LIPOSOMAL DOXORUBICIN ENHANCES THE RADIATION-INDUCED ABSCOPAL EFFECT BY PROMOTING THE RELEASE OF TUMOR CELL MITOCHONDRIAL DNA

Liqun Wang*, Ren Luo, Kateryna Onyshchenko, Gabriele Niedermann. University of Freiburg, Freiburg, Germany

**Background** Localized radiotherapy (RT) can cause a T cell-mediated abscopal effect on non-irradiated tumor lesions, particularly in combination with immune checkpoint blockade (ICB). By using syngeneic tumor models, we studied whether adding low-dose doxorubicin to RT and αPD-1 can enhance the RT-induced abscopal effect.

**Methods** In mice bearing bilateral subcutaneous tumors, the primary tumor was irradiated with 2 × 12 Gy (B16-CD133 melanoma model) or 3 × 8 Gy (MC38 colon carcinoma model). Liposomal doxorubicin (4 mg/kg) was given i.v. once together with RT; αPD1 was given weekly. Tumor growth and survival of mice were determined (5–9 mice per group). Depleting antibodies were used to elucidate whether the abscopal effect depended on CD8+ T cells. Tumor-specific CD8+ T cells were determined flow cytometrically using MHC tetramers and various antibodies. Mitochondrial DNA (mtDNA) was depleted in tumor cells with Zalcitabine. In vitro, extracellular (e)ATP release by tumor cells was determined by CellTiter-Glo® 2.0. Tumor cell production of type I Interferon (IFNb1) was measured by ELISA with/without incubation with cGAS-STING pathway inhibitors. CXCL10, cytosolic genomic DNA (gDNA), and cytosolic mtDNA were measured by qPCR.

**Results** Abscopal tumor control was as follows: RT/αPD-1/doxorubicin > doxorubicin/αPD-1 (p < 0.01) = RT/doxorubicin (p < 0.01) > RT/αPD-1 (p < 0.05) (B16 melanoma model); RT/αPD-1/doxorubicin > RT/αPD-1 (p < 0.01) = doxorubicin (p < 0.001) > doxorubicin/αPD-1 (p < 0.01) (MC38 colon carcinoma model). Experiments with various inhibitors of the cGAS/STING pathway showed that liposomal doxorubicin induced type I IFN through the cGAS/STING pathway (p < 0.05 with vs. without inhibitors). In mtDNA-depleted tumor cells, doxorubicin induced less cytosolic mtDNA (p < 0.001) (but not less cytosolic genomic DNA), less IFNb1 secretion (p < 0.05), less eATP release (p < 0.0001), and less CXCL10 (p < 0.0001) than in non-mtDNA-depleted tumor cells. Triple therapy with RT, αPD-1, and liposomal doxorubicin induced more mature dendritic cells (p < 0.05) and more tumor-specific CD8+ T cells (p < 0.01) compared to RT/αPD-1 and doxorubicin/αPD-1 therapy. When CD8+ T cells were depleted or mtDNA-depleted tumor cells were implanted, the doxorubicin-induced enhancement of the abscopal effect was abolished (p < 0.05).

**Conclusions** Single low-dose liposomal doxorubicin can substantially enhance the RT-induced abscopal effect in conjunction with αPD-1, mtDNA leakage induced by doxorubicin appears crucial for the doxorubicin-enhanced RT-induced abscopal effect. These findings may be helpful for the planning of clinical radiochemoimmunotherapy trials in (oligo) metastatic patients.

**Ethics Approval** All animal experiments were approved by the animal care committee of the Regierungspräsidium Freiburg (registration number: G18-066).