Tumor and Stromal Cell Biology

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DEPLETING NON-CANONICAL, CELL-INTRINSIC PD-L1 SIGNALS INDUCES SYNTHETIC LETHALITY TO SMALL MOLECULE DNA DAMAGE RESPONSE INHIBITORS IN AN IMMUNE INDEPENDENT AND DEPENDENT MANNER

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Background Tumor surface-expressed programmed death-ligand 1 (PD-L1) suppresses immunity when it engages programmed death-1 (PD-1) on anti-tumor immune cells in canonical PD-L1/PD-1.¹ Non-canonical, tumour-intrinsic PD-L1 signals can mediate treatment resistance²⁻⁶ but mechanisms remain incompletely understood. Targeting non-canonical, cell-intrinsic PD-L1 signals, especially modulation of the DNA damage response (DDR), remains largely untapped.

Methods We made PD-L1 knockout (PD-L1 KO) murine transplantable and human cell lines representing melanoma, bladder, and breast histologies. We used biochemical, genetic, and cell-biology techniques for mechanistic insights into tumor-intrinsic PD-L1 control of specific DDR and DNA repair pathways. We generated a novel inducible melanoma GEMM lacking PD-L1 only in melanocytes to corroborate DDR alterations observed in PD-L1 KO of established tumors.

Results Genetic tumor PD-L1 depletion destabilized Chk2 and impaired ATM/Chk2, but not ATR/Chk1 DDR. PD-L1KO increased DNA damage (γ H2AX) and impaired homologous recombination DNA repair (p-RPA32, BRCA1, RAD51 nuclear foci) and function (DR-GFP reporter). PD-L1 KO cells were significantly more sensitive versus controls to DDR inhibitors (DDRi) against ATR, Chk1, and PARP but not ATM in multiple human and mouse tumor models in vitro and in vivo in NSG mice. PD-1 independent, intracellular, not surface PD-L1 stabilized Chk2 protein with minimal Chk2 mRNA effect. Mechanistically, PD-L1 could directly complex with Chk2, protecting it from PIRH2-mediated polyubiquitination. PD-L1 N-terminal domains Ig-V and Ig-C but not the PD-L1 C-terminal tail co-IP'd with Chk2 and restored Chk1 inhibitor (Chk1i) treatment resistance. Tumor PD-L1 expression correlated with Chk1i sensitivity in 44 primary human small cell lung cancer cell lines, implicating tumor-intrinsic PD-L1 as a DDRi response biomarker. In WT mice, genetic PD-L1 depletion but not surface PD-L1 blockade with α PD-L1, sensitized immunotherapy-resistant, BRCA1-WT 4T1 tumors to PARP inhibitor (PARPi). PARPi effects were reduced on PD-L1 KO tumors in RAG2KO mice indicating immune-dependent DDRi efficacy. Tumor PD-L1 depletion, likely due to impaired DDR, enhanced PARPi induced tumor-intrinsic STING activation (e.g., p-TBK1, CCL5) suggesting potential to augment immunotherapies.

Conclusions We challenge the prevailing surface PD-L1 paradigm and establish a novel mechanism for cell-intrinsic PD-L1 control of the DDR and gene product expression. We identify therapeutic vulnerabilities from tumor PD-L1 depletion utilizing small molecule DDRi currently being tested in clinical trials. Data could explain α PD-L1/DDRi treatment resistance. Intracellular PD-L1 could be a pharmacologically targetable treatment target and/or response biomarker for selective DDRi alone plus other immunotherapies.

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