ACTIVITY OF THE TUMOR-INTRINSIC NLRP3 INFLAMMASOME PATHWAY PREDICTS FOR RESPONSE TO CHECKPOINT INHIBITOR IMMUNOTHERAPY IN MELANOMA PATIENTS

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Background We have previously determined that activation of a novel tumor-intrinsic NOD-, LRR- and pyrin domain-containing protein-3 (NLRP3) inflammasome-heat shock protein-70 (HSP70) signaling axis in response to PD-1 blockade triggers the recruitment of granulocytic myeloid-derived suppressor cells (PMN-MDSCs) into the tumor microenvironment, suppresses anti-tumor immunity and, in select settings, promotes tumor hyperprogression. We, therefore, sought to determine whether the activity of the tumor-intrinsic NLRP3-HSP70 pathway may correlate with anti-PD-1 response by interrogating clinical specimens derived from advanced melanoma patients undergoing anti-PD-1 monotherapy.

Methods Three independent approaches were utilized to measure the activity of the tumor-intrinsic NLRP3-HSP70 signaling pathway in 60 advanced melanoma patients undergoing either pembrolizumab or nivolumab monotherapy: 1. baseline week 0 plasma HSP70 levels were measured by ELISA, 2. germline PCR-based genotyping was performed to detect the single-nucleotide polymorphism (SNP), rs12239046, previously associated with enhanced NLRP3 expression, 3. PCR-based proximity ligation assay (PLA) analysis targeting the NLRP3-ASC proteins in baseline formalin-fixed paraffin-embedded tumor tissue specimens. Detection of the rs12239046 SNP was correlated with progression-free survival (PFS) while plasma HSP70 and NLRP3-ASC PLA levels were correlated with objective response (OR) based on RECIST1.1 assessment of week-12 CT imaging as well as PFS and overall survival (OS).

Results Our studies demonstrate that elevated baseline plasma HSP70 levels \((P = 0.0008)\) and elevated baseline tissue NLRP3-ASC PLA levels \((P = 0.0014)\) independently correlate with resistance to anti-PD-1 immunotherapy (ICI) based on week-12 OR in melanoma patients. Importantly, melanoma patients developing disease hyperprogression \((n=5)\) in response to ICI exhibited elevations in baseline plasma HSP70 levels \((P = <0.0001)\) and baseline tissue NLRP3-ASC PLA levels \((P = <0.0001)\) relative to patients with week-12 disease progression \((n=10)\). Above median baseline tissue NLRP3-ASC PLA levels were determined to correlate with both inferior PFS (HR 0.12, \(P = 0.0008\)) and OS (HR 0.16, \(P = 0.0456\)) in advanced melanoma patients undergoing ICI. Germline PCR detection of the rs12239046-C SNP was found to be associated with elevated plasma HSP70 levels and trended toward a correlation with inferior PFS (HR 0.50, \(P = 0.07\)).

Conclusions Baseline markers of the tumor-intrinsic NLRP3-HSP70 signaling pathway correlate with resistance and disease hyperprogression in melanoma patients undergoing anti-PD-1 immunotherapy. These data strongly support the important role of the tumor-intrinsic NLRP3 inflammasome in regulating responses to anti-PD-1 therapy and verify its relevance as a pharmacologic target to enhance immunotherapy efficacy. Expanded studies are warranted to confirm these findings in a larger patient cohort.