CANCER STEM CELLS AND PD-L1-EXPRESING EXOSOMES SUPPRESS ANTI-TUMOR EFFECTOR B CELLS

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Background We have identified anti-tumor effector B cells in adaptive immunotherapy. We also reported that cancer stem cell (CSC) vaccination induces CSC-specific T and B cell responses associated with the therapeutic efficacy which can be enhanced by anti-PD-L1 administration. Tumor cells were reported to suppress T cell function via secretion of exosomes that express PD-L1. However, the role of exosomal PD-L1 in CSC-B cell interaction is unknown.

Methods 4T1 and CT26 tumor-bearing mice were administered with anti-CD20 mAb to deplete B cells prior to anti-PD-L1 mAb administration. We isolated exosomes from D5 tumor cell culture media. To investigate if exosomal PD-L1 contributes to immunosuppression of B cells, we generated Rab27ako D5 cells to delete exosomes and PD-L1ko D5 cells by CRISPR/Cas9 gene editing system.

Results In vivo B cell depletion significantly abrogated the efficacy of anti-PD-L1 with more aggressive tumor growth, indicating the anti-tumor effect of anti-PD-L1 involves host B cells. We detected upregulated expression of PD-1 on activated B cells, and higher PD-L1 expression on ALDH^high^ CSCs than on ALDH^low^ non-CSCs. ALDH^high^ CSCs directly suppressed IgG production by purified normal spleen B cells in culture and the suppression could be blocked by anti-PD-L1 in a dose-dependent manner. Anti-PD-L1 also rescued CSC suppression on the IgG production by B cells isolated from the spleen of tumor-bearing mice. Administration of anti-PD-L1 partially recovered the humoral immune response, confirming the PD-L1/PD-1 pathway involvement in B cell suppression by CSCs. D5-derived exosomes suppressed B cell proliferation and IgG production in vitro, and administration of these exosomes promoted tumor growth and reduced animal survival, indicating the role of tumor-derived exosomes in B cell immunosuppression. Tumor-derived exosomes expressed PD-L1, but PD-L1 was absent in the exosomes isolated from the PD-L1^ko^ tumor cells as evident by western blot and flow cytometry. Deletion of Rab27a or PD-L1 did not affect the proliferation of the tumor cells in vitro. However, Rab27a^ko^ or PD-L1^ko^ resulted in significantly enhanced host anti-tumor immunity evident by reduced 4T1 tumor growth in vivo compared with the WT tumor. Furthermore, anti-PD-L1 therapy significantly reduced Rab27a^ko^ or PD-L1^ko^4T1 tumor growth and prolonged animal survival vs. the WT tumor control.

Conclusions PD-L1-expressing CSCs and exosomes suppress B cells via the PD-L1/PD-1 axis. Isolation of Rab27a^ko^ ALDH^high^ CSCs will help further characterize the suppression of CSC-derived exosomes on host B cells as well as T cells.