IMMUNE PROFILING OF KEAP1-DEFICIENT NSCLC: DEVELOPMENT OF THERAPEUTIC STRATEGIES TO OVERCOME RESISTANCE TO IMMUNOTHERAPY

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Background In LUAD, KEAP1 is the third most common tumor suppressor and loss-of-function mutations in KEAP1 commonly co-occur with STK11/LKB1 and KRAS mutations. KEAP1 protein that regulates the degradation of the antioxidant transcription factor NRF2. The role of STK11/LKB1 mutations in immunotherapy resistance has been characterized, however the mechanistic understanding of KEAP1 deficiency in shaping LUAD phenotype and therapy response is still very limited. Recent clinical data has been reported suggesting that mutations in STK11/LKB1 and KEAP1 are strongly associated with immune checkpoint blockade resistance in LUAD, particularly those with KRAS mutations. Nevertheless, the biology of KEAP1-deficient tumors and the immune suppression mechanisms are to be characterized.

Methods We have first validated response to anti-PD1 treatment in vivo using subcutaneous murine models, and performed a deep profiling and characterization of tumor microenvironment (TME) heterogeneity of KRAS-mutant (K) and LKB1 (KL), and/or KEAP1 deficient (KK and KKL) tumors using single-cell RNA sequencing (scRNA-seq) and multiplex staining. Data from pre-clinical models has been used to survey the immune genomic data available from the MD Anderson ICON study (a cohort of early stage lung cancer untreated 148 resected tumors) and TCGA lung cohorts to further validate our findings.

Results While K tumors showed significant response to anti-PD1 treatment, KEAP1 loss completely impaired therapeutic response to this immunotherapy. KEAP1-deficient tumors were characterized by low immune infiltration while displayed an enrichment of cancer associated fibroblasts (CAFs) and endothelial cells. scRNA-seq data indicated a significant reduction of T cell infiltration, in particular, CD8 and NK T cells, pronounced decreased of B cell population and a marked M2 macrophages polarization. Likewise, IHC and multiplex analysis of CD3 and F4/80 markers confirmed these previous findings. In TCGA lung cancer cohort, CD8B expression was dramatically decreased while MIF (macrophage migration inhibitory factor) was upregulated in KK compared to K LUADs tumors, and expression of KEAP1 inversely correlated with CD163, ARG2 and IL10, which are mainly secreted by macrophages. Concordantly, KEAP1-deficient pre-clinical tumors showed a significant upregulation of MIF expression and secretion, and CRISPR-Cas9 deletion of MIF dramatically impaired in vivo tumor growth in KK and KKL but not in K or KL models.

Conclusions These findings indicate that loss of KEAP1, alone or in combination with STK11/LKB1 alterations, unfavorably reprograms TME. These changes appear to be mediated at least in part through MIF upregulation, providing a potential therapeutic strategy for overcoming KEAP1-dependent resistance to immunotherapy.

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