1466 GALECTIN-1 ORCHESTRATES A HIERARCHICAL TUMOR TO STROMAL EXTRACELLULAR VESICLES SYSTEM THAT FOSTERS IMMUNE SUPPRESSION

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Background Extracellular vesicles (EVs) suppress effector cells and activate immunosuppressive cells in the 'escape phase' of tumor immunoediting. Tumor derived EVs contribute to the differentiation and expansion of immunosuppressive cell subsets. Bone marrow-derived cells (BMDCs) can uptake EVs released by 4T1 -a triple-negative breast cancer cell line- and induce myeloid-derived suppressor cells (MDSCs).[1] Immunosuppressive myeloid cells expansion can also be promoted by their own EV in an autocrine manner. Moreover, EVs secreted by immunosuppressive myeloid cells, may inherit their parental functions.[2] Nevertheless, the molecular circuits and mediators of tumor microenvironment EV release remain uncertain.

Methods We generated immunosuppressive myeloid cells by culturing mouse BMDCs with GM-CSF for three days in the presence of Gal1. Additionally, we cultured 4T1 WT and *Lgals1* knocked out cells. We purified Small EVs by size exclusion chromatography from conditioned medium of immunosuppressive myeloid cells (control and Gal1-treated) or 4T1 cells (WT or Gal1 KO). We further purified 4T1 EVs with CD63+ beads, a tumoral EV marker. ELISA, flow cytometry, miRNA sequencing, proteomic and metabolomic analyses verified EV identity. We performed functional assays by co-culturing tumoral or myeloid EVs with BMDC, activated T or B cells.

Results When exposed to EVs from 4T1 cells *in vitro*, BMDCs switched their differentiation pathway to a M-MDSCs phenotype ($CD11b^{+}Ly6G^{-}Ly6C^{hi}$) and showed higher immune checkpoint molecules expression, including PD-L1 ($p=0.005$) and VISTA ($p=0.003$). However, when exposed to tumoral EVs lacking Gal1, BMDCs reinforced a PMN-MDSCs phenotype ($CD11b^{+}Ly6G^{+}Ly6C^{lo}$). In contrast with $Gal1^{+}$ EVs, tumoral EVs lacking Gal1 failed to inhibit $CD4^{+}$ and $CD8^{+}$ T and B cell proliferation and activation. Gal1 is both in the cargo and corona of 4T1 EVs as revealed by flow cytometry and ELISA ($p=0.0032$). Moreover, EV production inhibition with GW4869 in 4T1 cells decreased Gal1 levels secreted ($p=0.037$). To analyze EV-derived Gal1 contribution as an MDSC autocrine signal, we added EVs from Gal1-treated immunosuppressive myeloid cells to new BMDCs and observed $CD11b^{+}$ cells expansion and higher VISTA expression on cells with M-MDSCs phenotype. Moreover, these EVs showed greater T cell-suppressive capacity and has different protein, metabolic, and miRNA cargo compared to control EVs.

Conclusions Here, we propose that $Gal1^{+}$ EVs released by tumoral cells interact with myeloid cells and potentiate their immunosuppressive properties, including the release of EV with autocrine and paracrine functions. Targeting Gal1 as a molecular mechanism of EV-mediated tumor development may help overcome therapy resistance.

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