TUMOR CELL-INTRINSIC MTORC1 SIGNALING THROUGH RAPTOR MAKES MELANOMA AND OVARIAN CANCER IMMUNOTHERAPY RESISTANT BY REGULATING INTERFERON-GAMMA RESPONSIVENESS AND PROMOTING TUMOR-INITIATING CELLS

¹Harshita Gupta*, ²Suresh Kari, ²Emily Salinas, ²Haiyan Bai, ²Erica Osta, ²Anand Kornepati, ²Juan Wang, ²Xinyue Zhang, ²Yidong Chen, ²Ratna Vadlamudi, ²Tyler Curiel. ¹UTHSCSA, San Antonio, TX, USA; ²UTHSA, San Antonio, TX, USA

Background Although immunotherapy can induce durable anti-tumor response in multiple cancers, immune checkpoint blockade (ICB) therapy resistance in ovarian cancer and melanoma remains problematic. Here, we report that tumor cell-intrinsic mTORC1 regulates ICB response through mTORC1 defining subunit Raptor (Rptor) by modulating interferon-gamma (IFN γ) resistance and tumor-initiating cell (TIC) virulence.

Methods We knocked down two distinct mTORC1 signaling components: Rptor (Rptorlo, aids in mTORC1 assembly) and Lamtor1 (Ltor1lo, docks mTORC1 on lysosomes) in murine ovarian cancer ID8agg and melanoma B16 cells. PD-L1 was CRISPR knocked out in B16 and human ovarian cancer line ES2. Mice with tumors were treated with a-PD-L1 $\pm$ a-CD8 antibody. TICs were estimated by flow-cytometry.¹

Results Rptorlo B16 and ID8agg, but not Ltor1lo B16 tumors grew slower and were a-PD-L1 responsive unlike control (ctrl) in WT mice. We noted that ctrl and Rptorlo B16 and ID8agg cells expressed similar surface PD-L1 in vitro. Thus, Rptor suppresses a-PD-L1 response in ICB-resistant tumors. Tumor immune analysis revealed increased CD8+ T cell% and a trend to increased IFN γ +CD8+ T cells in a-PD-L1 treated Rptorlo, but not ctrl B16. Rptorlo a-PD-L1 efficacy was lost with a-CD8 and in IFN γ knockout mice. In vitro, IFN γ suppressed Rptorlo ID8agg proliferation, unlike ctrl. These data suggested that lack of Rptor makes tumors ICB responsive, possibly by making tumors IFN γ -sensitive and increasing IFN γ +CD8+ T cells. Further, tumor and draining lymph node (DLN) TCF1+PD-1+ T cell stem cells (critical for aPD-L1/PD-1 success^{2 3}) were significantly higher in a-PD-L1 treated Rptorlo tumors. Thus, tumor Rptor status could regulate tumor microenvironment and distal DLN immune landscape on a-PD-L1 treatment. We previously published that mTORC1 promotes PD-L1-dependent tumor proliferation, TIC virulence^{1 4} PD-L1KO B16 and ES2 cells expressed similar total Rptor protein. However, lower levels of Rptor were loaded in mTOR complex in absence of PD-L1, as assessed by a-mTOR immunoprecipitation, suggesting that pro-tumorigenic Rptor functions were downstream of, and dependent on PD-L1. Successful Rptorlo aPD-L1 treatment reduced TIC in vivo, an effect reversed in absence of CD8+ T cells or host IFN γ . Inhibiting ID8agg mTORC1 with rapamycin reduced stemness genes oct4, nanog expression by QPCR. Further, ID8agg Rptorlo TIC formed significantly smaller tumors versus ctrl TIC in immune-compromised NSG mice, confirming their reduced virulence. Rptor, but not Ltor1, expression inversely correlated with tumor CD8+ infiltrate in IMvigor210 trial, and strongly with TIC gene signature in ovarian cancer patients.^{5 6}

Conclusions Tumor-cell intrinsic Rptor modulates ICB resistance, IFN γ responsiveness, immune microenvironment, and TIC virulence.

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Trial Registration N/A

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