A TUMOR-LUNG NLRP3-TLR4 DISTANT SIGNALING AXIS DRIVES IMMUNOTHERAPY RESISTANCE VIA G-CSF-DEPENDENT EXPANSION OF CIRCULATING PD1+ PMN-MDSCS

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Background The majority of solid tumor cancer patients do not benefit from current immunotherapy options due to the development of either primary or secondary resistance. We recently determined that the tumor-intrinsic NLRP3 inflammasome drives the recruitment of granulocytic myeloid-derived suppressor cells (PMN-MDSCs) and adaptive resistance to anti-PD-1 immunotherapy via a HSP70-TLR4 signaling axis. Prior studies have associated TLR4 gain-of-function mutations with inferior clinical outcomes while pulmonary inflammation has been correlated with anti-PD-1 resistance. How lung-expressed TLR4 and lung-derived factors influence tumor immunity at distant sites remains poorly understood.

Methods We engineered an inducible lung epithelial-specific TLR4 knock-out transgenic mouse model (SPC-TLR4 -/-) to investigate the impact of lung TLR4 on anti-PD-1 immunotherapy responses in BRAFV600ECDKN2A-/-PTEN-/- (YUMM1) melanoma and EO771 breast cancer syngeneic tumor models. Genetic silencing of NLRP3 and HSP70 was performed in tumor models. Immunohistochemistry, qrt-PCR, Western blot analysis, and multi-parameter flow cytometry was used to characterize tumor NLRP3-lung TLR4 crosstalk and its impact on anti-tumor immunity. Baseline tumor specimens and plasma samples derived from 40 stage IV melanoma patients undergoing anti-PD-1 immunotherapy were interrogated for intrinsic NLRP3 inflammasome activation levels by NLRP3-ASC proximity ligation and HSP70 ELISA assays. Objective responses were assessed by computed tomography imaging at week 12 of therapy based on RECIST1.1 criteria.

Results Anti-PD-1 immunotherapy expands circulating levels of PD-1+ PMN-MDSCs in mice harboring YUMM1 and EO771 breast cancer models. This was noted to correlate with elevated plasma levels of HSP70 and G-CSF. Both observations were ablated in mice harboring NLRP3-/- tumors as well as in SPC-TLR4 -/- hosts. Further studies found lung epithelial HSP70-TLR4 signaling to induce G-CSF release in a Wnt5a-dependent manner. Pharmacologic inhibition of HSP70 suppresses lung epithelial Wnt5a and G-CSF expression and inhibits the accumulation of PD-1+ PMN-MDSCs in the circulation. Further studies demonstrate the distant lung TLR4-Wnt5a-G-CSF axis promotes PD-1+ PMN-MDSC accumulation and primary tumor progression. Baseline tumor NLRP3 inflammasome activity (P = 0.0014) and plasma HSP70 levels (P = 0.0008) in stage IV melanoma patients independently correlate with elevated circulating PMNs and disease progression during anti-PD-1 immunotherapy.

Conclusions Together, these results describe a novel TLR4-Wnt5a-G-CSF signaling axis in the lung epithelium that induces the systemic expansion of PD-1+ PMN in response to activation of the tumor-intrinsic NLRP3 inflammasome. We conclude that this tumor-lung crosstalk supports primary tumor growth and contributes to anti-PD-1 immunotherapy resistance partially by serving as an anti-PD-1 antibody sink. Pharmacologic inhibition of NLRP3 and HSP70 represents promising strategies for overcoming anti-PD-1 resistance.