissue hypoxia seems to further intensify this process. Tumors produced by MDAMB231 PML KD cells showed lower necrotic content suggesting higher vascular supply. In agreement with this, CD31 immunocytochemistry showed significant increase of microvessel density in PML KD primary tumors.

Collectively, PML enhances the ability of neural stem cells to overcome degeneration-causing stimuli and opposes the metastatic activity of MDAMB231 breast cancer cells.